Precision Medicine Guided Use of TNF Inhibition in FSGS and Treatment Resistant Minimal Change Disease: Proof of Concept Target Engagement Study

Statistical Analysis Plan
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I give my approval for this SAP entitled "Precision Medicine Guided Use of TNF Inhibition in FSGS and Treatment Resistant Minimal Change Disease: Proof of Concept Target Engagement Study Statistical Analysis Plan," 31OCT2023.

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Contents

Abbreviations and Definitions

DSMB	Data Safety and Monitoring Board
eGFR	Estimated Glomerular Filtration Rate
FSGS	Focal Segmental Glomerulosclerosis
LLN	Lower Limit of Normal
MCP1	Monocyte Chemoattractant Protein 1
NEPTUNE	Nephrotic Syndrome Study Network
TIMP1	Tissue Inhibitor of Metalloproteinase 1
TNF	Tumor Necrosis Factor
TR-MCD	Treatment Resistant-Minimal Change Disease
uCr	Urinary Creatinine
uEGF	Urinary Epidermal Growth Factor
ULN	Upper Limit of Normal
UPCR	Urine Protein to Creatinine Ratio

1. Introduction

1.1 Preface

This Statistical Analysis Plan (SAP) describes statistical methods and analyses for the Precision Medicine Guided Use of TNF Inhibition in FSGS and Treatment Resistant Minimal Change Disease: Proof of Concept Target Engagement Study. This document should be read in tandem with the Precision Medicine Guided Use of TNF Inhibition in FSGS and Treatment Resistant Minimal Change Disease: Proof of Concept Target Engagement Study Protocol version 5.0, dated February 21, 2023. The SAP was finalized prior to the database lock.

The trial proposes to investigate whether urinary markers of intra-renal tumor necrosis factor (TNF) pathway activation in patients with focal segmental glomerulosclerosis (FSGS) or treatment resistant- minimal change disease (TR-MCD) are reduced with TNF inhibition via adalimumab.

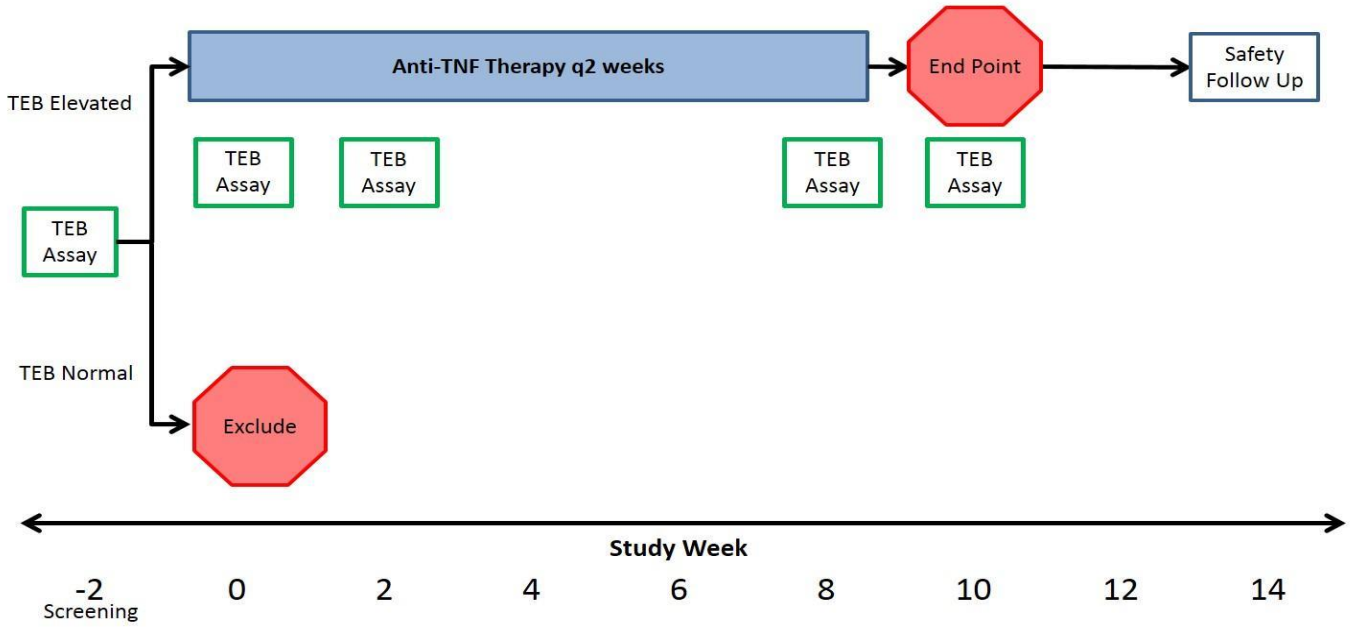
This proof-of-concept, multi-center, open-label clinical trial addresses the treatment of patients with clinically active FSGS or TR-MCD and urinary biomarkers of intra-renal TNF up-regulation, including urinary monocyte chemoattractant protein 1/urinary creatinine (urinary MCP1/uCr) and urinary tissue inhibitor of metalloproteinase 1/urinary creatinine (urinary TIMP1/uCr). These biomarkers have been shown to indicate intra-renal TNF upregulation. Up to eight patients, aged 6-80 years, were treated with 8 weeks of adalimumab therapy to assure a minimum evaluable study sample of six patients in this single-arm study. Adalimumab was dosed based on weight and administered subcutaneously every two weeks for 8 weeks (5 total doses). The urinary biomarkers MCP1/uCr and TIMP1/uCr were assessed throughout the study. The primary endpoints are the change in target engagement biomarker levels of urinary TIMP1/uCr and MCP1/uCr from baseline to study week 10. Key secondary outcomes include safety measures, serious adverse events, adverse events, percent change of estimated glomerular filtration rate (eGFR) and urine protein to creatinine ratio (UPCR) from baseline to week 10, and proportion of participants achieving a nadir UPCR less than 1.5 g/g and at least a 40% reduction from baseline at week 10.

1.2 Scope of the Analyses

The purpose of this document is to describe the statistical analyses to address the primary, secondary, and exploratory objectives to be conducted for this clinical trial. Ancillary analyses are not covered in this document.

2. Schematic of Study Design

Figure 1. Schematic of Study Design



TEB = Target Engagement Biomarker
TEB levels will be assessed at study weeks -2, 0, 2, 8, and 10

3. Study Aims and Hypotheses

3.1 Primary Aim and Hypothesis

The primary purpose of this proof-of-concept study is to test the hypothesis that TNF inhibition with adalimumab (Humira) in patients with up-regulation of the intra-renal TNF pathway results in reduction in urinary indicators of TNF pathway activation, specifically urinary MCP1/uCr or TIMP1/uCr levels.

Hypothesis:
TNF inhibition with adalimumab (Humira) in patients with up-regulation of the intra-renal TNF pathway results in reduction in urinary MCP1/uCr and/or TIMP1/uCr biomarker levels.

4. Endpoints

4.1 Primary Endpoints

- The change in urinary MCP1/uCr levels from baseline to study week 10, i.e., 2 weeks after the final dose of adalimumab
- The change in urinary TIMP1/uCr levels from baseline to study week 10, i.e., 2 weeks after the final dose of adalimumab

4.2 Secondary Endpoints

1. Incidence of serious adverse events, defined as the proportion of participants with treatment-emergent serious adverse events and the number of treatment-emergent serious adverse events, overall, by system organ classification, by relatedness to study medication, and by severity
2. Incidence of non-serious adverse events, defined as the proportion of participants with treatment-emergent non-serious adverse events and the number of treatment-emergent non-serious adverse events, overall, by system organ classification, by relatedness to study medication, and by severity
3. Incidence of adverse events leading to premature discontinuation of study medication, defined as the proportion of participants with treatment-emergent adverse events and the number of treatment-emergent adverse events that led to premature discontinuation of study medication, overall, by system organ classification, by relatedness to study medication, and by severity
4. Proportion of participants with abnormal laboratory tests for ALT, AST, total bilirubin, hemoglobin, hematocrit, and platelet count, proportion of participants with temperature ≥38.5˚C (indicating fever), and proportion of participants with any edema (over all body locations and by each location) over the course of the 10-week intervention period
5. Percent change in eGFR from baseline to week 10
6. Percent change in UPCR from baseline to week 10
7. Proportion of participants achieving a nadir UPCR less than 1.5 g/g and at least a 40% reduction from baseline at week 10

4.3 Exploratory Endpoints

1. In the subgroup of participants with kidney biopsy tissue collected within 12 months of study baseline, correlation coefficient between intra-renal gene expression of TNF (TNF activation score) [1] and urinary MCP1/uCr and TIMP1/uCr biomarker levels at baseline
2. Change from baseline to weeks 2, 4, and 8 in urinary MCP1/uCr
3. Change from baseline to weeks 2, 4, and 8 in urinary TIMP1/uCr
4. Percent change from baseline to weeks 2, 4, 8, and 10 in urinary MCP1/uCr
5. Percent change from baseline to weeks 2, 4, 8, and 10 in urinary TIMP1/uCr
6. Urinary MCP1/uCr values at baseline and weeks 2, 4, 8, and 10
7. Urinary TIMP1/uCr values at baseline and weeks 2, 4, 8, and 10
8. Changes in urinary epidermal growth factor normalized to urinary creatinine (uEGF/uCr) biomarker levels from baseline to week 10
9. Correlation coefficient between uEGF/uCr at baseline with changes and percent changes in urinary MCP1/uCr and urinary TIMP1/uCr biomarker levels and in eGFR and UPCR values at week 10.
10. Changes in FSGS-MCD Patient Reported Outcome (PRO) score from baseline to weeks 2, 4, 8 and 10

Endpoint Rationale:

The target engagement biomarkers, urinary MCP1/uCr and TIMP1/uCr, are the primary endpoints of this study as we hypothesize that the levels of this biomarkers at baseline reflect TNF up-regulation in the kidney and after TNF inhibition reflect target engagement. Neither eGFR nor urinary protein is a primary endpoint measure. However, we measure eGFR and urinary protein to provide estimates for future trials.

5. Study Methods

5.1 General Study Plan

This study is designed as a proof-of-concept multi-center, open-label single-arm clinical trial addressing the treatment of patients with FSGS or TR-MCD. Eligibility for the study is assessed during a 6-week screening period where bio-samples are used to assess TNF up-regulation and other laboratory and medical history criteria.

Patients are recruited from the active population of patients with FSGS or TR-MCD at participating sites. TR-MCD is defined as persistent proteinuria despite pre-trial immunomodulating treatment. Following informed consent and when appropriate, assent, a screening visit is conducted. Patients with elevated urinary TIMP1/uCr and MCP1/uCr levels and meeting all other eligibility criteria are scheduled to receive adalimumab every two weeks for 8 weeks (5 total doses).

A participant is considered to have completed the study if they complete the study through week 14 or if they withdraw prior to completion of the final study visit. Participants are followed in the study even if study medication is prematurely discontinued unless they withdraw their consent to do so. End of study is constituted by completion of the last visit for the final participant in the study protocol.

5.2 Inclusion-Exclusion Criteria and General Study Population

5.2.1 Participant Inclusion Criteria

1. Kidney biopsy confirmed FSGS or MCD
2. For Minimal Change Disease patients only, history of resistance to corticosteroid therapy
3. Increased urinary excretion of biomarkers of TNF activation (urinary MCP1/uCr and/or urinary TIMP1/uCr) at study screening
4. eGFR>30 ml/min/1.73 m^2 at screening
5. Urine protein to creatinine ratio ≥1.5 g/g at screening
6. Age 6-80 years
7. Weight >15kg
8. Stable therapy with angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and oral immunosuppression agents for at least 30 days prior to enrollment
9. Birth control use in females of child-bearing potential
10. Informed consent and assent if applicable

5.2.2 Participant Exclusion Criteria

1. Kidney or other solid organ or bone marrow transplant recipient
2. Allergy or intolerance to investigational agent
3. Secondary FSGS
4. Severe obesity, i.e., BMI >40 kg/m^2
5. Live virus vaccine in the past 3 months
6. Malignancy, current or in the past 5 years
7. Active local or systemic bacterial, fungal or viral infection
8. Active or latent Hepatitis B, Hepatitis C, HIV, or tuberculosis defined as quantiferon test positive
9. History of demyelinating disease, e.g., Multiple Sclerosis or Guillain-Barre
10. History of heart failure
11. Active liver disease, defined as AST or ALT > 2.5x Upper Limit of Normal
12. Systemic lupus erythematosus or ANA > 1:80
13. History of inflammatory bowel disease, e.g., ulcerative colitis or Crohns disease
14. Cyclophosphamide in past 90 days, Rituximab in the past 180 days
15. Pregnancy or nursing
16. Blood white blood cell count <4,500/mm^3; Hg <9 g/dL; Platelet count <150,000/mm^3 at enrollment. Use of an erythropoiesis stimulating agent will not be an exclusion criterion.
17. Concurrent use of interleukin-1 antagonist (Anakinra), other TNF blocking agent, methotrexate or abatacept
18. Diabetic nephropathy

5.3 Randomization and Blinding

This is a single-arm study and thus randomization and masking are not possible to minimize bias.

5.4 Study Assessments

The Schedule of Activities details the study procedures:

	Screening	Week 0	Week 2	Week 8	Week 10	Week 14
Visit window	*+/-2 DAYS*	*+/-2 DAYS*	*+/-2 DAYS*	*+/-2 DAYS*	*+/-2 DAYS*	*+/-4 DAYS*
Consent	X					
Medical History	X	X	X	X	X	X
Archived Kidney Biopsy Report Submission	X					
Archived Kidney Tissue sections submitted, if available*						
Concomitant Medications	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X**
Adverse Events Assessment		X	X	X	X	X
Vital Signs	X	X	X	X	X	X**
HRQOL assessment		X	X	X	X	
Comprehensive Metabolic Panel	X	X	X	X	X	X**

	Screening	Week 0	Week 2	Week 8	Week 10	Week 14
CBC with Platelets and Differential	X	X	X	X	X	X**
Urine Protein:Creatinine	X	X	X	X	X	X**
Assessment of Infections	X					
MCP1/TIMP1 (spot urine)	X	X	X	X	X	
Pregnancy Test	X	X	X	X		
Biorepository specimen	X*	X	X	X	X	
Adalimumab dosing		X	X	X		
* Do not submit if most recent biopsy specimen has already been submitted to NEPTUNE study; *DNA collected at screening only ** Optional procedures required if study visit is conducted in-person						

6. Outcomes

6.1 Primary Outcome Assessments

Spot urinary biospecimens to assay MCP1 (ng/mL), TIMP1 (ng/mL) and creatinine (mg/mL) are collected with spot urine tests at screening and weeks 0, 2, 8, and 10. Urine creatinine is used to normalize the urinary MCP1 and urinary TIMP1 by calculating the ratio of urinary MCP1 and urinary creatinine and the ratio of urinary TIMP1 and urinary creatinine.

Urinary MCP1 and Urinary TIMP1 are measured by ELISA methods [2]. There are no published reference ranges for the urinary MCP1/uCr nor urinary TIMP1/uCr levels; however, lower values for both biomarkers are associated with less intra-renal TNF activation. [1] Thus, either absolute or relative changes from baseline to post-treatment time points indicate TNF pathway target engagement by adalimumab and support the proof of concept of this trial.

6.2 Secondary Outcome Assessments

6.2.1 Safety measures

An adverse event is any untoward medical occurrence in a clinical study participant administered a medicinal (investigational or non-investigational) product. Adverse events, whether serious or non-serious, related or not related, are recorded after administration of adalimumab (i.e., are treatment-emergent events) until week 14. All serious adverse events that do resolve by the end of the study, or that do not resolve upon discontinuation of the subject's participation in the study, are followed until any of the following occurred:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status were available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (participant or health care practitioner refusal to provide additional information, or lost to follow-up after demonstration of due diligence with follow-up efforts).

Adverse events are categorized by severity and by relationship to study medication by the site investigators, and by system organ class by the coordinating center investigators.

The status of edema is important in this patient population as a manifestation of disease, so any edema (over all body locations and by each location) over the course of the 10-week intervention period is evaluated within the adverse event reporting as new or worse than prior to study.

As a clinically significant change, any fever event (temperature ≥38.5˚C) is recorded over the course of the 10-week intervention period.

6.2.2 Laboratory abnormalities

For ALT, AST, and total bilirubin, the laboratory result value is abnormal when it is greater than 2 x upper limit of normal (ULN). Laboratory result values either below the age-matched lower limit of normal (LLN) or above the age-matched upper limit of normal is defined as laboratory abnormalities for hemoglobin, hematocrit, and platelet count. The age-matched normal ranges of ALT, AST, total bilirubin, hemoglobin, hematocrit, and platelet count are provided below:

	Age Group	**Male**	**Female**
ALT	> or =6 year	7-55 U/L	7-45 U/L
AST	6-13 years	8-60 U/L	8-50 U/L
	> or =14 years	8-48 U/L	8-43 U/L
Total bilirubin	6 to 17 years	0.0-1.0 mg/dL	0.0-1.0 mg/dL
Hemoglobin	6-8 years	11.5-14.3 g/dL	11.5-14.3 g/dL
	9-10 years	11.8-14.7 g/dL	11.8-14.7 g/dL
	11-14 years	12.4-15.7 g/dL	11.9-14.8 g/dL
	15-17 years	13.3-16.9 g/dL	11.9-14.8 g/dL
	Adults	13.2-16.6 g/dL	11.6-15.0 g/dL
Hematocrit	6-7 years	34-42%	34-42%
	8-11 years	35-43%	35-43%
	12-15 years	38-47%	35-43%
	16-17 years	40-50%	35-43%
	Adults	38.3-48.6%	35.5-44.9%
Platelet count	6-9 years	187-400 x 10^9/L	187-400 x 10^9/L
	10-13 years	177-381 x 10^9/L	177-381 x 10^9/L
	14-17 years	139-320 x 10^9/L	158-362 x 10^9/L
	Adults	135-317 x 10^9/L	157-371 x 10^9/L

We calculate the proportion of participants with laboratory abnormalities at weeks 2, 4, 8 and 10, and overall (i.e., a participant experienced a lab abnormality at any visit).

6.3 Preliminary efficacy measures

eGFR (mL/min/1.73 m^2) is calculated from blood samples at screening and Weeks 0, 2, 8, 10 and 14 (if the study visit is conducted in person). Higher values generally indicate better kidney function. The calculation of eGFR could be found in Section 8.2.1.

UPCR (mg/mg) is calculated from urinary samples for proteinuria and creatinine at screening and Weeks 0, 2, 8, 10 and 14 (if the study visit is conducted in person). The preferred method for collection of urinary samples is through 24-hour urine collection, but first morning urine or spot urine is acceptable. The ratio of urine protein to urine creatinine (UPCR) is the secondary outcome measure of interest. Higher levels of UPCR indicate more significant kidney disease. In this pilot, proof-of-concept study, the normal range for UPCR is < 0.3 mg/mg for all ages.

6.4 Exploratory Outcome Measures

Intra-renal gene expression of TNF is derived from archived biopsy tissue of study participants or from the Nephrotic Syndrome Study Network (NEPTUNE) study data using the NEPTUNE study protocol and as published. [3] This parameter was only included for participants with biopsies within one year of consent in the TNF pilot study and is generated as a TNF activation score.

A TNF activation network was generated from expert curated interactions from NETPro annotations in the Genomatix Genome Analyzer database (Precigen Bioinformatics).[1] It was based on renal biopsy specimens available from more than 250 NEPTUNE participants with MCD and FSGS. From the database, causally downstream genes or proteins that increased expression from TNF exposure were used to generate a TNF activation score. Individual gene expression values were first Z-transformed. The TNF activation score for each participant was the average Z-score of the genes in their kidney RNA-seq profile and defines the intra-renal gene expression of TNF measure.

Urinary epidermal growth factor (uEGF, ng/ml) biomarker levels are assessed from spot urine specimens collected and normalized by urinary creatinine (mg/ml) at Screening, and Weeks 0, 2, 8, and 10. Higher levels of uEGF/uCr (ng/mg) indicate better kidney function.

The FSGS-MCD Patient Reported Outcome (PRO) score is still in development and scoring methods have not yet been finalized. The score will be derived from the FSGS-MCD PRO structured questionnaires completed by participants at in-person study visits at Weeks 0, 2, 8 and 10. The analyses of FSGS-MCD PRO scores will occur when the scoring methods have been developed and made available to the study investigators.

Assay Quality Methods:
Urinary MCP1, TIMP1, creatinine and EGF for all study participants and all time points for were assayed on the same plate to reduce batch effects.

7. Statistical Considerations

7.1 General Statistical and Analytical Plans

This is a pilot, proof-of-concept investigation to test the proposed biomarkers as indicators of target engagement. As such, no formal sample size calculations were performed, and analyses are primarily descriptive in nature. Our approach is to assess the biomarker change over time with TNF inhibition therapy within each patient and as a group. As the ELISA testing for this indication is new, the protocol uses an exploratory approach to assess individual and panel biomarker changes and correlations with clinical indicators of disease, including proteinuria, eGFR, and serum albumin.

This SAP document was finalized and approved prior to the database lock.

7.2 Analysis Dataset

All eligible participants who received at least one dose of adalimumab (as-treated population) are analyzed for primary, secondary, and exploratory endpoints.

7.3 Covariates and Subgroups

No adjustment for covariates is included in the analyses of the primary biomarker endpoints because of the limited sample size of this pilot, proof-of-concept study. No subgroup analyses are planned.

7.4 Missing Data

For the primary analysis of the primary endpoints, we will impute week 10 biomarker values using the week 8 biomarkers values, when needed. The remainder of our analyses uses a complete case approach (i.e., analyzing the observed values with no imputation of missing values). This method is valid under the missing completely at random assumption. We will assess the suitability of this assumption based on the extent and patterns of missingness, as well as clinical judgement.

7.5 Interim Analyses and Data Monitoring

No formal interim analyses were planned nor carried out for this study. The study was overseen by a Data and Safety Monitoring Board (DSMB) that reviewed the subject disposition, study conduct and safety data throughout the trial.

7.6 Multiple Testing

Statistical testing is conducted at the 0.05 significance level using two-tailed tests; two-sided p-values are reported. No adjustments for multiplicity are planned for this pilot study. Rather, inferences and decision-making are based primarily on the magnitude of treatment effects.

8. Summary of Study Conduct Data

Descriptive summary statistics are tabulated for baseline patient demographics and clinical characteristics (overall since this is a single-arm study). Continuous variables are summarized using descriptive statistics including n, mean, median, standard deviation, interquartile range (i.e., 1st and 3rd quartiles), and range (i.e., minimum and maximum). Qualitative variables will be summarized using counts and percentages. Unless otherwise specified, statistical analyses will be performed using SAS Version 9 or higher. Where appropriate, statistical tests will be conducted at the 0.05 significance level using two-tailed tests and p-values will be reported.

8.1 Subject Disposition

The number and percentage of participants consented, treated and withdrawn, along with reasons for withdrawal, are tabulated and summarized in a CONSORT diagram. Other disposition and study conduct information, including major protocol violations are summarized. Protocol deviations are provided in a listing. Duration of study follow-up is summarized.

8.2 Derived Variables

8.2.1 eGFR

The formulas used to calculate eGFR in adults and children are given below:

- CKID U25 age- and sex-dependent equation using serum creatinine (Pierce, 2021) [4] for patient whose age is younger than 25 years old:

  eGFR = k × (height/serum creatinine), with height in meters and serum creatinine in mg/dL

  For males, k is calculated as:

  For 1 to 11 years old: $39.0 \times 1.008^{(age-12)}$

  For 12 to 17 years old: $39.0 \times 1.045^{(age-12)}$

  For 18 to 25 years old: 50.8

  For females, k is calculated as:

  For 1 to 11 years old: $36.1 \times 1.008^{(age-12)}$

  For 12 to 17 years old: $36.1 \times 1.023^{(age-12)}$

  For 18 to 25 years old: 41.4

- CKD-EPI equation for patient whose age is greater than 25 years old [5]:

  $eGFRcr = 142 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.200} \times 0.9938^{Age} \times 1.012$ [if female]

  where:

  Scr = standardized serum creatinine in mg/dL

  $\kappa = 0.7$ (females) or 0.9 (males)

  $\alpha = -0.241$ (female) or -0.302 (male)

  $\min(Scr/\kappa, 1)$ is the minimum of Scr/κ or 1.0

  $\max(Scr/\kappa, 1)$ is the maximum of Scr/κ or 1.0

  Age (years)

8.2.2 Blood pressure index

The 2017 ACC/AHA hypertension guideline applied a lower threshold for defining hypertension and 130 (mmHg) /80 (mmHg) are the thresholds for hypertension in adult's guidelines. [6] An important update in the 2017 AAP CPG is a change in the definition of hypertension in children and adolescents: In children <13 years of age, hypertension is defined as systolic or

diastolic blood pressure ≥95th percentile on the new sex, age, and height tables. For adolescents ≥13 years of age, the 95th percentile approximates 130/80 mm Hg. Therefore, the 2017 AAP CPG has used the new adult blood pressure cut-points to define hypertension in adolescents ≥13 years of age. [7]

To allow for comparison across adults and children, systolic and diastolic blood pressure indices are calculated with the method below:

- For the patient whose age was 13 years or greater, the measured systolic blood pressure was divided by 130 (and the measured diastolic blood pressure was divided by 80)
- For the patient whose age was younger than 13 years, the measured systolic and the measured diastolic blood pressure were divided by the sex, age, and height specific 95th percentile systolic and diastolic blood pressure, respectively [8]

We use the Childhood Blood Pressure Macro-batch mode to calculate the specific 95th percentile blood pressure of the patient whose age is younger than 13 years. [9][10] Then the blood pressure index is calculated based on the method above. Blood pressure index $\geqslant 1$ indicates blood pressure in the hypertensive range and every 0.1-unit increase represents a 10% increase in blood pressure above the hypertensive range.

8.2.3 Laboratory abnormality variables

Elevation of ALT, AST or total bilirubin of > 2x ULN were prespecified as events of interest and were coded as 1 for values $\geq$ 2 x ULN, 0 for values below 2 x ULN. These are calculated for each participant at each time point.

Abnormal low values for hemoglobin, hematocrit, and platelet count were specified as events of interest and were coded as 1 for values < age-matched LLN and 0 otherwise. Abnormal high values for hemoglobin, hematocrit, and platelet counter were also specified as events of interested and were coded 1 for values > age-matched ULN and 0 otherwise. Any laboratory abnormality for these laboratory parameters were coded as 1 if abnormally low or high and 0 otherwise. These are calculated for each participant at each time point.

8.3 Demographic and Baseline Variables

Demographic and baseline variables for participants include: age in years at baseline, sex (male/female), ethnicity (Hispanic or Latino/not Hispanic or Latino/refused/missing), race (White/Black or African American/Asian/American Indian or Alaskan native/native Hawaiian or other Pacific Islander/refused/missing), duration of kidney disease (in months), baseline medication use (%), weight (kg), edema/no edema status, blood pressure index, eGFR (mL/min/1.73 m^2), UPCR (mg/mg), albumin (g/dL), hemoglobin (g/dL), WBC (k/mm^3), cholesterol (mg/dL), AST (U/L), ALT (U/L), total bilirubin (mg/dL), hematocrit (%), platelet count (K/uL), urinary MCP/uCr (ng/mg), urinary TIMP1/uCr (ng/mg) , uEGF/uCr (ng/mg), PRO Score, and TNF activation score.

8.4 Treatment Compliance

Compliance with study medication is summarized as the proportion of participants who received 1, 2, 3, 4, and (all) 5 doses of adalimumab. Descriptive statistics on the duration of adherence to study medication is summarized. The proportion of participants who permanently discontinue study medication is summarized with descriptive statistics and a listing provided of these participants with their reasons for permanent discontinuation of study medication.

9. Statistical Methods

9.1 Analyses of Primary Endpoints

The changes in urinary MCP1/uCr and urinary TIMP1/uCr levels from baseline to study week 10, i.e., 2 weeks after the final dose of adalimumab, are the primary endpoints. We provide tabular and graphical summaries of the changes from baseline to week 10 in urinary MCP1/uCr and in urinary TIMP1/uCr, including summaries at baseline and week 10. The changes from baseline to week 10 in urinary MCP1/uCr and urinary TIMP1/uCr levels will be tested using the Wilcoxon signed-rank test.

9.2 Analyses of Secondary Endpoints

Discrete safety outcomes (AEs, SAEs, AEs leading to premature discontinuation of study medication, and proportion of participants with abnormal laboratory tests, fever and new or worsening edema compared with baseline over the course of the 10-week treatment period) and discrete preliminary efficacy outcomes (proportion of participants achieving a nadir UPCR <1.5 g/g and at least a 40% reduction from baseline at week 10) are summarized descriptively.

Percent change in eGFR from baseline to week 10 and percent change in UPCR from baseline to week 10 are summarized descriptively in tabular and graphical formats, including baseline, follow-up and changes from baseline. Graphical summaries include spaghetti plots of participants' changes over time.

Clinical laboratory tests as described in Section 6.2.2 are summarized descriptively in tables and graphically with individual plots of laboratory value by visit with a reference line for LLN, ULN and 2xULN for those participants who have at least one abnormal value during the course of the study. A shift table (normal and abnormal results for screening and post-treatment values at each time point) for each clinical laboratory test of interest will be presented.

For discrete safety outcomes, frequency of events and proportion of participants experiencing events (i.e., treatment-emergent AEs and SAEs by body system, severity, and relatedness to adalimumab) will be summarized descriptively.

9.3 Tabulation of Individual Response Data

Listings of SAEs, AEs and AEs resulting in treatment discontinuation will be provided.

9.4 Exploratory Analyses

Spearman correlations and scatterplots are provided to assess the correlation of TNF activation score and urinary MCP1/uCr and urinary TIMP1/uCr biomarker levels at baseline, and the correlation of uEGF/uCr at baseline with changes and percent changes in urinary MCP1/uCr and urinary TIMP1/uCr biomarker levels and in eGFR and UPCR values at week 10.

Continuous outcomes (e.g., eGFR, uEGF/uCr biomarker levels) will be summarized descriptively, including baseline, follow-up and changes from baseline and graphically as spaghetti plots of participants' changes (absolute and relative) over time and values over time.

9.5 Sample Size

No formal sample size calculations were performed (either for estimation or hypothesis testing) because this study is a pilot investigation of urine biomarkers for target engagement of TNF inhibition. Rather, we assessed the magnitude of effects that can be detected in urine biomarkers using a paired t-test. With 7 patients, 80% power, and a two-sided type I error of 10%, we can detect an effect size (change from baseline to week 10 / SD) of 1.2 of changes in urinary TIMP1/uCr and of changes in urinary MCP1/uCr, with no adjustments for multiplicity (EAST 6.2).

10. Summary of Changes to the Protocol and/or SAP

The changes from the protocol-specified definitions of aims, outcomes and statistical analytic approaches are outlined below. These changes reflect advances in our knowledge of FSGS and MCD since the design of the study that were not incorporated as protocol amendments but were discussed during the formation of the Statistical Analysis Plan. These changes are documented herein and represent changes made prior to the database lock.

1. Change in the primary aim and hypothesis

Exclusion of the hypothesis that FSGS patients with up-regulation of the TNF pathway can be identified through intra-renal gene expression profiling and assay of urinary MCP1/uCr and urinary TIMP1/uCr levels by ELISA. This is demonstrated by the successful completion of the screening protocol that determined if they were included/eligible for drug.

Protocol: 3. OBJECTIVES AND PURPOSE

The primary purpose of this Proof of Concept study is to test the hypothesis that TNF inhibition with adalimumab (Humira) in patients with up-regulation of the intra-renal TNF pathway will result in reduction in urinary indicators of TNF pathway activation, specifically urinary MCP1/uCr and TIMP1/uCr levels.

Furthermore, this study is proposed to test the hypothesis that FSGS patients with up-regulation of the TNF pathway can be identified through intra-renal gene expression profiling and assay of urinary MCP1/uCr and TIMP1/Cur levels by ELISA.

SAP:
Section 1.1 Preface
This proof-of-concept, multi-center, open-label clinical trial addresses the treatment of patients with clinically active FSGS or TR-MCD and urinary biomarkers of intra-renal TNF up-regulation, including urinary monocyte chemoattractant protein 1/urinary creatinine (urinary MCP1/uCr) and urinary tissue inhibitor of metalloproteinase 1/urinary creatinine (urinary TIMP1/uCr).

Section 3.1 Primary Aim and Hypothesis.
The primary purpose of this proof-of-concept study is to test the hypothesis that TNF inhibition with adalimumab (Humira) in patients with up-regulation of the intra-renal TNF pathway results in reduction in urinary indicators of TNF pathway activation, specifically urinary MCP1/uCr or TIMP1/uCr levels.
Hypothesis:
TNF inhibition with adalimumab (Humira) in patients with up-regulation of the intra-renal TNF pathway will result in reduction in urinary MCP1/uCr and/or urinary TIMP1/uCr biomarker levels.

2. Inclusion of additional safety endpoints
Additional safety endpoints were added to the analysis that were not described in the protocol: AEs leading to premature discontinuation of study medication, proportion of participants with temperature ≥38.5˚C (indicating fever), and proportion of participants with new or worsening edema compared to baseline. They are standard safety parameters in this population.

Protocol: None

SAP: Section 4.2. Secondary Endpoints.

3. Incidence of adverse events leading to premature discontinuation of study medication, defined as the proportion of participants with treatment-emergent adverse events and the number of treatment-emergent adverse events that led to premature discontinuation of study medication, overall, by system organ classification, by relatedness to study medication, and by severity
4. Proportion of participants with abnormal laboratory tests for ALT, AST, total bilirubin, hemoglobin, hematocrit, and platelet count, proportion of participants with temperature ≥38.5˚C (indicating fever), and proportion of participants with new or worsening edema (over all body locations and by each location) over the course of the 10-week intervention period compared to baseline

3. Clarification of exploratory endpoints
The protocol included time to reduction in the two biomarkers, urinary MCP1/uCr and urinary TIMP1/uCr. The SAP clarifies that the trajectory of the biomarkers over the course of the study will provide the evaluation of the time to reduction.

Protocol: Section 4.2.3 EXPLORATORY ENDPOINTS.
Time to reduction of urinary MCP1/uCr and urinary TIMP1/uCr biomarkers from baseline

SAP: Section 4.3

2. Change from baseline to weeks 2, 4, and 8 in MCP1/uCr
3. Change from baseline to weeks 2, 4, and 8 in TIMP1/uCr
4. Percent change from baseline to weeks 2, 4, 8 and 10 in urinary MCP1/uCr
5. Percent change from baseline to weeks 2, 4, 8 and 10 in urinary TIMP1/uCr
6. Urinary MCP1/uCr values at baseline and weeks 2, 4, 8 and 10
7. Urinary TIMP1/uCr values at baseline and weeks 2, 4, 8 and 10
9. Correlation coefficient between uEGF/uCr at baseline with changes and percent changes in urinary MCP1/uCr and urinary TIMP1/uCr biomarker levels and in eGFR and UPCR values at week 10.

4. Change in analysis of the primary endpoints

The nonparametric approach was considered more appropriate for the small sample size.

Protocol: Section 10.3.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)**.**

The changes from baseline to week 10 in urinary MCP1/uCr and TIMP1/uCr levels will be tested using the paired t-test or Wilcoxon signed-rank test (if distributional assumptions are not met).

SAP: Section 9.1 Analyses of Primary Endpoints.

The changes from baseline to week 10 in urinary MCP1/uCr and urinary TIMP1/uCr levels will be tested using the Wilcoxon signed-rank test.

11. References

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12. List of Tables, Listings and Figures

All tables, listings and figures are saved in a separate file as supplementary material.

12.1 List of Tables

- Table 6. Duration of Adherence to Study Medication Summary. As-Treated Population
- Table 7. Demographic and Baseline Characteristics. As-Treated Population.
- Table 8. Change in urinary MCP1/uCr levels from Baseline to Week 10. As-Treated Population.
- Table 9. Change in urinary TIMP1/uCr levels from Baseline to Week 10. As-Treated Population.
- Table 10.1. Treatment-Emergent Serious Adverse Event (SAE) Summary. As-Treated Population.
- Table 10.2. Treatment-Emergent Serious Adverse Event (SAE) by System Organ Class and by Severity. As-Treated Population.
- Table 10.3. Treatment-Emergent Serious Adverse Events (SAE) by System Organ Class and by Relatedness to Study Medication. As-Treated Population.
- Table 11.1. Non-Serious Treatment-Emergent Adverse Event (AE) Summary. As-Treated Population.
- Table 11.2. Non-Serious Treatment-Emergent Adverse Events (AE) by System Organ Class and by Severity. As-Treated Population.
- Table 11.3. Non-Serious Treatment-Emergent Adverse Events (AE) by System Organ Class and by Relatedness to Study Medication. As-Treated Population.
- Table 12.1. Treatment-Emergent Adverse Events Leading to Premature Discontinuation of Study Medication Summary. As-Treated Population.
- Table 12.2. Treatment-Emergent Adverse Events Leading to Premature Discontinuation of Study Medication by System Organ Class and by Severity. As-Treated Population.
- Table 12.3. Non-Serious Treatment-Emergent Adverse Events Leading to Premature Discontinuation of Study Medication by System Organ Class and by Relatedness to Study Medication. As-Treated Population.
- Table 13. Proportion of participants with temperature ≥38.5˚C (indicating fever). As-Treated Population.
- Table 14. Proportion of participants with new or worsening edema (over all body locations and by each location) over the course of the 10-week intervention period compared to baseline. As-Treated Population.
- Table 15. Percent change in eGFR from baseline to week 10. As-Treated Population.
- Table 16. Percent change in UPCR from baseline to week 10. As-Treated Population.
- Table 17. Proportion of participants achieving a nadir UPCR <1.5 g/g and at least a 40% reduction from baseline at week 10. As-Treated Population.
- Table 18.1. Proportion of Participants with Abnormal Laboratory Tests for ALT, AST, Total Bilirubin, Hemoglobin, Hematocrit, and Platelet Count Over the Course of the 10-Week Intervention Period. As-Treated Population.
- Table 18.2. Shift Table for Normal and Abnormal Laboratory Test Changes from Screening to Weeks 0, 2, 8, 10, and 14 in ALT, AST, Total Bilirubin, Hemoglobin, Hematocrit, and Platelet Count. As-Treated Population.
- Table 19. Correlations between TNF activation score and urinary MCP1/uCr and TIMP1/uCr biomarker levels at baseline. As-Treated Population with Kidney Biopsy Tissue Collected within 12 Months of Study Baseline.

- Table 20.1. Change from baseline to weeks 2, 4, and 8 in urinary MCP1/uCr. As-Treated Population.
- Table 20.2. Percent change from baseline to weeks 2, 4, 8, and 10 in urinary MCP1/uCr. As-Treated Population.
- Table 20.3. Urinary MCP1/uCr values at baseline and weeks 2, 4, 8, and 10. As-Treated Population.
- Table 21.1. Change from baseline to weeks 2, 4, and 8 in urinary TIMP1/uCr. As-Treated Population.
- Table 21.2. Percent change from baseline to weeks 2, 4, 8, and 10 in urinary TIMP1/uCr. As-Treated Population.
- Table 21.3. Urinary TIMP1/uCr values at baseline and weeks 2, 4, 8, and 10. As-Treated Population.
- Table 22. Change in uEGF/uCr levels from baseline to weeks 2, 4, 8, and 10. As-Treated Population.
- Table 23. Correlation coefficient between uEGF/uCr at baseline with changes and percent changes in urinary MCP1/uCr and urinary TIMP1/uCr biomarker levels and in eGFR and UPCR values at week 10. As-Treated Population.
- Table 24. Changes in FSGS-MCD Patient Reported Outcome (PRO) score from baseline to weeks 2, 4, 8 and 10. As-Treated Population.

12.2 List of Listings

- Listing 1. Dosing Data. As-Treated Population.
- Listing 2. Reasons for Permanent Discontinuation of Study medication. As-Treated Population.
- Listing 3. Serious Adverse Events (SAEs). As-Treated Population.
- Listing 4. Non-Serious Adverse Events (AEs). As-Treated Population.
- Listing 5. Deaths. As-Treated Population.
- Listing 6. Adverse Events Leading to Premature Discontinuation of Study Medication. As-Treated Population.
- Listing 7. Protocol Deviations. All Subjects.

12.3 List of Figures

- Figure 1. CONSORT diagram. All Subjects.
- Figure 2.1. Spaghetti plot of changes from baseline to weeks 2, 4, 8, and 10 in urinary MCP1/uCr. As-Treated Population.
- Figure 2.2. Spaghetti plot of percent changes from baseline to weeks 2, 4, 8, and 10 in urinary MCP1/uCr. As-Treated Population.
- Figure 2.3. Spaghetti plot of urinary MCP1/uCr at baseline and weeks 0, 2, 4, 8, and 10. As-Treated Population.
- Figure 3.1. Spaghetti plot of changes from baseline to weeks 2, 4, 8, and 10 in urinary TIMP1/uCr. As-Treated Population.

- Figure 3.2. Spaghetti plot of percent changes from baseline to weeks 2, 4, 8, and 10 in urinary TIMP1/uCr. As-Treated Population.
- Figure 3.3. Spaghetti plot of urinary TIMP1/uCr at baseline and weeks 2, 4, 8, and 10. As-Treated Population.
- Figure 4.1. Spaghetti plot of changes from baseline to weeks 2, 4, 8, and 10 in eGFR. As-Treated Population.
- Figure 4.2. Spaghetti plot of percent changes from baseline to weeks 2, 4, 8, and 10 in eGFR. As-Treated Population.
- Figure 4.3. Spaghetti plot of eGFR at baseline and weeks 2, 4, 8, and 10. As-Treated Population.
- Figure 5.1. Spaghetti plot of changes from baseline to weeks 2, 4, 8, and 10 in UPCR. As-Treated Population.
- Figure 5.2. Spaghetti plot of percent changes from baseline to weeks 2, 4, 8, and 10 in UPCR. As-Treated Population.
- Figure 5.3. Spaghetti plot of UPCR at baseline and weeks 2, 4, 8, and 10. As-Treated Population.
- Figure 6.1. Spaghetti plot of participants with at least one abnormal laboratory value: ALT. As-Treated Population.
- Figure 6.2. Spaghetti plot of participants with at least one abnormal laboratory value: AST. As-Treated Population.
- Figure 6.3. Spaghetti plot of participants with at least one abnormal laboratory value: Total Bilirubin. As-Treated Population.
- Figure 6.4. Spaghetti plot of participants with at least one abnormal laboratory value: Hemoglobin. As-Treated Population.
- Figure 6.5. Spaghetti plot of participants with at least one abnormal laboratory value: Hematocrit. As-Treated Population.
- Figure 6.6. Spaghetti plot of participants with at least one abnormal laboratory value: Platelet Count. As-Treated Population.
- Figure 7.1. Scatterplot of TNF activation score and baseline urinary MCP1/uCr. As-Treated Population.
- Figure 7.2. Scatterplot of TNF activation score and baseline urinary TIMP1/uCr. As-Treated Population.
- Figure 8. Spaghetti plot of participants' uEGF/uCr from baseline to week 10. As-Treated Population.
- Figure9.1. Scatterplot of baseline uEGF/uCr and change from baseline to week 10 in urinary MCP1/uCr. As-Treated Population.
- Figure 9.2. Scatterplot of baseline uEGF/uCr and percent change from baseline to week 10 in urinary MCP1/uCr. As-Treated Population.
- Figure 10.1. Scatterplot of baseline uEGF/uCr and change from baseline to week 10 in urinary TMP1/uCr. As-Treated Population.
- Figure 10.2. Scatterplot of baseline uEGF/uCr and percent change from baseline to week 10 in urinary TMP1/uCr. As-Treated Population.

- Figure 11.1. Scatterplot of baseline uEGF/uCr and change from baseline to week 2, 8, and 10 in eGFR. As-Treated Population.
- Figure 11.2. Scatterplot of baseline uEGF/uCr and percent change from baseline to week 2, 8, and 10 in eGFR. As-Treated Population.
- Figure 12.1. Scatterplot of baseline uEGF/uCr and change from baseline to week 2, 8, and 10 in UPCR. As-Treated Population.
- Figure 12.3. Scatterplot of baseline uEGF/uCr and percent change from baseline to week 2, 8, and 10 in UPCR. As-Treated Population.