Precision Medicine Proof of Concept for TNF Inhibition in FSGS and Treatment Resistant Minimal Change Disease

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Sponsored by: University of Michigan
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LIST OF ABBREVIATIONS

DSMB	Data Safety and Monitoring Board
EGF	Epidermal Growth Factor
ESKD	End Stage Kidney Disease
FSGS	Focal Segmental Glomerulosclerosis
MCP1	Monocyte Chemoattractant Protein 1
TIMP1	Tissue Inhibitor of Metalloproteinase 1
MCD	Minimal Change Disease
TNF	Tumor Necrosis Factor
UPC	Urine Protein:Creatinine Ratio

STATEMENT OF COMPLIANCE

This trial will be conducted with Good Clinical Practice (GCP) and in accordance with the Code of Federal Regulations on the Protection of Human Subjects (21 CFR Part 50). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without documented approval from the applicable Institutional Review Boards (IRBs), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

PROTOCOL SUMMARY

Title:	Precision Medicine Proof of Concept for TNF inhibition in FSGS and Treatment Resistant Minimal Change Disease
Précis:	A Proof of Concept, multi-center, open label, clinical trial addressing the treatment of patients with focal segmental glomerulosclerosis (FSGS) or treatment resistant minimal change disease (TR-MCD). Up to 8 patients with intra-renal tumor necrosis factor (TNF) up-regulation will be treated with 8 weeks of adalimumab therapy. The primary assessment will be of the change in target engagement biomarker levels of urinary TIMP1/Cr or MCP1/Cr from baseline to study week 10. Key secondary outcomes include safety measures, serious adverse events, and adverse events.
Objectives:	This study is proposed to test the hypothesis that FSGS and TR-MCD patients with up-regulation of the TNF pathway can be identified through intra-renal gene expression profiling and assay of urine MCP1/Cr and TIMP1/Cr levels by ELISA. Furthermore, this study will 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 MCP1/Cr and/or TIMP1/Cr biomarker levels.
Endpoint	Primary Endpoint: The change in urine MCP1/Cr and TIMP1/Cr levels from baseline to study week 10, i.e. 2 weeks after the final dose of adalimumab Secondary Endpoints (1) Incidence of serious adverse events, adverse events and abnormal laboratory tests (2) Percent change of eGFR from baseline to week 10 (3) Percent change in urine protein:creatinine (UPC) from baseline to week 10 (4) Proportion of participants achieving a nadir UPC less than 1.5 g/g and at least a 40% reduction from baseline at week 10. Exploratory Endpoints (1) Correlation coefficient between intra-renal gene expression of TNF and urinary MCP1/Cr and TIMP1/Cr biomarker levels at baseline (2) Time to reduction of urinary MCP1/Cr and TIMP1/Cr biomarkers from baseline (3) Change in urinary EGF biomarker levels between baseline and week 10 (4) Change in Quality of Life score between baseline and week 10.
Population:	Up to eight patients with kidney biopsy confirmed FSGS or TR- MCD, increased urinary excretion of biomarkers of TNF activation (MCP1/Cr and TIMP1/Cr) at study screening, eGFR>30 ml/min/1.73 m^2 at enrollment, urine protein:creatinine (UPC) ratio ≥1.5 g/g at enrollment, age between

6-80 years, inclusive, and stable therapy with angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and oral immunosuppression agents for at least 30 days prior to enrollment will be enrolled and entered into the intervention phase of the study. Additional eligibility criteria are listed in the inclusion and exclusion criteria section below.

Phase: Proof of Concept/Phase 1b/2a

Number of Sites enrolling participants: Up to 8 sites in the USA will participate in this study.

Description of Study Agent :

Humira, adalimumab
Manufactured by Abbvie
Subcutaneous
A total of 5 doses will be administered during the trial at weeks 0, 2, 4, 6 and 8. Dosing will be based on weight:

Subject Weight	Adalimumab dose every other week
15kg to <30kg	20mg
≥30kg	40mg

Study Duration: Approximately 14 months

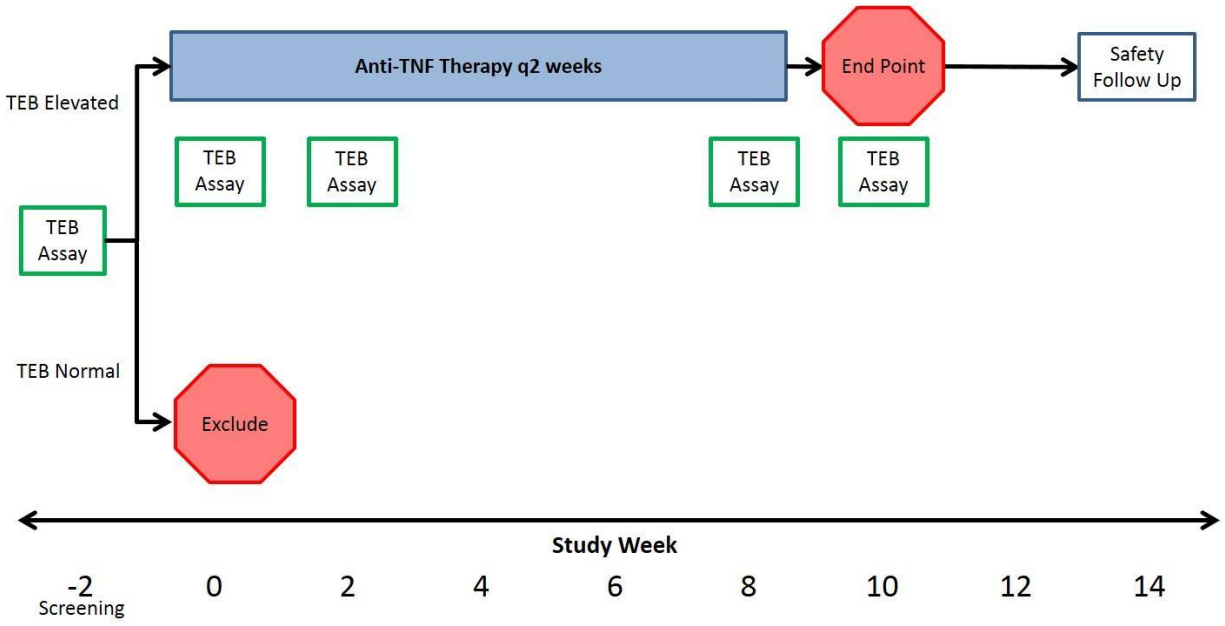
1. 7 month enrollment phase
2. 2 week screening, 8 week intervention and 6 week post intervention observation period for a total of 14 week participation per patient
3. 3 month analysis phase

Participant Duration: 16 weeks

1. 2 week screening period
2. 8 week intervention period
3. 6 week follow up post treatment

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

1 KEY ROLES

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

FSGS is a pattern of kidney injury that manifests as isolated, asymptomatic proteinuria or nephrotic syndrome. It is a serious condition and may lead to kidney failure in up to 50% of patients over a five to ten year period. Although primary FSGS is a rare entity, the incidence of the disease has been steadily rising over the last 30 years and it is generally the most common form of primary glomerular disease in all patient subgroups. Moreover, it one of the leading causes of chronic and end-stage kidney disease (ESKD), accounting for 5% of adult and 12% of pediatric ESRD cases.

The first-line therapy for primary FSGS, especially in those with nephrotic-range proteinuria, is usually corticosteroids. In patients who are unresponsive to steroids, calcineurin inhibitors (CNIs) are the recommended second-line therapy according to current KDIGO clinical guidelines[1]. Overall, approximately 20% of patients with FSGS will have a favorable response to corticosteroids and up to 40% will have clinical benefit from treatment with a calcineurin inhibitor. Other agents that have been tried with marginal success include mycophenolate mofetil and rituximab. Patients with treatment-resistant FSGS are at higher risk of progressing to ESKD.

Adjuvant therapies designed to reduce proteinuria in primary FSGS are mainly focused on agents that inhibit renin-angiotensin system including angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), or aldosterone antagonists. This is based on evidence that reduction in proteinuria in response to treatment is renoprotective and an independent predictor of kidney survival. These clinical data underscore the limitations of current therapy for FSGS and highlight the need to develop new strategies to safely and effectively achieve a reduction in proteinuria, either a partial or complete remission, preservation of kidney function and minimization of the adverse effects due to reduction of exposure to agents with little expectation of efficacy.

While there are many insults that result in FSGS, molecular profiling can result in identifiable subgroups of affected patients with discrete pathophysiological mechanisms that may inform a precision medicine driven next generation of treatment strategies for individuals with FSGS and treatment resistant MCD.

2.2 RATIONALE

Based on molecular profiling, informed therapy selection targeting the underlying disturbance has been used successfully in health care and biomedical research. When successful, this forms the basis of precision medicine for the treatment of selected patient populations.

The Nephrotic Syndrome Study Network (NEPTUNE, PI M. Kretzler) study investigators have used genome wide expression profiles of renal biopsy specimens available from more than 250 patients to identify molecular subgroups of NS[2]. Three distinct patient subgroups were identified and associated with long-term outcomes. Molecular pathway analysis of the subgroups with TR-MCD and FSGS showed activation of TNF as defined by causal inference network analysis of established downstream pathway members, including TIMP1 and MCP1 in one of the 3 subgroups. These patients presented with higher levels of proteinuria, lower levels of GFR, and more rapid progression of their glomerular disease. A TNF Z-score was computed for each NEPTUNE participant with MCD and FSGS. The score is the average Z-score of gene expression level across 145 TNF pathway genes from the tubulointerstitial compartment

of each participant's renal biopsy. Urinary MCP1 and TIMP1 were measured by ELISA from their baseline study visit and normalized to urine creatinine. Correlations of TNF gene expression Z-score with urinary biomarkers are shown in Figure 1 and distributions of these measures in boxplots from TNF z-score $\leq$ 0 vs > 0 are plotted in Figure 2.

Figure 2: Scatter plot of TNF z- score and urinary TIMP1/Cr, A: All FSGS and MCD patients (corr = 0.47, P<0.001) TNF z-score and urinary MCP1/Cr, B: All FSGS and MCD patients (corr = 0.51, P<0.001) TNF z-score and urinary TIMP1/Cr ng/mg. (L. Mariani, et al 2017)

A. B.

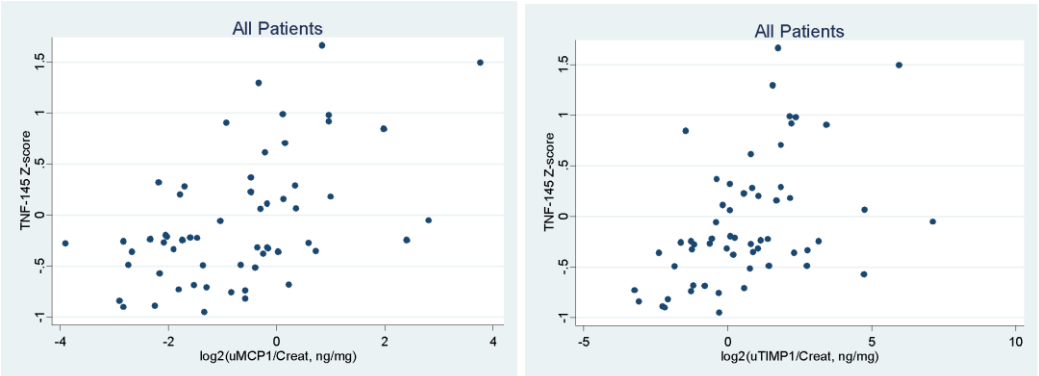
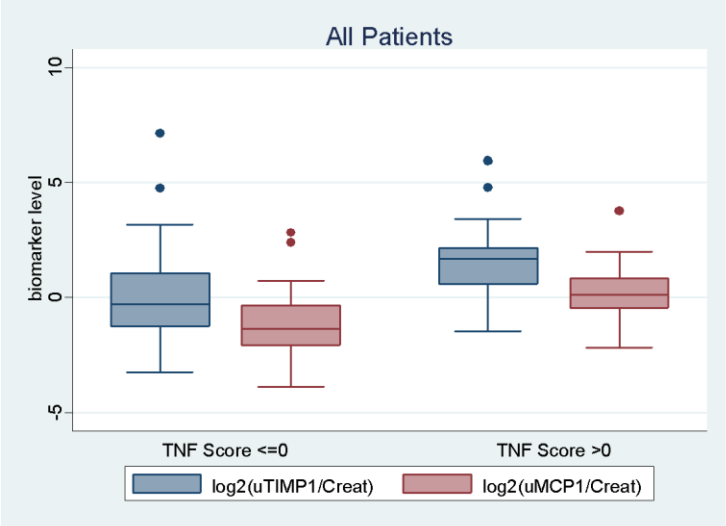


Figure 3: Distribution of biomarker level by TNF Z-score category. Difference in means was statistically significant (TIMP1, p-value 0.009; MCP1 p-value 0.001) (L. Mariani, et al 2017)



A logistic model was fit with the outcome of TNF Z-score >0 to assess the predictive ability of urinary biomarkers individually and combined. Results are shown in Table 1 for the subgroup of participants with eGFR >45. Model 3, which included both biomarkers as predictors had the highest positive predictive value and correct classification. Correctly classified was calculated by assigning those with an estimated probability of disease >= 50% as having disease and then calculating the % that are correctly classified as having and not having disease (true TNF Z-score > vs <= 0) using a patient sample with eGFR > 45 and either FSGS or MCD.

Table 1: Results of Multivariable Logistic Models of TNF Z-score >0 in NEPTUNE FSGS and MCD participants with eGFR>45

Patients with eGFR >45 (n = 49; 13 Events)	Model 1 c-statistic 0.79 PPV 33% correctly classified 69%		Model 2 c-statistic 0.79 PPV 67% Correctly classified 78%		Model 3 c-statistic 0.82 PPV 75% Correctly Classified 82%	
	OR	P-value	OR	P-value	OR	P-value
Log2(uTIMP/Cr)	1.79	0.017	--	--	1.54	0.07
Log2(uMCP/Cr)	--	--	2.31	0.008	1.81	0.08

Because both biomarkers contributed to greater predictive accuracy, both will be measured for patient selection into the clinical trial. The coefficients from the multivariable model will be used to calculate the odds of having a TNF Z-score >0 for each person. Odds are then converted to probability and patients with a probability >= 50% of having a positive TNF Z-score would be eligible for enrollment. Mathematically, the following equations will be used to calculate the probabilities for study inclusion:

1. Constant + [log2(uTIMP1/Cr) * Coef1] + [log2(uMCP1/Cr) * Coef2] = log Odds
2. Exp(log Odds) = Odds
3. Probability = Odds / (1 + Odds)

Using the following coefficients and constant from Model 3 in Table 2:

```
Logistic regression                             Number of obs   =         49
                                                LR chi2(2)      =      13.61
                                                Prob > chi2     =     0.0011
Log likelihood = -21.542865                     Pseudo R2       =     0.2401
```

tnf_cat	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
logMCPI_uCR_ngmg	.59214	.3381858	1.75	0.080	-.0706919	1.254972
log2timp1_uCr_ngmg	.4325066	.2401973	1.80	0.072	-.0382715	.9032848
_cons	-1.001269	.5091692	-1.97	0.049	-1.999222	-.0033157

i.e., log Odds = -1.001269 + 0.59214 * log2(uTIMP1/Cr) +0.4325066 * log2(uMCP1/Cr).

In NEPTUNE, these molecular pathway-defined patient subgroups characterized by high intra-renal TNF activation have documented persistence in proteinuria and reduced kidney function during prospective observation. Past studies of TNF inhibition in unselected treatment resistant FSGS patients demonstrated a reduction in proteinuria and preservation of estimated glomerular filtration rate (eGFR) in approximately 25% of studied patients[3]. In the FONT study, there were no clinical or laboratory findings that predicted response patterns to TNF-inhibition. This study also identified the accelerated clearance of the TNF monoclonal antibody in this patient population with significant proteinuria [4-6]. On average the Area Under the Curve (AUC) was 50% lower than that of comparison populations without proteinuria. In a parallel study of a small therapeutic compound, similar reduction in AUC in proteinuric patients with FSGS was documented suggesting that both large and small molecules are cleared more rapidly in patients with proteinuric kidney diseases such as FSGS and TR-MCD[7]. The recent findings within the NEPTUNE investigative community suggest that we may now have a mechanism to identify TNF-target relevant patients with primary nephrotic syndrome and use prior evidence to select a likely drug dosing regimen for this patient population.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

SERIOUS INFECTIONS

Some patients treated with Humira (adalimumab) are at increased risk for developing serious infections that may lead to hospitalization or death. Most patients who developed these infections were taking concomitant immunosuppressants such as methotrexate or corticosteroids.

Reported infections include:

Active tuberculosis (TB), including reactivation of latent TB. Patients with TB have frequently presented with disseminated or extrapulmonary disease. Study participants will be tested for TB before starting Humira.

Invasive fungal infections, including histoplasmosis, coccidioidomycosis, candidiasis, aspergillosis, blastomycosis, and pneumocystosis. Patients with histoplasmosis or other invasive fungal infections may present with disseminated, rather than localized, disease. Antigen and antibody testing for histoplasmosis may be negative in some patients with active infection. Consider empiric anti-fungal therapy in patients at risk for invasive fungal infections who develop severe systemic illness.
Bacterial, viral, and other infections due to opportunistic pathogens, including Legionella and Listeria.

MALIGNANCIES

Lymphoma and other malignancies, some fatal, have been reported in children and adolescent patients treated with TNF blockers, including Humira. Postmarketing cases of hepatosplenic T-cell lymphoma (HSTCL), a rare type of T-cell lymphoma, have been reported in patients treated with TNF blockers, including Humira. These cases have had a very aggressive disease course and have been fatal. The majority of reported TNF blocker cases have occurred in patients with Crohn's disease or ulcerative colitis and the majority were in adolescent and young adult males. Almost all of these patients had received treatment with azathioprine or 6-mercaptopurine concomitantly with a TNF blocker at or prior to diagnosis. It is uncertain whether the occurrence of HSTCL is related to use of a TNF blocker or a TNF blocker in combination with these other immunosuppressants.

HYPERSENSITIVITY

Anaphylaxis and angioneurotic edema have been reported following Humira administration. If a serious allergic reaction occurs, stop Humira and institute appropriate therapy.

HEPATITIS B VIRUS REACTIVATION

Use of TNF blockers, including Humira, may increase the risk of reactivation of hepatitis B virus (HBV) in patients who are chronic carriers. Some cases have been fatal. Study participants will be screened for HBV. Those with prior evidence of HBV infection will not be allowed to participate in this study.

NEUROLOGIC REACTIONS

TNF blockers, including Humira, have been associated with rare cases of new onset or exacerbation of central nervous system and peripheral demyelinating diseases, including multiple sclerosis, optic neuritis, and Guillain-Barré syndrome. Participants with these disorders will be excluded from the study. If any of these disorders develop, Humira will be discontinued.

HEMATOLOGIC REACTIONS

Rare reports of pancytopenia, including aplastic anemia, have been reported with TNF blockers. Medically significant cytopenia has been infrequently reported with Humira.

CONGESTIVE HEART FAILURE
Worsening and new onset congestive heart failure (CHF) has been reported with TNF blockers. Cases of worsening CHF have been observed with Humira. Participants with known CHF will be excluded from the study.

AUTOIMMUNITY
Treatment with Humira may result in the formation of autoantibodies and, rarely, in development of a lupus-like syndrome. Discontinue treatment if symptoms of a lupus-like syndrome develop.

IMMUNIZATIONS
Patients on Humira should not receive live vaccines. Pediatric patients, if possible, should be brought up to date with all immunizations before initiating Humira therapy. Adalimumab is actively transferred across the placenta during the third trimester of pregnancy and may affect immune response in the in utero exposed infant. The safety of administering live or live-attenuated vaccines in infants exposed to Humira in utero is unknown. Risks and benefits should be considered prior to vaccinating (live or live-attenuated) exposed infants.

ADVERSE REACTIONS
The most common adverse reactions in Humira clinical trials (>10%) were: infections (e.g., upper respiratory, sinusitis), injection site reactions, headache, and rash.

Directly relevant to this protocol**,** two small studies of TNF-inhibition with monoclonal antibody therapy in children and adults with multi-drug resistant FSGS have been reported.

Table 3. FONT trial summary of reported adverse events in 17 treated patients

Event	**Frequency (% of patients reporting)**
Severe Adverse Events	
Infection with hospitalization	30% (5/17)
Malignancy	0
Death	0
Other Adverse Events	
GI	24% (4/17)

Approaches to reduce risks associated with study drugs
Due to the frequent and sometimes serious risks associated with the study drug, specific measures have been instituted to minimize these risks and improve the risk:benefit ratio for participants. Based on past experience with the administration of immunosuppressive therapies to patients with FSGS and TR-MCD, the inclusion and exclusion criteria have been selected to enrich for subjects most-likely to benefit from therapy (see Inclusion Criteria) and to avoid those subjects who are less-likely to benefit or more likely to experience treatment-related toxicity (see Exclusion Criteria). Pregnancy status will be monitored throughout the study. In addition, frequent and routine laboratory monitoring will be carried out throughout the study protocol. The Clinical Investigator is responsible for reviewing and acting on adverse events in accordance with section 6.1.7.

2.3.2 POTENTIAL BENEFITS

In previous studies 4 of 17 (24%) of unselected patients with multi-drug resistant FSGS experienced a 50% reduction in proteinuria and preservation of eGFR with the use of TNF-inhibitor with monoclonal antibody therapy over a 16 week period[5].

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 urine MCP1/Cr and TIMP1/Cr 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 urine MCP1/Cr and TIMP1/Cr levels by ELISA.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This Proof of Concept, multi-center, open label, clinical trial addresses the treatment of patients with clinically active FSGS or TR-MCD. Up to eight patients with urinary MCP1/Cr and TIMP1/Cr urinary biomarker levels indicating intra-renal tumor necrosis factor (TNF) upregulation will be treated with 8 weeks of adalimumab therapy to assure a minimum evaluable study sample of six patients. The Humira will be dosed based on weight and administered subcutaneously every two weeks for 8 weeks (5 total doses). The urinary biomarkers MCP1/Cr and TIMP1/Cr will be assessed throughout the study. The primary assessment will be of the reduction in target engagement biomarker levels of urinary TIMP1/Cr or MCP1/Cr from baseline to study week 10. Key secondary outcomes include safety measures, serious adverse events, and adverse events.

4.2 ENDPOINTS

4.2.1 PRIMARY ENDPOINT

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

4.2.2 SECONDARY ENDPOINTS

(1) Incidence of serious adverse events, adverse events and abnormal laboratory tests
(2) Percent change of eGFR from baseline to week 10
(3) Percent change in UPC from baseline to week 10
(4) Proportion of participants achieving a nadir UPC less than 1.5 g/g and at least a 40% reduction from baseline at week 10.

4.2.3 EXPLORATORY ENDPOINTS

(1) Correlation coefficient between intra-renal gene expression of TNF and urinary MCP1/Cr and TIMP1/Cr biomarker levels at baseline
(2) Time to reduction of urinary MCP1/Cr and TIMP1/Cr biomarkers from baseline
(3) Change in urinary EGF biomarker levels between baseline and week 10
(4) Change in Quality of Life score between baseline and week 10.

Endpoint Rationale:
The target engagement biomarkers, urine MCP1/Cr and TIMP1/Cr, will be 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 will reflect target engagement. Neither eGFR nor urinary protein will be a primary endpoint measure. However, we will measure eGFR and urinary protein to provide estimates for future trials

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 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 (MCP1/Cr and/ or TIMP1/Cr) at study screening
4. eGFR>30 ml/min/1.73 m^2 at screening
5. Urine protein: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 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/mm3; Hg <9 g/dL; Platelet count <150,000/mm3 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 STRATEGIES FOR RECRUITMENT AND RETENTION

Patients will be recruited from the active population of patients with FSGS or TR-MCD w at participating sites. TR-MCD is defined as persistent proteinuria despite treatment. Following informed consent and when appropriate, assent, a screening visit will be conducted. Patients with elevated TIMP1/Cr and MCP1/Cr levels and meeting all other eligibility criteria will be scheduled to receive adalimumab every two weeks for 8 weeks (5 total doses). It is anticipated that only 25% of screened patients will be eligible for study participation based on TIMP1/Cr and MCP1/Cr levels. Eight eligible patients will begin study intervention with a goal to have a minimum of 6 patients fully completing the intervention phase and therefore contributing to the final analysis of the primary endpoint after 5 doses of adalimumab over an 8 week period.
Patients will be compensated for study participation to offset costs related to travel to study visits and incidentals.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Patients participating in this study may withdraw or be terminated for the following anticipated reasons.

- Ongoing participation in this research study is voluntary and patients may leave at any time without an identified cause or the need for explanation.
- Patients experiencing any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation such that continued participation in the study would not be in the best interest of the participant (as determined by the patient and/or study physician).
- Pregnancy and/or breast feeding
- Absolute contraindications to continued therapy with Humira.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Should a participant prematurely withdraw or be withdrawn from the study for any reason, he/she will be asked to return for key outcome determinations at study week 10 and final safety assessment at study week 14. At the week 10 visit any medication prescribed by their treating physician since leaving the study will be recorded, in addition to other required assessments. The participant's reason for withdrawal and their status at the time of withdrawal will be recorded. Should the participant die, the cause of death will be determined and recorded, if possible. This additional data will be utilized in the statistical analysis of the primary and secondary study outcomes as defined by the statistical plan.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

The investigators and/or other regulatory bodies reserve the right to terminate the study at any time. If this becomes necessary, appropriate procedures for continuing long-term follow-up and assuring the

adequate treatment and safety of the participating subjects will be arranged after review and approval by the Institutional Review Boards.

The DSMB will also provide external oversight concerning the safety and scientific integrity of the study for the duration of the clinical trial. The DSMB will review the progress of the study toward meeting enrollment goals, adverse and serious adverse event profiles, at regular intervals to occur at least twice annually. The DSMB may recommend at any time that the study should be terminated due to drug toxicity, patient safety, poor compliance and/or futility considerations. In such cases, their recommendations will be reviewed and discussed with the Executive Committee, which will make a final determination.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

Each site will be responsible for acquiring and distributing Humira to the study participants in accordance with all local institutional policies governing the prescription of drugs for research purposes.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Humira is supplied as a sterile, preservative-free solution of adalimumab for subcutaneous administration. The drug product is supplied as either a single-use, prefilled pen (Humira Pen), as a single-use, 1 mL prefilled glass syringe, or as a single-use institutional use vial. Enclosed within the pen is a single-use, 1 mL prefilled glass syringe. The solution of Humira is clear and colorless, with a pH of about 5.2.

Formulation and Strength: Any of the commercially available Humira formulations may be used in this study:

- Each 40 mg/0.4 mL prefilled syringe or prefilled pen delivers 0.4 mL (40 mg) of drug product.
- Each 40 mg/0.8 mL prefilled syringe or prefilled pen delivers 0.8 mL (40 mg) of drug product.
- Each 20 mg/0.2 mL prefilled syringe delivers 0.2 mL (20 mg) of drug product.
- Each 20 mg/0.4 mL prefilled syringe delivers 0.4 mL (20 mg) of drug product.

Labeling On-site: At the Clinical Site Research Pharmacy, patient specific labeling will be applied to Humira as this study drug will be provided in an open-label format to study participants. In addition to patient specific labeling information, the drug name, concentration, and date of preparation will be on the label prior to distribution. In accordance with §312.6 the drug will also contain a label with the statement “Caution: New Drug – Limited by Federal (or United States) law to investigational use.”

6.1.3 PRODUCT STORAGE AND STABILITY

Humira must be refrigerated at 36°F to 46°F (2°C to 8°C). DO NOT FREEZE. Do not use if frozen even if it has been thawed. Store in original carton until time of administration to protect from light. If needed, for example when traveling, Humira may be stored at room temperature up to a maximum of 77°F

(25°C) for a period of up to 14 days, with protection from light. Humira should be discarded if not used within the 14-day period. Do not use beyond the expiration date on the container. Record the date when Humira is first removed from the refrigerator in the spaces provided on the carton and dose tray. Do not store Humira in extreme heat or cold.

6.1.4 DOSING

Humira will be dosed based on weight at study weeks 0, 2, 4, 6, and 8. This dose has been selected in accordance with the recommended dose of Humira for patients 2 years of age and older with polyarticular juvenile idiopathic arthritis.

Subject Weight	Humira dose (every other week)
15kg to <30kg	20mg
≥30kg	40mg

6.1.5 ROUTE OF ADMINISTRATION

Humira will be given subcutaneously at every other week for 8 weeks (5 total doses).

6.1.6 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

If there is evidence of toxicity in abnormal laboratory tests or clinical adverse events that, in the judgment of the Investigator, could place the subject at increased risk, study drug administration should be interrupted and the investigator should notify the trial principal investigator at University of Michigan. Subjects may be considered eligible to receive further study medication treatment only after discussion with the study principal investigator at the University of Michigan.

6.1.7 DURATION OF THERAPY

The total duration of therapy will be 8 weeks with a single dose study drug given subcutaneously at study weeks 0, 2, 4, 6, and 8.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

6.2.1 RESPONSIBILITIES OF THE CLINICAL SITE PHARMACY

The dispensing pharmacy at each site will maintain an independent drug accountability log for all Humira that is dispensed for this study. The log must include at a minimum: the date dispensed, drug expiration date, lot number or other identifying number, and study participant receiving the drug.

6.2.2 THE SITE INVESTIGATOR RESPONSIBILITY

The site team will maintain a drug accountability log for each study patient to document subject number, visit number, date medication dispensed, and date medication returned. Any unused medication should be returned to the study site, documented, and disposed of in accordance with local policy.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

Biopsy report: The biopsy report from the qualifying kidney biopsy will have personal identifiers redacted, labeled with the study identification number and then submitted into the study database for review and confirmation of eligible pathology of FSGS or MCD.
Archived Kidney Biopsy Tissue: 4 unstained slides from archived paraffin block kidney tissue will be submitted for gene expression profiling when available. Patients who have submitted tissue from a kidney biopsy as part of NEPTUNE study participation will not be required to have additional tissue submitted as part of this study.
Health Related Quality of Life (HRQOL): The FSGS-MCD Patient Reported Outcome (PRO) assessment will be assessed in accordance with the schedule of events table (7.2.5). The FSGS-MCD PRO assessment should be completed by the participant at each in-person study visit.
Adverse events: Adverse event assessments will be done at each study visit as detailed in Section 9.
Complete medical history: The complete medical history will include a review of pertinent medical records covering details related to the patient's history of FSGS or TR-MCD provided by the patient and/or their referring physician(s).
Physical examination: A physical exam will include a review of systems and current physical findings
Interval medical history: A review of medications, interval symptoms, and any changes to the patient's medical history will be reviewed and documented.
Vital signs: Vital signs will include: blood pressure, temperature (^{o}C), height (cm), and weight (kg) will be measured.
Pregnancy Testing: For study participants of child-bearing potential, a urine pregnancy test will be performed at screening and each study visit prior to adalimumab administration.
Clinical Laboratories: A comprehensive set of laboratory results will be performed on blood and urine samples to evaluate any toxicity or adverse events associated with FSGS or TR-MCD or its treatment with the study drug.

- **Comprehensive metabolic panel (Comp)**: including serum electrolytes, BUN, creatinine, glucose, albumin, ALT, AST, alkaline phosphatase, total bilirubin, cholesterol, total protein
- **Complete blood count with differential (CBCD)**: including WBC, hemoglobin, hematocrit, platelet count, and differential cell count.
- **Urine protein/creatinine ratio:** 24-hour urine collection preferred, but first morning urine or spot urine is acceptable

Assessment of Infections:

- **Hepatitis B:** HBsAg, HBcAb IgG testing will be conducted at screening for all potentially eligible participants unless hepatitis screening has been conducted clinically within the past 45 days with documentation of results.
- **Hepatitis C:** HCAb screen will be conducted at screening for all potentially eligible participants unless hepatitis screening has been conducted clinically within the past 45 days with documentation of results.
- **HIV -1/HIV-2** antibody differentiation immunoassay will be conducted at screening for all potentially eligible participants unless HIV screening has been conducted clinically within the past 45 days with documentation of results.
- **Tuberculosis:** Quantiferon for tuberculosis should be measured unless tuberculosis screening has been conducted clinically within the past 45 days with documentation of results.

Serology:

- **Antinuclear cytoplasmic autoantibody (ANA):** should be measured at screening unless conducted clinically within the past 45 days with documentation of results.

Urine collection for target engagement biomarkers: A spot urine sample will be collected at each in person study visit before the administration of the study drug.
Optional Biological Specimen Repository: 10ml of Blood (serum, plasma, RNA and DNA (8.5 ml at the screening visit only)) and urine samples will be collected at each study visit for storage at the University of Michigan Biorepository. The specimens will be made available for approved investigations for future kidney disease related research.
Study drug reconciliation: Document subject number, visit number, date medication dispensed, and lot number at each visit in accordance with section 6.2.
Study drug administration: At week 0 the study team will administer Humira to the subject, and monitor the subject for 15 minutes post injection, recording blood pressure and heart rate at 15 minutes post injection. Subject/guardian will be taught how to administer Humira to themselves or their child. At week 2, the subject/guardian should administer Humira while study staff observes to ensure proper technique. An ice pack may be applied to the injection site to alleviate pain associated with the injection as needed.

7.2 STUDY SCHEDULE

The complete schedule for study visits is detailed below and summarized in the Study Flow Diagram (Figure 1) and Table of Study Visits (see below Schedule of Events, Section 7.2.5)

7.2.1 SCREENING VISIT

The screening visit will take place preferably within 2 weeks, but no more than 6 weeks prior to the baseline visit (Week 0)

At the screening visit:

a. Patient/Parent provides written informed consent and HIPAA authorization prior to any evaluation or testing. Minor participants should provide assent in accordance with IRB guidelines
b. A review of existing medical records and tests, provided by the patient or referring physician, will be performed to document the history of FSGS or TR-MCD and general medical history.
c. Vital signs including height, weight, temperature and blood pressure are obtained.
d. The patient undergoes a physical examination.
e. The patient has blood and spot urine collected for monitoring labs, including urine pregnancy test (if applicable).
f. A spot urine sample is obtained for measurement of target engagement biomarkers
g. If the patient consents, biorepository samples should be obtained
h. Archived kidney biopsy tissue is requested to be sent [when available and if not already submitted to the NEPTUNE study] to the laboratory at the University of Michigan for gene expression profiling for mechanisms of disease
i. Archived kidney biopsy report is anonymized and uploaded into the study electronic data capture system for central review

At Screening, a preliminary evaluation is performed and once screening is complete, if all inclusion and no exclusion criteria are met, the patient is deemed eligible and will be asked to return for the baseline visit for study drug initiation. Subjects who have not met study enrollment criteria will be considered a screen failure and their study participation will end.

Repeat Screening. If the patient is found to be ineligible, a repeat screening visit may be performed at the local clinician investigator's discretion.

Once eligibility is confirmed, the patient should be instructed to bring a 24-hour urine specimen to the baseline visit.

7.2.2 BASELINE/WEEK 0

The Baseline visit/Week 0 will occur within 6 weeks of the screening evaluation. The following procedures will take place at the baseline visit:

a) Interval medical history
b) Review of concomitant medications
c) Physical examination
d) Assessment of adverse events
e) Vital signs will be obtained including weight, blood pressure, and temperature
f) Subject to complete HRQOL assessment
g) 24-hour urine or first morning urine will be collected from the patient, along with blood samples for clinical laboratories, including urine pregnancy (if applicable)
h) Spot urine collection for biomarkers (TIMP1/Cr and MCP1/Cr).
i) Blood collection for the Biological Specimen Repository
j) Humira will be injected subcutaneously, monitor subject for 15 minutes post injection
k) Record heat rate and blood pressure 15 minutes post injection
l) Teaching on how to administer subcutaneous injections (to self or minor child)
m) Remind subject to bring a 24-hour urine sample (or first morning urine if 24 hour sample is not feasible) to the next study visit

7.2.3 FOLLOW-UP

Follow-up Visits (Weeks 2 and 8)

The following procedures will take place at each of the follow up visits

a) Interval medical history
b) Review of concomitant medications
c) Physical examination
d) Assessment of adverse events
e) Vital signs obtained including weight, blood pressure, and temperature
f) Subject to complete the HRQOL assessment
g) 24-hour urine or first morning urine will be collected from the patient, along with blood samples for clinical laboratories, including urine pregnancy (if applicable)
h) Spot urine collection for biomarkers (TIMP1/Cr and MCP1/Cr)
i) Blood collection for the Biological Specimen Repository
j) Subject to administer Humira to themselves (or child) under the guidance of study staff
k) Remind subject to bring a 24-hour urine sample (or first morning urine if 24 hour sample is not feasible) to the next study visit

Endpoint Visit (Weeks 10)

The week 10 follow-up visit includes a combination of toxicity monitoring and outcome assessments.

a) Interval medical history
b) Review of concomitant medications

c) Physical examination
d) Assessment of adverse events
e) Vital signs obtained including weight, blood pressure, and temperature
f) Subject to complete the HRQOL assessment
g) 24-hour urine or first morning urine will be collected from the patient, a fresh spot urine will be collected during the visit along with blood samples for clinical laboratories, including urine pregnancy (if applicable)
h) Spot urine collection for biomarkers (TIMP1/Cr and MCP1/Cr)
i) Blood biorepository sample collection

Safety Visit (Week 14) Phone visit or optional in-person study visit may be preformed

a) Interval medical history
b) Review of concomitant medications
c) Assessment of adverse events
d) If the optional in-person visit is scheduled:
    a. Physical examination
    b. Vital signs obtained including weight, blood pressure, and temperature
    c. Blood sample for clinical laboratories
    d. 24-hour urine or first morning urine will be collected from the patient, a fresh spot urine will be collected during the visit along with blood samples for clinical laboratories, including urine pregnancy (if applicable)

7.2.4 EARLY TERMINATION VISIT

Should a participant prematurely withdraw or be withdrawn from the study for any reason he/she will be asked to return for the key outcome determinations (week 10 assessments), as detailed above. In addition, at that time, any medication prescribed by their treating physician since leaving the study will be recorded. The participant will also be asked to participate in additional safety monitoring visits (week 14) as described in 7.2.3 if at least one dose of the study medication was administered. Should the participant die, the cause of death will be determined and recorded, if possible. This additional data will be utilized in the statistical analysis of the primary and secondary study outcomes and any missing data handled by appropriate statistical methods.

7.2.5 SCHEDULE OF EVENTS TABLE

	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**
CBC with Platelets and Differential	X	X	X	X	X	X**
Urine Protein:Creatinine [24 hour sample preferred]	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

7.3 JUSTIFICATION FOR SENSITIVE PROCEDURES

HIV, hepatitis B and hepatitis C testing is a part of the screening procedures as therapy with adalimumab is contraindicated for persons with active infections. Should HIV, hepatitis B, hepatitis C or tuberculosis/quantiferon testing be positive, the patient will be referred for clinical care through local treatment experts according to local standards of care and excluded from the study.

7.4 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

All concomitant prescription, over-the-counter and non-prescription medications taken during study participation will be recorded on the case report forms (CRFs) and checked for compatibility with the study drugs as defined in Section 7.5.

7.5 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

Patients should not receive live vaccines while taking Humira and for 3 months following the final adalimumab dose. Participants should not take anakinra, abatacept, or rituximab in combination with Humira.

7.6 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Humira is approved by the FDA for use in other health conditions. Consequently, it may be prescribed off label by the patient's local nephrologist per local clinical decision making.

8 ASSESSMENT OF SAFETY

All adverse events, whether serious or non-serious, related or not related, following exposure to adalimumab are to be documented by the investigator and recorded in the CRF and in the subject's source records. Investigators must record in the CRF their opinion concerning the relationship of the adverse event to the study drug.

All reported serious and non-serious adverse events should be followed up in accordance with clinical practice.

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable or unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non- investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonisation [ICH])

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Adverse events may include:
• Symptoms described by the patient
• Clinically significant changes in the patient's physical exam or other signs observed by the investigator or medical staff
• Test abnormalities (laboratory tests, ECG, X-rays, etc.) that reflect a change from baseline and/or that may result in changes in administration of investigational product or in an alteration in medical care (diagnostic or therapeutic)
• Conditions present at baseline that have either worsened or recurred following resolution

The patient will be evaluated for new AEs and the status of existing AEs at each study visit, or at any time contact is made with the patient outside of a scheduled visit. The Investigator may elicit symptoms using an open-ended question, followed by appropriate questions that clarify the patient's description of AEs.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An SAE is an AE that results in any of the following:
• Death: the patient died as a result of the event. The cause of death of a subject in a study within 30 days of the last dose of study drug, whether or not the event is expected or associated with the study drug, is considered a serious adverse event.
• Is life-threatening: An AE that places the patient, in the view of the Investigator or sponsor, at immediate risk of death from the AE as it occurred, i.e., does not include an AE that had it occurred in a more severe form, might have caused death.
• Requires inpatient hospitalization or prolongation of existing hospitalization:
  - Note: Planned or elective hospital admissions for treatment/procedures for a condition/disease that existed prior to signing the informed consent should be recorded in the patient's screening documents and will not be captured as SAEs. If, however, the admission or procedure occurs other than as planned due to a worsening of the condition, then the event should be recorded as a SAE. Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility) should not be recorded as an SAE.
• Persistent or significant disability/incapacity: An AE that results in a substantial disruption of a person's ability to conduct normal life functions.

• Pregnancy, or Congenital anomaly/birth defect: A pregnancy in an individual receiving the investigational product or congenital anomaly/birth defect that occurs in the offspring of a patient exposed to the investigational product.
• Other Medically Important Events: An AE that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition
Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.
Any situation that meets the criteria of a serious adverse event should be recorded on a Serious Adverse Event Report Form and be reported within 24 hours of becoming aware of the event to the IRB (per local policies), and the study Principal Investigator.
All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is 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 (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

8.1.3 PREGNANCY

Females of child bearing potential must use birth control for 30 days prior to the first dose of adalimumab through 30 days after the last dose of adalimumab.
All initial reports of pregnancy must be reported within 24 hours of becoming aware of the event using the Serious Adverse Event Form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomaly, ectopic pregnancy) are considered serious adverse events and must be reported using the Serious Adverse Event Form.
Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

Severity indicates the intensity of the event and should not be confused with seriousness (see sections 8.1.1 and 8.1.2 for assessing seriousness).
Severity Grading: The Investigator will assess the severity of all AEs/SAEs as Mild, Moderate, or Severe, based on the following definitions.
Definitions:
• Mild – Awareness of the event but easily tolerated
• Moderate – Discomfort enough to cause some interference with usual activity

• Severe – Inability to carry out usual activity

8.2.2 RELATIONSHIP TO STUDY AGENT

Assessment of the relationship between the AE and exposure to the investigational product is important for regulatory reporting and assists in the overall analysis of the safety information. For each AE/SAE the Investigator must determine whether, based on available evidence, there is a reasonable possibility that the study drug caused the event. Causal relationship to study drug administration is based on the following definitions:

• None: No relationship between the event and the administration of study drug. The event is related to other etiologies, such as concomitant medications or the patient's clinical state.

• Unlikely: The current state of knowledge indicates that a relationship to study drug is unlikely or the temporal relationship is such that study drug would not have had any reasonable association with the observed event.

• Possible: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the patient's clinical state or other modes of therapy administered to the patient.

• Related: The administration of study drug and AE were reasonably related in time and the AE was more likely explained by exposure to the study drug than by other causes. There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

A causality assessment must be provided for each AE/SAE recorded, even if there is only limited information at the time. Upon receipt of follow-up information, the Investigator may change his/her assessment of causality and amend the AE/SAE report accordingly.

For the purpose of safety analyses, all AEs that are classified as possible will be considered treatment-related events.

8.2.3 EXPECTEDNESS

An adverse event is considered unexpected if the nature or severity is not consistent with the applicable product reference safety information. See Appendix A.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Participants should be monitored for adverse events throughout the study with the final assessment occurring at week 14.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

All AEs will be captured from the time informed consent is signed through the week 14 visit. AEs should be recorded using appropriate medical terminology. When recording, it is preferable to provide a diagnosis. Sufficient information should be sought to assist the Investigator both in determining the diagnosis and in making a causality assessment.

Reporting should not be delayed pending receipt of all required information. If information is unavailable at the time of the initial report, the Investigator is expected to follow-up until the required information has been obtained

8.4.2 SERIOUS ADVERSE EVENT REPORTING

All SAEs including pregnancy must be reported to the study Coordinating Center University of Michigan through the Electronic Data Capture System, notification of the study Principal Investigator, and the

study IRB of record within 24 hours of the Investigator's first knowledge of the event, regardless of causal relationship. SAEs will be reported to the FDA in accordance with §312.32.
• A completed Clinical Study SAE Report Form containing a detailed written description of the event along with available supporting documents (e.g., discharge summary, autopsy report, diagnostic test results, etc.) should be submitted to the data coordinating center
• Additional information that is not available at the time the initial SAE Report Form was completed, must be promptly reviewed and provided to The University of Michigan within 48 hours of receipt. Full supporting documentation should be solicited by the investigative site even if the SAE occurred at another institution. Such documentation may include copies of relevant patient/hospital records, discharge summaries, laboratory/test results or autopsy reports.
• If, at any time after the patient has completed participation in the study, the Investigator or study staff becomes aware of an SAE that they suspect is related to the investigational product, then the event and any known details must be reported promptly to the University of Michigan based Principal Investigator and coordinating center.

8.5 STUDY HALTING RULES

As this study intends to treat a maximum of 8 patients in this study protocol, no halting rules have been defined a priori. Routine study safety monitoring will be performed by the study Data Safety and Monitoring Board.

8.6 SAFETY OVERSIGHT

Safety oversight will be provided by the study DSMB. Due to the small sample size, the DSMB chair will be notified of Serious Adverse Events until completion of the trial. The DSMB will review the trial protocol prior to enrollment initiation and with any substantial protocol amendments. Safety outcomes will be presented at each DSMB meeting. These include number of and proportion of subjects with SAEs (including segregation of those involving deaths), treatment emergent AEs, and discontinuation of study medication due to AEs. These presentations will be descriptive, with no formal inferential methods used. Serious Adverse Events and related or possibly related unexpected adverse events will be reported to the study IRB of record according to their reporting requirements. Participating trial sites will be notified of these events with an indication if a change in consent form is recommended.

9 CLINICAL MONITORING

Monitoring is the act of overseeing the progress of the study and of ensuring that it is conducted, recorded and reported in accordance with the protocol, standard operating procedures, good clinical practice standards, and the applicable regulatory requirements. Given the sample size, monitoring for this study will be done remotely.
Data entered into the case report forms will be reviewed for completion, integrity, and quality. Data reports will be shared with the sites and the steering committee. Data to be reviewed includes: enrollment, visit completion, data completeness, outstanding queries, biosample collection and shipping.
The study monitoring staff may conduct site management calls to ensure data compliance and data query resolution. Other topics may include:

- Protocol adherence
- Recruitment and retention strategies
- Regulatory document requirements
- Completeness of visits, forms, data, and samples

- Responses to data queries
- Electronic Case Report Forms (eCRFs) and source documents
- Study-specific training

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

This is a pilot, proof of concept investigation to test the proposed biomarkers as an indicator of target engagement. As such, no formal sample size calculations are performed and analyses are primarily descriptive in nature. Our approach will be 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 will use an exploratory approach to assess individual and panel biomarker change and correlation with clinical indicators of disease, including proteinuria, eGFR, and serum albumin.
A statistical analysis plan (SAP) will be written for the study that contains detailed descriptions of the analyses to be performed. The SAP will be finalized prior to database lock.

10.2 ANALYSIS DATASETS

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

10.3 DESCRIPTION OF STATISTICAL METHODS

10.3.1 GENERAL APPROACH

Analyses are primarily descriptive in nature in this pilot study. Continuous variables will be 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.

10.3.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The change in urine MCP1/Cr and TIMP1/Cr levels from baseline to study week 10, i.e. 2 weeks after the final dose of adalimumab, are the primary endpoints. We will provide tabular and graphical summaries of the changes from baseline to week 10 in MCP1/Cr and in TIMP1/Cr, including summaries at baseline and week 10. The changes from baseline to week 10 in urine MCP1/Cr and TIMP1/Cr levels will be tested using the paired t-test or Wilcoxon signed-rank test (if distributional assumptions are not met).

10.3.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Discrete outcomes (e.g., AEs, SAEs, proportion of participants achieving a nadir UPC <1.5 g/g and <40% from baseline) will be summarized descriptively. Continuous outcomes (e.g., changes in eGFR, change in proteinuria) will be summarized descriptively, including baseline, follow-up and changes from baseline.

10.3.4 SAFETY ANALYSES

Safety data, including frequency of events and proportion of participants experiencing events (e.g., treatment-emergent AEs and SAEs by body system, severity and relatedness to adalimumab), clinical laboratory tests, vital signs, and physical examinations, will be summarized descriptively.

10.3.5 ADHERENCE AND RETENTION ANALYSES

Subject disposition, including descriptive statistics on-treatment and on-study follow-up, will be reported. Listings of subject disposition, including reasons for early termination, will be reported. Other disposition and study conduct information, including major protocol violations, will be summarized.

Compliance with study medication will be assessed and summarized, including the proportion of participants who adhered to study treatment, the median duration of adherence to study treatment and the proportion of participants who permanently discontinued study medication.

10.3.6 BASELINE DESCRIPTIVE STATISTICS

Demographic data and baseline characteristics will be summarized.

10.3.7 TABULATION OF INDIVIDUAL RESPONSE DATA

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

10.3.8 EXPLORATORY ANALYSES

Spearman correlations and scatterplots will be provided to assess the correlation of intra-renal gene expression of TNF and urine biomarker levels at baseline. Continuous outcomes (e.g., changes in QOL outcomes, EGF biomarker levels) will be summarized descriptively, including baseline, follow-up and changes from baseline.

10.4 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 6 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 uTIMP1/Cr and of changes in uMCP1/Cr, with no adjustments for multiplicity (EAST 6.2).

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Every participating clinical site will maintain appropriate medical and research records for this trial, in compliance with GCP, regulatory and institutional requirements for the protection of confidentiality of participants. Participating clinical sites will also obtain institutional authorization for external monitoring by the Data Coordinating Center to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are defined as all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, participant's memory aids or evaluation checklists, pharmacy

dispensing records, recorded audio tapes, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents when the data is collected and recorded there as the primary source of information, but CRFs will not constitute the only form of source document information for this trial.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 ETHICAL STANDARD

The study will be monitored by clinical study monitors to assess adherence to good clinical practice and to this clinical study protocol.

The study may be audited by the regulatory authorities, the IRB, and/or the US Food and Drug Administration. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

12.2 INSTITUTIONAL REVIEW BOARD

This protocol, investigator's brochure, informed consent documents, and any other patient facing materials must be reviewed and approved in accordance with the IRB of Record's policies prior to any study procedures taking place.

12.3 INFORMED CONSENT PROCESS

Each site is responsible for ensuring that informed consent and assent, when applicable, is obtained from each patient using a current consent form according to the guidelines of its local IRB and in accordance with the Common Rule (45 CFR Part 46 subpart A, Protection of Human Subjects). The informed consent (ICF) and assent forms must be signed and dated by the patient or the legal guardian if applicable prior to initiation of any study-related activity. During the consent process, the study team will discuss the implications of participation in this study and that the drug dosing under study has not been studied previously. All participant questions should be fully answered before the ICF is signed. The signed ICF should be placed in the subject's research file and copies should be provided to the study participant as well as placed in the participant's medical chart according to local regulatory requirements.

The ICF as well as any other patient-facing documents, such as the quality of life forms or recruitment materials must be approved in accordance with the site's local IRB requirements.
Consenting Non-English Speaking Participants: Obtaining consent from non-English speaking participants should be conducted in accordance with IRB guidelines.

12.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant identifiers will only be known to the local study team. Any information that leaves the local study site should be identified with the participant's study ID number and no identifying information. Data must be kept in a secure location at the local site. The study database will be on a secure University of Michigan server.

12.5 FUTURE USE OF STORED SPECIMENS

The Steering Committee, including the study PI, PI from each participating site, and DCC PI, oversee all requests (both internal and external to the study) to access stored samples and data for ancillary studies directed toward the understanding of nephrotic syndrome, kidney disease, and/or the treatment protocol.

Once the steering committee for this clinical trial has completed their responsibilities (study closed, analysis complete, results reported) the stewardship of the stored biospecimens for future research will be under the NIH sponsored Nephrotic Syndrome Study Network (NEPTUNE) ancillary studies committee.

13 DATA HANDLING AND RECORD KEEPING

13.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data will be collected on study Case Report Forms and entered into the study electronic data base. Entered data will be assessed for completeness, accuracy, and timeliness. Queries will be distributed from the coordinating center to the study site personnel to maximize the data quality and accuracy.

13.2 STUDY RECORDS RETENTION

Study records will be maintained for 2 years after completion of the trial.

13.3 PROTOCOL DEVIATIONS

Protocol deviations will be reported to the study IRB and the study coordinating center. Every effort will be made to avoid protocol deviations except in the setting of patient safety.

13.4 PUBLICATION AND DATA SHARING POLICY

This trial is expected to be reported as a scientific publication and will follow the publication and data sharing policies of the NEPTUNE study and the Kidney Research Network (KRN). (NEPTUNE-Study.org and kidneyresearchnetwork.org)

14 CONFLICT OF INTEREST POLICY

Participating investigators and research personnel are responsible to maintain compliance with local and national conflict of interest policies.

15 LITERATURE REFERENCES

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3. Trachtman H, Vento S, Herreshoff E, et al. Efficacy of galactose and adalimumab in patients with resistant focal segmental glomerulosclerosis: report of the font clinical trial group. *BMC nephrology.* 2015;16:111.
4. Ternant D, Paintaud G, Trachtman H, Gipson DS, Joy MS. A possible influence of age on absorption and elimination of adalimumab in focal segmental glomerulosclerosis (FSGS). *European journal of clinical pharmacology.* Feb 2016;72(2):253-255.

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6. Roberts BV, Susano I, Gipson DS, Trachtman H, Joy MS. Contribution of renal and non-renal clearance on increased total clearance of adalimumab in glomerular disease. *Journal of clinical pharmacology.* Sep 2013;53(9):919-924.
7. Joy MS, Gipson DS, Dike M, et al. Phase I trial of rosiglitazone in FSGS: I. Report of the FONT Study Group. *Clinical journal of the American Society of Nephrology : CJASN.* Jan 2009;4(1):39-47.

APPENDIX

A. Humira Product Information

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use HUMIRA safely and effectively. See full prescribing information for HUMIRA.

HUMIRA (adalimumab) Injection, Solution for Subcutaneous use
Initial U.S. Approval: 2002

WARNING: RISK OF SERIOUS INFECTIONS
See full prescribing information for complete boxed warning.
Tuberculosis (TB), invasive fungal, and other opportunistic infections, some fatal, have occurred in patients treated with HUMIRA. Perform test for latent TB; if positive, start treatment for TB prior to starting HUMIRA. Monitor all patients for active TB during treatment, even if initial latent TB test is negative (5.1)

RECENT MAJOR CHANGES

Indications and Usage, Crohn's Disease (1.4)	2/2007
Indications and Usage, Plaque Psoriasis (1.5)	1/2008
Dosage and Administration, Crohn's Disease (2.2)	7/2007
Dosage and Administration, Plaque Psoriasis (2.3)	1/2008
Warnings and Precautions, Serious Infections (5.1)	2/2007
Warnings and Precautions, Malignancies (5.2)	1/2008
Warnings and Precautions, Immunizations (5.10)	2/2007

INDICATIONS AND USAGE

HUMIRA is a tumor necrosis factor (TNF) blocker indicated for treatment of:
Rheumatoid Arthritis (RA) (1.1)
- Reducing signs and symptoms, inducing major clinical response, inhibiting the progression of structural damage, and improving physical function in adult patients with moderately to severely active disease.

Psoriatic Arthritis (1.2)
- Reducing signs and symptoms of active arthritis, inhibiting the progression of structural damage, and improving physical function.

Ankylosing Spondylitis (1.3)
- Reducing signs and symptoms in patients with active disease.

Crohn's Disease (1.4)
- Reducing signs and symptoms and inducing and maintaining clinical remission in adult patients with moderately to severely active Crohn's disease who have had an inadequate response to conventional therapy. Reducing signs and symptoms and inducing clinical remission in these patients if they have also lost response to or are intolerant to infliximab.

Plaque Psoriasis (1.5)
- The treatment of adult patients with moderate to severe chronic plaque psoriasis who are candidates for systemic therapy or phototherapy, and when other systemic therapies are medically less appropriate.

DOSAGE AND ADMINISTRATION

HUMIRA is administered by subcutaneous injection.
Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis (2.1)
- 40 mg every other week. Some patients with RA not receiving methotrexate may benefit from increasing the frequency to 40 mg every week.

Crohn's Disease (2.2)
- Initial dose (Day 1) is 160 mg (four 40 mg injections in one day or two 40 mg injections per day for two consecutive days), followed by 80 mg two weeks later (Day 15). Two weeks later (Day 29) begin a maintenance dose of 40 mg every other week.

Plaque Psoriasis (2.3)
- 80 mg initial dose, followed by 40 mg every other week starting one week after initial dose.

DOSAGE FORMS AND STRENGTHS
- 40 mg/0.8 mL in a single-use prefilled pen (HUMIRA Pen) (3)
- 40 mg/0.8 mL in a single-use prefilled glass syringe (3)

CONTRAINDICATIONS
- None (4)

WARNINGS AND PRECAUTIONS
- Serious infections – do not start HUMIRA during an active infection. If an infection develops, monitor carefully, and stop HUMIRA if infection becomes serious (5.1)
- Malignancies – are seen more often than in controls, and lymphoma is seen more often than in the general population (5.2)
- Anaphylaxis or serious allergic reactions may occur (5.3)
- Hepatitis B virus reactivation – monitor HBV carriers during and several months after therapy. If reactivation occurs, stop HUMIRA and begin anti-viral therapy (5.4)
- Demyelinating disease, exacerbation or new onset, may occur (5.5)
- Cytopenias, pancytopenia – advise patients to seek immediate medical attention if symptoms develop, and consider stopping HUMIRA (5.6)
- Heart failure, worsening or new onset, may occur (5.8)
- Lupus-like syndrome – stop HUMIRA if syndrome develops (5.9)

ADVERSE REACTIONS

Most common adverse reactions (incidence ≥10%): infections (e.g. upper respiratory, sinusitis), injection site reactions, headache and rash (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Abbott Laboratories at 1-800-633-9110 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

DRUG INTERACTIONS
- Anakinra – increased risk of serious infection (5.7, 7.1)
- Live vaccines – should not be given with HUMIRA (5.10, 7.2)

USE IN SPECIFIC POPULATIONS
- Pregnancy: Physicians are encouraged to enroll pregnant patients in the HUMIRA pregnancy registry by calling 1-877-311-8972 (8.1)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling

Revised Jan 2008