THE NEPHROTIC SYNDROME STUDY NETWORK (NEPTUNE)

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PROTOCOL—VERSION 4.1

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FOREWORD

This fourth version of the **NEP**hrotic Syndrome S**TU**dy **NE**twork (NEPTUNE) Protocol was prepared in accordance with scientific objectives specified of the cohort studies in the original grant application # 1U54DK083912 initially funded by the National Institutes of Health from 09/08/2009 to 06/30/2014 and subsequently by grant application #2U52DK083912 funded from 09/08/2014 to 6/30/2019. Major modifications to this protocol were made following the majority meeting of cohort studies PIs in Baltimore, Maryland on September 29, 2014. Future modifications will be made as approved by a simple majority of the voting members of the Steering Committee and according to procedures specified in the study management section of this protocol.

1. INTRODUCTION

1.A. Overview

Idiopathic Nephrotic Syndrome (NS) is a rare disease syndrome responsible for approximately 12% of all causes of end-stage kidney disease (ESRD) and up to 20% of ESRD in children (1). Treatment strategies for Focal and Segmental Glomerulosclerosis (FSGS), Minimal Change Disease (MCD) and Membranous Nephropathy (MN), the major causes of NS, include high dose prolonged steroid therapy, cyclophosphamide, cyclosporine A, tacrolimus, mycophenolate mofetil and other immunosuppressive agents, which all carry significant side effects (2,3). Failure to obtain remission using the current treatment approaches frequently results in progression to ESRD with its associated costs, morbidities, and mortality (1). In the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry, half of the pediatric patients with Steroid Resistant Nephrotic Syndrome required renal replacement therapy within two years of being enrolled in the disease registry (4). FSGS also has a high recurrence rate following kidney transplantation (30-40%) and is the most common recurrent disease leading to allograft loss (5).

The prevailing classification of Nephrotic Syndrome categorizes patients into FSGS, MCD, MN, and childhood onset nephrotic syndrome (NS) if in the absence of other underlying causes, glomerular histology shows a specific histological pattern. This classification does not adequately predict the heterogeneous natural history of patients with FSGS, MCD, and MN (6). Major advances in understanding the pathogenesis of FSGS and MCD have come over the last ten years from the identification of several mutated genes responsible for causing Steroid Resistant Nephrotic Syndrome (SRNS) presenting with FSGS or MCD histopathology in humans and model organisms. These functionally distinct genetic disorders can present with indistinguishable FSGS lesions on histology confirming the presence of heterogeneous pathogenic mechanisms under the current histological diagnoses (7-13).

The limited understanding of FSGS, MCD, and MN biology in humans has necessitated a descriptive classification system in which heterogeneous disorders are grouped together. This invariably consigns these heterogeneous patients to the same therapeutic approaches, which use blunt immunosuppressive drugs that lack a clear biological basis, are often not beneficial, and are complicated by significant toxicity. The foregoing shortcomings make a strong case that concerted and innovative investigational strategies combining basic science, translational, and clinical methods should be employed to study FSGS, MCD, and MN. It is for these reasons that the Nephrotic Syndrome Study Network is established to conduct clinical and translational research in patients with FSGS/MCD and MN.

NEPTUNE Overall Objectives, in which the cohort studies defined in this protocol are embedded, consist of:

1. Establish a collaborative, integrated, and cost-effective investigational infrastructure to conduct clinical and translational research in FSGS, MCD, MN and childhood onset NS;
2. Perform a longitudinal observational cohort study of patients who present with biopsy-proven FSGS, MCD or MN;
3. Perform a longitudinal observational cohort study of children who present with childhood onset NS;
4. Administer a pilot and ancillary projects program that selects, supports, and coordinates studies that employ the unique resources, clinical data, or specimens assembled by NEPTUNE;
5. Implement a training program designed for advanced post-doctoral and junior faculty trainees, or established investigators interested in redirecting their investigative focus, who are preparing to become independent investigators in clinical and translational research in human glomerular disease;
6. In collaboration with the NIH Rare Disease Clinical Research Network Data Management Coordinating Center (DMCC) and two well-established disease-specific lay foundations (NephCure Kidney International and Halpin Foundation), develop and maintain a web-based platform for the exchange of information for lay people, physicians, and scientists interested in FSGS, MN, or MCD.

NEPTUNE Guiding Principles to implement the above objectives:

1. Collaboration: Collaboration among all participants within the Consortium, and between NEPTUNE and external investigators conducting pilot or ancillary studies using Consortium resources is essential to the success of the program.
2. Integration: NEPTUNE understands — and will take advantage of — the dramatic benefits to advancing glomerular disease science inherent in employing a multi-disciplinary approach.
3. Sharing: NEPTUNE will serve as a resource to the community of scientists and lay persons interested in studying FSGS, MN, MCD or childhood onset NS. The Consortium's ability to share its systematically collected data, biological specimens, and infrastructure for future ancillary studies is as important to advancing this field as studies conducted directly by NEPTUNE.
4. Networking: The existence of a network of investigators and clinical study sites with experience in glomerular disease research; and subsequently maintaining that network, will dramatically facilitate organizing and conducting future clinical studies by investigators in the public and private sectors.

1.B. Synopsis of Study Design

The proposed design is a two-armed multicenter prospective cohort study with un-blinded standardized evaluation of pre-specified study outcomes in two defined separate and parallel cohorts of study participants. Sub-studies addressing specific research questions will be nested in the main cohorts. Pilot interventional clinical trials will also be performed on subsets of participants of the main cohorts. Up to two thousand participants will be enrolled into the NEPTUNE longitudinal cohorts over the enrollment period from the initial start date of December 11, 2009. The minimum follow-up period will be 36 months and subsequent annual in-person visits and/or chart abstraction for laboratories, vital and end-stage renal disease status may be conducted pending additional funding. The study will be comprised of two arms: Cohort A, a biopsy group composed of two main cohorts and Cohort B, a treatment-naïve, non-biopsy group of children less than 19 years of age. In the Cohort A (biopsy group), the two main cohorts will be specified by histopathologic criteria obtained from a baseline renal biopsy and pre-specified inclusion criteria. The first main cohort will be comprised of participants with histopathologic features consistent with Focal and Segmental Glomerulosclerosis (FSGS) or Minimal Change Disease (MCD) and the second histopathology cohort of Membranous Nephropathy (MN). Combined these Cohort A subsets are targeted to include a minimum of 300 participants to be enrolled under Protocol V4.1. These new enrollees will augment those enrolled under previous NEPTUNE protocol versions. Participants will be enrolled at academic clinical centers in the United States and Canada. The sampling frame is a consecutive series of all eligible and consenting patients undergoing diagnostic renal biopsy for any glomerular disease with the exception of renal disease due to systemic diseases such as lupus erythematosus (SLE), diabetes mellitus or multiple myeloma. A recruit-to-replace strategy will be utilized to ensure that a minimum sample size of 600 participants with FSGS, MCD and MN and 36 months of observation is preserved by the end of the recruitment period. After an initial pre-screening, participants will undergo a screening/eligibility visit, baseline visit, biopsy visit, and subsequently four month-interval follow-up visits during year one and thereafter, six month-interval follow-up visits after biopsy. Each participant will experience 9-10 study visits depending on date of enrollment and subsequent chart review and/or annual visits as funding and study timeline permits. Procedures to be performed during study visits include diagnostic baseline kidney biopsy, laboratory studies, blood and urine collection for biorepository, limited clinical examination, and administration of questionnaires. The primary study outcome of the two main biopsy cohorts are a change in urinary protein excretion and renal function. Several secondary outcomes will be evaluated including but not limited to composite of death, metabolic and hematologic complications, malignancies and infections. Descriptive and hypothesis-driven data analysis of the biopsy cohort studies will be performed at 30, 45 and 60 months of the study. For the FSGS/MCD cohort, a sample size of 250 participants is sufficient at two-sided alpha level of 0.05 to accurately predict the primary clinical outcome of Complete/Partial Remission vs. No Remission of urinary protein excretion (UPE) with a power of 75%-96%. For the MN cohort, a sample size of 200 participants is sufficient at two-sided alpha level of 0.05 to accurately predict the primary clinical outcome of Complete/Partial Remission vs. No Remission of urinary protein excretion (UPE) with a power of 70%-94%.

Cohort B, the treatment-naïve, non-biopsy group, will consist of a minimum of 200 children less than 19 years of age with incident nephrotic syndrome (NS) to establish a prospective, multi-ethnic longitudinal cohort that incorporates detailed phenotyping, patient reported information (PRO) and standardized collection of biosamples. The main goal of this group is to extend the molecular medicine analysis concept to children with incident NS from the onset of the illness and throughout its course. It will address questions that are germane to the diagnosis and management of children with incident NS. Children who meet the inclusion criteria will be enrolled over 30 months with a minimum follow-up of 36 months from academic centers in North America. A kidney biopsy will not be required for enrollment. Should a biopsy become clinically indicated, these participants will be re-consented for kidney biopsy tissue but will not be required to consent to remain in the study. The sampling frame is a consecutive sample of all eligible and consenting participants. A recruit-to-replace strategy will be followed, enrolling up to 400 cNEPTUNE Cohort B participants to ensure that the minimum sample size of 120 actively followed participants is preserved at 100% at the end of the enrollment phase.

Data management, specimen collection, shipping and archiving will be managed with a web-based clinical research data management system using the Arbor Research Collaborative for Health (Arbor Research) proprietary ArborLink system, built specifically for the needs of NEPTUNE. This data entry platform represents a fully relational clinical research database with study management, detailed reporting and auditing capabilities. The organizational structure will be comprised of a Steering Committee of investigators, a representative study coordinator, NephCure and Halpin Foundation representatives, the NIDDK Histopathological Archive, the Pathology Reading Center, and the NIH Project Officer(s). The Steering Committee will be responsible for the overall management of the study. An initial complement of 25 clinical centers will be responsible for performing the main cohort studies at their respective sites, supplemented by additional sites as approved by the Steering Committee. The central hub of research operations will be the University of Michigan, which is the primary awardee of the NEPTUNE grant. The University of Michigan, in conjunction with Arbor Research, will operate a Project Administrative Unit, a Data Analysis and Clinical Studies Coordinating Center (DACC). Additionally, The University of Michigan will operate as a central biochemical laboratory and the Network Biorepository. A Scientific Advisory Committee (SAC) of internationally renowned experts will provide oversight along with a Data and Safety Management Board.

The Nephrotic Syndrome Study Network (NEPTUNE) has been created and charged with the purpose of accomplishing the specified study goals. NEPTUNE is comprised of individuals with outstanding dedication and multi-disciplinary investigative expertise. NEPTUNE came into being in large part due to the unflinching support, and significant commitment from the NephCure Kidney International, previously known as The NephCure Foundation, which is a lay research non-profit foundation devoted to understanding and improving the treatment of FSGS. NEPTUNE will operate in full with NephCure. The Halpin Foundation is also a lay research foundation that is supportive, committed and fully engaged with NEPTUNE. NEPTUNE was initially funded primarily through a 5-year grant award (U54 DK083912) from the NIDDK and ORD ($6.25 million) with additional funding from NephCure ($2.0 million) and the Regents of the University of Michigan ($2.0 million). It has received a subsequent 5 year grant award (U52 DK083912-06) from the NIDDK ($5.81 millions) and the Regents of The University of Michigan ($4.2 million).

1.C. Specific Aims – Cohort A (Biopsy Group)

Participants presenting with Nephrotic Syndrome will be initially enrolled into two main concurrent cohort studies, namely the FSGS/MCD Cohort Study and the MN Cohort Study. The specific aims and research hypotheses of each of the main cohort studies and the combined cohort are outlined below.

1.C.1. Aims Related to FSGS and MCD

Specific Aim 1: To determine the rates and predictors of clinical remission of FSGS/MCD.

Hypothesis 1: After accounting for the types, duration, and frequency of immunomodulating therapy, the probability of remission of FSGS/MCD defined as change in urinary protein excretion and/or renal function

will be predicted by baseline demographic, clinical, genetic and histopathological characteristics at the time of presentation.

Specific Aim 2: To identify gene expression profiles from renal biopsies of participants with FSGS/MCD that can be used to classify participants into distinct molecular subgroups.

Hypothesis 2: Genome-wide molecular profiles obtained from renal biopsy tissue at the time of diagnosis of FSGS/MCD will independently predict the rate of remission of FSGS/MCD defined as change in urinary protein excretion and change in renal function.

Specific Aim 3: To identify the transcriptional networks that are associated with individual genetic causes of FSGS/MCD; namely, mutations in *NPHS1, NPHS2, LAMB2, PLCE1/NPHS3* and *WT1*.

Hypothesis 3: Individual genetic causes of FSGS/MCD activate specific transcriptional pathways in renal tissue and the molecular characterization of these transcriptional pathways may serve as novel therapeutic targets with a high likelihood of more complete and sustained remission of FSGS/MCD.

1.C.2. Aims Related to MN

Specific Aim 4: To determine the rates and predictors of clinical remission of MN.

Hypothesis 4: After accounting for the types, duration, and frequency of immunomodulating therapy, the probability of remission of MN, defined as change in urinary protein excretion and/or renal function, will be predicted by baseline demographic, clinical, genetic and histopathological characteristics at the time of presentation.

Specific Aim 5: To identify gene expression profiles from renal biopsies of participants with MN that can be used to classify participants into distinct molecular subgroups.

Hypothesis 5: Genome-wide molecular profiles obtained from renal biopsy tissue at the time of diagnosis of MN will independently predict the rate of remission of MN defined as change in urinary protein excretion and/or renal function.

1.C.3. Aims Related to the Combined Histopathology Cohorts (FSGS, MCD, MN)

Specific Aim 6: To determine the effect of adjuvant (non-immunomodulating) therapy on the clinical remission of Nephrotic Syndrome.

Hypothesis 6: The use of non-immunomodulating therapy (including antihypertensives, lipid lowering, antithrombotic agents and dietary manipulation) will have an independent effect on the probability of remission of Nephrotic Syndrome defined as change in urinary protein excretion and/or renal function.

Specific Aim 7: To determine the rates of major medical complications in patients treated for Nephrotic Syndrome.

Hypothesis 7: The composite rates of major medical complications (vascular thrombosis, complications of kidney biopsy procedure, infection, and death) will vary according to the baseline characteristics and the types of treatment in patients with Nephrotic Syndrome.

Specific Aim 8: To evaluate the quality of life and its trends in participants undergoing treatment for Nephrotic Syndrome.

Hypothesis 8: The diagnosis of Nephrotic Syndrome, its treatment and clinical outcomes will have a varied measurable effect on the quality of life of participants with Nephrotic Syndrome.

Specific Aim 9: Clinical and molecular stratification of NS via a systems genetics approach. Sub-aim 1A will identify shared and specific molecular subgroups in NS using genome-wide renal tissue gene expression profiles and determine associations between baseline characteristics and clinical outcomes in these subpopulations. Sub-aim 1B will define the association between baseline characteristics, clinical outcomes, and gene expression signatures in individuals with a defined monogenic form of NS or with single copies of deleterious alleles of known NS genes.

Hypothesis 9: A: Functionally defined subgroups of NS will exhibit specific genetic, demographic, clinical, and histopathological baseline characteristics and specific adjusted hazard rates for: (a) change in urinary protein excretion (classified as remission versus no remission); and (b) change in renal function (categorical

renal function events). **B:** Patients with known disease-causing variants and those with rare variants of uncertain significance in these genes will differ in characteristics and outcomes from those participants with SRNS who do not have rare variants in these genes.

Specific Aim 10: To test association of APOL1 risk genotype with baseline characteristics, quantitative histology, and clinical outcomes in AA NS participants and to use whole kidney expression data to discover mechanisms of APOL1-associated kidney disease.

Hypothesis 10: APOL1 risk genotypes will have clinical utility as biomarkers for outcomes in AA NS participants and integrated analysis of whole genome transcriptomes and genetic variation in this population will permit discovery of the molecular mechanisms responsible for pathogenicity.

Specific Aim 11: To define non-invasive molecular predictors of outcome in functionally defined subgroups of NS. Candidate biomarkers, both discovered by NEPTUNE as published by others, will be tested in the NS cohort as biomarkers for renal outcomes and responses to therapy. Biomarker signatures will be linked to intra-renal genetic and molecular pathways.

Hypothesis 11: Both the change in urinary protein excretion classified as a binary response (remission versus no remission) and change in renal function will correlate in an independent fashion with a distinct set of biomarkers in molecularly defined NS subpopulations.

Specific Aim 12: To identify therapeutic targets based on the functional definition of NS. Clinical, histopathological, genetic and molecular information available in functionally defined NEPTUNE cohort patients will be used to identify molecular pathways as key drivers of NS and its subgroups. Intra-renal pathways will be linked with genetic and biomarker profiles in the same individuals to identify potential target engagement markers and candidate predictors of treatment response.

Hypothesis 12: A systems genetics approach linking genetic variance with intra-renal transcriptional pathways will allow identification of therapeutic targets and associated biomarkers for further experimental and clinical validation. In public-private partnerships, proof-of-concept studies will be focused on participants with molecular profiles that suggest NS is being driven by a shared set of "drugable pathways", taking optimal advantage of the comprehensive information and infrastructure developed by NEPTUNE.

1.C.4. Specific Aims – Cohort B (Treatment-naïve Non-Biopsy Group (cNEPTUNE))

To support a true paradigm shift from the current taxonomy that will enhance our understanding of disease biology, disease course and therapeutic options for children with new-onset Nephrotic Syndrome (NS) from the presentation of the illness and throughout its course, an incident children's NS cohort (cNEPTUNE) will be established. The overarching goal of this cohort is to advance the care of children with NS towards precision medicine by integrating molecular, genetic, clinical and patient reported data to understand disease patterns and design diagnostic and therapeutic solutions.

Pediatric nephrology practice provides care for children and adolescents up to age 21 years. At clinical intake, adolescents through their senior year in high school (age ranging 17-19 years) are often referred by pediatricians to pediatric subspecialists. Pediatric subspecialists treat first and based on initial response to therapy, decide to or not to conduct a diagnostic kidney biopsy. This treat first approach is most common in pre-adolescents but is continued in some practices in adolescents. Consequently, NEPTUNE Cohort B with the intention to enroll children and adolescents who enter into nephrology care at the time of disease presentation are eligible to enroll. We will use a common age < 19 as the upper age of eligibility to align with these practice patterns.

1.C.5. Aims Related to the cNEPTUNE cohort

Specific Aim 1: To establish a cohort of pediatric participants with incident NS with collection of biosamples to:

Aim 1a: Describe the relationship between patterns of relapse and remission and demographics, patient reported information, and prospectively collected clinical and laboratory data.

Aim 1b: Examine the association between patient reported *outcomes* and nephrotic syndrome disease activity and therapy in children with NS.

Hypothesis 1: Use of patient reported *information* captured with state-of-the-art communications technology will enhance the accuracy of participant characterization and enable better delineation of factors that influence the clinical course of disease, complications of treatment, and patient reported outcomes.

Specific Aim 2: To identify and characterize genes and molecular pathways that functionally define subgroups of pediatric NS

Hypothesis 2a: Functionally defined molecular subgroups of children with primary NS that are clinically relevant can be defined based on podocoyte, genetic, immunologic, and inflammation related markers.

Hypothesis 2b: Biomarkers in genetic and molecularly defined subgroups will predict clinical outcomes, including response, proteinuria remission and relapse patterns, and loss of kidney function.

Specific Aim 3: To identify novel therapeutic targets based on the functional definition of NS that may be suitable for future therapy development

Hypothesis 3: A systems biology approach linking disease pathways will allow the identification of therapeutic targets and associated biomarkers to monitor the response to treatment and to anticipate alterations in disease activity for future validation.

2. BACKGROUND

2.A. Statement of the Problem

Primary non-inflammatory glomerular diseases that include Focal and Segmental Glomerulosclerosis (FSGS), Minimal Change Disease (MCD), and Membranous Nephropathy (MN) are rare diseases that cause serious morbidity and high mortality. Despite their rarity, this disease group generates an enormous individual and societal economic burden, accounting for approximately 12% of prevalent ESRD cases (2005) at an annual cost of more than $3 billion in the United States alone (1). The true incidence and prevalence of these diseases is unknown since no population-based study has been conducted to date. Nevertheless, extrapolation from incidence data from the Olmstead County, Minnesota study (1994-03) suggests an incidence of 5400 and 3000 cases per year of FSGS and MN respectively, in the U.S. (14). Consistent with these estimates, the United States Renal Data Systems (USRDS) reported an incidence of ESRD of 2866 per year for FSGS and 566 cases per year for MN. (1). The reported incidence of ESRD due to FSGS and MN is likely to be an underestimation since more than 40,000 cases of ESRD per year are due to glomerular disease "without known histology" and ESRD due to hypertension with unspecified renal disease. The majority of these poorly characterized ESRD cases may be due to FSGS and MN since these are the leading glomerular disorders causing ESRD in most single center, histologically well-characterized series.

The clinical classifications of FSGS, MCD and MN do not appear to be optimally informative because diagnostic classification and rationale for treatments is based largely on observed histopathology rather than a system based on an understanding of the molecular underpinnings of these diseases. From a clinical perspective, it is clear that histopathology alone does not adequately predict the heterogeneous natural history or divergent response to therapy of individuals within a given glomerular histopathological category (15). It has been hypothesized that this clinical heterogeneity might be caused by heterogeneity of molecular mechanisms that each present with indistinguishable histopathology and clinical phenotype. Indeed, recent identification of several specific monogenetic disorders provides indisputable evidence that multiple specific disease processes can present clinically with indistinguishable FSGS histopathology (6,16-19). Therefore, we postulate that descriptive renal histopathology taxonomy labeled as FSGS or MN may represent several distinctive diseases with diverse molecular fingerprints and varied underlying pathophysiologic processes.

Our limited understanding of the biology of FSGS, MCD, and MN and the descriptive classification system may be partly responsible for the unsatisfactory therapeutic approaches currently used for these diseases. Current therapies lack a clear biological basis, are ineffective in many patients and are complicated by significant toxicity (20-31).

Given the large multidimensional burden imposed by FSGS, MCD and MN and the lack of effective therapy for the majority of patients, it is necessary to conduct studies that integrate the recent advances in basic science, employ translational techniques of molecular medicine, and evaluate new therapies to address the serious obstacles mitigating the effective treatment of patients with FSGS, MCD, and MN. We propose that several major barriers must be overcome. Among these barriers is the absence of specific biomarkers of glomerular disease that would allow refined, biologically relevant sub-classification of glomerular histopathology. New glomerular disease biomarkers could potentially improve our ability to predict the natural history of these diseases, allow for better tailoring of current therapies and lead to the identification of new therapeutic targets as well as provide the tools for early detection of these diseases.

Importantly, a robust multidisciplinary investigative infrastructure is presently lacking that would facilitate the assemblage of robust samples of participants for testing of new therapies and for the collection of human biological material and clinical data necessary for the identification and validation of biomarkers of FSGS, MCD, and MN. Given the low incidence and prevalence of these glomerular disease patients, a productive and cost-effective mechanism for identifying and recruiting clinical participants is critical to successful implementation of both therapeutic interventional trials and biomarker development studies.

Background of NEPTUNE: There has been long-standing recognition among investigators that the formation of a collaborative research network is necessary to advance clinical research in several glomerular diseases including FSGS/MCD and MN. Regional efforts to form referral networks, particularly by Drs. Ron Falk and Charles Jennette at UNC, Dr. Daniel Cattran at the University of Toronto, Dr. Frederick Kaskel in the Mid-Atlantic states, and Dr. Fernando Fervenza at the Mayo Clinic, succeeded in archiving many cases for valuable cohort studies and conducting seminal interventional trials. In 2005, Drs. David Salant and Lawrence Holzman, with backing from the NephCure Foundation and the NIDDK, organized a Glomerular Disease Workshop that convened international leaders to prioritize research efforts in this field. The published recommendations of the workshop (http://www.niddk.nih.gov/fund/other/glomerular/Summary-Report.pdf) noted inter alia that: (1) "Identification of biomarkers of glomerular disease by a variety of approaches has great potential to advance both basic and clinical research in glomerular diseases"; (2) "Meaningful clinical investigations of glomerular diseases are scarce due to the relative rarity of individual glomerular disorders, limited infrastructure and limited funding. In addition, glomerular disease-specific outcome measures have not been identified for the various morphologic forms of glomerular disease, other than measures such as serum creatinine. It would therefore be useful for investigators to perform studies that would evaluate new methods of assessing clinical outcomes in glomerular disease. Such new outcome measures could significantly reduce both patient sample size and follow-up time for interventional trials of glomerular disease;" and (3) "Participants were concerned about the absence of a concerted national effort to organize and facilitate clinical studies in glomerular disease. Several breakout groups suggested that establishing a cooperative network among nephrology groups at academic medical centers might provide a cost effective means of fostering glomerular disease clinical research." (32)

To build foregoing pioneering efforts, NEPTUNE is established as a multidisciplinary research and education platform that brings together clinical and translational scientists, and lay research and patient education foundations, aimed at improving understanding and identifying promising treatments for FSGS, MCD, MN And childhood onset nephrotic syndrome.

2.A.1. Rationale for the FSGS/MCD Study

The FSGS/MCD Cohort Study aims to develop biomarkers that lead to refined, biologically relevant classification of the variants of FSGS/MCD, which can be used to plan therapeutic clinical studies. The new molecular biomarkers might also predict disease natural history, allow proper selection and prediction of response to specific therapeutic intervention, allow early detection of disease, or provide indicators of disease activity.

Future studies of FSGS/MCD will borrow extensively from the field of oncology in which comprehensive molecular analysis of tumor tissue has led to the definition of cancer-specific molecular fingerprints representing different disease mechanisms or states which are indistinguishable when the classical histological characterization of neoplastic lesions is employed (33-35). In oncology, molecular signatures are currently used as diagnostic parameters to predict disease course and response to therapy (36). Standards are currently set to include genomic markers into routine practice (http://www.egappreviews.org). The NEPTUNE investigators and others have begun to apply these molecular techniques to glomerular diseases. Preliminary gene expression data suggest the presence of a common molecular pathway activated in chronic kidney disease (37). Investigators have also shown that focused cDNA microarray studies can identify sets of mRNA markers that differentiate intra-renal inflammatory states in renal tissues. In concert, Schmid et al showed that a podocyte-specific mRNA marker panel can identify distinct patterns of response to therapeutic interventions in Nephrotic Syndrome (38). Additionally, molecular techniques were used to define transcriptional networks that correlate with severity of clinical course in patients with diabetic nephropathy (39). Molecular fingerprinting tools have been used on renal biopsy tissue and leukocytes to define mRNA signatures in patients with IgA nephritis (40,41) and in patients with vasculitis (42).

Thus, the experience in oncology and initial studies in nephrology, including studies from the proposing group indicate that it is feasible to use molecular phenotyping to broaden our understanding of these diseases. This will alter our approach to diagnostic classification, disease prediction, definition of patient cohorts for clinical trials and identification of therapeutic targets in FSGS/MCD (43,44). It will provide a patient cohort with integrated clinical and molecular phenotypes as a backbone for further molecular

analysis of these diseases in pilot and ancillary studies above and beyond the specific aims defined in these cohort studies.

2.A.2. Rationale for the MN Study

Although MN is a rare disease, it is an important cause of Nephrotic Syndrome (NS) in Caucasian adults (45,46). MN and its health consequences are associated with catastrophic complications and the clinical outcomes include ESRD in a significant fraction of patients. MN accounts for a minimum of 0.7% of prevalent ESRD cases (1), which is likely a significant underestimate given that the majority of ESRD patients would not have experienced a diagnostic biopsy at the time of presentation and thus many cases of MN would have been missed (1,47). Extrapolated from disease incidence reported in Olmstead County, Minnesota (1994-03) where incident MN occurred at 1.0 case/100,000 population per year, the incidence of MN in the USA is approximately 3000 cases per year (48). The total cost for management and treatment of MN and its associated co-morbidities, and the economic impact on patients' lives are unknown, but considered to be substantial. As for FSGS, the clinical classification of MN is inadequate because it is based largely on observed histopathology rather than an understanding of the molecular mechanism of the disease, contributing to the clinical heterogeneity of outcomes in MN and limited success of the therapeutic approaches (49).

In up to two-thirds of MN cases, the etiology is not known and the idiopathic classification is applied to this group of patients. In the remaining one-third of cases, the term 'secondary MN' is applied because this form of the disease is associated with infections (i.e. Hepatitis B and C, streptococcal infection, syphilis), exposures to toxic agents (heavy metals, hydrocarbons, solvents, formaldehyde and certain drugs) and other systemic diseases such as systemic lupus erythematosus (SLE) (50,51). Only a few etiological studies of MN have been undertaken, and these studies showed that many infectious agents and environmental exposures may be associated with the development of MN (51). The major barriers that have impeded significant progress in the search for an effective therapy for MN include the absence of specific biomarkers of glomerular disease that form the basis of biologically relevant sub-classification and the inability to define functional underpinnings of the heterogeneity evident in the current histopathological classification of disease. The significant and poorly understood heterogeneity of MN also hampers the ability to define the effect of treatment and the ability to conduct clinical trials with standardized entry criteria. A primary objective of NEPTUNE and the proposed MN Cohort study is to apply molecular techniques to identify new MN biomarkers that can reliably predict natural history, allowing for a more refined classification scheme.

Thus, the experience in oncology and in initial studies in nephrology, including studies from the proposing group as delineated in the rationale for the FSGS cohort study, indicate that it is feasible to perform molecular phenotyping to improve our diagnosis and therapy in MN. These studies will form the basis for diagnostic classification, disease prediction, definition of patient cohorts for clinical trials, and identification of therapeutic targets in MN (43,44).

2.A.3. Rationale for cNEPTUNE

The majority of young children with new-onset NS have disease onset and flares that often follow an infectious or allergic event. They may have resolution, persistence or disease progression to ESKD. At onset, children with NS are routinely exposed to 8-12 weeks of corticosteroids as a diagnostic and treatment intervention [2]. The short term clinical outcomes following this approach are: 35% infrequently relapsing NS (IRNS), 40% chronic frequently relapsing/steroid dependent NS (FRNS/SDNS), and 25% steroid resistance (SRNS). Over time, an additional 20% of previously labeled IRNS or FRNS/SDNS patients will develop late SRNS[3]. There are no proven methods to predict disease course at the onset of NS. The armamentarium of non-specific therapies for FRNS/SDNS to alleviate steroid toxicity includes cyclosporine, tacrolimus, mycophenolate mofetil, cyclophosphamide, and rituximab, all of which carry significant side effects. These same medications are administered to patients with steroid resistant disease with a dismal rate of success in achieving sustained remission. Severe adverse events include peritonitis, pneumonia, septicemia, thromboembolism, diabetes, and acute kidney injury and they complicate 16% of the 5,000 NS-associated hospitalizations in US children annually [4]. Refractory NS is one of the leading causes of end stage kidney disease (ESKD) in children [5]. There is growing consensus that the steroid response-based taxonomy of NS is

inadequate because (A) it exposes children to toxic therapies despite a low likelihood of response in 25% and a poor, chronically relapsing, response in an additional 40%; (B) it does not reflect the molecular basis of the disease, (C) it inadequately predicts the heterogeneous natural history, and (D) it does not uniformly guide treatment to prevent deterioriation of kidney function. The shortcomings argue persuasively for concerted and innovative investigational strategies that can be applied to the study of childhood onset NS. This effort would culminate in the development of non-invasive methods to classify participants into homogeneous cohorts with a definable course and treatment strategy. It is for this reason that the NEPTUNE investigative team, hereby submit a plan to conduct cNEPTUNE.

In oncology, gene expression signatures are currently used as diagnostic parameters to predict disease course and response to therapy [6] and guidelines have been established to include genomic markers into routine practice (http://www.egappreviews.org). In an analogous manner, cNEPTUNE aims to develop clinically meaningful subgroups and biomarkers based on molecular function leading to a refined, biologically relevant classification scheme for childhood onset NS which can be used to plan interventional strategies.

Previous studies have profiled a variety of molecules in patients with NS in an attempt to differentiate patients with steroid responsive and steroid resistant NS or those with progressive decline in kidney function. These include studies of cytokine levels, signs of inflammation and immune response modulators, markers of fibrosis, changes in microRNA levels, podocyte abnormalities, and epigenetic changes [7-16]. In aggregate, these studies support the hypothesis that children with incident NS have a spectrum of abnormalities and not a single cause of disease. Future studies of childhood onset NS as proposed in this application will utilize similar molecular profiling approaches developed in NEPTUNE and leverage gene expression profiling from kidney tissue samples obtained in the current NEPTUNE cohort from children who were treatment naïve at the time of biopsy.

The proposed pediatric cohort offers the opportunity to systematically assess a wide range of factors based on the published literature and emerging data from NEPTUNE. This enhances the likelihood of defining useful functionally-based molecular subgroups relevant for the child with incident NS and ensuring external validity of the findings. Success of these efforts will result in progress toward a goal of *Precision Medicine* by defining the molecular basis of pediatric NS [1].

2.A.4. Molecular Analysis and Prediction of Renal Function

A principal goal of all cohort studies is to identify and validate molecular predictors of outcomes. The following data provide early preliminary evidence that the molecular discovery approach will be feasible.

Gene expression profiles from 72 genome-wide arrays obtained from six different glomerular diseases were correlated with the glomerular filtration rate at time of biopsy (eGFR, estimated using the MDRD formula (52)). A marker panel of 40 mRNAs was identified that correlated with the glomerular filtration rate of all patients studied independent of histopathology (cross-validated, r=0.78, p<0.001). In a second series of 50 arrays obtained from renal biopsies of patients with MN, FSGS, lupus nephritis and controls, the same marker set performed equally well with a cross-validated correlation coefficient ($r=0.69$, $p<0.001$). The same mRNA marker panel allowed the classification of CKD stage III-V versus stage I-II with a sensitivity of 0.73, specificity of 0.82, negative and positive predictive value of 0.61 and 0.89 respectively. Furthermore, in an independent cohort of 44 patients with MCD, IgA, HTN or DN and 36 months of follow-up, the ability of the above renal biopsy marker set to estimate GFR three years after renal biopsy was tested and shown to correctly predict CKD stage three years after biopsy in 34 of 44 patients with a sensitivity of 0.77, specificity of 0.75, negative and positive predictive value of 0.77 and 0.75, respectively.

Even more relevant to the proposed observational cohort studies, Dr. Kretzler and colleagues generated glomerular gene expression profiles from a cohort of 18 MN patients and controls (pre-transplant biopsies from living related donor kidneys). Using these genome-wide expression profiles for unsupervised cluster analysis of MN patients, these glomerular gene expression profiles segregated patients within MN into three sub-groups. Patients in MN sub-group 2 compared to sub-groups 1 and 3 exhibited a trend towards more severe glomerulosclerosis scores (6 versus 0, p<0.1). While this study of MN patient biopsy tissue did not have sufficient follow-up data to allow evaluation of the correlation of subgroups defined by marker profile with clinical outcomes, these results suggest that this experimental approach will be useful in the context of the current study.

In an independent study, 10 FSGS patients with associated longitudinal follow-up were evaluated by glomerular gene expression profiling (mean follow-up: 27 months [range 12-60]). Within this group, 5 FSGS patients exhibited complete or partial remission of proteinuria while 5 patients experienced no remission. Expression profiling identified 99 differentially expressed mRNAs that segregated the two groups (Bonferroni corrected T-test, $p<0.05$). In a second, independently profiled test group of 9 FSGS patients, five with and four without remission of proteinuria during 24 months of follow-up, this 99 mRNA marker set correctly classified seven of nine patients into remission or progression groups. Although these preliminary studies were limited by the small number of participants available, they predict that the investigational strategy proposed below using the much larger NEPTUNE MN cohort will allow the generation of predictive marker panels from biopsy tissue.

2.A.5. The Role of Genetics in FSGS and MCD

Major advances in understanding the pathogenesis of FSGS and MCD have come over the last ten years from the identification of several mutated genes responsible for causing Steroid Resistant Nephrotic Syndrome (SRNS) presenting with FSGS or MCD histopathology in humans and model organisms. Each of these genes is "monogenic" (i.e., a mutation of only one of these genes in a given patient is sufficient to cause SRNS). Both recessive inheritance patterns (e.g., *NPHS1*, *NPHS2*, *LAMB2*, *PLCE1*) and dominant patterns (e.g., *WT1*, *ACTN4*, *CD2AP*, *TRPC6*) have been identified (7-13). These functionally distinct genetic disorders can present with indistinguishable FSGS lesions on histology confirming the presence of heterogeneous pathogenic mechanisms under the current histological diagnosis of FSGS (7-13).

The same holds true for MCD, as specific monogenetic diseases that may present as FSGS can also present early in the course of disease as foot process effacement alone (MCD) or diffuse mesangial sclerosis (13,53), providing rationale to group these diseases as related entities in the FSGS/MCD cohort. Defining the associated clinical and molecular phenotypes in patients with genetic variances in a prospective manner will allow us to define associated clinical phenotypes and molecular pathways in this subcohort of FSGS/MCD patients.

2.A.6. Factors Associated with Clinical Outcomes in Nephrotic Syndrome

Evaluating the prognosis is critical in making the decision regarding when and what to use in terms of treatment; e.g., conservative versus immunosuppressive treatment in patients with NS (54-56). An accurate predictor of outcome of patients would allow separating those patients who are likely to have a long-term renal survival from those who are likely to progress to end stage renal disease. This would allow us to target immunosuppressive treatment to patients at high-risk of renal disease progression. However, finding useful markers that predict this last group has been difficult. Many individual factors such as advanced age, male gender, and selected biopsy findings; e.g., degree of interstitial fibrosis, glomerulosclerosis, vascular damage, or the percentage of glomeruli with Focal and Segmental Glomerulosclerosis, have all been found to be predictors of prognosis and/or response to immunosuppressive therapy in patients with NS (57). In addition, the degree of proteinuria may also predict those who are most likely to progress. Pei et al observed a 47% risk for progression in patients with proteinuria >4g/24h for longer than 18 months and a 66% risk in patients with proteinuria >8g/24h for more than 6 months (58). Urinary excretion ratios of α1-microglobulin, β2-microglobulin, IgM and IgG have also been found to be strong predictors of outcome in MN (59-62). Determining these ratios has been found helpful in assessing the severity of overall renal injury and to predict those who are most likely to respond to immunosuppressive therapy (63). Unfortunately, quantification of urinary α1-microglobulin, β2-microglobulin, IgM and IgG is not widely available and thus limits their clinical use. There is also concern with the establishment of cutoff values for these ratios because they may reflect activity only at certain times during the course of the disease and under certain conditions, which may vary widely over time and be independent of the activity of the primary disease. The degree of renal impairment at presentation has also been found to correlate with long term renal survival. However, renal function at presentation is widely variable and may be independent of disease severity. A recent study by Hladunewich et al using GFR determination by inulin clearances indicated that, in patients with MN with nephrotic range proteinuria, presenting GFR may be artificially low (64). Estimating renal function by using a serum creatinine value is also problematic in these patients because in NS there is an increase in the tubular secretion of creatinine which may result in a marked

overestimation of the GFR (62). Thus, the use of immunosuppressive treatment limited only to those patients who exhibit deterioration in renal function, based on reaching a determined serum creatinine threshold; e.g., ≥1.5 mg/dl, may result in delaying treatment beyond the point where reversible renal disease is still present.

Thus far, the best model for the identification of MN patients at risk was developed with data derived from the Toronto Glomerulonephritis Registry (58,65). This model takes into consideration the initial creatinine clearance (CrCl), the slope of the CrCl and the lowest level of proteinuria during a 6-month observation period. This risk score assessment has good performance characteristics and to date is the only one validated in two geographically diverse MN populations; one from Italy, the other from Finland (54). Based on data using this model, patients who present with a normal CrCl, proteinuria ≤4g/24h, and stable renal function over a 6 month observation period, have an excellent long-term prognosis. Patients whose CrCl remains unchanged during 6 months of observation, but continue to have proteinuria >4g but <8g/24h, have a 55% probability of developing chronic renal insufficiency and patients with persistent proteinuria >8g/24h, independent of the degree of renal dysfunction, have a 66-80% probability of progression to chronic renal failure within 10 years. On the other hand, patients with MN who were never nephrotic have an excellent long-term renal survival. A review of this algorithm was recently published (54).

Additional factors considered to be associated with outcomes specific to the MN cohort are heavy metal exposures, chronic infections and malignancies. In preliminary unpublished data obtained at the University of North Carolina Kidney Center a trend for a higher frequency of any type of infection in the year prior to MN disease onset compared to those with other glomerular diseases was observed. Oral infections, but not UTI or sinus infections were associated with MN. Two or more years of occupational heavy metal exposure was also highly associated with development of MN. These results indicate that a broader range of infections than originally thought may be associated with MN and that exposure to heavy metals may also play an important role in developing MN. However, the influence of these exposures on disease presentation and outcomes is not known. Data will be collected and analyses (pending additional funding) will be performed on all study cohorts with respect to these considerations.

Response Measurements: The best accepted responses are improved renal survival and complete remission (CR) of proteinuria. About 30% of MN cases will relapse subsequent to a CR (66). The great majority who do, however, will relapse to sub-nephrotic range of proteinuria, and will have stable long-term function. More recently partial remission (PR) has been also recognized as a positive outcome. A recent review on the 350 nephrotic patients with MN found that the 10-year renal survival was 100% in the CR group, 90% in the PR group, and 45% in the no remission group (67). Patients in CR or PR have a similar rate of decline: -1.5 ml/min/year in the CR group and -2 ml/min/year in the PR group. In contrast, the no remission group lost GFR at a rate of -10 ml/min/year. Thus, both CR and PR appear to be excellent predictors of long-term renal survival. In the two largest studies of patients with MN who achieved CR, only a few developed mild renal insufficiency, over a long observation period, and none progressed to ESRD.

2.B. Significance of Proposed Research

There is a paucity of data on the incidence, prevalence, clinical course and outcomes of FSGS, MCD, and MN. There is a shortage of effective or curative therapy. Limited application of modern investigational tools, specifically molecular phenotyping signifies a “translational gap” in the understanding and treatment of FSGS, MCD, MN and childhood onset NS. The proposed study will bring to bear on these diseases an interdisciplinary and international panel of investigators to study a large cohort of incident patients. The study proposed by this consortium will address the critical areas of deficiencies cited in the foregoing review of FSGS/MCD, MN and childhood onset NS. As all cohort participants have the opportunity to contribute biopsy tissue for analysis, the cohort studies are designed to answer the following questions: (a) Can tissue-based mRNA signatures be used to risk stratify participants who present with FSGS, MCD, MN and childhood onset NS; and (b) Can mRNA profiles in combination with clinical information be used to develop clinically useful and potentially non-invasive biomarkers of progression of FSGS/MCD,MN and childhood onset NS? Addressing these needs will allow us to define more homogeneous cohorts of patients, a critical prerequisite for successful interventional trials in rare disease cohorts.

Although this project relies initially on renal tissue for development of molecular biomarkers, the obvious limitation of this invasive diagnostic procedure can be overcome in the near future by exploiting the fact that intra-renal marker RNAs encode proteins expressed in the kidney, some of which are secreted or shed into the blood stream or glomerular ultrafiltrate, making these molecules accessible to specific assays in serum or urine. It may also be possible to develop biomarkers from the cellular compartments (exosomes) or entire cells (i.e., podocytes) encoding these mRNA and carrying the corresponding proteins that are shed into the urine. Lastly, given that progressive kidney disease is associated with significant inflammatory responses within the kidney, a significant proportion of the intra-renal mRNA profiles are potentially derivable from infiltrating leukocytes, as has been shown for IgA nephritis among others (45). Examining the transcriptome of circulating leukocytes for these intra-renal marker profiles might allow the definition of a specific subset of gene expression signatures associated with the activity of FSGS/MCD, and MN.

An imperative factor in educating patients identified as NEPTUNE participants and others challenged with living with nephrotic syndrome is providing access to resources and support for these diseases. Currently resources and information about these rare diseases are sparse and support can be difficult to obtain, especially phased with a lifelong rare disease diagnosis. To advance these goals, the patient advocacy group, NephCure Kidney International, is an active partner in the NEPTUNE study. As a patient advocacy group their goal to connect with NEPTUNE participants and nephrotic syndrome patients will be facilitated by reaching out to potential participants during the study consent process. These patients will be offered the option to have their contact information shared with NephCure for outreach by NephCure to inform them about available resources and support.

In summary, the cohort studies will initially rely on renal-tissue based gene expressing profiling studies to identify molecular fingerprints that can complement clinical characteristics and histologic parameters to develop informative functional categories of FSGS, MCD, and MN, to identify prognostic indicators and set the stage for the exploitation of new targets. The findings of the FSGS/MCD, and MN cohort studies will also form a platform for developing non-invasive biomarkers out of urine and blood for FSGS, MCD, and MN in future pilot studies which will be extended to the cNEPTUNE cohort. These approaches hold the promise of improving the health of tens of thousands of current and future adults and children affected by proteinuric kidney diseases. In this way, it is possible to begin to confine to the relics of history, the prevailing lamentable lack of understanding of FSGS, MCD, MN and childhood onset NS and the painful scarcity of satisfactory therapeutic options.

3. STUDY METHODS

3.A. Study Design Overview

A prospective parallel cohort study generating three groups of participants, represented by two arms, will be performed in NEPTUNE. The two groups are: Cohort A which includes (1) the FSGS/MCD Cohort; and (2) the MN Cohort; as well as Cohort B – a non-biopsy, treatment-naïve, pediatric cohort less than 19 years of age, cNEPTUNE. The sample size for the combined FSGS/MCD and MN Cohorts is a minimum of 600 participants, with a minimum of 300 new patients recruited under Protocol V4.1 respectively. The sample size for the third group, cNEPTUNE, will be a minimum of 200 participants. Participants will be recruited into each subgroup concurrently. All participants who meet the inclusion criteria at the participating centers will be enrolled if the participants or their legally authorized representative(s) provide comprehensive written informed consent. A recruit-to-replace strategy will be employed throughout the enrollment phase. Cohort A study visits including screening/eligibility, baseline, biopsy, and follow-up visits are depicted in Appendix 8A. Study visits for Cohort B, cNEPTUNE, including screening/eligibility, baseline, follow-up visits, and SMS texting are shown in Appendix 8B.

3.A.1. Cohort A: FSGS/MCD and MN Cohorts

Pre-Screening: Potential participants will be identified by study team staff reviewing the medical records of patients being seen in clinic for diagnosis of kidney disease or patients presenting for a diagnostic renal biopsy. If necessary, a partial waiver of informed consent will be sought from the institutional review board to pre-screen these potential participants. Once pre-screened and identified, potentially eligible patients will be approached by a clinical caregiver to request permission for a study team member to initiate discussion of the NEPTUNE study.

Screening/Eligibility Visit [V_{SE}]: As an introduction to the NEPTUNE study, a study team member will meet with a potential participant, assess enthusiasm for the study, complete an eligibility questionnaire with the potential participant, and obtain informed consent if appropriate. If informed consent is obtained, an eligibility urine sample will be obtained for central laboratory processing at this first study contact, if not concurrent with the Biopsy Visit [V_{BX}]. The eligibility visit will last 30-45 minutes.

Baseline Visit [V1]: This visit may be combined with the screening/eligibility visit and should occur within 45 days of the diagnostic renal biopsy. At this visit, informed consent will be obtained and a participant identification number (ID) will be assigned if not done prior. Blood, urine and nail specimens will be collected and the study questionnaires will be administered. The duration of this visit is estimated to be 90 minutes.

Biopsy Visit [V_{BX}]: A small blood (40 cc) sample and a spot urine specimen will be collected prior to the kidney biopsy. Since the kidney biopsy is a clinically indicated procedure and not a study procedure, it must be ensured that adequate renal tissue is obtained for diagnosis. One additional renal tissue core (a core is the amount of predominantly cortical renal tissue retrieved from the bevel after a single pass of the biopsy needle into the kidney) will be set aside for the purpose of this study. The number of passes to attempt to retrieve adequate tissue for clinical and research purposes will not exceed 5. Should tissue submitted for clinical diagnosis be deemed sufficient after completion of pathology analysis, the specimen set aside for research will be used for the NEPTUNE study. However, if insufficient tissue is obtained for clinical diagnosis, tissue set aside for research will be returned for diagnostic analysis. The additional time necessary to procure a renal tissue core and perform related study procedures is estimated to be 60 minutes; however, varying clinical protocols at different institutions will dictate the total time for the renal biopsy procedure (pre- and post-operative procedures).

Follow-up Visits [V2-8]: Blood and urine specimens will be collected at each follow-up visit. Study questionnaires will be administered at pre-defined visits as well (see Appendix 8A, Cohort B Visit Schedule). Medication changes will also be documented throughout the duration of the study at the follow-up visits. In the first year, follow-up

visits will occur at four month intervals, and subsequently every six months until month 36 for the targeted diseases. The duration of each follow-up visit is estimated to be 45-60 minutes.

Screening, Baseline, and Biopsy Visits may occur independently and sequentially or simultaneously to minimize burden to participants and study personnel.

3.A.2. Cohort B: cNEPTUNE – Treatment-naïve, non-biopsy Cohort

Pre-Screening: Potential participants will be identified by study team reviewing the medical records of patients presenting for new-onset nephrotic syndrome (NS) with proteinuria or albuminuria. If necessary, a partial waiver of informed consent will be sought from the institutional review board to pre-screen these potential participants.

Enrollment Visit [V1]: As an introduction to the NEPTUNE study, a study team member will meet with a potential participant and assess enthusiasm for the study. This visit will occur at initial presentation of the incident patient to the nephrologist. At this visit, informed consent and/or assent will be obtained and participant identification number (ID) will be assigned. Physical examination, blood and urine will be collected and the study questionnaire will be completed no later than 30 days after the initiation of immunosuppression therapy. Participants will be enrolled in the Short Messaging System (SMS) for subsequent collection of patient reported information (PRI) via text messaging.

Follow-up Visits [V2-9]: Vital signs, blood and urine specimens will be collected and study questionnaire will be completed at each follow-up visit (week 6, months 4 and 12 and then every 6 months thereafter) until the end of the study. There will be a phone visit at Month 8 to collect participant disease status, medication exposure, address questions, and confirm the next study visit date. Procedures and visit schedule are depicted in Appendix B.

Biopsy Visit [V_{BX}]: A biopsy is not required for entry into cNEPTUNE. However, if a biopsy becomes clinically indicated at any point in the study, the participant will be approached for consent to collect an extra core of kidney tissue. Participants who have a kidney biopsy will remain in the cNEPTUNE cohort and will continue with SMS monitoring and be counted towards the target of 120 patients. Participants who have a kidney biopsy will be seen every 4 months for the year following biopsy and then will be seen every 6 months thereafter.

Disease Status Home Monitoring: Collection of patient reported information (PRI) will be captured from the home environment using text messaging prompts. Automatic text messages will consist of daily messages sent for the first 3 months, and weekly for the subsequent 9 months. Information gathered will include home urine protein test results, nephrotic syndrome triggers (allergies, infection, stressors), edema status, consumption of prescribed immunosuppression, and nephrotic syndrome related absences from work/school. Alerts will be sent to the study team if the patient has $\geq$2+ urine protein for 3 days after being negative/trace, new onset swelling in the abdomen, genitals, or anasarca or if the patient/parent fails to respond to text messages for a week. Either the patient ($\geq$12yrs) or parent may respond to the text messages at the discretion of the parent/child dyad with approval from the local study team and IRB.

Home Urine Protein Monitoring: Home urine protein testing using Chemstix™ will be performed daily for the first 90 days, weekly for the next 9 months (total 1 year) Results of this testing will be reported with SMS text messaging system and confirmed at study visits. Study participants will be supplied Chemstix™ as part of the cNEPTUNE cohort study.

3.B. Study Patient Population and Participant Distribution

Each of the Clinical Centers will enroll participants during the recruitment period. A recruit-to-replace enrollment strategy will be implemented during the entire enrollment period to ensure that the final sample size is preserved.

These diagnostic criteria are based on a uniformly accepted minimum requirement for clinical diagnosis of the respective conditions in North America. As standardized guidelines and findings from new studies become available, it is anticipated that the definitions for these glomerular disorders will evolve.

Participants initially enrolled in the NEPTUNE study whose post-enrollment renal biopsy histopathology does not fulfill the histopathologic criteria of FSGS/MCD or MN will no longer be followed in the study with the exception of cNEPTUNE where a child will continue to be followed in the cNEPTUNE cohort until the completion of the 12 month visit if the kidney biopsy is not consistent with the FSGS, MCD, or MN pathology criteria. cNEPTUNE participants with biopsy fulfilling the FSGS, MCD or MN pathology criteria will remain in long-term cNEPTUNE study observation as outlined in section 3.D.1.

The study populations will include a racially and ethnically diverse group of adult and pediatric participants. The following biopsy criteria have been used to define selected conditions in the cohorts' study participants:

3.B.1. FSGS/MCD Cohort

A participant is defined as having FSGS or MCD in the presence of significant proteinuria (>1.5 g/g creatinine in a spot urine sample or ≥ 1500mg/24 hour, or an albuminia equivalency as determined by the local site PI) and a disease course requiring renal biopsy. Additionally, a verified renal histopathologist's report of renal biopsy tissue that includes one of the following categories:

- Minimal Change Disease
- Minimal Change Nephrotic Syndrome
- Glomerular minimal changes with Nephrotic Syndrome
- Extensive foot process effacement suggestive/consistent with Minimal Change Disease or Minimal Change Nephrotic Syndrome
- Focal Segmental Glomerulosclerosis, any variant according to the Columbia classification
- Collapsing Glomerulopathy, any etiology
- Tip Lesion (not necessarily FSGS variant)
- Diffuse Mesangial Sclerosis

The histological classification will be reviewed by the pathology committee to verify accuracy (see Appendix 8C, Methodology for Pathological Evaluation of Podocyte Injury and Segmental Sclerosis with Proteinuria in the FSGS/MCD Cohort, for detailed characterization of FSGS/MCD biopsies).

3.B.2. MN Cohort

A participant is defined as having MN in the presence of significant proteinuria at presentation (>1.5 g/g creatinine in a spot urine sample or ≥1500mg/24 hour, or an albuminuria equivalency as determined by the local site PI) and a verified histopathologist's report of renal biopsy tissue that includes one of the following categories:

- Membranous Glomerulopathy or Glomerulonephritis
- Idiopathic Membranous Glomerulopathy or Glomerulonephritis
- Membranous Glomerulopathy or Glomerulonephritis, idiopathic form

The histological classification will be reviewed by the pathology committee to verify accuracy (see Appendix 8D, Methodology for Pathology Analysis for Membranous Nephropathy for detailed characterization of MN biopsies).

3.B.3. cNEPTUNE (Cohort B Mod)

Participants enrolled in cNEPTUNE with subsequent clinically indicated kidney biopsy will participate fully in the biopsy component of the protocol. Consent will be obtained. The biopsy tissue will be collected, pathology report and slides will be reviewed for pathology classification of NS according to the criteria in 3.B.1 and 3.B.2. Children enrolled in cNEPTUNE with kidney biopsies that do not meet FSGS, MCD or MN criteria will be classified by pathology diagnosis and retained in the cNEPTUNE cohort to complete all protocol defined study visits and procedures. Entry Age Range is consistent with Cohort B eligibility.

There is no lower age limit for inclusion/exclusion in the NEPTUNE study for the FSGS/MCD and MN cohorts. Adults < 80 will be approached for inclusion. Participants must be < 19 years of age for consideration into cNEPTUNE.

3.B.4. Inclusion Criteria for Cohort A

Potential participants for the FSGS/MCD and MN Cohorts will be required to fulfill the following criteria:

- A new diagnosis of FSGS or MCD or MN according to characteristic light, electron (EM), and immunofluorescence microscopy (IM), with presence of at least five glomeruli per biopsy available for analysis. Biopsy slides will be reviewed and diagnosis confirmed by 2 study pathologists according to standardized criteria developed by the pathology committee (see Appendices C and D);
- Documented urinary protein excretion ≥1500 mg/24 hours or spot protein: creatinine ratio equivalent at the time of diagnosis or within 3 months of the screening/eligibility visit. Alternatively, an albuminuria equivalency determined by the local site PI;
- < 80 years of age
- Completion of V1 (baseline visit) within 45 days of V_{BX} (biopsy visit)
- Informed Consent

3.B.5. Inclusion criteria for Cohort B

Potential participants for Cohort B, cNEPTUNE will be required to fulfill the following criteria:

- <30 days of treatment for nephrotic syndrome
- Proteinuria/Nephrotic defined as:
  - Urinalysis with >2+ protein AND edema OR
  - Urinalysis with >2+ protein AND serum albumin < 3 OR
  - Urine protein:creatinine ratio >2 AND serum albumin <3
- Age <19 years
- Informed Consent and Assent when applicable

3.B.6. Exclusion Criteria for Cohort A

- Prior solid organ transplant
- A clinical diagnosis of FSGS/MCD or MN without diagnostic renal biopsy
- Clinical, serological or histological evidence of systemic lupus erythematosus (SLE) as defined by the ARA criteria. Patients with membranous in combination with SLE will be excluded because this entity is well defined within the International Society of Nephrology/Renal Pathology Society categories of lupus nephritis, and frequently overlaps with other classification categories of SLE nephritis (68)

- Clinical or histological evidence of other renal diseases (Alport, Nail Patella, Diabetic Nephropathy, monoclonal gammopathy (multiple myelomas), genito-urinary malformations with vesico-uretheral reflux or renal dysplasia)
- Known systemic disease diagnosis at time of enrollment with life expectancy less than 6 months
- Unwillingness or inability to give a comprehensive informed consent
- Unwillingness to comply with study procedures and visit schedule
- Institutionalized individuals (e.g., prisoners)
- Laboratory information unavailable prior to consent and biopsy procedure subsequently supporting exclusion criteria will deem a participant ineligible

Consented individuals determined to be ineligible post-biopsy procedure will be requested to consent for their tissue and samples to remain part of the NEPTUNE biorepository. These samples may serve as non-nephrotic control samples for future studies and consenting participants will be compensated up to $50 or as allowed by local IRBs.

3.B.7. Exclusion Criteria for Cohort B: cNEPTUNE

- End Stage Kidney Disease (ESKD) defined as the need for chronic dialysis or kidney transplant
- Prior solid organ or bone marrow transplant
- Secondary NS (systemic lupus erythematosus (SLE), vasculitis, Henoch Schonlein Purpura, Hepatitis B, C or HIV nephropathy)
- Clinical or histological evidence of other renal diseases (Alport syndrome, Nail Patella syndrome, Diabetic Nephropathy, monoclonal gammopathy (multiple myelomas), genito-urinary malformations with vesico-uretheral reflux or renal dysplasia)
- Known systemic disease diagnosis at time of enrollment with life expectancy less than 6 months
- Unwillingness or inability to give a comprehensive informed consent
- Unwillingness to comply with study procedures and visit schedule
- Institutionalized individuals (e.g., prisoners)

3.B.8. Recruitment Targets

The recruitment target for each clinical center is based on the volume and characteristics of patients treated at the clinical centers for each of the two main disease groups during the calendar years -2007, within the initial 5 years of the NEPTUNE study, during the years 2011 to 2013 for cNEPTUNE, and a realistic assessment of the study requirements by the principal investigator at each participating clinical center.

Target ranges were identified based on the anticipated distribution of these attributes among the source population at the clinical centers, and the required composition of the cohort so as to be able to achieve the desired composition of the subcohort. If the observed proportion in a center falls outside the target range for a given variable, the center will be directed to adjust recruitment goals accordingly. Adherence of each center to the recruitment goals will be monitored monthly at the DACC. For some attributes such as race, a similar distribution was not feasible at all study sites.

3.B.9. Racial/Ethnic Distribution

A goal of recruitment is to have a broad racial/ethnic representation from three major groups (i.e., White, African-American, and Latino/Hispanic). A small number of Asian/Pacific Islanders and Native Americans who may be available in the source populations at the clinical research centers will also be included. Tables 1a and 1b present the anticipated racial/ethnic distribution of participants in the two cohort studies at the completion of enrollment.

Table 1a. Race/Ethnicity Distribution in the FSGS/MCD Cohort

Race	**Proportions**
White	59%
African-American/Black	33%
Other	8%
Ethnic Group	
Hispanic	26%

Table 1b. Racial/Ethnic Distribution in the MN Cohort

Race	**Proportions**
White	68%
African-American/Black	18%
Other	14%
Ethnic Group	
Hispanic	17%

Table 1c. Racial/Ethnic Distribution in the cNEPTUNE Cohort

Race	**Proportions**
White	62%
African-American/Black	30%
Other	8%
Ethnic Group	
Hispanic	25%

3.B.10. FSGS/MCD and MN Pediatric Population

Children (age <18 years) meeting general eligibility criteria are eligible and encouraged to participate. Therapy may have been prescribed prior to the qualifying biopsy and is not an exclusion criterion; however, details of prior therapy, response to prior therapy and duration of proteinuria will be collected at enrollment for future analysis.

3.C. Contact Schedule and Participant Procedures – Cohort A: FSGS/MCD/MN

3.C.1. Overview of the Participant Visit and Contact Schedule

An important and necessary criterion for enrollment in cohort A is histologic confirmation of clinical diagnosis of glomerular disease on kidney tissue obtained from a kidney biopsy procedure. If participants are enrolled after the histologic confirmation of renal disease, the opportunity to obtain renal tissue for this research will be lost. Thus, it is necessary to obtain informed consent from the participant prior to the diagnostic kidney biopsy procedure so that an additional renal tissue core can be procured during the biopsy for the purpose of this research. To accomplish this objective, a vigilant screening process needs to be implemented for patients who are presenting *de novo* with a clinical syndrome consistent with FSGS, MCD or MN and who require a diagnostic kidney biopsy procedure. The recruitment effort will concentrate on screening potential study participants from the point at which participants are referred to the nephrology clinic, to the interventional radiology program for kidney biopsy procedure where applicable, and to the pathologist charged with evaluating newly procured kidney biopsy materials. Two enrollment scenarios are expected:

Preferentially, Screening/eligibility visit [V_{SE}] may occur at the time of the Baseline visit [V1] followed by the Biopsy visit [V_{BX}]. The biopsy visit should occur ±45 days of the Baseline Visit.

Alternatively, The Biopsy visit [V_{BX}] may occur at the time of Screening/eligibility visit [V_{SE}] prior to the Baseline visit ([V_{SE}, V_{BX}] followed by [V1]). The baseline visit should occur ±45 days of the Biopsy Visit.

Participants not meeting this enrollment regimen will no longer be considered "enrolled" participant and will not be followed.

Nonetheless, the recruitment strategy, which will be outlined in the Manual of Procedures, is designed to: 1) maximize recruitment of the targeted populations at all sites, 2) minimize participant and coordinator burden; and 3) achieve the highest possible level of safety for study participants.

In the first year, follow-up visits are expected to occur every four months after the baseline visit (see Appendices 8A and 8B, Visit Schedules), and subsequently every six months until the end of the study. Additionally, an unscheduled Relapse Visit can occur once annually per participant should a participant be scheduled to see their primary nephrologist giving an unscheduled visit window for each patient/year.In addition, the study protocol makes provision to include interval measures of renal function obtained for clinical purposes to be included in the study database.

3.C.2. Cohort A: Procedures During Screening/Eligibility [V_{SE}], Baseline [V1] and Biopsy [V_{BX}] Visits

General Principles: The Screening/Eligibility, Baseline, and Biopsy Visits period is expected to last at most 45 days around the time of renal biopsy. Before entering Baseline, participants will be screened to ensure that they meet the inclusion criteria and that none of the exclusion criteria are present. A de-identified screening log will be maintained in the study database.

Participants in the NEPTUNE cohorts will be followed for an initial period of up to 36 months or longer pending additional resources and depending on the date of enrollment. The following section describes the procedures to be implemented during the screening, baseline and follow-up phases of each cohort study.

3.C.2.a. Pre-screening

We will use a Forward Operating Study Subject (Participant) Awareness (FOSSA) System. The FOSSA System details that study personnel are trained to initiate screening at several points in the healthcare system where the opportunity exists to identify potential study participants. Study personnel screen medical records, appointment schedules and laboratory databases as consistent with local IRB protocols to identify patients who appear to meet inclusion/exclusion criteria.

3.C.2.a.(a) Screening/Eligibility [V_{SE}]

This visit serves as an introductory visit for pre-screened individuals who appear to be potential candidates for the NEPTUNE study. Ideally, this will occur at the time of a nephrology clinic visit or consultation. During this visit the potential candidate will complete an eligibility assessment and the study team member will determine level of interest. Informed consent may be obtained at this visit or during Baseline Visit [V2] (below). If the potential participant is prepared to consent and meets all study criteria, [V1] and [V2] can be in succession on the same day.

Individuals consenting to be NEPTUNE participants and who are only completing [V1] during the initial study contact will provide a spot urine sample to serve as the baseline proteinuria measure.

All individuals approached will also be informed of the Nephrotic Syndrome Study Network Contact Registry overseen through the Rare Diseases Clinical Research Network (RDCRN) Registry. Please see section 6.B.7 for more information.

Additionally, all individuals consenting to be NEPTUNE participants will be given the option to opt-in/-out to release their contact information (limited to their name, home address, phone and email address) to NephCure Kidney International. This patient advocacy group will provide patient education materials and resources to consenting individuals.

3.C.2.a.(b) Baseline Visit [V1

If not already obtained, the informed consent process will take place at this visit. A participant will formally enter the study and be assigned a study number. Additional information to be obtained includes participant contact and next of kin information, residence census tract, social security number, details about the participant's healthcare providers, documentation for permission to obtain medical records with appropriate signature(s), demographic information, a detailed medical history with medication use, and a limited physical examination (blood pressure, presence of edema, height and weight).

The data and specimens obtained at this visit will serve as the baseline study data for the purpose of data analysis and biochemical investigations, if [V2] occurs prior to biopsy visit [V3]. The Baseline Visit will include the following biospecimen procurement:

- Fasting blood draw (Adult: 100 cc, pediatrics by weight according to Table 2, page 38) for the NEPTUNE Biorepository and central biochemical laboratory samples including lipid profile, glucose, total cholesterol and triglycerides. Blood volumes will be reduced in pediatric participants and in all participants, adult and pediatric, with a hematocrit below 28%.

  If the baseline visit occurs on the same day as biopsy visit [V_{BX}], the blood draw will be deferred to a subsequent day, but not later than 45 days after the procedure. See Manual of Procedures for itemized list of baseline blood specimens to be drawn.
- Central biochemical laboratory 24-hour urine for Protein, Albumin, and Creatinine
- A "clean catch" research urine sample will be collected from all participants. Approximately 50 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository as outlined in the Manual of Procedures

Additionally, interim local laboratory results, between study visits, should be obtained from the participant's medical chart for:

- CBC [Hemoglobin, Hematocrit, WBC, MCV, MCH, MCHC, Platelets]
- Metabolic panel [Albumin, Bicarbonate, Total Bilirubin, Calcium, Carbon Dioxide, Chloride, Creatinine, Glucose, Alkaline Phosphatase, Potassium, Total Protein, Sodium, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Magnesium, Phosphorus, Urea Nitrogen]
- Spot urine assay for Creatinine, Protein, Albumin, Protein:Creatinine Ratio

- 24-hour Urine Creatinine Clearance, Total Creatinine and Protein
- Urinanalysis [Color, Appearance, Specific Gravity, pH, Leukocyte Esterase, Nitrite, Protein, Glucose, Ketones, Urobilin, Bilirubin, Blood, Sediment]
- Other laboratory results, as available from clinical records including: Cholesterol, Cystatin C, [C-Reactive Protein (CRP), Neutrophil Cytoplasmic Antibody (ANCA), Extractable Nuclear Antibodies (ENA), Nuclear Antibody Screen by Multiplexed Immunoassay (ANA), DNA Antibody Double-Stranded (Anti dsDNA), Cryoglobulins, Complements 3 and 4, Glomerular Basement Membrane Antibody (Anti-GBM), Anti Shiga-like Toxin (Anti-SLT)]
- Virology results as available from clinical records including: [Hepatitis B, Hepatitis C, Epstein Barr Viral Capsid Antigen, IgM (EBV), Cytomegalovirus Antibody: IgG and IgM (CMV), Human Immunodeficiency Virus: Proviral DNA by PCR Qualitative and Quantitative RNA Assay (HIV), Hantavirus, Parvovirus]

At this visit, participants will also be administered the Patient Reported Outcome Measurement Information System (PROMIS) quality of life questionnaire and medication adherence questionnaire.

3.C.2.a.(c) Biopsy Visit [V_{BX}]

This visit is concurrent with the kidney biopsy procedure. Prior to the biopsy procedure, a 40 cc blood sample will be obtained for the NEPTUNE biorepository to provide pre-treatment samples of plasma, serum, and RNA profiles. A spot urine will also be collected pre-biopsy for storage in the study biorepository. If [V_{BX}] is concurrent with the first study contact, the urine sample will be used as the eligibility proteinuria measure. During the biopsy procedure, an additional renal core will be obtained for research purposes if and only if it has been confirmed that sufficient tissue is available for diagnostic purposes. The number of passes attempted for the clinical and research cores will not exceed 5 attempts. This tissue will be stored with the NEPTUNE study coordinator until it is confirmed that adequate tissue necessary for histologic diagnosis of renal disease has been received, processed, and the treating physician, in consultation with the renal pathologist, has determined there is no longer clinical need for this sample, at which time it will be released for research use in the NEPTUNE study.

Participants will be assigned to an initial study cohort (FSGS/MCD or MN) by the Pathology Review Committee.

3.C.2.a.(d) cNEPTUNE – Clinically indicated biopsy

Participants in the cNEPTUNE cohort who have a clinically indicated need for a biopsy will be consented specifically to obtain biopsy tissue for research, and will otherwise follow the procedures outlined above. cNEPTUNE participants receiving a kidney biopsy will continue to be followed in the cNEPTUNE cohort. These participants will be seen every 4 months following their clinically indicated biopsy for the first year, and then every 6 months thereafter.

Alternative Enrollment Schedule from Network Practices

At sites where networks of nephrology practices are utilized for recruitment, the Biopsy Visit, [V_{BX}], may be combined with [V_{SE}]. In this recruitment scenario consent must be obtained prior to securing renal tissue for the NEPTUNE Biorepository and Baseline Visit [V1] will occur at the time of [V_{BX}] or within 45 days of the procedure. As specified above, the baseline biobank blood draw cannot occur on the day of renal biopsy.

Follow-up Visits [V2-V8] **General Principles:** The purposes for follow-up visits are to maximize participant retention; document the participant's clinical status including general well-being, the development of new symptoms or physical findings and course of the underlying disease; account for possible adverse events and side effects related to standard-of-care medication use; evaluate abnormal laboratory values; and identify participants who reach a study outcome.

If a visit or procedure is missed, the visit should be rescheduled as soon as possible within the allotted interval. An interval of ± one month is targeted for all protocol visits.

If a visit or procedure is unable to be performed within the range of the study visit prior to the following visit's window, it will be documented as "missed". Study data that is able to be obtained (e.g., local labs, updates from the electronic medical record, etc), should be documented in the missed visit's case report forms.

Follow-up visits in year one will occur in person at four-month intervals (months 4, 8 and 12) after the Baseline Visit [V1]. Thereafter, follow-up visits [V5-9] will occur at six-month intervals through [V8], the 36-month follow-up. Subsequently, with the identification of additional funding, annual follow-up visits [V9-] will occur once per year. During the follow-up phase, participants will continue to be contacted by telephone as necessary between scheduled follow-up visits to update contact information, ascertain interim medical history and potential outcome events, and to assess health resource utilization. The V2- visits are to occur within a range of three months before and up to three months after the scheduled timeline according to the visit calendar.

The follow-up visits [V2-8] will include updates to the participant's medical history, concomitant medications, pregnancy history, a limited physical examination to include blood pressure, edema, and weight. Participants <21 years of age will also be measured for height. The follow-up visits [V9-] will be as inclusive of study data elements as possible, except in the case where these visits occur as either a phone call update or a chart data extraction.

The population respective Quality of Life questionnaires will be administered at each in-person study visit.

In addition, the following biospecimens will be collected at in-person visits:

- Fasting blood draw (Adult: 65 cc – see Table 2 for pediatric blood volumes) for the NEPTUNE Biorepository and central biochemical laboratory studies.
- Spot urine for Creatinine, Protein, Albumin assay at Central biochemical laboratory.
- Central biochemical laboratory 24-hour urine for Protein, Albumin, and Creatinine.
- A "clean catch" research urine sample will be collected from all participants. Approximately 50 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository as outlined in the Manual of Procedures.

Local laboratory results from within ± 90 days of the follow-up visit should be obtained from the participant's medical chart for:

- CBC [Hemoglobin, Hematocrit, WBC, MCV, MCH, MCHC, Platelets]
- Metabolic Panel [Albumin, Total Bilirubin, Calcium, Carbon Dioxide, Chloride, Creatinine, Glucose, Alkaline Phosphatase, Potassium, Total Protein, Sodium, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT)], Magnesium (if available), Phosphorus (if available), Urea Nitrogen, and Cholesterol
- Spot urine assay for Creatinine, Protein, Albumin, Protein:Creatinine Ratio
- 24-hour Urine Creatinine Clearance, Total Creatinine and Protein
- Urinanalysis [Color, Appearance, Specific Gravity, pH, Leukocyte Esterase, Nitrite, Protein, Glucose, Ketones, Urobilin, Bilirubin, Blood, Sediment]
- Virology results as available from clinical records including: [Hepatitis B, Hepatitis C, Epstein Barr Viral Capsid Antigen, IgM (EBV), Cytomegalovirus Antibody: IgG and IgM (CMV), Human Immunodeficiency Virus: Proviral DNA by PCR Qualitative and Quantitative RNA Assay (HIV), Hantavirus, Parvovirus]

The following updates will be performed at the follow-up visits that occur annually after the Biopsy Visit [V4, V6, V8]:

- Contact Information
- Demographics
- Family History
- Update healthcare providers (physician, practice and hospital)
- Update Permission to Obtain Medical Records
- NEPTUNE Contact Registry Information (if not previously offered)

3.D. Contact Schedule and Participant Procedures – Cohort B: cNEPTUNE

Overview of the Participant Visit and Contact Schedule An important and necessary criterion for enrollment in the Cohort B, cNEPTUNE study is a limited exposure to pharmacologic treatment. If participants are enrolled prior to 30 days of pharmacologic treatment, the opportunity to observe the natural progression of nephrotic syndrome can best be preserved. Thus, it is necessary to obtain informed consent from the participant as soon as possible after initial onset of clinically diagnosed nephrotic syndrome. To accomplish this objective, a vigilant screening process will be implemented for patients who are presenting *de novo* with a clinical syndrome consistent with FSGS, MCD or MN and who require nephrotic syndrome treatment. The recruitment effort will concentrate on screening potential study participants from the point at which participants are referred to the nephrology clinic or seen in emergency rooms for suspected nephrotic syndrome.

This recruitment strategy, which will be outlined in the Manual of Procedures, is designed to: 1) maximize recruitment of the targeted populations at all sites, 2)

minimize participant and coordinator burden; and 3) achieve the highest possible level of safety for study participants.

In the first year, follow-up visits will occur at 6 weeks, 4 months and 12 months post enrollment, and then every six months until the end of the study (see Appendix B, Cohort B cNEPTUNE Visit Schedule), . Additionally, a phone-only visit is to be conducted at study month 8 and an unscheduled Relapse Visit can occur once annually per participant should a participant be scheduled to see their primary nephrologist during an unscheduled window. However, the study protocol makes provision to include interval measures of renal function obtained for clinical purposes to be included in the study database.

3.D.1. Procedures During Enrollment Visit [V1]

General Principles: The Screening/Eligibility and Baseline Visits period is expected to last at most 45 days. In most cases, screening/eligibility and baseline will occur at the same visit due to the desire to enroll children before NS therapy is initiated. Before entering Baseline visit component, participants will be screened to ensure that they meet the inclusion criteria and that none of the exclusion criteria are present.

Participants in the cNEPTUNE main cohort studies will be followed for an initial period of up to 36 months or 18 months post biopsy, whichever is longer. Follow up may be extended pending additional resources and depending on the date of enrollment. The following section describes the procedures to be implemented during the screening, baseline and follow-up phases of each cohort study.

3.D.1.a. Enrollment

We will use a Forward Operating Study Subject (Participant) Awareness (FOSSA) System. The FOSSA System details that study personnel are trained to initiate screening at several points in the healthcare system where the opportunity exists to identify potential study participants. Study personnel screen medical records, appointment schedules and laboratory databases as consistent with local IRB protocols to identify patients who appear to meet inclusion/exclusion criteria.

This visit serves as an introductory visit for pre-screened individuals who appear to be potential candidates for the Cohort B, cNEPTUNE study. Ideally, this will occur at the time of a nephrology clinic visit or consultation. During this visit the potential candidate will complete an eligibility assessment and the study team member will determine level of interest. Informed consent will be obtained at this visit.

All individuals approached will also be informed of the Nephrotic Syndrome Study Network Contact Registry overseen through the Rare Diseases Clinical Research Network (RDCRN) Registry. Please see section 6.B.7 for more information.

Additionally, all individuals consenting to be cNEPTUNE participants, and their guardians for minor subjects, will be given the option to opt-in/-out to release their contact information (limited to their name, home address, phone and email address) to The NephCure Foundation. This patient advocacy group will provide patient education materials and resources to consenting individuals.

3.D.1.a.(a) Baseline Visit [V1]

If not already obtained, the informed consent process will take place at this visit. A participant will formally enter the study and be assigned a study number. Additional information to be obtained includes participant contact and next of kin information, details about the participant's healthcare providers, documentation for permission to obtain medical records with appropriate signature(s), demographic information, residence census tract, social security number, a detailed medical history with

medication use, and a limited physical examination (blood pressure, presence of edema, height and weight).

The data and specimens obtained at this visit will serve as the baseline study data for the purpose of data analysis and biochemical investigations. The Baseline Visit will include the following biospecimen procurement:

- Blood draw by weight according to Table 2, page 34 for the NEPTUNE Biorepository and central biochemical laboratory samples including lipid profile, glucose, total cholesterol and triglycerides. Blood volumes will be reduced in pediatric participants and in all participants, adult and pediatric, with a hematocrit below 28%.
- Central biochemical laboratory 24-hour urine for Protein, Albumin, and Creatinine. A timed urine collection will be substituted for 24-hour specimen when subject is unable to collect 24 hour specimen.
- A "clean catch" research urine sample will be collected from all participants. Approximately 50 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository as outlined in the Manual of Procedures to serve as the baseline proteinuria measure

Additionally, interim local laboratory results between study visits, should be obtained from the participant's medical chart.

- CBC [Hemoglobin, Hematocrit, WBC, MCV, MCH, MCHC, Platelets]
- Metabolic panel [Albumin, Bicarbonate, Total Bilirubin, Calcium, Carbon Dioxide, Chloride, Creatinine, Glucose, Alkaline Phosphatase, Potassium, Total Protein, Sodium, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Magnesium, Phosphorus, Urea Nitrogen]
- Spot urine assay for Creatinine, Protein, Albumin, Protein:Creatinine Ratio
- 24-hour Urine Creatinine Clearance, Total Creatinine and Protein
- Urinalysis [Color, Appearance, Specific Gravity, pH, Leukocyte Esterase, Nitrite, Protein, Glucose, Ketones, Urobilin, Bilirubin, Blood, Sediment]
- Other laboratory results, as available from clinical records including: Cystatin C, Cholesterol, [C-Reactive Protein (CRP), Neutrophil Cytoplasmic Antibody (ANCA), Extractable Nuclear Antibodies (ENA), Nuclear Antibody Screen by Multiplexed Immunoassay (ANA), DNA Antibody Double-Stranded (Anti dsDNA), Cryoglobulins, Complements 3 and 4, Glomerular Basement Membrane Antibody (Anti-GBM), Anti Shiga-like Toxin (Anti-SLT)]
- Virology results as available from clinical records including: [Hepatitis B, Hepatitis C, Epstein Barr Viral Capsid Antigen, IgM (EBV), Cytomegalovirus Antibody: IgG and IgM (CMV), Human Immunodeficiency Virus: Proviral DNA by PCR Qualitative and Quantitative RNA Assay (HIV), Hantavirus, Parvovirus]

At this visit, participants will also be administered the PROMIS and medication adherence Questionnaire, and enrolled into the SMS text messaging system. Parents will be asked to complete these questionnaires for pediatric subjects age 10 and under. Children and adult subjects will self-report for ages 8 years and greater.

3.D.1.a.(b) Follow-up Visits [V2-V9] per study calendar;

General Principles: The purposes for follow-up visits are to maximize participant retention; document the participant's clinical status including general well-being, the development of new symptoms or physical findings and course of the underlying disease; account for possible adverse events and side effects related to standard-of-care medication use; evaluate abnormal laboratory values; and identify participants who reach a study outcome.

If a visit or procedure is missed, the visit should be rescheduled as soon as possible within the allotted interval. An interval of ± one month is targeted for all protocol visits.

If a visit or procedure is unable to be performed within the range of the study visit prior to the following visit's window, it will be documented as "missed". Study data that is able to be obtained (e.g., local labs, updates from the electronic medical record, etc), should be documented in the missed visit's case report forms.

Follow-up visits in year one will occur in person at 6 weeks, 4 months, and 12 months post enrollment. Thereafter, in-person follow-up visits will occur at six-month intervals through the 36-month follow-up. There is an additional phone visit at month 8. Additionally, an unscheduled Relapse Visit can occur once annually should a participant be scheduled to see their primary nephrologist.

During the follow-up phase, participants will continue to be contacted by telephone as necessary between scheduled follow-up visits to update contact information, ascertain interim medical history and potential outcome events, and to assess health resource utilization.

The follow-up visits will include updates to the participant's medical history, concomitant medications, pregnancy history, a limited physical examination to include blood pressure, edema, weight and height.

The PROMIS assessment will be administered at each in-person study visit, including relapse visits.

In addition, the following biospecimens will be collected at in-person visits:

- Fasting blood draw (Table 2 for pediatric blood volumes) for the NEPTUNE Biorepository and central biochemical laboratory studies.
- Spot urine for Creatinine, Protein, Albumin assay at Central biochemical laboratory.
- Central biochemical laboratory 24-hour urine for Protein, Albumin, and Creatinine or timed urine collection.
- A "clean catch" research urine sample will be collected from all participants. Approximately 50 cc of the "clean catch" urine will be processed and transferred to the NEPTUNE Biorepository as outlined in the Manual of Procedures.

Interim local laboratory results between follow-up visits should be obtained from the participant's medical chart for:

- CBC [Hemoglobin, Hematocrit, WBC, MCV, MCH, MCHC, Platelets]
- Metabolic Panel [Albumin, Total Bilirubin, Calcium, Carbon Dioxide, Chloride, Creatinine, Glucose, Alkaline Phosphatase, Potassium, Total Protein, Sodium, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT)], Magnesium (if available), Phosphorus (if available), Urea Nitrogen, and Cholesterol, Cystatin C
- Spot urine assay for Creatinine, Protein, Albumin, Protein:Creatinine Ratio
- 24-hour Urine Creatinine Clearance, Total Creatinine and Protein
- Urinalysis [Color, Appearance, Specific Gravity, pH, Leukocyte Esterase, Nitrite, Protein, Glucose, Ketones, Urobilin, Bilirubin, Blood, Sediment]
- Virology results as available from clinical records including: [Hepatitis B, Hepatitis C, Epstein Barr Viral Capsid Antigen, IgM (EBV), Cytomegalovirus Antibody: IgG and IgM (CMV), Human Immunodeficiency Virus: Proviral DNA by PCR Qualitative and Quantitative RNA Assay (HIV), Hantavirus, Parvovirus]

The following updates will be performed at the follow-up visits that occur annually after enrollment:

- Contact Information
- Demographics
- Family History
- Update healthcare providers (physician, practice and hospital)
- Permission to Obtain Medical Records
- NEPTUNE Contact Registry Information (if not previously offered)

Table 2. Blood draw volumes

Adults (age 18+):

Visit	Blood Draw Volume
Biopsy [V_{BX}]	40 mL
Baseline [V1]	100 mL
Follow-up [V2-8]	65 mL
Relapse Visit (V_R)	40 mL

Children as follows by weight:

Visit	Blood Draw Volume by Weight (in pounds)		
	< 20 pounds	21-51 pounds	> 52 pounds
Biopsy [VBX]	10 cc	20 cc	40 cc
Enrollment [V1]	20 cc	50 cc	100 cc
Follow-up [V2-9]	20 cc	50 cc	65 cc
Relapse Visit [V_R]	10 cc	20 cc	40 cc

For participants with a clinically reported hematocrit < 28% the blood draw should be reduced as indicated in the Manual of Procedures.

3.D.1.a.(a) Biopsy Visit [V_{BX}]

If a biopsy is clinically indicated for a participant in cNEPTUNE, the participant should be consented to have an additional core of biopsy tissue taken for research purposes. If the participant consents, prior to the biopsy procedure, a blood sample (consistent with table 2, above) will be obtained for the NEPTUNE biorepository. A spot urine will also be collected pre-biopsy for storage in the study biorepository. During the biopsy procedure, an additional renal core will be obtained for research purposes if and only if it has been confirmed that sufficient tissue is available for diagnostic purposes. The number of attempts to obtain the clinical and research cores will not exceed 5. This tissue will be stored with the NEPTUNE study coordinator until it is confirmed that adequate tissue necessary for histologic diagnosis of renal disease has been received, processed, and the treating physician, in consultation with the renal pathologist, has determined there is no longer clinical need for this sample, at which time it will be released for research use in the NEPTUNE study.

Participant pathology classification will be assigned by the Pathology Review Committee.

cNEPTUNE participants receiving a kidney biopsy will still continue to be followed in the cNEPTUNE cohort. These participants will be seen every 4 months following their clinically indicated biopsy for the first year, and then every 6 months thereafter.

3.D.1.a.(b) SMS System

cNEPTUNE participants will take part in the SMS text messaging system for the initial 12 months of NEPTUNE participation. They will be enrolled at the time of consent. Information gathered by the research coordinator at the time of enrollment will include:

- Phone number
- Text message recipient (patient or parent/guardian)
- Preferred time of day for texts
- Preferred day of week for weekly texts
- Local study team contact email for alert notifications

This information will be inputted into NEPTUNE-Link and will be automatically transferred to the SMS system. Participant responses to text messages will be returned to NEPTUNE-Link for the local study team to review.

Participants will receive text messages asking about home urine protein monitoring results, relapse triggers (infections, allergies, stressors), edema status and location, consumption of prescribed immunosuppressive medications and nephrotic syndrome related absences from school/work. Parent or child ($\geq$12yrs) can respond to text messages at the discretion of the parent/child dyad (with approval from coordinator/local PI). The SMS alert system may be paused for planned vacations or during hospitalizations by the site study coordinator. SMS pause start and end dates will be entered into the NEPTUNE-Link SMS administration CRF to implement the pause.

Alerts will be sent to the email addresses designated in NEPTUNE-Link for items such as dipstick $\leq$2+ for 3 days after being trace/negative, abdominal or genital edema, anasarca, if the patient reports not taking immunosuppressive medications, or if there is no response for one week without prior notification to the study team to "pause" the system.

3.E. Study Data

Extensive data will be collected during the NEPTUNE cohort studies. These data descriptions are provided in the following six sections:

1) Primary Outcome Variables
2) Secondary Outcome Variables
3) Intra-renal Gene Expression Exposure Variables
4) Histopathology Variables
5) Overview Biochemical Measures
6) Other Exploratory Exposure and Intermediate Outcome Variables

3.E.1. Primary Outcome variables

The two primary outcome variables are:

(1) Event rate of change in urinary proteinuria excretion (defined as remission, partial remission and non-remission); and

(2) Rate of change in renal function defined as:

(a) 25 mls/min/1.73m^2 reduction in follow-up estimated GFR (using the 4-variable MDRD equation for ages ≥18 years and modified Schwartz for ages <18 years) compared to baseline estimated GFR

(b) 50% decline in follow-up estimated GFR compared to baseline measurement

(c) End stage renal disease defined as estimated GFR ≤10cc/min, initiation of maintenance dialysis or preemptive kidney transplantation).

3.E.1.a. Proteinuria

Twenty-four-hour urinary excretion of total protein, albumin, and creatinine will be measured during Study Visits 2, 4-6, 8, 10, and 12. Because of the significant inconvenience to the participants in collecting the 24-hour urine sample, the effort and time required for 24-hour urine collection will be reimbursed at no less than $50.00 per each 24-hr urine collection in addition to the reimbursement for attending a study visit. In addition, the ratio of the urinary protein or albumin (by specific immunoassay) to creatinine ratio will be measured from a spot urine specimen at selected study visits (see visit calendar). The urine protein:creatinine ratio and urine albumin:creatinine ratio will also be measured on the 24-hour specimens.

Rationale for using Proteinuria as a Primary Outcome Variable: It may take 5-10 years before one can clearly establish loss of function in patients with FSGS/MCD and MN (69,70). The choice of change in urinary protein excretion (UPE) as a primary study outcome is controversial and may be questioned by the reader. Moreover, the FDA has not accepted UPE as a surrogate for ESRD in registration or indication clinical trials. Nonetheless, there is cogent justification for choosing UPE as a primary outcome variable in the NEPTUNE Study.

UPE appears to be a valid surrogate outcome for several reasons. Many studies have implicated increased persistent UPE as a major risk factor in the development of progressive tubular injury, interstitial fibrosis, and GFR loss (21,57,71-76).

While proteinuria is considered a surrogate end point for progression of chronic kidney disease in general, proteinuria is the hallmark of idiopathic glomerulopathies such as FSGS, MCD, and MN and indeed a direct correlation has been identified experimentally between specific abnormalities in the glomerular filter and proteinuria (73). Reductions in proteinuria have served well as a primary outcome measure in therapeutic trials of these disorders (66, 68). Most importantly, risk of progression and ultimate outcome for both children and adults with FSGS was independently associated with the degree of sustained proteinuria over time (69,70).

The justification for collapsing complete and partial remission into one primary outcome category is as follows. Complete remission is associated with an excellent long-term prognosis in FSGS, MCD, and MN with renal survival rates between 90 and 100% (15,75,77). Partial

remission (PR), defined as 50% reduction in proteinuria combined with a reduction below <-3.5 g/d has been shown to independently predict renal survival (adjusted time-dependent hazard ratio for renal failure, 0.48; 95% confidence interval, 0.24 to 0.96; P<0.04, (66, 68). This relationship holds for patients with FSGS, MCD, and MN (20,78-80). Thus, PR is an important therapeutic target which holds implications for both progression rate and renal survival (67). Reviews describing these issues have been published recently by NEPTUNE investigators (21,81). The preceding lines of evidence provide a strong basis to conclude that remission of proteinuria (either CR or PR) is a valid surrogate primary outcome for both improved renal survival and slower rate of progression of renal disease, and that this outcome is an important therapeutic target for the clinician. We are aware that proteinuria might correlate with the cumulative level of glomerular segmental scarring and for this reason might not be the optimal parameter to measure disease activity particularly in patients with advanced disease. Work from members of this group has indicated podocyte depletion to be a more proximal event in disease pathogenesis in several model systems and humans (82-84). Future pilot or ancillary studies in this network will focus on techniques to monitor the level of podocyte loss in this cohort using urine derived podocyte mRNAs, podocyte specific markers in exosomes, and unbiased urinary screening approaches. Future integration of proteinuria data and data obtained from these ancillary studies could allow the definition of new primary outcomes.

For proteinuria quantification, random spot urinary protein to urinary creatinine ratio (UP:C) has been extensively evaluated. It is a sensitive test for the detection of proteinuria, but has intrinsic limitations for proteinuria quantification in the nephrotic range (85-88), which is expected to be the case in many of the study participants. To combine the advantages of both approaches and to be comparable with published studies (89), the proposed primary outcome for this study will be the use of a urine protein:creatinine ratio obtained from 24-hour urine collection in the adult cohort and incontinent children. In children below five years of age, spot urine will be used. Please see Table 3 for definitions of remission used in this study.

Table 3. Definition of the Primary Outcome for the FSGS/MCD and MN Cohort Studies

Proteinuria (U:C) After Observation Period	Standard Definition	Definitions for the Cohorts
U:C ≤ 0.3g	Complete Remission (CR)	Complete/Partial Remission
Reduction in U:C of >50% plus final P:C ≤3.5g but >0.3g	Partial Remission (PR)	
Reduction in U:C of >50% with final P:C>3.5g	Limited Response (LR)	No Response
Reduction in U:C of <50%. (include increase in U:C <50%)	Non-Response (NR)	
Proteinuria increases by >50%	Progressive Proteinuria (PP)	
New development of nephrotic range proteinuria, i.e., >3.5 P:C after reaching a complete or partial remission	Relapse	Relapse

3.E.1.b. Renal Function

The main renal function measures in the cohort studies include serum creatinine, serum Cystatin C, a 24-hour urinary clearance of creatinine, and estimated GFR using the MDRD equation for adults and modified Schwartz formula for continent children under 18 years. Serum Cystatin C assay will be deferred until funds for ancillary studies become available. The schedule of these measures is summarized in Table 4.

Table 4. Schedule of Renal Function Measurements in the NEPTUNE main cohorts

Measure	Collection Schedule
Serum creatinine	Every visit
24-hour urinary creatinine excretion	Every visit
Estimated GFR	Every visit
Serum Cystatin C	Every visit*

* Funds pending

3.E.1.b.(a) Creatinine-based Measures

In clinical practice and large-scale epidemiological research projects the serum creatinine, estimated GFR and timed urinary creatinine clearance have been used for diagnosis and monitoring the progression of renal disease (90-92). These three measures will be ascertained in the current study visit. Factors other than GFR—including generation, tubular secretion, and extrarenal elimination of creatinine—affect serum creatinine concentration. Therefore, use of these serum creatinine or creatinine clearance determinations may not always provide an accurate estimate of renal function (92,93). The most widely utilized estimating equation (4-variable MDRD) may not be sensitive enough to detect progression of renal disease during the time frame of the study. The NEPTUNE will use the three measures of renal function (serum creatinine, estimated GFR and timed urinary creatinine clearance) in an attempt to compensate for the inherent limitations of each single measurement by itself. Ideally, the more precisely measured GFR would be employed but the expense, logistics and participant burden are prohibitive in the current study. The timed urinary creatinine clearance will be performed on a 24-hour urine collection. Because of the significant inconvenience to the participants in collecting a 24-hour urine sample, only six 24-hr urine collections are mandatory and these must include the Baseline Visit [V2], at least one annual follow-up visit [V6, V8, V10, V13] and the final follow-up visit ([V13]) depending on the time of enrollment into the study. Where urinary incontinence prohibits a full 24 hour urine collection, a best alternative urine specimen is to be collected as either a maximum timed urine collection (e.g. 6 to 12 hours), first morning urine collection or random urine collection.

3.E.1.b.(b) Cystatin C

Cystatin C has recently been proposed as a valuable marker of renal function and has been validated in large-scale epidemiological studies. Cystatins comprise a group of proteinase inhibitors, widely distributed in tissues and body fluids that form tight complexes with cysteine proteases. Cystatin C, a secreted molecule of this family, is increased in patients with malignant diseases, is related to insufficient renal function and appears to be a better marker of renal function than creatinine. Cystatins are secreted by many nucleated cells and the serum concentration is not as dependent on muscle bulk and nutritional state as serum creatinine. Serum concentrations of Cystatin C have been found to be potentially useful markers of renal function without most of the drawbacks of serum creatinine and the FDA has approved two assay techniques. Therefore, provisions will be made to set aside blood samples to measure serum Cystatin C concentrations as an additional measure of renal function if and when additional funds become available from ancillary proposals.

Justification for Choosing Renal Function as Primary Outcome Variable: We find it necessary to submit a defense for the use of the absolute reduction in GFR decline (time to event) rather than the rate of change (slope of GFR) as secondary renal function outcome in the FSGS/MCD and MN studies. Our rationales are straightforward. First, the absolute change in GFR is the more clinically important parameter. Secondly, the sample sizes of 250 and 200 participants, respectively, and duration of follow-up is not sufficient for a robust statistical power to estimate slope of GFR as an outcome. Thirdly, our experience in previous clinical studies causes us to be wary of the assumption of linear deterioration in renal function that underlies a slope-based analysis. This assumption is an empiric claim that has not always been substantiated. An estimated slope may be particularly erroneous when patients display a non-linear or otherwise erratic pattern of deterioration. In some cases, standard regression techniques may yield numerically large slopes that are not statistically different from zero. Missing measurements create instability in slope measurements. Also, slope evaluation techniques are poorly suited to censoring for dropouts and

missed data points, in part because the participation time is not appropriately weighted. For these reasons, time to ESRD provides a secondary outcome that obviates most of the problems inherent in a slope-based analysis. Time to ESRD or 50% decline in baseline GFR or 25 ml/min/1.73m^2 decline in baseline GFR is particularly attractive as a secondary outcome for the FSGS/MCD study because, in general, time to a specified event constitutes a clearer outcome than a slope. In addition, time to event analysis does not force a functional form (i.e. linear decline) on the process of progression. It is likely that the analytic problems associated with a particular measurement technique are minimized when comparing values for an individual over time. Censoring methods are readily applicable to participants who depart early. Lastly, time to ESRD (i.e. initiation of renal replacement therapy for usual clinical indications) is the most important clinical and economic consequence of FSGS/MCD and MN. This time can be ascertained for participants even after formal completion of the proposed study.

3.E.2. Secondary Outcome Variables

Composite of the following events will be considered as secondary outcomes of the NEPTUNE main cohort study. Additional CRFs or appropriate source documentation may be required for further clarification and/or event adjudication for the following 'secondary outcomes of special interest':

(1) Quality of Life: Patient-reported outcome will be assessed using the Patient Reported Outcome Measurement Information System (PROMIS) fro ages 8 and above and parent proxy PROMIS measures will be completed for ages 0-10. Overlap in ages 8 to10 years allows the complete ascertainment of this assessment in children who may find self reporting difficult due to delayed reading skills.

(2) New Onset Diabetes: New-onset diabetes is a diagnosis of diabetes as indicated by one or more of the following that was not present at the time of NEPTUNE enrollment.

- a) Documented diagnosis of diabetes in the medical record
- b) Casual (non-fasting) blood glucose > 200 mg/dL
- c) Fasting blood glucose >126 mg/dL
- d) 2 hour glucose > 200 after oral glucose tolerance test
- e) chronic use (> 6 months) of hypoglycemic therapy outside of pregnancy
- f) Hemoglobin A1C ≥ 6.5% (94)

(3) Malignancies: Any cancer diagnosis of the skin, hematopoietic system, or solid organ after enrollment in NEPTUNE

(4) Infections, Serious and Systemic: Infections including one of the following:

- a) Documented diagnosis of infection of the skin or subcutaneous tissue (e.g. cellulitis), vascular system, peritoneum, or any vital organ requiring the use of parenteral antibiotics and/or oral antibiotics alone or in combination for a treatment interval of ≥72 hours.
- b) Hospitalization for treatment of infection

(5) Thromboembolic Events: Documented diagnosis of one of the following:

- a) Embolic cerebrovascular accident
- b) Deep venous thrombosis
- c) Renal vein thrombosis or
- d) Pulmonary embolus

(6) Hospitalization: Documented hospital admission, including observation for ≥24 hours.

(7) Emergency Department/ Observation Unit Visit: Documented visit to an emergency department or observation unit that does not lead to hospitalization and is less than 24 hours.

(8) Acute Kidney Injury: Documented diagnosis of acute kidney injury as defined by the KDIGO criteria (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney inter., Suppl. 2012; 2: 1–138) and/or renal failure requiring renal replacement therapy <3 months.

(9) End-Stage Renal Disease (ESRD): Documented chronic dialysis or transplant shall be considered ESRD for the purposes of the NEPTUNE study, a final renal outcome.

(10) Death:

a) Infection-related Death. Documentation of death that is secondary to infection or sepsis.

b) Cardiovascular/Cerebrovascular-related Death: Documentation of death from at least one of the following:

i) Sudden death

ii) Myocardial infarction

iii) Congestive heart failure

iv) Primary intractable serious arrhythmia

v) Peripheral vascular disease

vi) Ischemic cerebrovascular accident

vii) Hemorrhagic cerebrovascular accident

viii) Thromboembolic event (e.g. pulmonary embolus, embolic cerebrovascular accident)

c) Malignancy-associated Death: Documentation of death secondary to cancer

d) Other Death: Documentation of death that does not fall into the above categories (e.g. primary respiratory failure).

3.E.2.a. Potential Data Sources and Ascertainment Strategy

Data Sources: The NEPTUNE study participant contact pattern includes study visits and interim phone calls on an as-needed basis. For selected sites that have access to electronic administrative or billing records, regular searches of those data sources will be performed (e.g., quarterly) for their center's study participants. We will also explore the feasibility of using additional databases (National Death Index, USRDS and SRTR) to identify outcomes, although it is clearly recognized that there is often a long delay (e.g., 12-18 months or longer) in getting access to these files, which are updated annually and require additional governmental approval. In preparation for use of national data sources, the patients will be consented for the collection of the social security number for use in assessing vital status, ESRD status and transplant status using these national data sources.

Outcome Search Strategy: For potential clinical events that lead to hospitalization, we will acquire information from the study participant or their self-assigned proxy regarding the dates (admission and discharge) and hospital (name, address, phone number) where the patient was hospitalized. The scope of medical record acquisition is limited to the following events: hospitalizations, infectious episode requiring parenteral antibiotic therapy, outpatient cardiac catheterization, outpatient ECHO (MUGA or nuclear perfusion imaging), new onset heart failure, extended (>24 hour) ER or observation unit stay.

For potential clinical events that do not lead to hospitalization, we will acquire information from the study participant or their self-assigned proxy regarding the date of the potential event

and the location where the patient received treatment (e.g., outpatient clinic/surgicenter, address, phone number, and treating physician).

Patient Reported Information using SMS (text messaging): Participants in the cNEPTUNE cohort will report results of home urine protein testing, edema status, medication adherence, presence of infections, stressors, or allergies, and work and school absences. Tailored text messages will be sent to the designated parent/patient throughout the initial year of study participation.

3.E.3. Intra-renal Gene Expression Exposure Variables

Intra-renal steady state mRNA levels are currently providing the first opportunity to obtain truly comprehensive functional information of renal tissue during the progression of kidney disease. Gene expression regulation reflects an integration of specific environmental exposures, the genetic disposition of an individual and the current disease stage and activity. Alterations in mRNAs can not only be used as molecular fingerprints, but can also provide information on disease processes when integrated with prior knowledge and sequence base information on the specific mRNAs. This approach thereby provides a fundamentally novel category of parameters for disease (subtype) definition.

The technology for renal biopsy based gene expression profiling is well established and integration with clinical variables has been documented by members of this consortium for renal diseases.

Compartment specific gene expression profiles will be obtained from all renal biopsies and integrated with clinical variables to predict primary and secondary outcomes as defined below. Vice versa, patients will be grouped into molecularly defined subgroups according to their similarity in gene expression profiles using cluster and principle component analysis. These subgroups will be compared for differences in exposure variables and outcomes.

Gene expression profiles will be compiled into transcriptional regulatory networks using a suite of tools developed by the Applied Systems Biology Core (ASBC) affiliated with the NEPTUNE. The ASBC in the O'Brien Renal Center at the University of Michigan will be responsible for providing access and guidance to the NEPTUNE researcher for optimal integration of this unique resource into their ongoing research efforts in Nephrotic Syndrome in a disease specific context. Aggregated, de-identified data sets will be made available to the research community at large using the kidney specific systems biology search engine Nephromine (www.nephromine.org).

3.E.4. Histopathologic Variables

Light microscopy slides (all levels available) and electron micrographs and immunofluorescence photographs (or recorded results in the pathology report) will be shipped to and scanned by the NIDDK Histopathological Archive personnel, returned to the recruiting site, and reviewed by two reference pathologists (members of the pathology committee) per each case. More than one pathologist is required to score each case to test reproducibility. A third pathologist will be consulted for quality control if a significant discrepancy occurs. Discordance will occur when any difference in number (>10%) for any specific glomerular lesion is recorded by two different pathologists. The same criterion will apply to the evaluation of tubulointerstitial damage and vascular disease. All cases with discordance will then be reviewed simultaneously by all three members of the pathology committee to reach consensus and to identify the specific reason for the initial disagreement. A decision will be made when two out of three pathologists agree on any given point of discordance. In those cases where agreement cannot be reached by the members of the pathology committee, the pathology advisory board will be directly involved in the decision process, and decision will be made when the majority (at least four of the six total members of the pathology committee and pathology advisory board) reaches a consensus.

The original diagnosis and quantitative analysis will be recorded according to criteria described in the pathology scoring criteria appendix in the study protocol.

Quantitative analysis will be performed using conventional morphologic analysis and experimental morphologic analysis.

For a detailed description and rationale of histological parameters evaluated see Appendices B and C, Methodology for Pathologic Analysis.

3.E.5. Overview of Biochemical Measures

A number of conditions dictate the performance of assays for biological measures at the time blood samples are obtained. These include the inability to perform assays on specimens stored for prolonged periods of time (e.g., fibrinogen, PTH, troponin I), and the desire to provide immediate feedback to participants and their physicians to enhance recruitment and retention (e.g., metabolic panel). We will continue to seek funds by developing ancillary studies to perform these measurements in the cohort study participants as soon as feasible.

All cohort participants will provide fasting blood specimens in six month intervals providing the capability of performing core and ancillary biochemical assays as frequently as twice yearly on specimens that will be collected and stored in the NEPTUNE Biorepository.

The baseline blood specimen has been shown to be the rate limiting resource for ancillary and pilot studies in multiple prospective cohort studies of renal disease (African American Study of Kidney Disease and Hypertension – AASK, Chronic Renal Insufficiency Cohort – CRIC, and the Focal and Segmental Glomerulosclerosis Clinical Trial – FSGS-CT, among others). The NEPTUNE protocol asks for a reduced amount of baseline blood samples obtained as in the NIDDK-funded CRIC study with a blood collection of 100 cc at the baseline visit in adult participants. Blood volumes are adjusted by weight for pediatric participants according to national guidelines (i.e., maximum of 30 ml per 3 month period in children 6-10 kg). See blood volume table on page 26. The total volume collected will be reduced at the follow-up visits for adults and children. If a patient has a hematocrit below 28%, total blood volume removed will be reduced to not exceed 0.5% of whole blood volume. A clean catch urine sample will be collected at every clinic visit for a variety of chemistry testing (e.g., electrolytes, protein and creatinine concentrations, or proteomic testing). See Table 5 for a listing of potential biochemical measures.

Table 5. Potential Biochemical Measures (Pending Available Funding)

Serum Measures	
Metabolic panel including: Albumin Bicarbonate Total Bilirubin Calcium Carbon Dioxide Chloride Creatinine Glucose Alkaline Phosphatase Potassium Total Protein Sodium Aspartate Aminotransferase (AST) Alanine Aminotransferase (ALT) Total Cholesterol LDL Cholesterol HDL Cholesterol Triglycerides Urea Nitrogen CBC including: Hemoglobin Hematocrit WBC (with differential) Platelet Count MCV MCH MCHC	Pending Available Funding: Magnesium Phosphorus Cystatin C Hgb A1C Homocysteine Troponin I iPTH Fibrinogen Uric Acid Advanced Glycation Endproducts (AGE) Lipoprotein (a) Apolipoproteins Inflammatory marker CRP/hs-CRP Inflammatory marker sICAM Vitamins A, B6, B12, C, E Folate Zinc Transferrin Insulin Insulin-like Growth Factor-1 Carotenoids Total Body Nitrogen Plasminogen Activator Inhibitor (PAI-1) TGF – Alpha and Beta TNF – Alpha Asymmetric Dimethyl-arginine (ADMA) Procollagen - 1 Iron and Iron-binding Capacity Ferritin Concentration Renin Aldosterone Prealbumin
Urinary Measures	
Creatinine Protein Albumin Urea nitrogen	Urinary Isoprostanes Sodium Potassium

3.E.5.a. Glycemic Control

Pending available funding, Hgb A1C concentrations will be measured at the baseline visit. Thereafter, this measure will be repeated on diabetic patients. Insulin and advanced glycation endproducts (AGE), may also be measured as markers of glucose control among cohort participants with diabetes mellitus.

3.E.5.b. Lipids and Lipoproteins

Total cholesterol, triglycerides, HDL and LDL cholesterol will be measured. In addition, Lipoprotein (a) and Apolipoprotein-B will be obtained pending available funding.

3.E.5.c. Markers of Inflammation

Pending available funding, levels of highly sensitive C-reactive protein (hs-CRP) will be measured at baseline. Levels of hs-CRP and other inflammatory markers including soluble intercellular adhesion molecule 1 (sICAM) will be assayed at additional times during follow-up.

3.E.5.d. Nutritional Status

Nutritional status will be assessed using a combination of clinical evaluation and biochemical markers if funds become available. Serum creatinine, albumin, bicarbonate, calcium, total cholesterol and triglyceride values will be used to evaluate nutritional status. Pending available

funding and storage capability, additional laboratory values may be measured for this purpose such as serum prealbumin, vitamins A, B6, B12, C, E, and folate, zinc, C-reactive protein, transferrin, CRP and insulin-like growth factor-1 concentrations, carotenoids, total body nitrogen and urinary electrolyte excretion.

3.E.5.e. Hemostatic/Prothrombotic Factors

Fibrinogen will be measured at baseline in all participants as part of the NEPTUNE if funds are available. Pending available funding, plasminogen activator inhibitor-1 (PAI-1) will be measured as a marker of hemostatic/prothrombotic activity.

3.E.5.f. Measures of Myocyte Injury

Troponin I will be measured at baseline in all participants if funds are available. Troponin, a direct measure of myocyte injury, is generally considered to be the most sensitive and specific measurement available (95). Most experts in the field consider it to be close to 100% sensitive and specific for cardiac ischemia in the right clinical setting (as troponin should not be found in the bloodstream normally).

3.E.5.g. Oxidative Stress

Pending available funding, oxidative stress will be estimated by measurement of urinary isoprostanes using one of the aliquots from the 24-hour urine collection.

3.E.5.h. Cytokines

Measures of TGF-alpha, TGF-beta and other cytokines will be obtained on stored specimens pending available funding. Significant information on the role of cytokines, particularly TGF-beta in diabetes, testifies to their utility in predicting worsening of renal function. TNF-alpha is less well understood, but appears to be important in CVD progression.

3.E.5.i. RAAS

Measures of plasma renin activity (PRA), angiotensin II (Ang II) and plasma/urinary aldosterone levels will be obtained on stored specimens pending available funding.

3.E.5.j. Endothelial Function

Pending available funding, endothelial function will be assessed by the determination of plasma concentration of asymmetric dimethyl-arginine (ADMA).

3.E.5.k. Fibrosis

Pending available funding, fibrosing predisposition will be assessed by measurement of serum concentrations of procollagen-1.

3.E.5.l. Heavy Metal Toxicity

Pending available funding, exposure to heavy metals (e.g., lead and cadmium) and body accumulation will be assessed through assay of trace metal concentrations in nail clippings.

3.E.5.m. Iron Status

Pending available funding, iron status will be assessed by serum iron and iron-binding capacity and serum ferritin concentrations. The importance of anemia and iron status has been demonstrated in both CKD progression and CVD.

3.E.5.n. Other Biochemical Measures

Electrolytes (Sodium, Potassium, Chloride, Bicarbonate), BUN, Creatinine, Glucose, AST, ALT, Total Bilirubin, Total Protein, Calcium, and CBC will be measured annually. A urine dipstick will be recorded annually for qualitative glucose, protein, and hematuria determinations. Magnesium, Phosphorous, Uric acid, and iPTH will be measured beyond baseline pending available funding.

3.E.5.o. Genotyping

Each patient enrolled in the NEPTUNE will be offered the opportunity to undergo mutational analysis by exon sequencing performed by The University of Michigan Genetics Laboratory. Mutation analysis will be obtained for the following Nephrotic Syndrome genes with recessive inheritance pattern: *NPHS1, NPHS2, LAMB2, PLCE1/NPHS3* and for the Nephrotic Syndrome genes with dominant inheritance pattern: *WT1* (and if indicated by family history also the extremely rare causative genes *TRPC6*, *ACTN4* and *CD2AP*). If no mutations are detected in these genes, the patient's DNA will be examined by exon capture and exon sequencing to detect mutations in so far unknown nephrosis-causing genes. If sibling cases with Nephrotic Syndrome become available in the study, they will be examined by homozygosity mapping with consecutive exon capture and exon sequencing. Genes postulated to infer risk of progression of glomerular disease (including MYH9) will also be considered.

3.E.5.p. Cell Line Growth and Immortalization

Pending available funding, cell immortalization of blood sample leukocytes will be pursued. Immortalized leukocytes may provide the raw material to understand the cellular and molecular mechanism of kidney disease pathways.

Schedule of Biologic Specimen Collection: Biologic specimens collected during the course of the cohort studies include blood, 24-hour urine, random spot urine, nail clippings, and renal biopsy tissue. The schedule of these collections is shown in Table 6.

Table 6. Schedule of Biologic Specimen Collection in Cohort Studies

Measure	Collection Schedule
Blood specimen	Every visit
Renal biopsy tissue	Biopsy Visit and as clinically indicated
24-hour urine specimen	Every visit
Spot urine specimen	Every visit

3.E.6. Other Exploratory Exposure and Intermediate Outcome Variables

3.E.6.a. Sociodemographic and Medical History

Data on age, gender, detailed race/ethnicity, education and income will be collected at study baseline. Patient residential census tract will be collected at baseline. A directed history will be obtained to determine associated factors, family history of renal and cardiovascular disease, prior cardiovascular disease and risk factors, coexisting morbidity, and health behaviors (see Manual of Procedures).

3.E.6.b. Medications and Vaccination Use

Participants are asked to list all prescription and over-the-counter medications they have taken since last study visit, and any vaccines they have received since their last study visit. (see Manual of Procedures).

3.E.6.c. Quality of Life

A Quality of Life (QOL) assessment is being performed using the PROMIS questionnaire. It will be completed by all age participants (Parent report for ages < 10 years and self report for all others 8+ years of age) (see Manual of Procedures).

3.E.6.d. Health Care Resource Utilization

Health care resource utilization data are being collected using administrative claims data (e.g. in the subset of Medicare-eligible patients) and data obtained directly from cohort participants

at the follow-up visits. If funding becomes available, this resource will be comprehensively developed by our study team.

3.E.6.e. Medication Adherence Measure

Patient self – report for age 8 years and greater and parent proxy for age 0 to 10 years will contain questions on adherence to medicine regimens prescribed for kidney disease and reasons for missing medications.

3.E.6.f. Occupational Exposures to Heavy Metals

Pending additional funding, exposure risks to heavy metals will be assessed in both cohorts in a structured questionnaire currently being developed by the NEPTUNE under the leadership of the University of North Carolina.

3.E.6.g. Pregnancy History

Pending additional funding, a detailed pregnancy history including pregnancy outcomes will be assessed in both cohorts in the NEPTUNE study by direct participant questioning as well as chart review as needed.

3.F. Statistical Analysis

3.F.1. General Methods for Statistical Analysis

We plan to use standard descriptive statistics to characterize the overall study population and subgroups of interest both at baseline and during follow-up. Summary statistics such as means, medians, standard deviations, and ranges will be produced for measured variables. Frequencies will be tabulated for categorical and ordinal variables. Graphical methods will be used extensively to examine distributions, identify potential influential points, and guide in data transformations if warranted. For outcomes collected longitudinally, and to examine associations among various measures, scatterplots and grouped boxplots will be produced to examine assumptions of linearity, symmetry, and homoscedascity. We can also easily calculate the rate and adjusted hazard rate of the main two clinical outcomes (change in UPE and renal function) using regression methods.

3.F.2. Analysis During the Adaptive Recruitment Phase

The study has several recruitment goals regarding the distribution of various groups, including the distribution of serum creatinine or eGFR, age and other characteristics. At some clinical centers, these measures will be available in advance from automated electronic databases; at others, the data will be available only at the time of the initial clinic visit.

In the sections above, we specified recruitment targets by histological diagnosis. During the course of recruitment, we will examine repeatedly these characteristics by center using the web-based, real-time monitoring function of NEPTUNELink. If, after completion of the recruitment of 300 participants, the distributions of these variables are close to the target ranges specified above, recruitment will be continued as previously described. If not, participants with some levels of these variables were oversampled and others undersampled and corrective measures will be taken to ensure adequate representation of both cohorts. Related statistical analysis will account for this unbalanced sampling through appropriate weighting schemes.

During the protocol revision for Version 3.0, the DACC regarded the number of diagnoses entered into the OG Cohort (Other Glomerulopathies) to be higher than anticipated. In an effort to maintain statistical power for the cohorts of interest (FSGS/MCD and MN) for the study's goals, the overall recruitment target goal was adjusted to 600 to reach the projected targets of 250 and 200 for the FSGS/MCD and MN cohorts, respectively.

Other measures, including screen failure for consented individuals determined to have exclusion criteria present post-enrollment, have been taken to compensate for over-enrolling in the OG group. The OG group will not be further followed, but samples and data obtained will remain available for comparative analyses.

3.F.3. Special Considerations for the Analysis of Molecular Biomarkers

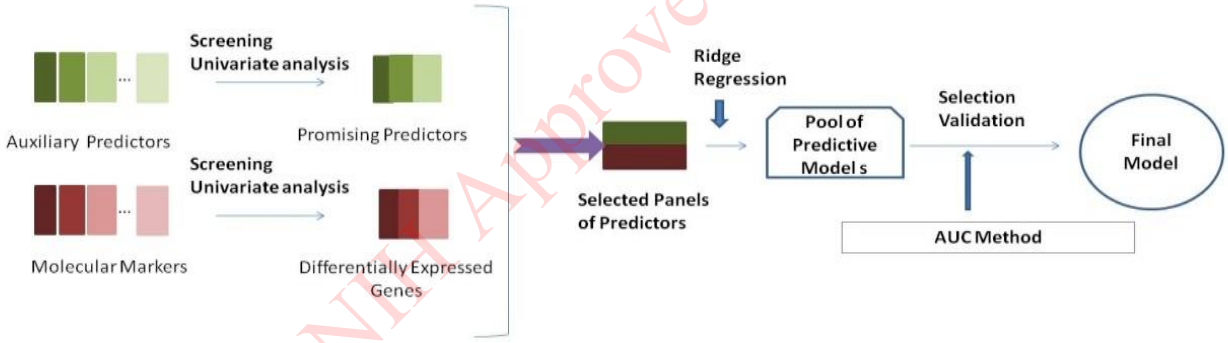
The analysis of molecular biomarkers aims to identify promising genetic signatures used in the building of a prediction model for clinical outcomes. To screen the large number of potential predictors without losing analytical power, we would proceed in a hypothesis generating manner. Regression analysis of the clinical endpoint on biomarkers allows us to obtain P-values in the testing of their association, and a threshold value of false discovery rate would be set to determine a pool of potentially important biomarkers. These selected biomarkers will then be analyzed and grouped according to the relevance of their biological functions available in various databases. For example, biomarkers in a common transcriptional network may form a molecular signature panel. Such various panels can be further tested for the association with the clinical endpoint via the method of ridge regression. Ridge regression enables us to bypass the analytic difficulty due to highly correlated biomarkers in the model and hence obtain robust results.

3.F.4. Prediction Model Combining Molecular Biomarkers and Auxiliary Variables

The primary objective of the data analysis is to build a predictive model that uses both molecular biomarkers and auxiliary predictors to classify patients into one of two clinical states of the primary endpoint, Complete Remission/Partial Remission (CR/PR) and No Remission/Relapse (NR/R). The second endpoint is the presence or absence of the loss of renal function. In addition to 20,000 molecular markers, there are 74 auxiliary predictors collected from both cohort studies, including 38 histopathology variables, 15 laboratory variables, 15 clinical and demographic information variables, and 6 treatment variables.

Given the large number of predictors, we take a three-stage data analysis strategy consisting of variable screening, model building, and model validation. Figure 1 shows a schematic flow of steps for the model building.

Figure 1: Schematic illustration of analytic steps taken to model building.



The first stage of analysis is a variable screening analysis, in which we perform univariate analysis. The aim of this study is to evaluate basic distributional characteristics of each predictor and pairwise correlation between the response and each predictor, as well as that between each pair of predictors. In this analysis, we also examine if transformations on some predictors are needed and if high-order predictors such as interactions and polynomial predictors are potentially useful predictors.

At the second stage of analysis, we build a predictive model for the clinical endpoint. First, we perform univariate logistic regression of the clinical endpoint on each of the predictors to detect promising predictors. To deal with the multiplicity in the test, we control a common significance level α_m on molecular markers and α_c on auxiliary predictors. At different levels of α_m and α_c, we obtain several sets of molecular and auxiliary predictors. For example, we may control α_m at 10^{-8} and α_c at 10^{-4} according to the Bonferroni's conservative type I error control. Using a set of the selected predictors, we invoke ridge logistic regression to build a predictive model, which essentially provides a linear classifier to determine the membership of a patient with a clinical class.

At the final stage of model building, we use the ROC curve method to select the final model from a small pool of candidate predictive models. To do this, we split the sample into the training set and the test

set at (2/3n):(1/3n) (96-98) randomly with multiple rounds, say 100. For the sample size 200, the split is 135:65, and for the sample size of 250, the split is 170:80. At each round, we use the training set to build a predictive model and the test set to evaluate its performance of prediction by the AUC, acronym for the area under the receiver operating characteristic (ROC) curve. We use the averaged AUC to determine the final predictor model with the best averaged prediction power.

3.F.5. Sample Size and Power Considerations

3.F.5.a. Overview

The primary aim of this study is to build up the prediction model, so our sample size and power calculation should be based on this goal. Following Dobbin and Simon (96), we adopt the expected probability of correct classification (PCC) as the measure of predictive power, which is approximately equal to the AUC, acronym for the area under the receiver operating characteristic (ROC) curve. This study focuses on two rare diseases: FSGS/MCD and MN, so we use PCC to evaluate the sample size of these two diseases, respectively.

3.F.5.b. Sample Size and Power Related to the FSGS/MCD Cohort Study

PCC is the probability of correct classification obtained on an infinite sample size and should be the gold standard of the best power. FSGS and MCD are rare diseases; it is unrealistic to find very large sample sizes to approximate the result under an infinite sample size. However, we will be able to find the PCC under realistic sample sizes, and we use PCC* to denote it. PPC* is the expected probability of correct classification with the optimal linear classifier constructed from the true molecular markers and auxiliary predictors. The results given in Table 6 are based on a simplified analysis with conservative assumptions. We screen 20,000 genes (probes) and 75 auxiliary predictors, in which there are r_m true gene expression markers and r_c true auxiliary predictors for the clinical endpoint. Ratio δ_m/σ_m is the average effect size of a single molecular marker, and the relative effect size of the auxiliary predictor to the molecular marker is 0.5. Also, ρ is the correlation between molecular markers and auxiliary predictors, and $\rho=0$ is assumed in Table 7 (3.N-2 below).

Table 7.Probability of Correct Classification

$r_m = 5$, $r_c = 10$				
δ_m/σ_m	PCC*	PCC(n=100)	PCC(n=150)	PCC(n=200)
0.2	0.788	0.638	0.667	0.687
0.4	0.941	0.876	0.892	0.896
$r_m = 50$, $r_c = 10$				
δ_m/σ_m	PCC*	PCC(n=100)	PCC(n=150)	PCC(n=200)
0.2	0.941	0.638	0.695	0.753
0.4	0.999	0.987	0.995	0.996
$r_m = 100$, $r_c = 10$				
δ_m/σ_m	PCC*	PCC(n=100)	PCC(n=150)	PCC(n=200)
0.2	0.982	0.672	0.766	0.837
0.4	0.99999	0.9994	0.99990	0.99994

For example, if we have 50 molecular markers and 10 auxiliary variables, from the sample size of 200, we will be able to reach a 99.6% PCC* with the average effect size of a single molecular marker to be 0.4, and the relative effect size of the auxiliary predictor to the molecular marker to be 0.5. This 99.6% PCC* almost reaches the optimum PCC of 99.9%, yet it is very realistic because the sample size of 200 falls in our recruitment targets.

It is noted that the inclusion of patients without the specified diseases of interest will not affect the power of classifier designed for this study as long as the required sample sizes given

in Table 7 are met. Such additional information from the third cohort might be useful in the development of a different classifier in an auxiliary study where the clinical status of these patients is of interest.

3.F.5.c. Sample Size and Power Related to the MN Cohort Study

Under the same assumption given in the power calculation for the FSGS/MCD cohort study, we can also calculate the power of the MN cohort study in the same fashion. And the result provided in Table 7 above (3.N-1) can also be applied to the MN cohort study.

For example, if we have 100 molecular markers and 10 auxiliary variables, from under the sample size of 150, the PCC* is 76.6% with δ_m/σ_m being 0.2 and the relative effect size of the auxiliary being 0.5. If we increase the sample size from 150 to 200, we can find an increase of PCC* from 76.6% to 83.7%.

3.F.5.d. Sample Size and Power Related to the cNEPTUNE Study

The analytic plan for this cohort is to identify groups of participants by clinical relapse and remission patterns as well as functional, molecular and genetic profiles, with a goal to relate those groups to clinically meaningful outcomes, including patient reported outcomes. As these subgroups are yet to be defined by the data obtained by the cohort, we present a range of exposures and minimum detectable odds ratios below. In the pediatric population, and as seen in the current NEPTUNE cohort, rates of clinically significant decline in kidney function, defined as a 40% drop in eGFR or ESRD, ranged from 0.00 to 0.16 per person-year, and as such, the study is not primarily powered to this event.

The statistical power calculations are based on the following assumptions. We conservatively assume a loss of 10% of the available follow-up due to participant retention issues. We assume a power of 80%, an alpha of 0.05, an intra-cluster correlation of 0.05, and a between-facility normalized standard deviation of the sample size of 0.15.

Power was computed for a range of sample sizes, event rates, and exposure percentages, representing between- and within-group comparisons and outcomes as described within each aim (Table H). For example, the 50% v. 50% exposure would correspond to an analysis across the entire sample between participants above v. below the median of a continuous covariate. Other percentages correspond to group comparisons of specific biomarker samples or among specific populations (e.g. participants with specified biomarkers, functionally defined subgroups). In each row, the minimum detectable odds ratio (MDOR) using logistics regression is provided with a varying outcome prevalence.

Table H. Power calculations

Exposure Percentages		Logistic analyses	
Group A	Group B	Outcome Prevalence	MDOR
50%	50%	35%-45%	2.3
		20%	2.7
20%	80%	35%-45%	2.8
		20%	3.1
20%	40%	35%-45%	3.1
		20%	3.6
10%	50%	35%-45%	6.4-6.9
		20%	6.7

3.F.5.e. Time to Event Analyses

Cox regression will be applied to analyze the time to event data of the primary or secondary endpoints collected in both cohort studies. This analysis allows us to assess association of the disease hazard rate with either clinical covariates of interest or genetic signature panels. Moreover, a survival distribution function of emission or that of renal function loss can be estimated under different patient profiles. A possible complication in the analysis is that patients may experience recurrences of remission, partial remission or no remission during the follow-up period. Thus, it is also of interest to perform an analysis of recurrent event history data, and to compare the results with those obtained from the only endpoint-based time to event analysis.

3.F.6. Data Management Infrastructure

The consortium data collection and management will be accomplished using Arbor Research ArborLink, regulatory-compliant, web-based clinical research information system. ArborLink is licensed and supported by the Arbor Research Collaborative for Health (Arbor Research); its use by NEPTUNE will be supported by Arbor Research. A NEPTUNE specific version of ArborLink will be henceforth called NEPTUNE-Link. Among its many attributes, this system allows systematic remote web-based clinical and laboratory data collection at study participant encounter sites, and provides patient tracking, and data management capability.

The SMS system will be managed using the NEPTUNE-Link data management system to supply information for message tailoring, Intervision, Inc. for routing of SMS messages to designated patient/parent recipient and return of messaging responses for storage to the NEPTUNE data storage at Arbor Research, Ann Arbor, MI.

As mandated by the funding agency, select data will be transferred to the central Data Management and Coordination Center (DMCC) of the RDCRN.

4. HUMAN SUBJECT CONSIDERATIONS

4.A. NEPTUNE Cohort Participant Considerations

4.A.1. Overall Recruitment and Retention Plan

Patients with signs and symptoms of kidney disease consistent with FSGS, MCD, MN or proteinuric renal disease who present for patient care at the participating clinical centers will be the primary study population targeted for enrollment into the NEPTUNE study. The NEPTUNE comprises clinical centers dispersed across the United States of America and the province of Ontario. These clinical centers cover the major population centers in all the regions of the continental US and the largest province in Canada. The volume of new kidney disease patients evaluated and treated annually at the participating clinical centers exceeds 50,000. Specifically, more than 1500 new participants (subjects) who fit the diagnosis of FSGS, MCD, and MN are seen annually at these clinical centers. It is not expected that the volume of activity at these the clinical centers will drop during the recruitment phase for the proposed study. The NEPTUNE team has developed a recruitment plan which entails the following two phases: (1) Pre-screening and identification of potential study participants; (2) Screening and verification of eligibility criteria. Details of these recruitment phases will be outlined in the Manual of Procedures but we hereby provide a brief description of the 4-phase recruitment plan to sufficiently evaluate its soundness. These phases may occur independently and sequentially or simultaneously, to ensure minimal patient burden.

Pre-Screening: The study eligibility criteria are based on current literature and are quite specific to the clinical definition of FSGS/MCD, MN and childhood onset NS. These criteria will be supplemented by manual review of medical records by study personnel who will be centrally and locally trained on recruitment techniques. Dissemination of the study and solicitations to participate in the study will also be facilitated by word of mouth, personal contacts and informational brochures directed to all the physicians in the adult and pediatric nephrology programs of the participating clinical centers and their nephrology referral base. Additional information regarding the study for incident childhood onset NS will be directed toward the Emergency Department and primary care community. The study team member will conduct a checklist assessment of eligibility criteria against the most recent clinical data that is available on the potential participant.

Screening/Verification of Eligibility Criteria: If the participant is deemed potentially eligible by concurrence of a study team member and a physician investigator, the eligibility criteria source documentation form will be initiated without personal health information (PHI). The next step is ascertaining if the potential participant agrees to discuss the NEPTUNE study further with a study team member. Once a potential participant agrees to contact/interaction by a NEPTUNE study team member initial eligibility can be confirmed.

At this visit, the first procedure is to confirm the eligibility criteria with the potential participant and to ascertain willingness to continue with the study process. A physician investigator and/or an experienced study coordinator will then provide all necessary information and a full explanation of the study to the potential participant in accordance with all the requirements of the respective Institutional Review Board and all applicable local, state and federal human participant research regulations. An open, non-coercive dialogue will be conducted with the participant. Patients are considered enrolled and thereby become study participants after a voluntary execution of the IRB-approved written comprehensive consent document. Upon granting of consent, a participant is formally enrolled in the NEPTUNE study and a study number will be assigned. Assent will also be requested from minor participants according to local IRB approved processes and procedures.

4.A.2. Monitoring of Recruitment

The DACC will institute a verifiable process to monitor screening and recruitment at the clinical centers. Timely detection of recruitment deficiency will be a paramount objective of the DACC. Each clinical center will be required to maintain a screening and recruitment log on-site at the clinical center. The recruitment log will also to be used to document the magnitude and reasons for non-participation by eligible patients with a clinical diagnosis of FSGS, MCD, and MN.

Recruitment reports for all the clinical centers will be developed by the DACC for regular review of the Steering Committee and will be included in the progress report to the NIH/NIDDK Project Officer. Monthly recruitment conference calls will be conducted with all the clinical centers in the consortium during which center-specific recruitment obstacles will be discussed together with potential and testable solutions. Recruitment strategies that are effective at one clinical center will be adapted at the other clinical centers whenever such concepts are transplantable.

Data from recruitment logs will be used to construct center-specific graphs of observed and expected recruitment profiles. The recruitment graph is a useful diagnostic tool and an important feedback mechanism that the Steering Committee and DACC will use to develop steps to remedy recruitment deficiency. It is anticipated that all clinical centers will have secured IRB approval by May 1, 2010 and that the training of study personnel at a central location will occur in May 2010.

4.A.2.a. Logistical Considerations

- The complexity of the clinical syndrome that constitutes FSGS, MCD and MN is such that diagnosis and treatment occur within nephrology programs at tertiary academic medical centers. Thus the majority of potential study participants will be efficiently engaged with subspecialty nephrology clinical practices rather than through primary care or other specialty clinics and it is for this reason that the recruitment campaign for this study will be focused on nephrology practices. For the same reasons, we would not use at-large measures such as mass mailing, radio/television announcements, newspaper advertising and screening with serum creatinine measurements at health fairs for recruitment because these are likely to be of low yield.
- Children with incident NS will indeed be identified in the local primary care or emergency department communities. These children are typically referred to pediatric nephrologists for specialty care including initial evaluation, initial treatment and education. This initial identification and referral period will be the focus of eligible pediatric patient identification and screening efforts.
- In order to capture renal biopsy tissue for the cohorts, it is necessary to identify potential study participants prior to the time of a clinically indicated renal biopsy. We propose to use a Forward Operating Study Subject Awareness (FOSSA) System. The FOSSA System entails that study personnel are trained to initiate screening at several points in the healthcare system where the opportunity exists to identify potential study participants. Study personnel screen medical records, appointment schedules and laboratory databases as consistent with local IRB protocols to identify patients who meet any of the following criteria: (1) new onset of proteinuria; (2) new referral with a probable diagnosis of glomerulonephritis and (3) scheduled for kidney biopsy. As part of FOSSA, all major nephrology practices that serve as referral bases for the participating clinical centers will be encouraged to implement the three screening steps of FOSSA with their new patients.
- Each participating clinical center will be required to designate a physician investigator who will be responsible for the recruitment and retention at the clinical centers. The designated investigator and study coordinator responsible for implementing the recruitment protocol will be expected to serve on the study wide recruitment and retention committee.
- The time lost from work, travel and incidental expenses associated with the recruitment and follow-up visits will be reimbursed up to $100 per participant. Time and effort needed for 24-hour urine collection will be reimbursed at $50.00 for each satisfactory 24-hour urine collection.
- Several participating clinical centers see a large number of their potential study participants as referrals from significant distances. To accommodate this common scenario and where it is not possible to plan ahead with these participants, they

would be offered an extra day of stay to conduct the study visit and the study would financially bear the necessary per diem expenses.

The NEPTUNE investigators are fully cognizant of the fact that the greatest challenge to the proposed study is the timely attainment of the recruitment goals. Thus, provisions have been made in the budget for a resource intensive recruitment plan and activities. We anticipate significant logistical impediments to achieving the recruitment goals and would deal with these problems expeditiously before the integrity of the study is imminently jeopardized. Unanticipated recruitment problems that are not immediately rectifiable would be met with rapid escalation of alternative strategies.

4.A.2.b. Alternative Recruitment Strategies

- Additional Clinical Centers: Three centers in the U.S. (Houston, Winston-Salem and Chicago) have been added to the study network. Academic nephrology programs in these two cities account for hundreds of new FSGS, MCD and MN diagnoses yearly. If proposed recruitment centers falter during the first year, additional clinical centers from large metropolitan areas would be sought to participate in the study. Working relationships currently exist between the proposing teams and the nephrology teams at several large national academic nephrology service providers. These clinical centers are not included in the current application due to limited resources. With unlimited resources, it would have been preferable to blanket recruitment operations across all available centers; however, no study has unlimited resources and operational efficiency and fiscal prudence dictate a plan with the minimum necessary overhead costs should be implemented first. If it becomes necessary to initiate recruitment at these additional clinical centers, resources would be shifted from the non-performing clinical centers originally stated. Clinical centers in Western Europe, Australia and Brazil, as sources of caucasian and black study participants, are also in place as alternative clinical centers that may be called upon to buttress shortfall in the original plan.

4.A.3. Recruitment of Blacks and Pediatric FSGS Patients

Two special populations bear a disproportionate burden with respect to FSGS as reflected in higher prevalence of disease: African Americans (AA) and the pediatric age group. Ideally, a sampling frame in which these two populations are overrepresented in the study cohort will enhance the ability of the proposing teams to test race-specific and pediatric age group-specific research hypotheses. We did not specify oversampling of the AA population because of the success in recruitment of AAs into the initial NEPTUNE cohorts and the large sample size necessary and the limited resources available to expand this to a distribution beyond that representing the racial distribution of individuals with the diseases of interest. A modest size pediatric cohort has been added to NEPTUNE as the cNEPTUNE cohort B which will only include children with incident disease. The study team envisions that future ancillary studies with supplementary recruitment of additional AA and pediatric participants would be pursued if the current proposal is successful in meeting its recruitment target. Notwithstanding this deferment, we would implement several steps to ensure that a significant fraction of the NEPTUNE participants in the current study are of AA race and pediatric age group, respectively. The steps to ensure this objective are: (1) inclusion of several pediatric nephrology programs in the participating clinical centers; (2) inclusion of clinical centers located in cities with two of the three largest AA populations in the US (Detroit and New York). The study team monitors the proportion of AA and pediatric participants enrolled as part of the recruitment monitoring plan and will be prepared to institute additional measures if either of these two important populations are underrepresented in the recruited cohort by study month 10 (one-third of the way through the 30-month recruitment period).

4.B. Retention Plan

In order to protect the power for statistical analyses of main research hypotheses, it would be necessary to maintain a retention rate of >85% by the end of the study. This means that the study cannot afford more than an annual average loss to follow-up rate exceeding 5%. Two periods are most vulnerable to patient dropout. Initially, patients

who have not fully anticipated the effort needed to comply with the study protocol may drop out in their first year of enrollment as the study requirements overwhelm them. Therefore, we implement a "recruit-to-replace" strategy during the entire 30-month recruitment phase to ensure that early attrition does not jeopardize study power. A slightly higher rate of dropout is to be expected in the last two years of the study when many patients become "fatigued" from the protocol requirements, thus an initial recruit-to-replace will also ensure that the study has enough spend down to spare during the last two years of the study. Specific measures that will be taken to enhance retention include: (a) regular contact with referring physician practices; (b) use of trained, certified study personnel to perform study visits and; (c) meeting of investigator with each study participant at least once annually during one of the follow-up visits. Established practices such as mailings and personal telephone calls to remind patients about forthcoming appointments will also be incorporated. The study team will promptly provide results of study tests to referring physicians, pending additional funding. Participants will be encouraged to include family members in their follow-up visits since this may enhance the participant support without extra costs. Follow-up visit appointments will be scheduled with large appointment windows to allow for more flexibility and participants' convenience.

The prevailing economic downturn coupled with the fact that many participants will be referred to the clinical centers from long distances, leads to an expectation that a significant proportion of the study participants will have fiscal limitations in meeting out-of-pocket travel expenses and in fact their prescribed medications. Studies in more economically advantaged populations also suggest that travel cost is an impediment to participation in clinical research when travel distance to/from the clinic is >100 miles. The NEPTUNE Steering Committee may vote to implement several measures to assist study participants:

- Pharmaceutical companies will be solicited through their patient assistance program for bulk supply of medications commonly prescribed to patients with kidney disease including antihypertensive, anti-lipid and antidiabetic drugs. These medications will be distributed by the DACC to the participating clinical centers for the use of study participants who need such assistance. We found this measure to be helpful in maintaining retention for other clinical studies.
- Participant reimbursement initially set at $100 will be supplemented with an additional mileage reimbursement for participants traveling more than 100 miles to the clinical center for study visits.
- Per diem expenses for overnight accommodation will be provided to study participants whose travel calls for this, particularly during seasons of inclement weather.
- Participating clinical centers will be encouraged to offer evening and weekend appointments to make it possible for participants with work and other obligations that limit their flexibility to attend study visits during regular business hours (8:00 AM – 5:00 PM Monday-Friday).
- Mailing of appointment reminders, formal study participation anniversary greetings, newsletters and intermittent courtesy calls, courtesy visits to the participants during hospitalization and recognition of major life events are additional measures that will be undertaken to embrace study participants and hopefully retain their participation.
- Participants for whom relocation brings about a change of providers and an excessive travel burden for completion of study visits may continue in the study by completing phone call visits, on a case-by-case basis with permission from the DACC. In such instances, biological specimens and processing will be coordinated with a local laboratory and shipped directly to the NEPTUNE Biorepository.
- Discomfort that can result from study procedures will be rigorously minimized. For example, many of the participants may be on immunosuppressants with myelosuppressive adverse effect as part of the treatment for FSGS leading to high risk of anemia. Therefore, phlebotomy for study purposes will not withdraw more than 250 cc annually. Adult participants with hematocrit <28% will follow the pediatric volumes at that particular study visit (see Manual of Procedures for pediatric blood draw reductions). Participants with hematocrit <25% will have study phlebotomy waived for that particular study visit.

Retention of participants is central to the internal validity of the study and will be an extraordinarily high priority of the investigators and staff. A key element is a pleasant, attentive and responsive staff that provides a reasonably flexible visit schedule. Other clinical center features that promote high retention rates include local tracking systems; frequent staff meetings; free and convenient parking; personal contacts through birthday cards, holiday cards, sympathy cards and flowers; small gifts at visits; and modest monetary incentives.

There exist limited research data on participant retention in non-therapeutic clinical studies similar to the proposed cohort studies. Lack of therapeutic interventions may heighten the perception of no benefit in participation on the part of the study participant. The study team is acutely sensitive to this issue and would continue to evaluate new retention methods that can be tested and implemented in the cohort studies. The aggressive retention measures that are proposed herein will, in practice and in principle, always give way to the welfare of the study participants who are, first and foremost, patients. Respect, care and empathy for the circumstances of the participants will be the overriding consideration in all aspects of the recruitment and retention plan laid out by the proposing team.

4.C. Participant Withdrawal

It is anticipated that over the course of time, participants may withdraw from the study. This may occur officially by formal notification from the participant to the investigator, or unofficially when a participant cannot be reached via the usual methods of contact. Every effort will be made to acquire complete data on all participants.

As a longitudinal, observational study, missed study visits and unanticipated loss of contact are not deemed dangerous to the study participants.

Participants who relocate to an area from which it is no longer feasible to travel to the clinical center will be asked to permit study personnel to contact them annually for a telephone contact follow-up. Centers may offer inducements to participants who drop-out or relocate in the form of additional travel reimbursement in return for their continued participation. A participant relocating to an area close to a different NEPTUNE clinical center may transfer to that center.

4.D. Clinical Management of Participants

Investigators recognize the obligation and importance of reporting research information to the health care providers of participants. It is essential that investigators report clinically actionable information to the appropriate provider in a timely fashion. A procedure for reporting such information from participating reading centers to NEPTUNE investigators will be implemented and described in the Manual of Procedures.

4.E. Transmission of Study Findings and Response Time

An IRB approved summary of patient status including urine protein, kidney function and blood pressure control status is provided to the patient as a study "SnapShot."

4.F. Clinical Alerts

Participants and their physicians will be notified as soon as possible if potentially serious medical problems are identified during any of the study visits although this is unlikely because study visits will typically coincide with routine clinic visits for most participants.

4.G. Referral for Clinical Care of Participants Lacking a Nephrologist

Every effort will be made to identify a primary treating nephrologist physician for each participant. In cases in which participants are unable to identify a primary nephrologist, the participant will be offered assistance in finding one. Each clinical center will establish a referral plan to accommodate participants with and without insurance. Clinical centers will develop a list of subsidized health centers for participants who are under- or uninsured and when necessary, clinical centers will direct the participant to social service agencies.

4.H. Standards of Care

Guidelines outlining current medical standards of clinical care are provided on the NEPTUNE study website as a resource for practicing clinicians. Participants will receive an explanation of these guidelines in conjunction with their test results. Participants will be encouraged to discuss results with their primary care physicians. Clinical center personnel and investigators will be accessible to answer additional questions.

4.I. Ethical Issues

4.I.1. Potential Risks to Participants

A minimal physical risk to participants arises from the diagnostic procedures for the collection of blood specimens, which require venipuncture of approximately 100 cc blood during the baseline visit and will require venipuncture of approximately 65 cc blood bi-annually in subsequent visits (see Table 2, page 26

for pediatric blood draw volumes). With venipuncture there is minimal risk of hematoma or infection. There is also a risk of dizziness/lightheadedness and rarely of fainting from blood draw.

A minimal physical risk of potential bleeding or injury to the kidney is added with the collection of an additional core of kidney biopsy tissue at the time of the diagnostic biopsy. This risk is considered small as the participant will be undergoing kidney biopsy for clinical indications. If the investigator/local physician collecting the kidney biopsy determines that the collection of an additional core of kidney tissue unduly increases the risk to the patient, this sample will not be collected. In these situations, residual kidney tissue from the clinical biopsy will be requested if available.

Risk/Benefit Assessment: While there is no immediate direct benefit to the individual participants of this study, there is considerable potential benefit to future patients and to society as a whole if molecular predictors of outcomes are identified. Identification of such predictors would potentially lead to interventions that may improve long-term outcomes for these diseases.

4.I.2. Formal Review of Current Literature Regarding Additional Biopsy Pass

A medline search for "*kidney, biopsy, complication"* returned approximately 900 references from which 11 studies addressed the relationship between the number of needle passes and biopsy complications (10 on native and/or transplant patients, 1 on transplant patients only); two of these studies concerned children. The studies were based on more than 2000 percutaneous needle biopsies of native kidneys. Biopsies were done by both radiologists and nephrologists, and used both TruCut-type and spring-loaded biopsy guns.

Formal statistical testing showed no relationship between the number of passes (or cores) and bleeding/complication risk in eight of these studies (99-107). Another study (108) stated that there was "no significant" difference in the number of passes/cores between biopsies complicated by and not complicated by bleeding, but no numbers were provided. One smaller study (99) reported a "slightly higher" number of passes (3.6 vs 2.8) between biopsies with complications or not; again no statistics were reported. One study (109) reported a borderline significant difference between no/mild complications and moderate complications with respect to the number of punctures (4.5±1.6 vs 5.5±1.7, P=0.052). Multivariate logistic regression; however, showed the risk of moderate complications to be significant only if the number of needle punctures exceeded five.

It seems self-evident that an increased number of needle passes would be associated with an increased risk of bleeding. However, a review of 11 published studies between 1992 and 2005 (99-109) which reported data on the relationship between needle biopsy complications and the number of needle passes, showed no significant relationship in nine of the studies. In the only study to show a (borderline) statistically significant greater number of needle passes in biopsies with moderate complications, when controlling for other variables, the complication risk was only greater when the number of passes exceeded five. Thus, the existing evidence does not support the proposition that three biopsy punctures/cores is associated with a higher risk of bleeding than two punctures/cores.

The above studies did suggest an increase in risk with renal dysfunction, severe hypertension and possibly some coagulation abnormalities. The other principle risk of the biopsy procedure is relatedto sedation. However, the number of biopsy needle passes is not related to kidney biopsy risk based on the 11 published studies..

4.I.3. Confidentiality

Protection of participants depends on the joint activities of all Clinical Centers and the Administrative Unit. Extensive efforts will be made to ensure that participants' confidentiality is maintained. Each participant is assigned a unique study identification number and is never tracked through the study by name, medical record number, or other personal identifier. A log of the participant names, participant ID numbers, and pertinent registration information (e.g. home address, telephone number, and emergency contact information) is maintained in a locked area at each clinical site. The staff at the DACC do not have access to this log. Only the participant ID number and date of birth are provided to the DACC staff. Any communication between the DACC and clinical sites regarding participant data occurs via the participant

ID number. Any forms or documents sent to the DACC, IRB or Regulatory Authorities will have all personal identifiers removed.

Authorized representatives of the Sponsor, the ORD and the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), participating clinical institution, as well as the IRB, have access to and may copy both medical records and records from participation in this study consistent with the policy of the NIH Certificate of Confidentiality. Such access is necessary to insure the accuracy of the findings and the safety and welfare of participants. If any publication or presentations result from this research, participants will not be identified by name or other personal identifier. All research reports, articles, and presentations will report only aggregate findings.

4.I.4. Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify the participant in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless the participant has consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if the participant consents to the disclosure, including for their medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research participants.

The Certificate cannot be used to refuse a request for information from personnel of the United States federal or state government agency sponsoring the project that is needed for auditing or program evaluation by the U.S. Department of Health and Human Services and/or the National Institutes of Health, which is funding this project [if FDA regulated, also include the following] or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). The Certificate of Confidentiality does not prevent a participant from voluntarily releasing information about themselves or their involvement in this research. If a participant wants research information released to an insurer, medical care provider, or any other person not connected with the research, the participant must provide consent to allow the researchers to release it.

4.J. Even with the Certificate of Confidentiality, the investigators continue to have ethical obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person. The Certificate of Confidentiality will also not be used to prevent disclosure as required by federal, state, or local law, such as reports of child abuse and neglect, or harm to self or others. Informed Consent

The consent process may differ somewhat by Clinical Center according to local Institutional Review Board (IRB) guidelines. Participants will continue to be asked to complete all study procedures. However, each study participant is able, during any study visit, to decline one or more data collection procedures without withdrawing from the study. This form covers all aspects of screening, baseline testing and subsequent follow-up visits.

Each Clinical Center will prepare an informed consent form and assent from where children will be enrolled following the guidelines of their local IRB and applicable regulations for Informed Consent (see Appendix 8.E for a sample Informed Consent document generated with the University of Michigan template as a reference for Clinical Centers). The initial Informed Consent and Assent form will be **signed** and **dated by the participant and or legal**

guardian before initiation of any study related activity and at a minimum, it will contain a description of the potential risks, benefits, expense to the participant, and alternative treatment.

Before signing the Informed Consent, the Research Coordinator will review the details of the consent form orally with the potential participant, and answer any questions the participant has concerning participation in the study. The original signed consent form will be stored in the participant study file at the Clinical Center, and a copy of the signed consent form will be given to the participant. Specifically, the following must be accomplished during the informed consent process:

- The participant must be informed that participation in the study is **voluntary** and that refusal to participate will involve no penalty or loss of benefits or negative impact on their medical care.
- The participant must be informed of the **purpose** of the study and that it involves **research**.
- The participant must be informed of any **alternative procedures,** if applicable.
- The participant must be informed of any reasonably foreseeable **risks**.
- The participant must be informed of any **benefits** from the research.
- An outline of safeguards to protect participant **confidentiality** must be included, as well as an indication of the participant's right to withdraw without penalty. This should be balanced with a discussion of the effect withdrawals have on the study, and the responsibility a participant has, within limits, to continue in the study if he or she decides to enroll.
- The participant must be informed for **whom to contact** for information about research participants' rights, information about the research study, and in the event of research-related injury.
- The participant must be informed as to whether or not **compensation** is offered for participation in the study and/or in the event of a medical injury.
- The participant must be informed that he/she will be notified of any significant **changes** in the protocol that might affect their willingness to continue in the study.

A separate section and/or signature page will be required for consent to collect a blood sample for genetic testing and storage of DNA. Participants may enroll in either of the two cohort studies but refuse to sign the genetic consent form without consequence to study eligibility.

The Health Insurance Portability and Accountability Act (HIPAA) became effective on April 14, 2003 and may affect the two cohort studies with regard to the use or disclosure of protected health information. Each institution may hold different requirements regarding HIPAA. Some institutions may request that this HIPAA language be inserted into the appropriate sections of the informed consent form; other institutions may require a stand alone document.

Participants will be asked to sign an additional form as required authorizing study personnel to request and review their medical records. This will permit access to medical record information that will enable investigators to inspect medical information and documents associated with reported clinical events. Appropriate revisions of the informed consent will be submitted to the local IRBs by each of the Clinical Centers for re-consenting processes of all participants at their first annual follow-up visit after implementation of this protocol revision.

It is the responsibility of the Principal and Co-Investigator(s) at each clinical center to provide their IRB with all pertinent materials and consent documents. Approval of the protocol, the informed consent form, and data collection forms/questionnaires must be obtained and forwarded to the University of Michigan, DACC before screening or enrolling participants. The Investigator also maintains the responsibility of initiating protocol re-approval, notification of protocol and/or consent form changes and termination of the study according to IRB requirements. The Administrative Unit monitors submission and annual renewal of these documents.

5. STUDY ORGANIZATION

5.A. Overview

The organizational components, decision making and communication structures of NEPTUNE have developed as described below.

- Project Administrative Unit
- Data Analysis and Clinical Studies Coordinating Center (DACC)
- 22 Clinical Centers (CC)
- NIDDK Project Scientists
- Biorepositories
- Central Biochemical Laboratory
- Central Digitalized Histopathological Archive
- Steering Committee
- Scientific Advisory Board
- Study Subcommittees

5.A.1. Project Administrative Unit

The Project Administrative Unit is located at the University of Michigan Health System. The Administrative Unit is directed by the Principal Investigator and Project Director, Dr. Matthias Kretzler. The Administrative Project Manager is the lead staff in charge of the Administrative Unit. The Associate Project Directors and Co-PIs (Drs. Gadegbeku and Gipson), Clinical Project Manager, and Administrative Project Manager will function within the Administrative Unit. The University of Michigan NEPTUNE Investigators, Research Assistants and Administrative Assistants will also function within the Administrative Unit. The Administrative Unit contact details are:

NEPTUNE Study Office
A520C Medical Science Research Building I
1150 W. Medical Center Drive
Ann Arbor, MI 48109
Phone: 734-615-5021 *or* 1-877-9-NEPTUNE
Fax: 734-615-6005

The responsibilities of the project administrative unit include:

1) Assure adherence to the goals and principles of the network
2) Prepare budgets and disbursement of funds
3) Prepare and process subcontract awards
4) Maintain records of all study finances, including pre- and post-award expenditures
5) Prepare progress, budget and communication reports
6) Prepare and submit all regulatory financial reports
7) Organize network meetings and conference calls
8) Support of interaction with other research organizations
9) Support and promote collaboration with other networks and consortia both nationally and internationally
10) Manage ongoing collaboration with the NephCure and Halpin Foundations
11) Provide all deliverables to the RDCRN Data Management and Coordinating Center (DMCC)

12) Perform other functions deemed necessary and assigned by the Project Director or his designees

5.A.2. Data Analysis and Clinical Studies Coordinating Center (DACC)

The DACC is located at the University of Michigan. The DACC will be directed by Dr. Kretzler (Project Director and PI). The study biostatistician (Dr. Peter Song) will function with the DACC. Dr. Kretzler, the Co-PIs (Drs. Gadegbeku, Gipson, and Holzman), Project Manager and Administrative Project Manager will function within the DACC. University of Michigan NEPTUNE Investigators, Research Assistants and Administrative Assistants will also function within the DACC. The data management infrastructure will be provided by Arbor Research Collaborative for Health as a subcontracting component of the University of Michigan based DACC.

The Data Coordinating Analysis Center (DACC) contributes content area expertise and shares in scientific leadership of the cohort studies and NEPTUNE through the Steering Committee as it identifies and prioritizes specific areas of investigation and subsequently refines and modifies NEPTUNE's scientific direction and emerging scientific knowledge. The DACC will assist in protocol development and preparation of scientific publications. The DACC will have the major responsibility of creating a database and data collection systems for the Clinical Centers, ongoing evaluation of data quality and performance monitoring of the Clinical Centers, and statistical analyses of the data.

The specific responsibilities of the DACC include:

1) Coordinate and support data linkage with the RDCRN Data Management and Coordinating Center (DMCC)
2) Develop and maintain network data collection from the Clinical Centers
3) Maintain functionality of NEPTUNELink (web-based clinical research information system)
4) Provide end-user support of NEPTUNELink to the clinical centers
5) Shepherd participant recruitment and retention at participating clinical centers
6) Monitor compliance with HIPAA regulations and maintain regulatory binders at all clinical centers
7) Provide data collection quality control
8) Coordinate analysis and reporting of data from main cohort and pilot studies,
9) Maintain and update study protocol and Manual of Procedures
10) Train and certify study personnel in NEPTUNE protocols
11) Manage protocol compliance and study operations at Clinical Centers
12) Perform other functions deemed necessary and assigned by the Project Director or his designees

5.A.3. Clinical Centers

Twelve founding clinical centers and 3 additional clinical centers (University of Pennsylvania, University of Washington at Seattle, and the Renal Disease Section of the NIDDK) comprise the consortium of clinical centers in NEPTUNE. In Fall 2011, three further sites were added to augment the study recruitment goals (Columbia University, Emory University, and Temple University). Winter 2012 enlisted site recruitment at the University of Illinois at Chicago, Stroger Hospital, and the University of Texas at Southwestern. In 2014, the network clinical centers are expanding to include Wake Forrest University Medical Center in North Carolina. The network was constituted based on the expertise of investigators and their commitment to the proposed study objectives as well as the size of target population, racial, gender, and age composition of their referral population base, their experience conducting clinical studies, and the quality of their existing clinical study infrastructure. Of note, the University of Toronto, UNC, Mayo Clinic, North Shore Long Island Jewish and Montefiore Hospital are hubs of pre-existent, well-functioning regional referral networks established for clinical research in glomerular disease.

The participating clinical centers and respective PI's are:

Clinical Center	Clinical Center PI	Clinical Center Co-Investigators
Children's Hospital of Los Angeles Los Angeles, CA	Lemley, Kevin	
Children's Mercy Hospital Kansas City, KS	Srivastava, Tarak	
Columbia University New York City, NY	Appelbaum, Gerald	Bomback, Andrew
Duke University Raleigh, NC	Wolf, Myles	Gbadegesin, Rasheed
Emory University Atlanta, GA	Greenbaum, Laurence	
Johns Hopkins Medical Institute Baltimore, MD	Atkinson, Meredith	
Los Angeles Biomedical Research Institute at Harbor UCLA Medical Center Torrance, CA	Adler, Sharon	
Kidney Disease Section, NIDDK, NIH Bethesda, MD	Kopp, Jeffrey	
MetroHealth Hospital at Case Western Medical Center/Cleveland Clinic Cleveland, OH	Sedor, John	Dell, Katherine
Montefiore Medical Center Bronx, NY	Kaskel, Frederick	
New York University School of Medicine New York, NY	Trachtman, Howard	Zhdanova, Olga
North Shore Long Island Jewish Health System Manhasset, NY	Sethna, Christina	
The Mayo Clinic Rochester, MN	Fervenza, Fernando	Lieske, John; Hogan, Marie
Temple University Philadelphia, PA	Gadegbeku, Crystal	Lee, Iris
University Health Network at Toronto General Hospital Toronto, Ontario, Canada	Cattran, Daniel	Reich, Heather, Hladunewich, Michelle
University of Illinois at Chicago, Stroger Hospital Chicago, IL	Perumal, Kalyani	
University of Miami Medical Center Miami, FL	Fornoni, Alessia	Zilleruelo, Gaston
University of Michigan Medical Center Ann Arbor, Michigan *(lead site)*	Kretzler, Matthias	Gipson, Debbie
University of Minnesota Minneapolis, MN	Nachman, Patrick	
University of North Carolina at Chapel Hill Chapel Hill, NC	Derebail, Vimal	Gibson, Keisha; Hogan, Susan
University of Pennsylvania	Holzman, Lawrence	Meyers, Kevin

Philadelphia, PA		
University of Southern California, Children's Hospital Los Angeles, CA	Lemley, Kevin	
University of Texas at Southwestern Dallas, TX	Kamalanathan Sambandam	Brown, Elizabeth
University of Washington Seattle Children's Hospital Providence Sacred Heart Seattle, WA	Nelson, Peter	Hingorani, Sangeeta, Tuttle, Katherine
Wake Forest Medical Center Winston-Salem, NC	Freedman, Barry	Lin, Jin Jar

The responsibilities of the clinical centers include:

1) Conduct the study according to the study protocol and applicable regulatory guidelines.
2) Conduct of particular aspects of the study may be delegated to qualified personnel
3) The clinical center PI is to oversee the overall study management at the site
4) Ensure that all the clinical center study staff are appropriately trained in study procedures
5) Screen, recruit, enroll and retain a designated number of study participants
6) Assess the accrual of study participants
7) Ensure participant confidentiality
8) Maintain appropriate study documentation
9) Enter and transfer data in a timely manner through NEPTUNELink
10) Procure, package and ship required specimens to the DACC and the study Biorepositories
11) Participate in NEPTUNE meetings and conference calls
12) Participate in ancillary studies
13) Provide budgetary estimates, financial reports and subcontract and subaward materials to the NEPTUNE Administrative Unit in a timely fashion
14) Develop and propose ancillary studies to the Steering Committee according to the present and emerging interest of the clinical center investigators

5.A.4. Specimen Biorepository

Tissue from (diagnostic) kidney biopsy of study participants as well as urine and blood components will be procured according to the study protocol and transferred to the NEPTUNE Biorepository for processing, storage and analysis. The NEPTUNE Biorepository is located at:

University of Michigan
A518 Medical Science Research Building I, SPC
1150 W. Medical Center Drive
Ann Arbor, MI 48109
(734) 615-5021 or 1-877-9-NEPTUNE

The Biorepository will be directed by the Project Director and PI (Dr. Kretzler), day-to-day operations will be attended to by a Biobank Manager.

The responsibilities of the Biorepository are:

1) Provide sample procurement kits to participating sites

2) Develop, train and implement sites in procurement procedures
3) Facilitate shipping to central biorepository
4) Facilitate shipping of samples to external biorepositories (Mayo and NIH)
5) Process, quality control and store biosamples
6) Distribute aliquotted biosamples to approved ancillary or pilot studies

In collaboration with the NIDDK, a small blood sample from participants will be shipped to the NIH Biorepository to include a DNA aliquot. The NIDDK is also requesting urine aliquots from all participants. These samples will be shipped to the NIDDK according to the manual of procedures.Biospecimens at the NIDDK Biorepository and samples will be maintained at:

NIDDK Biosample Repository
20301 Century Blvd., Building 6, Suite 400
Germantown, MD 20874
240-686-4703 or fax: 301-793-0353

The above-said samples will be provided to the NIDDK from the study approved blood and urine procurements and provide no additional health risk to study participants. This information will also be provided to potential participants during the consent process and they will have the opportunity to opt-out without study penalty.

5.A.5. Central Biochemical Laboratory

The central biochemical laboratory will be located at the University of Michigan Medical Center at the following address for blood specimens and urine samples not planned for analysis at the NEPTUNE Urine Biobank located at Mayo Clinic (see following):

University of Michigan Health System
Department of Pathology
2F367 University Hospital
1500 E. Medical Center Drive
Ann Arbor, MI 48109-5054

The Mayo Clinical Laboratories will perform quantification of proteinuria as part of the cohort studies for urine specimens and is located at:

Mayo Clinic
Department/Division of Nephrology
Stabile Building 7-03
Rochester, MN

Additional laboratory parameters of pilot and ancillary studies will be determined by the ancillary review committee and ancillary PI after approval of the studies by NEPTUNE.

5.A.6. NIDDK Histopathological Digital Archive

The Histopathological archive laboratory will be located at the intramural research program at the NIDDK in Bethesda (Drs. J. Kopp and S. Hewitt). It will be responsible for generation of digital histopathological images of the renal biopsy pathological (slide and images) specimens obtained from the cohort participants. It will provide an interface to the reference histopathologists and the DACC for analysis, annotation and integration of the histological information. This NEPTUNE resource will exist on an NIH server in Bethesda, MD and the primary contact as follows:

Jeffrey Kopp, MD
CAPT, USPHS

Kidney Disease Section, NIDDK, NIH
10/3N116, NIH
Bethesda, MD 20892-1268
301-594-3403

5.A.7. Steering Committee

The primary governing body of the study is the Steering Committee, which is comprised of each of the Principal Investigators at the Clinical Centers, the Principal Investigators at the DACC, the NIDDK Project Scientist, and a representative study coordinator. The Steering Committee will develop policies for the study pertaining to accessing patient data and specimens, ancillary studies, performance standards, publications and presentations. They will refine the study protocol, meet to discuss the progress of the study and resolve problems that arise. The Steering Committee may establish subcommittees on such topics as recruitment, measurement of renal function, risk factor assessment for renal disease, genetic studies, quality control, publications and ancillary studies. Small working groups may be established to prepare manuscripts, white papers, presentations, and other functions as needs determine.

5.A.8. Scientific Advisory Board

An independent group of experts in areas such as nephrology, clinical trials, rare disease studies and biomarker identification, who are not otherwise involved in the study, has been recruited to evaluate the proposed protocol and periodically review the progress of the study.

5.A.9. Study Subcommittees

The following subcommittees will be established to address specific study issues:

- Pilot and Ancillary Studies Committee
- Pathology Committee
- Genetics and Genomics Committee
- Publication Committee
- Training and Career Development Committee
- Quality Control Committee
- FSGS/MCD Cohort Study Committee
- MN Cohort Study Committee
- Outreach / Recruitment and Retention Committee
- Experimental Therapeutics and Clinical Trials Committees

5.B. NEPTUNE Study Policies

5.B.1. Ancillary Study Policy

To enhance the value of NEPTUNE, the Steering Committee welcomes proposals from individual investigators to carry out ancillary studies. Nevertheless, to protect the integrity of the NEPTUNE, such ancillary studies must be reviewed and approved by the Pilot and Ancillary Studies Committee and the Steering Committee before their inception or submission of a proposal for external funding consideration. The group has reviewed and approved a comprehensive study policy that clearly defines requirements and describes a process for review and approval of individual proposals.

An ancillary study may be developed based on information from cohort studies participants in an investigation or analysis which is relevant to NEPTUNE, but yet not performed using the current study protocol. Ancillary studies derive support from non-NEPTUNE study funds. A typical ancillary study will propose the collection of additional data not collected or analyzed as part of the routine NEPTUNE data set. Ancillary studies may be submitted by investigators within the NEPTUNE or investigators without a prior relationship to the NEPTUNE. Ancillary studies require external funding. Examples include studies

funded by investigator-initiated NIH research awards (R series awards, K series awards, and other career development awards) or grants from academic institutions or private sources (e.g. private foundations, pharmaceutical companies, etc.). Any ancillary study must have sufficient funding to cover the costs incurred by the NEPTUNE Clinical Centers and Laboratories (e.g. to process or ship samples), and by the DACC.

The NEPTUNE Ancillary Studies Policy will include a more detailed description.

5.B.2. Publication and Presentations

It will be the policy of the NEPTUNE study that preparation of all publications or presentations, other than materials prepared for local publicity purposes, must be assigned by the Steering Committee to specifically appointed writing committees, and that all such materials must be reviewed and approved by the Committee before publication. A detailed description of writing activities in NEPTUNE will be provided in a separate NEPTUNE Publication Policy.

5.B.3. Access to Study Data and Specimens

The Steering Committee will authorize access to study data and biospecimens. Investigators must submit a proposal requesting approval to access NEPTUNE data/specimens. A formal process is provided in the NEPTUNE Ancillary Study Policy. NEPTUNE data will be analyzed by members of the NEPTUNE Research Community, including investigators from institutions and industry outside of the Clinical Centers and DACC who have approved ancillary studies or approved analysis plans. In addition, the NEPTUNE Study will collaborate with the ORD and NIDDK to participate in their Central Repository for study specimens and data.

6. STUDY MANAGEMENT

6.A. Clinical Centers and Coordinating Center Responsibilities

It is the responsibility of each Clinical Center to conduct the cohort studies according to the study protocol and applicable regulatory guidelines. Conduct of particular aspects of the study may be delegated to qualified personnel; however, each Clinical Center Principal Investigator is responsible for overall study management. The Clinical Center study staff must be trained in all study procedures.

Each Clinical Center is responsible for screening, recruiting, enrolling and retaining a designated number of study participants. It is the responsibility of the Clinical Center study staff to assess their accrual, ensure participant confidentiality, maintain appropriate study documentation, enter and transfer data in a timely manner, and participate in the NEPTUNE study meetings and conference calls.

The Administrative Unit and the DACC at the University of Michigan is responsible for the overall study management, document control and distribution, study communications and data management and analysis. The DACC is responsible for establishing the Arbor Research NEPTUNELink database, developing a web-based data transmission system, assessing data quality and completeness throughout the study, and providing general assistance to the Clinical Centers to maintain long-term participation of the cohort study participants. The DACC also performs analyses as suggested by the Clinical Centers, the ORD and the NIDDK and Steering Committee Centers, as well as proposing original analyses to the collaborative group for their consideration. The Administrative Unit will prepare periodic reports on the progress of the study, including data on quality control, and interim and final results to the Steering Committee, the ORD, the NIDDK and the group of external advisors. The Administrative Unit will be responsible for arranging meetings and conference calls of the Steering Committee, meetings of the external advisors, and performing other administrative functions necessary to coordinate the efficient operation of the collaborative study group. The Administrative Unit will also establish, via subcontracts, central laboratories and additional clinical centers as deemed necessary by the study protocol. The Administrative Unit will also provide administrative coordination for any central repository to be established and directly supported by the ORD and or NIDDK, to store genetic material and other biological specimens obtained from cohort study participants. The Administrative Unit and DACC will work with the overall DMCC of the ORD network to ensure that data and study materials are provided in a timely fashion to the DMCC. The Administrative Unit and DACC will assist the DMCC in all its data management and administrative functions as stipulated in the RFA and as may be further directed by the ORD of the NIH.

6.A.1. Record Retention

Investigators will maintain study documents on-site and in an orderly fashion for a prescribed period of time, and will make available to the sponsor or the sponsor's representative the following documents: the signed study protocol, amendments, informed consent documents, and approval letters from the IRB, all primary source documentation, and all letters of correspondence. The DACC will maintain all study records for a period in accordance with their internal SOPs and applicable regulations.

6.B. Quality Assurance/Quality Control Activities

6.B.1. Personnel Training

The Administrative Unit and DACC will conduct a personnel training session and a certification session for staff who will perform clinical procedures before initiation of the protocol. This comprehensive training session includes all aspects of the protocol and Manual of Procedures (MOP) implementation such as staff-participant interaction, specimen handling, and data collection and entry procedures. Periodic conference calls and training sessions will be conducted to maintain standard application of procedures. All new personnel will be required to participate in a study training session.

6.B.2. Training and Certification Plans

Quality data collection and appropriate conduct of the study will require careful attention to the training of personnel at the Data Analysis and Clinical Studies Coordinating Center (DACC) and participating sites. Training and certification sessions for Clinical Center Study Coordinators and data entry personnel will be held prior to the initiation of participant recruitment. The protocol, forms and other materials will be

distributed to the appropriate personnel prior to the training session. Each clinical center's personnel will be trained centrally in the study requirements, standardized measurement of height, weight, and blood pressure, requirements for laboratory specimen collection including morning urine samples, counseling for adherence and the eliciting of information from study participants in a uniform and reproducible manner. During the training session, presentations will be made by staff members of the Project Administrative Unit, DACC, Biorepository, and the central biochemical laboratory. This training session will cover participant recruitment and participant eligibility and exclusion criteria. The study personnel will be shown how to enroll participants as uniformly as possible over time and ways to reach the recruitment goals in the allotted time period. The data to be collected and the procedures to be conducted at each visit will be reviewed in detail. Each of the data collection forms and the nature of the required information will be discussed in detail on an item by item basis. Entering data forms, responding to data discrepancy queries and general information about obtaining research quality data will also be covered during the training session.

6.B.3. Documentation: Protocol, Manual and Forms

Purpose of the Protocol: The protocol describes the study, explains which procedures will be done, why they will be done and how the results will be utilized and interpreted.

Manual of Procedures: The Manual of Procedures includes the detailed instructions for performing the procedures required by the protocol. Sections of the Manual of Procedures will be aimed toward the Project Administrative Unit, the data analysis and clinical studies coordinating center, the central biochemical laboratory, the biorepository and the Participating Center Study Coordinators.

Forms and Reports Manual: The Forms and Reports Manual includes forms to be used for study data collection with instructions for their use, and drafts of reports pertaining to enrolled participants.

6.B.4. Study Monitoring

The Administrative Unit will develop written standard operating procedures (SOPs) to ensure that all aspects of the study are conducted in a standard and uniform manner. These procedures are organized into a Manual of Procedures (MOP), which complies with the protocol, Good Clinical Practice (GCP) and applicable regulatory requirements. A data monitoring plan and schedule will be developed to assess protocol adherence. This plan will be presented to the Scientific Advisory Board for approval before implementation.

6.B.5. Other Quality Assurance Activities

The Administrative Unit will include a comprehensive Quality Assurance (QA) Plan in the Manual of Procedures that will consist of the following activities:

Personnel Training and Certification: As previously indicated, prior to cohort study enrollment a comprehensive training session will be conducted with all study personnel that will encompass all aspects of the study including communication, principles of Good Clinical Practice, study implementation and procedures, data entry and verification, test and specimen conduct and transfer.

Clinical Protocol and MOP Adherence and Auditing Activities: The DACC will request and verify specific information from clinical and reading centers to ensure the application of study procedures as they apply to participant safety, required intervals for timely conduct of procedures, appropriate documentation of data and specimens and compliance with SOPs. This information will take the form of a written report and may be acquired during clinical site monitoring visits.

Site Monitoring: Site monitoring may include a proportional assessment of information transfer from source documents (patient charts, lab reports, images) to Case Report Forms

(CRFs). A certain percentage of data will be reviewed to assess the accuracy of this process at each center. This information will take the form of a written report.

Database Auditing: A comparison of a certain percentage of data written on source documents to data entered into the electronic database provides information that describes and quantifies the accuracy of the data entry process and use of the data management system by personnel at each clinical center. This information will take the form of a written report.

Database Administration and Network Security: The DACC and Administrative Unit will establish SOPs for authorizing and documenting secure access to the study website, study documents and the electronic data management system (NEPTUNELink). These procedures ensure that only authorized personnel are able to view, access and modify study data.

Data Reporting: A set of standard reports will be developed to describe study activities that include accrual, study progress, and data quality. These reports will be developed using the NEPTUNELink system and provided to investigators, the ORD, the NIDDK and designated committees as appropriate.

Preparation and Integrity of Analysis Datasets: The DACC Database Administrator will create a set of standard data access descriptor/view files, which will be used in the generation of analysis datasets. As datasets are extracted from the main study database, they can be utilized separately from direct database processing and thereby protect the integrity of the data.

6.B.6. Website Development and Maintenance

The DACC, in conjunction with the RDCRN, will develop a study website for study-wide communication management, data and document management, and activity management and coordination. The study website will provide general information to the public, single-point restricted access to tools and information for investigators and clinical center study personnel including study resources and communication tools, as well as data entry and management tools. It will also provide an additional level of restricted access for DACC study personnel. The applied systems biology core inside the DACC has already released automated web-based data search tools (www.nephromine.org) and is currently establishing an internet portal for shared systems biology study using the molecular and clinical data generated in the study.

NEPTUNE study information will also be made available in collaboration with the RDRCN DMCC via the DMCC web site for RDCRN internal communication and community outreach.

6.B.7. Contact Registry

The Nephrotic Syndrome Study Network (NEPTUNE) Contact Registry is a method by which patients with Nephrotic Syndrome can register themselves in order to be contacted in the future about clinical research opportunities and updates on the progress of related research projects. Participation in the international Nephrotic Syndrome Patient Contact Registry helps NEPTUNE researchers inform patients and / or parents of patients about clinical research studies performed in our multi-center clinical research network. Patients who register in NEPTUNE's Contact Registry will be contacted periodically with research updates and with opportunities to participate in new or ongoing research projects.

Participant benefits of joining the contact registry include:

- Communication of open recruitment for clinical studies of nephrotic syndrome
- Notice of opening of new clinical sites doing research on rare diseases
- Information on activities from affiliated awareness and advocacy groups
- Future opportunities to participate in research

Patients and parents of patients with Nephrotic Syndrome are invited to join the NEPTUNE Contact Registry. All patients with Nephrotic Syndrome from the United States and around the world are invited to join via this public website. Any patient with a confirmed or suspected diagnosis of Nephrotic Syndrome,

including focal segmental glomerulosclerosis (FSGS), minimal change disease (MCD), membranous nephropathy (MN) or other kidney disease causing nephrotic syndrome can register at the RDCRN hosted and honest-broker managed website address: www.neptune-study.org

6.C. Data Management

The DACC will provide overall coordination, logistical support, and implementation for all aspects of the study protocol including data collection, data processing, tracking of participant recruitment, tracking of specimens, and training. The Arbor Research Collaborative for Health (Arbor Research), through its ArborLink team will provide support for clinical data management, and software systems development, deployment, and maintenance of a state-of-the-art world wide web-based data system that accommodates all scientific study data and permits tracking and coordination of all NEPTUNE activities within the framework of multidisciplinary project teams. The DACC Programmer has overall responsibility for establishing the technical strategies and monitoring the implementation activities within the DACC and at the participating clinical centers. The DACC biostatistical team in conjunction with Arbor Research, will provide all statistical programming and data management needs in the course of analyzing study data.

6.C.1. Electronic Clinical Research Data Management System (NEPTUNELink)

The web-based NEPTUNE-Link if the study instance to serve as the data entry platform. This will create a fully relational clinical research database with study management, detailed reporting, and auditing capabilities. The management, application, and data containing servers are isolated in separate segments, each protected with a dedicated internal firewall device. All data collected through the NEPTUNE-Link are initially stored in a Microsoft SQL server database, implemented with industry-standard practices to protect the security and integrity of these data. Audit trails are maintained for all NEPTUNE-Link activity and all changes to any data element in the system. Access within the network is controlled by these firewalls, with the rules implemented according to the principle of Least Access. Trained NEPTUNE study personnel with role based security access will input local study data through a web-based interface. All data with patient identifiers are stored in a SQL Server or SAS data formats. Though PHI are encrypted in NEPTUNE-Link storage, sites may use their decryption keys to create crosswalk files for linking to external data sources, such as the USRDS, National Death index or local HER, to obtain vital or ESKD status. NEPTUNE-Link is backed up to an Unitrends disk to disk backup device at least daily, with dismountable, AES-256 encrypted disk copies maintained in locked, fireproof containers and at a secure off-site location.

Local Data entry: NEPTUNELink is utilized for data submission by the study sites utilizing to the greatest degree possible a "paperless data collection method." When possible, data collected by the Clinical Center Coordinator is entered directly from the source document or participant to a data entry screen, bypassing the intermediate step of transcribing the data to a CRF thereby reducing entry error.

Central Data entry: Sites electing to have data entered into NEPTUNELink by the DACC may do so within a secure system only. A network will be established providing fax to scan documents for entry into NEPTUNELink by DACC data entry personnel.

6.C.2. Security

NEPTUNELink has been designed to prevent unauthorized access to data and to prevent data loss due to equipment failure or catastrophic events. Security procedures encompass user account management, user privilege assignment, data loss prevention (database backup), computer systems validation, and performance monitoring. User access is controlled by assignment of confidential usernames, passwords, and role assignment. The databases are hosted on secure Arbor Research computing servers and are restricted to only those individuals who are authorized to work on the study. Additional measures to prevent unauthorized external access to the database environment are employed using network firewall technologies. Arbor Research provides for daily protection against data loss through high speed, high capacity disk drives that are used to create disk to disk copies and simultaneous disk to tape copies of the data. The DACC and Arbor Research has Standard Operating Procedures established for authorizing and

documenting secure access to the study website, documents and NEPTUNELink. These procedures ensure that only authorized personnel are able to view, access and modify study data.

6.C.3. Standard Reports

In order to evaluate enrollment, the DACC will use the real-time study management capabilities of NEPTUNELink to generate monthly reports that described overall study accrual by site and demographic characteristics of the population.

On a quarterly basis, the DACC will generate reports that represent the study data collected as well as assessment of that data. The following list represents data and quality reports:

- Study accrual: overall, by site, compared to target
- Demographic characteristics of the population
- Participants screened versus enrolled
- Reasons for screening failure
- Enrollment and withdrawal reports
- Contact completion rates [Follow-up visits expected v. completed]
- Timely data entry reports

6.C.4. Data Safety Management Plan

The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual center IRB's for approval. Participant enrollment may only begin with IRB approved consent forms.

This is an observational study that meets the federal definition of minimal risk.

6.C.5. Study Oversight

The Study PI has primary oversight responsibility of this observational study. The NIH appointed Data/ Observational Safety Monitoring Board (D/OSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The D/OSMB will review accrual, patterns and frequencies of all adverse events, protocol compliance every 12 months. The D/OSMB makes recommendations to the NIH regarding the continuation status of the protocol.

Each site's Primary Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate report- detailed by severity, attribution (expected or unexpected), and relationship to the study procedures – will be available from the DMCC for site review. Adverse events will be reviewed no less than quarterly by the research team. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

6.C.5.a. Definitions and Standards

- The Rare Diseases Clinical Research Network defines an adverse event as: "…an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a Rare Diseases Clinical Research Network study."
- Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."
- Unexpected adverse events are defined as any adverse experience(s)…the specificity or severity of which is not consistent with the risks of information described in the protocol.
- Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study

- All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.
- For observational studies: Only those events associated with the conduct of the study and as defined above are reportable.

6.C.5.b. Adverse Events

Expected/Known Risks/Discomforts/Adverse Events Associated with Study Intervention and Procedures: Definition of Expected Adverse Events

Study Procedures:

- Venipuncture: Drawing blood causes discomfort when the hand or arm is stuck with the needle. A bruise may appear for a few days at the spot where the hand is stuck. There is a slight chance of infection. This is very unlikely. These risks are minimized by the use of trained personnel to draw blood. There is risk associated with light-headedness, dizziness and rarely fainting associated with blood draws.
- Kidney biopsy: When having a kidney biopsy for regular medical care, providing an additional core to the BioBank adds minimal risk to this procedure based on our review of the current literature. Participants' physicians will provide a separate consent. Participants' physicians will explain the reasons for a clinical biopsy. After all the studies needed for clinical care are finished, surplus samples will go to the NEPTUNE BioBank.

6.C.5.c. Reporting Timeline

Within 24 hours (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:

- Is considered life-threatening/disabling or results in death of subject

-OR-

- Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within 5 working days (of learning of the event).
- All other (suspected) reportable AEs must be reported to the RDCRN within 20 working days of the notification of the event or of the site becoming aware of the event.
- Local institutional reporting requirements to IRBs, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study PI.

6.C.6. RDCRN Adverse Event Data Management System (AEDAMS)

Upon entry of a serious adverse event into the NEPTUNELink system, the Study PI, site PIs, the Medical Review Officer, and NIDDK program officer will be immediately notified via email. This data will then be electronically transferred to the DMCC created Adverse Event Data Management System (AEDAMS).

Serious adverse events: The NIH appointed Medical Review Officer (MRO) will review causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The MRO may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be

presented in tabular form and given to the MRO and RDCRN DSMB on an annual basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all adverse events (serious/not serious and expected, unexpected) for site investigators and IRBs.

6.C.7. Study Discontinuation (Observational)

This study will not have study discontinuation rules as it is an observational study. The NIH and local IRB's have the authority to stop or suspend this trial at any time.

6.C.8. Subject Discontinuation

As an observational study, continued monitoring of every enrolled and eligible subject is anticipated unless withdrawal is requested for the indications listed below. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

Withdrawal of consent

Withdrawal by the participant

Withdrawal by the investigator

Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g.-mental status change).

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APPENDICES

8. APPENDICES

8.A. Visit Schedule

Procedures and Visit Schedule	Screen[a] /Eligibility	Biopsy	Base-line	4 Mos.	8 Mos.	12 Mos.	18 Mos.	24 Mos.	30 Mos.	36 Mos.	Relapse Visit
Visit Number	**[V_{SE}]***	**V_{BX}**	**[V1]**	**[V2]**	**[V3]**	**[V4]**	**[V5]**	**[V6]**	**[V7]**	**[V8-]**	
Eligibility Assessment	X										
Eligibility Confirmation			X								
Informed Consent	X		X								
Contact Information	X		X	X	X	X	X	X	X	X	
Demographics			X			X		X		X	
Baseline H&P			X								
Follow-up H&P				X	X	X	X	X	X	X	X
Medication Log		X									
Follow-up medication changes				X	X	X	X	X	X	X	X
Medication Adherence			X	X	X	X	X	X	X	X	X
PROMIS Survey			X	X	X	X	X	X	X	X	X
Biopsy Blood Specimens		X									
Biosample Baseline			X								
Biosample follow-up				X	X	X	X	X	X	X	X
24-hour urine			X	X	X	X	X	X	X	X	
Clean catch/ Urine dipstick (Spot)		X	X	X	X	X	X	X	X	X	X
Renal Biopsy tissue[b]		X									

** Screening, Enrollment, and Baseline Visits may occur one at a time, sequentially or concurrently (baseline blood draw cannot be performed on the day of renal biopsy).*

[a] Screen: Optional for post-biopsy consent

[b] To include 10 cc blood draw and pre-biopsy spot urine sample

Renal Biopsy (X#): At any time during the period of the study, participants receiving follow-up, surveillance, or other diagnostic renal biopsies, surplus tissue available for research should be obtained and banked with corresponding specimens

APPENDICES

8.B. Cohort B: cNEPTUNE Visit Schedule

Procedures and Visit Schedule	0 Mo	1.5 Mo	4 Mo	8 Mo Phone	12 Mo	18 Mo	24 Mo	30 Mo	36 Mo	RV
Informed Consent/Assent	X									
Demographics	X									
Physical Exam; Vital Signs	X	X	X		X	X	X	X	X	X
Medical and Family History	X	X	X		X	X	X	X	X	
Medication History	X	X	X	X	X	X	X	X	X	X
Questionnaires	X	X	X		X	X	X	X	X	X
Biosample Collection	X	X	X		X	X	X	X	X	X
Local Lab Results	X	X	X	X	X	X	X	X	X	X
Kidney Biopsy	***IF CLINICALLY INDICATED***									
Home Urine Protein	X	X	X	X	X					X
SMS (Text Messaging)	X	X	X	X	X					
Healthcare Utilization	X	X	X	X	X	X	X	X	X	X
Vital/ESRD Status	X	X	X	X	X	X	X	X	X	X

8.C. RV = Relapse VisitMethodology for Pathologic Evaluation of Podocyte Injury and Segmental Sclerosis with Proteinuria in the FSGS/MCD Cohort

8.C.1. Selection of cases

Patients will be selected based on the inclusion and exclusion criteria defined. All the following pathologic diagnoses as recorded in the biopsy report will be included and reviewed by the pathology committee to verify accuracy:

- Minimal change disease
- Minimal change nephrotic syndrome
- Glomerular minimal changes with nephrotic syndrome

- Extensive foot process effacement suggestive/consistent with minimal change disease or minimal change nephrotic syndrome
- Focal segmental glomerulosclerosis, any variant according to the Columbia classification
- Collapsing glomerulopathy, any etiology
- Tip lesion (including but not limited to FSGS variant)
- Diffuse mesangial sclerosis

The material to be evaluated minimally must include light microscopy and electron microscopy. Immunofluorescence is not an absolute criterion of inclusion.

8.C.2. Adequacy

Adequacy will be determined if at least 5 glomeruli are present in at least one of the slides from the paraffin embedded sections. The diagnostic lesion will need to be present in the material available for light microscopy (including the EM thick section, if submitted).

8.C.3. Review of pathologic material

The light microscopy slides (all levels available) and electron micrographs and immunofluorescence photographs (or recorded results in the pathology report) will be reviewed by two pathologists (members of the pathology committee) per each case. More than one pathologist is required to score each case to test reproducibility. The review of the pathology material will occur using the Aperio digital pathology system. All the light microscopic slides (including H&E, PAS, trichrome and silver) available for each case will be scanned at the Biobank Core site into the Aperio system at 20X resolution, stored at the central NEPTUNE server. Review of slides will be done remotely via the Aperio web access tool. The electron micrographs and immunofluorescence photographs will also be scanned or, if already received as digital images, will be integrated in the Aperio system and placed on the web for review by pathologists. Each biopsy will be reviewed by at least two renal pathologists of the consortium. A third pathologist will be consulted for quality control if a significant discrepancy occurs. Discordance will occur when any difference in number (>10%) for any specific glomerular lesion is recorded by two different pathologists. The same criterion will apply to the evaluation of tubulointerstitial damage and vascular disease. All cases with discordance will then be reviewed simultaneously by all 3 members of the pathology committee to reach consensus and to identify the specific reason for the intial disagreement. A decision will be made when 2/3 pathologists agree on any given point of discordance. Given the strict definitions for each morphologic lesion listed below, a low level of discordance is expected. However, in those cases where agreement cannot be reached by the members of the pathology committee, the pathology advisory board will be directly involved in the decision process, and decision will be made when the majority (at least 4 of the total of 6 members with the pathology commiitee and pathology advisory board) reaches a consensus. The use of the Aperio system will allow a quick and practical review of difficult cases by multiple pathologists at different centers. The Aperio digital pathology system has been tested in other studies and consortia and validated for quality assurance (1-3).

The original diagnosis and quantitative analysis will be recorded on the NEPTUNE server according to criteria described in the pathology scoring criteria appendix in the study protocol.

Quantitative analysis will be performed using conventional morphologic analysis and experimental morphologic analysis, according to the criteria listed below.

APPENDICES

8.C.4. Conventional Morphologic Analysis

8.C.4.a. Definition of Specific Morphologic Lesions by Histologic and Ultrastructural Analysis

1. Glomeruli:

First the total number of glomeruli will be determined using all levels available and scanned in the Aperio digital pathology system.

The number of glomeruli with specific morphologic features, as defined below, will be determined for each biopsy using all paraffin embedded sections available and thick sections (when available).

- ***No (minimal) changes***: none of the lesions below are present. A glomerulus with no morphologically detectable changes needs to be composed of at least 5 capillary loops in order to be counted. If a portion of a glomerulus with less than 5 capillary loops is present in a given section, it will not be included in the final counting of the total number of glomeruli nor will it be counted as a glomerulus with no or minimal changes.
- ***Global sclerosis with hyalinosis***: sclerosis involves 100% of the glomerular tuft and is accompanied by hyalinosis. Glomerular size is preserved or, compared to the glomeruli obtained in the same biopsy, increased or decreased by not more than 50%.
- ***Global sclerosis without hyalinosis***: sclerosis involves 100% of the glomerular tuft, with no accompanying hyalinosis. Glomerular size is preserved or, compared to the glomeruli obtained in the same biopsy, increased or decreased by not more than 50%.
- ***Obsolescent glomeruli***: Glomeruli are small and globally sclerotic without hyalinosis. Bowman's capsule is often absent. Obsolescent glomeruli are defined when glomerular size is decreased more than 50% compared to all other glomeruli in the same biopsy.
- ***Segmental sclerosis at the vascular pole with hyalinosis and/or foam cells***: Segmental solidification of the glomerular tuft is present with increased extracellular matrix and/or foam cells and/or hyalinosis and must be in continuity with the vascular pole. Hypertrophy of overlying epithelial cells may be seen. Adhesion to Bowman's Capsule may be present.
- ***Extended perihilar sclerosis with hyalinosis and/or foam cells***: Segmental solidification of the glomerular tuft with increased extracellular matrix and/or foam cells and/or hyalinosis is present, and must be in continuity with the vascular pole and involves an extended part of the glomerulus up to the tip but without involving the tip. Hypertrophy of overlying epithelial cells may be seen. Adhesion to Bowman's Capsule may be present.
- ***Segmental sclerosis at the vascular pole without hyalinosis and/or foam cells***: Segmental solidification of the glomerular tuft in continuity with the vascular pole with increased extracellular matrix is present without hyalinosis or foam cells. Hypertrophy of overlying epithelial cells may be seen. Adhesion to the Bowman's Capsule may be present.
- ***Extended perihilar sclerosis without hyalinosis and/or foam cells***: Segmental solidification of the glomerular tuft with increased extracellular matrix is present which must be in continuity with the vascular

pole and must involve an extended part of the glomerulus up to the tip but without involving the tip. Hypertrophy of overlying epithelial cells may be seen. Adhesion to Bowman's Capsule may be present.

- ***Segmental sclerosis away from the vascular and tubular poles with hyalinosis and/or foam cells***: Segmental solidification of the tuft with increased extracellular matrix and foam cells and/or hyalinosis is present. Hypertrophy of overlying epithelial cells may be seen. Adhesion to the Bowman's capsule may be present.
- ***Segmental sclerosis away from the vascular and tubular poles without hyalinosis and/or foam cells***: Segmental solidification of the tuft with increased extracellular matrix is present. Hypertrophy of overlying epithelial cells may be seen. Adhesion to Bowman's Capsule may be present. No hyalinosis or foam cells are present.
- ***Sclerosing tip lesion***: Solidification of the tuft at the tubular pole with increased extracellular matrix with or without adhesion to Bowman's Capsule is present without foam cells. Podocytes may be hypertrophic and attached to the epithelial cells at the tubular pole.
- ***Extended sclerosing tip lesion***: Solidification of the tuft at the tubular pole with increased extracellular matrix and adhesion to Bowman's Capsule which extends through a large portion of the glomerulus but does not involve the vascular pole. No foam cells are present. Podocytes are hypertrophic and attached to epithelial cells at the tubular pole.
- ***Diffuse mesangial sclerosis***: A generalized global increase (>50%) of mesangial matrix is present and may be accompanied by mild mesangial cell hypercellularity and hypertrophy of overlying epithelial cells.
- ***Proliferative Segmental collapse***: Wrinkling and folding of the GBM involving at least one glomerular lobule and less than 80% of the tuft is present. Wrinkling and folding of the GBM is accompanied by hypertrophy and hyperplasia of overlying epithelial cells (pseudocrescent) which may also contain protein reabsorption droplets and cytoplasmic vacuoles. A pseudocrescent is formed by at least 2 layers of epithelial cells.
- ***Proliferative Global collapse***: Wrinkling and folding of the GBM involving more than 80% of the tuft is present with hypertrophy and hyperplasia of overlying epithelial cells (pseudocrescents). A pseudocrescent is formed by at least 2 layers of epithelial cells.
- ***Non-proliferative collapse***: Segmental or global wrinkling and folding of the GBM without pseudocrescent formation is present (formerly called an ischemic type of collapse).
- ***Cellular Tip lesion***: Foam cells and/or hyaline material accumulation within the glomerular tuft at the tubular pole of the glomerulus are present, accompanied by hypertrophy of podocytes and/or bridging to the Bowman's capsule/proximal tubule take off area.
- ***Extended cellular tip lesion***: Foam cells and/or hyaline material accumulation are present within the glomerular tuft at the tubular pole of the glomerulus which extend through a large portion of the glomerulus

(>1/3 of the tuft) but do not involve the vascular pole, accompanied by hypertrophy of podocytes and/or bridging to the Bowman's capsule/proximal tubule take off area.

- ***Non-Tip Cellular lesions***: Endocapillary hypercellularity with podocyte hypertrophy are present. Hypercellularity may be due to foam cells, swollen endothelial cells or inflammatory cells and is not at the tip of the glomerulus.
- ***Mid tuft/central location***: Located neither at the tip, the perihilum, or the periphery of the tuft, with or without hyalinization and foam cells.
- ***Cannot determine location***: None of the above, with or without hyalinization and foam cells.

2. Podocytes:

Light microscopy (LM): The number of glomeruli with the following morphologic pathologic features (segmental and global) recognized on LM will be recorded**.**

- ***Hyperplasia by LM***: More than 2 layers of podocytes overlying the glomerular basement membranes are present.
- ***Halo by LM***: Detachment of podocytes from underlying GBM are present with intervening new loose basement membrane material (halo).

EM: Foot process effacement, microvillous transformation and condensation of the actin-based cytoskeleton will be recorded as % of glomerular area affected. Other parameters will be recorded as present or absent.

- ***Foot process effacement by EM***: % of glomerular capillary surface area affected by effacement.
- ***Loss of primary processes by EM*:** is present when the podocyte cell body sits directly on underlying GBM. This is generally accompanied by complete effacement (loss of foot processes).
- ***Condensation of the actin-based cytoskeleton by EM***: Electron dense cytoskeleton is reorganized and condensed at the GBM aspect of podocyte foot processes.
- ***Microvillous transformation by EM***: Cytoplasmic projections into the urinary space that emanate from the luminal side of podocyte membrane are present.

3. Deposits:

- ***Presence or absence of mesangial deposits by EM***: Mesangial deposits will be deemed to be present in those cases with positive staining for IgM on immunofluorescence. If no IgM is detected on immunofluorescence but electron dense deposits are noted on ultrastructural analysis, the case will not be considered suitable for study based on exclusion criteria.

4. Tubulo-interstitial damage:

The following parameters will be quantitated as an exact percentage of renal cortex involved in each pathologic feature. Interstitial inflammation will be separately evaluated in areas of fibrosis and non fibrotic parenchyma. Acute tubular damage will be evaluated semiquantitatively as mild, moderate or severe. The presence or absence of cortical microcysts will be determined.

- ***Chronic interstitial change*** is equivalent to interstitial fibrosis. Tubules are separated by collagen that stains blue on trichrome.
- ***Interstitial inflammation*** includes lymphocytes, monocytes, plasma cells. The presence of eosinophils in clusters representing more than 10% of inflammatory cells in a given inflamed area is recorded with an (*).
- ***Interstitial edema***: The interstitium is occupied by pale acellular material.
- ***Chronic tubulointerstitial damage*** is equivalent to tubular atrophy and generally accompanies interstitial fibrosis. It is defined by the presence of small tubules with thick tubular basement membranes or by thryroidization.
- ***Acute tubular damage*** is defined by the presence of tubular degenerative changes (flattening of the tubular epithelium, loss of proximal cell brush borders, pyknotic cells) plus tubular regenerative changes (hypertrophic epithelial cells with large nuclei and prominent nucleoli, mitotic activity).
- ***Microcysts*** are defined by the presence of dilated tubules (> twice the diameter of a normal proximal tubule) containing eosinophilic amorphous material, and is generally accompanied by scalloping of the cast profile. The epithelium lining the microcyst is generally flattened and does not reveal brush border.

5. Vascular disease:

The following 2 parameters will be scored:

- ***Arterial sclerosis* is** defined as thickening of the intima with fibrosis and/or duplication of the elastic lamina in interlobular and arcuate arteries.
- ***Arteriolar hyalinosis*** is defined as accumulation of hyaline material in the intima.

A semi-quantitative scoring system (0-3) will be used for each component (arterial sclerosis, arterial hyalinosis) according to the description below. The total vascular score will vary from 0 to 6.

0 absent

1 mild, focal (<25% of vessels)

2 moderate, ≥25% to 50% of vessels

3 severe, > 50% of vessels

8.C.4.b. Immunofluorescence

IgM: the presence of positive staining for IgM in the mesangium will be recorded. Positive immunofluorescence must be confirmed by the presence of mesangial deposits by electron microscopy.

8.C.5. Experimental Morphologic Analysis

Assessments of additional structural parameters will be performed as determined by the histopathology working group on the available material.

APPENDICES

8.C.5.a. Rational for Selection of the Above-Listed Parameters

1. Analysis of glomerular parameters

The use of the term "focal segmental sclerosis" (FSGS) has changed over the last 30 years (4). FSGS initially was used to define a lesion characterized by segmental accumulation of extracellular matrix in the glomerular tuft. With time other forms of glomerular damage characterized by endocapillary or extracapillary (podocyte) proliferation have been described as FSGS. A more formal morphologic classification of FSGS proposed in 2004 also includes many of the lesions described above as FSGS (5). In addition a classification based on podocyte injury characteristics (called podocytopathies) was proposed (6). This proposal included MCD and DMS as manifestations of podocyte injury and is based on morphologic features as well as on the etiology and pathogenic mechanisms underlying these heterogeneous group of diseases to better reflect pathogenesis and potential therapeutic response.

Because the glomerulus can react to injury in multiple ways, the pathology committee has structured the assessments to capture all possible morphologic manifestations of non-inflammatory glomerular injury so far described in association with nephrotic syndrome and proteinuria, to determine their correlation with molecular, clinical and therapeutic characteristics. The selection of the glomerular parameters listed above incorporates lesions that are considered part of the FSGS spectrum (morphologic classification) or of the podocytopathy spectrum (taxonomy of the podocytopathies). Some of these features are associated with better or worse prognoses compared to classic segmental sclerosis (defined as segmental accumulation of extracellular matrix) (7-9). Others are associated with genetically transmitted diseases, use of certain medications, or infections (6, 10). Some of the glomerular changes are commonly accepted signs of progression and disease chronicity (global sclerosis, obsolescent glomeruli, non-proliferative collapse), and can be present in all forms of glomerular damage associated with podocyte injury. Others are more restricted and not common to all forms of glomerular damage. There are features which represent active disease/damage (endocapillary and extracapillary proliferation with collapse)(10-13).

The glomerular size has also been associated with specific pathogenic mechanisms leading to segmental sclerosis, and for that reason it has been included as a study parameter (14). Some of the parameters listed are commonly analyzed by pathologists at routine biopsy evaluation (conventional morphologic analysis), while others typically are used for experimental studies (experimental morphologic analysis).

2. Analysis of podocyte parameters

Non-inflammatory glomerular diseases associated with nephrotic syndrome or proteinuria are known to be due to podocyte injury. Podocyte injury generally manifests as foot process effacement, but other morphologic changes of podocytes have been described and in some cases they appear to be associated with specific forms of glomerular injury. Podocyte injury may occur following a variety of insults, but podocytes have only a limited number of ways to react to injurious stimuli: they may undergo foot process effacement, hypertrophy and apoptosis, proliferation, or phenotypic changes that are detectable by electron microscopy such as condensation of the actin-based cytoskeleton, microvillous transformation, etc) (6, 14-16). Staining for GLEPP1 can be used as a podocyte marker because its expression in the podocyte cell body will highlight podocyte area, demonstrating podocyte hypertrophy which has been associated with FSGS in experimental models of podocyte injury (14, 17, 18). Some of the parameters listed are commonly analyzed by pathologists at routine biopsy evaluation (conventional morphologic analysis), while others are used for experimental studies (experimental morphologic analysis).

APPENDICES

3. Analysis of mesangial deposits

IgM nephropathy is a controversial entity, and morphologically can present as minimal change disease or mesangial hypercellularity (19). Some authors have suggested that it may be part of the spectrum of FSGS. Patients with IgM nephropathy present with proteinuria or nephrotic syndrome at the time of renal biopsy and appear to have a clinical course similar to FSGS patients, with high steroid resistance. In any case, it appears that the presence of mesangial deposits with IgM positive staining on immunofluorescence is a poor prognostic factor and needs to be further investigated (20-22). Assessment of mesangial deposits and immunofluorescence analysis are commonly performed in all renal biopsies during routine diagnostic work-up.

4. Analysis of the tubulointerstitial damage

Progression of any renal disease is not only dependent on the status of the glomeruli but the status of the tubulointerstitial compartment has a critical role in determining outcome. One known example is given by the routinely used activity or chronicity indices in the assessment of renal biopsies in patients with lupus nephritis (23-26). The status of the tubulointerstitial compartment is also commonly evaluated in transplant pathology (27). It is known, in some forms of non-inflammatory glomerular diseases with podocyte injury, that severe interstitial fibrosis and tubular atrophy (corresponding to chronicity index) are more common than in others, and are in general associated with poor outcome (28-30). An experimental model of renal disease also suggests that the status of the tubulointerstitium is the consequence of misdirected filtration from the glomerulus (31). In addition to chronic changes, acute changes (acute tubular injury, interstitial inflammation and microscysts) are more commonly associated with certain forms of glomerular and podocyte damage (28). Assessment of the status of the tubulointerstitium is commonly performed in all renal biopsies during routine diagnostic work-up.

5. Analysis of the vascular damage

It has been suggested that certain forms of glomerulosclerosis can be secondary to hypertension-induced hyperfiltration, and commonly are associated with glomerulomegaly (32, 33). The conclusion often made by pathologists during routine work-up regarding cause and effect of vascular disease and FSGS is an educated guess. However, reliable data are not available in human biopsies to support this mechanism for FSGS development or to classify some forms of glomerular disease as secondary to hyperfiltration. The analysis of the status of the vasculature together with glomerular and tubulointerstitial damage is necessary to investigate the potential association between chronic vascular damage, non-inflammatory glomerular diseases with podocyte injury and progression of kidney failure. The correlation of these morphologic parameters with molecular data is also critical to determine any association between the genetic background and/or molecular profile and the likelihood of developing segmental sclerosis and glomerulomegaly in patients with vascular damage.

Assessment of arteriosclerosis and arteriolar hyalinosis is commonly performed in all renal biopsies during routine diagnostic work-up.

8.C.6. References

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APPENDICES

8.C. Methodology for Pathology Analysis for Membranous Nephropathy

8.C.1. Selection of cases

Patients will be selected based on the inclusion and exclusion criteria listed in the cohort study description. All the following pathologic diagnoses as recorded in the biopsy report will be included and reviewed by the pathology committee to verify accuracy:

- Membranous glomerulopathy or glomerulonephritis
- Idiopathic membranous glomerulopathy or glomerulonephritis
- Membranous glomerulopathy or glomerulonephritis, idiopathic form
- Membranous glomerulopathy or glomerulonephritis, secondary form

The material to be evaluated must include light microscopy, immunofluorescence and electron microscopy.

8.C.2. Adequacy

Adequacy will be defined as availability of light microscopy (LM), electron microscopy (EM) and immunofluoresence (IM), with presence of at least 1 glomerulus per biopsy on light microscopy, 1 glomerulus on immunofluorescence and 1 glomerulus on electron microscopy.

8.C.3. Review of Pathologic Material

The light microscopy slides (all levels available), electron micrographs and immunofluorescence photographs (or recorded results in the pathology report) will be reviewed by two pathologists (members of the pathology committee) per each case. More than one pathologist will be required to score each case to test reproducibility. The review of the pathology material will occur using the Aperio digital pathology system. All the light microscopic slides (including H&E, PAS, trichrome and silver) available for each give case will be scanned at the Biobank Core site into the Aperio system at 20X resolution and stored at the central NEPTUNE server. Review of slides will be done remotely via the Aperio web access tool. The electron micrographs and immunofluorescence photographs will also be scanned or, if already received as digital images, will be integrated in the Aperio system and made available on the web for review by pathologists. Each biopsy will be reviewed by at least two renal pathologists of the consortium. A third pathologist will be consulted for quality control if a significant discrepancy occurs. Discordance will occur when any difference in number (>10%) for any specific glomerular lesion is recorded by two different pathologists. The same will apply to the evaluation of tubulointerstitial damage and vascular disease. All cases with discordance will also be reviewed simultaneously by all 3 members of the pathology committee to reach consensus and to identify the specific reason for any given discrepancy. A decision will be made when 2/3 pathologists agree on any given point of discordance. Given the strict definitions for each morphologic lesion listed below, a low level of discordance is expected. However, in those cases where agreement cannot be reached by the members of the pathology committee, the pathology advisory board will be directly involved in the decision process, and decision will be made when the majority (at least 4 of the total of 6 members with the pathology committee and pathology advisory board) reaches a consensus. The use of the Aperio system will allow a quick and practical review of difficult cases by multiple pathologists at different centers. The Aperio digital pathology system has been tested in other studies and consortia and validated for quality assurance (1-3).

The original diagnosis and quantitative analysis will be recorded on the NEPTUNE server according to criteria described in the pathology scoring criteria appendix in the study protocol.

Quantitative analysis will be performed using conventional morphologic analysis and experimental morphologic analysis, according to the criteria listed below.

8.C.4. Conventional Morphologic Analysis

8.C.4.a. Definition of Specific Morphologic Lesions by Histologic and Ultrastructural Analysis

1. Glomeruli:

First the total number of glomeruli will be determined using all sections available and scanned in the Aperio digital pathology system.

The number of glomeruli with specific morphologic features, as defined below, will be determined for each biopsy using all paraffin embedded sections available and thick sections (when available).

- ***No (minimal) changes***: none of the lesions below are present.
- ***Focal presence of spikes on silver stain:*** Spikes are defined as silver positive stains with an irregular profile on the outer side of the glomerular basement membranes and are deemed focal if present in <50% of the glomeruli.
- ***Diffuse presence of spikes on silver stain:*** Spikes are defined as silver positive stains with an irregular profile on the outer side of the glomerular basement membranes and are deemed focal if present in >50% of the glomeruli.
- The presence of ***leukocytes in glomerular capillaries*** are classified as 0-3 for each glomerulus with 0= none 1 = 1-7, 2=8-15, 3=>15 leukocytes per glomerulus.
- ***Focal and segmental mesangial hypercellularity*** are defined as > than 3 mesangial cells per mesangial lobule involving <50% of the visible mesangial region in a glomerulus and present in <50% of the glomeruli.
- ***Diffuse and segmental mesangial hypercellularity***: are defined as > than 3 mesangial cells per mesangial lobule involving <50% of the visible mesangial region in a glomerulus and present in >50% of the glomeruli.
- ***Focal and global mesangial hypercellularity***: are defined as > than 3 mesangial cells per mesangial lobule involving >50% of the visible mesangial region in a glomerulus and present in <50% of the glomeruli..
- ***Diffuse and global mesangial hypercellularity***: are defined as > than 3 mesangial cells per mesangial lobule involving >50% of the visible mesangial region in a glomerulus and present in >50% of the glomeruli.
- ***Segmental sclerosis*** is an exclusion criterion for entry of a diagnosis of membranous GN into the study.
- ***Global sclerosis with hyalinosis*** is defined as sclerosis involving 100% of the glomerular tuft accompanied by hyalinosis. Glomerular size is preserved, or increased or decreased by not more than 50% of the glomeruli in the same biopsy [??meaning??].
- ***Global sclerosis without hyalinosis***: is defined as sclerosis involving 100% of the glomerular tuft accompanied by hyalinosis. Glomerular size is preserved or increased or decreased by not more than 50% of the glomeruli in the same biopsy.
- ***Obsolescent glomeruli***: are defined as small globally sclerotic glomeruli without hyalinosis. Bowman's Capsule is often absent. The diameter of obsolescent glomeruli must be decreased by >50% compared to that of all other glomeruli in the same biopsy.

APPENDICES

2. **Podocytes:**
   - ***Foot process effacement by EM***: is described as the % of glomerular capillary surface area affected by effacement.
   - ***Loss of primary processes by EM*:** occurs when the podocyte cell body sits directly on the underlying GBM. This is generally accompanied by complete effacement (loss of foot processes).
   - ***Condensation of the actin-based cytoskeleton by EM***: is defined by the reorganization and condensation of the electron dense cytoskeleton at the GBM aspect of the podocyte foot process.
   - ***Microvillous transformation by EM***: are defined as cytoplasmic projections into the urinary space emanating from the luminal side of podocyte membrane.
3. **Deposits:**

   By immunofluroescence:
   - **Intensity** in a scale from 0-3 will be recorded as per pathology report, when possible digitalized images will also be reviewed. Because some pathologists use a scale from 0-4 and others a scale from 0-3, the intensity scale will be extracted from the pathology report as follow: 0=0, 1=1, 2=2, 3 and 4=3.
   - **Distribution and Location** will be recorded as subepithelial, subendothelial or mesangial and as segmental, if involving only a portion of the glomerulus or global if involving the entire glomerulus.
   - **Composition**: IgG and C3 positive stain will be recorded as described above. Other immunoglobulins (IgA and IgM) and complement fraction (C1q) will also be recorded as above.

   By electron microscopy:

The percentage of glomerular basement membrane involved by subepithelial deposits in each stage (I-IV) will be recorded; more than one stage may be present in a biopsy at one time. Mesangial deposits (including mesangial and paramesangial) will be recorded as present or absent. A minimum of 5 electron micrographs at 10,000X magnification will be reviewed.

- ***Subepithelial deposits Stage I:*** Electron dense deposits are present on the outer surface of the GBM.
- ***Subepithelial deposits Stage II:*** Electron dense deposits are present on the outer surface of the GBM and partially surrounded by extracellular matrix (spikes).
- ***Subepithelial deposits Stage III:*** Electron dense deposits are embedded in the extracellular matrix (intramembranous).
- ***Subepithelial deposits Stage IV:*** Electron dense deposits are partially reabsorbed and formed by irregular electron lucent areas and more electron dense areas.
- ***Deposit characteristics:*** The presence of transmembranous deposits and deposits with a concentric circular (nuclear pore) configuration will be noted as present or absent.
- ***Thickening of the GBM***: GBM thickness will be assessed on 10 cross sections of capillary loops at foci where there are no capillary wall deposits.
- ***Mesangial deposits:*** Mesangial deposits (including mesangial and paramesangial) will be recorded as present or absent.
- ***Subendothelial deposits***: Subendothelial deposits will be recorded as present or absent.

4. **Tubulointerstitial damage**

The following parameters will be quantitated as the exact % of renal cortex involved by each pathologic feature. Interstitial inflammation will be separately evaluated in areas of fibrosis and non fibrotic parenchyma. Acute tubular damage will be evaluated semiquantitatively as mild, moderate or severe. The presence or absence of cortical microcysts will be determined.

- ***Chronic interstitial change*** is equivalent to interstitial fibrosis. Tubules are separated by collagen that stains blue on trichrome.
- ***Interstitial inflammation*** includes lymphocytes, monocytes, plasma cells. The presence of eosinophils in clusters representing more than 10% of inflammatory cells in a given inflamed area is recorded with an (*).
- ***Interstitial edema***: The interstitium is occupied by pale acellular material.
- ***Chronic tubulointerstitial damage*** is equivalent to tubular atrophy and generally accompanies interstitial fibrosis. It is defined by the presence of small tubules with thick tubular basement membranes or by thryroidization.
- ***Acute tubular damage*** is defined by the presence of tubular degenerative changes (flattening of the tubular epithelium, loss of proximal cell brush borders, pyknotic cells) plus tubular regenerative changes (hypertrophic epithelial cells with large nuclei and prominent nucleoli, mitotic activity).

5. Vascular disease

The following 2 parameters will be scored:

- ***Arterial sclerosis* is** defined as thickening of the intima with fibrosis and/or duplication of the elastic lamina in interlobular and arcuate arteries.
- ***Arteriolar hyalinosis*** is defined as accumulation of hyaline material in the intima.

A semi-quantitative scoring system (0-3) will be used for each component (areterial sclerosis, arterial hyalinosis) according to the description below. The total vascular score will vary from 0 to 6.

0 absent
1 mild, focal (<25% of vessels)
2 moderate, ≥25% to 50% of vessels
3 severe, > 50% of vessels

8.C.5. Rational for Histopathology Evaluation

The morphologic classification of MN is based on conventional interpretation of specific histopathologic glomerular features. However, no specific data or criteria have been identified to date allowing categorization or classification which consistently predict outcome or response to therapy (4). It currently is not possible to identify genetically determined forms of MN by morphology (5), nor does the morphologic classification of MN provide information on biomarkers of each specific form of MN, of response to therapy or progression, nor does it facilitate assessment of specific underlying mechanisms for each disease that may be potential targets of new therapies. To overcome the current limitations in our evaluation of the renal biopsies in patients with MN, biopsies from patients initially selected based on a general diagnosis of MN by the renal biopsy report (see inclusion criteria) will be re-evaluated using descriptive morphological criteria. The morphologic features chosen for assessment in the study were selected based on possible associations with the pathogenesis, clinical presentation, progression or response to therapy in MN, which require further definitive analysis.

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8.C.5.a. Analysis of Glomerular Parameters

MN is an immune complex mediated glomerular lesion in which deposits most often are globally located in all capillary walls, particularly as the disease progresses. In a cohort of patients there may be segmental glomerular involvement with only a portion of the glomerulus demonstrating immune deposits; this has been identified more frequently in children and in association with mesangial deposits, suggesting a secondary form of MN (6). Mesangial hypercellularity is not a typical feature of "idiopathic" MN, but often is present in selected secondary forms of MN such as those associated with systemic lupus erythematosis and viral hepatitis (7). However, other forms of membranous nephropathy which may be considered secondary, such as those associated with malignancy or gold therapy, lack this increase in mesangial cellularity (8). Therefore, this is an important morphologic parameter to evaluate in any comprehensive assessment of MN, particularly as it relates to pathogenesis. Other features which may be reflective of clinical settings include the number of glomerular capillary leukocytes, which has been associated with underlying malignancy in patients with MN. It has been reported that the presence of more than 8 leukocytes per glomerulus is a marker of this form of secondary MN (9). Capillary basement membrane thickness is associated with the degree of proteinuria and serum creatinine concentration, and may play a role in clinical manifestations of MN (10). One goal of this approach is to identify general histopathologic parameters to be included in a predictor panel for the clinical endpoints of the study. As an immune complex mediated lesion, MN is not specifically associated with segmental glomerulosclerosis. However, glomerulosclerosis is associated with disease progression (11). The presence of focal and segmental glomerulosclerosis will be exclusionary criteria for the diagnosis of MN to ensure that molecular and other data are relevant to the disease process being studied. However, global glomerulosclerosis and glomerular obsolescence may reflect advanced glomerular damage with relevance to progression and therapeutic response so will be assessed. Some of the parameters listed are commonly analyzed by pathologists at routine biopsy evaluation (conventional morphologic analysis) while others are used for experimental studies (experimental morphologic analysis).

8.C.5.b. Analysis of Podocyte Parameters

Podocyte injury generally manifests as foot process effacement but other morphologic changes of podocytes may occur in association with forms of glomerular injury. Podocytes have only a limited number of ways to react to injurious stimuli including foot process effacement, hypertrophy and apoptosis, proliferation, or undergoing phenotypic changes such as condensation of the actin-based cytoskeleton, microvillous transformation, etc) that are detectable by electron microscopy (12, 13)(14, 15). In MN, podocytes respond to sublytic C5b-9 attack by enhanced production of extracellular matrix, forming "spikes", as well as other phenotypic changes such as disruption of the slit diaphragm and foot process effacement with resultant proteinuria (16, 17). Some of the parameters listed are commonly analyzed by pathologists at routine biopsy evaluation (conventional morphologic analysis) while others are used for experimental studies (experimental morphologic analysis).

8.C.5.b. Analysis of Deposits

The location, size and heterogeneity of the electron dense deposits in MN may have a bearing on the pathogenesis, course or therapeutic responsiveness of this disease and therefore will be evaluated in this study. The stage of MN, as defined ultrastructurally by the subepithelial deposit locations, surrounding basement membrane material and state of resorption (18), has been suggested to be a key factor in the potential for response to treatment (19). It also has been reported that patients with disease homogeneity with regard to stage are more likely to achieve complete remission although they tend to have more frequent disease relapse (20), and that deposit heterogeneity is an independent predicator of prognosis in MN (21). Capillary wall deposits in MN may have particular ultrastructural features, the significance of which is unknown. A limited number of cases have deposits with a circular nuclear pore appearance associated with autoantibodies in the patient's serum, which bind to structures at the nuclear periphery possibly reflecting a specific pathogenic mechanism for MN (22, 23). Transmembranous (penetrating) deposits have been suggested to be specific for systemic lupus erythematosis, even in the absence of features of the disease at the time of diagnosis of MN (24).

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Deposits in mesangial and/or subendothelial locations are not a typical feature of "idiopathic" MN, but are suggestive of a secondary form of this disease even in the absence of mesangial hypercellularity (11, 25). However, it is uncertain why some forms of secondary MN have deposits in other than subepithelial locations while others do not. Therefore, this parameter requires assessment to determine its significance in relation to other data being collected in this study. Renal biopsies also are assessed by immunofluorescence to determine the location, intensity, and composition of the immune complex deposits. Higher intensity of C3 quantitated by immunofluorescence has been related to increased proteinuria and a faster rate of loss of renal function in MN patients (20). Deposits in mesangial locations and those containing C1q are found in secondary forms of MN such as lupus nephritis (26). It is unknown whether these features are linked to other prognostic or pathogenic factors in MN. Some of the parameters listed are commonly analyzed by pathologists at routine biopsy evaluation (conventional morphologic analysis) while others are used for experimental studies (experimental morphologic analysis).

8.C.5.c. Analysis of Tubulointerstitial Damage

Progression of any renal disease is not only dependent on the status of the glomeruli but also on the status of the tubulointerstitial compartment. One known example is given by the routinely used activity or chronicity indices in the assessment of renal biopsies in patients with lupus nephritis (27-30). It has been shown in patients with MN that chronic tubulointerstitial lesions correlate with decreased renal function, higher blood pressure and lower renal survival (20). The degree of proteinuria and morphologic stage of MN also are significantly associated with tubulointerstitial injury (31, 32). Therefore, evaluation of this renal compartment is relevant in determining morphologic features for progression and response to therapy. Assessment of the status of the tubulointerstitium is commonly performed in all renal biopsies during routine diagnostic work-up.

8.C.5.d. Analysis of Vascular Disease

The analysis of the status of the vasculature together with glomerular and tubulointerstitial damage will be necessary to investigate the potential association between chronic vascular damage, glomerular injury and global sclerosis, and progression of kidney failure. Vascular sclerosis has been identified as a risk factor for reduced renal function at presentation and is a predictive factor for lower renal survival in patients with MN (20). Assessment of arteriosclerosis and arteriolar hyalinosis is commonly performed in all renal biopsies during routine diagnostic work-up.

8.C.6. References

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