



Kidney Precision Medicine Workshop

DoubleTree by Hilton Hotel
8120 Wisconsin Avenue
Bethesda, MD
May 23–25, 2016

Meeting Summary

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) convened a 2.5-day workshop to obtain input from a wide range of stakeholders—including academic researchers, bioethicists, clinicians, patients, and pharmaceutical industry representatives—on a proposed initiative to develop a precision medicine approach to treating acute kidney injury (AKI) and chronic kidney disease (CKD). In addition to hearing formal presentations, attendees participated in facilitated discussions in six breakout sessions to provide input to the NIDDK on the challenges and key issues relevant to the Kidney Precision Medicine Project (KPMP). Reports from these breakout sessions are summarized in this document. Detailed accounts of the discussions that took place during the sessions are provided in Appendices A through F. Prior to the breakout sessions, a panel of experts provided their perspectives on how to analyze kidney tissue, a summary of which also is included below.

Six Perspectives on How to Analyze Kidney Tissue

Dr. Andrew McMahon provided a perspective on kidney tissue analysis in the context of the GenitoUrinary Development Molecular Anatomy Project (GUDMAP). GUDMAP's goal is to develop a molecular-based anatomical understanding of the developing genitourinary system. The information and tools contained in GUDMAP include anatomical analyses of gene expression, molecular signatures of specific cell types and regions, transgenic mouse strains, and ontology to group data. Developmental data from mouse models are providing insights into human development. The first stage of GUDMAP was to develop detailed histological data of kidney development. Providing public access to high-resolution images was coupled with producing new tools for annotation of image files. The mouse anchor genes form a key data set of GUDMAP, providing single-marker identification of tissue types. Sixty percent replication has been found when comparing mouse anchor genes for tissue types to human. Differences, therefore, can be expected between mouse and human development. For example, distinct progenitor lineage compartments were found to exist in mouse and human tissues. One approach to resolving cell types that Dr. McMahon illustrated for a section of the glomerulus is using multiple hybridization chain reaction (HCR) probes. Antibodies have proved to be some of the best tools for characterizing structures in emerging anatomy, such as the initiation and later stages of nephrogenesis, and the technique is applicable to both mouse and human. GUDMAP is advancing from two-dimensional (2D) imaging to developing views of the three-dimensional (3D) architecture of kidney development by sectioning lobes of the human kidney, producing 3D models of the nephrogenic niche. In summary, GUDMAP has used mouse as a blueprint to understand the basis for human anatomy. Dr. McMahon predicted that a detailed understanding of development will be key in understanding the deep basis of disease (e.g., low nephron count is highly associated with CKD).

Dr. Stephen Hewitt argued for the need to redefine renal dysfunction. He stated that what is normal in tissue needs to be better defined, and “normal” may, in fact, be highly variable. To help redefine renal dysfunction, Dr. Hewitt endorsed conducting biopsies early in disease development and after treatment. In conducting biopsies, sufficient tissue is required.

Improved analytical technologies alone will not be sufficient for a new understanding of renal dysfunction. Instead, pathophysiology will drive a better understanding of disease. Evaluating kidney histopathology needs to be redefined, and all compartments of the kidney need to be included in this effort. After defining the lesions that have a pathophysiology relationship, the frequency and specificity of lesions will need to be understood.

Clinical trials—including Phases 1, 2, and 3—should be initiated constantly and kept small and short term in the discovery phase. Otherwise, the treatments being tested risk being out of date. Biological endpoints in addition to clinical responses need to be considered in clinical trials. In addition to clinical trials, new models are needed that can be credentialed by comparative physiology in terms of what maps to humans, and Dr. Hewitt proposed the dog as a model for studying kidney disease.

Dr. Katalin Susztak’s work focuses on tissue from nephrectomies. As part of the 1,000 Kidney Genetic, Genomics Project (1K2GP), her laboratory collects real-time updated clinical data (e.g., kidney function, cardiovascular disease, diabetes), histopathology, genotyping, transcriptome analysis of microdissected samples, epigenome analysis, and cell type-specific data. Her paradigm is that CKD is a gene-environmental disease. Dr. Susztak provided an overview of some of the research being conducted in her laboratory. In human diabetic and hypertensive kidney disease, specific changes in gene modules have been found. CKD is associated with structural changes (fibrosis) and functional traits in the kidney. Gene expression changes were found to be associated with fibrosis (immune system-related pathways) and estimated glomerular filtration rate (eGFR) (metabolic pathways). The study of conserved transcriptional changes between mouse and human kidney fibrosis is an approach to developing new target pathways for therapeutics. Genotype-driven gene expression in normal kidneys has been found to be associated with disease risk. Determining which genes are causal in kidney disease will allow targeting of new therapeutics.

Dr. Betty Diamond discussed practical considerations for single-cell analysis (SCA) of kidney tissue in lupus nephritis (LN) as part of the Accelerating Medicines Partnership (AMP) in LN. AMP/LN, was established to perform SCA of tissue, develop new molecular targets, stratify patients, identify predictors of response, and seek surrogates of kidney disease processes outside the kidney. Because LN is not as common as diabetic nephropathy, multiple sites were needed for sample collection. A common protocol was developed which involved freezing kidney biopsies at the site and subsequently digesting them enzymatically. The protocol was optimized to increase cell yield and viability. Disaggregating frozen tissue was found to be a better approach than disaggregating fresh tissue and then freezing the cells. Frozen and HypoThermosol[®]-preserved tissue were compared, and frozen tissue was found to perform as well and to be the more widely applicable approach. Although only between 5,000 and 20,000 cells are obtained per biopsy, their viability is good. Differences in gene expression were found between LN and tumor nephrectomy controls. Approximately 3,000 genes were identified, and the dominant cell type differed from normal kidney. The interferon-positive signature found in blood cells of patients with LN also has been found in urinary cells. SCA mostly produces good yields for gene expression. Generally, insufficient numbers of cells are obtained for performing both single-cell mass cytometry (CyTOF) and RNA sequencing (RNA-seq). The protocol allows collecting samples from multiple clinical sites worldwide and freezing them to avoid batch effects and allow processing together. It is anticipated that disease-specific signatures will be found and that kidney and urinary samples will be analyzed together. The remaining technical problem is to increase cell yield and recovery of all cell types, which would

allow the performance of RNA-seq on multiple cell populations and the combination of RNA-seq with other technologies.

Dr. Maria Gomez provided an overview of the Biomarker Enterprise to Attack DKD (BEAt-DKD), an Innovative Medicines Initiative (IMI) program comprising industrial and academic partners. The overall goals of the program include the following: (1) to identify targetable mechanisms and pathways under initiation and progression of diabetic kidney disease (DKD) and (2) to identify and validate biomarkers of disease progression and treatment responses. BEAt-DKD will gather data from large observational studies and clinical trials, including clinical samples, biopsy samples, and genetic data. The discovery work packages will entail identifying biomarkers from the observational prospective studies, validating the efficacy of the biomarkers in intervention studies, identifying mechanisms and pathways, and using biomarkers for imaging. Work also will be performed to validate and integrate the data. Regulatory concerns will be integrated from the start to facilitate translations to clinical practice. From each patient, anthropomorphic, laboratory, and clinical data will be collected. In addition to kidney biopsies, samples will be collected of plasma serum, circulating blood cells, and urinary vesicles; and magnetic resonance imaging (MRI) and ultrasound imaging will be performed.

Dr. Avi Rosenberg spoke on the use of targeted mass spectrometry for tissue analysis. He indicated the need to adapt technologies to formalin-fixed, paraffin-embedded (FFPE) samples. Different technologies require different sample types. Whole scrape sampling is applicable to homogeneous tumors and histologically simple tissue that meets analytical requirements; laser capture microdissection (LCM) is applicable to histologically diverse tissue or intermixed tumor and normal tissue; and expression microdissection (xMD) is useful for subcellular targets, cell-type specific analysis, and an intermixed population of cells. Abundant technologies exist for multiplexing nucleic acid analyses. For proteomic multiplexing analysis, targeted mass spectrometry using multiple reaction monitoring (MRM) is the state of the art. Discovery mass spectrometry provides no amplification and is biased toward the most abundant peptides and proteins. In contrast, MRM requires prior knowledge of proteins or peptides of interest, removes the bias toward high-abundance proteins or peptides, and provides semi-quantitative results. Dr. Rosenberg applied MRM to studying collagens, which are significantly conserved with common repeat elements. The expression of some collagens differs by tissue type. In kidney, fibrosis produces a unique collagen profile. Dr. Rosenberg compared the advantages and disadvantages of targeted mass spectrometry. Advantages include that the technology is multiplex, quantitative, and sensitive; can include post-translational modifications; and allows identification of approximately 500 peptides in 30 minutes. Disadvantages include proteome limitations in FFPE samples, expensive startup, the need for upfront validation, sample loss during processing, and the lack of amplification. Many pre-analytical factors—including those occurring during surgery (e.g., ischemic time), pathology and processing (e.g., fixation conditions, tissue processing, size of tissue sample), and sectioning and storage (e.g., storage conditions)—affect proteomic analysis of FFPE tissue.

Panel Discussion

The following points were made in the discussion with Dr. Hewitt about his perspective on analyzing kidney tissue:

- More samples are needed to develop biomarkers. The kidney needs to be considered as a whole organ.
- The problem of standardizing analysis of exosomes will require collaboration among researchers, perhaps in a workshop setting where approaches could be compared. Different assays have differing sensitivities.

The following points were made in the discussion with Dr. Diamond about SCA for LN:

- Stored urine samples exist for noncellular RNA analysis.
- The freeze and thaw protocol is being modified to improve yield. Improvements are being investigated by varying such factors as the division of samples prior to freezing, the length of time in cryostorage, and thawing protocols.

The following points were made in the discussion with Dr. Gomez about BEAt-DKD:

- Based on clinical chemistry results, five subgroups have been identified in the cohort. Patients who are insulin resistant are being followed most closely for kidney disease.
- Analysis is being performed on urinary vesicles to determine how well data agree with other sample types, including biopsy and nephrectomy tissue.
- Basic research is needed to better understand urinary vesicles. Evidence exists that they are heterogeneous, which will complicate the interpretation of RNA data from urinary vesicles.

The following points were made in the discussion with Dr. Rosenberg about targeted mass spectrometry:

- Detection limit improvements are needed. Current analytical techniques require large sample sizes (e.g., 50 mL urine samples for exosomes), thereby creating storage problems. Miniaturizing procedures is a promising approach for improving sensitivity.

Day 1 Breakout Group Reports

Tissue Interrogation Session

Dr. Rosenberg summarized the discussions of the Tissue Interrogation Breakout Group. The group began its session with short presentations on state-of-the-art tissue interrogation techniques. The group followed the presentations with discussions aimed at developing recommendations to the NIDDK on the next steps for tissue interrogation. Topics considered included heterogeneity within kidney tissue and cells, how deeply to interrogate tissue, and how to analyze and visualize results.

Dr. Rosenberg provided an overview of the short presentations. Dr. Nir Hacohen spoke about dissociation and analysis to identify cell types. SCA interrogates fewer cells than Drop-seq but at great depth. To improve detection of gene expression in cells with low expression, compounded genetic signatures from aggregated cells can be used. Dr. Susztak discussed epigenetics, genetics, and proteomic analyses of DKD biopsies. She emphasized the temporal nature of RNA and the utility of tumor nephrectomy specimens. Dr. Jeremy Norris described performing proteomics and lipidomics on whole renal tissue samples. He provided images of infection. In his discussion of multiplex expression approaches, Dr. McMahon summarized various approaches to multiplexed imaging, including CyTOF. Dr. Hewitt discussed object-oriented pathology and linking molecular pathology and physiology. He emphasized the need for a detailed path to establish ground truths and training for lesion-specific pathology. Dr. Rosenberg described rapid microdissection using immunohistochemical targeting of cellular and subcellular targets. Dr. Mark Knepper presented on deep sequencing of microdissected tubules, which has created a public resource of deep sequencing results for rat tubules. Dr. Gomez described a cell encyclopedia effort. Dr. Steve Potter presented on using gene signatures rather than individual genes to describe heterogeneity, possibly as markers for disease. Dr. Manjeri Venkatachalam provided a perspective on AKI, outlining factors that result in underrepresentation of proximal tubule cells under normal isolation techniques and

the effects of injury on proximal tubular cell markers, as well as noting that the tubular interstitial compartment has an activated signature in AKI. The results of a poster on laser microdissection and 3D reconstruction of immune cells also were discussed.

In the discussion, the group came to the conclusion that the next steps in tissue interrogation will involve an aggregation of technological approaches, noting that technologies are evolving rapidly compared to the timing for starting studies. The information gained from the different methods may be complementary in many cases, resulting in the need for using multiple approaches. Careful planning of the best ways to use limited tissue will be required, starting with nondestructive interrogation techniques so that multiple techniques can be performed on the same material. The group considered whether conservation of pathways versus disparate pathways characterize AKI, early CKD, and late CKD. The group observed that a biopsy represents a snapshot in time that needs to be placed in the context of disease progression.

The group provided the following responses to each of the charge questions:

- **How can we best obtain integrated knowledge about physiology and pathophysiology from biopsy samples?** Multidimensional data collection was called for, including genetic-epigenetic-transcript-protein-metabolite data; data from tissue blocks, single cells, and, possibly, dissected tissue; complete clinical and histological data; and data from different patient and sample populations (e.g., nephrectomies, rapid progression, clinical and molecular target-based samples).
- **How will we integrate data from complementary technologies?** Computational integration is feasible, as described in the presentation by Dr. Olga Troyanskaya.
- **Are analytic pathways different for AKI and CKDs (because the processes/cells might be different)?** Tissue and data collection should be the same so that researchers can integrate and compare pathways.
- **How deep should we go before diminishing returns?** Both deep and shallow approaches are needed. We need some samples/patients with very comprehensive analysis and large numbers with shallow approaches.
- **How do we deal with heterogeneity within kidney tissue and cells?** This question was answered in the presentations by Drs. Potter, McMahon, and Rahul Satija. SCA is needed.
- **How will we tie the data to sample site/assay noise/patient subgroups?** Data centers will be needed to address this issue.
- **How can we analyze and visualize results (and map back onto tissue)?** The data coordination and analysis group should direct this effort.
- **Where do we start? What are the benchmarks (hierarchy)? What are the downstream studies (animals, etc.)?** Starting with easily accessible material before going to more precious material was suggested. The suggestion was made to prioritize human studies because of the large differences with mouse.

In the breakout session, Dr. Srinivas Ravi Iyengar had the following suggestions: (1) Use graph-based databases for data integration; (2) perform detailed cell biology/physiology studies in organoid/tissue chip models using podocytes, collecting duct cells differentiated from induced pluripotent stem cells (iPSCs) from individual patients from whom biopsies are obtained; (3) determine the whole genome sequence of patients from whom biopsies are obtained; and (4) integrate network and dynamical models across scales.

Discussion

In the discussion of the breakout report, the following additional points were made:

- The data from biopsy samples will need to be leveraged with knowledge about pathological or clinical phenotypes.
- Having small groups perform analyses will help maintain high standards of quality.
- Data of different scales will need to be integrated.
- The effects of preservation methods on tissue interrogation were discussed. Methods for analyzing FFPE samples are needed. Biopsy samples could be split between FFPE preservation and other preservation technologies. Immunological preservation techniques interfere with proteomic analysis. Embedding in OCT[®] for sectioning frozen tissues suppresses ionization, preventing mass spectrophotometric analysis.
- Storage artifacts can affect the integrity of tissues. For example, FFPE samples are subject to hydrolysis and oxidation. RNA in FFPE samples is chemically fractured, however, not degraded, and might be useful for microdissection studies of RNA.
- Archival material represents a potential source of data.
- Phenotypes and subphenotypes for AKI are needed.
- Biomarkers are needed for clinical trial design. Developing biomarkers is a short-term goal that could be achieved by collecting biopsy samples.
- Proteomic data could be used to identify biomarkers.
- Frozen samples can be used for laser microdissection if they are of sufficient size and quality.
- Other fields could be studied for approaches to successfully leveraging complementary technologies (e.g., The Cancer Genome Atlas [TCGA]). Signals from kidney disease may prove to be more subtle than for cancer, however, and more difficult to discern using large databases.
- Obtaining native kidney biopsies was discussed. Approximately one-third of current “native” kidney biopsies are from patients with diabetes and might not be typical. Cadavers might be a supplemental source of native biopsies.
- The Clinical Laboratory Improvement Amendments (CLIA) standards require leftover tissue from clinical diagnoses to be retained for 10 years in case it is needed for diagnostic purposes. This requirement limits the use of clinical samples for research purposes.

Dr. Robert Star asked the group about first steps for tissue interrogation: that is, what analyses should be performed on all biopsy tissues? The following responses were made by the participants:

- Inventorying the data sets and samples that exist to coordinate independent efforts.
- Tailoring the types of analyses to the research goal, which could range from developing drugs to understanding kidney physiology to investigating the mechanisms of disease.

- Vigorously studying how best to preserve samples without compromising integrity (i.e., transport media, cryoprotectants).
- Developing graphical databases for integrating morphology and molecular information. Nephrotic Syndrome Study Network (NEPTUNE) uses such an interface to represent data.
- Performing genomic sequencing.
- Relating biopsy data to tubular physiology.
- Conducting prospective studies, particularly for AKI.
- Developing biomarkers useful for the timescale of drug development (i.e., 3 to 12 months).

Tissue Collection Session

Dr. David Salant summarized the discussions of the Tissue Collection Breakout Group. Regarding tissue procurement, the group was of the opinion that research questions will drive the technology. Once endpoints are known, what tissue to collect, how to preserve it, and how to analyze it will be determined. FFPE-preserved tissues were discussed. Although extensive archival tissue exists, the associated data are potentially noisy because of limitations of preservation. If prospective studies are performed to develop targets, archival tissue could be used for validation. Using single cells for sequencing was discussed. The technology addresses limitations on the amounts of tissue available for study. The approach is promising, but more validation and study of SCA are needed.

Discussion

In the discussion of the breakout report, the following additional points were made:

- FFPE samples can be used in creative ways (e.g., quantifying podocytes).
- SCA results need to be validated by structure and/or disease to provide medically useful results. Individual cells of the same cell type (e.g., podocytes) may vary in the extent to which they are diseased. Model systems, rather than human tissue, were suggested for use in validation studies, but large differences between human and animal tissues were cited as a reason to conduct validation studies with human tissue.
- Improved dissociation procedures are needed to improve SCA results. The current capture rate is low.
- Discoveries from genome-wide association studies (GWAS) and SCA need to be translated to the clinic to provide new, reimbursable diagnostic techniques.
- Use of transport media that do not allow collection of RNA data from samples still can allow collection of DNA data.
- Pristine data for each kidney cell type are needed before seeking to understand pathway data in substandard kidneys. Archival tissue can be used for validation after pathways associated with disease are identified.

- Currently, mass spectrometric analysis (e.g., for lipidomics) requires frozen rather than fixed tissue. Technologies are evolving that can use whole cells instead of cross-sections. Protocols that are not mercury-based also are being optimized for fixed tissue.
- The next step in lipidomics by mass spectrometry is to identify individual molecules.
- No special preservation techniques are needed for 3D analysis. For robotic screening with electron microscopy, however, prestaining is required before embedding tissue.
- Possible approaches to tissue acquisition include taking an extra core in addition to a diagnostic biopsy (e.g., NEPTUNE) and research biopsies. In NEPTUNE, the diagnostic biopsy preservation was performed according to the procedures of the local pathology laboratory, and the extra core was preserved in RNAlater[®].
- A participant advocated for regular analysis of patient samples by immunofluorescence, the benefits of which cannot be predicted in advance, and electron microscopy, which has been estimated to add valuable information in 18 percent of cases. Preservation of samples with RNAlater[®] interferes with immunofluorescence and electron microscopy but not light microscopy. Performing analyses in a staged approach was proposed to maximize benefits to patients.
- For AKI, it was suggested that samples be analyzed with all of the standard modalities.
- The group discussed the appropriate qualifications for staff members who will perform research biopsies. A participant recommended that each study site have a staff member dedicated to conducting biopsies. Past results suggest that this approach increases retention for repeat biopsies. For research biopsies, the person who performs biopsies should be committed to patient safety and part of the research team. To ensure adequate amounts of tissue, nephrologists should perform biopsies, not interventional radiologists.
- Maximizing patient safety was discussed. Limiting the number of centers in a study and standardizing biopsy protocols also were suggested. Based on one researcher's experience biopsying patients with diabetes, biopsies should be avoided for patients with a GFR less than 40 because of the risks of small kidneys and bleeding, but another participant argued against establishing a GFR threshold for biopsies, noting that patients with GFRs of 30 can still have enough cortex for a successful biopsy. A comment was made that because patients with AKI are rarely biopsied, establishing benchmarks for performing biopsies on such patients is difficult.
- Sequential biopsies were suggested (e.g., over a period of 10 years) to improve understanding of the early stages of CKD.
- Experimental protocols for biopsying patients with AKI are needed. Biopsies generally are not performed on patients in the intensive care unit (ICU) unless clinically necessary because of high risks to the patient. It was noted that some patients with AKI (e.g., those with drug-induced damage), however, do not have shrunken, scarred kidneys. A participant argued that a study collecting research biopsies should not start with patients who are very sick. As a renal fellow, he was instructed to perform biopsies on patients with AKI only if clear evidence existed that they did not have acute tubular necrosis (ATN). Transplant patients were suggested as candidates for biopsies. Dr. Salant summarized the criteria offered for biopsying patients with AKI: patients with clinical indications for a biopsy, patients who are not critically ill, transplant patients, and patients with unexplained AKI.

- Prioritizing tissue collection using a stereologic approach was suggested.
- The clinical benefits of biopsies were discussed. A participant indicated that in 30 to 40 percent of patients with AKI, biopsy results made a difference in their care. For example, high levels of oxalate is an indication for dietary management. He suggested that all patients with diabetes be biopsied.
- The suggestion was made to couple biopsies with metrics of pathophysiology (e.g., tubular function).
- Dr. Salant summarized potential criteria for biopsying patients with CKD: all patients with CKD, particularly those with diabetes and hypertension, with eGFR greater than 40 or 45.

Bioethics Session

Dr. Chirag Parikh indicated that in discussing bioethics, the breakout group began with research biopsies, which pose the more difficult bioethics questions than using leftover clinical material or biopsy material from extra cores. The group focused on AKI, which does not have a systematic effort in place to collect biopsies.

The question of which patients with AKI to biopsy was discussed. Because ATN is not well understood and much of the existing knowledge is incorrect, research should focus on patients with ATN. In cases of sepsis, which has high morbidity and mortality, biopsies should be performed early before many organs are involved. For contrast-induced nephropathy, a better understanding of causality is needed. Biopsies will improve the understanding of the role of immune factors in thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS).

Considerations for biopsies of patients with AKI include increasing the participation of nephrologists in the critical care team. Nephrologists need to participate in training on obtaining consent and need to be involved in the process of obtaining informed consent. To facilitate obtaining biopsies, nephrologists might partner with ICU and critical care specialists. For patients in the ICU, performing a transvenous rather than a transcutaneous biopsy should be considered.

The group had discussed performing research biopsies on patients with AKI. Issues of obtaining informed consent from incapacitated patients were raised. Risks in patients with AKI need to be quantified. More knowledge exists about complications in stable patients. Benefits need to be quantified as well. Potential benefits to family members are the easiest to describe, but the compelling nature of the research also needs to be communicated. A national protocol is needed because it is a high-risk procedure. Data and Safety Monitoring Board oversight will be required.

Increasing clinical indications for obtaining an extra core was discussed. These included surgery; a liver transplant, although often kidney injury accompanies the need for a liver transplant; deceased donors, although the sample population might not be representative; delayed graft function; and acute kidney disease.

For obtaining biopsies from patients with CKD, the group recommended early coordination with primary care physicians. As patients have a better understanding of their disease, they are more likely to agree to a biopsy.

Discussion

In the discussion of the breakout report, the following additional points were made:

- The group had difficulty reaching consensus on the bioethical issues surrounding biopsies.
- Drug development should prioritize clinical settings.
- Sepsis is a difficult medical condition for obtaining biopsy samples.
- A case for biopsies needs to be built based on what results can bring to the treatment and care of patients.
- For CKD, more biopsies are needed earlier in the disease course, but many patients at that time are not under the care of a nephrologist. As a result, more collaboration is needed between researchers and caregivers to prevent researchers being viewed as acting opportunistically. Building relationships between nephrologists and endocrinologists also was suggested. Caregivers can help select patients who are likely to understand the protocol and be dependable participants. Mr. Paul Conway also suggested that government agencies, such as the Centers for Medicare and Medicaid Services, might have tools to identify patients earlier in the disease process.
- Effort is needed on building the patient community so that patients approach researchers on their own initiative when a study opens. Kidney patients are not as “activated” as patients with such diseases as cystic fibrosis. Mr. Conway, President of the American Association of Kidney Patients (AAKP), suggested coordinating with such organizations as AAKP, the American Society of Nephrology, and the National Kidney Foundation (NKF) to build the patient community. Members of these organizations are a population to target to increase involvement in research that will have positive impacts on patient care. For example, NKF founded the Patient and Family Council, which includes in its goals educating and raising the awareness of individuals on dialysis and their families. Social media is used by patient advocacy organizations as an outlet for providing information about trials. In addition, these patient organizations are well placed to collaborate with other patient organizations of interest in kidney research, such as those representing patients with diabetes. The important role that patient advocacy can play in compelling Congress to recognize the need for more kidney research was discussed.
- Another participant observed that individuals with a family history of kidney disease or who are members of a population with high rates of kidney disease (e.g., the Pima American Indians) are strongly motivated to participate in research studies.
- The issue of obtaining normal kidney biopsy tissue was raised. Obtaining biopsy tissue from deceased donors is likely to become more difficult because it is perceived as increasing the discard rate for potential transplant organs because of potential discovery of scarring. Obtaining biopsies from deceased donors might be affected by whether the biopsies are part of clinical care or for research, as well as whether they are part of a limited postmortem exam.
- Obtaining biopsy tissue during open surgery was discussed. The timing and clinical setting of the sample might affect the tissue (e.g., before or after aneurysm repair). Concern was raised about the additional risk to the patient of performing a biopsy during a procedure like aneurysm repair.
- As in oncology, performing biopsies needs to become part of the culture in nephrology.
- The ethics of performing biopsies on patients with diabetes without albuminuria, as well as on people with a family history of type 2 diabetes who do not have CKD, had been debated by the group. A participant responded with examples that would build a case for performing biopsies:

markers being discovered in the patient population, interventions in animal models proving successful in the prevention of kidney disease evolution, or a genetic profile being found to be associated with a kidney disease phenotype. It was pointed out that lesions of diabetic nephropathy were found in biopsies of Pima Indians whose kidneys were functioning normally, but whether such knowledge benefits the patient is not clear.

- The benefit of altruism from volunteering for a kidney biopsy was portrayed as less clear than for donating a kidney.

It was suggested that if more interventions were available in AKI, the case for performing biopsies would be stronger. Dr. Parikh pointed out that few treatments have been developed in part because limited data from biopsied tissue are available.

Day 2 Breakout Group Reports

AKI Session

Dr. Lloyd Cantley summarized the discussions of the AKI breakout group. The group discussed defining the cohort without reaching a consensus. Transplants were suggested as a cohort. Advantages of this cohort include less strict guidelines for obtaining institutional review board (IRB) approval for deceased donors; living donors as a potential source for normal tissue; and the availability of follow-up biopsies for delayed graft function, although the source of such kidneys generally is deceased donors. Disadvantages include that AKI in deceased donors might not be representative because of such conditions as immunosuppression and ischemia. Biopsies might be performed on patients with contrast-induced nephropathy or kidney failure following coronary artery bypass grafting (CABG) surgery. Patients in the ICU without sepsis or hypotension were proposed for research-only biopsies, but not in the early stages of a study. Patients with late nonrecovery and patients with AKI of unknown etiology were suggested for clinically indicated biopsies. It was suggested that each center in a study select which groups to include in its cohort. The group emphasized that safety of the patient should be paramount to prevent bad outcomes from biopsies in a study, and the parameters that will determine eligibility for a study need to be incorporated into the IRB approval.

Clinical data would be maintained by a data coordinating center. Acquiring the right data will be key to assigning patients to the correct disease phenotype. The group recommended that the following data be acquired at the time of biopsy: demographics, exposures, medications and/or contrast agent, AKI risk parameters (e.g., blood pressure, bacteremia, toxin exposure, urine output), laboratory results (e.g., baseline eGFR prior to AKI, typical AKI laboratory results), imaging results, and prebiopsy clinical diagnosis. Longitudinal data to collect—preferably over a period of at least 5 years—include hospitalizations, renal replacement therapy or transplant, eGFR, medications, and laboratory results.

Biopsy protocols should be highly standardized. Although no consensus was reached, suggestions were made to collect the following samples, with analyses conducted according to standard operating procedures (SOPs) established for each sample and analysis type: serum (analyze for proteomics, biomarkers, and cytokines); nucleated blood cells (analyze for identification of circulating nucleated cell types and perform DNA genotyping); urine (analyze for proteomics and biomarkers); urinary exosomes; and intact urinary cells (analyze by single-cell RNA sequencing [scRNA-seq] and fluorescence-activated cell sorting [FACS]). The group recommended not collecting stool or saliva samples. For the biopsy, trained personnel should follow an SOP for the initial division at biopsy of tissue for diagnostic and research purposes to ensure adequate tissue and glomeruli for diagnostic hematoxylin and eosin (H&E) staining, immunofluorescence, and electron microscopy. The division of research material from a third core for analysis should be guided by the technology group. No clear consensus was reached on the

analyses that should be performed on the research material. Suggestions included RNA-seq and discovery proteomics; LCM, microdissection, multiplex expression, and Drop-seq RNA sequencing; metabolomics and lipidomics; and MRM and parallel reaction monitoring (PRM) mass spectrometry. RNA-seq and some form of discovery proteomics were the two types of analyses closest to core technologies according to the group, and the others were recognized as specialized techniques that individual centers might perform on their own samples.

Pathology will form the cornerstone of studies of AKI. The group recommended that pathology be performed locally and scored by a specified panel of investigators using a digital image repository residing in the data coordinating center. The following standards and SOPs will be required:

(1) standardization of tubular injury scoring for all tubular segments, (2) establishment of standardized tubule regeneration scoring, (3) standardized reporting of electron microscopy of tubules and vasculature, (4) an SOP for interstitial cell identification and quantification, and (5) an approach for 3D reconstruction both with and without immunofluorescence of the vascular compartment to identify changes. The group recommended convening a conference to establish these consensus standards.

The role of the data coordinating center was envisioned as establishing SOPs for collection and analysis; maintaining a clinical database for longitudinal information that will consist of de-identified data provided by local sites during the course of the study; maintaining a database for core data available to all researchers, including pathology, RNA-seq data, and proteomics data; and providing bioinformatics and pathway analysis from the full data set. The data coordinating center would fill a critical role in providing a database that would be available to all investigators. The center would ensure that the collection and analysis of all data are uniform.

Discussion

In the discussion on defining the cohort, the following additional points were made:

- In response to a question from Dr. Star, Dr. Cantley indicated that nephrotoxins (e.g., cisplatin) had not been discussed specifically by the group as a factor in determining cohorts.
- Dr. Cantley suggested that whether or not to include patients on dialysis in cohorts be left to the discretion of individual researchers.
- The issue of statistical significance was discussed. Dr. Cantley indicated that defining the cohort by diagnosis will determine the number of patients needed. AKI can be attributable to many possible diagnoses, complicating the choice of cohort. Without knowing pathways, differentiating among different disease types is problematic. It also was noted that common pathways might exist among disease types assigned to separate phenotypic bins. A participant commented that longitudinal data from multiple biopsies collected from the same patients over the course of the disease, as well as biomarkers linked to clinical outcomes, are needed to define different types of AKI.
- Studying a pig model of ischemia and sepsis was suggested as an alternative to trying to obtain IRB approval for biopsies from patients with these conditions, but Dr. Cantley was in favor of focusing on patients who are not high-risk to biopsy (e.g., late nonrecovery, AKI of unknown etiology) rather than animal models.
- The timing of biopsies relative to injury was not discussed by the group. Including large numbers of patients in studies, however, will allow study of the natural history of kidney diseases.

- A participant suggested collaborating with oncologists to obtain tissue samples from patients involved in experimental studies of pathway inhibitors.
- Patients with liver disease and elevated creatinine levels might be an additional cohort to target. Dr. Cantley indicated that collecting biopsies during liver transplants had been discussed by the group.
- Patients with contrast-induced nephropathy will represent a cohort that does not have underlying kidney disease.

In the discussion on clinical data, the following additional points were made:

- Several factors will help ensure patient safety. The need for imaging to guide the biopsy is important; therefore, collaborations with radiologists should be formed, and both a radiologist and a nephrologist should be present at biopsies.
- It was suggested that a nephrologist, rather than a radiologist, perform the biopsy to obtain a good sample.
- A biomarker for renal reserve is needed to supplement imaging data.

In the discussion on biopsy protocols, the following additional points were made:

- Extensive experience in performing kidney biopsies and membership on the investigative team were proposed as necessary qualifications to ensure patient safety and obtain enough reliable tissue. Dr. Cantley indicated that Dr. Michael Mauer specifically had advocated for having a member of the research team at each site dedicated to performing biopsies.
- Urine samples will be processed to analyze the urinary exosome, intact cells, and the supernatant.
- All centers might not be able to collect and analyze the suggested prebiopsy samples. Collecting and storing samples at each center for analysis elsewhere is an alternative. Dr. Cantley responded that the group had not reached a consensus on which prebiopsy samples would be useful across every biopsy.
- Prioritization of analyses to be performed on biopsy samples should be clear in the protocols in case of limitations from the amount of available tissue. A participant suggested considering the technological strengths of centers, as well as the number of patients recruited with different subtypes of AKI, when prioritizing analytical protocols.

In a general discussion of AKI, the following additional points were made:

- AKI studies should consider performing multiple biopsies, especially of patients who progress to CKD.
- Baseline biopsies could be performed on patients with diabetes or hypertension.
- Polarized light microscopy should be performed on biopsy samples for diagnostic purposes (e.g., to detect oxalate).

CKD Session

Dr. John Sedor summarized the discussions of the CKD Breakout Group. Although the session did not manage to cover all of the suggested questions in the allotted time, participants felt confident that the questions could be addressed because the several ongoing CKD studies—including Chronic Kidney Disease in Children (CKiD), Chronic Renal Insufficiency Cohort (CRIC), NEPTUNE, and others—provide the CKD community with a strong conceptual base and a set of tested protocols on which to build new research and therapeutic initiatives. Discussions focused on two general questions: (1) Which kinds of patients should be recruited for this project? and (2) What role should research biopsies play?

Participants did not reach a consensus on research goals; nevertheless, there were many points of agreement. First, discussants agreed on the importance of focusing on humans, rather than animal models, as research subjects. Dr. Sedor illustrated this point by discussing one of his trainee's patients: a 28-year-old pregnant woman who had lost her baby was diagnosed with end-stage focal segmental glomerulosclerosis (FSGS) and placed on dialysis. While wrestling with the personal consequences of her disease, she also was eager to do “anything I can to help other people”; she was one of the first patients recruited into NEPTUNE. Dr. Sedor drew two lessons from this patient: (1) Beyond dialysis and transplants, physicians still cannot do much for most CKD patients; and (2) patient experiences have much to teach the medical community. There are many altruistic patients who are eager to work with medical researchers to help solve the problem of CKD.

The group also expressed universal agreement on the importance of collecting tissue samples as material that is essential to a new understanding of kidney disease mechanisms and to identification of new biomarkers to assist in diagnosis and treatment of CKD. The group did not reach a consensus about the types of patients to be chosen, which molecular phenotypes to analyze, the timing of the samples with regard to stage of renal failure, and the numbers and kinds of biopsies to be performed. Although considerable support was voiced for inclusion of all CKDs within the study population, participants discussed the potential of targeting certain subpopulations as well.

The CKD session did not discuss specific biomarkers to be targeted, in part because other CKD studies, such as NEPTUNE and other consortia, have already identified some interesting biomarkers that might be worth following; these consortia also have developed experimental protocols on which the KPMP can build. There was support for an “agnostic” approach to identifying biomarkers—acquisition of a broad array of molecular information without preconceived notions about which pathways to target. The heterogeneity of CKD adds an additional argument for this data-first approach because different pathways are likely to be relevant to different forms of CKD. An unstated but overriding theme for all the discussion groups is that personalization of cancer treatment is a paradigm for use of precision medicine in treating the heterogeneous varieties of kidney disease. However, Dr. Sedor raised the possibility that the power and sensitivity of that approach may be less applicable to CKD than has been anticipated.

Two kinds of biopsies can provide tissue material for research: research biopsies and indication biopsies that were performed initially for clinical reasons but have the potential to offer data of value to researchers. Both kinds of biopsies were considered to be valuable. Because biopsies are not without risk, informed patient consent is essential, especially for research biopsies; so is the need for a doctor with deep experience and an excellent track record to perform those biopsies. That risk also dictates a thoughtful rationale for biopsy protocols and choice of patient volunteers.

A general consensus was reached that it was important to biopsy tissue at early stages of renal disease, but less agreement on the functional definition of “early” with regard to specific rate of kidney function. Although some valuable information could be derived from late-stage biopsies, the importance of patient safety issues, especially for research biopsies, as well as the presumably greater research value of tissue

from early-stage diseases, generally argue for research emphasis on collecting tissue samples from patients with early-stage renal disease.

The need for healthy, control tissue also was stressed, although there was less clarity on the ideal source for that tissue. Among the possibilities are samples from rejected transplanted kidneys and biopsies of donor kidneys before transplantation. As a different kind of control, Dr. Sedor advocated for attention to “slow progressors,” that is, those renal patients whose disease stays stable over time or progresses only slowly. He commented that there is as much to be learned from what keeps renal patients healthy as there is to be learned from what makes them sick.

Additional CKD subpopulations of interest include DKD (including the long-term Pima Indian study), the Mesoamerican nephropathy cluster, and pediatric populations. Research attention to the Mesoamerican cohort was considered premature at this point because so little is known. Although inclusion of pediatric patients would be desirable, several participants noted that changes in IRB policy (increasing restrictions on invasive procedures in children) are likely to constrain broad-scale studies of pediatric patients.

A strong consensus was achieved on the need for longitudinal studies of CKD. A key goal of the KPMP is to link the clinical data—that is, the outcomes—to molecular phenotypes. Because the typical disease progression of CKD is on the scale of 10 to 15 years, this goal is realizable only with in-depth longitudinal studies. For purposes of testing therapeutics, the slow progression of CKD poses an additional problem because the standard U.S. Food and Drug Administration (FDA) protocol for demonstrating drug outcomes is 5 years, a short period for CKD. Dr. Sedor expressed the hope that recognition by FDA of the special circumstances of CKD could be a key outcome of this project.

Participants agreed that, in theory, second biopsies can provide a valuable way to monitor progression of the disease, as well as specific effects of any therapeutic treatment, including possible disease regression. Although additional biopsies require patient consent, Dr. Mauer pointed out that a significant percentage of patients who volunteer for a first biopsy also will agree to a second, suggesting the feasibility for second samples from a useful subset of the patient cohort. Ironically, in practice, research biopsies may be a harder sell to physicians than to the patients themselves. Dr. Sedor said that even at this meeting, physicians expressed mixed feelings about second biopsies because of the potential of complications for second biopsies; a minority of participants admitted that they would not want to undergo second biopsies themselves.

Participants discussed the desirability of development of noninvasive methods, such as innovative imaging methods, as either a supplement or substitute for biopsies and other analytical methods. Several such methods are in the early stages of development.

The benefits of a broad database for the KPMP also was discussed. One way of expanding the database might be to build research biopsies into existing long-term studies, such as CRIC (which includes a new cohort on early-stage CKD) or the Preventing Early Renal Loss in diabetes (PERL) study (which is investigating the use of allopurinol to preserve kidney function in type 1 diabetes). Making use of electronic health records (EHRs) was recommended as a way to identify a broad range of research-patient candidates from across the country.

Discussion

In the discussion of the breakout report, the following additional points were made:

- Several participants suggested that data mining of archived clinical material could provide a valuable supplement to newly collected tissue material. Although agreeing in principle about the

value of such samples, some participants reminded their colleagues that older material will not necessarily be accessible to the modern molecular analysis planned for this study.

- The Pima Indian diabetes study has been targeting very early stages of renal disease, including research subjects who might not even realize that they have the disease. This special population is worth concerted study. Other underinvestigated subgroups also merit some focused attention.
- In comparative chemical evaluation of terminal renal disease, it is very important to standardize information across studies and across research centers.
- One logistical difficulty in biopsies of early-stage renal patients is that, unlike late-stage patients, they usually are still working, and biopsies require patients to take time off work.
- In addition to collecting new material, the research community should be interrogating standard samples in new ways.
- Patient respect is essential. Patient volunteers should be treated as colleagues in a clinical investigation.

Data Session

Dr. McMahon summarized the discussions of the Data Breakout Group.

A strong consensus was achieved that the first data priority should be development of a molecular map of the normal human kidney. This database would serve as a high-standard reference atlas for interrogating molecular data on the kidney, and it would provide a predictive molecular framework for mapping any particular biopsy sample to other information in the database. More broadly, by linking high-resolution molecular and cellular data with genomic and histological information, including data representative of genetic and demographic variability, this atlas would facilitate the development of a modern, more integrated conceptual understanding of the human kidney.

The first priority in data acquisition should be genomic information: not only deep genomic sequencing, but also transcriptional profiles of all RNA populations, as well as selected chromatin profiles of kidney tissue. Stored material also would enable secondary proteomic and lipidomic mapping approaches.

Information from normal kidney tissue is an essential foundation for the KPMP; thus, acquisition of such data is the first priority. Definition of “normal” is not necessarily straightforward, and merits further discussion, especially before making the important decision on which representative examples of normal tissue to include in the database. The group recommended that the normal biopsy material include samples from both males and females, a broad range of ages, and racial groups reflective of the general population to provide a complete picture of the range of normal activity of any given gene across the population. The potential source of normal biopsy material was left unresolved, although the possible use of pretransplant biopsies of donor kidneys, or rejected donor kidneys, was discussed.

Even with information on normal kidney tissue alone, a reference atlas would be immediately and broadly useful in connecting relevant research findings and in providing predictive markers to the research community. The atlas would evolve over time as additional data, and additional kinds of data, are added. Complementary secondary approaches also could be selected based on the value of the information to the research community. With this reference base, biopsy samples of diseased tissue could then be compared to reference material.

For maximum usefulness, reference data must not only include high-quality nucleic acid and chromatin data, but also preserve cellular relationships; for instance, information on proximal tubule cells should be accompanied by parallel information on neighboring interstitial and vascular cells. To realize the goal of spatial resolution of data, all these samples must be accompanied by high-resolution imaging; it is expected that imaging techniques will develop in concert with the evolution of genomic information over time.

The breakout group made no recommendations on approaches for acquiring these data. Consensus was reached, however, on the need for significant improvement in data quality over current cell dissociation procedures.

Organization, communication, and harmonization of research in this multi-institution collaboration is essential. The group suggested the formation of working groups to set consistent, “best-practice” protocols and standards to be followed by all participants for data storage, data sharing, data evaluation, tissue preservation, patient confidentiality, communication of research results, and the like. As an example, consistency in acquisition of even routine urine samples—such as time between sample acquisition and processing—is vital for separating biological variability from variability in sample handling. Dr. McMahon suggested that researchers use surrogates for human material for initial tests of protocols to ensure that procedures for data acquisition, sharing, and coordination are operating as well as possible before turning to patient material.

Discussion

- A participant asked whether normal material would be collected concurrently with diseased samples. For both scientific and logistical reasons, Dr. McMahon suggested that the reference atlas would proceed first. Several participants noted their enthusiasm for the concept of a molecular map of normal kidneys.
- Another participant noted that given the special challenges of AKI and CKD research programs, Dr. McMahon had described only about 10 percent of the effort that ultimately will be needed to integrate data on diseased kidneys with the reference atlas.
- One participant observed that this is an extraordinarily ambitious proposal with regard to potential rewards, although, like the Allen Brain Atlas, it is one that will require considerable resources to realize. Dr. McMahon replied that this project is actually more difficult than the Allen Brain Atlas because human brains are wired in a nicely reproducible way at the macrocellular level, whereas, unfortunately, kidneys are not.
- A participant offered the perspective that a molecular atlas is not enough. He added that it would be a shame to throw out the insights gained over many decades from classic cell biology. Dr. McMahon agreed, noting that the atlas is meant to be a scaffold to which other data could be added. He stated that in an ideal world, one could click on the standard histological section and be linked to related data, including data on cell structure.

Appendix A: Tissue Interrogation Session

Breakout Group Leaders

- Technology Chairs: Andy McMahon, Steve Potter, Katalin Susztak, and Srinivas Ravi Iyengar
- AKI Chairs: Manjeri Venkatachalam and Charlie Alpers
- CKD Chairs: Avi Rosenberg and Ray Harris
- NIDDK Representatives: Krystyna Rys-Sikora and Deborah Hoshizaki

Charge Questions

- How can we best obtain integrated knowledge about physiology and pathophysiology from biopsy samples?
- How will we integrate data from complementary technologies?
- Are analytic pathways different for AKI and CKDs (because the processes/cells might be different)?
- Are pathways sufficiently robust, scalable, and validatable?
- How deep should we go before diminishing returns?
- How do we deal with heterogeneity within kidney tissue and cells?
- How will we tie the data to sample site/assay noise/patient subgroups?
- How can we analyze and visualize results (and map back onto tissue?)?
- Where do we start?
- What are the benchmarks (hierarchy)?
- What are the downstream studies (animals, etc.)?

Short Presentations

SCA—Disassociation and Analysis to Identify Cell Types (Kidney Biopsies from LN) (Dr. Nir Hacohen)

As part of AMP, Dr. Hacohen and his colleagues have analyzed the immune infiltrate into kidneys from lupus patients and healthy donors, focusing mainly on scRNA-seq. Other technologies were considered, but scRNA-seq allows the determination the cellular activation states. scRNA-seq is contrasted with Drop-seq in that it sequences hundreds of cells at high depth, rather than thousands of cells at lower depth. Because kidney biopsies are so small, the number of CD45+ cells obtained from each sample is on the order of several thousand. If the Drop-seq approach were used, most of those cells would be lost. The sample of immune infiltrate was sorted into plates and RNA-seq results were obtained at high depth. This approach allows directly sorting cells in an unbiased way into different categories. Dr. Hacohen showed an example of the ability of the sorting technique to separate CD45+ cells with CD3, CD14, and other markers, which is similar to the type of results obtainable by FACS. Very few CD45– cells were

included. Marking sample results from each of four patients with a different color, some colors dominated in different cell types, showing that different categories of cells were present in infiltrate from different patients.

Dr. Hacohen described advantages and disadvantages of the technique. Advantages include the following: (1) unbiased discovery of cell types and states and (2) maximizing what can be learned from precious samples, which is relevant to bioethics concerns about obtaining biopsy samples. SCA allows researchers to find disease-associated signatures that span across cell types and have coherence within cell types, which is difficult to determine in whole tissue. Disadvantages include the following: (1) detecting genes at low expression levels is difficult, but this information can be recovered somewhat by aggregating cells; (2) spatial localization is lacking, but these studies can be followed up by taking the gene signatures and the main markers obtained and applying them either to different cores from the same patients or additional sections from the same patients (e.g., for tumor samples); (3) some technologies are expensive, but this is changing as the cost of sequencing libraries continues to decrease; and (4) the dissociation protocol may cause some alteration of the profile, but this effect is being studied in depth, and changes do not appear to be major. Dr. Hacohen noted that plate-based methods are very effective for analyzing CD45+ cells from kidney, but viability of non-immune cells needs improvement.

Lessons learned and factors to consider include the following: (1) optimization of sample preparation is essential because live cell dissociation is not feasible for a large number of samples; and (2) obtaining complex libraries for cell types that occur in small numbers is important.

Data on specific genes and their expression show that obtaining very deep data on expression is feasible with this technology.

SCA—Spatial Mapping (Dr. Steve Potter)

Dr. Potter indicated that his topic was how to go from dissociated cells, where all spatial information has been lost, to the ability to reconstruct a 3D, single-cell resolution atlas. Normally, cell binning is based on known markers, a rationale allowing localization of most cell types. When SCA is performed, however, not every gene expressed in a cell is detected because of biological and technical noise, requiring the use of a complex gene signature rather than a single gene. In spatial mapping of data from SCA, all results must be validated, typically by *in situ* hybridizations and immunological stains. Dr. Potter observed that most binning is performed based on markers defined by mouse. As Dr. McMahon had stated, most but not all mouse markers work in human, resulting in a need to define new markers in human. Single-molecule *in situ* hybridization is a very powerful procedure for validating single cell studies. Dr. Potter emphasized that before researchers can learn what is going wrong in disease, they need to have a base atlas to define “normal.” He predicted that no single normal kidney exists. Different “normals” will be found for young and old, females and males, and different races, and variation also is likely within these groups.

Epigenetics, Genetics and Proteomic Analyses of DKD Biopsies (Dr. Susztak)

The subject of Dr. Susztak’s research is causal pathways for human diabetic and hypertensive CKD. Partial nephrectomies and tumor nephrectomies provide a valuable source of tissue because they are accompanied by clinical (including time updated) and histological data; the samples are readily available and a large number of tissue samples can be collected; and they provide more relevant information to human kidney disease than mouse models. Disadvantages include the lack of controls for hypertension of diabetes in the absence of kidney disease. The method her research group uses for tissue interrogation is manual microdissection for glomeruli and tubules, providing enough material for genotyping, genome-wide cysteine methylation analysis, histone modification analysis, and RNA-seq. When determining causal pathways for CKD, genetic and epigenome data are needed to supplement gene expression data,

and phenotype analysis is needed to attempt to determine causality. On her wish list, Dr. Susztak included longitudinal data set enrichment for patients with rapid progression, increased sample size, single-cell data sets, greater emphasis on functionalization of GWAS hits to establish causality, and computational integration of different data sets.

Proteomics and Lipidomics on Whole Renal Tissue Samples (Dr. Jeremy Norris)

Dr. Norris provided an example of imaging mass spectrometry used to study kidney. His laboratory formed a collaboration with a microbiologist with the goal of imaging infection. As a model, they used infection in mouse. Imaging showed the formation of abscesses and a nutritional immunity reaction in which metals were sequestered by proteins away from bacteria. The infection was visualized by combining protein imaging (calprotectin) with metal imaging by inductively coupled plasma-mass spectrometry. In an infected animal, the image shows sites of abscesses, recruitment of calprotectin to those sites, and dysregulation of manganese and zinc at the infection sites.

Advantages of imaging mass spectrometry include that it is an excellent tool for discovery, is label free, requires no special reagents, is high throughput, is molecularly specific (e.g., can differentiate among thousands of proteins), produces multi-omic data, correlates with pathology images, has real translational potential, and has applications with diagnostic endpoints. In addition, matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) is FDA-approved for other applications.

Disadvantages include technical limitations (e.g., sensitivity and quantitation challenges); the perception that special expertise is needed, although Dr. Norris' group is actively involved in training, including involving pathologists; and the expense of the equipment, although as instruments are being developed, prices are decreasing.

Multiplex Expression Approaches (Dr. McMahon)

Dr. McMahon described the multiplex expression approaches of HCR, RNA tomography, and tissue mass cytometry. In HCR, small oligonucleotides are hybridized to RNA transcripts, and the signal is amplified using nucleic acid probes with attached fluorescent groups. The fluorescent groups are released by the chain reaction of hybridization. In an example of a combinatorial spatial expression study of the glomerulus, the juxtaglomerular cells, mesangial cells, podocytes, and vascular cells are depicted with different-colored fluorescent probes marking expression of different genetic markers. This technique can be used for relational and spatial mapping.

In RNA tomography, a piece of tissue is obtained (e.g., zebrafish embryo), the specimen is sectioned, and RNA expression of the sections is performed. These data are used to compute a 3D expression distribution of any transcript (e.g., the spatial distribution of Hedgehog signaling pathway components in a mammalian limb). The method is applicable to tissue with fixed axes, which does not apply to kidney.

Tissue mass cytometry involves staining tissue with a cocktail of antibodies labeled with different metal ions, irradiating the sample with an ultraviolet (UV) laser, and detecting the metal ions given off by mass cytometry. This method can provide a detailed analysis of approximately 100 different protein markers simultaneously within a spatial resolution of a single cell. A sample use would be to map the distribution of proteins in particular cells throughout a tumor.

For multiplex *in situ* hybridization, advantages include that it is a standard approach, it provides single-cell resolution, and RNA detection is standardized, whereas disadvantages include sensitivity (i.e., detects transcripts with moderate abundance and above), the need to develop custom probes, and the limitations of the range of fluorophores. Advantages of RNA tomography include detection of all genes and production of detailed spatial and transcriptional maps, whereas disadvantages include the requirement of

a defined reference axis and invariant anatomy and the fact that the technology is computationally intense. Advantages of mass cytometry include the quantitation of protein levels, the ability to analyze more than 100 proteins, and cell resolution, whereas the disadvantages include the need to validate selected antibodies and the fact that it is still an emerging technology.

Lessons learned from HCR include that it is relatively expensive and suited to multiple detection of a small set of probes; the sensitivity is in the moderate abundance range; cells with odd shapes are difficult to visualize; and fluorophore preference is to match shorter wavelengths to more abundant transcripts. The approach was successfully applied to glomerular imaging in a human fetal kidney section.

Object-oriented Pathology and Linking Molecular Pathology and Physiology (Dr. Hewitt)

Dr. Hewitt spoke on the need for a detailed pathology review of renal biopsy material to discover ground truths. A study was performed comparing the absolute number of glomeruli in a biopsy with the number pathologists estimated and found substantial discrepancies. The need exists to treat kidney as a multi-compartment organ in which the compartments are not independent and do not have good functional denominators. Dr. Hewitt argued that the glomerulus should not be used as an estimator of everything else in a biopsy. A consensus needs to be developed for diagnostic features, including sensitivity, specificity within biopsies, and temporal aspects (e.g., the presence of lesions within a biopsy in reference to the disease process). Lesion-specific features are needed that provide understanding of sensitivity, specificity, and temporal relationships. These features need to be recognizable by pathologists without requiring extensive training. A consensus should be developed for how to identify lesions. Dr. Hewitt cited another study in which pathologists were asked to identify mitosis in 50 images, and little agreement was found between pathologists in the results.

The conventional paradigm has served the field well, but too much is subjective. It provides information about what the disease is based on the pattern of injury, but provides little prognostic information. The field is moving toward an object-oriented paradigm, and more objective definitions of disease are being sought. Diagnosis with a small “d” becomes Diagnosis with a capital “D” when it incorporates diagnosis, prognosis, and predictive medicine. The diagnosis of the disease with a small “d” likely will retain some elements of subjective features. Prognosis likely will remain somewhat related to qualitative evaluations. Ultimately, however, moving to predictive medicine models based on enumeration and quantitation is needed. This shift will require an understanding of how good each measure is and how to measure it best, perhaps with the assistance of computerized diagnosis. The goals of predictive medicine are to apply knowledge of basic biology and molecular biology and link it to molecular and physiological relationships to obtain improved reproducibility among pathologists and among specialties. This effort will be extremely labor intensive, requiring extensive training and effort backed by substantial investment of resources.

xMD Techniques on Extracellular Matrix (Dr. Rosenberg)

In expression microdissection (xMD), tissue is immunohistochemically stained; a film is laid over it; the tissue is irradiated; the film bonds to target cells; and the film, enriched for the target of interest, is removed for further analysis. Dr. Rosenberg demonstrated the specificity of the technique in an image of gut tissue stained for cytokeratin. He illustrated the improved ability of xMD compared to macrodissection to discriminate between lymphoma and melanoma cells in a lymphoma/melanoma cell line by showing the strong signal of the melanoma mutation signature in xMD samples that was either not detected or detected at low levels in macrodissected samples.

Advantages of the technique are automation; speed (images can be obtained in less than 30 seconds); the use of standard slides; the ability to analyze FFPE or frozen material; very fine resolution, on the order of 1 μm ; consistency from slide to slide; the lack of a need for a microscope; the ability to enrich for DNA,

RNA, and protein; and equipment costs of about \$400. Disadvantages include tissue and slide storage and handling (slides must be desiccated once cut); the dependence of sensitivity on the ability to find a specific probe; the requirement for added treatment for RNA enrichment (i.e., the need for RNAase inhibitors); the need for histology and immunohistochemical support; and the limitation of detection technologies.

Examples of xMD applications are use of DNA to enrich for cardiac myocytes and pleuropulmonary blastoma, use of RNA to enrich for rare glial and neuronal populations, use of miRNA to discriminate between stroma and epithelium in the prostate, and use of shotgun proteomics in liver and intercalated discs and targeted proteomics in podocytes. Dr. Rosenberg compared an image of a section of mouse kidney glomerulus before xMD and an image of the xMD film on which podocytes were picked up specifically by cannabinoid receptor expression.

Deep Sequencing of Microdissected Tubules: Omics and Mapping Pathways (Dr. Mark Knepper)

Dr. Knepper's laboratory performs studies of renal tubule transcriptomics. Identifying the genes expressed in all cell types in the rodent kidney under standard conditions provides a starting point for understanding systems biology and basic physiology of the rodent kidney. He noted, however, that the degree to which such understanding will apply to the human kidney is an open question. His laboratory has made his findings on the transcriptomes of renal tubule sections in rat available online. The data are searchable by gene, yielding localized expression data (e.g., for aquaporins). Data are available for 15,000 genes expressed in the kidney tubule and can be downloaded for further analysis. The laboratory uses microdissection of rat and mouse kidney to obtain small samples (approximately 1,000 cells per sample) for RNA-seq. In samples with multiple cell types, single cell techniques are used to identify the transcriptome of the principal cells, such as intercalated cells from collecting ducts. Single-cell techniques involve obtaining cell surface markers enriched for particular cell types and performing Fluidigm-based RNA sequencing.

Dr. Knepper contrasted the two levels of approaches, single tubule and single cell. For single tubules, researchers can obtain the complete transcriptome (8,000 genes), and expertise in renal tubule microdissection is required (i.e., dissection under a microscope with forceps). Some segments need simple perfusion to free segments, but the microdissection usually is a short protocol and viable tissue is obtained. For single cells, the same depth of sequencing is not possible, and enrichment is needed for minor cell types. Cells are sorted with cell surface markers, and dissolution of the tissue is required.

BEAt-DKD Approaches for Tissue Interrogation (Dr. Gomez)

Dr. Gomez began by emphasizing the need for obtaining as much information as possible from the same patient. MRI and ultrasound data will be collected from the same patient on the same day biopsies are performed to facilitate validating imaging biomarkers. In animal studies, imaging and biopsy results have been shown to be well correlated, but few studies have been performed in humans.

Dr. Gomez also noted that nephrectomies provide a good opportunity to collect tissue. Approximately one-third of biopsies from nephrectomies are on patients who have diabetes, so this source will be included in BEAt-DKD. Obtaining biopsy tissue before nephrectomies will eliminate the effects of the stress of the surgery on the tissue. Epigenomic data will be correlated with data from urinary vesicles, which provide another source of data to compare to biopsy data.

A cell type-specific molecular encyclopedia is being developed using multiple approaches. Target cells include podocytes, glomerular epithelial cells, mesangial cells, and proximal tubule cells. These cells can be isolated from human urine. Both insulin-sensitive and insulin-resistant patients will be sampled.

Dr. Gomez presented some data on urinary vesicle cells. Urinary vesicle cells show a wide range of cell sizes but a homogeneous profile of expressed genes. In a pilot study of a small number of patients, significant differences were observed in expression in patients with microalbuminuria, macroalbuminuria, and controls.

Another interrogation technique that will be used on FFPE-fixed samples is the proximity ligation assay (PLA) approach. This technique provides quantitative data on signaling activity, which can be correlated to disease stage.

SCA—Dissociation Procedures (Dr. Srinivas Ravi Iyengar)

Dr. Iyengar described artifacts that can be introduced from dissociation procedures for SCA. Compared with normal tissue, many of the cells in tissue samples from patients with AKI are dead or injured. These cells are fragile and prone to break during isolation procedures. In early fibrosis, membranes thicken, and the digestion procedure needs to be adjusted. In late fibrosis, tubules will require more digestion time. These artifacts can lead to underrepresentation of particular cell types in SCA: fragile cells and cells trapped in collagen and thick basement membranes. In addition, RNA and protein alteration can result from digestion. Procedures such as LCM, however, examine tissue *in situ*.

Discussion

In the course of the discussion, the following points were made:

- Digestion of tissue was discussed. Aliquots of samples can be taken during the course of digestion to help overcome some of the problems of digestion in SCA. In general, the digestion period should be minimized. Cold-active proteases, such as those produced by cold-weather extremophile bacteria, are needed but are not commercially available.
- CD45 cells are difficult to capture using Drop-seq. A solution might be to add more beads to the sample. Some drops might have two beads, but this only would result in reading some cells twice.
- Many technologies are complementary. It was suggested that different studies should focus on different technologies. In comparing methods, standards are needed for colocalization.
- Layering of technologies was suggested as a solution for limited sample size. Samples analysis should begin with nondestructive technologies, such as imaging.
- Technologies are evolving very rapidly.
- Biomarker development was discussed by the group. A barrier to drug development is that current biomarkers are too long term. New biomarkers are needed to predict patient response within a shorter timeframe. In particular, biomarkers are needed to predict rapid progression. Although liquid biopsies are an attractive goal, circulating biomarkers will need to be correlated with changes in tissue, requiring the collection of biopsies for biomarker development. Long-term cohorts exist that have banked samples and outcome data (e.g., CRIC, NEPTUNE), but these studies have not yielded promising biomarkers to test.
- RNA sequencing of single cells was discussed. For collection of RNA data, RNAase inhibitors are used, and frozen tissue is preferred.
- The question arose of whether pathways are likely to be different in differing types of CKD. It was suggested that more divergence is likely in early-stage disease.

- Questions to consider in study design include determining an acceptable number of biological replicates and whether to obtain multiple biopsies.

Appendix B: Tissue Collection Session

Breakout Group Leaders

- Technology Chairs: Stephen Hewitt and Matthias Kretzler
- AKI Chairs: Lloyd Cantley and Joe Bonventre
- CKD Chairs: John Sedor, David Salant, Amy Mottl, and Mike Mauer
- NIDDK Representatives: Mike Flessner and Danny Gossett

Charge Questions

- What are the risks?
- What are the benefits?
- How do we adequately communicate with patients?
- What will be required to change the existing culture?
- How will patients be selected and recruited?
- Are there specific issues for AKI?
- Are there specific issues for CKDs?

Discussion

The session opened with a review of the questions to be discussed. If tissue is acquired from kidney biopsies, how much is needed? How should it be stored? How should the tissue quality be assessed? Are new tools needed to ensure that there will be samples from a large enough group of people to standardize going forward?

Dr. Mattias Kretzler explained that many new technologies are starting to gain traction. Much structural information can be captured now and made widely available. Technology working groups are developing these technologies and miniaturizing them. The AMP moved to a single-cell focus. In NEPTUNE, however, aggregate tissue still is used. Protocols now are using 5,000 to 8,000 live cells as a starting point; the challenge is to get viable cells. Cores are split, one half is stored in a fixative and the other half is frozen, and preserved samples are shipped overnight to a facility that can do single-cell analysis.

A participant asked if existing tissue material was being considered for research, noting that each participant's home institution probably retains many historical renal cell samples. He suggested that with the right bioinformatics approach, 100,000 samples could be analyzed. Dedicated material procured in the right environment could be used, but archival samples will be substantially noisier. Another participant agreed that the noise would be large, but added that a large enough number of samples could help eliminate the noise. Existing tissue samples are a source of data with no risk to patients and could be used in parallel to other research techniques. A participant suggested that archived tissues could be particularly helpful with rare diseases because it would take a long time to gather all that data from new patients. It was noted that rare diseases tend to have better phenotyping because they are rare and scientists have thought carefully about them.

Upcoming changes to the Common Rule will include serious restrictions on consent; that rule will not be retroactive, but many journals are saying they will not publish studies unless the consent is clear. A participant suggested that NEPTUNE be the model for how studies should be conducted with regard to consent in that it does not include any clinical kidney biopsies—all biopsies are research biopsies.

A participant mentioned RNAlater[®], noting that tissue is held back and used later if needed; he wondered why it was not being used for more purposes. Dr. Kretzler responded that several research networks are using it now, explaining that it is useful for light microscopy but not immunofluorescence. RNAlater[®] is also much more expensive, so funding agencies would have to assume enough of the cost for it to be feasible.

One participant noted that in his practice, every patient is approached to donate an extra core for a research study, so the majority of patients are contributing in one way or another to a research opportunity.

A participant asked if it is a foregone conclusion that the field is moving to a single-cell platform. He asked if scientists have considered alternatives that might be able to produce a single-cell phenotype but maintain the morphology. Dr. Kretzler explained that this is a question that is still under consideration, noting that the movement to a single-cell platform is not absolute.

A participant suggested that a dedicated research unit embedded into a clinical operation would shorten the distance between sample procurement and analysis.

It was suggested that longitudinal clinical phenotyping is an important issue for the group to address. In studies with small numbers of subjects, an even greater need for validation exists.

A participant asked about the size of biopsy cores, commenting that the average clinical biopsy seems to be much smaller than the recommended size. Dr. Kretzler noted that the protocols being used for the Transformative Research in Diabetic Nephropathy (TRIDENT) study and NEPTUNE are using is to generate an additional core; the clinical cores are taken first, and after clinical needs have been met, the physician declares the smaller core to be the research core.

Dr. Kretzler was asked how to prioritize the collection of tissue for research biopsies with patients who bleed. He noted that feedback from the ethical panel will be informative for this.

A participant explained his experience with protocol biopsies. He submitted tissue for light microscopy and asked to make three slides. Because the diagnosis was known, he asked that the tissue not necessary for clinical purposes be left untouched. He suggested that a protocol be written that provides for adequate clinical evaluation of protocol biopsy tissue while keeping most of it for research. He cautioned that RNAlater[®] has substantial effects on electron microscopy.

It was noted that getting an additional core from a clinical biopsy is an activity that could be performed at many locations. To maintain strict rules for safety, however, protocol biopsies should be performed by someone dedicated to that research procedure and the sample collection should not be spread across many sites.

One participant commented that clinical phenotyping will get out of control quickly unless it is well-planned from the beginning by people who know how to develop SOPs and use a standardized electronic record.

A participant questioned the ethics of returning to a former patient several years later for another biopsy, noting that this is not often done clinically. He recognized that a second biopsy can have enormous

statistical power, however, for removing noise. A participant described a study of 285 patients with a baseline kidney biopsy. A total of 254 patients completed the study, and only one patient refused a second biopsy because of their experience with the first, so in general, a poor initial biopsy experience is not an issue. He noted that protocol biopsies are frequently people who are committed to the cause. It was asked if it was difficult to gain IRB approval for these protocol biopsies. The referenced study involved seven IRBs in two countries, and none refused. It was noted that the subjects had no clinical indications of disease.

A participant asked about the best approach to using normal tissue for protocol development, technology development, or as a comparator to diseased tissue. Living kidney donors have real advantages in terms of not having had the illness.

The session moved to discussing AKI, noting that many issues are the same as those already discussed. One of the AKI chairs asked the participants if anyone thought an AKI biopsy could be research-only. He noted that the field stopped doing biopsies for AKI because the results were not affecting therapies and the data were not being used, but information always can be gained from a biopsy. The question was raised of whether a researcher seeking IRB approval for a research core for AKI is performing a research-only biopsy if the doctor always will return to the patient with clinical data.

Another participant asked if there is a reason other than research to do a biopsy in AKI patients. A respondent explained that the history of renal biopsy included all research that was performed when no therapy existed for the diseases. He noted that two cores are taken now because more information is acquired from electron microscopy and immunofluorescence than from H&E; he wondered why the field has not begun taking three cores now that the science has progressed significantly.

The participants discussed whether a biopsy provides significant information to justify its use without necessity for diagnosis. One participant suggested that the most obvious time to do a biopsy is when drug-induced AKI is suspected; another participant stated that nephrologists are fairly proficient at diagnosing ATN from nonbiopsy tests and, thus, a biopsy is not warranted. Another participant disagreed, explaining that ATN is not a diagnosis but a condition that can be caused by many possible problems. A biopsy to diagnose ATN might not end up being worthwhile, but surprise diagnoses always occur. It was pointed out that if there are no more clinical actions to be taken, the biopsy becomes a research biopsy. Another participant commented that it may be viewed as a research biopsy by the community—although perhaps not by such specialists as the session participants—but it will not change the actions taken, particularly if the researchers begin doing protocol biopsies early in the disease process. If clinically relevant information is obtained from a research biopsy, it is still a research biopsy; the best research biopsies may be those that also provide clinically relevant information when they can. In any case, the patients' consent still must be obtained, and they still must be made to understand that the tissue is used for research purposes but that information in the biopsy will return to the patient.

A participant asked if any early intervention studies exist that can be coupled to biopsies, such as Phase 1 studies focusing on ATN. A suggested model would be a renal biopsy that reveals only ATN, which would provide an opportunity for obtaining data from a subset of patients with a clinical reason for doing a biopsy who could then be asked to consent to some part of the tissue to be used for research. This might form the basis for interventions. Another participant suggested that this posed a clinical conundrum, as the actions taken would be very different if the patient has ATN versus some form of rejection.

The participants discussed the most “sellable” way to explain the need for biopsies to colleagues, noting that they probably tend to be overly conservative about conducting biopsies for AKI. Diagnostic reasons can be used to justify the procedure, and patients want to know what is happening with their disease. One participant observed that if 100 people with suspected ATN are biopsied, 5 to 10 percent will have something else wrong. If biopsies are not performed for AKI, the result is a self-fulfilling answer—if

researchers do not biopsy because they do not have a therapy, they will never have a therapy. Another participant suggested that the percentage of patients whose biopsies would reveal another issue is greater than 5 to 10 percent and perhaps closer to 25 to 30 percent. Another argument for performing a biopsy is that at a later stage, the procedure would show the conversion from AKI to CKD. Any diabetic with AKI also could be biopsied with justification. These could be potential pilots the group proposes to establish precedents for these types of biopsies. The consensus was that very strong arguments could be made for selective circumstances or the discovery of new therapeutics, but one could not advocate for biopsies on all AKI patients.

One participant remembered that at least one biopsy study with AKI had been done, noting that as always, it is worthwhile to return to the literature before stating that a particular type of study has not been done. A participant advocated for a pilot study to identify the cohort in which there is clinical equipoise. Another participant commented that he had recently worked on the consult service and biopsied four patients in 2 weeks because it was not clear why they had AKI. He noted that the line between clinical and research activities is flexible because in clinical practice, doctors must gain consent from patients and inform them that some procedures may not help them. The participant suggested that the line is arbitrarily drawn.

The session participants began to consider CKD questions. They discussed whether the NEPTUNE approach with indication biopsies to obtain an extra core was an appropriate model. It was suggested that the patient populations of interest are those who are not biopsied routinely, such as diabetic patients. One participant commented that there is a culture in which a biopsy of a diabetic that indicates only diabetes is an indication of a mistaken decision to perform a biopsy, and that culture has to go by the wayside.

In terms of moving the field forward, one participant commented on the need to educate colleagues in the field. Many clinicians are not aware of some important aspects of the process, so specialists must educate clinician colleagues and patients about the function of the kidneys. As education expands, it becomes easier to advocate for performing more biopsies, which is what is needed to make them more acceptable.

A participant commented that the important question has become how to “sell” the procedure and how to pass the IRB. He wondered where the knowledge of blindness and diabetes would be if ophthalmologists had been told that they only could do functional tests and could not look at the eye. Much damage occurs in diabetes before it is recognized.

It was suggested that nondiabetic patients with GFRs between 90 and 45 could be part of an intervention trial; the endpoint would be to examine what happens to fibrosis. The goal would be to affect a particular aspect driving the progression, but the intervention also could be studied for structural and molecular effects. A participant noted that the current methods would call for 2,000 patients in a CKD study who receive treatment in one arm versus the other for the standard of care. In such a study, very little is learned about how the conditions are actually working. A smaller scale study with renal biopsies could be a better way to try to understand the actual processes and would be an ethically easier design to support.

The participants discussed whether to recommend clinical or research biopsies, and the consensus was for both.

The participants debated how to collect patients with no evidence of kidney disease. One participant suggested that the missing piece is research biopsies on people with diabetes, including people with no manifestations of kidney disease—this would be an approach to learn about the early stages of the disease. One suggestion was to look at populations known to be at extremely high risk of developing DKD, but most diabetics do not develop it. The participants wondered how to convince an average adult type 2 diabetic with no albuminuria to undergo the tests. Validation for biomarkers was suggested, and another participant commented that researchers must target their resources, such as approaching patients

with a family history of diabetic end-stage renal disease (ESRD), significant retinopathy, or other risk factors that would increase the patient yield.

The group of patients who do not progress was discussed. One participant suggested that in a theoretical group of patients with 15 years of diabetes and normal kidney function levels, he would tell them about the possibility that they have protective mechanisms and that research has the potential to learn how to stimulate those mechanisms, which gives an at-risk person an opportunity to contribute. Another participant commented that cultured cells of people with and without nephropathy and normal cells were demonstrative; people who were protective were different from both fast-track people with nephropathy and normal controls. He commented that nephrologists are missing this population of people who never get sick, and they could be broadcasting an important message that doctors are not receiving.

A participant asked about the interim urine and blood specimens to collect to standardize across platforms, remembering that BEAt-DKD also collected MRIs. The response was that such an invasive procedure also should include a full complement of noninvasive elements. When the tissue signatures can be triangulated with a robust noninvasive element, the study can move into a larger sample size. The bottom line is to acquire all the longitudinal data and noncontroversial data available.

The group generally agreed that the field should move toward protocol biopsies in people with CKD to find earlier stage DKD. Many such patients do not have the classic symptoms. The participants discussed the cutoff point at which the disease is too far progressed, with some participants suggesting a GFR less than 30, some saying they would not accept GFRs less than 35, and others recommending 40 or 45. One participant commented that the lower the cutoff, the smaller the kidneys and the higher the risk. It was suggested that if the way the field thinks about tissue and research is to change, researchers have to err on the side of safety, and there are limits to what can be learned from scarred tissue.

Appendix C: Bioethics Session

Breakout Group Leaders

- AKI Chairs: Chirag Parikh and Steven Korbet
- AKI Patient: Sandra Palumbo
- CKD Chairs: William Knowler and Kathy Tuttle
- CKD Patients: Richard Knight and Paul Conway
- NIDDK Representatives: Paul Kimmel, Andy Narva, and Kevin Abbott

Charge Questions

- What are the risks?
- What are the benefits?
- How do we adequately communicate with patients?
- What will be required to change the existing culture?
- How will patients be selected and recruited?
- Are there specific issues for AKI?
- Are there specific issues for CKDs?

Discussion

A participant pointed out that this group has to determine how to get real biopsy tissue; how to ensure that this tissue can be used to improve the lives of patients; and how to do it in a way that is ethical when this procedure is known to be not completely safe.

One participant referred to three case categories discussed earlier in the day and proposed starting with the hardest cases first, offering these patients the opportunity to further understanding of the disease by participating in research-only biopsies. However, some clinicians may be unwilling to ask this of a patient under any circumstances. A participant commented that many earlier presentations referred to existing research-only biopsies for CKD, and it was explained that many of those studies, like NEPTUNE, are drawn from existing material and as such carry no additional risk.

Broader engagement of the medical community and outreach to that community is needed. A participant pointed out that sepsis is the most homogenous endotype that nephrologists see. Subgroups of people with ATN in persistent or higher stages also could be good candidates. She recommended that nephrologists form partnerships with other doctors and surgeons. Another participant noted that many patients who may be good candidates for biopsy are not under the care of nephrologists. Many people with ATN or AKI do not have it on their problem list and are unaware they have it until it becomes severe.

A former anesthesiologist commented that it is important for someone to take ownership of performing biopsies and the natural community to do so is the nephrologists. They should standardize procedures,

determine the hurdles, define the research goals, and perform the training. A participant noted that many nephrologists are not currently doing these kinds of procedures and are treating ESRD instead of focusing on prevention. Awareness is low because nephrologists have been less proactive and engaged as they should be.

A participant pointed out that ATN can have many causes, including sepsis, and patients with these issues are the sickest; these patients and their families will be hesitant to perform a biopsy because the risk outweighs the reward. The procedure must be considered in terms of logistical, clinical, and ethical issues. Logistical issues include consent; clinical issues include all medical considerations that would affect the procedure; and ethical issues include such questions as whether a researcher should proceed with a test for altruistic societal needs if it is likely to confer risks to a patient that are not minimal.

Risks versus benefits to patients were considered. A participant working in ethics commented that most of his work has nothing to do with informed consent and everything to do with risk/benefit considerations. He questioned what arguments nephrologists have constructed in the nonclinical space to convince the family, patient, or IRB that this procedure is reasonable, suggesting examples of animal models or new targets. An example in earlier discussions mentioned glioma patients with a 100 percent chance of death, thereby making the high risk associated with research-only tests acceptable. Sepsis patients have a 90 percent risk of death; although this is high, families or primary care physicians might not accept the risk of doing these procedures. A participant asked whether evidence exists that procedures performed on the kidney make a difference in these patients, suggesting that the first patients to test should be those with clear kidney disease and no other medical issues. A participant suggested that clinicians need to communicate the benefits because this procedure is predicated on risk/benefit considerations, wondering how much nephrologists must explain to patients about being one very small part of a very long process. It was pointed out that personal and societal benefits at the beginning of this process will be very low.

A participant pointed out that the group had been discussing personal benefit—which is absent or small for research biopsies—and societal benefit, but he proposed a third sphere of benefit, describing his work in American Indian communities, where everyone knows someone with diabetes. Members of these communities want to participate in research not for society at large, but for their own relatives and community. He proposed that such a benefit sphere could be found for any familial disease. Another participant pointed out that the cystic fibrosis community, for example, is very activated and engaged in any new trial; patients with kidney disease could learn from the engagement of other groups. A participant commented that the best example in modern medical history is HIV/AIDS. Activist groups knocked down barriers and showed that the disease can affect everybody, such as through the Ryan White campaign. Several initiatives are in progress for kidney issues, such as the Kidney Health Initiative, but how to connect to these and stimulate research interest is not clear.

The challenges of communication with patients and families about risk was discussed. The risk to a patient with AKI may be one death in 1,000 procedures, so it is important to determine how to talk about the risk honestly in establishing partnerships with patients. Another participant countered that the risk of death is not well known in many of these patients because studies have used stable patients and many real patients are less stable. A participant discussed the importance of making the patients partners in something that is not easy to understand. Patients often are critically ill, so the consent must be acquired from the family.

One participant raised pragmatic concerns of the appropriate training for the person who performs the biopsy. The participant noted that if the biopsy is performed in an academic hospital, the procedure could be done by trainees. Standards will need to be written with consideration for who performs the procedure and what their training is. A former IRB chair noted that he asks for 3 years of endoscopy data to

determine the best person to perform the procedure—it may not be the senior attending. The number of procedures done for clinical purposes can reasonably be translated to research-only procedures.

The appropriate authority for establishing safety protocols was discussed. Another participant described the process of developing a data coordinating center; the center frequently is the body that arranges protocols for safety and ethics. Considerations of who performs the procedure could be written into the funding announcement, or the study group could be charged with determining best policies.

The discussion turned to ways to biopsy patients in the ICU. The perspective was described for non-nephrologists: a patient in an ICU is consistent with an ATN diagnosis, and this situation should resolve within 3 weeks; if resolution has not occurred in that time, a biopsy will be needed. A participant pointed out that the most common phenotype in the ICU now is chronically ill patients, and kidney disease is the most common chronic organ disease. However, it is unclear whether the kidney is a bystander in the chronic failure process. Avoiding dialysis may be sufficient benefit to perform the procedure even if it has no effect on the rest of the disease.

One participant suggested that if the goal is to determine how to do research-only biopsies with new technologies, the group with the fewest number of other organs involved should be the group studied, which would provide a clearer view of the effects. A report from this committee might include considerations for performing the easiest study and considerations for studies on common life-threatening illnesses.

A participant wondered how to change the paradigm and get other kinds of doctors involved, suggesting that a coordinating center would include many integrated specialties. Another participant commented that nephrologists become involved too late in the process and are the only ones who do not participate in the currently popular health care team model. It was suggested that nephrologists should get involved with primary care physicians and catch problems early because patients still go to primary care physicians first. Another participant pointed out that the CKD initiative was intended to educate primary care physicians about CKD; at some point, primary care physicians have to refer the patient, but nephrologists could do better communicating which patients are the best candidates for referral.

The need for more education of patients and nephrologists was discussed. Patients need to be aware of potential issues, such as problems that may arise with contrast in certain situations. A participant proposed creating a learning health system with connections across different settings, commenting that nephrologists can learn a lot from oncology: Research occurs at the point of care, clinical trials are part of community practice, and there is no isolation of diseases or specialties. Research activities need to be incorporated into what is done for patients so that the burden of care can be reduced.

The issue of whether researchers have built up enough momentum with autopsy samples and other biopsies was raised. Several participants were in favor of expanding the use of existing samples, but others noted that biopsies done at the convenience of the patient and doctor will be very heterogeneous and potentially of questionable clinical relevance. A participant noted that his institution includes a program with diseased donors, so they are able to receive a biopsy when the organ is removed.

A participant wondered about the purpose of these studies, saying that her emphasis is the development of noninvasive tests and open organ registries so that patients can access their information.

Appendix D: AKI Session

Breakout Group Leaders

- Chair: Lloyd Cantley
- Other Discussion Leaders: Joseph V. Bonventre, Stephen Korbet, Manjeri Venkatachalam, Charlie Alpers, Robert “Skip” Nelson, Sandra Palumbo, and Nicholas Sadovnikoff
- NIDDK Representatives: Paul Kimmel and Kevin Abbott

Overall Goals

- Assuming we are able to get adequate tissue from a substantial number of subjects with AKI, what would you like to do with it, with what goal in mind?
- Kidney biopsy: Will it lead to new pathways and drug targets in AKI?
- What are the driver cells of interest? What are the driver pathways?

Charge Questions: Defining the Cohort

- Will research biopsy of patients with AKI be acceptable to patients?
- What patient populations should be included (questions, populations, endpoints, feasibility, safety)?

Discussion

The session opened with a reminder about the overall goals and questions provided for the discussion. The first questions posed to participants were (1) What should be started right now? and (2) What is optimal? These led to a conversation of possible “low-hanging fruit” that should be discussed first.

It was clarified that the NIDDK is most interested in the logistics of getting the right people—patients and doctors—into a clinical situation where the biopsy can be obtained and identifying what can be done with the tissue. The NIDDK also is interested in phases: what can be done in a 2- to 3-year timeframe versus what can be done in 5 years. This raises a question about what each phase would look like. In many ways, the output of this meeting will help determine a path forward for this issue, and one goal is to codify an explanation for patients on why this is being done.

A participant suggested that biopsies could be done across multiple centers and shipped to a central core where they could be compiled for sequencing and downloaded into a database with particular clinical components tagged and defined.

Potential research goals discussed included the following:

- Predictors that someone who presents with AKI will progress to CKD.
- Drug targets for a defined population or populations.

It was emphasized to the group that no request for applications (RFA) has been written yet. Typically, the NIDDK would have a data coordinating center, which would serve as the central intellectual repository of all tissue. That central system would be multidisciplinary: It would include pathologists, “big data”

specialists, small data clinical statisticians, nephrologists, ethicists, primary care doctors, and patients—all of whom would be involved with the central curation of the data that came from two to four clinical sites. The clinical sites would be tasked to recruit patients, organize logistics, work through ethical issues, address clinical problems, and obtain the best data from patients—including tissue—which would be placed into the central repository for analysis.

The disposition of the information from all the biopsies that have been done to date was discussed. No central data depository currently exists. It was suggested that the computing and interpretive power of current tools will lead to the creation of a grand repository of data with access for all researchers. It was noted that different data cataloging systems are in use, however, and these systems do not communicate easily.

Participants elaborated on the nontransferability of the data in clinics' EHR systems. When study patients come in, someone from the center will need to know that they have come to the clinic, get new data, and have someone transfer that data to the data center. A concern was raised about the usefulness of the biopsies without accompanying clinical data; a participant suggested that this consortium try to curate clinical data from EHRs. A participant suggested that a consortium could be developed with an AKI component, a CKD component, and a central data center to curate the data. One challenge would be finding a way to automate the transfer of basic patient demographic data (e.g., age, race, ethnicity, exposures, hospitalizations, drug history) from the clinic to the data center. It will increase the amount of effort required from the study coordinator, as well as the numbers of data errors introduced via transcription from the clinic's record into a case report form. It also was emphasized that the desired baseline clinical data must be identified.

One participant queried whether this group was expected to decide on all of the clinical data that needs to be saved around each biopsy or whether the central function of the proposed data coordination center will be to define the clinical information to be uploaded. An NIDDK representative explained that if this group could present the clinical scenarios and recommend the number of patients, how the study would function, and how safety will be addressed, that would be enough, adding that any suggestions on what the database might entail would lead to a richer discussion.

Participants discussed the potential duration of the study, as well as timing and types of follow-up. It was agreed that this would be determined by the decision on whether a study focused solely on AKI or whether it examined AKI and CKD together. Questions were raised about whether certain follow-up could be done during a multiyear study, and one presenter suggested including language in the RFA about maximizing follow-up during the funding period.

A lengthy debate ensued about how the size of the cohort would affect the usefulness of drug targets to the pharmaceutical industry. Proposed cohorts included a pool of all types of AKI patients, patients with a specific type of AKI, and both AKI and CKD patients. The group was reminded that the decisions from this session on how best to define the cohort would affect how NIDDK's call for research will be written.

Some participants advocated a very broad cohort, arguing that if the data collected were broad enough, it could easily be parsed into the correct "buckets" to obtain more specific data and compare them between a large number of subgroups (e.g., which patients do or do not have proteinuria, which do or do not have underlying diabetic disease) to examine whether any pathways are consistent across multiple subgroups. Other participants, however, were concerned that using too broad a cohort would lead to data that are not specific enough to answer anything. A participant suggested that this study could help clarify the current "gray zone" between AKI and CKD to determine which AKI patients might be more likely to develop CKD.

Some participants advocated using a narrow cohort to obtain more specific data from the start of the study. Others were concerned that using too small a cohort would produce results so specific that the pharmaceutical industry would consider pursuing the identified targets as too big an investment for too small a return.

Participants disagreed about whether a pharmaceutical company would be more likely to develop a drug for a population that was nearer critical condition or for one that was in the beginning stages of AKI. A drug for critical populations might have a stronger immediate impact, but one for an early-stage population would have a bigger long-term impact.

One participant asked whether any animal models of ATN mimic the pathways in humans. A respondent indicated that it is extraordinarily difficult to mimic what happens in AKI in animals. A brief discussion ensued regarding whether it might be useful to attempt models in such animals as nonhuman primates and guinea pigs.

A participant suggested that the transplant population would be easiest to reach to form a cohort, and the proposal was made that biopsies could be performed on transplanted kidneys at multiple stages, including pretransplant, during transplant on the operating table, and post-transplant.

A participant suggested that the pharmaceutical industry has said it needs short-term biomarkers immediately to help predict who might have a troublesome course.

One participant emphasized that specific attention must be paid to patients who have any kind of subclinical rejection phenomena.

A participant asked whether certain high-risk populations, such as bypass patients, ought to be considered for biopsies. Another participant cautioned that surgeons likely would be reluctant to perform biopsies on such patients, as their goal is to complete their procedure and close the patient as quickly as possible.

Proposed selection factors for cohorts include which type of AKI a patient has, whether the patient also has CKD, whether a patient is diabetic, and how damaged a kidney is.

A participant raised the question about how to convince patients to do so many biopsies, which could lead to patient concerns about potential trauma to the transplanted kidney, antirejection medication, and what the benefits might be of undergoing multiple biopsies. Another participant clarified that due to the position of a transplanted kidney, the kidney is more accessible and the biopsy would be different from a pretransplant biopsy. Several participants shared their opinions that patients would be willing because someone who has experienced renal failure and dialysis would understand the need to learn more to be able to help others, perhaps by delaying dialysis.

One participant added that a clinician could explain that a biopsy of a donor kidney could help quantitate the ways that kidney is imperfect and might affect how successful the transplant will be. Another participant raised the option that clinicians could inform a patient that the biopsy could provide data that might retrospectively reveal information about delayed effects of transplanting a kidney with particular biomarkers, which could affect the decisions of future patients about whether or not to accept a kidney with those biomarkers. A participant shared their experience that even though they had received approval from their own institution's IRB, the organ bank that the institution works with blocked such biopsies from being done. The organ bank is concerned that the discard rate will increase, which will lengthen the wait time for patients on the donor list and lead to more patients dying while waiting for a kidney. The organ bank in question might be more likely to approve biopsies if they are used only for research purposes and not for clinical decisions. Because of the complex ethical issues involved, this conversation was tabled and the group focused on other issues during the session.

A discussion occurred about what would happen once some biomarker data are obtained and some pathways identified: What would be done next for the proof of concept? Would it be *in vitro* or *ex vivo* work? Would it involve an animal model, or would it go straight into a Phase 0 or Phase 1 study? One presenter suggested that if a pathway is identified and it is clearly up- or downregulated in humans, it would correlate tightly with an outcome and could be incorporated into an animal model; once the animal model has determined whether or not a drug will work, then a Phase 0 trial would make sense. A proposed Phase 0 study's first year might involve obtaining 10 biopsies from the group of patients being considered. While the tissue is being stored in a repository during that first year, the scientific data cohort and the biostatistical center could set up SOPs. The analysis could wait until after SOPs are established.

One participant expressed a concern that the transplant group may be less willing to perform these extra biopsies than this group assumes. Another participant responded that some organizations already perform several biopsies, so a supporting case could be made.

One participant reminded the group that in contrast with other diseases (such as the American Heart Association or the American Diabetes Association), no advocacy organization for AKI exists.

Regarding a question about the current storage of biopsied specimens, a participant shared that if a patient does not consent to research at the time of their biopsy, scientists are not allowed to do research using that tissue. Another participant added the caveat that if a researcher is able to de-identify those specimens and prove that there is minimal or no risk to the patient, then those samples can be used if the researcher is able to get the permission from the IRB. In those cases, a researcher must follow CLIA standards and use those specimens only to the extent that some residual diagnostic tissue remains within the archive. It was emphasized that this would, however, preclude the possibility of following up on that patient's future progress because the tissue samples would have been de-identified. One participant shared that their organization includes a parallel EHR into which the patient information flows longitudinally, but all the identifying information is kept on the hospital side so that a de-identified, research-only health record is created. A participant noted that the upcoming Common Rule revision might include a change in consent to biospecimens.

The following analytical techniques were discussed for biopsy samples:

- Several participants agreed that it would be useful to perform RNA-seq on all biopsy samples, which could lead to a wealth of data for future clinical use. This is something that could be done across multiple centers.
- Some participants highlighted the types of data that could be gleaned from scRNA-seq, pointing out that this would be straightforward to implement. Others were concerned that scRNA-seq is dissociative and will not be feasible in diseased tissue. One participant suggested that such information could be matched against segment-specific transcriptomics.
- One participant suggested looking at transcriptomics. Another participant argued that a large volume of tissue is required for that—transcriptomics could be obtained from the biopsy, but it might require the use of an entire core.
- The suggestion was made to take sections and perform microdissection and transcription, which would still leave enough of a sample for CLIA purposes: CLIA stipulates that the entire clinical specimen cannot be used during research and that part of it must be archived. When a core is used only for research and not for clinical purposes, however, the entire core can be used.
- Several participants discussed mass spectrometry as a possibility.

- A few participants suggested label-free imaging.
- Another suggestion was made for basic discovery proteomics, which would take less than half of a core; the Mayo Clinic has done this on a very small section of tissue.
- One participant suggested obtaining tissue slices to perform such exercises as biomarker localization or to build on what the pathology looks like. It was agreed that that material could possibly come from the clinical core.

The possibility was raised that images could be captured during the biopsy tests and included in some sort of digital repository to ensure standardization and open access.

The question arose about how many cores would be collected during a biopsy, which would determine the number of tests that could be run. It was suggested that the collection of multiple cores also could be useful if a patient needs more analysis from a biopsy than was assumed beforehand because an extra core would be available already. One participant suggested a model where cores are split into samples, sending one sample to a centralized data repository, keeping one sample to use locally, and keeping one sample for the study.

Several participants agreed on the need for an SOP for the biopsy process, which would include a written record of the prebiopsy diagnosis (or top three probabilities, if the diagnosis is unclear) as part of the justification for the biopsy. Other possibilities for the biopsy SOP involve specifying the performance of immunohistochemistry or immunofluorescence analyses. Besides a uniform, standardized tissue collection process, the SOP also could include quality assurance/quality control mechanisms.

One participant raised the issue that SCA requires the sample cells to be dissociated, which takes away the context of proximity. It was pointed out that in some cases, if one area of a kidney is contributing to an upregulated signal, its neighboring areas could be reactively suppressing that signal, leading to a neutral reading.

Pathological analysis was discussed. A participant suggested examining the microvasculature of a sample, which would not be captured in a biopsy. Another raised the question about what level of pathological analysis should be done. Typically, only the worst-looking tubules are scored as having a particular degree of AKI; the participant queried whether the individual segments should be scored as well, or whether that might be too much detail. Another participant suggested performing a test to see whether that level of scoring would be feasible. Participants shared that in some institutions, a pathologist would come to the biopsy with a dissecting microscope and examine cores as they are extracted to perform an initial evaluation of some aspects of the cores and to separate what tissue is needed for the diagnosis and what would be available for research purposes. Other participants emphasized that some institutions do not have the enough staffing to do this at this time. One participant argued that this illustrates the need for pathology standardization.

The types of clinical samples and clinical sample analyses that should be coupled with biopsies was discussed. Suggestions included the following: urine, blood, stool, and saliva (for exomes). A few participants called attention to potential complications in examining urine specimens in the KPMP. It was noted that scRNA-seq analysis in urine and blood requires different sample handling than analysis for biomarkers. It was suggested that urine be stored in a way that allows measurement of proteins, lipids, and other analytes in the future. Performing genetic analysis, or at least obtaining the patient's consent to be able to perform whole-exome sequencing or whole-genome chromatin immunoprecipitation sequencing (ChIP-Seq) on the blood, was suggested. Isolating exomes was discussed. Such samples are fairly tolerant in terms of storage, but the techniques to prepare or store them are labor-intensive. How much clear data could be obtained was questioned.

An examination of correlations among biomarkers was proposed. This would allow for a more sophisticated analysis of the protocol biopsy, as the predictive nature of the protocol biopsy could go beyond the histology.

Clinical data to be collected was discussed. Information about creatinine levels, urine output, and drugs would be valuable. This information could be stored in the database with the samples. These data, together with transcriptomic data and perhaps exome sequencing, could help identify common pathways of injury, precipitating factors, and pathologic diagnoses.

The participants discussed IRB approval, focusing mainly on minimizing the risk to patients. One concern is to ensure that high-risk patients (e.g., ICU patients) and patients who need a surrogate (e.g., children, people who are cognitively impaired) are not pressured to give a biopsy. A participant opined that if the AKI community already knew about all forms of AKI except the more dangerous kinds (e.g., sepsis AKI patients), then it might be worth pursuing the higher risk options, but at this point that is not the case. A few participants discussed risk factors, such as how likely it is that an AKI patient would bleed during a biopsy. This is an elevated risk in transfusion patients.

One participant explained that an IRB would ask for data on the complication rate of doing biopsies on the assumption that it would be no worse than what has been done in a clinical setting. That data would then be evaluated against the proposed knowledge to be gained. It was emphasized that such data would need to be institution-specific to ensure that the institution and staff involved have the necessary experience.

One participant suggested surveying a group of patients to evaluate what percentage would support this kind of procedure if it would be helpful to future patients like them, giving them every opportunity to say “no,” and presenting an IRB with that statistic.

Appendix E: CKD Session

Breakout Group Leaders

- Discussion Leaders: Sus Waikar, William Knowler, Avi Rosenberg, Kathy Tuttle, Amy Mottl, Paul Conway, Maria Gomez, Ray Harris, John Sedor, Matthias Kretzler, David Salant, Jeffrey Kopp, and Brad Rovin
- NIDDK Representatives: Mike Flessner and Tracy Rankin

Overall Goals

- Assuming we are able to get adequate tissue from a substantial number of subjects with CKDs, what would you like to do with it and with what goal in mind?
- Using biopsy tissue as the diagnostic platform, what cellular processes or pathways would be most informative to establish CKD phenotypes?
- What molecular pathways in CKDs would be most amenable to a therapeutic target? Repair pathways? Transport and signaling pathways? Uremic solute pathways?

Charge Questions: Defining the Cohort

- Should nephrologists perform research biopsies or biopsies for indications, such as diabetes or long-term hypertension? When would it be best to biopsy during the course of “traditional” CKD for sub-type diagnosis?
- Will research biopsy of patients in early stages of CKDs be acceptable to patients? How about repeat biopsies for research purposes?

Charge Questions: Phasing the Study

- Introduction: KPMP Logic Model
- Task 1: Develop/test techniques/protocols
- Task 2: Pilot studies
- Task 3: Phase 2 patient study?
- Task 4: Phase 3 clinical study?

Discussion

The chairs reminded the participants of the overall goals and specific questions, noting that it is important to have good discussion about the three major points planned. This discussion will be distilled into tomorrow’s presentation.

A participant noted some challenges he has experienced, including that every scientist has a favorite molecule and would focus and drive tissue collection only to that if possible. In light of this tendency, it is important to sequentially manage tissue. Other participants responded that some consortia have done this, and their collected biopsies can be used and compared.

The participants considered what phenotypes should be collected from patients. One participant recommended creating goals and using these to select patient populations and determine the studies needed. It was suggested that new phenotypes could be created based on mechanism and function, rather than relying on existing suboptimal clinical phenotypes. Initially, the field seemed more focused on a single disease, but now it seems to be in a discovery mode, so it is necessary to get patients and samples and to determine ways to get phenotypes. Participants wondered what methods would be applicable to a smaller patient population or a broader range of CKDs and what populations it would be desirable to collect tissue from for discovery purposes. A participant asked if this line of reasoning directed the group to considerations of cellular mechanisms and the single-cell approach. One of the premises of NEPTUNE is that pathology classification has not been helpful and molecular methods are needed. Another participant asked if specific biomarkers or targets exist for different diseases within CKD.

A noted that many patients diagnosed with diabetic nephropathy had biopsies that disagreed with this diagnosis. About 20 to 40 percent of these were diagnosed with another condition.

The participants discussed whether the studies under the KPMP should include all patients or only a particular phenotype. The answer to this question depends on whether the study is focusing on early-stage or late-stage disease because early-stage samples will show many differences but late-stage samples will have converged to a similar presentation. The participants noted that the breakout group that had discussed tissue collection had concluded that early research biopsies are needed.

The chair asked the participants what goal would be desired when obtaining tissue. An early protocol biopsy and careful histopathology was noted to be effective in NEPTUNE for separating the population into subgroups. A study should begin with enough people to answer the questions under consideration.

One participant suggested that correlating histology with markers that reflect the disease progression would be highly valuable. This would allow doctors to identify the fast-progressing patients and learn which diseases or patients would progress more slowly.

The participants discussed development of new targets and the desire to study slow progressives. One participant noted that the KPMP has been funded with a significant amount of money but limited to a 5-year timeframe. He theorized that the first year would be spent developing protocols and determining how to analyze tissue and that the second and third years would be used for patient recruitment. This leaves scant time to study progression, so a plan is necessary to guide the researchers in developing justification for longer term follow-up of slow versus rapid progressives.

Before beginning a study under the KPMP, the researchers must decide which samples should be collected, given the limited budget and time. A participant noted that it is unlikely that a study starting fresh could get very far with such a budget, but the possibilities presented in this conference have been promising. If researchers are selective, different kinds of expertise could be contributed. Starting with the best characterized cohorts could make studies more feasible. Identifying what is already gathered could lead to shortcuts for a limited study.

A participant brought up slow progressives—“medalists”—as a potential population of people who have gone many years without complications. Another participant noted that there is a clear voice from the pharmaceutical industry for a focus on early diabetes to identify initiating mechanisms, as this is the stage of the disease likely to teach researchers the most about new therapeutic targets.

Large clinical trials in certain centers have subsets of patients within the trial willing to volunteer for kidney biopsies, providing a better understanding of the patient population and the varied nature of what is being studied. It was noted that many altruistic people are willing to volunteer for the procedure.

The chair asked the participants what cellular pathways would be most informative to establish CKD phenotypes using biopsy tissue, noting that late-stage diseases might involve a general fibrotic pathway while early-stage disease pathways likely would be more specific.

A participant offered an opinion about what types of patients pharmaceutical companies target first. Patients most likely to receive a new drug first are those with the most advanced disease. The participant suggested that she would first want to get information on patients with late-stage diseases because they have less time.

One participant expressed reservations about whether early-stage versus late-stage disease would have the same target. Another participant commented that the later stages of a disease should retain some of the same factors present in early stages. Practically speaking, from the patient's point of view, later stages are where the greatest impact could be made.

Regarding cost-effectiveness, every patient treated is a potential dialysis prevention.

A participant who works in drug development pointed out that many drugs are used before the long-term effects are known; it is preferable to use new treatments on later stage patients because this group has the most to gain and the least to lose. Another participant offered a different perspective, describing a late-stage kidney that is more than half scarred and highly unlikely to be significantly affected by drug therapies. This participant noted that even if the patients have “the most to gain,” it is unlikely that targeting the drug to them will help them. Earlier stage patients are actually the population with the most to gain because some kidney function can be salvaged.

The participants discussed the infrequency of stage 3 and stage 4 biopsies due to elevated risk; it was clarified that these patients would not receive protocol biopsies, but they could be biopsied for research purposes. An earlier breakout session theorized that the ideal target would be active earlier but still identifiable in later stage biopsies. Biopsies still should be performed early because good biomarkers have yet to be identified for later testing. Another participant asked whether any data confirm that the initiating mechanisms are still present in later stages. A participant commented that early biopsies would be tremendous for the insights, but the reality is that CKD progression is such that value would be very limited in 5-year or 10-year follow-up unless there also were 2-year follow-ups.

A participant theorized that the field experiences many failures because clinicians are treating stage 3 and stage 4 CKD; treatments may be more effective on a stage 2 population. Animal models may be indicating a more successful early treatment, despite a culture of treating later because of fast progressives. Another participant commented that the discussion is unfortunately being driven by the definition of endpoints accepted by the FDA. Using lupus as an example, trying to develop an endpoint acceptable to the FDA allows researchers to develop drugs. If a population has a deteriorating GFR at a certain rate, a reduction of this rate would be considered a success by clinicians but may not be acceptable by the FDA. If clinicians know that a patient can be kept off dialysis for 10 extra years by reducing this rate, everyone in this room would consider that a “win.” Knowing the endpoint would help in terms of desired results from biopsies for targets.

One participant commented that at more advanced stages, using people with high-risk progression for a trial is interesting, but the more important consideration is the risk that is going to be alleviated with medication. In high-risk patients, the biomarkers that are most important to identify are those that are treatment-specific. Another participant explained that clinicians still can do targeted therapies—if an effective antifibrotic was available, it would be useful for stage 3 and 4, and potentially earlier stages. The question is whether there are targets that will work at both early and late stages, and this answer will not be known until enough biopsy samples are acquired from both populations.

A participant asked how to define early-stage versus late-stage disease, noting that he had read papers indicating that interstitial inflammation correlates well with poor outcome and that glomerular lesions are seen more often in early stages. He theorized that the literature was suggesting glomerular lesions could result from some of the earlier disease pathways and interstitial disease is a later process. Another participant commented that studies of glomeruli would be a compelling reason to obtain early biopsies, and there may be important early changes in the interstitial compartment without obvious gross morphological changes.

Another participant theorized that many drugs could potentially target pathogenetic pathways that could be important. A counterargument was that many of those drugs are not suitable for long-term treatment.

A participant commented that histopathological data suggest that the inflammatory component is driving some aspect of fibrosis. He noted that many immunosuppressants are used in practice and wondered if these are playing a not-well-understood role in controlling the inflammation that is responsible for turnover. Single-cell approaches to understanding inflammation could be applied to other patterns of tubular interstitial disease secondary to primary glomerular diseases. The participant noted that significantly progressed fibrosis does not have many aspects that can be reversed.

Another participant suggested that the current state of the science does not know enough about which pathways or molecules to target, so there is necessary discovery in biopsies to be done. He suggested that obtaining a second biopsy may provide insights into changes in the molecular profile over time. Another participant added that the amount of available information in comparing a first biopsy to a second is incredibly enlightening and shows which molecular pathways are being touched or being left untouched by treatment. He noted that patients who were biopsied again already are developing unexpected chronicity and fibrosis, so inflammation is playing a role, and whatever is being treated with anti-inflammatory drugs is not turning this process off. Another participant noted that once a patient undergoes multiple biopsies, fear is no longer a consideration.

A participant stated that in conceptualizing studies, an imbalance between injury and repair should be considered. If one patient is progressing and one is not, the doctor has missed the critical component in the disease, so it is very important to be looking at the broad spectrum of therapy targets.

The consensus of the group was to focus on protocol biopsies; the ideal stage was not agreed upon, but most participants seemed to prefer early stages rather than late, with potential repeat biopsies if the patient is willing.

The participants discussed taking advantage of such opportunities as clinical trials that already are being conducted, biomarkers that already are well-characterized, and studies that already have been funded and for which patients already have been recruited. It was noted that the participants had had some debate about the quality of data from convenience samples but agreed on the great value of clinical samples that already have been collected—the two points of view do not have to be mutually exclusive. A participant commented that researchers ought to capitalize on existing tissues because of the limited amount of money and time.

It was suggested that kidney disease lends itself well to various disease controls.

The suggestion was made that a short timeframe favors cross-sectional studies.

A participant commented that there is no perfect biomarker for CKD, but there are many possible biomarkers. It is possible to use biomarkers that are less than optimal until the best biomarkers are defined. Another participant advocated for a study like NEPTUNE, which includes groups that have not been recruited widely, groups that are less common in the population, and rare diseases.

A participant commented that in addition to biopsies, the community should advocate collecting other samples—like serum, imaging, and so forth—and eventually looking for peripheral markers outside of biopsies. The example was presented of lupus patients receiving an MRI prior to the biopsy and researchers then trying to correlate the data.

A participant asked how ongoing therapies complicate biopsy-established assays, commenting that many different techniques are used to manage these diseases and wondering how those techniques affect the biopsies. In response, another participant noted that the question was important but not yet answered, explaining that a study like NEPTUNE is observational, and so patients were treated with whatever the clinician decides is optimal. Another participant commented that this approach adds a lot of noise, but researchers are aware of this.

The participants discussed whether it is feasible to study a biopsy before the patient is put under a standard of care or whether that would constitute malpractice. It was proposed that if the clinician is planning to put the patient on a therapy, the biopsy could be performed immediately prior to starting that therapy. A participant with expertise in IRB procedures explained that it would have to be a protocol biopsy and suggested that this could be a new initiative that might eventually lead to establishing the biopsy as a standard of care.

The chair noted that establishing a biopsy as a standard of care would be a culture shift in how patients with a kidney disease are approached and that the field may not be entirely ready for this. The early-disease patients are not yet in nephrology clinics—they are in primary care and not yet being treated by specialists. The chair noted that the discussion group contained doctors with many specialties and asked them to comment on whether their communities would accept their patients going to the clinic and being offered the chance to be in a study. One participant explained that she is not allowed to recruit for her research protocol studies in the internal medicine clinics, but family medicine will allow it. Internal medicine feels that the study is putting their patients at risk, and they also do not want to deter patients—some of whom may be indigent or have otherwise precarious situations—from coming to the clinic. Another participant commented that there is limited money for this research and suggested that perhaps only centers with a good relationship with their internal medicine departments should participate. A different participant noted that cross-disciplinary clinics have been found to be the most successful, and the participant conducting the study added that she also has found success in forming a joint diabetes-CKD clinic with colleagues, which allows patients to attend only one appointment for associated diseases. Another participant commented that other joint clinics have been successful, explaining that diabetes is sometimes more difficult because the diabetologist might deliver only patients who already have the disease, not those with the warning signs, but a nephrologist who develops a good relationship with the diabetes service may be referred patients at earlier stages. It was noted that early-stage CKD in nondiabetes forms is an even more challenging condition in which to find patients. A participant noted that the question revolves around incident versus prevalent patients. If patients are recruited from general practice and they have any degree of hypertension, they already will be on medication.

Appendix F: Data Session

Breakout Group Leaders

- Chair: Andy McMahon
- Other Discussion Leaders: Steve Potter, Sanjay Jain, and Chirag Parikh
- NIDDK Representatives: Rebekah Rasooly and Deborah Hoshizaki

Discussion

Question: *What types of data need to be collected or generated (clinical phenotype, cell and molecular omics, 2D histology, 3D images, super-resolution microscopy)?*

The breakout group discussed the types of data that need to be collected or generated. Formation of a task force to harmonize data collection specifications was suggested. Collection of the following types of data were discussed:

- Whole tissue versus cell type-specific samples. Whole tissue and cell type-specific samples are needed. Particular cell types could be prioritized.
- Imaging data. An imaging data hub with a hub master (similar to GUDMAP) could be created. Use of imaging data in diagnosis is one of the goals of precision medicine. Pathology will play a key role in translation.
- Genomic and transcriptomic data. Dr. McMahon suggested that collection of these data should be prioritized. Methods are sufficiently standardized to allow analysis by a wide range of researchers. Single nucleotide polymorphism (SNP) genotyping should be performed.
- Lipidomics, proteomics, and mass spectrometry data. These data would be supplemental to nucleic acid data because analytical techniques are less standardized. The KPMP should remain open to analysis by these techniques in the long term.
- Variation in promoter and exon usage.
- Epigenetic markers.

Question: *What types of curation and annotation are needed to make data accessible to the research community?*

Types of annotation were discussed. Histology data should be made available. For DNA data, annotation tools are standardized. The lack of standardized annotation for other technologies, such as mass spectrometry, makes the data difficult to use.

Issues of data curation were raised. GUDMAP has explored the challenge of quantifying imaging data. It was agreed that addition to processed image data, raw images should be retained in the KPMP for future analysis. Clinical follow-up data will be needed, but linking data to EHRs is not likely to be sufficiently precise. Research-grade follow-up data will be required, which will involve curation of data from EHRs and ensuring that it is machine-readable. An example of the need for manual curation of EHR data is that diagnoses can change. The data elements that will need to be research grade (e.g., serum creatinine) will need to be defined at the outset, as well as what curation will be performed blind. Data on other disease

endpoints associated with CKD and AKI, such as myocardial infarction, also should be collected, possibly as part of the EHR.

Retention of patients will allow collection of follow-up clinical samples. These samples are likely to be those that are noninvasive (e.g., blood, urine), rather than repeat biopsies. Interactions with patients will lead to increased retention. Incentivizing retention by returning information to patients was proposed, but this would require additional resources and might conflict with IRB regulations (e.g., for genomic data).

Curation and annotation of the data are likely to require a substantial investment. In the NIH Library of Integrated Network-based Cellular Signatures (LINCS) Program, approximately 10 percent of the funds are spent on data sharing and 20 percent on data coordination. Allocation of resources will depend on the degree to which the data hub controls curation versus the data collectors. Infrastructure (i.e., software) design could be centralized, but each collection center could store data on its own website and link the data to the data hub.

Creation of a data coordinating center was suggested. Curation and data generation priorities will need to be harmonized. In the LINCS Program, the data curators were computer scientists and the data generators were biologists, resulting in different priorities. The data coordinating center would have administrative functions to coordinate and oversee data generation. It could serve both the contributors of the data and the users, facilitating low- to high-grade analyses by a broad range of users.

Question: *What data needs to be collected up front to ensure later linkage to the United States Renal Data System (USRDS)?*

Collecting the following data up front was proposed:

- Comorbidities.
- Laboratory results. Including all laboratory results in a database would not be practical, but linking to EHRs might be an option for capturing clinical data without including too many clinical fields in a database.
- Acquisition parameters for sample collection. Relevant data include sample handling protocols and the time between collection and freezing.

Question: *What samples should be stored? How will they be stored?*

The group discussed sample storage. Cells need to retain viability when shipped overnight. For scRNA-seq, samples could be frozen onsite and shipped. AMP has studied factors affecting cell dissociation and transport thoroughly.

Specimens could be stored centrally or locally. Dr. Rasooly indicated a preference for centralized sample storage. Dr. Hoshizaki suggested a tissue core center to oversee sample procurement, which would be performed at the research project sites. The degree to which data-generating decisions are centralized will need to be determined. It was suggested that data-generating sites ship samples to the coordinating center, which would distribute them to the multiple analytical centers. A tracking system will be needed that includes quality control data that can be accessed in real time to quickly identify quality control concerns. In NEPTUNE, batch sample processing led to windows of time during which quality control could not be evaluated, but the problem was unavoidable. Transparency will be needed about factors that might affect quality control, such as changes in staff or sample procurement policies. One option would be to rely on a commercial entity for a tissue core center. For very large tissue hubs, disaster planning becomes an issue.

Dr. McMahon suggested the need to store a large portion of the samples collected for analysis by future technologies. In NEPTUNE, unstained tissue is recognized as a limited resource, and any request to analyze the tissue is subject to a rigorous review process by an expert panel that is knowledgeable about the resource and analytical techniques.

Question: *What are the advantages of a website versus a federated portal? Are there good examples to emulate? What are they?*

Online presentation of the data was discussed. The LINCS Program presents data generated by the program in the LINCS Data Portal (<http://lincsportal.ccs.miami.edu/dcic-portal/index.html>). These data can be searched by such aspects as drug (for drug effects), cell type, gene, cell line, assay, center, and release date. Epic software is not a good benchmark for an organized data repository.

Desirable characteristics for a KPMP database include integration of the data, searchability, and ease of use. Development of online meta-analysis tools will help ensure usability. Data usage also would be facilitated by providing matrices to organize data on input. The design of the relational database should be planned carefully to prevent bottlenecks in the ability to query the database. In NEPTUNE, querying nonstandardized genotype data proved to be challenging. The types of questions a database might answer are whether changes in pathways in a particular cell type exist between normal and disease states, which would require identifying the patient source by condition or disease state. In planning the database, the intended consumers (e.g., scientists seeking to develop hypotheses, biologists, computer scientists, drug developers) should be considered.

Disease classifications will need to be considered in constructing the database, including cases with multiple biopsies and nominal diagnoses. The issue was raised whether specific issues exist with particular diseases, such as DKD or FSGS, that would require them to be described by separate databases or whether all types of AKI and CKD should be included together. It was suggested that the clinical data structure developed in CRIC and NEPTUNE be used. A controlled vocabulary for clinical phenotypes will be needed for the KPMP.

The ability to link to other platforms is a desirable feature for the KPMP database. For example, tranSMART is a platform that allows scientists to integrate phenotype and annotated genotype information. TranSMART has had good uptake by clinician scientists, allowing them to define a cohort of interest and ask such questions as whether transcription in that cohort is regulated differently. It can be used as a tool to determine whether testing a hypothesis is feasible. Health Level Seven International

(HL7) language should be used. It will be important for the KPMP database to liaise with other databases, such as databases containing information about diabetes and the Big Data to Knowledge (BD2K) website.

Question: *How will we ensure confidentiality of data? How will we preserve the participant identity so that it can be linked to other efforts (without double counting)?*

Consent issues were raised. Obtaining consent for use of biopsy data is likely to become more restrictive in the future. Studies for the KPMP might follow existing models of consents. Efforts are being made to develop uniform consents at some institutions, such as the NCI Cancer Centers. Consent forms and research protocols should specify if patients will not benefit individually from studies and indicate that samples will be shared in the future.

Ensuring data confidentiality will be important in the KPMP. Existing software for de-identification of data could be leveraged.

Question: *What should be the sharing policy? When will data and samples be shared with the broader research community? Should data be shared upon validation (and prior to publication)?*

Data and sample sharing policy was discussed. Consent forms and research protocols should indicate that samples will be shared in the future. In response to a question about intellectual property, Dr. Hoshizaki indicated that all KPMP data likely will be shared after validation. Raw data should be publicly available. Dr. McMahon suggested that the NIDDK develop guidelines for data sharing to which investigators will need to agree as a condition for participating in data generation.

Returning data to the data-generating sites was discussed. It was observed that these sites are likely to invest their own resources in generating data for which they will not be reimbursed. Returning data early to the participants will increase motivation and help create a community. One sharing model would be for those who have contributed samples to have earlier access to the data generated. Different types of data could be shared on varying timescales. When NEPTUNE began, core data were accessible to all and uploaded in real time. Additional data that were ancillary to the core data were not made available until they were published. During the course of the project, however, it was found that including access to ancillary data with the core data increased collaboration and benefited all parties. For the Encyclopedia of DNA Elements (ENCODE) project, data were shared with the researchers who generated it, and a deadline was imposed by which they must publish before the data were made public. This approach allowed publication of the data first in high-impact journals. Thousands of articles based on those data were published once the data became public. A 6- to 12-month embargo on making data public was suggested. Young investigators especially need to publish. Building a research community around centralized resources could be a stated goal of the KPMP. Dr. Hoshizaki suggested that the consortium be run as an integrated group.

The KPMP will need to be as inclusive as possible because of the large investment of the NIDDK. Part of data sharing to the broader research community will involve the development of outreach tools. Training opportunities will need to be made available to the community in the use of the tools through workshops and other means.

A model was proposed for the KPMP in which the NIH would fund the development of a tissue resource from the cohort, and future study of those samples would continue under other funding mechanisms. Key to the success of this approach will be establishing a platform to capture the data.

Question: *Are there specific issues for AKI? CKDs?*

Capturing the complexity of disease states is a challenge. A database similar to the reference atlas could be developed to replicate particular conditions or disease states. A comparison with the reference atlas (e.g., heat maps of RNA-seq data) could reveal pathways indicative of disease states. In diseased kidney, changing cell phenotypes and hidden cell types (e.g., by fibrosis) pose additional challenges. Cell type heterogeneity will need to be characterized.

Large numbers of samples likely will be needed. Heterogeneity of disease typically is underestimated. A protocol will be needed to determine which patients' tissue samples are likely to be most informative to make maximum use of material, the collection of which puts patients at risk.

Other Discussion Points

Dr. McMahon raised the issue of the need for reference data. Such characteristics as age, sex, and race are likely to cause variations in normal kidney. A normal kidney reference manual actually might comprise a set of books—an atlas—each specific to age, sex, or race. To produce the atlas, whole kidney samples will be needed from a representative distribution of normal patients by age, sex, and race. The atlas would provide a range of gene expression levels that would be considered normal for each cell population and could be compared to specific patient samples. It was suggested that data in the atlas be linked to the 3D anatomical structure of the kidney. Dr. Rasooly recommended that such an atlas have the capability to be queried by cell type.

The length of time to develop baseline data was discussed. Collecting samples with representative distributions of age, race, and sex is not a trivial challenge, even when including samples from living donors and cancer nephrectomies. One estimate was approximately 2 years, but sample sizes will depend on the variability found.

Obtaining samples from healthy patients can be challenging. For example, one exclusionary condition in a study obtaining skin biopsies from healthy patients was lack of cardiac abnormalities, but approximately 50 percent of candidates were not able to be enrolled because of this criterion. For kidney biopsies, identifying donors without hypertension is likely to be difficult because of high rates in the general population. Body mass index might be another criterion that would eliminate many potential donors.

Possible sources of samples were discussed. Nephrectomies are a possible source of tissue samples. Typically, nephrectomies performed for localized kidney cancer result in a cure, and histologic, clinical, and follow-up data routinely are collected. Nephrectomy tissue might provide information about kidney disease as a result of diabetes or hypertension. Pretransplant biopsies might be a source of normal tissue.

The group discussed using existing data. If benchmarks are developed, these data could be used. Most SCA data exist for microdissected glomeruli and tubules. Thousands of samples exist for DKD, hypertensive kidney disease, FSGS, and nephrotic syndrome. Dr. McMahon responded that GUDMAP decided not to use existing data because of quality control concerns, although RNA-seq data for kidney is easier to perform than microarray data.

The feasibility of SCA was considered. Currently, SCA can be performed on normal tissue. Performing SCA on diseased kidney is very ambitious, but protocols for SCA are being developed. For reference, single-cell data can be compared to nondissociated tissue harvested at the same time. Dr. McMahon indicated that it is likely that rather than just storing whole tissue, performing LCM and SCA will be proposed before the details of SCA protocols are finalized. It was pointed out that new technologies for SCA are being developed, such as hydrogels, that might represent improvements over existing technologies (e.g., Drop-seq), but opposition was expressed to specifying the use of particular

technologies in the KPMP. Making the case for different technologies would be better left to the application process, and harvesting tissue for single-cell dissociation could be left as an option. A concern that dissociation studies from biopsies would not be able to provide sufficient analytical depth was raised. Dr. McMahon suggested that comparing scRNA-seq data from related cells would be valuable. Information from the structure of normal kidney will facilitate development of positional biomarkers. CyTOF could be performed to detect positional biomarkers, producing data on the range of cell types in a sample and their position in the kidney.

The group discussed quality control for analytical data. It was suggested that a given site specialize in a particular type of nucleic acid data. To minimize intersample variability, analyses should be performed by a dedicated team in large batches. If the same types of analyses are performed at multiple sites, resources will need to be invested for synchronization. As a result, limiting the number of sites (i.e., three to four) would be desirable. Rigorous training will be required for sample collection, preparation, and quality control. This approach was successful in the ENCODE project. Quality control measures will be needed that will apply to all of the sites. Starting with one site performing analyses and adding sites during the course of the study was suggested to minimize the amount of time invested in synchronization.

Including patient representatives on the KPMP steering committee was recommended. This would be the most effective approach to keeping patients engaged.