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Efficacy of Rituximab in Treatment Resistant Focal Segmental Glomerulosclerosis with Elevated SuPAR and Activation of Podocyte β3 Integrin

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Running Head: Rituximab in FSGS and an Assessment of SuPAR

ABSTRACT
Introduction: Severe, non-responsive, primary Focal Segmental Glomerulosclerosis (FSGS) can progress to end stage kidney disease (ESKD) in < 5 years. Soluble urokinase-type plasminogen activator receptor (suPAR) may contribute to podocyte effacement by activating podocyte β3 integrin. It has been reported as a potential permeability factor and biomarker for primary FSGS. Rituximab has demonstrated efficacy case reports and small series. Whether rituximab is efficacious in patients with treatment resistant FSGS in the context of high suPAR levels and evidence podocyte B3 integrin activation remains unknown.

Methods: In this non-blinded, open label pilot study, the safety and efficacy of Rituximab was assessed in treatment resistant adult patients with primary FSGS and a suPAR > 3500 pg ml⁻¹ with evidence of β3 integrin activation. Rituximab (1 gram) was given on Day 1 and 15. The primary outcome was proteinuria at 12 months.

Results: Only 13/38 screened patients qualified for the study of which 9 consented to participate. The baseline proteinuria and GFR were 7.70±4.61 g/day and 67±38 ml/min, respectively. A transient response at 6 months was noted in 2 patients without a parallel change in suPAR. At 12 months, there was no statistically significant improvement in proteinuria with all participants remaining nephrotic (7.27±7.30 g/day). GFR marginally declined to 60±38 ml/min with one patient progressing to ESKD. There were 2 serious infections, an infusion related reaction and leucopenia attributed to Rituximab.

Conclusion: Rituximab was ineffective when administered to adult patients with treatment resistant primary FSGS with a high suPAR and evidence of podocyte activation.

Keywords: Nephrotic Syndrome, Focal Segmental Glomerulosclerosis (FSGS), Soluble urokinase-type plasminogen activator receptor (suPAR), Rituximab, Treatment Resistance
Focal segmental glomerulosclerosis (FSGS) describes a renal histologic lesion caused by diverse etiologies and pathological processes, all of which can lead to podocyte injury and depletion. Patients with primary FSGS, typically present with nephrotic syndrome, focal and segmental lesions on light microscopy, no definable immune complex deposition on immunofluorescence microscopy and widespread foot process effacement on kidney biopsy electron microscopy (EM) examination. Spontaneous remissions are rare (<5%), and if patients are untreated and/or unresponsive to immunosuppression, the disease typically progresses to end stage kidney disease (ESKD) over 6-8 years in 50% of patients. Those with severe nephrotic syndrome (proteinuria > 10 grams) have a worse prognosis and can expect to progress to ESKD over 3-5 years if unresponsive. Further, the FSGS lesion recurs after transplantation in approximately a third of patients with severe primary disease contributing significantly to graft loss. Thus, primary FSGS poses a significant burden on patient health and well-being as well as health care resources.

In primary FSGS presenting with severe nephrotic syndrome, the presence of a circulating factor that results in podocyte effacement and disruption of the glomerular filtration barrier has been supported by several experimental and clinical studies. Recent insights into podocyte biology have identified soluble urokinase plasminogen activator receptor (suPAR), a myeloid cell derived circulating factor, which connects innate immune function to the maintenance of the slit diaphragm through its ability to form signaling complexes with other transmembrane proteins, including activation of podocyte αvβ3 integrin. Activation of this receptor and its downstream pathways result in activation of small GTPases (e.g., Rac1), leading to podocyte foot process effacement, proteinuria, glomerular damage and loss of renal function. In suPAR transgenic mice, variable amounts of renal disease with proteinuria, loss of kidney
function, and glomerulosclerosis, characteristic of FSGS, were noted. As such, both suPAR and evidence of activation of the podocyte β3 integrin have been proposed as a key mechanism for primary FSGS, and as potential biomarkers.

Immunosuppressive treatments have been shown to improve proteinuria and slow progression, but the side effects of current options that include high dose prolonged corticosteroids, cytotoxic agents and calcineurin inhibitors are significant, while efficacy is limited. 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. 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. This potential direct effect on podocyte integrity, independent of its known effect on selective depletion of the B cell clone, supports Rituximab as an attractive option to consider for the treatment of immunologically mediated FSGS. To date, only case reports and a small open-label trials exist to suggest that Rituximab might prove effective in patients with FSGS and these reports primarily included children and patients with post renal transplant recurrence wherein additional therapies were part of the treatment regimen.

In this non-blinded, open label pilot study, we tested the efficacy of Rituximab in steroid resistant or intolerant adults with primary FSGS patients. As part of the protocol, we also measured markers of disease activity, and restricted trial entry to patients with both high levels of serum suPAR and evidence of activation of the podocyte β3 integrin. In addition to reporting on the trial results, we report on the sizable population of FSGS patients who failed screening for
inclusion on the basis of these biomarkers as well as a control population with other forms of glomerular disease. We hypothesized that Rituximab may be an effective therapy in patients with FSGS and evidence of disease activation.

METHODS

Study subjects, inclusion and exclusion criteria

Participating patients were adults (> 18 years of age) with primary FSGS. All biopsy reports were reviewed by the investigators to confirm evidence of diffuse foot process effacement (>80%) on EM (MH, FF, SS). All study participants had proteinuria ≥ 3.0 g/24h and an estimated GFR ≥ 40 ml/min/1.73m², using the 4 variable MDRD equation as published in the NKF-CKD guidelines. The rationale for the GFR criteria was that patient with severely reduced GFR are more likely to have significant interstitial and glomerular scarring that would indicate irreversible injury. Finally, only patients with a suPAR > 3500 pg ml⁻¹ with evidence of β₃ integrin activation were included (micro-flow image; MFI > 1). All subjects provided informed consent as per the Declaration of Helsinki for Medical Research Involving Human Subjects.

Other exclusions included the 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 those with a secondary form of FSGS that can be associated with hyperfiltration injury (e.g., massive obesity, vesicoureteral reflux, or renal mass reduction). Also, patients with active infections, malignancy within the preceding 5 years, Type 1 or 2 diabetes mellitus were excluded. Women who were pregnant or nursing were excluded for safety reasons.

Definition of treatment resistant FSGS
Treatment resistance or intolerance was defined as persistent or increasing proteinuria (≥ 3.0 grams), despite ACE inhibitor/ARB treatment as tolerated and a minimum of 8 weeks of prednisone therapy, a trial of calcineurin inhibitor for ≥ 3 months, cytotoxic therapy and/or contraindication/intolerance to such therapy (e.g., osteoporosis/osteonecrosis). Patients exhibiting partial response to immunosuppressive treatment, but remaining nephrotic required a minimal washout period before initiating Rituximab to avoid over immunosuppression and consequent risk of serious adverse events. However, to avoid risk of worsening of their underlying FSGS disease, in those with some response, investigators were permitted to apply the following rules in regards to immunosuppressive therapies: cytotoxic therapy discontinued at least 6 months prior to initiation of Rituximab; ACTH and/or mycophenolate mofetil discontinued at least 30 days prior to initiation of rituximab; calcineurin inhibitors tapered and discontinued within 60 days after the first rituximab dose and prednisone reduced to ≤ 10 mg/day at least 30 days prior to receiving the rituximab infusion.

**Study design and protocol**

This was a non-blinded, open label study using rituximab provided by Genentech Pharmaceuticals. The study was performed at the Mayo Clinic and the University Health Network. Research ethics boards at both sites approved the study. Screening began in December of 2016 with enrollment during 2017 and 2018 and follow-up was completed by January 2019. The conduct and reporting of the study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cohort studies (Supplementary Table S1). Prior to initiating active therapy, target blood pressure (systolic <140 mm Hg) was achieved during a three-month run-in period. Angiotensin receptor blockers (ARB) were used preferentially because better tolerated, with minimal cough or angioedema. The dose was
increased at 2 weeks intervals until target blood pressure was achieved, or until intolerable side
effects occurred. Additional medication was added as necessary in patients whose blood
pressure control was not at target at the discretion of the attending nephrologist. As part of the
standard of care for patients with nephrotic syndrome and severe hyperlipidemia, patients were
started on a statin increased to the maximum recommended or tolerated dose. Finally, all patients
received dietary counseling to maintain a low salt diet (2-3g/day) and a dietary protein target
intake of 0.8 g/kg ideal body weight/day of protein throughout the duration of the study. Once
stabilized, further escalations to drugs that block the renin angiotensin system or the dose of lipid
lowering agents were not permitted. Dose reductions, however, were guided by side effects (i.e.,
hyperkalemia, hypotension, myalgia etc.).

Rituximab was infused intravenously on days one and fifteen at a dose of 1000 mg.
Established site infusion protocols were employed, but all patients received pre-infusion
treatment with acetaminophen 1000 mg po (give 30-60 min prior to rituximab),
diphenhydramine (Benadryl®) 50 mg oral (give 30-60 minutes prior to rituximab) and
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 were
started on double strength Bactrim three times a week (or its equivalent) for pneumocystis
pneumonia prophylaxis. This treatment continued until the B cells (CD19/20+) were replete
(>15 cells/microliter on peripheral blood flow cytometry).

At each visit, patients were questioned about their symptoms and possible side effects of
therapy. Physical examination included the measurement of blood pressure and body weight.
Fasting blood samples and aliquots from 24-hour urine collections were taken at baseline and
then at 1, 3, 6 and 12 months. Measurements included serum concentrations of creatinine,
electrolytes, liver function tests, blood glucose, cholesterol profile (triglycerides, HDL cholesterol, LDL cholesterol), albumin, immunoglobulins (IgG, IgM and IgA) as well as flow cytometry for CD 19 and 20. The glomerular filtration rate was estimated by the MDRD equation. Proteinuria was monitored by 24-hour urine collections with simultaneous urine creatinine measurements to ensure collection completeness. At each time point, blood was also sampled for suPAR and evidence of podocyte β3 integrin activation was obtained.

**Study outcomes**

The primary outcome measure was the change in proteinuria 12 months post treatment. Complete remission was defined as proteinuria < 0.3 g/day; partial remission as reduction in proteinuria by >50% with a final urine protein < 3.0 g/day, but >0.3 g/day; incomplete remission as reduction in proteinuria ≥50%, but residual proteinuria still >3.0g/d and no response defined as worsening serum creatinine > 30% above baseline and/or <50% reduction in proteinuria or worsening of proteinuria. Secondary outcomes measured at 1-, 3-, 6- and 12-months post therapy included changes from baseline suPAR level and activation of podocyte β3 integrin as indicated by relative AP5 activity, which is an antibody utilized to detect the active state of β3 integrin. Finally, changes in other measures of the nephrotic syndrome, including improvements in serum albumin and cholesterol profile as well as documented side effects and toxicity, were noted.

**Laboratory determinations**

Most values were determined by site-specific laboratory evaluations. Serum SuPAR concentration was determined by quantitative sandwich enzyme immunoassay technique (Quantikine® ELISA Human uPAR Immunoassay, R&D Systems and by Virgates) specified by manufacturer’s protocol. These samples were assessed in a blinded manner at a central laboratory (JR). An additional 20 samples from patients with nephrotic syndrome secondary to
other histologic causes than FSGS (e.g., membranous, MPGN) served as an additional control group. The baseline sample was run in real time as it defined inclusion, subsequent samples were batched and run at the end of the study. Activation of integrin in comparison to healthy controls is a measure that assesses the podocyte damaging effect of suPAR in the FSGS patient’s blood samples. To quantitatively examine the effect of FSGS patient sera on podocyte β3 integrin activity, a conditionally immortalized human podocyte cell line was cultured at 37 °C for 14 days for complete differentiation. The cells were then incubated in 5-10% of FSGS patient serum for 24 hours with recombinant suPAR protein as a positive control. Next, the cells were fixed with 4% paraformaldehyde (PFA) and proceeded for immunofluorescence staining for AP5 (Blood Center of Wisconsin) and paxillin (Millipore). After immunostaining, confocal (Leica) images were 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 were co-incubated with both FSGS sera and suPAR blocking antibody.

**Statistical analysis**

Descriptive statistics were calculated for all variables of interest. Continuous measures were summarized using mean ± standard deviation whereas categorical measures were summarized using counts and percentages. Paired testing was used to assess changes over time. The Fisher Exact Test was utilized to compare proportions. An analysis of variance was used to compare responses by dose with the Tukey test utilized for between group comparisons. A p value <0.05 deemed statistically significant. All analyses were carried out using STATA Version 16 (Statacorp, College Station, TX, USA).
RESULTS

Cohort screening and inclusion

Of the 38 FSGS patients screened for the study who met all clinical and histological criteria, only 13 qualified based on suPAR level cut off and cellular β3 integrin activation profile, of which 9 consented to participate. Non-consenting patients were either concerned about potential side effects associated with Rituximab or could not travel for study visits. Baseline characteristics of the patients who qualified for the study and those that did not are displayed in Table 1. Of the 25 excluded patients, 21 were excluded on the basis of a serum suPAR, 2 were excluded on the basis of inadequate AP5 activation while an additional 2 were excluded for not meeting either criterion. There were no significant clinical or other laboratory differences between included and excluded patients with all having parameters compatible with the nephrotic syndrome. Control samples included patients with other forms of kidney disease including membranous nephropathy, IgA Nephropathy and other disease entities (n=16). These patients were also not different by clinical or other laboratory parameters than the FSGS cohort with an average GFR of 73 ± 28 ml/min and an average proteinuria of 7.74 ± 3.84 g/day (Table 1).

Baseline characteristics of the treated cohort

The cohort had an average age of 37 ± 16 years. Approximately half of the cohort was male (56%) with the majority being Caucasian (67%). All, but one patient, were stable on either monotherapy (7 patients) or dual blockade (1 patient) of the renin angiotensin system (RAS) prior to the run-in phase with no noted changes in urine protein. The single patient not on RAS blockade did not tolerate the therapy due to hypotension. Prior exposure to multiple
immunosuppressive agents was noted in all patients. At the time of the baseline evaluation, immunosuppression regimens included high dose prednisone monotherapy (1 patient), calcineurin monotherapy (1 patient), prednisone with calcineurin inhibitor (1 patient), mycophenolate mofetil with calcineurin inhibitor (2 patients) or triple therapy with prednisone, calcineurin inhibitor and mycophenolate mofetil (4 patients). Other population baseline characteristics are displayed in Table 2. Mean 24-hour protein was 7.70 ± 4.61 g/day, mean serum albumin was 30±7 g/dL, and baseline eGFR was 67±38 ml/min. As per protocol, all baseline suPAR values were > 3500 pg ml⁻¹ with evidence of β₃ integrin activation with average values of 4120±1169 pg ml⁻¹ and 1.56±0.59, respectively.

**Treatment response**

There was no a significant change in urine protein at 12 months compared to the baseline value (7.70±4.61 versus 7.27±7.30 grams) with no patients in remission at 12 months. At 6 months one patient had a partial response and one a complete remission (Table 2). The single patient who responded had a normal GFR (Figure 1). In the others, GFR declined significantly from 67±38 to 60±38 ml/min with one patient progressing to ESKD (P=0.02) (Figure 1). As such, no measure of proteinuria was available at the 12-month follow-up visit. Other measures of the nephrotic syndrome, including albumin and LDL cholesterol also were not significantly different from baseline values at 12 months. In the overall cohort, blood pressure remained controlled throughout the trial. Despite the planned removal of other immunosuppressive agents, at the end of the trial 2 patients remained on prednisone ≥ 5 mg /day, 3 remained on calcineurin inhibitors and 3 remained on both as withdrawal of immunosuppression was deemed too precarious due to lack of any response to Rituximab. Rituximab therapy had no impact on the
chosen biomarkers with no significant change in either suPAR as measured by R&D or Virgates or activation of β3 integrin (Table 2)

**Adverse Events**

During the year of follow-up, there were 16 adverse events recorded of which 3 were deemed as serious. One patient was hospitalized with flu-like symptoms and severe vomiting resulting in acute kidney injury (AKI) that subsequently recovered, a second developed pneumonia that also recovered, and a third patient was hospitalized with a significant worsening in serum creatinine that progressed rapidly to ESKD in the context of persistent severe nephrotic syndrome. There were 2 adverse events deemed related to Rituximab therapy including an infusion related reaction and a decreased WBC that recovered without sequelae. Other adverse events included nausea, vomiting, headaches, dizziness, weakness, cramps, headache and a maculopapular rash, none of which were deemed by the investigator related to the Rituximab therapy based on the timing of the events.

**DISCUSSION**

Given the nature of suPAR as innate-immune circulating factor and the expanding use of Rituximab in autoimmune diseases, this study was designed assess the effectiveness of Rituximab in adult patients with treatment resistant primary FSGS, while incorporating information from plasma suPAR levels and the serum effects on podocyte beta 3 integrin activation. Of the 38 patients screened for the study who met clinical criteria, only 13 qualified based on a serum suPAR > 3500 pg ml\(^{-1}\) and evidence of β3 integrin activation (micro-flow image; MFI > 1). In the 9 FSGS patients who consented to participate, Rituximab was ineffective at producing a sustained remission. All patients at 12 months remained nephrotic with an overall
significant decline in GFR, including a single patient that progressed to ESKD. Only a single patient with a preserved GFR responded completely and another had a partial response by 6 months, but both relapsed by 12 months of follow-up (Figure 1). It is possible that these patients would have benefited from re-treatment with rituximab at month 6, but this was not part of the protocol. Adverse events were significant with 3 classified as serious.

Success with the use of Rituximab been noted in the pediatric literature and following post-transplant FSGS recurrence albeit in the context of multitargeted immunosuppression and often plasmapheresis. 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. 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. In another study, 54 children with idiopathic nephrotic syndrome dependent on either steroids or calcineurin inhibitors were randomized to receive either Rituximab or ongoing standard immunosuppression. 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. Similarly, in a study of both children and adults with steroid dependent or frequently relapsing nephrotic syndrome including 8 patients with FSGS, Rituximab decreased both the need for steroid maintenance and the number of relapses. These studies suggest that Rituximab can be a useful as a sparing or replacement immunosuppressive agent in FSGS noted to be responsive to other immunosuppressive therapies.

However even in steroid-resistant nephrotic syndrome, Rituximab may result in remission in children. In a recent systematic review that included 226 children, response to
Rituximab was noted in 39.2% of children with steroid-resistant nephrotic syndrome secondary to FSGS. Studies that include only adult patients are more limited. A recent systematic review and meta-analysis included 16 observational studies that described the outcome after Rituximab therapy in 51 adult patients with FSGS. The study noted a complete remission rate of 43% and a partial remission rate of 11%. The relapse rate of patients treated with Rituximab was 47% over a mean follow-up period of 18.7±9 months. Unlike our study, only a minority of the included FSGS patients were completely treatment resistant with most being steroid dependent or frequently relapsing FSGS. Our study, in contrast, included treatment resistant patients many on multitargeted immunosuppression. The lack of response to Rituximab was further illustrated by our inability to safely wean off other immunosuppressive treatments in the majority of the patients. The single patient who responded had a preserved GFR and had only failed steroid monotherapy, suggesting that in multi-drug resistant disease, Rituximab does not appear to be an effective immunosuppressive therapy. Further, 2 serious adverse events related to infections were noted.

In an effort to preselect the study patient group and to enrich the FSGS cohort with patients with high serum suPAR and activation of podocyte β3 integrin, we utilized a serum level of suPAR > 3500 pg ml⁻¹ with evidence of β3 integrin activation (MFI > 1). SuPAR has been previously demonstrated to be elevated in patients with FSGS with discriminatory power compared to other glomerular diseases. SuPAR may also reach very high levels 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 β3 integrin. Our control population, which included patients with proteinuria >3.0
g/24h due to other disease entities (e.g., IgA Nephropathy, Membranous Nephropathy) had lower levels of suPAR than those with primary FSGS who met the inclusion criteria (3212 ± 857 vs 4306 ± 888 pg ml⁻¹ respectively), but higher levels than those patients with FSGS that were screen failures (2679 ± 1033 pg ml⁻¹). None of these differences met statistical significance. This is similar to results noted using samples from the Neptune Cohort wherein significant overlap in the suPAR level was noted between patients with FSGS, minimal change disease, IgA nephropathy and membranous nephropathy.³⁵ In this mixed cohort, suPAR concentration at baseline inversely correlated with eGFR and the urine suPAR/creatinine ratio positively correlated with the urine protein/creatinine ratio.

Utilizing serum suPAR in nephrotic patients with biopsy proven, and otherwise multi-drug resistant FSGS did not prove a discriminatory biomarker, as noted by the high screen failure rate among equally nephrotic patients with biopsy proven FSGS. This result is consistent with recent meta-analysis that noted suPAR alone could not distinguish patients presenting with FSGS from those in remission.³⁶ β₃ integrin activation assays can provide additional insights into patient stratification because integrin activation is the downstream event triggering cellular injury and may be different based on different suPAR isoforms or proteolytic fragments that measure similar suPAR amounts, yet with a different biological response. Moreover, suPAR’s ability to serve as a biomarker for FSGS may require additional co-factor analysis such as genotype for APOL1³⁷ or the presence of anti-CD40 auto-antibodies.³⁸ This is further supported by recent large studies suggesting suPAR to be a robust marker of innate immune activation during inflammation,³⁹,⁴⁰ cardiovascular mortality⁴¹ and CKD incidence and progression.⁴¹ The Elisa system measuring suPAR is important when attempting to utilize suPAR level as a biomarker for FSGS. Although both the R&D Elisa and the Virogates Elisa have been shown to
be useful, the later showed an improved ability to separate FSGS from healthy patients or from patients with other glomerular diseases. While the cell-based integrin activation assay using podocytes exposed to patient serum has been employed on a case-by-case basis, this study highlights difficulties to evaluate integrin activation in higher throughput fashion and on repeated sampling. Perhaps other cell lines with engineered β3 integrin expression such as transfected K562 cells may be employed.

In summary, Rituximab does not appear to be effective in adult patients with severe treatment resistant FSGS with high serum suPAR and podocyte integrin activation. Furthermore, in FSGS when accompanied by severe nephrosis, Rituximab may be hazardous. Whether Rituximab in FSGS patients with lower suPAR/integrin activity assessment would be useful, requires study. Our study suggests that multi-drug resistant FSGS may be unique with respect to biomarkers and mechanistic pathways. Understanding the mechanisms in each patient through biomarker testing may ultimately guide more personalized treatment regimens that are more effective and avoid side effects.

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Registration Number: NCT01573533

DECLARATION

The results presented in this paper have not been published previously in whole or part, except in abstract format.

AUTHOR’S CONTRIBUTIONS
Michelle A. Hladunewich, MD – Study conception and design, recruitment, analysis and interpretation of data, drafting, revision and final approval of manuscript

Dan Cattran, MD - Study conception and design, recruitment, analysis and interpretation of data, drafting, revision and final approval of manuscript

Sanjeev M. Sethi, MD – Pathology review, drafting, revision and final approval of manuscript

Salim S Hayek, MD - Drafting, revision and final approval of manuscript

Jing Li, PhD – Analysis of biomarker samples and final approval of drafted manuscript

Changli Wei, PhD - Analysis of biomarker samples and final approval of drafted manuscript

Sarah Mullin, Bsc - Drafting, revision and final approval of manuscript

Heather N. Reich- Drafting, revision and final approval of manuscript

Jochen Reiser, MD Study conception and design, recruitment, analysis and interpretation of data, drafting, revision and final approval of manuscript

Fernando C. Fervenza MD Study conception and design, recruitment, analysis and interpretation of data, drafting, revision and final approval of manuscript

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**DISCLOSURES**

J.R is co-founder and co-chair of the scientific advisory board of Walden Biosciences. S.H. is a member of the scientific advisory board of Walden Biosciences.

**SUPPLEMENTARY MATERIAL**
Table S1 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Guidelines for Cohort Studies.

Supplementary information is available at KI Report’s Website

REFERENCES


Table 1: Baseline Characteristics in Screen-Failed and Eligible Patients

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<tr>
<td>SuPAR pg ml⁻¹ (Virgates)</td>
<td>6507±2284</td>
<td>7226±3811</td>
<td>7759±3811</td>
<td>7519±4359</td>
<td>6415±2320</td>
<td>0.32</td>
</tr>
<tr>
<td>AP5 Ratio</td>
<td>1.56±0.59</td>
<td>1.17±0.17</td>
<td>1.13±0.34</td>
<td>1.15±0.30</td>
<td>1.24±0.27</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Table 2: Treatment Response**

**Figure 1: Proteinuria and Corresponding Serum SuPAR Levels**

Urine protein levels at baseline, 1, 3, 6 and 12 months with corresponding SuPAR levels in three representative patients. Where panel CR is the patient with a complete remission at 6 months; PR is the patient with a partial remission at 6 months while NR demonstrates a patient with no response. Proteinuria is the solid line while SuPAR is the dashed line. There is no notable relation between the two values.
SupAR (pg/ml)

Proteinuria (g/day)

Baseline | 1 Month | 3 Months | 6 Months | 12 Months
--- | --- | --- | --- | ---
2,000 | 3,000 | 4,000 | 5,000 | 6,000

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