

A Pilot Study to Assess the Efficacy of  
Rituximab Therapy in Patients With  
Treatment Resistant Idiopathic Focal  
Segmental Glomerulosclerosis (FSGS):  
Integrating an Assessment of the  
Relevance of suPAR and Activation of  
Podocyte  $\beta$ 3 Integrin

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## **Rare Diseases Clinical Research Network**

### **Neptune Consortium**

**A Pilot Study to Assess the Efficacy of Rituximab Therapy in Patients with Treatment Resistant Idiopathic Focal Segmental Glomerulosclerosis (FSGS): Integrating an Assessment of suPAR and Activation of Podocyte  $\beta$ 3 Integrin**

**This protocol is for research purposes only, and should not be copied, redistributed or used for any other purpose. The procedures in this protocol are intended only for use by Consortium investigators in carefully controlled settings. The Chair of this study should be consulted before using or attempting any procedure in this protocol.**

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## Table of Contents

Participating Institutions/Investigators Table.....	2
1. Protocol Synopsis .....	6
1.1 Overview .....	9
2. Objective and Research Questions .....	9
3. Background.....	10
4. Study Design and Methods .....	13
4.1 Inclusion Criteria .....	13
4.2 Exclusion Criteria .....	14
4.3 Recruitment of Participants .....	15
4.5 Retention strategies .....	15
4.6 Data Elements.....	15
4.7 Schedule of Events .....	21
5. Safety Monitoring .....	22
5.1 Data and Safety Monitoring Plan.....	23
5.2 Study Oversight.....	23
5.3 Definitions and Standards .....	24
5.4 Expected/Known Risks/Discomforts/Adverse Events Associated with Study Intervention and Procedures: Definition of Expected Adverse Events.....	25
5.5 Reporting Guidelines.....	28
5.6 RDCRN Adverse Event Data Management System (AEDAMS).....	30
5.7 Study Discontinuation (Interventional) .....	31
5.8 Subject Discontinuation.....	32
5.9 Data Quality and Monitoring Measures.....	32
6. Statistical Considerations .....	32
7. Data Management.....	32
7.1 Registration.....	33
7.2 Data Entry .....	33
7.3 Data Quality Control .....	33
8. Human Subjects .....	34
8.1 GCP Statement.....	34
8.2 Risks .....	34

8.3 Benefits .....	34
8.4 Recruitment.....	34
8.5 Written Informed Consent.....	34
8.6 Process of Consent: .....	35
8.7 Certificate of Confidentiality .....	
9. References.....	36

## 1. Protocol Synopsis

### Interventional Synopsis

<b>Protocol Number:</b>	6803
<b>Protocol Title:</b>	A Pilot Study to Assess the Efficacy of Rituximab Therapy in Patients with Treatment Resistant Idiopathic Focal Segmental Glomerulosclerosis (FSGS): Integrating an Assessment of the Relevance of suPAR and Activation of Podocyte $\beta$ 3 Integrin
<b>Study Chair:</b>	Fernando C. Fervenza, MD
<b>Statistician:</b>	NA
<b>Consortium:</b>	Neptune
<b>Participating Sites:</b>	University Health Network, Sunnybrook Health Sciences Centre, Rush University Medical Center, Mayo Clinic College of Medicine, New York University Medical Center, Albert Einstein School of Medicine
<b>Activation Date:</b>	06/29/16
<b>Current Status:</b>	Active
<b>Sample Size:</b>	20
<b>Target Enrollment Period:</b>	2 years
<b>Study Design:</b>	Interventional Pilot
<b>Primary Study Objective:</b>	This is a pilot trial to assess the safety, feasibility and efficacy of Rituximab therapy in 20 adult and pediatric patients with either steroid and/or calcineurin inhibitor resistant focal segmental glomerulosclerosis (FSGS) or with a significant intolerance or contraindication to the use of these agents. In addition to clinical outcomes, levels of suPAR and $\beta$ <sub>3</sub> integrin activation will be assessed.
<b>Secondary Study Objective(s):</b>	Changes in the baseline levels of these potential biomarkers in response to treatment will be compared to clinical measures of efficacy.
<b>Study Population and Main Eligibility/ Exclusion Criteria:</b>	<p>Inclusion Criteria:</p> <ul style="list-style-type: none"> <li>• FSGS involving native kidneys with a diagnostic biopsy.</li> <li>• Patients &gt; 6 years of age and &lt; 80 years of age</li> <li>• suPAR &gt; 3500 pg/ ml</li> <li>• <math>\beta</math><sub>3</sub> integrin activation as indicated by relative AP5 activity &gt; 1 mean fluorescence intensity (MFI)</li> <li>• Treatment with an ACEI and/or ARB as tolerated for at least 3 months prior to enrollment with a target a systolic blood pressure <math>\leq</math> 140 mmHg and a diastolic</li> </ul>

	<p>pressure <math>\leq</math> 90 mmHg in adults and blood pressure readings less than the 95<sup>th</sup> percentile for age, gender and height or less than 120/80 mmHg (whichever is lower) in children in at least 75% of readings</p> <ul style="list-style-type: none"> <li>• Proteinuria <math>\geq</math> 3.0 grams as measured by 24-hour urine collection in adults or Urine Protein to creatinine ratio (Up/c) <math>\geq</math> 1.0 in the first morning urine in children, despite ACE inhibitor / ARB treatment as tolerated and a minimum of 8 weeks of prednisone therapy at <math>\geq</math> 1 mg/kg/day, a trial of calcineurin inhibitor for <math>\geq</math>3 months or a contraindication/intolerance to such therapy</li> <li>• Patients must have a minimal washout period before initiating Rituximab to avoid over immunosuppression and consequent risk of morbidity.</li> <li>• Negative serum pregnancy test (for women of child bearing age)</li> <li>• Men and women of reproductive potential must agree to use an acceptable method of birth control during treatment and for twelve months (1 year) after completion of the trial</li> <li>• Able and willing to give written informed consent and comply with study requirements</li> </ul> <p>Exclusion Criteria:</p> <ul style="list-style-type: none"> <li>• Estimated GFR <math>&lt;</math> 40 ml/min per 1.73m<sup>2</sup>. The rationale is that patients with advanced renal failure may progress rapidly towards ESRD.</li> <li>• Collapsing variant of FSGS, as it is rare and has been associated with an aggressive course</li> <li>• Patients with medical conditions that may cause FSGS (e.g. HIV, lymphoma, heroin use) or have a secondary form of FSGS due to hyperfiltration injury (eg. massive obesity, vesicoureteral reflux, or renal mass reduction)</li> <li>• Type 1 or type 2 diabetes mellitus as diabetic glomerulosclerosis may be contributing to proteinuria in these patients</li> <li>• History of serious recurrent or chronic infection</li> <li>• Presence or suspicion of active infection including HIV, Hepatitis B and HCV with positive tests for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb), hepatitis B virus (HBV), hepatitis C serology, or HIV serology. TB screening should be conducted on anyone who is at high risk for TB (i.e., clinical suspicion of TB, from country where TB common, or lives/works where TB more common). Absolute neutrophil count <math>&lt;</math> 1.5 x10<sup>3</sup>/mL</li> <li>• Known active infection requiring hospitalization or</li> </ul>
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	<p>treatment with intravenous antibiotics within 4 weeks or oral antibiotics within 2 weeks of the study initiation</p> <ul style="list-style-type: none"> <li>• Patients in receipt of a live vaccine within 4 weeks of the study initiation</li> <li>• Concomitant malignancies or previous malignancies within the last five years, with the exception of adequately treated basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix</li> <li>• Previous Treatment with a B-cell depleting antibody</li> <li>• History of severe allergic reactions to humanized or murine monoclonal antibodies</li> <li>• Treatment with any investigational agent within 4 weeks of the study initiation</li> <li>• History of major psychiatric disorder, drug or alcohol abuse within the previous 6 months</li> <li>• Any other disease, metabolic dysfunction, physical examination finding or clinical laboratory that provides a reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that may affect the interpretation of the results or render the patient at high risk from treatment complications</li> </ul>
<b>Treatment</b>	
<b>Agent:</b>	Rituximab
<b>Dosage, schedule, route of administration:</b>	Rituximab will be infused intravenously on days one and fifteen at a dose of 375 mg/m <sup>2</sup> up to a maximum of 1 gram per dose in children and at a dose of 1000 mg on days 1 and 15 in adults.
<b>Safety Issues:</b>	Participants will be monitored for infusion-related reactions.
<b>Primary Outcome Measures:</b>	<p>The major outcomes (given the short duration of the study) will be categorized by changes in proteinuria (with stable renal function) The primary endpoint will be determined by patient status at 12 months of follow-up defined as</p> <p>In Adults:</p> <ul style="list-style-type: none"> <li>• Complete Remission – Proteinuria &lt; 0.3 g/d daily</li> <li>• Partial Remission – Improvement in proteinuria by &gt; 50% and to a level between 0.3-3.5g/d</li> <li>• Incomplete Remission – Improvement in proteinuria equal to or &gt;50%, but residual proteinuria still &gt;3.5g/d</li> </ul> <p>In Children:</p> <ul style="list-style-type: none"> <li>• Complete Remission – Up/c &lt; 0.2</li> <li>• Partial Remission – Improvement in proteinuria by &gt; 50% and to a Up/c value of 0.2-1</li> <li>• Incomplete Remission – Improvement in proteinuria equal to or &gt;50%, but residual Up/c &gt;1</li> <li>• No Response:</li> </ul>

	<ul style="list-style-type: none"> <li>Worsening of renal function (serum creatinine &gt; 30% above baseline) without significant improvement in proteinuria (&lt;50% improvement) or no improvement or worsening in proteinuria</li> </ul>
<b>Secondary Outcome Measures:</b>	<ul style="list-style-type: none"> <li>Change from baseline in suPAR level and in podocyte <math>\beta</math>3 integrin activity as indicated by relative AP5 after 1, 3, 6 and 12 months activity</li> <li>Duration of complete or partial remission following treatment</li> </ul>
<b>Statistical Considerations (sample size and analysis plan):</b>	NA
<b>Sponsors (federal, state, foundation and industry)</b>	Neptune Pilot Study Program - National Institutes of Health Genentech Pharmaceuticals

## 1.1 Overview

The incidence of focal segmental glomerulosclerosis (FSGS) is increasing. Novel insights into the pathogenesis of disease suggest the presence of a circulating factor (suPAR) that ultimately could damage renal podocytes. This factor may distinguish idiopathic FSGS from secondary causes of glomerular damage. Currently available treatment options for idiopathic FSGS are often ineffective and/or associated with significant toxicity. Rituximab may be a potential therapy as beyond its potential for immunomodulation, it may also have a direct effect at the level of the renal podocyte.

This is a pilot trial to assess the safety, feasibility and efficacy of Rituximab therapy in 20 adult and pediatric patients with either steroid and/or calcineurin inhibitor resistant focal segmental glomerulosclerosis (FSGS) or with a significant intolerance or contraindication to the use of these agents. In addition to clinical criteria, elevated levels of suPAR and  $\beta$ 3 integrin activity as will define inclusion in an effort to establish a more homogeneous patient population and to understand the relationship of this permeability factor to response to treatment. Changes in the baseline levels of potential biomarkers in response to treatment will be compared to clinical measures of efficacy.

## 2. Objective and Research Questions

This is pilot trial (Phase 2). The overall objective of this study is to assess the safety, feasibility and efficacy of Rituximab therapy in 20 adult and pediatric patients with either steroid and/or calcineurin inhibitor resistant focal segmental glomerulosclerosis (FSGS) or with a significant

intolerance or contraindication to the use of these agents. In addition to clinical criteria, elevated levels of suPAR and  $\beta$ 3 integrin activity will define inclusion. We hypothesize that Rituximab will be effective at lowering proteinuria in this patient population and changes in these biomarkers will mirror response or lack thereof.

**The major outcomes** (given the short duration of the study) will be categorized by changes in proteinuria (with stable renal function). The primary endpoint will be determined by patient status at 12 months of follow-up defined as:

***In Adults:***

- Complete Remission – Proteinuria < 0.3 g/d daily
- Partial Remission – Improvement in proteinuria by > 50% and to a level between 0.3-3.5g/d
- Incomplete Remission – Improvement in proteinuria equal to or >50%, but residual proteinuria still >3.5g/d

***In Children:***

- Complete Remission – Up/c < 0.2
- Partial Remission – Improvement in proteinuria by > 50% and to a Up/c value of 0.2-1
- Incomplete Remission – Improvement in proteinuria equal to or >50%, but residual Up/c >1

***Or in Both Groups:***

- Worsening of renal function (serum creatinine > 30% above baseline) without significant improvement in proteinuria (<50% improvement) or no improvement in proteinuria (<50%) or worsening of proteinuria ie. No response

**Secondary outcomes** will include:

- Change from baseline suPAR level and activation of podocyte  $\beta$ 3 integrin as indicated by relative AP5 activity (MFI > 1) after 1, 3, 6 and 12 months
- Duration of complete or partial remission following treatment

### **3. Background**

#### ***Focal Segmental Glomerulosclerosis (FSGS)***

The incidence of FSGS has increased and it is now the most common cause of nephrotic syndrome in North American adults and children<sup>1</sup>. In a recent study, FSGS accounted for 17% of all renal biopsies representing a 13-fold increase in the incidence of this renal lesion over the past two-three decades<sup>2</sup>. Spontaneous remissions are rare in patients with nephrotic range proteinuria (<5%), and if untreated and/or unresponsive, the disease typically progresses to end stage renal disease (ESRD) over 6-8 years in 50% of patients<sup>3-5</sup>. Those with severe nephrotic syndrome (proteinuria > 10 grams) have a much worse prognosis and can expect to progress to ESRD over approximately 3 years<sup>3-5</sup>. Further, FSGS recurs after transplantation in approximately 30% of patients contributing significantly to loss of graft function. Thus, FSGS poses a significant strain on patient health and well-being as well as health care resources.

The pathophysiology of FSGS is unclear. It is a disease that primarily targets the glomerular epithelial cell or podocyte and the presence of a circulating factor that results in podocyte

effacement and disruption of the glomerular filtration barrier has been suggested by indirect evidence for over 3 decades. Evidence supporting the presence of a circulating factor is derived from clinical cases that have reported virtual immediate recurrence of massive proteinuria following anastomosis of the donor artery to the recipient vessels at the time of renal transplantation. There have also been studies using animal models that have been able to demonstrate that serum from patients with FSGS can increase glomerular permeability to albumin<sup>6-8</sup>. Utilizing an in vitro assay, Savin and colleagues demonstrated significantly increased albumin permeability [P(alb)] of isolated glomeruli when exposed to plasma from patients with FSGS<sup>8</sup>, yet the specific molecular characteristics and mechanism of action of this permeability factor has remained elusive.

Recent insights into podocyte biology have identified a urokinase receptor (uPar) integral to the maintenance of the slit diaphragm through its ability to form signalling complexes with other transmembrane proteins including lipid-dependent activation of  $\alpha\beta3$  integrin<sup>9</sup>. Activation of this receptor and its downstream pathways result in hypermotility of podocyte foot processes, podocyte effacement, proteinuria, glomerular damage and loss of renal function. uPAR is a glycosyl-phosphatidylinositol (GPI)-anchored three-domain (DI, DII and DIII, as numbered from the N terminus) protein, which can be released from the plasma membrane as a soluble molecule (suPAR) by cleavage of the GPI anchor<sup>10</sup>. suPAR has been demonstrated to be elevated in patients with FSGS and is highest in patients with recurrent disease post-transplantation. Further, this 20-50 kDa circulating protein can be partially removed by plasmapheresis, and beneficial responses in proteinuria can be observed in cases where suPAR levels drop below a certain threshold where there is reduced activation of the podocyte  $\beta3$  integrin.

Immunosuppressive treatments have been shown to improve proteinuria and slow progression to ESRD, but side effects of the current options including high dose prolonged corticosteroids, cytotoxic agents and calcineurin inhibitors are significant and rates of treatment failure and relapses are common. Steroid resistance even to prolonged treatment is present in up to 50% of patients<sup>11-14</sup>, and such courses of high dose steroids can result in significant side-effects including, but not limited to, cataracts, skin thinning, acne, diabetes, osteoporosis/osteonecrosis and weight gain. Cytotoxic agents can result in increased rates of infections, propensity to late onset malignancy and infertility. Calcineurin inhibitors have been utilized successfully as either first line therapy in those at high risk of developing steroid-related side effects, or as second line therapy in corticosteroid resistant cases<sup>14, 15</sup>. However, treatment trials with these agents have shown a significant relapse rate (as high as 50%) and longer term or maintenance therapy with these drugs raises concerns with respect to nephrotoxicity and hypertension among other adverse effects<sup>16</sup>.

In summary, our current therapeutic options, although often at least partially successful, have significant adverse effects that limit their long-term utilization. Further, this broad-based, non-specific approach also suffers from the lack of any early indicator that success in either controlling or terminating the disease process is being achieved. To date only response in proteinuria is available to guide therapy and an improved understanding in podocyte specific pathobiology would allow for an earlier assessment preventing unnecessary exposure to potentially toxic immunosuppression.

## **Rituximab**

Rituximab is a genetically engineered, chimeric, murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant pre-B and mature B cells. The CD20 antigen is not expressed on hematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissues. Thus, it has an impressive safety record when compared to classic cytotoxic agents. In vitro studies have demonstrated that the Fc portion of Rituximab binds human complement and can lead to cell lysis of the targeted cell through complement-dependent cytotoxicity and it has been demonstrated that Rituximab mediates antibody-dependent cellular cytotoxicity. Most recently, Rituximab been shown to be effective in the treatment of idiopathic membranous nephropathy and may work at least in part by depletion of the autoantibody to the podocyte located antigen PLA2R<sup>17</sup>. Further, Rituximab may have a direct podocyte modulating effect via cross reactivity with sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) protein and regulation of acid sphingomyelinase (ASMase) essential for the lipid-raft compartmentalization of the podocyte plasma membrane as well as for the organization and signaling of podocytes in general<sup>18</sup>. This potential direct effect on podocytes independent of its known effect on selective depletion of the B cell clone may make it a very effective option to consider for the treatment of idiopathic FSGS.

To date, only case reports and a small open-label trial exist to suggest that Rituximab might prove effective in patients with FSGS and these reports included primarily children and renal transplant recipients<sup>19-25</sup>. In one study, the safety and efficacy of Rituximab was assessed in a multicenter series of 22 patients, aged 6 –22 years with severe steroid-dependent nephrotic syndrome or steroid-resistant, but cyclosporin-sensitive idiopathic nephrotic syndrome. Patient were treated with two to four infusions of Rituximab. Remission was induced in three of the seven proteinuric patients, and one or more immunosuppressive treatments could be withdrawn in 19 patients (85%), with no relapse of proteinuria<sup>26</sup>. More recently 54 children with idiopathic nephrotic syndrome dependent on either steroids or calcineurin inhibitors were randomized to receive either Rituximab or ongoing standard immunosuppression<sup>27</sup>. The experimental arm had a relapse rate of 18% compared to 48% in the control arm at 3 months and had a significantly higher probability of being free of long-term immunosuppression. Although success has been noted in the pediatric literature, results have been mixed in adult patients with either idiopathic or recurrent disease post transplantation<sup>19-25</sup>.

## ***Integration of New Basic and Clinical Information into an Intervention Study of Patients with Idiopathic FSGS***

Treatment options and the assessment of efficacy have been limited by the complex pathogenesis of the disease including the inability to identify and measure in a precise manner these unknown serum factors. We have recently noted that suPAR is elevated in two-thirds of subjects with primary FSGS, but not in individuals with other glomerular diseases. Part of the foundation of this work was based on mouse models wherein we explored the effects of suPAR on kidney function and morphology. We determined that circulating suPAR activates podocyte  $\beta_3$  integrin in both native and grafted kidneys, which results in foot process effacement, proteinuria and FSGS-like glomerulopathy. These morphologic changes and subsequent

laboratory findings of renal disease only develop when suPAR activates podocyte  $\beta_3$  integrin. Our work suggests that suPAR is produced by neutrophils, monocytes and perhaps other cells, such as T cells, (although the precise source of suPAR is still unknown) and released into the circulation. It then enters the kidney glomeruli, binds and activates  $\beta_3$  integrin via a moiety on domain 2, one of the major proteins anchoring podocytes to the underlying glomerular basement membrane (GBM). Increased plasma levels of suPAR lead to increased  $\beta_3$  integrin activation, thus leading to the podocyte dysfunction, effacement and proteinuria characteristic of FSGS. We found increased integrin  $\beta_3$  activity in podocytes from failing kidney allografts due to recurrent FSGS. In addition, we determined that a higher concentration of suPAR before transplantation results in an increased risk for recurrence of FSGS after transplantation. We also found that suPAR-rich FSGS serum activates integrin  $\beta_3$  in human podocytes, and that co-incubation with uPAR antibody or cycloRGDfV reduces integrin activity. We have demonstrated in preliminary data that Rituximab may affect integrin  $\beta_3$  activity in a SMPDL-3b dependent manner and preservation of podocyte expression of SMPDL-3b by Rituximab is likely to correlate to the activation of integrin  $\beta_3$ . Thus, it appears that the disease can be abrogated by lowering serum suPAR concentrations through plasmapheresis, or by interfering with the suPAR- $\beta_3$  integrin interaction through antibodies and small molecules targeting either uPAR, SMPDL-3b or  $\beta_3$  integrin.

The SuPAR and renal function relationship was recently examined longitudinally in over 3500 patients recruited from the Emory Cardiovascular Biobank. SuPAR levels were found to be correlated with both baseline eGFR ( $r=-0.45$ ,  $p<0.0001$ ) and urine protein ( $r=0.22$ ,  $p<0.0001$ ), further those patients with normal renal function and the highest quartile of suPAR level had a three-fold increased incidence of CKD. SuPAR levels were independently correlated with renal function, decrease in eGFR and incident kidney disease<sup>28</sup>.

All of this data suggests that serum suPAR is a circulating factor that may cause FSGS<sup>10</sup>. This variation in suPAR and activation of podocyte  $\beta_3$  integrin may also help explain the variation in the responses that have been seen with the various therapeutics options employed to date in the treatment of idiopathic FSGS treatment and can now be tested at the clinical level. Further, this pathogenic mechanism is particularly intriguing due to the recent description of the action of Rituximab's direct podocyte modulating effect via cross reactivity with SMPDL-3b protein and regulation of ASMase essential for the organization and signaling of podocytes<sup>18</sup>.

Thus we propose integrating a pilot project testing the efficacy of Rituximab in steroid resistant or intolerant patients with idiopathic FSGS patients and the newly described permeability factor by restricting trial entry to patients with elevated levels of suPAR.

## 4. Study Design and Methods

### 4.1 Inclusion Criteria

#### Inclusion Criteria

- FSGS involving native kidneys with a diagnostic biopsy.
- Patients must be > 6 years of age and < 80 years of age
- suPAR > 3500 pg ml<sup>-1</sup>

- $\beta_3$  integrin activation as indicated by mean fluorescence intensity of AP5 (MFI > 1) corrected by paxillin FMI, followed by correction from normal controls. Activation of integrin in comparison to healthy controls is a measure to assess for a podocyte damaging effect of suPAR in the FSGS patients' blood samples.
- Treatment with an ACEI and/or ARB as tolerated for at least 3 months prior to enrollment to with a target a systolic blood pressure  $\leq$  140 mmHg and a diastolic pressure  $\leq$  90 mmHg in adults and blood pressure readings less than the 95<sup>th</sup> percentile for age, gender and height or less than 120/80 mmHg (whichever is lower) in children in at least 75% of readings
- Proteinuria  $\geq$  3.0 grams in adults, or urine protein to creatinine ratio  $\geq$  1.0 in the first morning urine in children, despite ACE inhibitor / ARB treatment as tolerated and a minimum of 8 weeks of prednisone therapy, a trial of calcineurin inhibitor for  $\geq$  3 months and/ or contraindication/intolerance to such therapy (diabetes, osteoporosis/osteonecrosis, age >60, BMI  $\geq$ 35)
- Patients with treatment resistant FSGS, patients exhibiting partial response to immunosuppressive treatment but remaining nephrotic
- Patients must have a minimal washout period before initiating Rituximab to avoid over immunosuppression and consequent risk of morbidity. However, to avoid risk of worsening of their underlying FSGS disease in those with partial response these patients are to discontinued or reduced other immunosuppressive therapies as follows:
  - o Cytotoxic therapy discontinued at least 6 months prior to initiation of Rituximab
  - o ACTH and/or MMF discontinued at least 30 days prior to initiation of Rituximab
  - o CNI tapered and discontinued beginning 60 days after the first Rituximab dose
  - o Prednisone reduced to  $\leq$  10 mg/day 30 days prior to Rituximab
- Negative serum pregnancy test (for women of child bearing age)
- Men and women of reproductive potential must agree to use an acceptable method of birth control during treatment and for twelve months (1 year) after completion of the trial
- Able and willing to give written informed consent and comply with study requirements

## 4.2 Exclusion Criteria

### Exclusion Criteria

- Estimated GFR < 40 ml/min per1.73m<sup>2</sup>. The rationale is that patients with advanced renal failure may progress rapidly towards ESRD.
- Collapsing variant of FSGS, as it is rare and has been associated with an aggressive course
- Patients with medical conditions that may cause FSGS (e.g. HIV, lymphoma, heroin use) or have a secondary form of FSGS due to hyperfiltration injury (massive obesity, vesicoureteral reflux, or renal mass reduction)
- Type 1 or type 2 diabetes mellitus as diabetic glomerulosclerosis may be contributing to proteinuria in these patients
- History of serious recurrent or chronic infection
- Presence or suspicion of active infection including HIV, Hepatitis B and HCV Positive tests for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb), hepatitis B virus (HBV), hepatitis C serology, or HIV serology. Testing for HIV, Hepatitis B and C should have occurred <2 years prior to enrollment into the study. Potential participants who are at high risk for TB should be tested. TB disease screening should be conducted on any potential study patient 1) for whom there is any clinical suspicion of TB, 2) who comes from

a country where TB disease is common (most countries in Latin America, the Caribbean, Africa, Asia, Eastern Europe and Russia) or 3) who lives or works where TB disease is more common (homeless shelters, prisons or jails or some nursing homes).

- 
- Absolute neutrophil count  $< 1.5 \times 10^3/\text{mL}$
- Known active infection requiring hospitalization or treatment with intravenous antibiotics within 4 weeks or oral antibiotics within 2 weeks of the study initiation
- Patients in receipt of a live vaccine within 4 weeks of the study initiation
- Concomitant malignancies or previous malignancies within the last five years, with the exception of adequately treated basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix
- Previous Treatment with a B-cell depleting antibody
- History of severe allergic reactions to humanized or murine monoclonal antibodies
- Treatment with any investigational agent within 4 weeks of the study initiation
- History of major psychiatric disorder, drug or alcohol abuse within the previous 6 months
- Any other disease, metabolic dysfunction, physical examination finding or clinical laboratory that provides a reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that may affect the interpretation of the results or render the patient at high risk from treatment complications

### 4.3 Recruitment of Participants

Participants will be identified and enrolled from the 6801 NEPTUNE study, the contact registry already in place as well as from prevalent patients at the involved sites. Coordinators from sites already involved in the NEPTUNE consortium will review these potential patient populations to find eligible candidates and ensure a seamless transition to this active therapeutic program. Both children and adult sites will participate. Involved centers have large, diverse patient populations.

### 4.5 Retention strategies

Given the small size of the trial and the fact that participants have resistant disease we do not expect any issues with respect to retention, as all participants will be followed closely by their care team.

### 4.6 Data Elements

#### Therapeutic Intervention

Rituximab will be infused intravenously on days one and fifteen at a dose of  $375 \text{ mg}/\text{m}^2$  up to a maximum of 1 gram per dose in children and at a dose of 1000 mg on days 1 and 15 in adults.

#### Clinical and Laboratory Evaluations

##### **Pre-Treatment Baseline Evaluation:**

- Hematology: complete blood count (CBC) with differential and platelet count (*within 6 weeks of treatment*).

- Serum Chemistries: BUN, creatinine, total bilirubin, alkaline phosphatase, total protein, albumin, AST, ALT, calcium, cholesterol profile, glucose, sodium, potassium, uric acid, bicarbonate and fasting cholesterol (total cholesterol, LDH, HDL, triglycerides) (*within 6 weeks of treatment*).
- Urine Chemistries:
  - 24 hour measurement of urinary sodium, protein and creatinine or protein to creatinine ratio in children if unable to complete a 24 hours urine collection. For patients who would like to begin urine collection prior to the pre-treatment baseline evaluation visit, oral consent will be obtained over the phone and a urine collection kit will be provided. Oral will be documented by signing and dating the oral consent script.
  - Random urine (UA) for study research laboratory measurement of suPAR, AP5, creatinine and albumin.
- Flow Cytometry for CD 19 and 20 (*Day 1*)
- Immunoglobulins (IgG, IgM and IgA) (*Day 1*)
- Serology Testing: Hepatitis B, Hepatitis C, HIV (< 2 years prior to enrollment in the study)
- Serum pregnancy test for women of childbearing potential at baseline (*within 2 weeks of treatment*)
- Serum and urine suPAR concentration and activation of podocyte  $\beta$ 3 integrin

Pediatric patients will have blood drawn limited to 3 mL/kg as per recommended NIH guidelines. As necessary, cholesterol profile may be omitted in pediatric patients. Further, determination of infectious status can occur at a separate blood draw.

If any of the above mentioned tests, procedures or evaluations were completed as standard of care within 6 weeks of baseline, participants need not repeat the investigations. Site investigator will review and consider completed evaluations to determine if appropriate to use for baseline evaluation and if repeat evaluations are necessary.

### ***Rituximab Infusion (Day 1 and Day 15)***

See below procedure for Rituximab infusion in adult patients or follow your site's SOP as required. Pediatric sites will follow locally established protocols

### **Dose Day 1**

At the Day 1 visit, the site coordinator should do the following:

- Ask the participant if he/she has any questions or concerns.
- Clinical Assessments- use source documents to collect information for each of the clinical assessments.
  - a. Collect subject's medical history (any changes since last visit)
  - b. Ask the subject about his/her concomitant medications (changes since last visit)
  - c. Ask the participant if they have experienced any adverse events since last visit
  - d. Urine pregnancy test for women unless participant has had tubal ligation, hysterectomy or is post-menopausal for 2 years.
- Ensure that PCM/charge nurse has confirmed that adequate resources are available to monitor patient during and following infusion.
- Confirm that pre-treatment screening has been completed.

- Hold all antihypertensive medications 12 hours before and on day of infusion.

**Monitoring:**

- Vital signs at baseline, then every 30 minutes during infusion, and 1 hour following completion of infusion.
- Monitor during infusion, and for up to 2 hours after completion of infusion for adverse effects: fever (temperature  $\geq 38^{\circ}\text{C}$ ), chills, rigors, rash, urticaria, flushing, hypotension, bronchospasm, dyspnea, angioedema, acute pain in chest, back or extremities, rhinitis, nausea, weakness, headache.
- Stop infusion and notify on-call physician and investigator, if any of the following are observed:
  - Any allergic or other adverse effects (see above).
  - Systolic BP falls below 100 mm Hg or decreases by 20% from baseline.
  - Respiration rate greater than 20.

*See section below for Management of Infusion Reactions.*

Pre-Medications: Give prior to Rituximab infusion

- acetaminophen 1000 mg po (give 30-60 min prior to rituximab).
- diphenhydramine (Benadryl®) 50 mg oral (give 30-60 minutes prior to rituximab).
- Methylprednisolone 100 mg (SoluMedrol®) in 0.9% NaCL to total 50 mL (200mL/hr completed 30 minutes prior to the start of Rituximab infusion).

Following Rituximab infusion, patients will be started on double strength Bactrim three times a week (or its equivalent) for pneumocystis pneumonia prophylaxis. This treatment will continue until the B cells (CD19/20+) have been repleted ( $>15$  cells/microliter on peripheral blood flow cytometry). In the rare event that B cells (CD19/20+) are not replete by the 6 Month visit, flow cytometry should be repeated at Month 9 and Bactrim (or its equivalent) continued until point of repletion.

FIRST of two Rituximab infusions:

- Infuse rituximab through a dedicated IV line after pre-medications administered.
  - 1,000 mg of Rituximab in 0.9% NaCL to total of 1000mL.
  - Start infusion at 50 mL/hr for the first hour.
  - If infusion tolerated, increase rate by 50 mL/hr every 30 minutes to a maximum of 400 mL/hr as tolerated, until completed.
  - 0.9% NaCL , 20mL flush after infusion. Use same rate as Rituximab infusion.
- Enter data collected on source documents into the e-CRF's online.
  - Retain all informed consent documents, materials from visit, and source documents in an organized fashion in a secured, double-locked room.

**Dose Day 15**

At the Day 15 visit, the site coordinator should do the following:

- Ask the participant if he/she has any questions or concerns.
- Clinical Assessments- use source documents to collect information for each of the clinical assessments.
  - a. Collect subject's medical history (any changes since last visit)
  - b. Ask the subject about his/her concomitant medications (changes since last visit)
  - c. Ask the participant if they have experienced any adverse events since last visit
  - d. Urine pregnancy test for women unless participant has had tubal ligation, hysterectomy or is post-menopausal for 2 years.

Use the following procedure for Rituximab infusion (or as per your site's SOP as required):

- Ensure that PCM/charge nurse has confirmed that adequate resources are available to monitor patient during and following infusion.
- Confirm that pre-treatment screening has been completed.
- Hold all antihypertensive medications 12 hours before and on day of infusion.

Monitoring:

- Vital signs at baseline, then every 30 minutes during infusion, and 1 hour following completion of infusion.
- Monitor during infusion, and for up to 2 hours after completion of infusion for adverse effects: fever (temperature  $\geq 38^{\circ}\text{C}$ ), chills, rigors, rash, urticaria, flushing, hypotension, bronchospasm, dyspnea, angioedema, acute pain in chest, back or extremities, rhinitis, nausea, weakness, headache.
- Stop infusion and notify on-call physician and investigator, if any of the following are observed:
  - Any allergic or other adverse effects (see above).
  - Systolic BP falls below 100 mm Hg or decreases by 20% from baseline.
  - Respiration rate greater than 20.

*See section below for Management of Infusion Reactions.*

Pre-Medications: Give prior to rituximab infusion

- acetaminophen 1000 mg po (give 30 min prior to rituximab).
- diphenhydramine (Benadryl®) 50 mg oral (give 30-60 minutes prior to rituximab).
- Methylprednisolone 100 mg (SoluMedrol®) in 0.9% NaCL to total 50 mL (200mL/hr completed 30-60 minutes prior to the start of Rituximab infusion).

**SECOND of 2 rituximab infusions** to be given 2 weeks following first infusion:

- Infuse rituximab through a dedicated IV line after pre-medications administered.
- 1,000 mg of Rituximab in 0.9% NaCL to total of 1000mL.

***If first infusion was tolerated without allergic reactions:***

- Start infusion at 100 mL/hr
- If tolerated, increase rate by 100 mL/hr every 30 minutes to a maximum of

400 mL/hr until completed.

- 0.9% NaCl , 20mL flush after infusion. Use same rate as Rituximab infusion.

***If first infusion was NOT tolerated:***

- Start infusion at 50 mL/hr .
- If tolerated, increase rate by 50 mL/hr every 30 minutes to a maximum of
- 400 mL/h until completed.
- 0.9% NaCl , 20mL flush after infusion. Use same rate as Rituximab infusion.

- Enter data collected on source documents into the e-CRF's online.
- Retain all informed consent documents, materials from visit, and source documents in an organized fashion in a secured, double-locked room.

**Management of Infusion-Related Reactions to Rituximab**

**Signs & Symptoms**

- Fever (temp  $\geq 38^{\circ}\text{C}$ ), chills, rigors
- Flushing, hypotension
- Bronchospasm, dyspnea, angioedema
- Rash, urticaria, angioedema, rhinitis
- Pain in chest, back or extremities
- Nausea, headache, weakness

**Management**

Stop infusion and notify on-call physician and PI if the above symptoms are observed, if systolic BP falls below 100 mmHg or decreases by 20% from baseline, or if respirations are greater than 20. If hypersensitivity or infusion-related reaction develops, the infusion may be slowed or interrupted. Symptoms may be treated with diphenhydramine, acetaminophen, or IV saline or bronchodilators, if warranted.

If patient experiences a mild reaction that is deemed by the investigator to be clinically significant, the infusion rate should be reduced to half the rate that was used at the time of the reaction. Once the reaction has resolved, the infusion should be kept at the reduced rate for an additional 30 minutes. If the reduced rate is tolerated, then the infusion rate may be increased to the next closest rate on the infusion schedule.

Patients who experience a moderate to severe reaction should have their infusion interrupted immediately and should receive aggressive symptomatic treatment. The infusion should not be restarted before all the symptoms have disappeared, and then at half the rate. If the reduced rate is tolerated for 30 minutes, then the infusion rate may be increased to the next closest rate on the infusion schedule.

***Post –Treatment Evaluation (at 1, 3, 6 12 months and possible unscheduled visit):***

- Hematology: complete blood count (CBC) with differential and platelet count.
- Serum Chemistries:

- BUN, creatinine, total bilirubin, alkaline phosphatase, total protein, albumin, AST, ALT, calcium, glucose, sodium, potassium, uric acid, bicarbonate and fasting cholesterol (total cholesterol, LDL, HDL, triglycerides)
- Urine Chemistries: 24 hour measurement of urinary sodium, protein and creatinine or protein to creatinine ratio in children if unable to complete a 24 hours urine collection.
- Random urine (UA) for study research laboratory measurement of suPAR, AP5, creatinine and albumin. Flow Cytometry for CD 19 and 20
- Immunoglobulins (IgG, IgM and IgA)

Post-treatment evaluation may occur at a visit not specified in the test and procedure schedule in the event that a patient contacts the physician with symptoms of relapse. Upon such event, the patient would come in for an “unscheduled visit” and complete the evaluation above.

As with the pre-treatment evaluation, pediatric patients will have blood drawn limited to 3 mL/kg as per recommended NIH guidelines. As necessary, the cholesterol profile may be omitted in pediatric patients.

#### **Treatment Failure and Criteria for Early Study Withdrawal**

Patients demonstrating a significant worsening of renal function (serum creatinine > 30% above baseline) or a significant worsening of urine protein that results in worsening symptoms of the nephrotic syndrome can exit the trial at their physicians discretion after review with the lead investigators (Drs Fervenza, Cattran and/or Hladunewich).

#### **Serum and Urine suPAR Measurement**

SuPAR concentration will be determined by quantitative sandwich enzyme immunoassay technique (Quantikine® ELISA Human uPAR Immunoassay, R&D Systems) specified by manufacturer’s protocol. These samples will be assessed in a blinded manner and repeated on the same aliquot in an independent laboratory at the end of the trial. Further an additional 20 samples from patients with nephrotic syndrome secondary to other histologic causes (eg membranous, MPGN) than FSGS will serve as an additional “control” group. Although the baseline sample will be run in real time as it defines inclusion, subsequent samples will be batched and run at the end of the study.

#### **Determination of Podocyte $\beta$ 3 Integrin Activity**

To quantitatively examine the effect of FSGS patient sera on podocyte  $\beta$ 3 integrin activity, a human podocyte cell line is cultured at 37 °C for 14 days for complete differentiation. The cells are then incubated in 5-10% of FSGS patient serum for 24 hours with recombinant suPAR protein as a positive control. Next, the cells are fixed with 4% paraformaldehyde (PFA) and proceeded for immunofluorescence staining for AP5 (Blood Center of Wisconsin) and paxillin (Millipore). After immunostaining, confocal (Leica) images are taken to quantify the AP5 and paxillin intensity for each sample treatment. Paxillin signal is used to correct AP5 signal. The relative AP5 signal (AP5/paxillin ratio) from each patient serum is then normalized against that of normal blood donor included in each assay for final report. To control for suPAR specificity, the cells will be co-incubated with both FSGS sera and suPAR blocking antibody.

Aliquots of the baseline research blood and urine samples will be sent to Dr. Reiser’s laboratory

immediately. All research blood and urine collected subsequent to baseline will be sent to Dr. Reiser's and an independent laboratory at the end of the study. Research blood and urine tests, subsequent to test run on baseline collection will be performed as a batch, after study completion, as dictated by budgetary constraints. All suPAR and AP5 measurements will be performed in Dr. Reiser's laboratory and confirmed in an independent laboratory.

### Research Sample Procedures

Research blood and urine samples will be collected at baseline, 1, 3, 6 and 12 months. Blood samples will be collected in red top serum tubes, centrifuged at 2,000 x g for 10 minutes at 4°C and sera immediately harvested. Fresh urine samples will be drawn from a random urine collection, centrifuged at 2,000 x g for 10 min and supernatant harvested. To eliminate and minimize freeze thaw, both serum and urine samples will be divided into 500uL aliquots. Aliquots will be stored at minus 80°.

Samples will be labeled in a manner so as to blind laboratory assessment investigators to both patient and time-point. Aliquots of the baseline research blood and urine samples will be sent to Dr. Reiser's laboratory immediately, as suPAR and  $\beta_3$  integrin activation results determine eligibility. All research blood and urine collected subsequent to baseline will be sent to Dr. Reiser's and an independent laboratory at the end of the study. In addition, a site stored aliquot of the baseline sample will be shipped along with these other samples to assess replication at the end of the study. These assessments will be performed as a batch after study completion as dictated by budgetary constraints. Baseline and subsequent samples will be sent to Dr. Changli Wei at Rush University.

## 4.7 Schedule of Events

Tests	Baseline	Day 1	Day 15	1 month	3 months	6 months	12 months	Unscheduled visit <sup>5</sup>
Informed Consent	X*							
History and Exam	√*			√	√	√	√	√
Complete blood count (CBC) with differential <sup>1</sup>	√*			√	√	√	√	√
Serum Chemistries <sup>2</sup> : BUN, Creatinine, Total bilirubin, Alkaline phosphatase, Total protein, Albumin, AST, ALT, Calcium, Glucose, Sodium, Potassium,	√*			√	√	√	√	√

Uric acid, Bicarbonate, Total Cholesterol (LDH, HDL, Triglycerides)								
Serum pregnancy	X							
Urine Pregnancy <sup>3</sup>		X	X					
Hold all antihypertensive medications 12 hours before and on day of infusion		X	X					
Pre-infusion Medication: <ul style="list-style-type: none"> <li>• Acetaminophen</li> <li>• Diphenhydramine</li> <li>• Methylprednisolone</li> </ul>		X	X					
Rituximab Infusion		X	X					
Double strength Bactrim three times a week (or its equivalent) for pneumocystis pneumonia prophylaxis.		X	X	X	X	X	X	X
CD 19 and 20		X		X	X	X	X	X
Immunoglobulins (IgG, IgM and IgA)		X		X	X	X	X	X
24 hour creatinine for CrCl	√*			√	√	√	√	√
24 hour urine protein OR 24 hour Uprot/Ucreat	√*			√	√	√	√	√
24 hour urinary Na+	√*			√	√	√	√	√
Research Labs (blood and random urine) <sup>4</sup>	X*			X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X

\*within 6 weeks of infusion

√ Standard of Care

<sup>1,2</sup> Approximately 50mL for adults and 3mL/kg for pediatric patients. At baseline (Time 0), if patients had any Time 0

study specific labs drawn clinically at Mayo within 30 days, those values may be appropriate to use upon the investigator's review. The exception to this is serum pregnancy test which must be completed within 2 weeks of Time 0.

<sup>3</sup> Urine pregnancy test for women unless participant has had tubal ligation, hysterectomy or is post-menopausal for 2 years.

<sup>4</sup> Blood and urine to be used for research purposes, see Appendix A. Research Sample Procedures.

<sup>5</sup> Post-treatment evaluation may occur at a visit not specified in the test and procedure schedule in the event that a patient contacts the physician with symptoms of relapse.

## 5. Safety Monitoring

All adverse events will be reported centrally and reviewed by the Study Chair. Either Dr. Hladunewich [REDACTED] or Dr. Cattran [REDACTED] will be available 24 hours a day, seven days a week to study investigators. If a significant reaction or serious adverse event occurs during or following the first Rituximab infusion, the second infusion may be modified or may not proceed at the direction of the study chair. All adverse events will be reported back to the study investigators and to the DSMB who would subsequently make recommendations with respect to discontinuation of the trial and disclosure to study participants.

### 5.1 Data and Safety Monitoring Plan

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

This is an interventional pilot study that meets the federal definition of moderate risk.

### 5.2 Study Oversight

The Study Chair has primary oversight responsibility of this clinical trial. The appointed Data (Observational) Safety Monitoring Board (D/OSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The D/OSMB will review accrual, patterns and frequencies of all adverse events, protocol compliance every interventional "6 months". The D/OSMB makes recommendations to the NIH regarding the continuation status of the protocol.

Each site's Primary Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate report- detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed immediately by the research team and reported as detailed below (see section 5.5). A separate report detailing protocol compliance will also be available from the DMCC for site review on a monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

### 5.3 Definitions and Standards

The Rare Diseases Clinical Research Network defines an adverse event as: "...an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a Rare Diseases Clinical Research Network study."

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with Idiopathic Focal Segmental Glomerulosclerosis (FSGS) that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

#### **Serious Adverse Events**

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

#### **Assessment of Adverse Events**

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to Rituximab (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes - There is a plausible temporal relationship between the onset of the AE and administration of the study drug, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response

to the study drug; and/or the AE abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.

No - Evidence exists that the AE has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to study drug administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study. Expected adverse events are also listed or characterized in the Product Monograph or current Investigator Brochure.

An unexpected adverse event is defined as any adverse experience...the specificity or severity of which is not consistent with the risks of information described in the protocol. Unexpected adverse events are those not listed in the Product Monograph (P.M) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.M. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.M. or I.B. only referred to elevated hepatic enzymes or hepatitis.

All reported adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE), developed and maintained by CTEP at National Cancer Institute.

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

##### Study Drug/Intervention: Drug Information for Rituximab:

Rituximab has been associated with infusion-related reactions, which may be related to the release of cytokines and/or other chemical mediators. These reactions may involve fever and chills; skin rash and itching; flushing; nausea and vomiting; fatigue; headache; cold-like symptoms; sensation of tongue or throat swelling; and difficulty breathing. These conditions usually manifest from 30 minutes to 2 hours after the start of a Rituximab infusion. These symptoms are more commonly seen during the first infusion of Rituximab. Infusion related side effects have occurred in 77% of patients during the first infusion when the drug was given to treat Non-Hodgkin's Lymphoma, but only 7% were considered to be severe reactions. A mild to moderate change in blood pressure may also occur during the infusion. In a previous study in patients with rheumatoid arthritis, 18-30% of the patients had a decrease in blood pressure with the first infusion of Rituximab, whereas a mild to moderate increase in blood pressure occurred in up to a quarter of patients (8-25%).

In about 1 in 1000 patients, serious side effects may occur. These serious side effects include:

- Chest pain (approximately 1 in 1,000)
- Abnormal heart beat (approximately 1 in 1,000)
- Severe shortness of breath (approximately 1 in 10,000)
- Severe skin rash (approximately 1 in 10,000)
- Lung inflammation (swelling and redness inside the lungs; approximately 1 in 10,000)

- Heart failure (approximately 1 in 10,000)
- Kidney failure from rapid killing of tumor cells (approximately 1 in 10,000; seen only in cancer patients)

In the most rare circumstances (1 in 10,000) death can result, related to the infusion.

Other side effects, not necessarily occurring during the Rituximab infusion, may include the following:

- Anemia or low blood count
- Low platelet count (uncommon)
- Joint pain
- Infection
- Flu-like symptoms
- Decreased appetite (uncommon)
- Fatigue
- Sleep problems (uncommon)
- High blood pressure (uncommon)
- Bowel Perforation (development of a hole in the small or large intestine) or Bowel Obstruction (blockage of the small or large intestine). To date, 4 cases have occurred in Canada following the use of Rituximab for the treatment of either Lymphoma or Arthritis

### **Cardiovascular Events**

Because hypotension may occur during a Rituximab infusion, consideration should be given to withholding anti-hypertensive medications 12 hours prior to the Rituximab infusion. Angina pectoris, cardiac arrhythmias such as atrial flutter and fibrillation, heart failure, and myocardial infarction have occurred in patients treated with Rituximab. Therefore, patients with a history of cardiac disease should be monitored closely.

### **Infections**

Given the mechanism of action of Rituximab and the knowledge that B cells play an important role in maintaining normal immune response, patients may have an increased risk of infection following Rituximab therapy. Rituximab should not be administered to patients with an active infection or to severely immunocompromised patients (e.g., those in whom levels of CD4 or CD8 are very low). Physicians should exercise caution when considering the use of Rituximab in patients with a history of recurring or chronic infections or with underlying conditions that may further predispose patients to serious infection. Patients who develop infection following Rituximab therapy should be promptly evaluated and treated appropriately. If a patient experiences a new infection during the intervening time between any of the four Rituximab infusions, the subsequent infusion should be delayed (up to a maximum of 14 days) until the infection has completely resolved and treatment with any anti-infective medications has been completed. Receipt of further Rituximab infusions is at the discretion of the investigator. Remaining infusions should be scheduled so that they are given 1 week apart.

### **Hepatitis B Infections**

Cases of hepatitis B reactivation, including those with a fatal outcome, have been reported in

patients with RA, GPA, and MPA treated with Rituximab. Screening for HBsAg, HBcAb, HBV, and hepatitis C serology must be performed in all patients before initiation of treatment with Rituximab. Patients with positive hepatitis serology will be excluded from the study. Any patient who experiences a de novo hepatitis infection during the study will be immediately withdrawn from Rituximab study therapy and will be followed until the common closeout date or, if required, for longer in Extended Follow-Up (see Section 3.1.4).

### Progressive Multifocal Leukoencephalopathy

Cases of fatal PML have been reported following use of Rituximab for the treatment of autoimmune diseases, including RA. Several, but not all, of the reported cases had potential risk factors for PML, including the underlying disease, long-term immunosuppressive therapy, or chemotherapy. PML has also been reported in patients with autoimmune disease not treated with Rituximab. Physicians treating patients with autoimmune diseases should consider PML in the differential diagnosis of patients reporting neurological symptoms which may include clumsiness, progressive weakness, visual, speech, and sometimes, personality changes. If PML is considered then a neurological consultation should be obtained. If PML is diagnosed in a patient receiving Rituximab, no further infusions of rituximab should be administered; reductions in concomitant immunosuppressive therapy and appropriate treatment including antiviral therapy should be considered. There are no known interventions that can reliably prevent PML or adequately treat PML should it occur.

### Neutropenia

Decreases in neutrophils have been observed in adult patients with GPA and MPA following treatment with Rituximab. Neutropenia was not associated with an observed increase in serious infection in such patients. Patients with an ANC  $< 1.5 \times 10^3/\mu\text{L}$  at screening will not be enrolled in the study. The risk mitigation strategies for neutropenia occurring during the study are summarized below.

#### Risk Mitigation for Neutropenia

ANC (cells x $10^3/\mu\text{L}$ )	Action
0.5 – $< 1.5$	Interrupt dosing.  When ANC increases to $\geq 1.5 \times 10^3/\mu\text{L}$ , resume dosing as clinically appropriate.
$\leq 0.5$	Permanently discontinue study drug and follow patient with safety visits to look for signs of infection until $\text{ANC} \geq 1.5 \times 10^3/\mu\text{L}$ . If count does not return to $\geq 1.5 \times 10^3/\mu\text{L}$ within 2 months (or sooner if deemed necessary by the investigator), a hematologic consult should be requested and the patient followed until resolution or

stabilization.

ANC = absolute neutrophil count.

### **Hypogammaglobulinaemia**

Decreases in IgA, IgG, and IgM have been observed in adult patients with GPA and MPA following treatment with rituximab. No increased rate in overall infections or serious infections was observed after the development of low IgA, IgG, or IgM levels. Assessment of the Roche safety database and published literature reveals that development of hypogammaglobulinemia after rituximab treatment in pediatric patients with various underlying disease has been observed, and, in some cases, this has been reported as severe and long term requiring long-term IVIg treatment. However, attribution of these events to rituximab is limited by potential alternate etiologies, such as common variable immunodeficiency disease, severity of underlying diseases, use of rituximab as a last resort after exposure to other immunosuppressive agents, and limited clinical data including lack of randomized clinical trials, retrospective data collection, and lack of detailed safety data reporting. Importantly, the majority of these cases were not reported to have been associated with serious infections. The investigator should closely monitor the patient's immunoglobulin levels during the study. If immunoglobulin levels decrease below the LLN, dosing should be interrupted or discontinued, as clinically appropriate. IVIg (restricted to replacement dosing for hypogammaglobulinemia) should be given at the discretion of the investigator.

The safety of immunization with any vaccine, particularly live viral vaccines, following Rituximab therapy has not been studied and it is not recommended that any live viral vaccines be given within 4 weeks of Rituximab infusion.

It is also unknown what affect Rituximab will have on a developing fetus, sperm, or a breast-fed infant. For this reason, study participants are advised to use adequate contraception and avoid pregnancy during participation in the study and for a period of 12 months after receiving Rituximab

### **Study Procedures:**

Venipuncture: The risks of drawing blood include temporary pain, bruising, or rarely infection at the site of the needle stick. Participants may experience similar discomfort from the needle used for the infusions of Rituximab.

## **5.5 Reporting Guidelines**

### **Adverse Event Reporting Period**

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and ends 30 days after study discontinuation/termination. After this period, investigators should only report SAEs that are attributed to prior study treatment.

### **Procedures for Eliciting, Recording, and Reporting Adverse Events**

#### **Eliciting Adverse Events**

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"

- “Have you had any new or changed health problems since you were last here?”

### **Specific Instructions for Recording Adverse Events**

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

#### **a. *Diagnosis vs. Signs and Symptoms***

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

#### **b. *Deaths***

All deaths that occur during the protocol-specified AE reporting period (see Section 5.1.2), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report “Unexplained Death”.

#### **c. *Preexisting Medical Conditions***

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

#### **d. *Hospitalizations for Medical or Surgical Procedures***

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

#### **e. *Pregnancy***

If a female subject becomes pregnant while receiving investigational therapy or within 12 months after the last dose of study drug, a report should be completed and expeditiously

submitted to the sponsor who will report this information to Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the study drug should be reported as an SAE.

**f. Post-Study Adverse Events**

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior study drug exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

**g. AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product**

- Progressive Multifocal Leukoencephalopathy (John Cunningham Virus)
- Malignancy Events
- Posterior Reversible Encephalopathy Syndrome
- Hepatobiliary Events

These events should be reported to the sponsor as soon as site is aware, as they must be further communicated to Genentech by the sponsor within a 15 day time period.

**Serious Adverse Events**

- *Within **24 hours** (of learning of the event), investigators must report to the sponsor any reportable Serious Adverse Event (SAE) that:*
  - *Is considered life-threatening/disabling or results in death of subject*
- OR-
- *Is Unexpected/Unanticipated*
- *Investigators must report all other reportable SAEs within **5 working days** (of learning of the event).*
- *All other (suspected) reportable AEs must be reported to the RDCRN within **20 working days** of the notification of the event or of the site becoming aware of the event.*

Local institutional reporting requirements to IRBs, any GCRC oversight committee and the country specific regulatory authorities, if appropriate, remain the responsibility of the treating physician and the Study Chair.

**5.6 RDCRN Adverse Event Data Management System (AEDAMS)**

Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, site PIs, the Medical Review Officer, and any additional agencies (if applicable- industry sponsor, CTEP, etc) of any reported adverse events via email.

Serious adverse events: The NIH appointed Medical Review Officer (MRO) determines causality (definitely not related, probably not related, possibly related, probably related,

definitely related) of the adverse event. The MRO may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MRO and RDCRN DSMB on a “bi-annual” basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all adverse events (serious/not serious and expected, unexpected) for site investigators, IRBs, and country specific regulatory authorities. This information will also be communicated directly to Genetech.

The sponsor is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to the appropriate regulatory authority (FDA or Health Canada), appropriate IRB(s), and Genentech, Inc. in accordance with GCP and regulatory guidelines.

## **5.7 Study Discontinuation (Interventional)**

The NIH, DSMB and local IRB's (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met

### **Cessation of Trial**

Patients with a documented reaction to rituximab or a serious infection secondary to immunosuppression will be allowed to exit the trial. Patients with greater than 30% increase in serum creatinine without improvement in proteinuria and without alternative reasons beyond the their natives kidney disease progression eg NSAID, infection, volume depletion etc that occurs beyond the first three months post rituximab will also be allowed to exit the study in order that alternative therapy can be considered.

- Accrual has been met
- The study objectives have been met
- The Study Chair / Study Investigators believe it is not safe for the study to continue
- The DSMB suspends or closes the trial
- The NIH suspends or closes the trial
- The FDA or Health Canada suspends or closes the trial

## 5.8 Subject Discontinuation

An intent to treat approach will be used. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g..severe infusion related reaction)

## 5.9 Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The RDCRN DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.
- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

## 6. Statistical Considerations

This is a small pilot study to primarily assess safety for the use of Rituximab in idiopathic FSGS. There is no preliminary data available to perform a power calculation, therefore the number of participants was chosen based on available drug and funding. However, paired parametric or non-parametric testing, where appropriate, will be utilized to assess for any meaningful differences from the baseline values for all variables of interest (serum creatinine, urine protein, serum albumin, cholesterol profiles as well as serum and urine suPAR values. The results of this pilot trial are expected to assist in the design of larger future randomized trials.

## 7. Data Management

As much as possible data quality is assessed at the data entry point using intelligent on-line forms. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures

are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

All study data will be collected via systems created in collaboration with the RDCRN Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

## **7.1 Registration**

Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system, the system will assign a participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers will be required to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant. In this fashion, no personal identifiers would be accessible to the DMCC.

## **7.2 Data Entry**

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

## **7.3 Data Quality Control**

As much as possible data quality is assessed at the data entry point. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

## **8. Human Subjects**

### **8.1 GCP Statement**

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

### **8.2 Risks**

The potential risks of this study are:

In addition to the risks related to Rituximab administration and venipuncture listed in Section 5.4, participants may have risks related to the disclosure of personal health information collected during this study. In order to minimize the possibility of a breach of confidentiality, all data and samples will be de-identified and stored in a secure manner.

### **8.3 Benefits**

The potential benefits of this study are:

Participants may or may not receive any direct medical benefit from participation in this study. However, it is possible that Rituximab may result in a remission of kidney disease. Further, any abnormalities detected during the study will be communicated directly to participants and their physicians to aid in their treatment plan. Information learned from this study may benefit other patients with FSGS in the future.

### **8.4 Recruitment**

Potentially eligible patients will be approached by an individual within their circle of care (nurse, physician, etc). For patients interested in participating, the Investigator or designate will then obtain written informed consent. The informed consent process will be conducted in accordance with ICH/GCP guidelines.

### **8.5 Written Informed Consent**

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

## **8.6 Process of Consent:**

The informed consent process will begin when a potentially eligible patient is approached by an appropriately trained and delegated member of the study staff to discuss participation in this study. After reviewing the Informed Consent form, the potential participant must be given ample time to ask questions and decide upon participation. The study staff must ensure comprehension of the material reviewed and then ascertain the potential participant's willingness to participate. The informed consent process is ongoing, and it is the responsibility of the investigator to ensure that participants continue to consent throughout the course of the study. New information that is relevant to the participant's ongoing consent will be communicated to study participants throughout the study. The informed consent form will be revised, as appropriate, and ongoing participants re-consented or given the opportunity to decline further participation.

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