Statistical Analysis Plan (SAP):

Pediatric Longitudinal Glomerular Disease: a PNRC Multi-Institutional Cohort Study

NCT: Not Yet Assigned

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STATISTICAL ANALYSIS PLAN

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PROJECT TITLE:	Pediatric Longitudinal Glomerular Disease: a PNRC Multi-Institutional Cohort Study Get PLUGGED IN
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SAP History (Summary of substantial modifications):

Version Number	Version Date	SAP section number	Description of change:	Brief rationale	Revisions made by:
1.00	3-Feb-26	N/A	Initial draft	N/A	N/A
1.01	13-Apr-26	All sections	Edited cohorts, clarified outcome definitions and modified analysis plan	Initial draft revised based on comments from Novartis	Ernest Amankwah
1.02	30-Apr-26	2.2 4.3, 4.4	Relevance of subgroups clarified; all potential covariates listed and cause of death clarified	revised based on comments from Novartis	Ernest Amankwah

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1. STUDY OBJECTIVES

1.1. PRIMARY OBJECTIVES

1. To characterize the longitudinal trajectory and progression of proteinuria, measured by urine protein–creatinine ratio (UPCR), following diagnosis and to identify baseline demographic and clinical factors associated with changes in proteinuria over time
2. To characterize longitudinal changes in kidney function as measured by estimated glomerular filtration rate (eGFR) and to identify baseline demographic and clinical factors associated with changes in kidney function over time.

1.2. SECONDARY OBJECTIVES

1. To estimate overall survival and identify baseline demographic and clinical factors associated with risk of death during follow-up.
2. To determine the incidence and timing of progression to renal failure and to identify prognostic factors associated with risk of progression.
3. To characterize the incidence and timing of renal transplantation and describe clinical characteristics associated with progression to transplant.
4. To describe longitudinal growth patterns (e.g., height, weight, BMI z-scores) and identify disease-related factors associated with impaired growth trajectories.
5. To characterize developmental progression, measured over time and identify clinical factors associated with delays or deviations from expected developmental milestones.
6. To evaluate educational outcomes over follow-up and examine associations between disease severity and academic achievement.
7. To quantify school absenteeism and examine its relationship with disease burden and clinical progression.
8. To estimate the frequency and timing of unplanned hospitalizations and identify factors associated with unplanned hospitalizations.
9. To characterize the incidence and types of infections during follow-up and evaluate clinical factors associated with increased infection risk.
10. To describe longitudinal changes in histopathological findings and evaluate associations between pathological features and disease progression.

1.3. EXPLORATORY OBJECTIVE

1. To characterize longitudinal changes in health-related quality of life, following diagnosis and to identify baseline demographic and clinical factors associated with changes in patient reported outcomes over time

2. STUDY DESIGN

This study is a national multi-center mixed retrospective-prospective cohort study design that will enroll incident and prevalent "cases" of glomerular diseases (i.e., patients) while under the care of pediatric nephrologists and followed forward in time from present to future, with simultaneous collection of data. Because both incident and prevalent cases are eligible, such that patients may be enrolled with various lag times following the time of initial diagnosis of glomerular disease, data of interest that have been generated through routine clinical care between the time of diagnosis and the time of enrollment will be retrospectively collected. In both phases of data collection for a given participant (prospective and retrospective), data will be abstracted from the medical record and entered into a REDCap database for the study. Any data collected from study-specific source records (e.g., patient reported outcomes or Quality of Life instruments not obtained/utilized in routine clinical care) will be designated as such in the cohort-specific Schedule of Assessments (Appendix A). The initial duration of the study will be 5 years. Given the study's landmark status as the first and only North American multi-disease multi-institutional longitudinal cohort study in the field of pediatric nephrology, there is no planned end date to the study at this time.

2.1. TREATMENT GROUPS

Single arm (GD cases)

2.2. STUDY POPULATION

The target population for this study is children who have been diagnosed at < 18 years of age with incident or prevalent documented glomerular disease at participating PNRC U.S. sites.

The Primary population will include children with biopsy-proven glomerular disease.

The Secondary population will include children with a clinical diagnosis of glomerular disease who haven't been biopsied [ex. Post-Infectious Glomerulonephritis or Post-Streptococcal Glomerulonephritis (PIGN) and Idiopathic Nephrotic Syndrome (INS)]

Inclusion Criteria

1. Incident or prevalent, objectively diagnosed (via biopsy and/or clinical diagnosis) glomerular disease
2. Birth to 17 years, 364 days at time of biopsy and/or diagnosis
3. Signed and dated written informed consent provided by the participant's parent(s) (or legal guardian) and the participant's assent in accordance with International Council for Harmonization Good Clinical Practice (ICH-GCP) and local site laws/regulations prior to admission to the trial.

Assent will be sought according to the participant's age, level of maturity, competence and capacity

Exclusion Criteria

1. Declined (or unable to provide informed consent, + informed assent of minors) to participate
2. End Stage Kidney Disease (Stage 5 CKD, eGFR $\leq$ 15mL/min/1.73m^2)

The study population will be further categorized into the following prespecified disease cohorts:

1. IgA Nephropathy
2. IgA Vasculitis
3. Idiopathic Immune Complex Glomerulonephritis (ex: IC-MPGN, Full house GN without diagnosis of Lupus, etc.)
4. C3 Glomerulopathy
5. Membranous Nephropathy
6. Focal and Segmental Glomerulosclerosis
7. Minimal Change Disease
8. Infection-related or post-infectious Glomerulonephritis (with or without biopsy)
9. Lupus Nephritis
10. Alport Syndrome

Additional cohort-specific eligibility criteria are detailed in the protocol.

2.3. EXPOSURES OF INTEREST

GD

3. OUTCOME VARIABLES

3.1. PRIMARY OUTCOMES

3.1.1. Percent change in urine protein-creatinine ratio (as a measure of proteinuria) from baseline (date of the diagnostic biopsy or date of the official clinical diagnosis in the non-biopsied patients) to follow up. Follow up will be every 6 months (±3 months) after diagnosis

3.1.2. Percent change in estimated glomerular filtration rate (eGFR) from baseline to follow up

3.2. SECONDARY OUTCOMES

3.2.1. Death from any cause

3.2.2. Renal failure (including dialysis-dependence)

3.2.3. Renal transplantation

3.2.4. Changes in weight and height rowth trajectory, derived from height and weight
3.2.5. Key developmental milestones derived from QOL survey of caregiver
3.2.6 Key educational milestones, derived from QOL survey of caregiver
3.2.7 Caregiver effect: Missed days of school and work for caregiver
3.2.8. Unplanned Hospitalization – duration and frequency including AKI (reason for admission)
3.2.9. Infections (Urine, Lunch, Ear, Skin/Tissue, URI and others)
3.2.10. Histopathological Response – Serial biopsy reports and images

3.3. EXPLORATORY OUTCOME(S)

Health-related Quality of Life (HR-QOL, employing the PROMIS QOL instrument validated in children)

3.4. OTHER PARAMETERS

3.4.1. Demographic and clinical characteristics at baseline

- Demographics (e.g., age, weight, body mass index (BMI), gender, race, ethnicity)
- Hospitalization details, type of pediatric department (e.g., neonatology, cardio-surgery, intensive care unit (ICU), hematology) and procedures related to disease diagnostic modalities
- Medical history including concomitant medications history
- Baseline conditions
- Disease characteristics, such as type of GD, GD characteristics obtained according to standard diagnostic modalities and local protocols
- Risk factors for GD
- Description of treatment modalities
- Initial DE dosage and formulation
- Results of lab tests (if available)

4. STATISTICAL METHODOLOGY

4.1. GENERAL METHODOLOGY

Data analyses for this study will be performed by the Data Coordinating Center for Multicenter Studies in the Johns Hopkins All Children's Institute for Clinical and Translational Research at study completion, once the database has been locked and the Statistical Analysis Plan has been finalized and approved by the study team. The first

statistical analyses will be performed after two years of study initiation. All analyses will be performed on overall study population and by type of GD (where numbers permit) using SAS® version 9.4 or higher (SAS Institute, Cary, NC, USA).

4.1.1. Population for analysis

The analytic population for all analyses will consist of all enrolled patients with at least six months of follow up data.

4.1.2. Participant flow

The flow of patients, including the overall patients screened, excluded patients, patients enrolled, and patients retained in the analytic dataset will be presented in a CONSORT-type diagram.

4.1.3. Handling of missing data

The frequency and patterns of missing data will be examined, and if deemed appropriate, the potential impact of missing data will be evaluated using sensitivity analyses and multiple imputation. Multiple imputation models will include outcome and baseline variables in a single imputation model and 50 complete data sets will be imputed using chained equations

4.2. SUMMARY OF DEMOGRAPHICS AND CLINICAL CHARACTERISTICS AT BASELINE

Demographic and baseline clinical characteristics of the study population (all enrolled participants) as well as the analytic population (see above, 4.1.1) will be summarized overall and by age group (<1, 1-<6, 6-<13, 13-<18 years), sex and type of GD. Continuous variables will be summarized as means with standard deviations or medians with interquartile ranges and compared between age groups on an exploratory basis using ANOVA or Kruskal-Wallis test as appropriate. Categorical variables will be summarized as counts with percentages and compared between age groups on an exploratory basis using the Chi-squared test or Fisher's exact test as appropriate.

4.3. ANALYSIS OF THE PRIMARY OUTCOMES

Protein-creatinine ratio (UPCR) will be analyzed as a continuous variable and summarized at baseline and follow up timepoints post-diagnosis of index GD using median and interquartile range in the analytic population. The ratio will also be reported by type of GD, age group and sex. Baseline UPCR will be defined as the first value obtained after diagnosis. Participants will contribute repeated UPCR measurements until death, loss to follow-up, or administrative censoring. The association between clinical characteristics and change in UPCR over the study period will be evaluated using a multivariable mixed-effects model with random intercepts and slopes to account for within-participant and -site correlations. Time will be modelled using spline functions to allow for nonlinear trajectories and both baseline and time-varying covariates will be included in the models. Potential confounding factors that will be included in the multivariable model will be selected based on prior literature and clinical knowledge and will include: age, sex, race/ethnicity, disease severity, weight/BMI, medications, and major comorbidities.

eGFR, calculated from serum creatinine using the CKD-EPI equation, will be analyzed as a continuous outcome measure in a similar manner as UPCR. If UPCR and/or eGFR is skewed, we will log transform and analyze them on the log scale

4.4. ANALYSIS OF THE SECONDARY OUTCOMES

4.4.1. All cause mortality will be defined as death from any cause after diagnosis. Overall survival will be estimated using Kaplan-Meier method. Median survival time and survival probabilities at clinically relevant time point (e.g. 1,3 and 5 year) will be reported with 95% confidence intervals. Association between clinical characteristics and mortality will be evaluated using a multivariable Cox proportional hazards regression with prespecified baseline and time-varying covariates (see below). Survival time will be defined as time from diagnosis of GD to death and survivors will be censored at the last day of follow up or end of study, whichever occurs first. The proportional hazard assumption will be checked using Schoenfeld residuals. Potential covariates will be selected based on prior literature and clinical knowledge and will include: age at diagnosis, sex, race/ethnicity, disease severity, weight/BMI, medications, eGFR, UPCR, and major comorbidities.

4.4.2. Renal failure (including dialysis-dependence). The incidence of renal failure will be summarized with the corresponding 95% confidence interval for each disease group. The association between clinical characteristics and time to renal failure will be evaluated using Fine and Gray subdistribution hazard model with prespecified covariates (see above); accounting for mortality (not related to renal failure) and renal transplantation as competing risks. Time to renal failure will be defined as time from diagnosis of GD to renal failure and survivors who do not develop renal failure will be censored at the last day of follow up or end of study, whichever occurs first. Potential clinical factors will be determined as indicated above.

4.4.3. Renal transplantation. The incidence of renal transplantation will be summarized with the corresponding 95% confidence interval for each disease group. Association between clinical characteristics and time to renal transplantation will be evaluated with Fine and Gray subdistribution hazard model with prespecified covariates; accounting for mortality (not related to renal transplantation) and renal failure as competing risks. Time to renal transplantation will be defined as time from diagnosis of GD to the first occurrence of renal transplantation and survivors who do not receive renal transplantation will be censored at the last day of follow up or end of study, whichever occurs first. Potential clinical factors will be determined as indicated above.

4.4.4. Growth trajectory will be defined as change in height and weight over time. We will calculate height-for-weight Z score, weight-for-height Z score and BMI Z-score over time. The association between prespecified clinical characteristics and growth trajectory over the study period will be evaluated using a multivariable mixed-effects model with random intercepts and slopes to account for within-participant and -site correlations. Time will be modelled using spline functions to allow for nonlinear

trajectories and both baseline and time-varying covariates will be included in the models. Potential factors will be determined as indicated above.

4.4.5 Time to attainment of key developmental milestones (derived from QOL survey of caregiver) will be summarized using mean and standard deviation or median and interquartile range. The proportion of patients with delayed development at baseline (first assessment after diagnosis) and follow up will also be reported. The association between clinical factors and time to attainment of key developmental milestones will be evaluated with multivariable Cox regression models (with prespecified baseline and time-varying covariates), where the sample size is adequate).

4.4.6 Time to attainment of key educational milestones will be summarized using mean and standard deviation or median and interquartile range. The proportion of patients with delayed educational attainment at baseline (first assessment after diagnosis) and follow up will also be reported. The association between clinical factors and time to attainment of key educational milestones will be evaluated with multivariable Cox regression models (with prespecified baseline and time-varying covariates), where the sample size is adequate)

4.4.7 Caregiver effect: Missed school days will be summarized using descriptive statistics (mean and standard deviation or median and interquartile range as appropriate)

4.4.8 Unplanned Hospitalization will be summarized using descriptive statistics. The association between clinical factors and number of unplanned hospitalizations will be examined with a multivariable negative binomial regression model

4.4.9 The incidence and type of infection will be reported with the corresponding 95% CI at each follow up time point. The association between clinical factors and infection will be evaluated using multivariable mixed models with random effects for patient and site

4.4.10 Histopathological Response will each be summarized in the analytic population during the exposure period using descriptive statistics. Distributions will be compared across age groups where appropriate, using Chi-Square or Fisher's exact test, for categorical variables, and Kruskal-Wallis for continuous variables

4.5. ANALYSIS OF THE EXPLORATORY OUTCOME

Health-related quality of life (HRQoL) measured using PROMIS® instruments (e.g., Physical Function, Fatigue, Pain Interference) will be analyzed as T-scores (mean 50, SD 10), with higher scores indicating better or worse health depending on the domain.

PROMIS domain scores will be summarized at each time point using means (SD). Changes in PROMIS scores over time will be evaluated using linear mixed-effects models with random intercepts and slopes to account for within-participant and -site correlations. Secondary analyses will focus on clinically important differences. The proportion of participants with improvement or decline will be reported with 95%CI.

4.6 SAMPLE SIZE JUSTIFICATION

The pediatric population (age 0-17 yrs.) in the United States is estimated at 74 million. It is estimated that 0.5% of U.S. children (0-17 yrs.) have moderate chronic kidney disease (CKD) (NHANES Annual Data Report | USRDS), resulting in ~370,000 children with CKD. In children, it is estimated that 14% of CKD (eGFR <75) cases are glomerular[4]. Therefore, approximately 52,000 children have glomerular disease with CKD in the U.S. We conservatively estimate to enroll 1000 patients at 20 sites in the first two years of the study based on historical data from study sites. We will continue to enroll after two years with increased number of sites.

5. REFERENCES

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