

Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Fervenza FC, Appel GB, Barbour SJ, et al. Rituximab or cyclosporine in the treatment of membranous nephropathy. N Engl J Med 2019;381:36-46. DOI: 10.1056/NEJMoa1814427

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes

STUDY TITLE:

MEmbrous Nephropathy Trial Of Rituximab (MENTOR)

A Multi-Center Randomized Controlled Trial of Rituximab Versus Cyclosporine in the Treatment of Idiopathic Membranous Nephropathy (IMN)

Study Drugs

Rituximab and Cyclosporine

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SPECIFIC AIMS:

The specific aims of this Phase III trial are to test the hypothesis;

1. That B cell targeting with Rituximab **is more effective than** Cyclosporine in inducing **long-term remission** (complete or partial) of proteinuria in patients with IMN.
2. That B cell targeting with Rituximab **reduces the number of relapses (efficacy in sustaining remission) and increases the time to relapse when compared with treatment with** Cyclosporine.
3. That B cell targeting with Rituximab **is as effective as** Cyclosporine in inducing **complete or partial remission** of proteinuria in patients with IMN during the active treatment phase.
4. That B cell targeting with Rituximab has a better side effect profile and improved quality of life when compared with treatment with Cyclosporine in patients with IMN.

BACKGROUND AND SIGNIFICANCE

IMN is a common immune-mediated glomerular disease and remains the leading cause of Nephrotic Syndrome (NS) in Caucasian adults.¹ Although in most patients the disease progresses relatively slowly, approximately 40% of patients eventually develop End Stage Renal Disease (ESRD).² Because of its frequency, it remains the 2nd or 3rd cause of a primary glomerulopathy leading to ESRD.³ Patients with IMN who remain nephrotic are at an increased risk for thromboembolic⁴ and cardiovascular (CV) events.^{5, 6} Available immunosuppressive therapies including corticosteroids, alkylating agents, and calcineurin inhibitors (Cyclosporine), appear to be at least partially successful in reducing proteinuria in IMN, but their use is controversial and is associated with a number of adverse effects and a high relapse rate, thus tempering their use in IMN. (reviewed in ⁷) The significant risks associated with current immunosuppressive therapies are important in a disease where up to 30% of IMN patients are said to achieve spontaneous remission of proteinuria, enjoy long-term renal survival and, should not be treated with immunosuppressive therapy. This information is, however, misleading, since the percentage of patients that undergo spontaneous remission is much lower when patients are selected with higher grades of proteinuria at presentation e.g. proteinuria >8g/24h.⁸ Similarly, although Schieppati et al. reported a 72% renal survival at eight years for 100 untreated patients with IMN, in this study, 37% of patients were non-nephrotic; a cohort well known to rarely progress to renal failure and hence a built-in bias towards a better outcome. Indeed the overall proteinuria was relatively low (56% of patients had proteinuria <5g/24h), and the median follow-up was only 39 months. The follow-up time is an important issue given that the natural history of the disease process tends to be slow even in the worst cases. The final limitation was that all deaths were excluded from the analysis.⁹ However despite the favorable elements of this cohort, 25% reached ESRD by the end of 8 years. One approach to this has been to provide immunosuppression treatment only to those subjects identified as being at higher risk of progression: males, severe proteinuria, impaired renal function, a high prevalence of secondary lesions of focal and segmental sclerosis, and prominent tubulointerstitial damage on renal biopsy. Our experience, and others, however, suggests this approach is seriously flawed.¹⁰ There is a substantial fraction of patients, who progress to chronic kidney disease (CKD), that are not identified by these predictors and are thus unjustifiably excluded from therapy. A second approach has been to treat only those patients who are exhibiting deteriorating kidney function.^{11, 12} These trials have been deemed successful because proteinuria and

azotemia were diminished.¹¹⁻¹³ Careful review of the data however, shows that reversal of azotemia is almost always incomplete and often transient suggesting that the decline in GFR is merely attenuated and not arrested. In addition, we believe that a prolonged run-in (i.e., treatment-free) period of 12 or more months would be required to prospectively confirm this type of truly progressive IMN, but that this in turn would result in a significant and probable irreversible loss of nephrons and ultrafiltration capacity.¹⁴ This can be illustrated by the study of Polanco et al.⁸ These authors suggested that patients that were treated conservatively and who did not go into spontaneous remission had final creatinine = 2.4 ± 2.2 mg/dL, and eGFR 53 ± 35 ml/min.⁸ However, we believe this information is inaccurate. Taking into consideration that the mean age at presentation was 51 years and the mean follow up was 69 months (making these patients ~56 years at follow-up) the eGFR should be ~30 ml/min if males and ~22 ml/min if females, indicating a significant loss of function with just waiting. The paper further underestimates chronic kidney disease because their end point was restricted to the absence of chronic dialysis or need for renal transplantation. If we add loss of kidney function (as measured by change in GFR) their disease course would indicate a significantly higher incidence of CKD over time supporting our contention that prolonging the wait time for spontaneous remission results in patients paying a heavy price.

Why do so many patients still progress to ESRD? There is growing evidence implicating proteinuria as a major player in the development of progressive tubular injury, interstitial fibrosis, and GFR loss. The higher the sustained levels of proteinuria, the faster the decline in renal function,¹⁵ a relationship that is true not only for patients with IMN but for other proteinuric renal diseases including FSGS and diabetic nephropathy.¹⁶⁻¹⁹ The reverse appears also to be true. There is strong evidence to support that remission of proteinuria (either complete or partial remission) is a valid surrogate end point for both improved renal survival and slower rate of progression of renal disease, and that this reduction is an important therapeutic target for the clinician. This view is in line with the position statement of the NKF and NIDDKD indicating that proteinuria can be used as a surrogate marker and principal endpoint in clinical trials in proteinuric renal disease where GFR-based declines in function take too long to be of practical use.²⁰ Proteinuria is also a marker for CV risk.^{5, 21-24} Recent post-hoc analyses of the diabetic trials RENAAL, IDNT and others, have shown that proteinuria determines renal outcome^{25, 26} and CV outcome.²⁷⁻²⁹ The link between chronic renal failure (CRF) and CV disease is so strong that over 40% of patients starting dialysis already have evidence of CV disease.^{30, 31} This information is important in the present study since all patients will have high grade proteinuria $>5\text{g}/24\text{h}$, a scenario almost universally associated with marked abnormalities in their lipid profile (high total cholesterol, normal or low levels of HDL and increased LDL).^{5, 6} Apart from hyperlipemia, patients with IMN and nephrotic syndrome (NS) are also at risk for thromboembolic events, with a prevalence rate as high as 50%, and a mortality rate within this group as high as 42%.^{4, 32} These data serve to emphasize the importance of other common life-defining sequelae of membranous nephropathy in these patients, in addition to their risk of renal failure.

There is no standard specific treatment for IMN. Initial therapy should be supportive and involves restricting dietary protein intake, controlling blood pressure, hyperlipidemia, and edema. The ideal target for blood pressure is not firmly established but current recommendations suggest that 130/80 mm Hg should be the treatment goal. There are only limited data to support a lower target of 125/75 mm Hg if there is proteinuria >1 g/d.

Reducing protein intake to about 0.6-0.8 g/kg ideal body weight per day also tends to decrease proteinuria.³³ ACEi and/or ARBs are effective anti-hypertensive agents that may also reduce proteinuria in both diabetic and nondiabetic chronic nephropathy patients and slow progression of renal disease independent of blood pressure control.³⁴ This is the rationale for making these drugs the preferred agents to treat hypertension in proteinuric renal diseases. However, evidence that such therapy is beneficial in IMN is weak, largely inferential, and the following issues need to be considered: 1) The degree of renal protection is related to the degree of proteinuria reduction and if proteinuria is not lowered, the benefit is substantially attenuated.^{35, 36} In the RENAAL trial the renal protective effect of angiotensin II blockade in patients with diabetic nephropathy was nearly fully explained by its anti-proteinuric effect.²⁶ 2) In patients with IMN, the anti-proteinuric effect is modest (<30% decrease) and is more significant in patients with lower levels of proteinuria.³⁷⁻³⁹ 3) Thus, in contrast to diabetic renal diseases, ACEi may not offer the same degree of renal protection to patients with IMN.⁴⁰ In fact, studies by du Buf-Vereijken et al.⁴¹ and in a review by Troyanov et al.,⁴² the use of ACEi or ARBs by multivariate analysis did not show an independent value in determining the prognosis of patients with IMN. More recently, Praga et al. showed additional evidence that in patients with NS, (the majority with IMN), ACEi were ineffective in reducing proteinuria, and that this failure to respond in IMN patients was associated with poor renal function outcomes.^{43, 44} 4) In patients in which a significant anti-proteinuric response is observed, the effect is usually seen within 2 months of initiation of angiotensin II blockade therapy.³⁷ Although a relative reduction of proteinuria is always a positive result, the aim of anti-proteinuric therapy is to reduce it as close as possible to normal levels (complete remission (CR)). Reaching this goal in patients with proteinuria > 5g/24 using conservative treatment with ACEi or ARBs seems unrealistic, even when these drugs are used at the highest dose. Taken all together, in the past decade relatively little progress has been made in the treatment of patients with IMN, and up to 40% of the patients will still progress to end stage renal failure. Agents that result in a higher response and lower relapse rates, as well as fewer adverse effects, are truly needed.

Current conservative therapy

Initial therapy should be supportive and involves restricting dietary protein intake, controlling blood pressure, hyperlipidemia and edema (see above). In patients that do have an anti-proteinuric response to angiotensin II blockade therapy, the effect is usually seen within 2 months of initiation of the medication and tends to be modest.³⁷ Although a relative reduction of proteinuria is an important effect, the aim of anti-proteinuric therapy is to reduce as close as possible to normal levels (complete remission). Reaching this goal in patients with sustained proteinuria > 5g/24 using conservative treatment with ACEi or ARBs is unlikely, even when these drugs are used at the highest dose. The role of immunosuppressive agents in the management of patients with IMN thus remains a critical issue. Our immunosuppressive treatment armamentarium in this regard has evolved but is still limited. Agents that result in a higher response rate with a lower relapse rate and fewer adverse effects are truly needed and thus the focus for our proposed randomized controlled trial.

Current Options for Immunosuppression in IMN

Cyclosporine (CSA)

Cyclosporine is a calcineurin inhibitor (CNI) that exerts its immunosuppressive effect by blocking the production of IL-2, IL-3 and IFN- γ , resulting in a reduction of T-

lymphocyte helpers/inducers and cytotoxic cell function.⁴⁵ This immunosuppressive effect is probably not the only anti-proteinuric effect of CSA since it is known to have both hemodynamic effects and to act on the podocyte structure. The latter effect has been demonstrated *in vitro* to alter glomerular permeability⁴⁶ by a direct effect on the actin cytoskeleton (and therefore the shape) of podocytes.⁴⁷ This is postulated to protect podocytes from immune injury and may be part of the explanation behind the observation of its significant benefit in certain immunologic glomerular disorders such as in IMN and minimal change despite significantly less exposure compared to solid organ transplant recipients. Cyclosporine has been extensively used in the treatment of proteinuric glomerular diseases and has been proven to be effective in inducing remission of idiopathic nephrotic syndrome in children and adults.⁴⁸ It has been used successfully in patients with IMN and nephrotic syndrome resistant to corticosteroids and/or cytotoxic drugs and has been tried in some of the glomerular disorders even before exposure to cytotoxic therapy because of its significantly different side effect profile.^{39, 49-55}

In the most relevant randomized controlled trial, 51 patients with steroid-resistant nephrotic syndrome and preserved renal function (creatinine clearance [CrCl] > 42 ml/min/1.73m²) were randomized to CSA or placebo treatment, with both groups also receiving low-dose prednisone (0.15 mg/kg/day).⁵¹ The duration of treatment was 26 weeks followed by a tapering off period over 4 weeks. At 26 weeks a significantly higher remission rate was observed in patients treated with CSA -compared to placebo- (75 % versus 22%, P. <0.001). Among the CSA-treated patients with remission, 90% showed partial remission and 10% complete remission. Twelve months after discontinuing the drug a significant percentage of the CSA treated patients remained in remission (39%) compared to the placebo treated patients (39 versus 13%; p = 0.007).⁵¹

The effectiveness of a more prolonged course of CSA in inducing remission of proteinuria has been recently documented in a study where CSA was given for 12 months and an 80% remission in proteinuria (complete plus partial) was observed.⁵⁵ It was indicated in this paper that CSA combined with prednisone is more effective than CSA monotherapy in maintaining remission in IMN.⁵⁵ However careful review of their data suggests that the group that remained in remission following tapering of the CSA had substantially higher trough levels, both in the monotherapy and in the combined CSA plus prednisone group, compared to the relapse group suggesting that this may have been the reason for the more sustained remission. This would support our trial proposal i.e. CSA alone (no prednisone is required). In earlier studies⁵¹ low dose prednisone was added to CSA because historical data in patients with MCD/FSGS had suggested higher remission rates with combination therapy and that perhaps the combination would reduce the nephrotoxicity of CSA. A recent review of these early studies and the more current ones suggest that this combination is not necessary.⁴⁸ Further support that CNI monotherapy is effective is illustrated in the recent RCT in MN using Tacrolimus. This study showed virtually identical remission rates in proteinuria as in the original CSA plus prednisone trial (see above) using Tacrolimus monotherapy. Their entry criteria required the presence of nephrotic syndrome resistant to ACE inhibitor therapy, steroids or the combination of steroids with cytotoxic drugs. Their relapse rate following discontinuation of the drug was similar to that of the RCT with CSA.⁵⁶ CSA has also been shown to be of benefit in inducing remission of nephrotic syndrome and preserving renal function in patients with IMN associated with progressive renal failure. In a prospective randomized controlled trial, 64 patients were placed on a restricted protein diet and followed closely for 12 months. Patients that demonstrated significant progression of their disease during

this observation period (n = 17) determined by an absolute loss in CrCl > 8 ml/min/year and persistent nephrotic range proteinuria were randomly assigned to either CSA 3.5 mg/kg/day or placebo for 12 months.⁵⁷ A significant reduction of proteinuria was observed only in CSA-treated patients and the remission was sustained in 75% of these patients followed on average for 21 months.⁵⁷

Thus, CSA represents an effective therapy for inducing remission of nephrotic syndrome in patients with IMN. It leads to remission of proteinuria in the majority of nephrotic patients with IMN but with less potential serious toxicity compared to the use of cytotoxic/alkylating agents. There is also more experience with CSA (versus other CNI) in the US and Canada and for these reasons it has been chosen as the comparator arm for our study.

Patients with IMN that do progress do so over years and follow-up in RCTs with CNI therapy have not been of sufficient duration to illustrate improvements in renal survival. However there is well documented recent data that indicates that both complete and partial remission in proteinuria is associated with improved renal survival. Complete remission was associated with a 100% 10-year survival and partial remission (defined as more than a 50% decrease from baseline and attaining a urine protein level of <3.5 g per day) was associated with a 90% 10 year renal survival compared to 50% kidney survival in those that fail to remit. In addition, in this same study PR as measured by slope of CCR, resulted in a slowing of progression rate of >80 % compared to non remitters.⁴²

Toxicity of cyclosporine

Cyclosporine is a potent immunosuppressive agent and is associated with significant risk of both short and long-term toxicity. In the majority of cases these adverse effects are associated with both total daily dose and total exposure. Precise incidences of these toxicities are hard to ascertain from the literature given the rarity of studies where these agents are used as monotherapy and their use for a limited duration as proposed in this trial. There is however undoubtedly an acute effect on filtration function and delayed effects on renal vasculature that have been well documented and a recent review summarized both short and long-term toxicities associated with this agent.⁵⁸ Hypertension, overgrowth of body hair and gingiva hyperplasia, mild tremor, infections, elevated bilirubin levels and mild nausea represent the most frequently observed short-term adverse effects related to CSA.⁵⁸ These are seen in up to 20 to 30% of the treated population but are usually tolerated and/or treatable with additional agents (such as additional anti-hypertension drugs) or by dose reduction.⁴⁸

Cyclophosphamide (CYC)

In IMN, experimental data suggests that B cells are involved in the pathogenesis of the disease.⁵⁹ To date, the best proven long-term therapy for patients with IMN consists of the combined use of corticosteroids and CYC, the Ponticelli's protocol. However, the potential side effects with the use of 9g of methylprednisolone mandated by this protocol as well as the potential risk of other serious side effects associated with the use of cytotoxic agents (such as bone marrow toxicity, severe infections, gonadal dysfunction) combined with the long-term risk of malignancy associated with CYC has left many physicians reluctant to use this regimen. It should also be remembered that IMN is a relapsing and remitting disease and even with the use of this therapy, relapse rates remain high. In Ponticelli et al.'s randomized 6 month study in patients with IMN comparing methylprednisolone plus chlorambucil versus methylprednisolone plus CYC the overall

relapse rate was 24% (21/87) and similar in both arms. These relapses occurred between 6 and 30 months post treatment, with the majority within 18 months.⁶⁰ An additional ~10% of patients had to stop treatment and were discontinued from the study because of adverse effects. The risk of malignancy with CYC is substantial with the hazard of neoplasia roughly correlated to the cumulative dose and duration of cytotoxic therapy.⁶¹⁻⁶³ Faurschou et al. recently investigated the incidence of malignancies associated with CYC exposure in a cohort of 293 patients with Wegener's granulomatosis.⁶⁴ The risk of malignancy was not increased for patients who never received CYC or for patients treated with cumulative CYC doses ≤ 36 g.⁶⁴ In contrast, high risks of leukemia (SIR 59.0, 95% CI 12-172) and bladder cancer (SIR 9.5, 95% CI 2.6-24) were observed for patients treated with cumulative CYC doses >36 g. These data would suggest that a patient who weighs 80kg and is treated with CYC at a dose of 2.5 mg/kg/day would exceed this threshold after 2 standard courses of the Ponticelli's protocol. Since IMN is a remitting and relapsing disease with the potential for significant cumulative CYC dosing, there is a substantial risk of late-occurring, serious malignancies. The other major concern limiting CYC utility is related to its gonadal toxicity. Data indicates that ovarian failure is seen in female patients of any age receiving a cumulative dose of as little as 28 g of CYC. In addition, these authors found that age at the onset of therapy was an additional independent factor associated with sterility.^{65, 66} Although male infertility is harder to assess, studies have demonstrated that doses above 7.5 g/m² of CYC can result in permanent oligospermia.⁶⁷ For these reasons, the majority of the academic centers in the US have considered CYC treatments too toxic and relegated the use of Ponticelli's protocol to rescue therapy in patients who have failed less toxic immunosuppressive therapies.

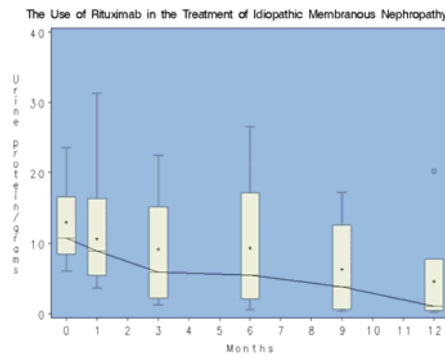
INNOVATION

A new approach to therapy: the case for targeting B cells: In IMN, experimental data suggest that B cells are involved in the pathogenesis of the disease.⁵⁹ To date, the best proven therapy for patients with IMN consists of the combined use of corticosteroids and cyclophosphamide (CYC). The mechanism of action of CYC includes suppression of various stages of the B cell cycle including B cell activation, proliferation, and differentiation and inhibition of immunoglobulin secretion, supporting the hypothesis that B cell abnormalities are involved in the pathogenesis of IMN.^{68, 69} Given the key role of IgG antibodies in IMN⁷⁰, it is reasonable to postulate that suppression of antibody production that targets glomerular antigens by depleting B cells may improve or even resolve the glomerular pathology.. This approach of stopping the initiating pathogenic event could potentially result in resolution of the disease process. **This is the theory underlying the application of selective B cell targeting with Rituximab (RTX) in IMN. Our hypothesis is that it will prove at least equal to, or even superior to current therapies, both in the production of short term and long term control of the NS and be safer than any of the other current regimens used to treat IMN**

PRELIMINARY STUDIES

Based on this rationale, we conducted a pilot trial in 15 newly-biopsied patients (<3 years) with IMN and proteinuria >5 g/24h despite ACEi/ARB use for >3 months and systolic BP <130 mmHg.⁷¹ Mean baseline creatinine was 1.4 mg/dl. Thirteen males and 2 females, median age 47 (range 33-63), were treated with RTX (1g) on days 1 and 15. At

six months, patients who remained with proteinuria $>3\text{g}/24\text{h}$ received a second identical course of RTX. Baseline proteinuria of $13.0 \pm 5.7\text{g}/24\text{h}$ (range 8.4-23.5) decreased to $6.0 \pm 7.0\text{g}/24\text{h}$ (range 0.2-20) at 12 months (mean \pm SD) (Figure 1). In the fourteen patients who completed 12 months follow-up complete remission (proteinuria $<0.3\text{g}/24\text{h}$) was achieved in 2 patients and partial remission ($<3\text{g}/24\text{h}$) in 7 patients. The mean drop in proteinuria from baseline to 12 months was $6.2 \pm 5.1\text{g}$ ($p=.002$, paired t-test). In 5 of these 7 patients, proteinuria was $<1.5\text{g}/24\text{h}$.



At 18 months follow up 3 of these 7 PR patients achieved CR of their proteinuria.

(Figure 1)

Five patients did not respond. There were a limited number of minor side-effects (see below).

Initial CD20+ B cell depletion was seen in all patients. However, at 3 months, CD20+ B cells were starting to recover with five patients demonstrating $>35\text{ cells}/\mu\text{l}$ (range 35-152).⁷¹ These data contrast with previous work by Ruggenenti et al. using the lymphoma regimen where RTX is given weekly ($375\text{ mg}/\text{m}^2$) for 4 weeks.² Using that regimen, B cell depletion was maintained up to 12 months post treatment and their proteinuria reduction was more rapid and complete. Similarly our studies using this standard lymphoma protocol in patients with ANCA vasculitis demonstrated that peripheral blood B-cell depletion was consistently achieved and maintained for at least six months. Pharmacokinetic (PK) analysis had showed that RTX levels in our original 2-dose regimen were 50% lower compared to studies in non-proteinuric conditions. This could potentially result in undertreatment. Based on these results, we conducted an additional study based on the premise that in patients with MN, 4 weekly doses of RTX would result in more effective B cell depletion, a higher remission rate while still maintaining the same safety profile as patients treated with RTX dosed at $1\text{g} \times 2$. Twenty patients (including 11 patients that had failed alternative immunosuppressive therapy) with IMN and proteinuria $>5\text{g}/24\text{h}$ received RTX ($375\text{mg}/\text{m}^2 \times 4$), with retreatment at 6 months regardless of proteinuria response. A detailed pharmacokinetics study was conducted in concert with immunological analyses of the adaptive immune compartment (T and B cells) to ascertain the impact of RTX on lymphocyte subpopulations. Baseline proteinuria of $11.9 \pm 4.9\text{g}/24\text{h}$ decreased to $4.2 \pm 3.8\text{g}/24\text{h}$ and $2.0 \pm 1.7\text{g}/24\text{h}$ at 12 and 24 months, respectively ($p<0.001$) while creatinine clearance increased from 72.4 ± 33 at baseline to $88.4 \pm 31.5\text{ ml}/\text{min}/1.73\text{m}^2$ at 24 months ($p=0.02$) (Figure 2A/B).

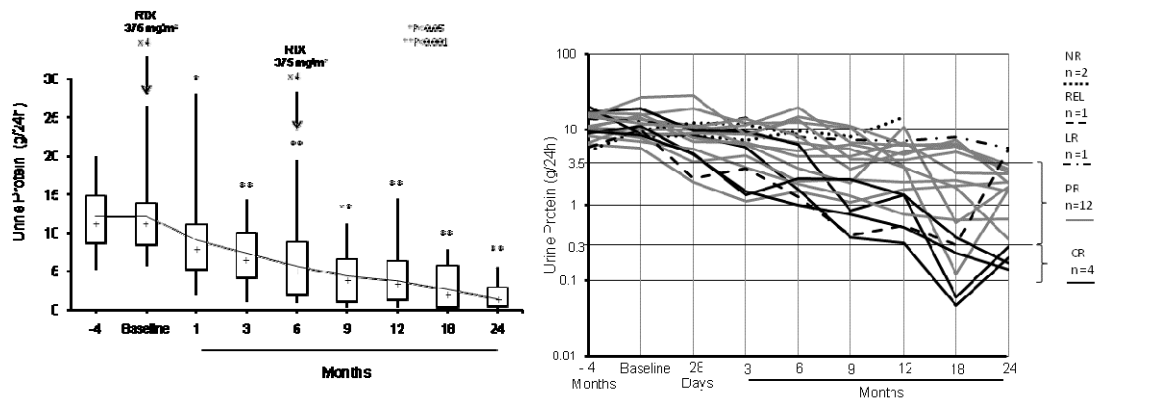


Figure 2. A. Box plots of urine protein by months since start of RTX therapy. The top and bottom of the box are the estimated 75th and 25th percentiles, respectively. The intermediate horizontal line and "+" sign represent the median and mean urine protein, respectively. The vertical lines extend to the largest (smallest) data point that is within 1.5 times the inter quartile range (75th-25th percentile) above the 75th percentile (or below the 25th). The square symbol identifies points outside of this range. The number of patients with follow-up at 0, 1, 3, 6, 9, 12, 18, and 24 months are: 20, 20, 20, 19, 18, and 18, respectively. * $P<0.05$, ** $P<0.001$; B. Longitudinal effect of RTX on proteinuria (log transformed).

Of 18 patients who completed 24-months follow up, 4 are in complete remission, 12 are in partial remission (CR + PR = 80%), 1 had a limited response ($>50\%$ drop in P but $>3.5\text{g}/24\text{h}$) and 1 patient relapsed from a partial remission. When interpreting these results we should take into account that $>50\%$ of these patients had failed previous immunosuppressive therapy. This study also emphasizes that proteinuria is reduced gradually and that it may take several months to reach its nadir. This observation is in agreement with previous reports in patients with IMN treated with prednisone in combination with a cytotoxic agent, but was seen without the short-term toxicity observed with alkylating agents.⁶⁰ Kidney function remained stable or improved in all patients. These results were observed despite the fact that in patients with nephrotic syndrome (NS), serum albumin levels influence tubular creatinine secretion resulting in an endogenous creatinine clearance that overestimates GFR in NS.⁷² In addition, previous investigators have indicated that the onset of IMN is accompanied by a severe depression in hydraulic permeability of the glomerular capillary wall. This is partially offset by enhancement of filtration surface area and by profound depression of glomerular oncotic pressure). This can result in the GFR initially remaining in the normal range or at worst, depressed by $<50\%$ in moderate IMN. In severe IMN these investigators found this mechanism was significantly worsened by a marked reduction in functional glomerular number and a GFR depression of $>50\%$. These abnormalities were presumably reversed with RTX treatment and resolution of the NS. However, an additional explanation could have been that a number of patients had their angiotensin II blockade reduced or discontinued with time, altering the renal hemodynamics and thereby contributing to the significant increase in creatinine clearance. The important summary point in this regard is this type of GFR improvement by reduced RASS blockade would not be accompanied by a decrease in proteinuria. RTX therapy represents a substantial advantage versus the risk for nephrotoxicity and other short-term toxicities associated with the use of calcineurin inhibitors (CNI). In addition RTX treatment assures patient adherence (since it is given by IV infusions). This is in contrast to the necessary monitoring required during the treatment with Cyclosporine. This makes

RTX an easier option from the perspective of physician management and potentially offers a better quality-of-life for the patient. Cost estimates are also worth addressing. Although RTX is more expensive upfront the annual treatment costs compared to Cyclosporine are similar. Perhaps most importantly, the effect of RTX appears to be sustained as only 1 patient in our recent pilot (5%) relapsed within the 2 year follow up period. A similar long term preservation of proteinuria reduction was observed in our first study⁷¹ with only 1 patient relapsing at 36 months. Thus, in the 35 patients included in our 2 studies, only 2 relapses occurred, and in one case proteinuria relapsed into the sub nephrotic range. These results ($2/35 = 6\%$ relapse) are similar if not better than those reported by Ponticelli et al. ($21/87=24\%$) (63), and are substantially better than those reported with CNi treatment. In addition they are significantly better than the most recently suggested IMN treatment option of prednisone combined with MMF. In these studies a $>40\%$ relapse rate has been reported shortly after discontinuing therapy.^{51, 56, 74}

Serum RTX levels using the four dose regimen were similar to those obtained with 2 doses of RTX. Four doses of RTX did result in more effective B cell depletion but proteinuria reduction was basically identical to the results obtained using RTX 1000mg on days 1 and 15. Thus, we believe that the two dose regimen with retreatment at 6 months should be used in a randomized-control trial comparing RTX to Cyclosporine (the standard of care for IMN in the US). We believe that RTX will prove equal or superior to Cyclosporine in the treatment of MN and could represent the new standard of care for patients with this disease.

Toxicity of Rituximab

No dose-limiting effects were observed in our two Phase I/II studies evaluating safety and efficacy of RTX in IMN. The most commonly observed side effects were infusion related (flu-like symptoms, chills/rigors, fever, fatigue, headache, hypotension, nausea, leukopenia, angioedema and pruritus) and typically responded to an interruption of the infusion and resumption at a slower rate.⁷¹ Other side effects included: 1) serum sickness-like syndrome (n=1); hair loss and thinning (n=2); one case of community acquired pneumonia three months after the first infusion that resolved with oral antibiotic treatment (this patient was retreated without complications); 4) herpes zoster reactivation (n=1) treated with oral antiviral drugs with full recovery. Similar to the study by Ruggenenti et al., no patient had a major drug-related adverse event.²

Anti PLA2R levels and response to treatment

RTX offers an advantage in MN over nonselective immunosuppressive agents such as CYC since it primarily targets B cells. However, changes in CD20+ B cell number in general has not consistently paralleled the response in terms of either reduction in proteinuria or improvement in the clinical phenotype of the nephrotic syndrome.^{71, 75} This variation strongly suggests that the monitoring of the CD 20 count will not prove to be the ideal instrument for determining treatment dose and/or duration. It would be substantially better if we could monitor the specific effect of immunosuppressive therapy on the pathogenic antibodies in IMN. A recent discovery indicates that autoantibodies to the M-type phospholipase A2 receptor (PLA2R) may represent such a specific marker of idiopathic IMN. This autoantibody has been found in greater than 70% of idiopathic IMN patients.⁷⁶ When the analysis is limited to new-onset, nephrotic patients with idiopathic MN, this sensitivity increases even further. These investigators have shown that PLA2R is a transmembrane constituent of the human podocyte (the most likely target cell in MN) and anti-PLA2R antibodies are predominantly of the IgG4 subclass and co-

localize with IgG4 in the sub-epithelial deposits which are pathognomonic of the disease. In their observations, the presence of anti-PLA2R correlates well with disease activity, disappearing with a spontaneous or treatment-induced remission, and reappearing with a relapse of IMN.

We also have studied whether the immunologic changes in serum anti-PLA2R levels parallel the clinical reduction in proteinuria in response to RTX in patients with MN. Serial serum samples from our 2 cohorts of MN patients treated with RTX (cohort 1 treated with 1 gm of RTX at d.1 and d.15) and cohort 2 (treated with 4 weekly doses of 375 mg RTX), were assayed for anti-PLA2R antibodies.⁷⁷ Ten out of 15 (67%) patients in cohort 1 and 16/20 (80%) in cohort 2 had anti-PLA2R reactivity in their baseline serum samples. When the 2 cohorts were combined, the baseline distribution of cases with anti-PLA2R positivity was not significantly different when compared to the final clinical groupings of complete remission (CR), partial (PR) limited or no remission (NR), ($p=0.61$). However, an anti-PLA2R level below 500 arbitrary units at 9-12 months after initiation of treatment was significantly different among these groups: in the complete remission (CR) group 100% had a reduction to this level, in the PR group 88% had reduction to this level but in the limited response (LR) group only 50% had a reduction and in those with no response only 17% had a reduction to this level. ($p=0.006$). Median proteinuria at 9, 12, and 15-18 months was consistently lower in those subjects with undetectable or very low anti-PLA2R levels versus those with higher levels. In the group in which anti-PLA₂R disappeared, 86% were in complete or partial remission at the final time point : 4 complete remissions (CR), 14 partial remissions (PR), 1 limited remission (LR), and one non-responder (NR). Perhaps most relevant was, in those patients with decreases in anti-PLA2R, the decline almost always preceded the reduction in proteinuria by months. In addition, 1 patient who had attained remission and who had become anti-PLA2R negative following RTX became antibody positive at the time of his relapse. Thus, in at least these two cohorts of MN patients with anti-PLA2R antibodies at the start of treatment, post-RTX anti-PLA2R levels correlated with and seemed to precede clinical response to RTX (Figure 3). These results suggest that monitoring anti-PLA2R autoantibody levels may provide a window onto the immunologic effects of treatment on the course of IMN, and allow a more specific and an earlier means of determining treatment effectiveness compared to the clinical response of decreasing proteinuria. This observation is understandable given the nature of the deposits and proposed pathophysiology of IMN. Even if the B cells producing anti-PLA2R were completely eliminated and all circulating anti-PLA2R removed, the immune deposits would persist in the subepithelial space until cleared. Supportive evidence of this was seen in repeat kidney biopsies performed in RTX-treated IMN patients after they had entered complete clinical remission.⁷⁸ There are other important implications to the antibody story to consider if they truly parallel immunologic disease activity. Secondary chronic changes in IMN such as focal sclerosis or interstitial damage from prolonged disease activity may lead to indefinite persistence of low-level proteinuria, despite full clearance of immune deposits. This is the case seen in kidneys made proteinuric in the Heymann nephritis model and subsequently transplanted into naïve hosts.⁷⁹ This may explain our observation that not only does the decline in proteinuria lag behind that of anti-PLA2R, but also that the median proteinuria never reaches zero even by 24 months. It may also explain why most clinical responses are partial remissions (rather than complete) in the group that cleared anti-PLA2R. It will be important to see whether these patients will continue to have a further slow decline in proteinuria with further follow-up, similar to patients with IMN who undergo spontaneous remission.⁸ It may also help to explain why even patients

with only partial remissions are still associated with good long term outcomes, i.e. in these cases the residual proteinuria may be explained by the residual kidney scars rather than the persistence of the immunologic disease.⁴²

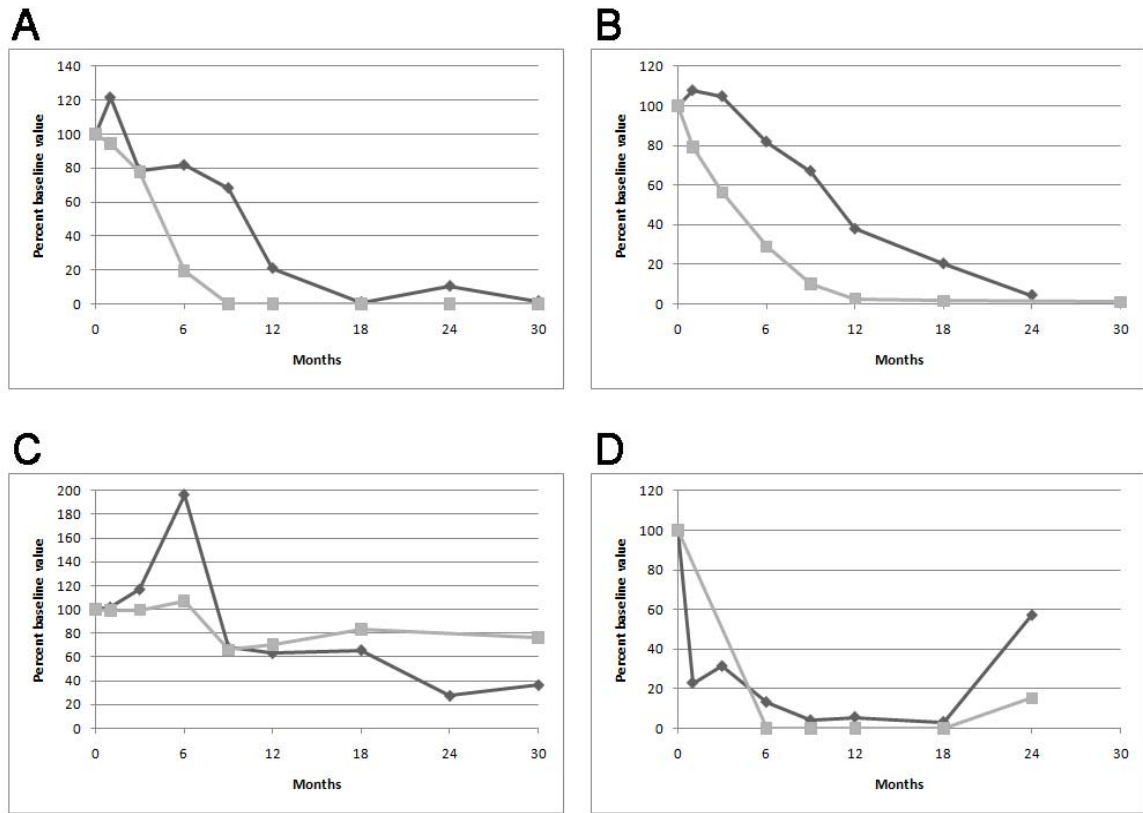


Figure 3. Representative plots of anti-PLA2R (gray squares) and proteinuria (black diamonds) versus time following initial RTX treatment. Values are plotted as percent of baseline value. Panel A and B depicts the typical reduction and disappearance of anti-PLA2R followed by resolution of proteinuria exhibited by the majority of patients. Panel C is representative of patients in whom anti-PLA2R did not substantially decline following treatment and the associated with persistence of proteinuria. Panel D depicts the single patient whose anti-PLA2R level returned with relapse of his disease after having initially disappeared.

This incomplete mirroring of immunological improvement by clinical status is an important issue. As mentioned, proteinuria could remain at a sub-nephrotic level in spite of the absence of immunological disease activity. In this situation, further immunosuppression would have no benefit but would have continued toxicity risk. In contrast, at a similar level of proteinuria, immunological activity, as detected by the continuing presence of circulating anti-PLA2R could be ongoing. In this setting, an increase or change in immunosuppressive regimen may be the best strategy. Ultimately, if confirmed anti-PLA2R levels may become an important surrogate assessment of disease activity and of treatment outcome. We propose to expand these observations by measuring anti-PLA2R levels in all patients enrolled into the current trial. This in itself will be a major innovation for the results of these studies have the potential to create a

new paradigm for monitoring and treatment in patients with IMN. If confirmed, the autoantibody level can be added to predictive algorithms and have the potential to influence future treatment protocols and lead to improved care of patients with IMN.

DNA Testing

Recent data show that in patients with membranous nephropathy, mutations in HLA-DQA1 and PLA2R1 alleles are associated with an increased risk for developing membranous nephropathy.⁸⁰ One of these alleles, the PLA2R1, is in fact the gene for the PLA2 receptor. In our pilot studies, ~70% of the patients with membranous nephropathy had antibodies present in circulation against PLA2 receptor.⁷⁷ Thus we would like to evaluate a linkage between the presence of these antibodies and potential genetic mutations in this group of patients. We will ask for the patients consent to further evaluate this linkage by collecting samples of blood to perform additional testing as part of their informed consent form (ICF).

OVERALL RESEARCH DESIGN AND METHODS:

The goal of this proposal is to conduct a prospective randomized controlled phase III study comparing RTX to Cyclosporine (CSA) in the treatment of patients with idiopathic IMN. Once a patient with idiopathic IMN and proteinuria >5g/24h is identified, meets other entry criteria and consents to the study, he/she will receive a minimum of 3 months of conservative therapy aimed at maximizing Angiotensin II blockade (run-in phase). If at the end of this period the patient still meets entry criteria he/she will be randomized into a 12-month treatment period, and a subsequent follow-up of 12 months. Efficacy of treatment will be assessed by remission status (based on changes in proteinuria) at 24 months from randomization. Patient safety will be assessed via collection of adverse events data and evaluation of pre- and post-treatment laboratory data. At the 6-month post-randomization visit, patients who have been randomized to either CSA or RTX but who do not have a reduction in proteinuria >30% (confirmed on repeat measurements within 2 weeks) will be considered treatment failures and exit the study. Data from that point onward will be censored. Patients in the RTX group who have a reduction in proteinuria of equal to or >30% at 6 months will be given another identical course of RTX (1g x 2). This treatment regimen was chosen given our initial data that suggested a percentage of our cohort appeared to respond to an additional course of therapy. Data from our 2 pilot studies showed that RTX is out of the circulation by month 2 in the majority of patients and B cells have recovered by months 3 in patients treated with RTX 1g x 2 (the regimen chosen for the study). Thus, at 6 months, patients in the RTX arm are completely, at least as best as we can measure, free of immunosuppression, and hence the second course should not be associated with additive toxicity. The same definition of response will apply to the CSA arm at 6 months. An equal to or >30% reduction in proteinuria will dictate continuation of the CSA for an additional 6 months (total of 12 months of full dose CSA). This assessment at six months allows a balance between risk-benefit in both arms. The protocol provides similar exposure to immunosuppression in both arms and allows an early exit for patients related to safety issues by providing a lack of efficacy (futility) end point at 6 months.

Primary endpoint.

CR or PR (defined as per table 1) at 24 months after randomization will be the primary endpoint. This will be assessed in the protocol adherent patients.

Secondary endpoints.

1. Relapse state at month 24 after randomization (Urine Protein) (UP)>3.5 after earlier CR or PR)
2. AntiPLA2R levels
3. Quality of life as measured by modified KDQOL
4. Adverse events
5. ESRD
6. CR or PR, and CR alone at 6, 12, 18, and 24 months after randomization
7. Time to CR or PR
8. Effect of treatment on renal function, as assessed by slope of creatinine clearance from baseline to 24 months.

| Table 1. Definition of remission status at 24 months | |
|---|--|
| Remission status | Proteinuria (UP g/24 hours) after treatment |
| Complete remission (CR) | UP \leq 0.3 g and serum albumin \geq 3.5g/dl |
| Partial remission (PR) | Reduction in baseline UP of $> 50\%$ plus final UP \leq 3.5 g but $>0.3\text{g}$ |
| Non-response (NR) | Reduction in baseline UP of $< 30\%$. (includes increase in UP) |
| Relapse | Development of nephrotic range proteinuria following CR or PR, i.e. $>3.5\text{g/d}$ |

Patient recruitment

The study population will be comprised of individuals with biopsy-proven idiopathic MN. Total study size will be 126 patients, enrolled among up to 25 participating sites. Potential candidates for the study will be identified by the investigators using existing databases and clinical trial networks. The P.I. or the study coordinator will contact these potential candidates and if the preliminary interview indicates their willingness to participate, a consent form will be reviewed with the patient. If a potential candidate remains interested, the consent form will be signed, and the patient enrolled in the study. The institutional review board at each participating site will approve the recruitment process and consent forms. The Mayo Nephrology Collaborative Group (MNCG) will post information on its web site describing the study and identifying local contacts at the collaborating centers. Each site involved may have their own methods of informing and recruiting potentially eligible patients but in all cases will have IRB approval before embarking on this process. Each site will be expected to randomize approximately 6-10 patients over the recruitment period. If, based on site estimates of the IMN population that would be eligible for this study, we enroll approximately 30% of the annual population in a single year our recruitment will be complete. We have assembled a consortium for this trial with a population that well exceeds the enrollment needs based upon the multi-year enrollment plan for this trial.

Recruitment strategy

Potential candidates for the study will be identified by the P.I at each site using existing research and clinical databases and clinical trial networks. With local IRB approval, electronic health records and pathology clinical data bases will be used for cohort discovery at each institution. Study investigators will also contact local research volunteer registries and local patient advocacy groups and request they provide information about this trial to their participants/members. Each participating site will be encouraged to accept referrals of study patients identified from national patient volunteer registries such as researchmatch.org and the Office of Rare Disease Research sponsored patient contact registry. The Mayo Nephrology Collaborative Group (MNCG) will post

information on its web site describing the study and identifying local contacts at the collaborating centers. Regional advertising will also be carried out via our ongoing participation in the NEPTUNE trial (sponsored by the NIDDK/ODR.) through its collaborative network of 18 academic centers in North America. The P.I. or the study coordinator will contact potential study candidates and if the preliminary interview indicates their willingness to participate, a consent form will be reviewed with the patient. If a potential candidate remains interested, the consent form will be signed, and the patient enrolled in the study. According to our site estimates, only 18% of the available population will need to be eligible and willing to participate in the trial to achieve the target sample size.

Potential Challenges and Solutions

Patient recruitment is often slower than anticipated in clinical studies. The team of clinical centers engaged in this trial, including the primary Mayo Clinic site, have significant experience in recruitment and conduct of clinical trials. Past recruitment success at Mayo into phase I and II trials of RTX in adults with IMN is documented by the enrollment of 15 patients in less than 6 months and 20 patients in 11 months.^{71, 75} At Mayo Clinic alone, an average of 50 cases of MN a year have been diagnosed for the past 5 years based on kidney biopsy registry. The projected available population is > 200% of the recruitment goal.

If enrollment at some of the involved clinical sites is slower than expected, we have the option to recruit additional patients from the other participating sites or add, for example, additional sites from the Neptune Consortium to our trial.

Inclusion Criteria

- Idiopathic MN diagnosed by renal biopsy (original biopsy needs to include light, immunofluorescence and electron microscopy); pathology report must be adjudicated by a study PI (Dr. Fervenza or Dr. Catturan, or documented delegate) prior to randomization.
- Age 18-80 years
- If female, must be post-menopausal, surgically sterile or practicing a medically approved method of contraception (with exception of no birth-control pill given the potential for increased risk of thromboembolism in the nephrotic setting).
- Treatment with an ACEi and/or ARB, for ≥ 3 months prior to randomization and adequate blood pressure control (BP <130/80 mm Hg in >75% of the readings). Patients with documented evidence of >3 months treatment with maximal angiotensin II blockade, on an HMG-CoA reductase inhibitor, and BP control (BP <130/80 mm Hg in >75% of the readings) who remain with proteinuria >5g/24h may enter the study immediately and be randomized to RTX/CSA without the need of the run-in/conservative phase of the study. However, in addition these patients must have a documented <50% reduction in proteinuria compared to previous 24 hr proteinuria or Uprot/UCr ratio estimates during this period of ACEi and/or ARB treatment otherwise they must fulfill the run-in requirement,
 - (Please refer to manual of operation for clarification of tests mandated for patients who are randomized without the run-in period)
- Proteinuria $\geq 5\text{g}/24\text{h}$ on two 24-hour urine collection collected within 14 days of each other despite Ang II blockade for ≥ 3 months as described above.

- Estimated GFR ≥ 40 ml/min/1.73m² while taking ACEi/ARB therapy. The GFR will be estimated using the 4 variable MDRD equation as published in the NKF-CKD guidelines.⁸¹ This approach is adopted, rather than the much more expensive and more invasive techniques (e.g. inulin or iothalamate clearance) since the likelihood of detecting significant changes in GFR in this short term study is remote regardless of which method is chosen. At entry into the study and at set time points thereafter patients will also have a 24h urine collection for calculation of CrCl and proteinuria.

Exclusion Criteria

- Age <18 years or >80 years.
- Estimated GFR <40 ml/min/1.73m². The rationale for the criteria is that patients with severe reduction in GFR are likely to have significant interstitial and glomerular scarring and are less likely to benefit from treatment.
- Patient must be off prednisone or mycophenolate mofetil for >1 month and alkylating agents for >6 months. The rationale is to minimize the potential confounding effect of delayed benefits from previous immunosuppressive agents and to reduce the risk of too much immunosuppression from the combined previous drug exposure plus trial drug exposure, e.g. infections.
- Patients with presence of active infection or a secondary cause of IMN (e.g. hepatitis B, SLE, medications, malignancies). Testing for HIV, Hepatitis B and C should have occurred <2 years prior to enrollment into the study. Screening for malignancy should be carried out according to standard guideline recommendations.
- Type 1 or 2 diabetes mellitus: to exclude proteinuria secondary to diabetic nephropathy. Patients who have recent history of steroid induced diabetes but no evidence on renal biopsy performed within 6 months of entry into the study are eligible for enrollment.
- Pregnancy or breast feeding (for safety reasons).
- History of resistance to CSA or RTX. Patients who previously responded to CSA with either a CR or PR but relapsed off CSA are eligible.

Randomized Treatment Groups: Once all entry criteria have been satisfied and confirmed, patients will be randomized to treatment with RTX or Cyclosporine. Randomization will be performed by study site staff through the electronic case report form (eCRF.) The randomization list will be stratified by site, and generated by the Data Coordinating Center (DCC) using random permuted blocks of variable size.

Rituximab: Patients randomized to the RTX arm will receive 1000 mg IV on Days 1 and 15. Patients who achieve complete remission at 6 months will not be retreated. A second course of RTX 1000 mg IV will be administered at study month 6 for individuals who have not achieved a complete remission, but have achieved at least a > 30% reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not a CR). Dosing at study month 6 will be independent of CD19+ B cell count. The rationale for retreating patients who have had an equal to or > 30% reduction in proteinuria at 6 months is based on our experience in our pilot studies (first study using 1000 mg on Days 1 and 15 and second study using 4 weekly doses of 375mg/m²) where an increase in the proteinuria remission rate was achieved after a second course of treatment. In our 2 studies repeated courses of RTX were not associated with additive adverse effects in comparison to their first course. If after six months the reduction in proteinuria is less than 30% compared to baseline the RTX treatment will not be repeated, the patient will exit from the study and will be considered a failure of therapy.

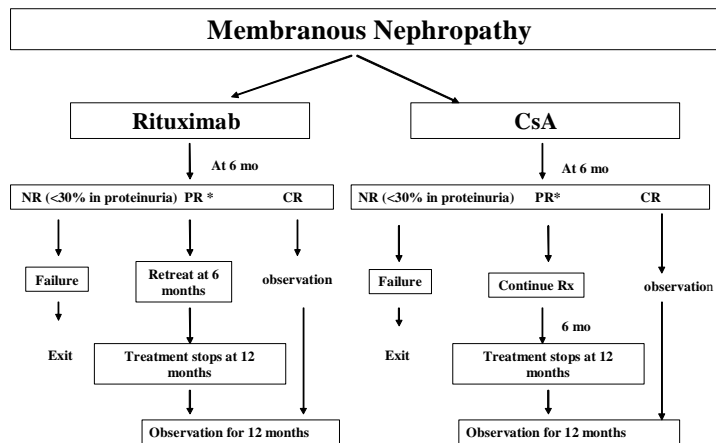
Dosage and Administration of Rituximab: The first infusion of both courses (Day 1 and Day 168) of RTX will be administered IV at an initial rate of 50 mg/hr. All patients will be premedicated with acetaminophen (1g) and diphenhydramine HCl (50 mg) by mouth from 30 to 60 minutes prior to the start of an infusion. Premedication with steroids (100 mg methylprednisone IV) will also be given 30 minutes prior to the first infusion of each series of RTX (Day 1 and Day 168). If a hypersensitivity or infusion-related reaction does not occur, the infusion rate will be escalated by 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If a hypersensitivity (nonIgE-mediated) or infusion-related reaction develops, the infusion will be temporarily slowed or interrupted. Treatment of infusion-related symptoms with additional diphenhydramine and acetaminophen will be recommended. Additional treatment with bronchodilators or IV saline may be indicated. The infusion may be continued at one-half the previous infusion rate upon improvement of the patient's symptoms. If the infusion was well tolerated, subsequent infusions (Day 15 and Day 182) may be administered at an initial rate of 100 mg/hr and increased by 100 mg/hr increments at 30-minute intervals, to a maximum of 400 mg/hr as tolerated. If the first infusion was not tolerated, the guidelines for the first infusion should be followed for all subsequent infusions. Patients administered an antihistamine for treatment or prevention of infusion related reactions will be given appropriate warnings about drowsiness and impairment of driving ability prior to discharge. Participants will be observed for 1 hour post infusion. There will be a +/- 3 day window for each study visit to account for weekends, holidays, and scheduling conflicts.

Monitoring of Rituximab Effects

The numbers of CD20/CD19+ B cells in the peripheral blood will be quantified by flow cytometry using peripheral blood leukocytes. Flow cytometry will be performed pre-RTX treatment and at regular intervals following administration of RTX (see Table 2b: Test Schedule and Monitoring for Rituximab Treatment Arm). These assays will allow us to follow the level of B-cell depletion, kinetics of B-cell reconstitution and the composition of cells that re-populate the B-cell pool after treatment with RTX as well as helping to ensure dosing adequacy in these patients.

Cyclosporine: Patients randomized to the Cyclosporine arm will be started at a dose of CsA = 3.5 mg/kg/day p.o. divided into 2 equal doses given at 12 hour intervals. Target trough CsA blood levels, as determined in whole blood by HPLC, are 125 to 175 ng/ml. Patients will have their doses adjusted according to their blood levels of CSA as monitored every 2 weeks until the target trough level is reached. If a complete remission is achieved by six months, CSA will be tapered by approximately 1/3 of the maintenance dose monthly and hence discontinued after three months. If there has been at least an equal to or >30% reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not complete remission) the CSA will be continued for an additional six months. A persistent and otherwise unexplained increase in serum creatinine >30% will prompt an approximate 25% dose reduction of CSA e.g. aiming for 25% reduction in CSA trough level. For example, if trough CSA was 175 ng/ml reduce to 130 ng/ml; and if with this dose reduction the creatinine does not return to baseline levels within 3 weeks, then a second dose reduction of approximately 25% with a similar reduction in CSA trough level (e.g. if CSA trough level was 100 ng/ml, reduce to 75 ng/ml) will be used. If the creatinine does not fall to within 30% of baseline values with this second dose reduction, the drug will be discontinued. If after six months the

reduction in proteinuria is less than 30% compared to baseline the drug will be discontinued, the patient will exit from the study and be considered a failure of therapy. At the end of 12 months, Cyclosporine will be tapered by 1/3 of the maintenance dose monthly and hence discontinued after 3 months. Serum potassium level will be checked at the initiation of Cyclosporine in conjunction with blood draw for CSA level. If Cyclosporine is increased during treatment, potassium will be rechecked two weeks post increase in CSA dosage.



* here defined as equal to or > 30% reduction in baseline proteinuria

Figure 4. Schematic representation of the study design.

Blinding

This will be an open-label study with neither patients nor physicians blinded. Blinding, for this study, was considered difficult and unnecessary and would significantly add to the cost of the study. The need to provide RTX intravenously would require that the CSA arm patients would have to receive an IV infusion of saline which seems unjustifiable from the scientific and budgetary points of view. Furthermore, with the exception of quality of life measures, all endpoints are based on objective (laboratory) criteria. Having the physicians in the study blinded to CSA level also introduces a potential risk factor that's unnecessary in our estimation. Documentation of cyclosporine levels will ensure that patients are taking the drug at the appropriate time and achieving the prescribed levels indicated in the protocol.

Pharmaceutical logistics

RTX will be provided by Genentech (see appended letter of support). Although Cyclosporine is recognized by insurance service providers as standard treatment for patients with IMN, participants randomized to the cyclosporine arm will receive the drug free of charge. Appropriate quantities of the medication will be supplied to the individual from the individual sites at Time 0 and Months 3, 6, 9, and 12.

Screening visit

The Screening visit will consist of meeting with any potential candidate for the study, reviewing eligibility criteria, including laboratory requirements as outlined in Tables 2a and 2b, reviewing and signing the informed consent and starting the patient on the initial step of the protocol, i.e. maximize angiotensin II blockade as described in the run-in phase below. If any of the exams, tests or procedures involved in this study were completed as routine standard of care within 30 days of screening, participants do not need to repeat them. It will be up to the study doctor to review and consider if the previous findings are appropriate/adequate to use otherwise they will be repeated.

Patient monitoring and evaluation.

Cyclosporine trough level measurements will be done every 2 weeks until levels are stable and at target. Potassium will also be checked 2 weeks after initiation of Cyclosporine, in conjunction with CSA level. If Cyclosporine is increased during treatment, potassium will be rechecked at the 2 week time point. Cyclosporine levels will also be assessed whenever serum creatinine rises by >30% without a recognizable reason such as volume depletion, additional drugs, sepsis, etc. Patients will be followed for 2 years following randomization to monitor for the occurrence of adverse events, late remissions, relapses, GFR changes and development of end stage renal disease. The first 12 months of the study will be considered as the treatment period while the remaining 12 months will be considered as an observational period including the final 3 months of tapering to discontinuation. Patients who cannot tolerate the medications or who discontinue study medication will continue to receive follow-up as scheduled with study laboratory testing limited to serum creatinine and 24h Uprot/UCr ratio at the same time intervals as those continuing in the trial. However, the therapeutic/management plan for these patients who exit the study will be solely at the discretion of the managing nephrologist.

Laboratory testing

In order to contain costs all clinical tests will be carried out locally at the recruiting centers. However, at critical time points: Time 0, 6, 12, 18, and 24 months from start of treatment, recruiting centers will ship to Mayo Clinic, Rochester, Minnesota, 2 blood samples and 2 urine samples (aliquots obtained from two 24h urine collection) for determination of serum creatinines, serum albumins and urinary protein/creatinine ratios. Blood and urine kits will be provided by Mayo Clinic, Rochester, Minnesota, for these samples along with shipping costs (see Lab Manual). The Central Lab results (Mayo Clinic, Rochester, MN) will be used for statistical purposes since this will ensure a standard methodology is applied to the critical (for the trial analysis) laboratory assessments. By measuring the volume of the 24-hour urine collection we will also be able to calculate both clearance and the 24-hour urine protein from the aliquot. Proteinuria results at Time 0, 6, 12, 18 and 24 months will be taken as the mean of two 24-hour urine collections done back to back, and the mean of these 2 collections will be used to determine CR, PR, or NR.

In all patients, CBC, serum creatinine, electrolytes, albumin, urinalysis, 24-hour Uprot/UCr ratio, and creatinine clearance will be evaluated at each study visit. In patients receiving RTX, additional tests will include: quantification of lymphocyte subsets by flow cytometry, serum immunoglobulin levels, and determination of humanized anti-chimeric antibodies (HACA) at study visits Day 1, 6, and 9 months. HACA will be performed free of charge by Genentech using established techniques.

Anti-PLA2R Assay

We will collect serum samples (5 mL), at Time 0, and months 3, 6, 9, 12, 18, and 24 for the measurement of anti-PLA2R antibodies. Recent evidence suggests that the presence of autoantibodies to the M-type phospholipase A2 receptor (PLA2R) is a specific marker of idiopathic MN, found in greater than 70% of patients.⁷⁶ When the analysis is limited to new-onset, nephrotic patients with idiopathic MN, this sensitivity increases further. The presence of anti-PLA2R correlates well with disease activity, disappearing with a spontaneous or treatment-induced remission, and reappearing with a relapse of MN.

We intend to explore the correlation of anti-PLA2R and disease activity by carefully looking at anti-PLA2R titer in response to RTX/cyclosporin and the achievement of partial or complete remission. In those patients that have no remission from their disease in response to RTX, we will determine if circulating anti-PLA2R is still present which might suggest the need for a second course of RTX or alternate immunosuppressive therapy.

Serum samples from all 126 subjects will be collected at baseline and months 3, 6, 9, 12, 18, and 24. Serum samples will be collected at the individual sites by the study coordinator and stored in a dedicated -20° freezer until a point when they can be shipped in bulk to Dr. Fervenza at Mayo Clinic, Rochester, Minnesota (see Appendix B). Mayo Clinic will then send the samples in bulk to Dr. Laurence Beck at Boston University or to Dr. Paul Brenchley at the Manchester Royal Infirmary in the UK. The sera will be tested in batches for reactivity toward immobilized recombinant PLA2R by western blot (the assay currently in use) or ELISA (expected to be in use at the time this proposal is in effect), with appropriate positive and negative controls. Upon completion of the study, we will correlate response to treatment in each group to the baseline presence and/or titer of anti-PLA2R. A major hypothesis to be addressed is whether there is a difference in treatment response between those subjects who are initially anti-PLA2R negative versus those who possess circulating anti-PLA2R. In those subjects who are initially positive for anti-PLA2R, we will also correlate the primary endpoint (remission status) to anti-PLA2R titers throughout and at the end of the study.

Investigators in the study will be blind to the results of anti-PLA2R antibodies until the study is completed. The rationale is to avoid bias in the treatment of these patients. Although ideally patients would be stratified to the different arm based on the results of their initial anti-PLA2R test in order to avoid potential imbalance this will not be possible for practical and financial reasons. Given the relatively small number expected to be negative, it is unlikely this will bias the balance between positive and negative patients at randomization.

Histopathology

Due to the expertise of the pathologists at each of the clinical centers and to the characteristic findings on the renal biopsy in MN, a review of the biopsy slides will not be required prior to randomization at a Central Pathology Laboratory. However, a report documenting the results of the histology review including, light microscopy, immunofluorescence and electron microscopy results, as well as percentage of global and segmental glomerular sclerosis, tubulo-interstitial index, and immunofluorescence findings will be submitted to the DCC and reviewed by the study P.I prior to randomization. Renal pathology will be centrally reviewed at the end of the study by Dr. Sanjeev Sethi, from the Pathology Department at Mayo Clinic Rochester.

Quality of life measures

Psychosocial response to illness and its treatment may play an important role in overall adjustment and illness management. Research from a variety of chronic illness populations, including steroid resistant FSGS and chronic kidney disease (CKD), has consistently linked physical and psychosocial variables with health outcomes. These findings highlight the significant burden experienced by adults with uncontrolled nephrotic syndrome. Little is known about the impact of MN or its treatment on patient reported outcomes. Due to the severity of this disease and the potential risk for progression in these patients, psychosocial factors may play an important role in understanding their response to treatment. In addition to evaluating the safety and efficacy of RTX and CSA, it is essential to assess the individual's perception of his/her quality of life relative to disease status and therapeutic response. Quality of life will be assessed by patient self report using the modified KDQOL questionnaire form at Time 0, 6, 12 and 24 months.

Table 2a: Test Schedule and Monitoring for Cyclosporine Treatment Arm

| Tests | <u>Screening</u> | Time 0 | Day 15 | Day 28 | 3 mo | 6 mo | 9 mo | 12 mo | 18 mo | 24 mo |
|---|------------------|-----------|--------|--------|------|-----------|------|-----------|-----------|-----------|
| History/Exam | X | X | | | X | X | X | X | X | X |
| CBC | X | X | | | X | X | X | X | X | X |
| BUN,Cr,Lytes, Albumin (including potassium) | X | X | X | | X | X | X | X | X | X |
| CPK | | X | | | X | | | | | |
| CsA levels † | | | X | X | X | X | X | X | | |
| Lipid panel | | X | | | | X | | X | | X |
| Pregnancy | X | X | | | | X | | | | |
| Anti-PLA2R antibodies | X | X | | | X | X | X | X | X | X |
| DNA sample | | X | | | | | | | | |
| Potassium and Cr ¹ | | | X | X | | | | | | |
| UA | X | X | | | | X | | X | | X |
| Uprot/Ucr 24h | X | XX | | | X | XX | X | XX | XX | XX |
| 24h creatinine for CrCl | X | X | | | | X | | X | | X |
| 24h urine urea | | X | | | | X | | X | | X |
| 24h urinary Na ⁺ | | X | | | | X | | X | | X |
| Quality of life-KDQOL | | X | | | | X | | X | | X |
| Gene Expression | | X | | | | | | X | | X |

1. Potassium, Cr checked at 2 weeks post Cyclosporine initiation. If Cyclosporine is increased during treatment, potassium, Cr and CsA will be rechecked after 2 weeks

†Patient will have blood levels of CSA monitored every 2 weeks until target is reached and then as per above schedule.

Table 2b: Test Schedule and Monitoring for Rituximab Treatment Arm

| Tests | <u>Screening</u> | Time 0 | Day 1 | Day 15 | Day 28 | 3 mo | 6 mo | Day 168 | Day 182 | 9 mo | 12 mo | 18 mo | 24 mo |
|---|------------------|-----------|-------|--------|--------|------|-----------|---------|---------|------|-----------|-----------|-----------|
| History/Exam | X | X | | | | X | X | | | X | X | X | X |
| CBC | X | X | | | | X | X | | | X | X | X | X |
| BUN,Cr, Lytes(including potassium)/Alb | X | X | | | | X | X | | | X | X | X | X |
| CPK | | X | | | | X | | | | | | | |
| PBFC ¹ | | | X | | X | | X | | | | X | | |
| Serum IgG, IgA and IgM | | | X | | | | X | | | | X | | X |
| Lipid panel | | X | | | | | X | | | | X | | X |
| HACA ² | | | X | | | | X | | | X | | | |
| Pregnancy | X | X | | | | | X | | | | | | |
| Anti-PLA2R antibodies | X | X | | | | X | X | | | X | X | X | X |
| DNA Sample | | X | | | | | | | | | | | |
| UA | X | X | | | | | X | | | | X | | X |
| Uprot/Ucr 24h | X | XX | | | | X | XX | | | X | XX | XX | XX |
| 24h creatinine for CrCl | X | X | | | | | X | | | | X | | X |
| 24h urine urea | | X | | | | | X | | | | X | | X |
| 24h urinary Na ⁺ | | X | | | | | X | | | | X | | X |
| Pre-infusion medication (i.e.Tylenol, Benadryl) | | | X | X | | | | X | X | | | | |
| Pre-infusion medication (i.e. | | | X | | | | | X | | | | | |

| | | | | | | | | | | | | | |
|---------------------------|--|---|---|---|--|--|---|---|---|--|---|--|---|
| methyprednisone) | | | | | | | | | | | | | |
| Rituximab | | | X | X | | | | X | X | | | | |
| Quality of life- KDQOL | | X | | | | | X | | | | X | | X |
| Gene Expression | | X | | | | | | | | | X | | X |

1. PBFC: Peripheral blood flow cytometry. Quantitate B and T cell subsets;
2. HACA: human anti-chimeric antibodies; pre-infusion on Days 1 and at 6 months and 9 months.

Run In Phase – Common Therapy for Both Arms

Blood pressure control and angiotensin II blockade: The target blood pressure (<130/80mmHg in >75% of the readings; but not <100 mmHg systolic) is chosen based on recent recommendations by the JNC VII.⁸² The first step will be the introduction of an ACE Inhibitor (ACEi). Because this part of the study aims to maximize Ang II blockade, ACEi dose will be increased every 2 weeks until the maximum tolerated/FDA approved dose is achieved or until intolerable side effects occur (e.g. development of postural hypotension, light headed, hyperkalemia, etc). Once ACEi dose has been maximized and there are no observable side-effects and/or blood pressure is not at target, a long acting ARB will be added. The ARB dose will be increased every 2 weeks to achieve the maximum tolerated or maximum approved dosage.

Patients whose blood pressure control is not at target will receive additional medications in the following order: a loop diuretic, a cardioselective β -blocker, a non-dihydropyridine calcium channel blocker (CCB), and clonidine. The selection of these drugs adheres to the recommendations of the JNC VII.⁸² The choice of a non-dihydropyridine CCB was made because of concerns that dihydropyridine-type CCB may obscure the anti-proteinuric effects of the above therapy. In order to further ensure that any potential adverse effect is minimized we have limited CCB to be used as a fifth agent, and to be used only when the combination of an ARB/ACEi, a diuretic, and a β -blocker have failed to bring BP to target level. Recent data have shown that an aldosterone or renin antagonist have significant antiproteinuric effects. The reason we do not include these agents as part of the treatment protocol is out of concern for the development of severe hyperkalemia when all (ACEi, ARB, a direct renin inhibitor and aldosterone antagonist) these agents are used in combination. This side effect is likely to be further accentuated in the Cyclosporine group and we want to avoid an imbalance of RAS blockade between the arms of the study.

Concomitant Treatment

At the start of the run-in/conservative phase of the study and as part of the standard of care for patients with nephrotic syndrome and significant hyperlipidemia, patients will be started on atorvastatin 10 mg a day (or its equivalent with the exception of rosuvastatin (crestor) which is to be avoided given the associated marked increase in the area under the curve (AUC) when used in conjunction with CSA). If tolerated clinically and, as evidenced by the lack of persistent elevation of liver transaminase >3x upper limit of normal or high CK, or clinical rhabdomyolysis), the dose will be increased according to the recently published KDOQI-dyslipidemia guidelines.⁸³ The dose should not be increased above the maximum of 40 mg/day. The rationale for not using a higher statin dose is because of the risk of developing proteinuria with the use of statins at high doses, and the added risk of rhabdomyolysis in the CSA group. Patients will remain at the highest tolerated dose for the entire duration of the study. Serum lipids will be measured at Time 0 and every 3 months thereafter. High sodium intake (e.g. >200 mm Na/d or 4.6 g sodium/d) can significantly impair the beneficial effects of Ang II blockade.⁸⁴ Therefore patients will be instructed to follow a low salt diet (2-3g/day). Patients will also receive dietary counseling at enrollment as part of their standard of care. Patients will be advised of a dietary protein target intake of 0.8-1.0 g/kg ideal body weight/day of

high quality protein and will be encouraged to maintain the same diet throughout the duration of the study. Patients with proteinuria >10g/24h and serum albumin <2g/dl should be considered for prophylactic anticoagulation.

Stopping points for medications:

1. For Cyclosporine: At 6 months, patients who failed therapy (as previously defined) will have CSA dose tapered and discontinued.
After 12 months of therapy, regardless of the degree of proteinuria, Cyclosporine will be tapered and discontinued as described above.
2. Patients in the RTX arm who have failed therapy at 6 months (as previously defined) will not have a repeat treatment course.
After the second course of RTX therapy at six months they will have no further RTX therapy regardless of their proteinuria level
3. Significant potential life threatening infection.
4. Persistent elevation of liver enzymes >2 x normal (despite CSA arm dose reduction).
5. Persistent elevation of serum potassium >5.8 mEq/l despite diet changes, ACEi/ARB reduction, diuretic dose increase (and in the CSA arm dose reduction).
6. A rise in serum creatinine by >30% above baseline that persists in the absence of secondary causes such as volume depletion or use of potential nephrotoxic agents (and despite adjustments in the CSA dose)
7. Persistent hypertension in either arm, i.e. supine blood pressure >160 mmHg systolic or >90 mmHg diastolic, despite institution of maximum antihypertensive therapy (and despite adjustments of CSA dose)
8. Other clinically relevant adverse effects not resolved by reduction in dosage of test medications.
9. Development of any malignancy or lymphoproliferative disorder.
10. Pregnancy.
11. The appearance of an independent disease whose standard therapy requires continuous administration of significant amounts of corticosteroids, other immunosuppressive agents or plasma exchange therapy.
12. Serious concomitant disease with an expected survival of less than 4 years.
13. At the wish of the patient.
14. The study will be placed on hold if 3 of the first 5 patients enrolled and treated with RTX or CSA develop severe opportunistic infections, or experience Grade 3-4 SAEs.

Patients who choose to discontinue study medication or are withdrawn because of adverse events will be strongly encouraged to continue follow up examination to the study termination.

Ancillary studies:

Quantitative gene expression analysis

Quantitative gene expression analysis using real-time PCR and Microarray technology in peripheral blood mononuclear cells: There is little information on global gene expression

changes that occur during active nephrotic syndrome secondary to IMN and subsequent to remission of this disease. Furthermore, there is limited information on how Rituximab and Cyclosporine affects gene expression of potentially pathogenic genes. As part of this study, peripheral blood will be collected at Time 0, 12 and 24 months after therapy. Following treatment with Rituximab and Cyclosporine administration, B-cell-specific genes will be assessed to track the extent of treatment in the B-cell pool gene expression. Both quantitative TaqMan real-time polymerase chain reaction and gene microarray (Affymetrix U133 chips) will be used to evaluate gene expression profiles. Real-time PCR will be used only to confirm the findings of the gene chip for genes with statistical significance.

Staining original renal biopsy for CD20 + B cells

We will obtain each patient's permission to review renal biopsy slides and to obtain the renal biopsy paraffin block in order to obtain biopsy tissue for staining with antibody against CD20 positive cells and anti-APL2R (3 slides, 3 μ thick). The purpose is to stain sections from biopsy tissue with an antibody against CD20 positive cells and to look for receptor density to APL2R. These may be the same cells that are in circulation and that are depleted by Rituximab. We want to examine the hypothesis that there is a correlation between the number of CD20 positive B cells infiltrating the kidney and the patient's response to therapy. Preliminary results suggest that staining for CD20 and APL2R antibodies are increased in kidney biopsy of patients with MN. All patients by this juncture will have completed the therapeutic part of the study and will have already received rituximab or CSA treatment as part of study so this ancillary project will have no therapeutic or management implications.

Immunohistochemistry methods

Immunohistochemical staining will be performed at the Department of Pathology, Mayo Clinic, Rochester, Minnesota, by Dr. Sanjeev Sethi. Formalin-fixed, paraffin embedded sections will be cut onto coated glass slides. Slides will be incubated with monoclonal anti-CD20 and APL2R primary antibody at 1:1000 dilution (DAKO). Heat induced antigen retrieval will be used. Sections will be incubated at 20C overnight and rinsed. Endogenous peroxidase activity will be prevented by pretreating all sections with 3% hydrogen peroxide. Sections will be incubated with a secondary rabbit anti-mouse antibody linked with avidin-biotin complex. Sections will be counterstained with hematoxylin. CD20 positive cells (B-cells) will be counted and the density of positive cells per area of renal cortex will be calculated utilizing Image Pro Plus (Media Cybernetics) computer image analysis software.

Statistical Methods and Analysis:

Sample size estimation: The primary study goal is to compare the long-term efficacy of RTX (given at baseline and repeated at 6 months) with CSA (12 months treatment + 3 months taper) using proteinuria response 24 months after randomization. Proteinuria positive response will be defined as attaining and maintaining CR or PR at 24 months after randomization. We propose to establish that the RTX treatment is non-inferior to the

CSA. RTX will be considered non-inferior to CSA if the response rate of the RTX arm is at most 15% worse than that of CSA arm. In addition we will compare the quality of life (QOL) and adverse event (AE) profile over the course of the treatment period. The preliminary data has indicated that CSA is effective in inducing a CR/PR of proteinuria in between 60 and 75% of MN cases.^{48, 51} However, nephrotic syndrome (NS) relapses may be as high as 50% once CSA is discontinued.⁸⁵ Thus, we estimate a CR/PR rate in patients treated with CSA of 30-50% at 24 months after randomization.⁸⁵ Similar remission and relapse rates with the use of Tacrolimus (another calcineurin inhibitor) have been reported by Praga et al.⁵⁶ In this latter study, almost half of the MN patients had a relapse of the nephrotic syndrome after tacrolimus was discontinued.⁵⁶ On the other hand, based on the long term follow up on the 35 patients treated with RTX from our 2 studies, we estimate the relapse rate to be <10% at 24 months.^{71, 75} We observed a CR/PR rate at 12 months, very similar to the rate for calcineurin inhibitors i.e. 60%, and in our second study completed recently, we observed an 80% CR/PR rate at 24 months, implying that RTX had a ~20% failure rate with 95% (CI 6% to 44%). To be conservative for the sample size estimation, we considered a maintained CR/PR rate at the low end of our previous experience, 55% CR/PR for RTX at 24 months and at the high end of our experience with CSA (CNI therapy) CR/PR for CSA of 45%. We propose a non-inferiority trial with non-inferiority margin $\delta = 15\%$, with the null hypothesis of $\pi_{\text{RTX}} - \pi_{\text{cyclosporine}} < \delta$, where π_{RTX} and $\pi_{\text{cyclosporine}}$ are the proportions of patients with CR/PR at 24 months in treatment arms of RTX and CSA, respectively. Under these assumptions, and a one sided alpha of 0.025, enrollment of 63 evaluable patients per study arm is required to achieve 80% power to show that the RTX is not inferior. For the intent to treat (ITT) analysis we will consider all patients lost to follow up to be non-responders so no correction to the sample size is needed for dropouts. With 63 patients per arm we will have 80% power to detect moderate differences of .6 standard deviations for continuous measures or around .25 for proportions for the comparisons of the QOL and AEs.

Statistical and Analytical Plan: All demographics and entry laboratory data will be summarized by treatment group. Frequency distributions will be used to describe categorical values and basic summary statistics (mean, standard deviation, median, and inter-quartile range) will be used to describe continuous values. To provide more reliable estimates and minimize the “regression to the mean effect” duplicate urine measurements obtained at baseline (Time 0), 6, 12, 18 and 24 months will be averaged. The chi-square test and logistic regression will also be used to compare the percent of patients with remission at 6, 12, 18, and 24 months adjusting for treatment center and the baseline proteinuria (UP<8g/24h vs >=8g/24h). Odds ratios and associated 95% confidence intervals will be estimated. The formal test for the primary endpoint will be based on the significance of the treatment group factor in the logistic regression model for UP failure at month 24. The Wilcoxon rank-sum test and ordinal logistic regression will be used to compare the ordered remission status outcome (1=CR, 2=PR, 3=NR) between treatment groups. Longitudinal methods for categorical outcomes (e.g. generalized estimating equation or GEE models) will be used to compare remission status profiles between treatment arms. Unless otherwise noted, all tests will be two-sided with alpha level 0.05.

For repeated measures such as urine protein, individual rates of change will be estimated using within-patient linear regression analyses (including data from all visits). Because of the non-linearity of the changes in proteinuria log transformation estimates will be used. Renal function readings will be censored at initiation of dialysis or renal transplant. Additionally, mixed effects models, assuming a random center effect, and a random slope of creatinine clearance and/or reciprocal of creatinine and intercept for each patient will be fit using data from all visits. These models allow comparison of the average slope between groups, while taking into account that each patient's slope may be based on a variable number of readings. Treatment group comparisons regarding quality-of-life scales will be done using repeated measures analyses and mixed effects models.

Adverse events (both patient and event counts) will be tabulated by body system, severity and, for each severity, by investigator-assessed relationship to study drug. Group comparisons for adverse events with 4 or more occurrences will be done using chi-square or Fisher's exact test. The last RTX injection is at 6 months and the tapering of Cyclosporine will be completed by month 15. Hence, the adverse events analyses will focus on events through month 18, allowing these additional months for potential lingering Cyclosporine or RTX complications.

The analysis of the primary endpoint (urine protein failure at month 24) will be intent-to-treat (ITT), and will include all randomized subjects in the analysis. For those without a 24 month visit, the 18 month visit results will be used if available, otherwise they will be assumed to have failed at 24 months. Per protocol (PP) analyses will also be done including only those subjects who receive a full course of study medication and who have a 24 month visit.

Interim analyses: No interim analyses for efficacy will be performed during the trial, however safety data will be compiled and reviewed approximately every six months or at the request of the Data Safety Monitoring Board.

Investigator and Coordinator Training: In order to make efficient use of funding, annual investigator and coordinator meetings will be coordinated with national nephrology meetings where possible. It is expected that the majority of the training will occur through web-based technology and will be coordinated through the data management and coordinating Center.

Data management and quality assurance: Study data, including enrollment and monitoring data, will be maintained on a secure password protected database accessible to all study centers from the world-wide-web. Each center will enter data for their patients. Quality control will be ensured by oversight by the AHRC coordinating center, who will review the electronic files of all patients on a regular basis for completeness.

Quality and completeness of data entry will be monitored on a weekly basis during the initial 6 months of study enrollment and biweekly thereafter. Data quality reports will be generated monthly for review by the study Data Quality Assurance Committee, St. Michael's Hospital/University of Toronto. Data queries generated by identification of incomplete or inconsistent data will be raised directly within the electronic eCRF and should be resolved by the study coordinator or PI in a timely manner. Corrections or changes in the data management system are tracked with the retention of the original data and the corrected data with the date of data entry and submitting personnel. Sites with persistent delays or difficulties in data capture will be provided additional study based training. Study data will be stored within the data centre of St. Michael's Hospital/University of Toronto with an off-site daily back-up system.

Analysis of the primary and secondary outcomes will be conducted at the data coordinating center (DCC). Additional analyses requested by ancillary investigators will be conducted when feasible within the scope of this trial funding. The DCC will implement the trial policy for data sharing and ancillary studies. A fee structure will be established for ancillary studies that are beyond the scope of this trial.

Data security:

All clinical data will be processed in a secure electronic environment that includes virus protection, and restricted access. Electronically stored data are subject to extensive security measures including virus detection, and restricted access. Security measures in place for the database management system proposed for this study include: browser security, firewall protection, user name/password protection, user re-authentication, and inactivity time-out. The database will not contain study participant name, address or medical record number. Patient data will be identified only by study code. Study data, including enrollment and monitoring data, will be maintained on a secure password protected database. Quality control will take place at time of data entry (range, consistency checks) and will be ensured by oversight by the P.I. and Quality Assurance and Clinical Management Workgroup which will review the files of all patients on a regular basis for completeness.

The **Data Coordinating Center (DCC)** is located at the Applied Health Research Center at St. Michael's Hospital/University of Toronto and consists of coordinating center manager, project manager, regulatory manager, and administrative support. The DCC will be responsible for facilitating all aspects of the study planning, training, implementation and analysis phases.

PROTECTION FOR HUMAN SUBJECTS

One hundred and twenty-six patients with MN will participate in the study. Their participation will consist of up to 13 visits over a 2-year period. The only clinical specimens obtained will be blood and urine. The age range for participation is 18-80

years. RTX will be provided to the study participants by Genentech and the cyclosporine will be provided to that arm free of charge as per protocol.

Protection of human subjects:

1. Informed consent process. Patients who are candidates for the study will review and sign an informed consent form, to allow review of their history and physical exam including blood pressure estimates, as well as serum/urinary chemistries and medication history.

2. Measures to reduce risks and discomforts associated with study drug.

RTX infusions will be administered at centers with the following patient safety equipment and supplies: oxygen, oral and endotracheal airways and intubation equipment, epinephrine 1:1000 solution for intravenous or endotracheal injection, antihistamines, corticosteroids, intravenous infusion solutions, tubing, catheters, tape, and defibrillator with electrocardiogram monitor. Patients will be pre-medicated with acetaminophen (1g) and diphenhydramine HCl (50 mg) by mouth from 30 to 60 minutes prior to the start of an infusion. Premedication with steroids (100 mg methylprednisone IV) will also be given 30 minutes prior to the first infusion of each series of RTX (Day 1 and Day 168). Patients administered an antihistamine for the treatment or prevention of infusion-related reactions will be given appropriate warnings about drowsiness and impairment of driving ability prior to discharge. Patients will be instructed how and when to take their cyclosporine, taught about the most common drug interactions and to contact their coordinator/study physician about potential interaction with over-the-counter medications and / or intercurrent illnesses requiring additional treatment prescribed by others. They will also be informed about the need to maintain the proper time interval prior to assessment of their cyclosporine drug levels.

3. Adverse event reporting. Adverse event reports, including SAEs, will be sent to the respective IRB as per local requirements. As part of the reporting structure, SAEs will be reported to the FDA, Health Canada, Genentech Drug Safety and the Genentech medical science liaison as stipulated below. An adverse event summary form will be completed and will be summarized for each IRB, the FDA and Health Canada annually. Adverse events will be coded and graded using the Common Toxicity Criterion v.3.0 (ctep.cancer.gov/reporting/ctc.html).

Regulatory issues:

1. **Inclusion of women:** Women make up approximately 30-50% of the population of patients with IMN and are expected to be enrolled in numbers in proportion to this normal distribution.
2. **Inclusion of minorities:** Individuals of all races and ethnicities are at risk for MN. Based on the ethnic composition of the patients followed at, and referred to the Mayo Clinic the population served will include African-Americans, Asian, and Hispanic subjects proportional to that in the general US and Canadian population. This will ensure adequate enrollment within the different racial groups.
3. **Inclusion of children:** It is desirable to include children whenever possible in clinical research studies. The rationale to exclude children in this trial is that: a) the disease is rare in children; b) there are no published natural history studies in children but the

general impression is that the disease is benign in this patient population; c) no studies on specific immunosuppressive treatment in this age group have been published, d) many cases of MN in children are secondary to SLE and positive laboratory markers are often delayed beyond the point of clinical presentation. We do not want this possibility to contaminate and confound the study population. In the absence of safety and efficacy data we would not like to expose children to an unproven therapy.

Data Safety Monitoring Board (DSMB): The DSMB will include 1 chair person, clinical experts in nephrology, epidemiology and trial design, and a biostatistician. We will develop a charter for the DSMB and the board will be responsible for evaluating the study design and progress of the study. The board will be provided data on a regular basis to monitor patient safety. Meetings will be conducted on a semi-annual schedule by conference call.

Benefits:

Patients might expect to gain the following benefit from study participation: an opportunity to be exposed to experimental medicine that may be effective for their kidney disease. Patients will be made aware of the possibility of adverse events known to be associated with RTX (and CsA) and the possibility of previously unrecognized adverse events. All patients will benefit from close follow-up and monitoring of their disease process in terms of both efficacy and safety in both arms of the study.

Data sharing plan:

Results from the study will be published in both abstract and in manuscript form as soon as feasible. Three years after completion of the study, data and remaining samples will be made available to the research community for appropriate and well-designed post hoc studies. Proposals for research projects will be reviewed by the Operations committee and presented to all the study investigators for approval.

ESTIMATED DURATION OF THE STUDY

The estimated duration of the study, given a 24-month enrollment period, a 2-year follow-up for each patient, and 1 year for data analysis is approximately 60 months.

ANTICIPATED RESULTS

We believe RTX will be as effective as Cyclosporine in inducing complete and partial remissions in this patient population with membranous nephropathy.

PUBLICATION POLICY

A policy similar to the NIH policy on publication of study results will apply to this study. Details regarding policy statements may be found on the website at <http://www1.od.nih.gov/oma/manualchapters>. Any abstract or manuscript must be submitted to Genentech four weeks prior to submission as per contract.

APPENDIX A
INSTRUCTIONS FOR COLLECTING AND STORING
SPECIMENS FOR RITUXAN (Rituxan®) HACA ASSAYS

Rituximab HACA Assays

Rituximab serial determinations of the presence of antibodies to Human Anti-Chimeric Antibody (HACA) in Investigator Sponsored Trial (IST) study subjects will be performed by Covance Bio analytical Services, Chantilly, VA.

Serial HACA Sera Collection Schedule:

Sera should be collected for HACA assessments prior to the rituximab infusion on Days 1 and 6 months and 9 months (non-infusion day).

RITUXIMAB HACA SERA COLLECTION PROCEDURES

Sample Collection Requirements: *(Provided by Covance Bio analytical Services)*

1. Non-sterile 3.5ml red/gray top round bottomed, polypropylene sample tubes (12mm x 55mm) with push plug polystyrene caps.
2. 1" x 2" sample Label to fit the above referenced tubes.

Serum Collection and Shipping Instructions

1. Draw 3-5 mL of blood (yielding ~1.5 mL of serum) into a red/gray top Vacutainer tubes (for each sample designated in the study flow chart appendix and the study procedure text) using standard venipuncture techniques. If the Vacutainer tube has clot activator, invert tube 5 times *gently* to mix.
2. Label the Vacutainer tubes with the patient's identification (patient number and patient initials), date and time of blood draw (dd-MMM-yy format for the date i.e. 01-JAN-04 and 24:00 hour clock format for the time).
3. Allow the blood clot upright at room temperature for **30** minutes. (If using plain red top Vacutainer with no clot activator, allow blood to clot upright at room temperature for **60** minutes)
4. Centrifuge the sample to isolate the serum (supernatant) from the red blood cells at 1000 x g (approx. 2000 rpm) for at least 10 minutes.

5. Completely fill out the Covance Laboratories, Inc. provided sample label (YELLOW for HACA Rituximab Levels) with the corresponding patient identification, date and time of blood draw (dd-MMM-yy format for the date i.e. 01-JAN-04 and 24:00 hour clock format for the time) and affix it to one of the room temperature polypropylene sample tubes provided. Use scotch tape to secure the label.
6. Draw off the supernatant and pipette a 1.0-1.5 ml aliquot of serum into the already labeled polypropylene sample tubes.
7. Freeze and store the samples upright at or below -20°C as soon as possible.
8. Batch and ship the specimens to Covance at the address provided below.
Avoid deliveries on weekends and on holidays.
9. Please be sure to use 25-30 pounds of Dry Ice when shipping the samples to ensure receipt in a frozen state. Make sure the specimens are protected by the provided packaging material to prevent breakage by the dry ice. (NOTE: Ice Packs will not sufficiently freeze the samples during shipment.)
10. A completed Rituxan Specimen Shipping Invoice MUST accompany all the samples being shipped. Failure to do so can result in delays or the inability to process the samples.
11. Ship specimens on a quarterly basis using over night shipping via Federal Express, using the supplied pre-paid FEDEX air bills to:

Kurt J. Reber
Specimen Coordinator
Immunochemistry Services
Covance Laboratoires, Inc.
3635 Concorde Pkwy, Suite 100
Chantilly, Virginia 20151-1130
Specimen.Coordinator@Covance.com
Work: (703)245-2200 ext 2803
Work Cellular: (703)409-9796

Bulk Sample and Shipping supplies will be provided under separate cover and shipment.

APPENDIX B

INSTRUCTIONS FOR COLLECTING AND STORING Anti-PLA2R SERUM SAMPLES

1. Draw 5 mL of blood into plain red top Vacutainer tube using standard venipuncture techniques. Invert tube 5-10 times gently to mix.
2. Allow blood to clot upright at room temperature for 30 minutes.
3. Centrifuge the sample at approximately 3000 rpm (2000g) for 15 minutes.
4. Write the date of the blood draw (dd-MMM-yyyy [i.e., 01-JAN-2003] format for the date) on the provided label and attach the label (piggy-backed) to the provided polypropylene tubes.
5. Draw off the supernatant and pipette 500 uL serum into each of the 4 polypropylene sample tubes.
6. Freeze the sample upright at or below -20C as soon as possible.
7. For shipping, put samples in biohazard bag.
8. Provided patient sample card should be placed in separate bag and shipped in container as well.
9. Place samples into shipping container filled with 10 pounds of dry ice.
10. Batch ship samples to the following address:

BAP Freezer Facilities
Mayo Clinic, Stabile SL-39
150 Third Street, SW
Rochester, MN 55905

APPENDIX C
INSTRUCTIONS FOR DNA SAMPLE

1. Draw blood into two 10mL EDTA tubes.
2. No processing is necessary.
3. The tubes should be sent to Mayo Clinic Rochester at room temperature (do not freeze).
4. Place tubes directly in the provided Styrofoam container. Insert Styrofoam container into cardboard box and tape securely so that the box does not open during transport.
5. Sample needs to be sent to mail on day of collection. If blood is drawn on a Friday, check “For Saturday Delivery” box.
6. Place box in Federal Express envelope. Attach pre-printed airbill and send by Federal Express. If you do not have local pickup services, please call 1-800-238-5355 to arrange for pickup by Federal Express.
7. FedEx Airbill: Please complete all information in Box 1 (institution, address, etc.).

Send samples to: BAP Freezer Facility
 Stabile SL-39
 150 Third Street SW
 Rochester, MN 55902

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STUDY TITLE:

MEmbranous Nephropathy Trial Of Rituximab (MENTOR)

A Multi-Center Randomized Controlled Trial of Rituximab versus Cyclosporine in the Treatment of Idiopathic Membranous Nephropathy (IMN)

Study Drugs

Rituximab and Cyclosporine

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Sponsor Investigator

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1.1 Overview

This prospective, randomized, controlled phase III nephrology study will determine whether rituximab is non-inferior to cyclosporine in inducing long-term remission of proteinuria in patients with idiopathic membranous nephropathy (IMN.) Subjects are randomized to open-label courses of treatment: IV rituximab (RTX), 1000 mg (2 infusions, 14 days apart), or oral cyclosporine (CSA) 3.5 to 5 mg/kg/day. Subjects are evaluated at 6 months; those that meet treatment response criteria will continue study medication and receive either 2 further infusions of RTX or a further 6 months of daily CSA treatment. Throughout the study period, laboratory and safety parameters will be collected and documented.

Study Hypothesis: B cell targeting with Rituximab is non-inferior to Cyclosporine in inducing long-term remission of proteinuria.

Comparison(s): IV rituximab (RTX), 1000 mg (2 infusions, 14 days apart; repeated at 6 months if a substantial reduction in proteinuria (equal to or >25%) is seen at 6 months) or oral cyclosporine (CSA) 3.5 to 5 mg/kg/day for 6 months (continued for another 6 months if a substantial reduction in proteinuria (equal to or >25%) is seen at 6 months).

SPECIFIC AIMS:

The specific aims of this Phase III trial are to test the hypothesis;

1. That B cell targeting with Rituximab **is non-inferior to** Cyclosporine in inducing **long-term remission** (complete or partial) of proteinuria in patients with IMN.
2. That B cell targeting with Rituximab **reduces the number of relapses (efficacy in sustaining remission) and increases the time to relapse when compared with treatment with** Cyclosporine.
3. That B cell targeting with Rituximab **is as effective as** Cyclosporine in inducing **complete or partial remission** of proteinuria in patients with IMN during the active treatment phase.
4. That B cell targeting with Rituximab has a better side effect profile and improved quality of life when compared with treatment with Cyclosporine in patients with IMN.

BACKGROUND AND SIGNIFICANCE

IMN is a common immune-mediated glomerular disease and remains the leading cause of Nephrotic Syndrome (NS) in Caucasian adults.¹ Although in most patients the disease progresses relatively slowly, approximately 40% of patients eventually develop End Stage Renal Disease (ESRD).² Because of its frequency, it remains the 2nd or 3rd cause of a primary glomerulopathy leading to ESRD.³ Patients with IMN who remain nephrotic are at an increased risk for thromboembolic⁴ and cardiovascular (CV) events.^{5, 6} Available immunosuppressive therapies including corticosteroids, alkylating agents, and calcineurin inhibitors (Cyclosporine), appear to be at least partially successful in reducing proteinuria in IMN, but their use is controversial and is associated with a number of adverse effects and a high relapse rate, thus tempering their use in IMN. (reviewed in ⁷) The significant risks associated with current immunosuppressive therapies are important in a disease where up to 30% of IMN patients are said to achieve spontaneous remission of proteinuria, enjoy long-term renal survival and, should not be treated with immunosuppressive therapy. This information is, however, misleading, since the percentage of patients that undergo spontaneous remission is much lower when patients are selected with higher grades of proteinuria at presentation e.g. proteinuria >8g/24h.⁸ Similarly, although Schieppati et al. reported a 72% renal survival at eight years for 100 untreated patients with IMN, in this study, 37% of patients were non-nephrotic; a cohort well known to rarely progress to renal failure and hence a built-in bias towards a better outcome. Indeed the overall proteinuria was relatively low (56% of patients had proteinuria <5g/24h), and the median follow-up was only 39 months. The follow-up time is an important issue given that the natural history of the disease process tends to be slow even in the worst cases. The final limitation was that all deaths were excluded from the analysis.⁹ However despite the favorable elements of this cohort, 25% reached ESRD by the end of 8 years. One approach to this has been to provide immunosuppression treatment only to those subjects identified as being at higher risk of progression: males, severe proteinuria, impaired renal function, a high prevalence of secondary lesions of focal and segmental sclerosis, and prominent tubulointerstitial damage on renal biopsy. Our experience, and others, however, suggests this approach is seriously flawed.¹⁰ There is a substantial fraction of patients, who progress to chronic kidney disease (CKD), that are not identified by these predictors and are thus unjustifiably excluded from therapy. A second approach has been to treat only those patients who are exhibiting deteriorating kidney function.^{11, 12} These trials have been deemed successful because proteinuria and

azotemia were diminished.¹¹⁻¹³ Careful review of the data however, shows that reversal of azotemia is almost always incomplete and often transient suggesting that the decline in GFR is merely attenuated and not arrested. In addition, we believe that a prolonged run-in (i.e., treatment-free) period of 12 or more months would be required to prospectively confirm this type of truly progressive IMN, but that this in turn would result in a significant and probable irreversible loss of nephrons and ultrafiltration capacity.¹⁴ This can be illustrated by the study of Polanco et al.⁸ These authors suggested that patients that were treated conservatively and who did not go into spontaneous remission had final creatinine = 2.4 ± 2.2 mg/dL, and eGFR 53 ± 35 ml/min.⁸ However, we believe this information is inaccurate. Taking into consideration that the mean age at presentation was 51 years and the mean follow up was 69 months (making these patients ~56 years at follow-up) the eGFR should be ~30 ml/min if male and ~22 ml/min if female, indicating a significant loss of function with just waiting. The paper further underestimates chronic kidney disease because their end point was restricted to the absence of chronic dialysis or need for renal transplantation. If we add loss of kidney function (as measured by change in GFR) their disease course would indicate a significantly higher incidence of CKD over time supporting our contention that prolonging the wait time for spontaneous remission results in patients paying a heavy price.

Why do so many patients still progress to ESRD? There is growing evidence implicating proteinuria as a major player in the development of progressive tubular injury, interstitial fibrosis, and GFR loss. The higher the sustained levels of proteinuria, the faster the decline in renal function,¹⁵ a relationship that is true not only for patients with IMN but for other proteinuric renal diseases including FSGS and diabetic nephropathy.¹⁶⁻¹⁹ The reverse appears also to be true. There is strong evidence to support that remission of proteinuria (either complete or partial remission) is a valid surrogate end point for both improved renal survival and slower rate of progression of renal disease, and that this reduction is an important therapeutic target for the clinician. This view is in line with the position statement of the NKF and NIDDKD indicating that proteinuria can be used as a surrogate marker and principal endpoint in clinical trials in proteinuric renal disease where GFR-based declines in function take too long to be of practical use.²⁰ Proteinuria is also a marker for CV risk.^{5, 21-24} Recent post-hoc analyses of the diabetic trials RENAAL, IDNT and others, have shown that proteinuria determines renal outcome^{25, 26} and CV outcome.²⁷⁻²⁹ The link between chronic renal failure (CRF) and CV disease is so strong that over 40% of patients starting dialysis already have evidence of CV disease.^{30, 31} This information is important in the present study since all patients will have high grade proteinuria >5g/24h, a scenario almost universally associated with marked abnormalities in their lipid profile (high total cholesterol, normal or low levels of HDL and increased LDL).^{5, 6} Apart from hyperlipemia, patients with IMN and nephrotic syndrome (NS) are also at risk for thromboembolic events, with prevalence rate as high as 50% and a mortality rate within this group as high as 42%.^{4, 32} These data serve to emphasize the importance of other common life-defining sequelae of membranous nephropathy in these patients, in addition to their risk of renal failure.

There is no standard specific treatment for IMN. Initial therapy should be supportive and involves restricting dietary protein intake, controlling blood pressure, hyperlipidemia, and edema. The ideal target for blood pressure is not firmly established but current recommendations suggest that 130/80 mm Hg should be the treatment goal. There are only limited data to support a lower target of 125/75 mm Hg if there is proteinuria >1 g/d. Reducing protein intake to about 0.6-0.8 g/kg ideal body weight per day also tends to

decrease proteinuria.³³ ACEi and/or ARBs are effective anti-hypertensive agents that may also reduce proteinuria in both diabetic and non-diabetic chronic nephropathy patients and slow progression of renal disease independent of blood pressure control.³⁴ This is the rationale for making these drugs the preferred agents to treat hypertension in proteinuric renal diseases. However, evidence that such therapy is beneficial in IMN is weak, largely inferential, and the following issues need to be considered: 1) The degree of renal protection is related to the degree of proteinuria reduction and if proteinuria is not lowered, the benefit is substantially attenuated.^{35, 36} In the RENAAL trial the renal protective effect of angiotensin II blockade in patients with diabetic nephropathy was nearly fully explained by its anti-proteinuric effect.²⁶ 2) In patients with IMN, the anti-proteinuric effect is modest (<30% decrease) and is more significant in patients with lower levels of proteinuria.³⁷⁻³⁹ 3) Thus, in contrast to diabetic renal diseases, ACEi may not offer the same degree of renal protection to patients with IMN.⁴⁰ In fact, studies by du Buf-Vereijken et al.⁴¹ and in a review by Troyanov et al.,⁴² the use of ACEi or ARBs by multivariate analysis did not show an independent value in determining the prognosis of patients with IMN. More recently, Praga et al. showed additional evidence that in patients with NS, (the majority with IMN), ACEi were ineffective in reducing proteinuria, and that this failure to respond in IMN patients was associated with poor renal function outcomes.^{43, 44} 4) In patients in which a significant anti-proteinuric response is observed, the effect is usually seen within 2 months of initiation of angiotensin II blockade therapy.³⁷ Although a relative reduction of proteinuria is always a positive result, the aim of anti-proteinuric therapy is to reduce it as close as possible to normal levels (complete remission (CR)). Reaching this goal in patients with proteinuria > 5g/24 using conservative treatment with ACEi or ARBs seems unrealistic, even when these drugs are used at the highest dose. Taken all together, in the past decade relatively little progress has been made in the treatment of patients with IMN, and up to 40% of the patients will still progress to end stage renal failure. Agents that result in a higher response and lower relapse rates, as well as fewer adverse effects, are truly needed.

Current conservative therapy

Initial therapy should be supportive and involves restricting dietary protein intake, controlling blood pressure, hyperlipidemia and edema (see above). In patients that do have an anti-proteinuric response to angiotensin II blockade therapy, the effect is usually seen within 2 months of initiation of the medication and tends to be modest.³⁷ Although a relative reduction of proteinuria is an important effect, the aim of anti-proteinuric therapy is to reduce as close as possible to normal levels (complete remission). Reaching this goal in patients with sustained proteinuria > 5g/24 using conservative treatment with ACEi and/or ARBs is unlikely, even when these drugs are used at the highest dose. The role of immunosuppressive agents in the management of patients with IMN thus remains a critical issue. Our immunosuppressive treatment armamentarium in this regard has evolved but is still limited. Agents that result in a higher response rate with a lower relapse rate and fewer adverse effects are truly needed and thus the focus for our proposed randomized controlled trial.

Current Options for Immunosuppression in IMN

Cyclosporine (CSA)

Cyclosporine is a calcineurin inhibitor (CNI) that exerts its immunosuppressive effect by blocking the production of IL-2, IL-3 and IFN- γ , resulting in a reduction of T-lymphocyte helpers/inducers and cytotoxic cell function.⁴⁵ This immunosuppressive

effect is probably not the only anti-proteinuric effect of CSA since it is known to have both hemodynamic effects and to act on the podocyte structure. The latter effect has been demonstrated *in vitro* to alter glomerular permeability⁴⁶ by a direct effect on the actin cytoskeleton (and therefore the shape) of podocytes.⁴⁷ This is postulated to protect podocytes from immune injury and may be part of the explanation behind the observation of its significant benefit in certain immunologic glomerular disorders such as in IMN and minimal change despite significantly less exposure compared to solid organ transplant recipients. Cyclosporine has been extensively used in the treatment of proteinuric glomerular diseases and has been proven to be effective in inducing remission of idiopathic nephrotic syndrome in children and adults.⁴⁸ It has been used successfully in patients with IMN and nephrotic syndrome resistant to corticosteroids and/or cytotoxic drugs and has been tried in some of the glomerular disorders even before exposure to cytotoxic therapy because of its significantly different side effect profile.^{39, 49-55}

In the most relevant randomized controlled trial, 51 patients with steroid-resistant nephrotic syndrome and preserved renal function (creatinine clearance [CrCl] > 42 ml/min/1.73m²) were randomized to CSA or placebo treatment, with both groups also receiving low-dose prednisone (0.15 mg/kg/day).⁵¹ The duration of treatment was 26 weeks followed by a tapering off period over 4 weeks. At 26 weeks a significantly higher remission rate was observed in patients treated with CSA -compared to placebo- (75 % versus 22%, P. <0.001). Among the CSA-treated patients with remission, 90% showed partial remission and 10% complete remission. Twelve months after discontinuing the drug a significant percentage of the CSA treated patients remained in remission (39%) compared to the placebo treated patients (13%; p = 0.007).⁵¹

The effectiveness of a more prolonged course of CSA in inducing remission of proteinuria has been recently documented in a study where CSA was given for 12 months and an 80% remission in proteinuria (complete plus partial) was observed.⁵⁵ It was indicated in this paper that CSA combined with prednisone is more effective than CSA monotherapy in maintaining remission in IMN.⁵⁵ However careful review of their data suggests that the group that remained in remission following tapering of the CSA had substantially higher trough levels, both in the monotherapy and in the combined CSA plus prednisone group, compared to the relapse group suggesting that this may have been the reason for the more sustained remission. This would support our trial proposal i.e. CSA alone (no prednisone is required). In earlier studies⁵¹ low dose prednisone was added to CSA because historical data in patients with MCD/FSGS had suggested higher remission rates with combination therapy and that perhaps the combination would reduce the nephrotoxicity of CSA. A recent review of these early studies and the more current ones suggest that this combination is not necessary.⁴⁸ Further support that CNI monotherapy is effective is illustrated in the recent RCT in MN using Tacrolimus. This study showed virtually identical remission rates in proteinuria as in the original CSA plus prednisone trial (see above) using Tacrolimus monotherapy. Their entry criteria required the presence of nephrotic syndrome resistant to ACE inhibitor therapy, steroids or the combination of steroids with cytotoxic drugs. Their relapse rate following discontinuation of the drug was similar to that of the RCT with CSA.⁵⁶ CSA has also been shown to be of benefit in inducing remission of nephrotic syndrome and preserving renal function in patients with IMN associated with progressive renal failure. In a prospective randomized controlled trial, 64 patients were placed on a restricted protein diet and followed closely for 12 months. Patients that demonstrated significant progression of their disease during this observation period (n = 17) determined by an absolute loss in CrCl > 8 ml/min/year

and persistent nephrotic range proteinuria were randomly assigned to either CSA 3.5 mg/kg/day or placebo for 12 months.⁵⁷ A significant reduction of proteinuria was observed only in CSA-treated patients and the remission was sustained in 75% of these patients followed on average for 21 months.⁵⁷

Thus, CSA represents an effective therapy for inducing remission of nephrotic syndrome in patients with IMN. It leads to remission of proteinuria in the majority of nephrotic patients with IMN but with less potential serious toxicity compared to the use of cytotoxic/alkylating agents. There is also more experience with CSA (versus other CNI) in the US and Canada and for these reasons it has been chosen as the comparator arm for our study.

Patients with IMN that do progress do so over years and follow-up in RCTs with CNI therapy have not been of sufficient duration to illustrate improvements in renal survival. However there is well documented recent data that indicates that both complete and partial remission in proteinuria is associated with improved renal survival. Complete remission was associated with a 100% 10-year survival and partial remission (defined as more than a 50% decrease from baseline and attaining a urine protein level of <3.5 g per day) was associated with a 90% 10 year renal survival compared to 50% kidney survival in those that fail to remit. In addition, in this same study PR as measured by slope of CCR, resulted in a slowing of progression rate of >80 % compared to non-remitters.⁴²

Toxicity of cyclosporine

Cyclosporine is a potent immunosuppressive agent and is associated with significant risk of both short and long-term toxicity. In the majority of cases these adverse effects are associated with both total daily dose and total exposure. Precise incidences of these toxicities are hard to ascertain from the literature given the rarity of studies where these agents are used as monotherapy and their use for a limited duration as proposed in this trial. There is however undoubtedly an acute effect on filtration function and delayed effects on renal vasculature that have been well documented and a recent review summarized both short and long-term toxicities associated with this agent.⁵⁸ Hypertension, overgrowth of body hair and gingiva hyperplasia, mild tremor, infections, elevated bilirubin levels and mild nausea represent the most frequently observed short-term adverse effects related to CSA.⁵⁸ These are seen in up to 20 to 30% of the treated population but are usually tolerated and/or treatable with additional agents (such as additional anti-hypertension drugs) or by dose reduction.⁴⁸

Cyclophosphamide (CYC)

In IMN, experimental data suggests that B cells are involved in the pathogenesis of the disease.⁵⁹ To date, the best proven long-term therapy for patients with IMN consists of the combined use of corticosteroids and CYC, the Ponticelli's protocol. However, the potential side effects with the use of 9g of methylprednisolone mandated by this protocol as well as the potential risk of other serious side effects associated with the use of cytotoxic agents (such as bone marrow toxicity, severe infections, gonadal dysfunction) combined with the long-term risk of malignancy associated with CYC has left many physicians reluctant to use this regimen. It should also be remembered that IMN is a relapsing and remitting disease and even with the use of this therapy, relapse rates remain high. In Ponticelli et al.'s randomized 6 month study in patients with IMN comparing methylprednisolone plus chlorambucil versus methylprednisolone plus CYC the overall relapse rate was 24% (21/87) and similar in both arms. These relapses occurred between

6 and 30 months post treatment, with the majority within 18 months.⁶⁰ An additional ~10% of patients had to stop treatment and were discontinued from the study because of adverse effects. The risk of malignancy with CYC is substantial with the hazard of neoplasia roughly correlated to the cumulative dose and duration of cytotoxic therapy.⁶¹⁻⁶³ Faurschou et al. recently investigated the incidence of malignancies associated with CYC exposure in a cohort of 293 patients with Wegener's granulomatosis.⁶⁴ The risk of malignancy was not increased for patients who never received CYC or for patients treated with cumulative CYC doses ≤ 36 g.⁶⁴ In contrast, high risks of leukemia (SIR 59.0, 95% CI 12-172) and bladder cancer (SIR 9.5, 95% CI 2.6-24) were observed for patients treated with cumulative CYC doses >36 g. These data would suggest that a patient who weighs 80kg and is treated with CYC at a dose of 2.5 mg/kg/day would exceed this threshold after 2 standard courses of the Ponticelli's protocol. Since IMN is a remitting and relapsing disease with the potential for significant cumulative CYC dosing, there is a substantial risk of late-occurring, serious malignancies. The other major concern limiting CYC utility is related to its gonadal toxicity. Data indicates that ovarian failure is seen in female patients of any age receiving a cumulative dose of as little as 28 g of CYC. In addition, these authors found that age at the onset of therapy was an additional independent factor associated with sterility.^{65, 66} Although male infertility is harder to assess, studies have demonstrated that doses above 7.5 g/m² of CYC can result in permanent oligospermia.⁶⁷ For these reasons, the majority of the academic centers in the US have considered CYC treatments too toxic and relegated the use of Ponticelli's protocol to rescue therapy in patients who have failed less toxic immunosuppressive therapies.

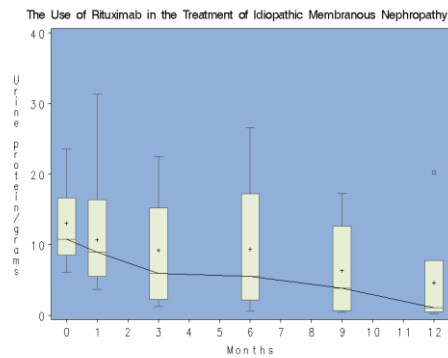
INNOVATION

A new approach to therapy: the case for targeting B cells: In IMN, experimental data suggest that B cells are involved in the pathogenesis of the disease.⁵⁹ To date, the best proven therapy for patients with IMN consists of the combined use of corticosteroids and cyclophosphamide (CYC). The mechanism of action of CYC includes suppression of various stages of the B cell cycle including B cell activation, proliferation, and differentiation and inhibition of immunoglobulin secretion, supporting the hypothesis that B cell abnormalities are involved in the pathogenesis of IMN.^{68, 69} Given the key role of IgG antibodies in IMN⁷⁰, it is reasonable to postulate that suppression of antibody production that targets glomerular antigens by depleting B cells may improve or even resolve the glomerular pathology. This approach of stopping the initiating pathogenic event could potentially result in resolution of the disease process. **This is the theory underlying the application of selective B cell targeting with Rituximab (RTX) in IMN. Our hypothesis is that it will prove at least equal to, or even superior to current therapies, both in the production of short term and long term control of the NS and be safer than any of the other current regimens used to treat IMN.**

PRELIMINARY STUDIES

Based on this rationale, we conducted a pilot trial in 15 newly-biopsied patients (<3 years) with IMN and proteinuria >5 g/24h despite ACEi/ARB use for >3 months and systolic BP <130 mmHg.⁷¹ Mean baseline creatinine was 1.4 mg/dl. Thirteen males and 2 females, median age 47 (range 33-63), were treated with RTX (1g) on days 1 and 15. At six months, patients who remained with proteinuria >3 g/24 received a second identical

course of RTX. Baseline proteinuria of $13.0 \pm 5.7 \text{ g/24h}$ (range 8.4-23.5) decreased to $6.0 \pm 7.0 \text{ g/24h}$ (range 0.2-20) at 12 months (mean \pm SD) (Figure 1). In the fourteen patients who completed 12 months follow-up complete remission (proteinuria $<0.3 \text{ g/24h}$) was achieved in 2 patients and partial remission ($<3 \text{ g/24h}$) in 7 patients. The mean drop in proteinuria from baseline to 12 months was $6.2 \pm 5.1 \text{ g}$ ($p=.002$, paired t-test). In 5 of these 7 patients, proteinuria was $<1.5 \text{ g/24h}$.



At 18 months follow up 3 of these 7 PR patients achieved CR of their proteinuria.

(Figure 1)

Five patients did not respond. There were a limited number of minor side-effects (see below).

Initial CD20+ B cell depletion was seen in all patients. However, at 3 months, CD20+ B cells were starting to recover with five patients demonstrating $>35 \text{ cells}/\mu\text{l}$ (range 35-152).⁷¹ These data contrast with previous work by Ruggenenti et al. using the lymphoma regimen where RTX is given weekly (375 mg/m^2) for 4 weeks.² Using that regimen, B cell depletion was maintained up to 12 months post treatment and their proteinuria reduction was more rapid and complete. Similarly our studies using this standard lymphoma protocol in patients with ANCA vasculitis demonstrated that peripheral blood B-cell depletion was consistently achieved and maintained for at least six months. Pharmacokinetic (PK) analysis had showed that RTX levels in our original 2-dose regimen were 50% lower compared to studies in non-proteinuric conditions. This could potentially result in undertreatment. Based on these results, we conducted an additional study based on the premise that in patients with MN, 4 weekly doses of RTX would result in more effective B cell depletion, a higher remission rate while still maintaining the same safety profile as patients treated with RTX dosed at $1 \text{ g} \times 2$. Twenty patients (including 11 patients that had failed alternative immunosuppressive therapy) with IMN and proteinuria $>5 \text{ g/24h}$ received RTX ($375 \text{ mg/m}^2 \times 4$), with retreatment at 6 months regardless of proteinuria response. A detailed pharmacokinetics study was conducted in concert with immunological analyses of the adaptive immune compartment (T and B cells) to ascertain the impact of RTX on lymphocyte subpopulations. Baseline proteinuria of $11.9 \pm 4.9 \text{ g/24h}$ decreased to $4.2 \pm 3.8 \text{ g/24h}$ and $2.0 \pm 1.7 \text{ g/24h}$ at 12 and 24 months, respectively ($p<0.001$) while creatinine clearance increased from 72.4 ± 33 at baseline to $88.4 \pm 31.5 \text{ ml/min/1.73m}^2$ at 24 months ($p=0.02$) (Figure 2A/B).

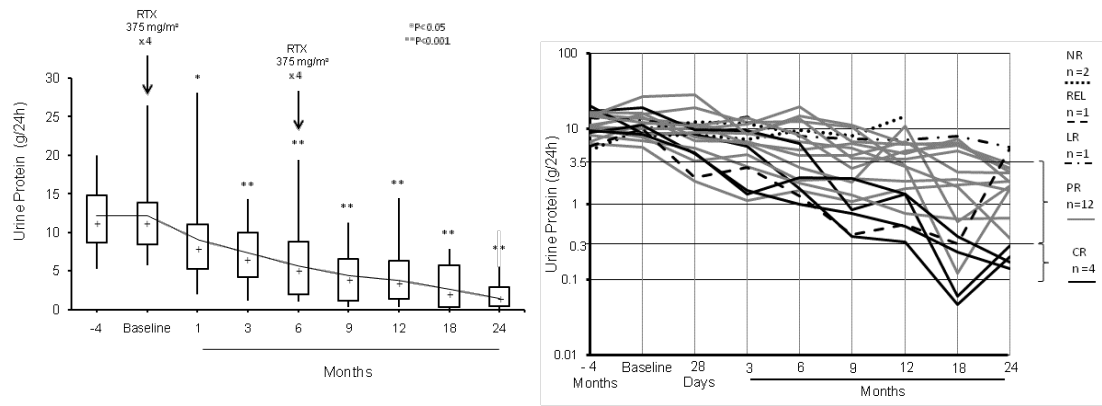


Figure 2. A. Box plots of urine protein by months since start of RTX therapy. The top and bottom of the box are the estimated 75th and 25th percentiles, respectively. The intermediate horizontal line and "+" sign represent the median and mean urine protein, respectively. The vertical lines extend to the largest (smallest) data point that is within 1.5 times the inter quartile range (75th-25th percentile) above the 75th percentile (or below the 25th). The square symbol identifies points outside of this range. The number of patients with follow-up at 0, 1, 3, 6, 9, 12, 18, and 24 months are: 20, 20, 20, 19, 18, and 18, respectively. * $P<0.05$, ** $P<0.001$; B. Longitudinal effect of RTX on proteinuria (log transformed).

Of 18 patients who completed 24-months follow up, 4 are in complete remission, 12 are in partial remission (CR + PR = 80%), 1 had a limited response ($>50\%$ drop in P but $>3.5\text{g}/24\text{h}$) and 1 patient relapsed from a partial remission. When interpreting these results we should take into account that $>50\%$ of these patients had failed previous immunosuppressive therapy. This study also emphasizes that proteinuria is reduced gradually and that it may take several months to reach its nadir. This observation is in agreement with previous reports in patients with IMN treated with prednisone in combination with a cytotoxic agent, but was seen without the short-term toxicity observed with alkylating agents.⁶⁰ Kidney function remained stable or improved in all patients. These results were observed despite the fact that in patients with nephrotic syndrome (NS), serum albumin levels influence tubular creatinine secretion resulting in an endogenous creatinine clearance that overestimates GFR in NS.⁷² In addition, previous investigators have indicated that the onset of IMN is accompanied by a severe depression in hydraulic permeability of the glomerular capillary wall. This is partially offset by enhancement of filtration surface area and by profound depression of glomerular oncotic pressure. This can result in the GFR initially remaining in the normal range or at worst, depressed by $<50\%$ in moderate IMN. In severe IMN these investigators found this mechanism was significantly worsened by a marked reduction in functional glomerular number and a GFR depression of $>50\%$. These abnormalities were presumably reversed with RTX treatment and resolution of the NS. However, an additional explanation could have been that a number of patients had their angiotensin II blockade reduced or discontinued with time, altering the renal hemodynamics and thereby contributing to the significant increase in creatinine clearance. The important summary point in this regard is this type of GFR improvement by reduced RASS blockade would not be accompanied by a decrease in proteinuria. RTX therapy represents a substantial advantage versus the risk for nephrotoxicity and other short-term toxicities associated with the use of calcineurin inhibitors (CNI). In addition RTX treatment assures patient adherence (since it is given by IV infusions). This is in contrast to the necessary monitoring required during the treatment with Cyclosporine. This makes

RTX an easier option from the perspective of physician management and potentially offers a better quality-of-life for the patient. Cost estimates are also worth addressing. Although RTX is more expensive upfront the annual treatment costs compared to Cyclosporine are similar. Perhaps most importantly, the effect of RTX appears to be sustained as only 1 patient in our recent pilot (5%) relapsed within the 2 year follow up period. A similar long term preservation of proteinuria reduction was observed in our first study⁷¹ with only 1 patient relapsing at 36 months. Thus, in the 35 patients included in our 2 studies, only 2 relapses occurred, and in one case proteinuria relapsed into the sub nephrotic range. These results ($2/35 = 6\%$ relapse) are similar if not better than those reported by Ponticelli et al. ($21/87=24\%$) (63), and are substantially better than those reported with CNi treatment. In addition they are significantly better than the most recently suggested IMN treatment option of prednisone combined with MMF. In these studies a >40% relapse rate has been reported shortly after discontinuing therapy.^{51, 56, 74}

Serum RTX levels using the four dose regimen were similar to those obtained with 2 doses of RTX. Four doses of RTX did result in more effective B cell depletion but proteinuria reduction was basically identical to the results obtained using RTX 1000mg on days 1 and 15. Thus, we believe that the two dose regimen with retreatment at 6 months should be used in a randomized-control trial comparing RTX to Cyclosporine (the standard of care for IMN in the US). We believe that RTX will prove equal or superior to Cyclosporine in the treatment of MN and could represent the new standard of care for patients with this disease.

Toxicity of Rituximab

No dose-limiting effects were observed in our two Phase I/II studies evaluating safety and efficacy of RTX in IMN. The most commonly observed side effects were infusion related (flu-like symptoms, chills/rigors, fever, fatigue, headache, hypotension, nausea, leukopenia, angioedema and pruritus) and typically responded to an interruption of the infusion and resumption at a slower rate.⁷¹ Other side effects included: 1) serum sickness-like syndrome (n=1); hair loss and thinning (n=2); one case of community acquired pneumonia three months after the first infusion that resolved with oral antibiotic treatment (this patient was retreated without complications); 4) herpes zoster reactivation (n=1) treated with oral antiviral drugs with full recovery. Similar to the study by Ruggenti et al., no patient had a major drug-related adverse event.²

Anti PLA2R levels and response to treatment

RTX offers an advantage in MN over nonselective immunosuppressive agents such as CYC since it primarily targets B cells. However, changes in CD20+ B cell number in general have not consistently paralleled the response in terms of either reduction in proteinuria or improvement in the clinical phenotype of the nephrotic syndrome.^{71, 75} This variation strongly suggests that the monitoring of the CD 20 count will not prove to be the ideal instrument for determining treatment dose and/or duration. It would be substantially better if we could monitor the specific effect of immunosuppressive therapy on the pathogenic antibodies in IMN. A recent discovery indicates that autoantibodies to the M-type phospholipase A2 receptor (PLA2R) may represent such a specific marker of IMN. This autoantibody has been found in greater than 70% of IMN patients.⁷⁶ When the analysis is limited to new-onset, nephrotic patients with IMN, this sensitivity increases even further. These investigators have shown that PLA2R is a transmembrane constituent of the human podocyte (the most likely target cell in MN) and anti-PLA2R antibodies are predominantly of the IgG4 subclass and co-localize with IgG4 in the sub-

epithelial deposits which are pathognomonic of the disease. In their observations, the presence of anti-PLA2R correlates well with disease activity, disappearing with a spontaneous or treatment-induced remission, and reappearing with a relapse of IMN.

We also have studied whether the immunologic changes in serum anti-PLA2R levels parallel the clinical reduction in proteinuria in response to RTX in patients with MN. Serial serum samples from our 2 cohorts of MN patients treated with RTX (cohort 1 treated with 1 g of RTX at d.1 and d.15) and cohort 2 (treated with 4 weekly doses of 375 mg RTX), were assayed for anti-PLA2R antibodies.⁷⁷ Ten out of 15 (67%) patients in cohort 1 and 16/20 (80%) in cohort 2 had anti-PLA2R reactivity in their baseline serum samples. When the 2 cohorts were combined, the baseline distribution of cases with anti-PLA2R positivity was not significantly different when compared to the final clinical groupings of complete remission (CR), partial (PR) limited or no remission (NR), ($p=0.61$). However, an anti-PLA2R level below 500 arbitrary units at 9-12 months after initiation of treatment was significantly different among these groups: in the complete remission (CR) group 100% had a reduction to this level, in the PR group 88% had reduction to this level but in the limited response (LR) group only 50% had a reduction and in those with no response only 17% had a reduction to this level. ($p=0.006$). Median proteinuria at 9, 12, and 15-18 months was consistently lower in those subjects with undetectable or very low anti-PLA2R levels versus those with higher levels. In the group in which anti-PLA₂R disappeared, 86% were in complete or partial remission at the final time point: 4 complete remissions (CR), 14 partial remissions (PR), 1 limited remission (LR), and one non-responder (NR). Perhaps most relevant was, in those patients with decreases in anti-PLA2R, the decline almost always preceded the reduction in proteinuria by months. In addition, 1 patient who had attained remission and who had become anti-PLA2R negative following RTX became antibody positive at the time of his relapse. Thus, in at least these two cohorts of MN patients with anti-PLA2R antibodies at the start of treatment, post-RTX anti-PLA2R levels correlated with and seemed to precede clinical response to RTX (Figure 3). These results suggest that monitoring anti-PLA2R autoantibody levels may provide a window onto the immunologic effects of treatment on the course of IMN, and allow a more specific and an earlier means of determining treatment effectiveness compared to the clinical response of decreasing proteinuria. This observation is understandable given the nature of the deposits and proposed pathophysiology of IMN. Even if the B cells producing anti-PLA2R were completely eliminated and all circulating anti-PLA2R removed, the immune deposits would persist in the subepithelial space until cleared. Supportive evidence of this was seen in repeat kidney biopsies performed in RTX-treated IMN patients after they had entered complete clinical remission.⁷⁸ There are other important implications to the antibody story to consider if they truly parallel immunologic disease activity. Secondary chronic changes in IMN such as focal sclerosis or interstitial damage from prolonged disease activity may lead to indefinite persistence of low-level proteinuria, despite full clearance of immune deposits. This is the case seen in kidneys made proteinuric in the Heymann nephritis model and subsequently transplanted into naïve hosts.⁷⁹ This may explain our observation that not only does the decline in proteinuria lag behind that of anti-PLA2R, but also that the median proteinuria never reaches zero even by 24 months. It may also explain why most clinical responses are partial remissions (rather than complete) in the group that cleared anti-PLA2R. It will be important to see whether these patients will continue to have a further slow decline in proteinuria with further follow-up, similar to patients with IMN who undergo spontaneous remission.⁸ It may also help to explain why even patients with only partial remissions are still associated with good long term outcomes, i.e. in

these cases the residual proteinuria may be explained by the residual kidney scars rather than the persistence of the immunologic disease.⁴²

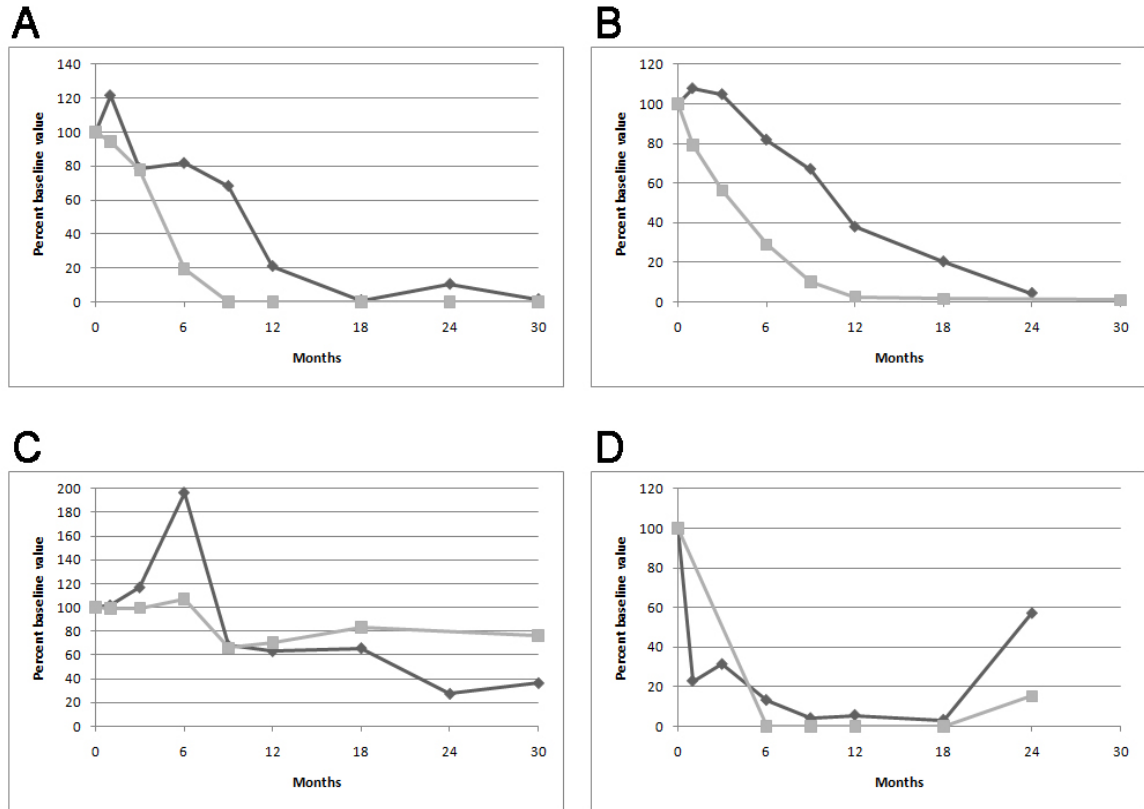


Figure 3. Representative plots of anti-PLA2R (gray squares) and proteinuria (black diamonds) versus time following initial RTX treatment. Values are plotted as percent of baseline value. Panel A and B depicts the typical reduction and disappearance of anti-PLA2R followed by resolution of proteinuria exhibited by the majority of patients. Panel C is representative of patients in whom anti-PLA2R did not substantially decline following treatment and the associated with persistence of proteinuria. Panel D depicts the single patient whose anti-PLA2R level returned with relapse of his disease after having initially disappeared.

This incomplete mirroring of immunological improvement by clinical status is an important issue. As mentioned, proteinuria could remain at a sub-nephrotic level in spite of the absence of immunological disease activity. In this situation, further immunosuppression would have no benefit but would have continued toxicity risk. In contrast, at a similar level of proteinuria, immunological activity, as detected by the continuing presence of circulating anti-PLA2R could be ongoing. In this setting, an increase or change in immunosuppressive regimen may be the best strategy. Ultimately, if confirmed anti-PLA2R levels may become an important surrogate assessment of disease activity and of treatment outcome. We propose to expand these observations by measuring anti-PLA2R levels in all patients enrolled into the current trial. This in itself will be a major innovation for the results of these studies have the potential to create a new paradigm for monitoring and treatment in patients with IMN. If confirmed, the

autoantibody level can be added to predictive algorithms and have the potential to influence future treatment protocols and lead to improved care of patients with IMN.

OVERALL RESEARCH DESIGN AND METHODS:

The goal of this proposal is to conduct a prospective randomized controlled phase III study comparing Rituximab (RTX) to Cyclosporine (CSA) in the treatment of patients with IMN. Once a patient with IMN and proteinuria $\geq 5\text{g}/24\text{h}$ is identified, meets other entry criteria and consents to the study, he/she will receive a minimum of 3 months of conservative therapy aimed at maximizing Angiotensin II blockade (run-in phase). If at the end of this period the patient still meets entry criteria he/she will be randomized into a 12-month treatment period, and a subsequent follow-up of 12 months. Efficacy of treatment will be assessed by remission status (based on changes in proteinuria) at 24 months from randomization. Patient safety will be assessed via collection of adverse event data and evaluation of pre- and post-treatment laboratory data. At the 6-month post-randomization visit, patients who have been randomized to either CSA or RTX but who do not have a reduction in proteinuria $\geq 25\%$ (confirmed on repeat measurements within 2 weeks) will be considered treatment failures and exit the study. Data from that point onward will be censored. Patients in the RTX group who have a reduction in proteinuria of equal to or $>25\%$ at 6 months will be given another identical course of RTX (1g x 2). This treatment regimen was chosen given our initial data that suggested a percentage of our cohort appeared to respond to an additional course of therapy. Data from our 2 pilot studies showed that RTX is out of the circulation by month 2 in the majority of patients and B cells have recovered by month 3 in patients treated with RTX 1g x 2 (the regimen chosen for the study). Thus, at 6 months, patients in the RTX arm are completely, at least as best as we can measure, free of immunosuppression, and hence the second course should not be associated with additive toxicity. The same definition of response will apply to the CSA arm at 6 months. An equal to or $>25\%$ reduction in proteinuria will dictate continuation of the CSA for an additional 6 months (total of 12 months of full dose CSA). This assessment at six months allows a balance between risk-benefit in both arms. The protocol provides similar exposure to immunosuppression in both arms and allows an early exit for patients related to safety issues by providing a lack of efficacy (futility) end point at 6 months.

Primary endpoint

CR or PR (defined as per table 1) at 24 months after randomization will be the primary endpoint. This will be assessed in an intention to treat (ITT) analysis.

Secondary endpoints

1. Relapse state at month 24 after randomization (Urine Protein (UP) $> 3.5\text{ g}/24\text{h}$ after earlier CR or PR)
2. Anti-PLA2R levels
3. Quality of life as measured by modified KDQOL
4. Adverse events
5. ESRD
6. CR or PR, and CR alone at 6, 12, 18, and 24 months after randomization
7. Time to CR or PR
8. Effect of treatment on renal function, as assessed by slope of creatinine clearance from baseline to 24 months.

| Table 1. Definition of remission status | |
|--|---|
| Remission status | Proteinuria (UP g/24 hours) after treatment |
| Complete remission (CR) | UP \leq 0.3 g/24h and serum albumin \geq 3.5g/dl |
| Partial remission (PR) | Reduction in baseline UP of \geq 50% plus final UP \leq 3.5 g/24h but $>$ 0.3 g/24h |
| Non-response (NR) | Reduction in baseline UP of $<$ 25% (includes increase in UP) |
| Relapse | Development of nephrotic range proteinuria following CR or PR, i.e. $>$ 3.5g/24h |

Patient recruitment

The study population will be comprised of individuals with biopsy-proven idiopathic MN. Total study size will be 126 patients, enrolled among up to 25 participating sites. Potential candidates for the study will be identified by the investigators using existing databases and clinical trial networks. The P.I. or the study coordinator will contact these potential candidates and if the preliminary interview indicates their willingness to participate, a consent form will be reviewed with the patient. If a potential candidate remains interested, the consent form will be signed, and the patient enrolled in the study. The institutional review board at each participating site will approve the recruitment process and consent forms. The Mayo Nephrology Collaborative Group (MNCG) will post information on its web site describing the study and identifying local contacts at the collaborating centers. Each site involved may have their own methods of informing and recruiting potentially eligible patients but in all cases will have IRB approval before embarking on this process. Each site will be expected to randomize approximately 6-10 patients over the recruitment period. If, based on site estimates of the IMN population that would be eligible for this study, we enroll approximately 30% of the annual population in a single year our recruitment will be complete. We have assembled a consortium for this trial with a population that well exceeds the enrollment needs based upon the multi-year enrollment plan for this trial.

Recruitment strategy

Potential candidates for the study will be identified by the P.I. at each site using existing research and clinical databases and clinical trial networks. With local IRB approval, electronic health records and pathology clinical data bases will be used for cohort discovery at each institution. Study investigators will also contact local research volunteer registries and local patient advocacy groups and request they provide information about this trial to their participants/members. Each participating site will be encouraged to accept referrals of study patients identified from national patient volunteer registries such as researchmatch.org and the Office of Rare Disease Research sponsored patient contact registry. Regional advertising will also be carried out via our ongoing participation in the NEPTUNE trial (sponsored by the NIDDK/ODR.) through its collaborative network of 18 academic centers in North America. The P.I. or the study coordinator will contact potential study candidates and if the preliminary interview indicates their willingness to participate, a consent form will be reviewed with the patient. If a potential candidate remains interested, the consent form will be signed, and the patient enrolled in the study. According to our site estimates, only 18% of the available population will need to be eligible and willing to participate in the trial to achieve the target sample size.

Potential Challenges and Solutions

Patient recruitment is often slower than anticipated in clinical studies. The team of clinical centers engaged in this trial, including the primary Mayo Clinic site, has significant experience in recruitment and conduct of clinical trials. Past recruitment success at Mayo into phase I and II trials of RTX in adults with IMN is documented by the enrollment of 15 patients in less than 6 months and 20 patients in 11 months.^{71, 75} At Mayo Clinic alone, an average of 50 cases of MN a year have been diagnosed for the past 5 years based on kidney biopsy registry. The projected available population is > 200% of the recruitment goal.

If enrollment at some of the involved clinical sites is slower than expected, we have the option to recruit additional patients from the other participating sites or add, for example, additional sites from the Neptune Consortium to our trial.

Inclusion Criteria

- Idiopathic MN diagnosed by renal biopsy (original biopsy needs to include light, immunofluorescence and electron microscopy); pathology report must be adjudicated by a study PI (Dr. Fervenza or Dr. Cattran, or documented delegate) prior to randomization.
- Age 18-80 years inclusive
- If female, must be post-menopausal, surgically sterile or practicing a medically approved method of contraception (with exception of no birth-control pill given the potential for increased risk of thromboembolism in the nephrotic setting).
- Patient must be off prednisone or mycophenolate mofetil for >1 month and alkylating agents for >6 months. The rationale is to minimize the potential confounding effect of delayed benefits from previous immunosuppressive agents and to reduce the risk of too much immunosuppression from the combined previous drug exposure plus trial drug exposure, e.g. infections.
- Treatment with an ACEi and/or ARB, for ≥ 3 months prior to randomization and adequate blood pressure control (target BP is <130/80 mm Hg in >75% of the readings, but subjects with BP <140/80 mmHg in >75% of the readings will be eligible).

OR

If patient is intolerant to even a very low dose of either ACEi or ARB therapy, approval for participation in the trial has been obtained from the study PI(s) prior to randomization.

Patients with documented evidence of ≥ 3 months treatment with maximal angiotensin II blockade, on an HMG-CoA reductase inhibitor, and BP control (BP <140/80 mm Hg in >75% of the readings) who remain with proteinuria $\geq 5\text{g}/24\text{h}$ and meet the other eligibility criteria (as confirmed at the Time 0 visit by the central lab results) may enter the treatment phase of the study and be randomized to RTX/CSA without the need of the run-in/conservative phase of the study. However, in addition these patients must have a documented <50% reduction in proteinuria compared to previous 24 hr proteinuria or Uprot/Ucrea ratio estimates during this period of ACEi and/or ARB treatment otherwise they must fulfill the run-in requirement.

- (Please refer to manual of operation for clarification of tests mandated for patients who are randomized without the run-in period)
- Proteinuria $\geq 5\text{g}/24\text{h}$ using the average from two 24-hour urine collections collected within 14 days of each other despite Ang II blockade for ≥ 3 months as described above.

- Estimated GFR ≥ 40 ml/min/1.73m² while taking ACEi/ARB therapy OR quantified endogenous creatinine clearance ≥ 40 ml/min based on a 24 hour urine collection. The GFR will be estimated using the 4 variable MDRD equation as published in the NKF-CKD guidelines.⁸¹ This approach is adopted, rather than the much more expensive and more invasive techniques (e.g. inulin or iothalamate clearance) since the likelihood of detecting significant changes in GFR in this short term study is remote regardless of which method is chosen. At entry into the study and at set time points thereafter patients will also have a 24h urine collection for calculation of CrCl and proteinuria.

Exclusion Criteria

- Patients with presence of active infection or a secondary cause of IMN (e.g. hepatitis B, SLE, medications, malignancies). Testing for HIV, Hepatitis B and C should have occurred < 2 years prior to enrollment into the study. Screening for malignancy should be carried out according to standard guideline recommendations.
- Type 1 or 2 diabetes mellitus: to exclude proteinuria secondary to diabetic nephropathy. Patients who have recent history of steroid induced diabetes but no evidence on renal biopsy performed within 6 months of entry into the study are eligible for enrollment.
- Pregnancy or breast feeding (for safety reasons).
- History of resistance to CSA (or other calcineurin inhibitors, e.g. tacrolimus), RTX or alkylating agents (e.g. Cytoxan). Patients who previously responded to CSA/CNI, RTX or alkylating agents with either a CR or PR but relapsed off CSA/CNI after 3 months, or relapsed off RTX or alkylating agent after 6 months, are eligible.

Randomized Treatment Groups: Once all entry criteria have been satisfied and confirmed, patients will be randomized to treatment with Rituximab or Cyclosporine. Randomization will be performed by study site staff through the electronic case report form (eCRF.) The randomization list will be stratified by site, and generated by the Data Management and Coordinating Center (DMCC) using random permuted blocks of variable size.

Rituximab: Patients randomized to the RTX arm will receive 1000 mg IV on Days 1 and 15. Patients who achieve complete remission at 6 months will not be retreated. A second course of RTX 1000 mg IV will be administered at study month 6 for individuals who have not achieved a complete remission, but have achieved at least a $\geq 25\%$ reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not a CR). Dosing at study month 6 will be independent of CD19/20+ B cell count. The rationale for retreating patients who have had an equal to or $> 25\%$ reduction in proteinuria at 6 months is based on our experience in our pilot studies (first study using 1000 mg on Days 1 and 15 and second study using 4 weekly doses of 375mg/m²) where an increase in the proteinuria remission rate was achieved after a second course of treatment. In our 2 studies repeated courses of RTX were not associated with additive adverse effects in comparison to their first course. If after six months the reduction in proteinuria is less than 25% compared to baseline the RTX treatment will not be repeated, the patient will exit from the study and will be considered a failure of therapy.

Dosage and Administration of Rituximab: The first infusion of both courses (Day 1 and Day 181) of RTX will be administered IV at an initial rate of 50 mg/hr. All patients will be premedicated with acetaminophen (1g) and diphenhydramine HCl (50 mg) by mouth from 30 to 60 minutes prior to the start of an infusion. Premedication with

steroids (100 mg methylprednisolone IV) will also be given 30 minutes prior to the first infusion of each series of RTX (Day 1 and Day 181). If a hypersensitivity or infusion-related reaction does not occur, the infusion rate will be escalated by 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If a hypersensitivity (non-IgE-mediated) or infusion-related reaction develops, the infusion will be temporarily slowed or interrupted. Treatment of infusion-related symptoms with additional diphenhydramine and acetaminophen will be recommended. Additional treatment with bronchodilators or IV saline may be indicated. The infusion may be continued at one-half the previous infusion rate upon improvement of the patient's symptoms. If the infusion was well tolerated, subsequent infusions (Day 15 and Day 195) may be administered at an initial rate of 100 mg/hr and increased by 100 mg/hr increments at 30-minute intervals, to a maximum of 400 mg/hr as tolerated. If the first infusion was not tolerated, the guidelines for the first infusion should be followed for all subsequent infusions. Patients administered an antihistamine for treatment or prevention of infusion related reactions will be given appropriate warnings about drowsiness and impairment of driving ability prior to discharge. Participants will be observed for 1 hour post infusion. Although most study visits have a window of +/- 10 days, the 2nd infusion in each series (i.e., Day 15 and Day 195) will have a more restricted window of +/- 3 days. Specifically, the 2nd infusion in each series should occur 14 days +/- 3 days from the time of the 1st infusion in the series. The window is to account for weekends, holidays, and scheduling conflicts.

Monitoring of Rituximab Effects

The numbers of CD19/20+ B cells in the peripheral blood will be quantified by flow cytometry using peripheral blood leukocytes. Flow cytometry will be performed pre-RTX treatment and at regular intervals following administration of RTX (see Table 2b: Test Schedule and Monitoring for Rituximab Treatment Arm). These assays will allow us to follow the level of B-cell depletion, kinetics of B-cell reconstitution and the composition of cells that re-populate the B-cell pool after treatment with RTX as well as helping to ensure dosing adequacy in these patients.

Cyclosporine: Neoral brand (Novartis) is the preferred Cyclosporine product for this trial. Participating sites must contact the DMCC *prior* to dispensing any other brand to study participants as not all brands of Cyclosporine are bio-equivalent. Patients randomized to the Cyclosporine arm will be started at a dose of CSA = 3.5 mg/kg/day p.o. divided into 2 equal doses given at 12 hour intervals. Target trough CSA blood levels, as determined in whole blood by HPLC, are 125 to 175 ng/ml. Patients will have their doses adjusted according to their blood levels of CSA as monitored every 2 weeks +/- 3 days until the target trough level is reached. After reaching target, CSA trough levels will continue to be checked as per the visit schedule (Table 2a). If a complete remission is achieved by six months, CSA will be tapered by approximately 1/3 of the maintenance dose monthly and hence discontinued after two months. If there has been at least an equal to or >25% reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not complete remission) the CSA will be continued for an additional six months. A persistent and otherwise unexplained increase in serum creatinine >30% will prompt an approximate 25% dose reduction of CSA, aiming for a corresponding 25% reduction in CSA trough level (for example, if trough CSA was 175 ng/ml the goal would be to reduce it to 130 ng/ml by reducing the dose by approximately 25% - dose reduction is approximate because CSA is only available in

specific dose strengths (25mg, 100mg) and therefore some rounding may be required). If with this dose reduction the creatinine does not return to within 30% of baseline levels within 3 weeks, then a second dose reduction of approximately 25% with a similar reduction in CSA trough level (e.g. if CSA trough level was 100 ng/ml, reduce to 75 ng/ml) will be used. If the creatinine does not fall to within 30% of baseline values with this second dose reduction, the drug will be discontinued. If after six months the reduction in proteinuria is less than 25% compared to baseline the drug will be discontinued, the patient will exit from the study and be considered a failure of therapy. At the end of 12 months, Cyclosporine will be tapered by 1/3 of the maintenance dose monthly and hence discontinued after 2 months. Serum potassium and serum creatinine levels will be checked at the initiation of Cyclosporine in conjunction with blood draw for CSA level. If Cyclosporine dose is changed during treatment, potassium, creatinine and CSA levels will be rechecked every two weeks +/- 3 days post increase/decrease in CSA dosage until levels are stable and at target.

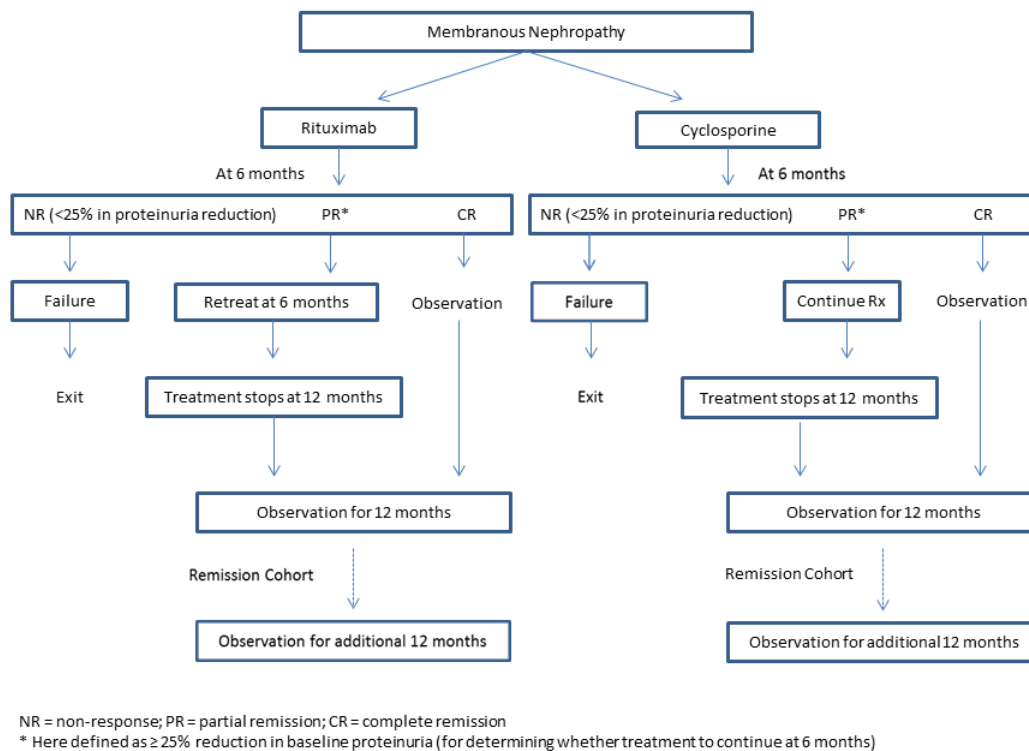


Figure 4. Schematic representation of the study design.

Blinding

This will be an open-label study with neither patients nor physicians blinded. Blinding, for this study, was considered difficult and unnecessary and would significantly add to the cost of the study. The need to provide RTX intravenously would require that the CSA arm patients would have to receive an IV infusion of saline which seems unjustifiable from the scientific and budgetary points of view. Furthermore, with the exception of quality of life measures, all endpoints are based on objective (laboratory) criteria. Having the physicians in the study blinded to CSA level also introduces a potential risk factor

that's unnecessary in our estimation. Documentation of cyclosporine levels will ensure that patients are taking the drug at the appropriate time and achieving the prescribed levels indicated in the protocol.

Pharmaceutical logistics

RTX will be provided by Genentech. Although Cyclosporine is recognized by insurance service providers as standard treatment for patients with IMN, participants randomized to the cyclosporine arm will receive the drug free of charge. Neoral brand (Novartis) is the preferred Cyclosporine product for this trial. If it is not possible to source Neoral from the local hospital pharmacy, please contact the DMCC prior to dispensing any study drug as not all brands of Cyclosporine are bio-equivalent. Appropriate quantities of the medication will be supplied to the individual from the individual sites following randomization and again at months 3, 6, 9, and 12 and may also be supplied at other time points should dose adjustments be required.

Screening visit

The Screening visit will consist of meeting with any potential candidate for the study, reviewing eligibility criteria, including laboratory requirements (as outlined below and in Tables 2a and 2b), and reviewing and signing the informed consent form.

Candidates who have not yet been exposed to conservative therapy will be started on the initial step of the protocol, i.e. maximize angiotensin II blockade as described in the run-in phase below. If any of the exams, tests or procedures involved in this visit were completed as routine standard of care within 30 days of screening, participants do not need to repeat them. It will be up to the study doctor to review and consider if the previous findings are appropriate/adequate to use otherwise they will be repeated. In addition to data from this screening visit, retrospective data from at least one additional time point within the previous year will be collected whenever possible. Retrospective data collection needs to include proteinuria, urine creatinine, creatinine clearance, Uprot/Ucrea ratio, systolic blood pressure, diastolic blood pressure, serum creatinine, and serum albumin values.

Candidates who have already been on conservative therapy for 3 months or more without a documented decrease in proteinuria of >50% as compared to a value from within the last 3 to 12 months do not need to fulfill the run-in requirement as long as their local labs indicate that proteinuria remains $\geq 5\text{g}/24\text{h}$. These individuals should proceed directly to full screening as part of the Time 0 visit and do not require a formal screening visit. Review of the eligibility criteria, lab requirements, and the review and signing of the informed consent form will take place at the Time 0 visit for these individuals. However, retrospective data from at least two previous time points (approximately 3 months prior to obtaining consent plus one more time point within the previous year) will still be collected whenever possible. Retrospective data collection needs to include proteinuria, urine creatinine, creatinine clearance, Uprot/Ucrea ratio, systolic blood pressure, diastolic blood pressure, serum creatinine, and serum albumin values.

Patient monitoring and evaluation

Patients will be followed for 2 years following randomization to monitor for the occurrence of adverse events, late remissions, relapses, GFR changes and development of end stage renal disease. The first 12 months of the study will be considered as the treatment period while the remaining 12 months will be considered as an observational

period including the period of tapering to discontinuation in the Cyclosporine arm. In addition, as described later in the protocol (see ancillary studies) we will seek permission to follow the remission cohort (i.e., those in PR or CR at 12 months or CR at 6 months as well as those who are not in remission but have a $\geq 50\%$ reduction in proteinuria from baseline by month 12) for an additional 12 months of observation beyond the previous end point of observation of 24 months to monitor for relapses and changes in PLA2R.

For those patients in the Cyclosporine arm, **Cyclosporine trough level measurements will be done every 2 weeks +/- 3 days after initiation of Cyclosporine until levels are stable and at target. Potassium and creatinine will also be checked in conjunction with CSA level. If Cyclosporine dose is changed during treatment, potassium, creatinine and CSA level will be rechecked every 2 weeks +/- 3 days until levels are stable and at target.** Cyclosporine levels will also be assessed whenever serum creatinine rises by $>30\%$ without a recognizable reason such as volume depletion, additional drugs, sepsis, etc. Patients who cannot tolerate the medications and/or who are treatment failures at 6 months will exit the study at 6 months (i.e., the 6 month visit and accompanying data collection should still occur). Patients who go in to complete remission will discontinue study drug but will continue with full visit follow-ups until month 24 as scheduled. However, the therapeutic/management plan for these patients will be solely at the discretion of the managing nephrologist although while in CR no further immunosuppressive therapy is recommended. Likewise, patients who relapse from either a partial or complete remission or who are not in partial or complete remission by the end of the treatment period (i.e., 12 months) will continue with the visit schedule, but the therapeutic/management plan will be solely at the discretion of the managing nephrologist. Finally, for patients who temporarily stop taking Cyclosporine, there are three possible scenarios:

1. Patients who discontinue Cyclosporine for < 2 weeks may simply be restarted on the study drug and continue to fully participate in the trial. The treatment is to be restarted at its former dose and the patient returned to the regularly scheduled follow-ups including the periodic sampling for assessing potassium, creatinine and CSA trough levels.
2. Patients who discontinue Cyclosporine for ≥ 2 weeks, but < 4 weeks may also be restarted on the study drug at its former dose and fully participate in the trial, but the temporary discontinuance will be logged as a protocol deviation and the missed treatment days will be made up at the end of month 12. This is to ensure that all patients receive approximately 12 months of treatment with study drug.
3. Patients who discontinue Cyclosporine for ≥ 4 weeks will not be restarted on study drug, but will continue with full visit follow-ups until month 24 as scheduled. The therapeutic/management plan will be solely at the discretion of the managing nephrologist.

Laboratory testing

In order to contain costs all clinical tests will be carried out locally at the recruiting centers. However, at critical time points: Time 0, and 6, 12, 18, and 24 months from start of treatment, recruiting centers will ship to Mayo Clinic, Rochester, Minnesota, 2 blood samples and 2 urine samples (aliquots obtained from two 24h urine collections) for determination of serum creatinines, serum albumins and urinary protein/creatinine ratios. Blood and urine kits will be provided by Mayo Clinic, Rochester, Minnesota, for these samples along with shipping costs (see Lab Manual). The Central Lab results (Mayo Clinic, Rochester, MN) will be used for statistical purposes since this will ensure a

standard methodology is applied to the critical (for the trial analysis) laboratory assessments. By measuring the volume of the 24-hour urine collection we will also be able to calculate both clearance and the 24-hour urine protein from the aliquot. Proteinuria results at Time 0, 6, 12, 18 and 24 months will be taken as the mean of two 24-hour urine collections done back to back (within 14 days of each other), and the mean of these 2 collections will be used to determine CR, PR, or NR.

In order to control for the possibility of under collection or over collection of urine, a maximum 20% variation in total 24h urine creatinine (mg/specimen) will be allowed between the two urine collections required at Time 0, 6 months, 12 months, 18 months, and 24 months. A difference of greater than 20% will necessitate a third urine collection (also to be sent to the central lab), with the values from one of the two previous collections being discarded. We will use the subject's weight to approximate the expected total urine creatinine (mg/kg) to determine which of the previous collections is closest to the truth and which should be discarded. It is anticipated that a 3rd urine collection will be needed in <10% of cases. Proteinuria will be calculated as the mean of these two 24-hour urine collections.

In all patients, CBC with differential, serum creatinine, electrolytes, albumin, urinalysis, 24-hour Uprot/Ucrea ratio, and creatinine clearance will be evaluated as noted in Tables 2a and 2b. In patients receiving RTX, additional tests will include: quantification of lymphocyte subsets by flow cytometry, serum immunoglobulin levels, and determination of humanized anti-chimeric antibodies (HACA) at study visits Day 1, 6 months, and 9 months. HACA will be performed free of charge by Genentech using established techniques.

Anti-PLA2R Assay

Recent evidence suggests that the presence of auto-antibodies to the M-type phospholipase A2 receptor (PLA2R) is a specific marker of idiopathic MN, found in greater than 70% of patients.⁷⁶ When the analysis is limited to new-onset, nephrotic patients with idiopathic MN, this sensitivity increases further. The presence of anti-PLA2R correlates well with disease activity, disappearing with a spontaneous or treatment-induced remission, and reappearing with a relapse of MN.

We intend to explore the correlation of anti-PLA2R and disease activity by carefully looking at anti-PLA2R titer in response to RTX/CSA and the achievement of partial or complete remission. In those patients that have no remission from their disease in response to RTX/CSA, we will determine if circulating anti-PLA2R is still present which might suggest the need for a second course of RTX, continued treatment with CSA, or alternate immunosuppressive therapy. Furthermore, in those patients that are in complete remission at 6 months, partial or complete remission at 12 months, , or have a $\geq 50\%$ reduction in proteinuria from baseline by 12 months (i.e., the MENTOR remission cohort), we intend to explore the following critically important questions:

1. The relationship between the rate of increase of anti-PLA2R titer and patient relapse
2. The relationship between the absolute change in anti-PLA2R titer (from baseline/remission point) and clinical phenotype (i.e., response/relapse time)
3. The predictive capacity of changes in anti-PLA2R titer with regard to relapse and/or durability of remission

4. The timing between changes in anti-PLA2R titer and any subsequent clinical relapse

Serum samples from all 126 subjects will be collected at the Screening visit (when applicable), Time 0, and months 3, 6, 9, 12, 18, and 24. Serum samples will be collected at the individual sites by the study coordinator and stored in a dedicated -20° freezer until a point when they can be shipped in bulk to Dr. Fervenza at Mayo Clinic, Rochester, Minnesota. Mayo Clinic will then send the samples in bulk to Dr. Paul Brenchley at the Manchester Royal Infirmary in the UK. The sera will be tested in batches for reactivity toward immobilized recombinant PLA2R by ELISA, with appropriate positive and negative controls. Upon completion of the study, we will correlate response to treatment in each group to the baseline presence and/or titer of anti-PLA2R. A major hypothesis to be addressed is whether there is a difference in treatment response between those subjects who are initially anti-PLA2R negative versus those who possess circulating anti-PLA2R. In those subjects who are initially positive for anti-PLA2R, we will also correlate the primary endpoint (remission status) to anti-PLA2R titers throughout and at the end of the study.

For those in the remission cohort (see ancillary studies), additional serum samples will be collected from those that achieve partial or complete remission or $\geq 50\%$ reduction in proteinuria by 12 months at additional time points as follows:

- For patients in the CSA arm that are in partial or complete remission or $\geq 50\%$ reduction in proteinuria at 12 months additional samples will be collected at months 13, 14, 15, 16, 17, 20, 22, 28, 32, and 36; for those who achieve complete remission at 6 months additional samples will be collected at months 7, 8, 10, and 11 then at 14, 16, 20, 22, 28, 32, and 36.
- For patients in the RTX arm that achieve partial or complete remission or $\geq 50\%$ reduction in proteinuria at 12 months additional samples will be collected at months 14, 16, 20, 22, 26, 28, 30, 32, 34, and 36; for those in the RTX arm that have achieved complete remission at 6 months, additional samples will be collected at months 10, 14, 16, 20, 22, 26, 28, 30, 32, 34, and 36.

Whole blood for these particular serum samples may be collected at a peripheral lab and shipped to the individual study site within 48 hours for processing and storage in a -20° freezer as described in the ancillary studies section later in this protocol.

Investigators in the study will be blind to the results of anti-PLA2R antibodies until the study is completed. The rationale is to avoid bias in the treatment of these patients. Although ideally patients would be stratified to the different arms based on the results of their initial anti-PLA2R test in order to avoid potential imbalance this will not be possible for practical and financial reasons. Given the relatively small number expected to be negative, it is unlikely this will bias the balance between positive and negative patients at randomization.

Histopathology

Prior to randomization, a report documenting the results of the histology review including, light microscopy, immunofluorescence and electron microscopy results, as well as percentage of global and segmental glomerular sclerosis, tubulo-interstitial index,

and immunofluorescence findings will be submitted to the DMCC and reviewed by the study P.I.

Following randomization, biopsy slides will be centrally reviewed on an on-going basis (but as soon as possible following randomization) by the Pathology Department at Mayo Clinic Rochester and/or the Pathology Department at the University Hospital Network, Toronto.

Quality of life measures

Psychosocial response to illness and its treatment may play an important role in overall adjustment and illness management. Research from a variety of chronic illness populations, including steroid resistant FSGS and chronic kidney disease (CKD), has consistently linked physical and psychosocial variables with health outcomes. These findings highlight the significant burden experienced by adults with uncontrolled nephrotic syndrome. Little is known about the impact of MN or its treatment on patient reported outcomes. Due to the severity of this disease and the potential risk for progression in these patients, psychosocial factors may play an important role in understanding their response to treatment. In addition to evaluating the safety and efficacy of RTX and CSA, it is essential to assess the individual's perception of his/her quality of life relative to disease status and therapeutic response. Quality of life will be assessed by patient self-report using the modified KDQOL questionnaire form at Time 0, 6, 12 and 24 months.

Table 2a: Test Schedule and Monitoring for Cyclosporine Treatment Arm

| Tests/Assessments | Screen | T0 | Day 15 | Day 28 | Day 90/ 3m | Day 180/ 6m | Day 210/ 7m | Day 240/ 8m | Day 270/ 9m | Day 300/ 10m | Day 330/ 11m | Day 365/ 12m | Day 395/ 13m | Day 425/ 14m | Day 455/ 15m | Day 485/ 16m | Day 515/ 17m | Day 545/ 18m | Day 605/ 20m | Day 665/ 22m | Day 730/ 24m | Day 850/ 28m | Day 970/ 32m | Day 1095/ 36m |
|---|--------|----|--------|--------|------------|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|------------------|----------------|------------------|----------------|--------------|------------------|------------------|--------------|------------------|------------------|------------------|
| Randomization | | X | | | | | | | | | | | | | | | | | | | | | | |
| History/Exam | X | X | | | X | X | | | X | | | X | | | | | | X | | | X | | | |
| CBC with differential | X | X | | | X | X | | | X | | | X | | | | | | X | | | X | | | |
| BUN, Cr, Lytes, Albumin (including potassium) | X | X | | | X | X | | | X | | | X | | | | | | X | | | X | | | |
| CPK | | | X | | X | | | | | | | | | | | | | | | | | | | |
| CSA level † | | | X | X | X | X | | | X | | | X | | | | | | | | | | | | |
| Lipid panel | | X | | | | X | | | | | | X | | | | | | | | | X | | | |
| Serum Pregnancy | X | X | | | | X | | | | | | | | | | | | | | | | | | |
| Anti-PLA2R antibodies | X | X | | | X | X | X ^d | X ^d | X | X ^d | X ^d | X | X ^c | X ^{c,d} | X ^c | X ^{c,d} | X ^c | X | X ^{c,d} | X ^{c,d} | X | X ^{c,d} | X ^{c,d} | X ^{c,d} |
| DNA sample | | X | | | | | | | | | | | | | | | | | | | | | | |
| Gene Expression | | X | | | | | | | | | | X | | | | | | | | | X | | | |
| Potassium and Cr ¹ | | | X | X | | | | | | | | | | | | | | | | | | | | |
| UA | X | X | | | | X | | | | | | X | | | | | | | | | X | | | |
| Uprot/Ucrea 24h | X | XX | | | X | XX | | | X | | | XX | | | | | | XX | | | XX | | | |
| Uprot/Ucrea (spot urine) | | | | | | | X ^d | X ^d | | X ^d | X ^d | | X ^c | X ^{c,d} | X ^c | X ^{c,d} | X ^c | | X ^{c,d} | X ^{c,d} | | X ^{c,d} | X ^{c,d} | X ^{c,d} |
| 24h creatinine for CrCl | X | X | | | | X | | | | | | X | | | | | | | | | X | | | |
| 24h urine urea | | X | | | | X | | | | | | X | | | | | | | | | X | | | |
| 24h urinary Na ⁺ | | X | | | | X | | | | | | X | | | | | | | | | X | | | |
| Quality of life-KDQOL | | X | | | | X | | | | | | X | | | | | | | | | X | | | |
| Adverse Events | | | X | X | X | X | | | X | | | X | | X | | | | X | | | X | | | |
| Dispense/Return Medication | | X | X | X | X | X | | | X ^a | | | X ^a | X ^a | X ^{a,b} | | | | | | | | | | |

¹ Potassium, Cr checked at 2 weeks +/- 3 days post Cyclosporine initiation. If Cyclosporine is increased or decreased during treatment, potassium, Cr and CSA will be rechecked after 2 weeks +/- 3 days

†Patient will have blood levels of CSA monitored every 2 weeks +/- 3 days until target is reached and then as per above schedule.

^a Only for participants who continue on CSA at 6 months

^b Telephone or in-clinic visit to confirm successful down-titration of CSA for patients who continue on medication to 12 months (if by phone, medication should be returned at 18 month visit)

^c For participants in the MENTOR remission cohort who are in PR or CR at 12 months or have a ≥50% reduction in proteinuria from baseline by 12 months

^d For participants in the MENTOR remission cohort who are in CR at 6 months

Table 2b: Test Schedule and Monitoring for Rituximab Treatment Arm

| Tests/Assessments | Screen | T0 | Day 1 | Day 15 | Day 28 | Day 90/ 3m | Day 180/ 6m | Day 181 | Day 195 | Day 270/ 9m | Day 300/ 10m | Day 365/ 12m | Day 425/ 14m | Day 485/ 16m | Day 545/ 18m | Day 605/ 20m | Day 665/ 22m | Day 730/ 24m | Day 790/ 26m | Day 850/ 28m | Day 910/ 30m | Day 970/ 32m | Day 1030/ 34m | Day 1095/ 36m |
|---|--------|----|-------|--------|--------|---------------|----------------|---------|---------|----------------|-----------------|-----------------|------------------|------------------|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Randomization | | X | | | | | | | | | | | | | | | | | | | | | | |
| History/Exam | X | X | | | | X | X | | | X | | X | | | X | | | X | | | | | | |
| CBC with differential | X | X | | | | X | X | | | X | | X | | | X | | | X | | | | | | |
| BUN, Cr, Lytes (including potassium) / Albumin | X | X | | | | X | X | | | X | | X | | | X | | | X | | | | | | |
| PBFC ¹ | | | X | | X | | X | | | | | X ³ | | | | | | | | | | | | |
| Serum IgG, IgA and IgM | | | X | | | | X | | | | | X | | | | | | X | | | | | | |
| Lipid panel | | X | | | | | X | | | | | X | | | | | | X | | | | | | |
| HACA ² | | | X | | | | X | | | X | | | | | | | | | | | | | | |
| Serum Pregnancy | X | X | | | | | X | | | | | | | | | | | | | | | | | |
| Urine Pregnancy | | | X | X | | | | X | X | | | | | | | | | | | | | | | |
| Anti-PLA2R antibodies | X | X | | | | X | X | | | X | X ^d | X | X ^{c,d} | X ^{c,d} | X | X ^{c,d} | X ^{c,d} | X | X ^{c,d} | X ^{c,d} | X ^{c,d} | X ^{c,d} | X ^{c,d} | X ^{c,d} |
| DNA Sample | | X | | | | | | | | | | | | | | | | | | | | | | |
| Gene Expression | | X | | | | | | | | | | X | | | | | | X | | | | | | |
| UA | X | X | | | | | X | | | | | X | | | | | | X | | | | | | |
| Uprot/Ucrea 24h | X | XX | | | | X | XX | | | X | | XX | | | XX | | | XX | | | | | | |
| Uprot/Ucrea (spot urine) | | | | | | | | | | | X ^d | | X ^{c,d} | X ^{c,d} | | | X ^{c,d} | X ^{c,d} | | X ^{c,d} | X ^{c,d} | X ^{c,d} | X ^{c,d} | X ^{c,d} |
| 24h creatinine for CrCl | X | X | | | | | X | | | | | X | | | | | | X | | | | | | |
| 24h urine urea | | X | | | | | X | | | | | X | | | | | | X | | | | | | |
| 24h urinary Na ⁺ | | X | | | | | X | | | | | X | | | | | | X | | | | | | |
| Pre-infusion medication (i.e. Tylenol, Benadryl) | | | X | X | | | | X | X | | | | | | | | | | | | | | | |
| Pre-infusion medication (i.e. methylprednisolone) | | | X | | | | | X | | | | | | | | | | | | | | | | |
| Rituximab Infusion | | | X | X | | | | X | X | | | | | | | | | | | | | | | |
| Quality of life- KDQOL | | X | | | | | X | | | | | X | | | | | | X | | | | | | |
| Adverse Events | | | X | X | X | X | X | X | X | X | | X | | | X | | | X | | | | | | |

1. PBFC: Peripheral blood flow cytometry. Quantitate B and T cell subsets;

2. HACA: human anti-chimeric antibodies; pre-infusion on Days 1 and at 6 months and 9 months.

3. In the rare case where B cells (CD19/20+) are not replete at Month 12, PBFC will be repeated at Month 18

^c For participants in the MENTOR remission cohort who are in PR or CR at 12 months or have a $\geq 50\%$ reduction in proteinuria from baseline by 12 months

^d For participants in the MENTOR remission cohort who are in CR at 6 months

Run In Phase – Common Therapy for Both Arms

The purpose of the run-in phase is to determine if an individual's IMN can be adequately controlled through conservative, non-immunosuppressive treatment. If proteinuria can be brought below 5g/24h after 3 months or more of conservative therapy, the individual will not be randomized to one of the study's treatment arms. In the rare case where a patient is intolerant to even a very low dose of ACEi or ARB therapy, participation in the randomized treatment component of the trial may still be possible (provided the patient still fulfills the other entry criteria), but will require review and approval by the study PI(s) prior to randomization. .

Blood pressure control and angiotensin II blockade: The target blood pressure (<130/80mmHg in >75% of the readings; but not <100 mmHg systolic) is chosen based on recent recommendations by the JNC VII.⁸² The first step will be the introduction of an ACE Inhibitor (ACEi). Because this part of the study aims to maximize Ang II blockade, ACEi dose will be increased every 2 weeks until the maximum tolerated/FDA approved dose is achieved or until intolerable side effects occur (e.g. development of postural hypotension, light headed, hyperkalemia, etc). Once ACEi dose has been maximized and there are no observable side-effects and/or blood pressure is not at target, a long acting ARB will be added. The ARB dose will be increased every 2 weeks to achieve the maximum tolerated or maximum approved dosage.

For patients whose blood pressure control is still not at target, it is recommended that they receive additional medications in the following order: a loop diuretic, a cardioselective β -blocker, a non-dihydropyridine calcium channel blocker (CCB), and clonidine. The selection of these drugs adheres to the recommendations of the JNC VII.⁸² The choice of a non-dihydropyridine CCB was made because of concerns that dihydropyridine-type CCB may obscure the anti-proteinuric effects of the above therapy. In order to further ensure that any potential adverse effect is minimized we have limited CCB to be used as a fifth agent, and to be used only when the combination of an ARB/ACEi, a diuretic, and a β -blocker have failed to bring BP to target level. Recent data have shown that an aldosterone or renin antagonist have significant antiproteinuric effects. The reason we do not include these agents as part of the treatment protocol is out of concern for the development of severe hyperkalemia when all (ACEi, ARB, a direct renin inhibitor and aldosterone antagonist) these agents are used in combination. This side effect is likely to be further accentuated in the Cyclosporine group and we want to avoid an imbalance of RAS blockade between the arms of the study.

Concomitant Treatment

At the start of the run-in/conservative phase of the study (or at the start of the treatment phase of the study for those patients who are run-in exempt) and as part of the standard of care for patients with nephrotic syndrome and significant hyperlipidemia, patients will be started on atorvastatin 10 mg a day (or its equivalent with the **exception of rosuvastatin** (Crestor) which is to be avoided given the associated marked increase in the area under the curve (AUC) when used in conjunction with CSA). *Patients who are currently taking rosuvastatin (Crestor) and are randomized to the CSA treatment arm will need to be changed to atorvastatin or its equivalent prior to or at the time of starting the*

cyclosporine. If tolerated clinically and, as evidenced by the lack of persistent elevation of liver transaminase >3x upper limit of normal or high CK, or clinical rhabdomyolysis, the dose will be increased according to the recently published KDOQI-dyslipidemia guidelines.⁸³ The dose should not be increased above the maximum of 40 mg/day. The rationale for not using a higher statin dose is because of the risk of developing proteinuria with the use of statins at high doses, and the added risk of rhabdomyolysis in the CSA group. Patients will remain at the highest tolerated dose for the entire duration of the study. Serum lipids will be measured at Time 0 and every 3 months thereafter. High sodium intake (e.g. >200 mm Na/d or 4.6 g sodium/d) can significantly impair the beneficial effects of Ang II blockade.⁸⁴ Therefore patients will be instructed to follow a low salt diet (2-3g/day). Patients will also receive dietary counseling at enrollment as part of their standard of care. Patients will be advised of a dietary protein target intake of 0.8-1.0 g/kg ideal body weight/day of high quality protein and will be encouraged to maintain the same diet throughout the duration of the study. Patients with proteinuria >10g/24h and serum albumin <2g/dl should be considered for prophylactic anticoagulation.

Patients randomized to receive Rituximab should be started on single strength Bactrim one a day (or its equivalent) for pneumocystis pneumonia prophylaxis. This treatment will continue until the end of the study treatment phase (12 months) and the B cells (CD19/20+) have been repleted (>15 cells/microliter on peripheral blood flow cytometry). B cells (CD19/20+) are expected to be replete by Month 12. In the rare event that B cells (CD19/20+) are not replete by Month 12, flow cytometry should be repeated at Month 18 (see table 2b) and Bactrim (or its equivalent) continued to that point of repletion.

Stopping points for medications:

1. For Cyclosporine: At 6 months, patients in complete remission (as previously defined) will have CSA dose tapered and discontinued while those who fail therapy will have CSA discontinued immediately (no need to taper the dose).
After 12 months of therapy, regardless of the degree of proteinuria, Cyclosporine will be tapered and discontinued as described above.
2. Patients in the RTX arm who have either failed therapy or are in complete remission at 6 months (as previously defined) will not have a repeat treatment course.
After the second course of RTX therapy at six months they will have no further RTX therapy regardless of their proteinuria level
3. Significant potential life threatening infection.
4. Persistent elevation of liver enzymes >2 x normal (despite CSA arm dose reduction).
5. Persistent elevation of serum potassium >6.0 mEq/l despite diet changes, ACEi/ARB reduction, diuretic dose increase (and in the CSA arm dose reduction).
6. A rise in serum creatinine by >30% above baseline that persists in the absence of secondary causes such as volume depletion or use of potential nephrotoxic agents (and despite adjustments in the CSA dose)
7. Persistent hypertension in either arm, i.e. supine blood pressure >160 mmHg systolic or >90 mmHg diastolic, despite institution of maximum antihypertensive therapy (and despite adjustments of CSA dose)

8. Other clinically relevant adverse effects not resolved by reduction in dosage of test medications.
9. Development of any malignancy or lymphoproliferative disorder.
10. Pregnancy.
11. The appearance of an independent disease whose standard therapy requires continuous administration of significant amounts of corticosteroids, other immunosuppressive agents or plasma exchange therapy.
12. Serious concomitant disease with an expected survival of less than 4 years.
13. At the wish of the patient.
14. The study will be placed on hold if 3 of the first 5 patients enrolled and treated with RTX or CSA develop severe opportunistic infections, or experience Grade 3-4 SAEs.

Patients who choose to discontinue study medication or are withdrawn because of adverse events will be strongly encouraged to continue follow up examination to the study termination.

Ancillary studies:

1. DNA Testing

Recent data show that in patients with membranous nephropathy, mutations in HLA-DQA1 and PLA2R1 alleles are associated with an increased risk for developing membranous nephropathy.⁸⁰ One of these alleles, the PLA2R1, is in fact the gene for the PLA2 receptor. In our pilot studies, ~70% of the patients with membranous nephropathy had antibodies present in circulation against PLA2 receptor.⁷⁷ Thus we would like to evaluate a linkage between the presence of these antibodies and potential genetic mutations in this group of patients. We will ask for the patients consent to further evaluate this linkage by collecting samples of blood to perform additional testing as part of their informed consent form (ICF).

2. Quantitative gene expression analysis

Quantitative gene expression analysis using real-time PCR and Microarray technology in peripheral blood mononuclear cells: There is little information on global gene expression changes that occur during active nephrotic syndrome secondary to IMN and subsequent to remission of this disease. Furthermore, there is limited information on how Rituximab and Cyclosporine affects gene expression of potentially pathogenic genes. As part of this study, peripheral blood will be collected at Time 0, 12 and 24 months after therapy. Following treatment with Rituximab and Cyclosporine administration, B-cell-specific genes will be assessed to track the extent of treatment in the B-cell pool gene expression. Both quantitative TaqMan real-time polymerase chain reaction and gene microarray (Affymetrix U133 chips) will be used to evaluate gene expression profiles. Real-time PCR will be used only to confirm the findings of the gene chip for genes with statistical significance.

3. Staining original renal biopsy for CD20 + B cells

We will obtain each patient's permission to review renal biopsy slides and to obtain 3 unstained slides, 3 microns thick for staining with antibody against CD20 positive cells and anti-APL2R. The purpose is to stain sections from biopsy tissue with an antibody against CD20 positive cells and to look for receptor density to APL2R. These may be the same cells that are in circulation and that are depleted by Rituximab. We want to examine the hypothesis that there is a correlation between the number of CD20 positive B cells infiltrating the kidney and the patient's response to therapy. Preliminary results suggest that staining for CD20 and APL2R antibodies are increased in kidney biopsy of patients with MN. All patients by this juncture will have completed the therapeutic part of the study and will have already received rituximab or CSA treatment as part of study so this ancillary project will have no therapeutic or management implications.

Immunohistochemistry methods

Immunohistochemical staining will be performed at the Department of Pathology, Mayo Clinic, Rochester, Minnesota, by Dr. Sanjeev Sethi and/or by Dr. Carmen Avila-Casado at University Health Network, Toronto, Ontario. Formalin-fixed, paraffin embedded sections will be cut onto coated glass slides. Slides will be incubated with monoclonal anti-CD20 and APL2R primary antibody at 1:1000 dilution (DAKO). Heat induced antigen retrieval will be used. Sections will be incubated at 20C overnight and rinsed. Endogenous peroxidase activity will be prevented by pretreating all sections with 3% hydrogen peroxide. Sections will be incubated with a secondary rabbit anti-mouse antibody linked with avidin-biotin complex. Sections will be counterstained with hematoxylin. CD20 positive cells (B-cells) will be counted and the density of positive cells per area of renal cortex will be calculated utilizing Image Pro Plus (Media Cybernetics) computer image analysis software.

4. The MENTOR Remission Cohort

Participation in this ancillary study will be restricted to patients in the MENTOR cohort who respond to immunosuppression with partial or complete remission at month 12 (as defined in Table 1), complete remission at 6 months, or $\geq 50\%$ reduction in proteinuria from baseline by month 12. At the completion of the immunosuppression treatment component of the protocol, the expectation is that 70% of all randomized IMN cases ($N=126 \times 70\% = 88$ patients) will experience both a clinical (complete or partial proteinuria remission) and immunological remission. The immunologic remission is expected to be documented by anti-PLA2R levels significantly reduced from baseline with most levels in the normal range (less than 40U). This population is estimated to be 75% of the initial PLA2R + cohort ($75\% \times 88 = 66$ patients). The great percentage of these patients will reach this remission at the 12 month study point. There will be rare cases of CR by 6 months. Our anticipated/expected rate of proteinuria relapse will be between 30% and 50% between month 12 and month 36 (20-30% in those with CR at 6 months). Although the clinical proteinuria remission status at 24 months remains the main determinant of the RCT, the remission cohort (with an extension up to month 36 for periodic sampling of anti-PLA2R titer and urinary protein/creatinine ratios only) will provide a unique and probably one-time only opportunity in which to study a number of biomarkers of the immunological mechanism as predictors of clinical relapse. Patients will be followed as per protocol for at least 12 months of observation (i.e., to month 24)

after achieving proteinuria remission or $\geq 50\%$ proteinuria reduction, but for those in the remission cohort we will extend this up to month 36 (a maximum observation time of 24 months post treatment phase) in those that remain in CR or PR. Those who relapse between months 24 and 36 will only be followed until their relapse which is defined as development of nephrotic range proteinuria ($> 3.5\text{g/day}$) following CR or PR.

In addition, we are now able to measure other components of the immunological elements of primary/idiopathic MN activity and are now better able to more precisely define the remission state when combining these additional biomarkers of the immune remission state with PLA2R titres. These additional biomarkers include soluble PLA2R. A detectable level of soluble PLA2R antigen (sPLA2R) is present in normal serum as a result of specific secretion of PLA2R and likely cleavage of the extracellular membrane receptor, and is likely to be a characteristic of the normal, remission state of patients with idiopathic MN. We hypothesize that as patients experience immunological relapse, their biomarker status will change from sPLA2R +ve, anti-PLA2R -ve to sPLA2R -ve, anti-PLA2R +ve. The timeframe of this change needs to be established and our remission cohort provides a unique opportunity to study this hypothesis. This change could vary over weeks dependent on the level of sPLA2R and the rate of production of anti-PLA2R. In addition, there may be a distinct phase of soluble circulating immune complexes where the patient is apparently seronegative for both free sPLA2R and free anti-PLA2R until excess of anti-PLA2R antibodies dominate. This MENTOR Remission cohort offers a unique opportunity to investigate:

- a) the transition from immunological remission to relapse
- b) the link between immunological relapse and clinical relapse (proteinuria)
- c) additional biomarkers of relapse.

The benefit of this study will be the improved capacity to predict those patients at high risk of clinical relapse and the potential to institute therapy potentially in advance of clinical relapse. It may also allow us to determine if the changing immunologic profile can predict the severity of relapse and provide the opportunity to consider, in the future, matching this profile to preventive strategies and/or early and less aggressive immunosuppressive interventions and/or gauge when risk-benefit favours reducing immunosuppressive therapy.

We will obtain each patient's permission to obtain 5mL of blood at designated time points (up to a maximum of 11 extra samples in total) up until month 36 or until relapse (see Tables 2a and 2b for visit schedule) to be used for PLA2R and sPLA2R testing. In addition, a urine sample will be collected to determine relapse by measuring urine protein/creatinine ratios. Samples may be collected at a peripheral lab where possible in order to minimize the demands on patient travel time, but blood will need to be sent to study sites for processing and storage (-20 degrees C) within 48 hours of being drawn. Serum samples will be kept in a dedicated freezer until a point when they can be shipped in bulk to Dr. Fervenza at Mayo Clinic, Rochester, Minnesota. Mayo Clinic will then send the samples in bulk to Dr. Paul Brenchley at the Manchester Royal Infirmary in the UK.

Statistical Methods and Analysis:

Sample size estimation: The primary study goal is to compare the long-term efficacy of RTX (given at baseline and repeated at 6 months) with CSA (12 months treatment + 2 months taper) using proteinuria response 24 months after randomization. Proteinuria positive response will be defined as attaining and maintaining CR or PR at 24 months after randomization. We propose to establish that the RTX treatment is non-inferior to the CSA. RTX will be considered non-inferior to CSA if the response rate of the RTX arm is at most 15% worse than that of CSA arm. In addition we will compare the quality of life (QOL) and adverse event (AE) profile over the course of the treatment period. The preliminary data has indicated that CSA is effective in inducing a CR/PR of proteinuria in between 60 and 75% of MN cases.^{48, 51} However, nephrotic syndrome (NS) relapses may be as high as 50% once CSA is discontinued.⁸⁵ Thus, we estimate a CR/PR rate in patients treated with CSA of 30-50% at 24 months after randomization.⁸⁵ Similar remission and relapse rates with the use of Tacrolimus (another calcineurin inhibitor) have been reported by Praga et al.⁵⁶ In this latter study, almost half of the MN patients had a relapse of the nephrotic syndrome after tacrolimus was discontinued.⁵⁶ On the other hand, based on the long term follow up on the 35 patients treated with RTX from our 2 studies, we estimate the relapse rate to be <10% at 24 months.^{71, 75} We observed a CR/PR rate at 12 months, very similar to the rate for calcineurin inhibitors i.e. 60%, and in our second study completed recently, we observed an 80% CR/PR rate at 24 months, implying that RTX had a ~20% failure rate with 95% (CI 6% to 44%). To be conservative for the sample size estimation, we considered a maintained CR/PR rate at the low end of our previous experience, 55% CR/PR for RTX at 24 months and at the high end of our experience with CSA (CNI therapy) CR/PR for CSA of 45%. We propose a non-inferiority trial with non-inferiority margin $\delta = 15\%$, with the null hypothesis of $\pi_{\text{RTX}} - \pi_{\text{cyclosporine}} < \delta$, where π_{RTX} and $\pi_{\text{cyclosporine}}$ are the proportions of patients with CR/PR at 24 months in treatment arms of RTX and CSA, respectively. Under these assumptions, and a one sided alpha of 0.025, enrollment of 63 evaluable patients per study arm is required to achieve 80% power to show that the RTX is not inferior. For the intent to treat (ITT) analysis we will consider all patients lost to follow up to be non-responders so no correction to the sample size is needed for dropouts. With 63 patients per arm we will have 80% power to detect moderate differences of .6 standard deviations for continuous measures or around .25 for proportions for the comparisons of the QOL and AEs.

Statistical and Analytical Plan: All demographics and entry laboratory data will be summarized by treatment group. Frequency distributions will be used to describe categorical values and basic summary statistics (mean, standard deviation, median, and inter-quartile range) will be used to describe continuous values. To provide more reliable estimates and minimize the “regression to the mean effect” duplicate urine measurements obtained at baseline (Time 0), 6, 12, 18 and 24 months will be averaged. The chi-square test and logistic regression will also be used to compare the percent of patients with remission at 6, 12, 18, and 24 months adjusting for treatment center and the baseline

proteinuria (UP<8g/24h vs ≥8g/24h). Odds ratios and associated 95% confidence intervals will be estimated. The formal test for the primary endpoint will be based on the significance of the treatment group factor in the logistic regression model for UP failure at month 24. The Wilcoxon rank-sum test and ordinal logistic regression will be used to compare the ordered remission status outcome (1=CR, 2=PR, 3=NR) between treatment groups. Longitudinal methods for categorical outcomes (e.g. generalized estimating equation or GEE models) will be used to compare remission status profiles between treatment arms. Unless otherwise noted, all tests will be two-sided with alpha level 0.05.

For repeated measures such as urine protein, individual rates of change will be estimated using within-patient linear regression analyses (including data from all visits). Because of the non-linearity of the changes in proteinuria log transformation estimates will be used. Renal function readings will be censored at initiation of dialysis or renal transplant. Additionally, mixed effects models, assuming a random center effect, and a random slope of creatinine clearance and/or reciprocal of creatinine and intercept for each patient will be fit using data from all visits. These models allow comparison of the average slope between groups, while taking into account that each patient's slope may be based on a variable number of readings. Treatment group comparisons regarding quality-of-life scales will be done using repeated measures analyses and mixed effects models.

Adverse events (both patient and event counts) will be tabulated by body system, severity and, for each severity, by investigator-assessed relationship to study drug. Group comparisons for adverse events with 4 or more occurrences will be done using chi-square or Fisher's exact test. The last RTX injection is at 6 months and the tapering of Cyclosporine will be completed by the end of month 14. Hence, the adverse events analyses will focus on events through month 18, allowing these additional months for potential lingering Cyclosporine or RTX complications.

The analysis of the primary endpoint (urine protein failure at month 24) will be intent-to-treat (ITT), and will include all randomized subjects in the analysis. For those without a 24 month visit, the 18 month visit results will be used if available, otherwise they will be assumed to have failed at 24 months. Per protocol (PP) analyses will also be done including only those subjects who receive a full course of study medication and who have a 24 month visit.

Interim analyses: No interim analyses for efficacy will be performed during the trial, however safety data will be compiled and reviewed approximately every six months or at the request of the Data and Safety Monitoring Board.

Investigator and Coordinator Training: In order to make efficient use of funding, annual investigator and coordinator meetings will be coordinated with national nephrology meetings where possible. It is expected that the majority of the training will occur through web-based technology and will be coordinated through the data management and coordinating Center.

The **Data Management and Coordinating Center (DMCC)** is located at the Applied Health Research Center (AHRC) at St. Michael's Hospital/University of Toronto. The DMCC will be responsible for facilitating all aspects of the study planning, training, implementation, data collection and analysis.

The clinical information collected for this study will be stored at the DMCC at the Applied Health Research Centre of St. Michael's Hospital in Toronto, ON, Canada and also sent to a federal data repository held by the Office of Rare Diseases Research (ORDR) and located at the University of South Florida (USF) in Tampa, FL.

The DMCC database and the USF database use several layers of protection for the clinical data stored there. These databases meet all of the local and federal security requirements for research datacenters. Participant information is stored only using a study ID.

Data management and quality assurance: Study data, including enrollment and monitoring data, will be maintained on a secure password protected database accessible to all study centers from the world-wide-web. Each center will enter data for their patients. Quality control will be ensured by oversight by the AHRC coordinating center, who will review the electronic files of all patients on a regular basis for completeness.

Quality and completeness of data entry will be monitored on a weekly basis during the initial 6 months of study enrollment and biweekly thereafter. Data quality reports will be generated monthly for review by the study Data Quality Assurance Committee, St. Michael's Hospital/University of Toronto. Data queries generated by identification of incomplete or inconsistent data will be raised directly within the electronic eCRF and should be resolved by the study coordinator or PI in a timely manner. Corrections or changes in the data management system are tracked with the retention of the original data and the corrected data with the date of data entry and submitting personnel. Sites with persistent delays or difficulties in data capture will be provided additional study based training. Study data will be stored within the data centre of St. Michael's Hospital/University of Toronto with an off-site daily back-up system.

Analysis of the primary and secondary outcomes will be conducted at the DMCC. Additional analyses requested by ancillary investigators will be conducted when feasible within the scope of this trial funding. The DMCC will implement the trial policy for data sharing and ancillary studies. A fee structure will be established for ancillary studies that are beyond the scope of this trial.

Data security:

All clinical data will be processed in a secure electronic environment that includes virus protection, and restricted access. Electronically stored data are subject to extensive security measures including virus detection, and restricted access. Security measures in place for the database management system proposed for this study include: browser security, firewall protection, user name/password protection, user re-authentication, and

inactivity time-out. The database will not contain study participant name, address or medical record number. Patient data will be identified only by study code. Study data, including enrollment and monitoring data, will be maintained on a secure password protected database. Quality control will take place at time of data entry (range, consistency checks) and will be ensured by oversight by the P.I. and Quality Assurance and Clinical Management Workgroup which will review the files of all patients on a regular basis for completeness.

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 and the University of South Florida before accrual can occur from the clinical site.

Demographic and adverse event data collected as part of this trial will be transferred to the USF on a monthly basis throughout the trial, and uploaded into the data repository dbGaP (database of Genotypes and Phenotypes).

A system of coded identifiers will be used to protect participant confidentiality and safety. Each participant enrolled will be assigned a local subject identifier by the DMCC which is a combination of the site 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 data is sent to the USF, the system will assign a participant ID number. Thus each participant will have two codes: the local code used to identify the patient in the eCRF and in source documentation and which is linked to personal identifiers, and a second code assigned by the USF. For all data transfers to the USF 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 USF.

PROTECTION FOR HUMAN SUBJECTS

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.

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

One hundred and twenty-six patients with IMN will participate in the study. Their participation will consist of up to 13 visits over a 2-year period. The only clinical specimens obtained will be blood and urine. The age range for participation is 18-80

years. RTX will be provided to the study participants by Genentech and the cyclosporine will be provided to that arm free of charge as per protocol.

Protection of human subjects:

1. Informed consent process. Patients who are candidates for the study will review and sign an informed consent form, to allow review of their history and physical exam including blood pressure estimates, as well as serum/urinary chemistries and medication history.

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.

2. Measures to reduce risks and discomforts associated with study drug.

RTX infusions will be administered at centers with the following patient safety equipment and supplies: oxygen, oral and endotracheal airways and intubation equipment, epinephrine 1:1000 solution for intravenous or endotracheal injection, antihistamines, corticosteroids, intravenous infusion solutions, tubing, catheters, tape, and defibrillator with electrocardiogram monitor. Patients will be pre-medicated with acetaminophen (1g) and diphenhydramine HCl (50 mg) by mouth from 30 to 60 minutes prior to the start of an infusion. Premedication with steroids (100 mg methylprednisolone IV) will also be given 30 minutes prior to the first infusion of each series of RTX (Day 1 and Day 181). Patients administered an antihistamine for the treatment or prevention of infusion-related reactions will be given appropriate warnings about drowsiness and impairment of driving ability prior to discharge. Patients will be instructed how and when to take their cyclosporine, taught about the most common drug interactions and to contact their coordinator/study physician about potential interaction with over-the-counter medications and / or intercurrent illnesses requiring additional treatment prescribed by others. They will also be informed about the need to maintain the proper time interval prior to assessment of their cyclosporine drug levels.

3. Adverse event reporting. Each site's Principal 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 at

least every 6 months by the research team. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

An adverse event is defined as: "...an unfavorable and unintended sign, symptom or disease associated with a participant's participation in the study."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

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 or product monograph.

Expected adverse events are those that are identified in the research protocol or product monograph as having been previously associated with or having the potential to arise as a consequence of participation in the study.

All adverse events that occur between Time 0 and study termination will be reported. All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

- Within **24 hours** (of learning of the event), investigators must report any 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 SAEs within **5 working days** (of learning of the event).
- All other AEs must be reported to the DMCC 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 FDA, if appropriate, remain the responsibility of the treating physician.

As part of the reporting structure, SAEs will be reported to Health Canada, Genentech Drug Safety and the Genentech medical science liaison. An adverse event summary form will be completed and will be summarized for each IRB and Health Canada annually.

Sites will enter all Adverse Event data into the study eCRF. Upon entry of a serious adverse event, the system will notify the DMCC by email. The DMCC will in turn notify the study PIs, the designated medical monitor and any other designated personnel by email. The medical monitor will then review the SAE data to determine causality (definitely not related, probably not related, possibly related, probably related, definitely

related) of the serious adverse event. The medical monitor [and, if applicable, sponsor] may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the serious adverse event. A back-up notification system is in place so that any delays in review by the medical monitor beyond a specified period of time are forwarded to a secondary reviewer. The eCRF maintains audit trails and stores data (and data updated) related to any adverse event in the study.

Study Discontinuation

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

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

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. Reasons for subject discontinuation include:

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site

Regulatory issues:

1. **Inclusion of women:** Women make up approximately 30-50% of the population of patients with IMN and are expected to be enrolled in numbers in proportion to this normal distribution.
2. **Inclusion of minorities:** Individuals of all races and ethnicities are at risk for MN. Based on the ethnic composition of the patients followed at, and referred to the Mayo Clinic the population served will include African-Americans, Asian, and Hispanic subjects proportional to that in the general US and Canadian population. This will ensure adequate enrollment within the different racial groups.
3. **Inclusion of children:** It is desirable to include children whenever possible in clinical research studies. The rationale to exclude children in this trial is that: a) the disease is rare in children; b) there are no published natural history studies in children but the general impression is that the disease is benign in this patient population; c) no studies on specific immunosuppressive treatment in this age group have been published, d) many cases of MN in children are secondary to SLE and positive laboratory markers are often delayed beyond the point of clinical presentation. We do not want this possibility to contaminate and confound the study population. In the

absence of safety and efficacy data we would not like to expose children to an unproven therapy.

Data Safety Monitoring Board (DSMB): The DSMB will include 1 chair person, clinical experts in nephrology, epidemiology and trial design, and a biostatistician. We will develop a charter for the DSMB and the board will be responsible for evaluating the study design and progress of the study. The board will be provided data on a regular basis to monitor patient safety. Meetings will be conducted on a semi-annual schedule by conference call.

Benefits:

Patients might expect to gain the following benefit from study participation: an opportunity to be exposed to experimental medicine that may be effective for their kidney disease. Patients will be made aware of the possibility of adverse events known to be associated with RTX (and CSA) and the possibility of previously unrecognized adverse events. All patients will benefit from close follow-up and monitoring of their disease process in terms of both efficacy and safety in both arms of the study.

Data sharing plan:

Results from the study will be published in both abstract and in manuscript form as soon as feasible. Three years after completion of the study, data and remaining samples will be made available to the research community for appropriate and well-designed post hoc studies. Proposals for research projects will be reviewed by the Operations committee and presented to all the study investigators for approval.

ESTIMATED DURATION OF THE STUDY

The estimated duration of the study, given a 24-month enrollment period, a 2-year follow-up for each patient, and 1 year for data analysis is approximately 60 months. For those patients in the MENTOR remission cohort, their participation will be extended up to 3-years follow-up from the first day of study drug.

ANTICIPATED RESULTS

We believe RTX will be as effective as Cyclosporine in inducing complete and partial remissions in this patient population with membranous nephropathy.

PUBLICATION POLICY

A policy similar to the NIH policy on publication of study results will apply to this study. Details regarding policy statements may be found on the website at <http://www1.od.nih.gov/oma/manualchapters>. Any abstract or manuscript must be submitted to Genentech four weeks prior to submission as per contract.

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Protocol: Membranous Nephropathy Trial Of Rituximab (MENTOR)

Version: 6.0, May 3, 2012

Summary of Changes

An amendment has been drafted to the MENTOR study protocol. The following is a summary of key changes to the previous version of the protocol (5.0, March 22, 2012) including the rationale for each change. Formatting changes and minor administrative changes (typos, grammatical changes, minor clarifications in wording) are not included in this table.

| Original Text | Modified Text | Rationale |
|---|---|---|
| Cover Page | | |
| [Text Addition] | Genentech, Incorporated | Genentech Inc. has been identified as a source of funding for this study |
| Biogen IDEC Pharmaceuticals Corporation | [Text Removal] | Biogen is the drug manufacturer, but the financial funding is coming through Genentech. |
| [Text Addition] | <u>Food and Drug Administration IND Exemption</u> Number: 109567 | |
| Laboratory Testing | | |
| In all patients, CBC, serum creatinine, electrolytes, albumin, urinalysis, 24-hour Uprot/UCr ratio, and creatinine clearance will be evaluated at each study visit. | In all patients, CBC, serum creatinine, electrolytes, albumin, urinalysis, 24-hour Uprot/UCr ratio, and creatinine clearance will be evaluated as noted in Tables 2a and 2b. | Clarification that labs will be performed in accordance with the visit schedule outlined in the Tables. |
| Anti-PLA2R Assay | | |
| We will collect serum samples (5 mL), at Time 0, and months 3, 6, 9, 12, 18, and 24 for the measurement of anti-PLA2R antibodies. | We will collect serum samples (5 mL), at Screening , Time 0, and months 3, 6, 9, 12, 18, and 24 for the measurement of anti-PLA2R antibodies. | Additional serum sample collection specified for the screening visit in order to facilitate comparisons between pre- and post-treatment antibody levels |

Protocol: Membranous Nephropathy Trial Of Rituximab (MENTOR)

Version: 7.0, May 17, 2012

Summary of Changes

An amendment has been drafted to the MENTOR study protocol. The following is a summary of key changes to the previous version of the protocol (6.0, May 3, 2012) including the rationale for each change. Formatting changes and minor administrative changes (typos, grammatical changes, minor clarifications in wording) are not included in this table.

| Original Text | Modified Text | Rationale |
|--|---|---|
| Inclusion Criteria | | |
| [Moved from Exclusion Criteria] | Patient must be off prednisone or mycophenolate mofetil for >1 month and alkylating agents for >6 months. The rationale is to minimize the potential confounding effect of delayed benefits from previous immunosuppressive agents and to reduce the risk of too much immunosuppression from the combined previous drug exposure plus trial drug exposure, e.g. infections. | The rationale is to minimize the potential confounding effect of delayed benefits from previous immunosuppressive agents and to reduce the risk of too much immunosuppression from the combined previous drug exposure plus trial drug exposure, e.g. infections. |
| Exclusion Criteria | | |
| Age <18 years or >80 years | [Text deleted] | Age requirements are already part of the inclusion criteria |
| Laboratory Testing | | |
| Proteinuria results at Time 0, 6, 12, 18 and 24 months will be taken as the mean of two 24-hour urine collections done back to back, and the mean of these 2 collections will be used to determined CR, PR, or NR. | Proteinuria results at Time 0, 6, 12, 18 and 24 months will be taken as the mean of two 24-hour urine collections done back to back (within 14 days of each other), and the mean of these 2 collections will be used to determined CR, PR, or NR. | Clarification of the timing of the 2 24-hour urine collections. |
| Concomitant Treatment | | |
| [Text Addition] | Patients who are currently taking rosuvastatin (crestor) and are randomized to the CSA treatment arm will need to be changed to atorvastatin or its equivalent. | Clarification of acceptable concomitant statin treatment for those in the CSA arm. |
| Appendices | | |
| | [Text Deleted] | All appendices were |

| | | |
|--|--|--|
| | | removed from the protocol and have been inserted into the study lab manual as they deal with sample collection and shipping. |
|--|--|--|

Protocol: Membranous Nephropathy Trial Of Rituximab (MENTOR)

Version: 8.0, August 21, 2012

Summary of Changes

An amendment has been drafted to the MENTOR study protocol. The following is a summary of key changes to the previous version of the protocol (7.0, May 17, 2012) including the rationale for each change. Formatting changes and minor administrative changes (typos, grammatical changes, minor clarifications in wording) are not included in this table.

| Original Text | Modified Text | Rationale |
|-------------------|--|--|
| Cover Page | | |
| [Text Addition] | K.Thorpe (Co-I) | Kevin Thorpe, a statistician, has been added to the list of committee members. |
| [Text Addition] | NEPTUNE6804 | |
| [Text Addition] | ClinicalTrials.gov identifier: NCT01180036 | |
| Overview | | |
| [Text Addition] | <p>1.1 Overview</p> <p>This prospective, randomized, controlled phase III nephrology study will determine whether rituximab is non inferior to cyclosporine in inducing long-term remission of proteinurea in patients with idiopathic membranous nephropathy (IMN). Subjects are randomized to open-label courses of treatment: IV rituximab (RTX), 1000 mg (2 infusions, 14 days apart), or oral cyclosporine (CSA) 3.5 to 5 mg/kg/day. Subjects are evaluated at 6 months; those that meet treatment response criteria will continue study medication and receive either 2 further infusions of RTX or a further 6 months of daily CSA treatment. Throughout the study period, laboratory and safety parameters will be collected and documented.</p> <p>Study Hypothesis: B cell targeting with Rituximab is more effective than Cyclosporine in inducing long term</p> | A summary of the study has been added as per NIH's requirement |

| | | |
|---|--|---|
| | remission of proteinuria. Comparison(s): IV rituximab (RTX), 1000 mg (2 infusions, 14 days apart; repeated at 6 months or oral cyclosporine (CSA) 3.5 to 5 mg/kg/day from 6-12 months if a substantial reduction in proteinuria(equal to or >30%) is seen at 6 months. | |
| DNA Testing | | |
| Recent data show that in patients with membranous nephropathy, mutations in HLA-DQA1 and PLA2R1 alleles are associated with an increased risk for developing membranous nephropathy. ⁸⁰ One of these alleles, the PLA2R1, is in fact the gene for the PLA2 receptor. In our pilot studies, ~70% of the patients with membranous nephropathy had antibodies present in circulation against PLA2 receptor. ⁷⁷ Thus we would like to evaluate a linkage between the presence of these antibodies and potential genetic mutations in this group of patients. We will ask for the patients consent to further evaluate this linkage by collecting samples of blood to perform additional testing as part of their informed consent form (ICF). | [No change] | Moved to section entitled “Ancillary studies” as the DNA testing is optional. Individuals can participate in the main study without consenting to participate in the ancillary studies. |
| Inclusion Criteria | | |
| Treatment with an ACEi and/or ARB, for ≥3 months prior to randomization and adequate blood pressure control (target BP is <130/80 mm Hg in >75% of | Treatment with an ACEi and/or ARB, for ≥3 months prior to randomization and adequate blood pressure control (target BP is <130/80 mm Hg in >75% of the readings, but subjects with BP <140/80 mmHg in >75% of the readings | While the target BP is <130/80 mmHg, some variation from the target is acceptable as long as it’s not more than a 10 mmHg systolic increase (i.e., |

| | | |
|--|--|---|
| <p>the readings). Patients with documented evidence of >3 months treatment with maximal angiotensin II blockade, on an HMG-CoA reductase inhibitor, and BP control (BP <130/80 mm Hg in >75% of the readings) who remain with proteinuria >5g/24h may enter the study immediately and be randomized to RTX/CSA without the need of the run-in/conservative phase of the study. However, in addition these patients must have a documented <50% reduction in proteinuria compared to previous 24 hr proteinuria or Uprot/UCr ratio estimates during this period of ACEi and/or ARB treatment otherwise they must fulfill the run-in requirement,</p> <ul style="list-style-type: none"> ○ (Please refer to manual of operation for clarification of tests mandated for patients who are randomized without the run-in period) | <p>will be eligible). Patients with documented evidence of >3 months treatment with maximal angiotensin II blockade, on an HMG-CoA reductase inhibitor, and BP control (BP <140/80 mm Hg in >75% of the readings) who remain with proteinuria >5g/24h may enter the study immediately and be randomized to RTX/CSA without the need of the run-in/conservative phase of the study. However, in addition these patients must have a documented <50% reduction in proteinuria compared to previous 24 hr proteinuria or Uprot/UCr ratio estimates during this period of ACEi and/or ARB treatment otherwise they must fulfill the run-in requirement,</p> <ul style="list-style-type: none"> ○ (Please refer to manual of operation for clarification of tests mandated for patients who are randomized without the run-in period) | <p>140/80).</p> |
| <p>Estimated GFR ≥ 40 ml/min/1.73m² while taking ACEi/ARB therapy. The GFR will be estimated using the 4 variable MDRD equation as published in the NKF-CKD guidelines.⁸¹ This approach is adopted, rather than the much more expensive and more invasive techniques (e.g. inulin or iothalamate clearance) since the</p> | <p>Estimated GFR ≥ 40 ml/min/1.73m² while taking ACEi/ARB therapy OR quantified endogenous creatinine clearance >40 ml/min based on a 24 hour urine collection. The GFR will be estimated using the 4 variable MDRD equation as published in the NKF-CKD guidelines.⁸¹ This approach is adopted, rather than the much more expensive and more invasive techniques (e.g. inulin or iothalamate clearance) since the likelihood of detecting significant changes in GFR in this short term study</p> | <p>Either the eGFR or CrCl will suggest whether the patient has sufficient reserve to handle the study treatment. Furthermore, it is not clearly understood which of these measures best reflects true GFR.</p> |

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| likelihood of detecting significant changes in GFR in this short term study is remote regardless of which method is chosen. At entry into the study and at set time points thereafter patients will also have a 24h urine collection for calculation of CrCl and proteinuria. | is remote regardless of which method is chosen. At entry into the study and at set time points thereafter patients will also have a 24h urine collection for calculation of CrCl and proteinuria. | |
| Exclusion Criteria | | |
| Estimated GFR <40 ml/min/1.73m ² . The rationale for the criteria is that patients with severe reduction in GFR are likely to have significant interstitial and glomerular scarring and are less likely to benefit from treatment. | [Text deleted] | See change to inclusion criteria above. |
| History of resistance to CSA or RTX. Patients who previously responded to CSA with either a CR or PR but relapsed off CSA are eligible. | History of resistance to CSA (or other calcineurin inhibitors, e.g. tacrolimus) or RTX. Patients who previously responded to CSA/CNI or RTX with either a CR or PR but relapsed off CSA/CNI after 3 months, or relapsed off RTX after 6 months, are eligible. | Resistance to other CNIs and eligible duration before relapse has been specified for clarification. |
| Rituximab | | |
| Dosing at study month 6 will be independent of CD19+ B cell count. | Dosing at study month 6 will be independent of CD19/20+ B cell count. | CD19/20+ has been added for clarification. |
| Dosage and Administration of Rituximab | | |
| The first infusion of both courses (Day 1 and Day 168) of RTX will be administered IV at an initial rate of 50 mg/hr. | The first infusion of both courses (Day 1 and Day 181) of RTX will be administered IV at an initial rate of 50 mg/hr. | The 2 nd round of infusions will not be administered until after the 6 month (Day 180) central lab results have been received and it has been determined whether or not the patient has failed therapy. |
| If the infusion was well tolerated, subsequent infusions (Day 15 and Day 182) may be administered at an initial rate of 100 mg/hr | If the infusion was well tolerated, subsequent infusions (Day 15 and Day 195) may be administered at an initial rate of 100 mg/hr and increased by 100 mg/hr increments at 30-minute | The day of the 2 nd infusion in each series was also adjusted (see above). |

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| and increased by 100 mg/hr increments at 30-minute intervals, to a maximum of 400 mg/hr as tolerated. | intervals, to a maximum of 400 mg/hr as tolerated. | |
| There will be a +/- 3 day window for each study visit to account for weekends, holidays, and scheduling conflicts. | Although most study visits have a window of +/- 10 days, the 2 nd infusion in each series (i.e., Day 15 and Day 195) will have a more restricted window of +/- 3 days. Specifically, the 2 nd infusion in each series should occur 14 days +/- 3 days from the time of the 1 st infusion in the series. The window is to account for weekends, holidays, and scheduling conflicts. | The 1 st infusion in each series cannot occur until the central lab results are received and reviewed so we have allowed greater flexibility in the timing of this visit. However, the 2 nd infusion should occur as close as possible to 2 weeks (14 days) from the 1 st infusion so the window is smaller. |
| Cyclosporine | | |
| CSA will be tapered by approximately 1/3 of the maintenance dose monthly and hence discontinued after three months. | CSA will be tapered by approximately 1/3 of the maintenance dose monthly and hence discontinued after two months. | CSA dose tapering period is changed to 2 months. Since initial 1/3 dose reduction will occur at the beginning of tapering period, we only require 2 months to discontinue CSA. |
| Pharmaceutical logistics | | |
| Appropriate quantities of the medication will be supplied to the individual from the individual sites at Time 0 and Months 3, 6, 9, and 12. | Appropriate quantities of the medication will be supplied to the individual from the individual sites following randomization and again at months 3, 6, 9, and 12. | Time 0 has been clarified to “following randomization” |
| Anti-PLA2R Assay | | |
| Mayo Clinic will then send the samples in bulk to Dr. Laurence Beck at Boston University or to Dr. Paul Brenchley at the Manchester Royal Infirmary in the UK. | Mayo Clinic will then send the samples in bulk to Dr. Paul Brenchley at the Manchester Royal Infirmary in the UK. | The sample will be sent to Dr. Paul Brenchley in UK only. |
| Table 2a: Test Schedule and Monitoring for Cyclosporine Treatment Arm | | |
| [Text addition] | -Day # added for each visit -Randomization, Adverse Events, Dispense/Return Medication rows and Day 455 (14 mo) column have been added to the table. | CSA treatment schedule table has been updated to provide more clarification |
| [Footnote #1] 1. Potassium, Cr checked at 2 weeks post | [Footnote #1] 1. Potassium, Cr checked at 2 weeks +/- 3 days post Cyclosporine | +/- 3 days has been added to specify the window for the |

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| Cyclosporine initiation. If Cyclosporine is increased during treatment, potassium, Cr and CsA will be rechecked after 2 weeks | initiation. If Cyclosporine is increased during treatment, potassium, Cr and CsA will be rechecked after 2 weeks +/- 3 days | study visit. |
| [Text addition] | ^a Only for participants who continue on CSA at 6 months ^b Telephone or in-clinic visit to confirm successful down-titration of CSA for patients who continue on medication to 12 months (if by phone, medication should be returned at 18 month visit) | 2 footnotes have been added for newly added rows |
| Table 2b: Test Schedule and Monitoring for Rituximab Treatment Arm | | |
| [Text addition] | [Footnote #3]: 3. In the rare case where B cells (CD19/20+ cells) are not replete at Month 12, PBFC will be repeated at Month 18 | B cells will be considered replete when >15 cells/microliter. This is expected to occur by Month 12 or sooner, but in rare cases, it may take longer. This is a precautionary measure. |
| [Text addition] | -Day # added for each visit -Randomization, Adverse Events rows have been added to the table. | RTX treatment schedule table has been updated to provide more clarification |
| Day 168/Day 182 | Day 181/Day 195 | RTX infusion dates have been changed. Please see above. |
| Concomitant Treatment | | |
| Patients in both study arms will also be started on single strength Bactrim one a day (or its equivalent) for pneumocystis pneumonia prophylaxis. This treatment will continue until study medication is stopped, up to 15 months. | Patients randomized to receive Rituximab should be started on single strength Bactrim one a day (or its equivalent) for pneumocystis pneumonia prophylaxis. This treatment will continue until study medication is stopped and B cells (CD 19/20+) have been repleted (>15 cells/microliter on peripheral blood flow cytometry). These B cells are expected to be replete by Month 12. In the rare event that these B cells are not replete by Month 12, flow cytometry should be repeated at Month 18 (see table 2b). | Only patients in the Rituximab arm require treatment with Bactrim. In rare cases, B cell populations may take longer to recover than expected. |
| Stopping points for medications | | |
| 5. Persistent elevation of serum potassium <u>>5.8</u> | 5. Persistent elevation of serum potassium <u>>6.0 mEq/l</u> despite diet | Per Executive Committee |

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| mEq/l despite diet changes, ACEi/ARB reduction, diuretic dose increase (and in the CSA arm dose reduction). | changes, ACEi/ARB reduction, diuretic dose increase (and in the CSA arm dose reduction). | |
| DMCC | | |
| <p>The Data Coordinating Center (DCC) is located at the Applied Health Research Center at St. Michael's Hospital/University of Toronto and consists of coordinating center manager, project manager, regulatory manager, and administrative support. The DCC will be responsible for facilitating all aspects of the study planning, training, implementation and analysis phases.</p> | <p>The Data Management and Coordinating Center (DMCC) is located at the Applied Health Research Center at St. Michael's Hospital/University of Toronto. The DMCC will be responsible for facilitating all aspects of the study planning, training, implementation, data collection and analysis.</p> <p>The clinical information collected for this study will be stored at the DMCC at the Applied Health Research Centre of St. Michael's Hospital in Toronto, ON, Canada and also sent to a federal data repository held by the Office of Rare Diseases Network (ORDN) and located at the University of South Florida (USF) in Tampa, FL.</p> <p>The DMCC database and the USF database use several layers of protection for the clinical data stored there. These databases meet all of the local and federal security requirements for research datacenters. Participant information is stored only using a study ID.</p> | Text addition to explain the nature of our collaboration with the ORDN. |
| Data security | | |
| [Text addition] | 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 and the University of South Florida before accrual can occur from the clinical site. | Clarification of the data transfer process and how privacy will be protected. |

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| | <p>Demographic and adverse event data collected as part of this trial will be transferred to the USF on a monthly basis throughout the trial, and uploaded into the data repository dbGaP (database of Genotypes and Phenotypes).</p> <p>A system of coded identifiers will be used to protect participant confidentiality and safety. Each participant enrolled will be assigned a local subject identifier by the DMCC which is a combination of the site 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 data is sent to the USF, the system will assign a participant ID number. Thus each participant will have two codes: the local code used to identify the patient in the eCRF and in source documentation and which is linked to personal identifiers, and a second code assigned by the USF. For all data transfers to the USF 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 USF.</p> | |
| PROTECTION FOR HUMAN SUBJECTS | | |
| [Text addition] | <p>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.</p> <p>The study protocol will be reviewed and approved by the National Institutes of</p> | <p>Clarification of GCP practices and the fact that the protocol will be reviewed and approved by the NIH.</p> |

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| | Health (NIH) before submission to individual center IRBs for approval. Participant enrollment may only begin with IRB approved consent forms. | |
| Protection of human subjects: 1. Informed consent process | | |
| [Text Addition] | 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. | Clarification of the consent process. |
| Protection of human subjects: 3. Adverse event reporting | | |
| [Text addition] | Each site's Principal 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 [how often] by the research team. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports. An <u>adverse event</u> is defined as: "...an | Clarification of adverse event reporting process. |

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| | <p>unfavorable and unintended sign, symptom or disease associated with a participant's participation in the study."</p> <p><u>Serious adverse events</u> include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."</p> <p>An <u>unexpected adverse event</u> is defined as any adverse experience...the specificity or severity of which is not consistent with the risks of information described in the protocol.</p> <p><u>Expected adverse events</u> 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</p> <p>All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.</p> <ul style="list-style-type: none"> • Within <u>24 hours</u> (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that: <ul style="list-style-type: none"> ○ Is considered life-threatening/disabling or results in death of subject -OR- ○ Is Unexpected/Unanticipated • Investigators must report all other reportable SAEs within <u>5 working days</u> (of learning of the event). • All other (suspected) reportable AEs must be reported to the RDCRN | |
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| | <p>within <u>20 working days</u> of the notification of the event or of the site becoming aware of the event.</p> <p>Local institutional reporting requirements to IRBs, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician.</p> | |
| [Text addition] | <p>Sites will enter all Adverse Event data into the study eCRF. Upon entry of a serious adverse event, the system will notify the study team, study PIs, the designated Medical Review Officer (MRO) and any other designated personnel by email. The MRO will then review the SAE data to determine causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the serious adverse event. The MRO [and, if applicable, sponsor] may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the serious 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 eCRF maintains audit trails and stores data (and data updated) related to any adverse event in the study.</p> | Clarification of adverse event reporting process. |
| Study Discontinuation | | |
| [Text addition] | <p>The Study Sponsor, DSMB and local IRBs (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:</p> <ul style="list-style-type: none"> • Accrual has been met • The study objectives have been met • The Study Investigators believe it is not safe for the study to continue • The DSMB suspends or closes | Clarification of study discontinuation rules. |

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| | the trial | |
| <i>Subject Discontinuation</i> | | |
| [Text addition] | <p>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.</p> <ul style="list-style-type: none"> • Withdrawal of consent • Withdrawal by the participant • Withdrawal by the investigator • Intercurrent illness or event that precludes further visits to the study site | Clarification of reasons for subject discontinuation |

Protocol: Membranous Nephropathy Trial Of Rituximab (MENTOR)

Version: 9.0, 30-Jul-2013

Summary of Changes

An amendment has been drafted to the MENTOR study protocol. The following is a summary of key changes to the previous version of the protocol (8.0, August 21, 2012) including the rationale for each change. Formatting changes and minor administrative changes (typos, grammatical changes, minor clarifications in wording) are not included in this table.

| Original Text | Modified Text | Rationale |
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| Overview | | |
| Study Hypothesis: B cell targeting with Rituximab is more effective than Cyclosporine in inducing long term remission of proteinuria. | Study Hypothesis: B cell targeting with Rituximab is non-inferior to Cyclosporine in inducing long-term remission of proteinuria. | Wording revised to be consistent with preceding paragraph and with the sample size estimation described later in the protocol. |
| Comparison(s): IV rituximab (RTX), 1000 mg (2 infusions, 14 days apart; repeated at 6 months or oral cyclosporine (CSA) 3.5 to 5 mg/kg/day from 6-12 months if a substantial reduction in proteinuria (equal to or >30%) is seen at 6 months. | Comparison(s): IV rituximab (RTX), 1000 mg (2 infusions, 14 days apart; repeated at 6 months if a substantial reduction in proteinuria (equal to or >25%) is seen at 6 months) or oral cyclosporine (CSA) 3.5 to 5 mg/kg/day for 6 months (continued for another 6 months if a substantial reduction in proteinuria (equal to or >25%) is seen at 6 months). | Wording revised for clarity. Also, continued treatment with study drug now requires a reduction in proteinuria of $\geq 25\%$ at 6 months rather than $\geq 30\%$. The initial cut-off of 30% was somewhat arbitrary (best guess based on previous pilot studies) with the idea being to avoid exposing patients to additional immunosuppressant therapy if the treatment is obviously not working. On the other hand, it's unfair to stop treatment in patients who are showing some response to treatment and it has been suggested that 30% is too high a cut-off (based on recent feedback from treating clinicians who have seen a handful of cases with reductions in the 28% range). |
| Specific Aims | | |
| 1. That B cell targeting with | 1. That B cell targeting with | Wording revised to be |

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| Rituximab is more effective than Cyclosporine in inducing long-term remission (complete or partial) of proteinuria in patients with IMN. | Rituximab is non-inferior to Cyclosporine in inducing long-term remission (complete or partial) of proteinuria in patients with IMN. | consistent with the overview (see above) and the sample size estimation described later in the protocol. |
| Overall Research Design and Methods | | |
| At the 6-month post-randomization visit, patients who have been randomized to either CSA or RTX but who do not have a reduction in proteinuria $\geq 30\%$ (confirmed on repeat measurements within 2 weeks) will be considered treatment failures and exit the study. Data from that point onward will be censored. Patients in the RTX group who have a reduction in proteinuria of equal to or $>30\%$ at 6 months will be given another identical course of RTX (1g x 2). [....] The same definition of response will apply to the CSA arm at 6 months. An equal to or $>30\%$ reduction in proteinuria will dictate continuation of the CSA for an additional 6 months (total of 12 months of full dose CSA). | At the 6-month post-randomization visit, patients who have been randomized to either CSA or RTX but who do not have a reduction in proteinuria $\geq 25\%$ (confirmed on repeat measurements within 2 weeks) will be considered treatment failures and exit the study. Data from that point onward will be censored. Patients in the RTX group who have a reduction in proteinuria of equal to or $>25\%$ at 6 months will be given another identical course of RTX (1g x 2). [...] The same definition of response will apply to the CSA arm at 6 months. An equal to or $>25\%$ reduction in proteinuria will dictate continuation of the CSA for an additional 6 months (total of 12 months of full dose CSA). | The initial cut-off of 30% was somewhat arbitrary (best guess based on previous pilot studies) with the idea being to avoid exposing patients to additional immunosuppressant therapy if the treatment is obviously not working. On the other hand, it's unfair to stop treatment in patients who are showing some response to treatment and it has been suggested that 30% is too high a cut-off (based on recent feedback from treating clinicians who have seen a handful of cases with reductions in the 28% range). |
| Primary Endpoint (see also Table 1) | | |
| CR or PR (defined as per table 1) at 24 months after randomization will be the primary endpoint. This will be assessed in the protocol adherent patients. | CR or PR (defined as per table 1) at 24 months after randomization will be the primary endpoint. This will be assessed in an intention to treat (ITT) analysis . | Changed to intention to treat analysis to be consistent with the statistical and analytical plan described later in the protocol. |
| Inclusion Criteria | | |
| Treatment with an ACEi and/or ARB, for ≥ 3 months prior to randomization and | Treatment with an ACEi and/or ARB, for ≥ 3 months prior to randomization and adequate blood | Cases of patients with documented intolerance to ACEi/ARB therapy are |

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| <p>adequate blood pressure control (target BP is <130/80 mm Hg in >75% of the readings, but subjects with BP <140/80 mmHg in >75% of the readings will be eligible).</p> <p>Patients with documented evidence of >3 months treatment with maximal angiotensin II blockade, on an HMG-CoA reductase inhibitor, and BP control (BP <140/80 mm Hg in >75% of the readings) who remain with proteinuria >5g/24h may enter the study immediately and be randomized to RTX/CSA without the need of the run-in/conservative phase of the study. However, in addition these patients must have a documented <50% reduction in proteinuria compared to previous 24 hr proteinuria or Uprot/UCrea ratio estimates during this period of ACEi and/or ARB treatment otherwise they must fulfill the run-in requirement,</p> | <p>pressure control (target BP is <130/80 mm Hg in >75% of the readings, but subjects with BP <140/80 mmHg in >75% of the readings will be eligible).</p> <p>OR</p> <p>If patient is intolerant to even a very low dose of either ACEi or ARB therapy, approval for participation in the trial has been obtained from the study PI(s) prior to randomization.</p> <p>Patients with documented evidence of ≥3 months treatment with maximal angiotensin II blockade, on an HMG-CoA reductase inhibitor, and BP control (BP <140/80 mm Hg in >75% of the readings) who remain with proteinuria ≥5g/24h and meet the other eligibility criteria (as confirmed at the Time 0 visit by the central lab results) may enter the treatment phase of the study and be randomized to RTX/CSA without the need of the run-in/conservative phase of the study. However, in addition these patients must have a documented <50% reduction in proteinuria compared to previous 24 hr proteinuria or Uprot/UCrea ratio estimates during this period of ACEi and/or ARB treatment otherwise they must fulfill the run-in requirement.</p> | <p>expected to be very rare. The purpose of exposing patients to treatment with ACEi and/or ARB therapy prior to randomizing them to study drug is to see if they will respond to conservative therapy and can thereby avoid more aggressive treatment with immunosuppressive drugs. However, given that conservative therapy is not an option for patients who are intolerant, it would be unfair to exclude these patients from participation in the trial and from access to potentially helpful immunosuppressant drugs.</p> <p>Other revisions to this criterion are for clarity only.</p> |
| <p>Exclusion Criteria</p> | | |
| <p>History of resistance to CSA (or other calcineurin inhibitors, e.g. tacrolimus) or RTX. Patients who previously responded to CSA/CNI or RTX with either a CR or PR but relapsed off CSA/CNI after 3 months, or relapsed off RTX after 6 months, are</p> | <p>History of resistance to CSA (or other calcineurin inhibitors, e.g. tacrolimus), RTX or alkylating agents (e.g. Cytoxan). Patients who previously responded to CSA/CNI, RTX or alkylating agents with either a CR or PR but relapsed off CSA/CNI after 3 months, or relapsed off RTX or alkylating agent</p> | <p>In addition to CSA/CNI or RTX, IMN patients may also have been previously exposed to alkylating agents. Only patients with a reasonable possibility of responding to study drug and who have had time to wash out any previous treatment from their system</p> |

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| eligible. | after 6 months, are eligible. | should be considered for randomization. |
| Rituximab | | |
| A second course of RTX 1000 mg IV will be administered at study month 6 for individuals who have not achieved a complete remission, but have achieved at least a $\geq 30\%$ reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not a CR). Dosing at study month 6 will be independent of CD19/20+ B cell count. The rationale for retreating patients who have had an equal to or $> 30\%$ reduction in proteinuria at 6 months is based on our experience in our pilot studies (first study using 1000 mg on Days 1 and 15 and second study using 4 weekly doses of $375\text{mg}/\text{m}^2$) where an increase in the proteinuria remission rate was achieved after a second course of treatment. In our 2 studies repeated courses of RTX were not associated with additive adverse effects in comparison to their first course. If after six months the reduction in proteinuria is less than 30% compared to baseline the RTX treatment will not be repeated, the patient will exit from the study and will be considered a failure of therapy. | A second course of RTX 1000 mg IV will be administered at study month 6 for individuals who have not achieved a complete remission, but have achieved at least a $\geq 25\%$ reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not a CR). Dosing at study month 6 will be independent of CD19/20+ B cell count. The rationale for retreating patients who have had an equal to or $> 25\%$ reduction in proteinuria at 6 months is based on our experience in our pilot studies (first study using 1000 mg on Days 1 and 15 and second study using 4 weekly doses of $375\text{mg}/\text{m}^2$) where an increase in the proteinuria remission rate was achieved after a second course of treatment. In our 2 studies repeated courses of RTX were not associated with additive adverse effects in comparison to their first course. If after six months the reduction in proteinuria is less than 25% compared to baseline the RTX treatment will not be repeated, the patient will exit from the study and will be considered a failure of therapy. | The initial cut-off of 30% was somewhat arbitrary (best guess based on previous pilot studies) with the idea being to avoid exposing patients to additional immunosuppressant therapy if the treatment is obviously not working. On the other hand, it's unfair to stop treatment in patients who are showing some response to treatment and it has been suggested that 30% is too high a cut-off (based on recent feedback from treating clinicians who have seen a handful of cases with reductions in the 28% range). |
| Cyclosporine | | |
| [Text addition] | Neoral brand (Novartis) is the preferred Cyclosporine product for this trial. Participating sites must contact the DMCC <i>prior</i> to dispensing any other brand to study | While the preference for Neoral is explained in the study's Manual of Operations, the DSMB suggested that it be included in the protocol as |

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| | participants as not all brands of Cyclosporine are bio-equivalent. | well. |
| Patients will have their doses adjusted according to their blood levels of CSA as monitored every 2 weeks until the target trough level is reached. | Patients will have their doses adjusted according to their blood levels of CSA as monitored every 2 weeks +/- 3 days until the target trough level is reached. | Clarification of visit window. |
| If there has been at least an equal to or >30% reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not complete remission) the CSA will be continued for an additional six months. | If there has been at least an equal to or >25% reduction compared to their central laboratory (Mayo Clinic Rochester) baseline (Time 0) proteinuria (but not complete remission) the CSA will be continued for an additional six months. | See above. |
| A persistent and otherwise unexplained increase in serum creatinine >30% will prompt an approximate 25% dose reduction of CSA, aiming for 25% reduction in CSA trough level. For example, if trough CSA was 175 ng/ml reduce to 130 ng/ml and if with this dose reduction the creatinine does not return to baseline levels within 3 weeks, then a second dose reduction [...] | A persistent and otherwise unexplained increase in serum creatinine >30% will prompt an approximate 25% dose reduction of CSA, aiming for a corresponding 25% reduction in CSA trough level (for example, if trough CSA was 175 ng/ml the goal would be to reduce it to 130 ng/ml by reducing the dose by approximately 25% - dose reduction is approximate because CSA is only available in specific dose strengths (25mg, 100mg) and therefore some rounding may be required). If with this dose reduction the creatinine does not return to within 30% of baseline levels within 3 weeks, then a second dose reduction [...] | Clarification of process of dose reduction. |
| Serum potassium level will be checked at the initiation of Cyclosporine in conjunction with blood draw for CSA level. If Cyclosporine is increased during treatment, potassium will be rechecked two weeks post increase in CSA dosage. | Serum potassium and serum creatinine levels will be checked at the initiation of Cyclosporine in conjunction with blood draw for CSA level. If Cyclosporine dose is changed during treatment, potassium, creatinine and CSA levels will be rechecked two weeks +/- 3 days post increase/decrease in CSA dosage. | Wording revised for consistency with the test schedule in Table 2a. Also, for safety and to ensure that CSA level remains at target, parameters should be rechecked every time there is a change in CSA dose (i.e., either increase OR decrease). |

| Pharmaceutical logistics | | |
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| [Text addition] | Neoral brand (Novartis) is the preferred Cyclosporine product for this trial. If it is not possible to source Neoral from the local hospital pharmacy, please contact the DMCC prior to dispensing any study drug as not all brands of Cyclosporine are bio-equivalent. | While the preference for Neoral is explained in the study's Manual of Operations, the DSMB suggested that it be included in the protocol as well. |
| Appropriate quantities of the medication will be supplied to the individual from the individual sites following randomization and again at months 3, 6, 9, and 12. | Appropriate quantities of the medication will be supplied to the individual from the individual sites following randomization and again at months 3, 6, 9, and 12 and may also be supplied at other time points should dose adjustments be required. | Some flexibility is needed as multiple dose adjustments may be required, particularly during the early part of the treatment phase. All dispensed CSA will be tracked in the accountability log of the eCRF. |
| Screening Visit | | |
| [...] and starting the patient on the initial step of the protocol, i.e. maximize angiotensin II blockade as described in the run-in phase below. | Candidates who have not yet been exposed to conservative therapy will be started on the initial step of the protocol, i.e. maximize angiotensin II blockade as described in the run-in phase below. [...] In addition to data from this screening visit, retrospective data from at least one additional time point within the previous year will be collected whenever possible. Retrospective data collection needs to include proteinuria, urine creatinine, creatinine clearance, Uprot/Ucrea ratio, systolic blood pressure, diastolic blood pressure, serum creatinine, and serum albumin values. | Given that some patients will require the run-in phase while others will not, clarification has been provided regarding the screening requirements for each of these two possibilities. In particular, certain lab values from within the previous year are requested regardless of whether or not the run-in is required. The purpose is to verify disease trajectory, proteinuria trends, and study eligibility. |
| [Text addition] | Candidates who have already been on conservative therapy for 3 months or more without a documented decrease in proteinuria of >50% as compared to a value from 12 months ago (or a pro-rated percent reduction if the time period is less than a year, for instance a decrease in proteinuria of >25% as compared to a value from 6 months ago – see MOP for further details) do not need to fulfill the run-in requirement as long | Given that some patients will require the run-in phase while others will not, clarification has been provided regarding the screening requirements for each of these two possibilities. In particular, certain lab values from within the previous year are requested regardless of whether or not the run-in is required. The purpose is to |

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| | <p>as their local labs indicate that proteinuria remains $\geq 5\text{g}/24\text{h}$. These individuals should proceed directly to full screening as part of the Time 0 visit and do not require a formal screening visit. Review of the eligibility criteria, lab requirements, and the review and signing of the informed consent form will take place at the Time 0 visit for these individuals. However, retrospective data from at least two previous time points (approximately 3 months prior to obtaining consent plus one more time point within the previous year) will still be collected whenever possible. Retrospective data collection needs to include proteinuria, urine creatinine, creatinine clearance, Uprot/Ucrea ratio, systolic blood pressure, diastolic blood pressure, serum creatinine, and serum albumin values.</p> | <p>verify disease trajectory, proteinuria trends, and study eligibility.</p> |
| Patient Monitoring and Evaluation | | |
| <p>Patients will be followed for 2 years following randomization to monitor for the occurrence of adverse events, late remissions, relapses, GFR changes and development of end stage renal disease. The first 12 months of the study will be considered as the treatment period while the remaining 12 months will be considered as an observational period including the period of tapering to discontinuation in the Cyclosporine arm.</p> | <p>Patients will be followed for 2 years following randomization to monitor for the occurrence of adverse events, late remissions, relapses, GFR changes and development of end stage renal disease. The first 12 months of the study will be considered as the treatment period while the remaining 12 months will be considered as an observational period including the period of tapering to discontinuation in the Cyclosporine arm.</p> | <p>No change to text. However, text relocated from later in the paragraph to the beginning.</p> |
| <p>Cyclosporine trough level measurements will be done every 2 weeks until levels are stable and at target. Potassium will also</p> | <p>Cyclosporine trough level measurements will be done every 2 weeks +/- 3 days until levels are stable and at target. Potassium and creatinine will also be checked 2</p> | <p>Wording revised for consistency with the test schedule in Table 2a. Also, for safety and to ensure that CSA level remains at target,</p> |

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| <p>be checked 2 weeks after initiation of Cyclosporine, in conjunction with CSA level. If Cyclosporine is increased during treatment, potassium will be rechecked at the 2 week time point.</p> | <p>weeks +/- 3 days after initiation of Cyclosporine, in conjunction with CSA level. If Cyclosporine dose is changed during treatment, potassium and creatinine will be rechecked at the 2 week +/- 3 days time point.</p> | <p>parameters should be rechecked every time there is a change in CSA dose (i.e., either increase OR decrease).</p> |
| <p>Patients who cannot tolerate the medications or who discontinue study medication will continue to receive follow-up as scheduled with study laboratory testing limited to serum creatinine and 24h Uprot/UCr ratio at the same time intervals as those continuing in the trial. However, the therapeutic/management plan for these patients who exit the study will be solely at the discretion of the managing nephrologist.</p> | <p>Patients who cannot tolerate the medications or who are treatment failures at 6 months will exit the study. Patients who go in to complete remission will discontinue study drug but will continue with full visit follow-ups until month 24 as scheduled. However, the therapeutic/management plan for these patients will be solely at the discretion of the managing nephrologist. Likewise, patients who relapse from either a partial or complete remission will continue with the visit schedule, but will be managed at the discretion of the treating nephrologist. Finally, for patients who temporarily stop taking Cyclosporine, there are three possible scenarios:</p> <ol style="list-style-type: none"> 1. Patients who discontinue Cyclosporine for < 2 weeks may simply be restarted on the study drug and continue to fully participate in the trial. The treatment is to be restarted at its former dose and the patient returned to the regularly scheduled follow-ups including the periodic sampling for assessing potassium, creatinine and CSA trough levels. 2. Patients who discontinue Cyclosporine for ≥ 2 weeks, but < 4 weeks may also be restarted on the study drug at its former dose and fully participate in the trial, but the | <p>As part of the study endpoints, it is important to follow patients in remission and also to see how many relapse and when.</p> <p>Patients randomized to the Cyclosporine arm are meant to take the study drug for 12 months + 2 months of tapering. In order to ensure that all Cyclosporine patients receive approximately the same amount of drug, any stoppages of ≥ 2 weeks (but less than 4 weeks) will need to be made up at the end of month 12. However, if patients stop drug for > 4 weeks then they will not be restarted on Cyclosporine, but will continue to be followed for observation.</p> |

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| | <p>temporary discontinuance will be logged as a protocol deviation and the missed treatment days will be made up at the end of month 12. This is to ensure that all patients receive approximately 12 months of treatment with study drug.</p> <p>3. Patients who discontinue Cyclosporine for ≥ 4 weeks will not be restarted on study drug, but will continue with full visit follow-ups until month 24 as scheduled. The therapeutic/management plan will be solely at the discretion of the managing nephrologist.</p> | |
| Laboratory Testing | | |
| [Text addition] | <p>In order to control for the possibility of under collection or over collection of urine, a maximum 20% variation in total 24h urine creatinine (mg/specimen) will be allowed between the two urine collections required at Time 0, 6 months, 12 months, 18 months, and 24 months. A difference of greater than 20% will necessitate a third urine collection (also to be sent to the central lab), with the values from one of the two previous collections being discarded. We will use the subject's weight to approximate the expected total urine creatinine (mg/kg) to determine which of the previous collections is closest to the truth and which should be discarded. It is anticipated that a 3rd urine collection will be needed in <10% of cases. Proteinuria will be calculated as the mean of these two 24-hour urine collections.</p> | <p>In response to some observed inconsistencies in urine collection, we are allowing for the possibility that a 3rd collection may be needed in order to ensure the validity of the data being reported. Proteinuria values will be of particular importance when analyzing the study endpoints.</p> |
| Anti-PLA2R Assay | | |
| Serum samples from all 126 subjects will be collected at Screening, Time 0, and | Serum samples from all 126 subjects will be collected at the Screening visit (when applicable), Time 0, and | For run-in exempt patients, the first serum sample for the anti-PLA2R assay may not be |

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| months 3, 6, 9, 12, 18, and 24. | months 3, 6, 9, 12, 18, and 24. | collected until Time 0. |
| Histopathology | | |
| Renal pathology will be centrally reviewed at the end of the study by Dr. Sanjeev Sethi, from the Pathology Department at Mayo Clinic Rochester. | Following randomization, biopsy slides will be centrally reviewed on an on-going basis (but as soon as possible following randomization) by the Pathology Department at Mayo Clinic Rochester and/or the Pathology Department at the University Hospital Network, Toronto. | Clarification of the central review that is required for the study. Rather than wait for the end of the study (when it may be more difficult to access the slides), the central review will be done on a rolling basis as soon as possible following randomization. We anticipate that the pathologists at Mayo and UHN will work together to accomplish the review promptly so that slides can be returned to their respective centers. |
| Tables 2a and 2b | | |
| [Text deletion] | | The collection of adverse events at Time 0 was removed from both tables. Adverse events occurring prior to and up to Time 0 do not need to be reported as the patient will not yet have started study drug. All adverse events occurring subsequent to Time 0 and until study termination should be reported. |
| Run In Phase – Common Therapy for Both Arms | | |
| [Text addition] | The purpose of the run-in phase is to determine if an individual's IMN can be adequately controlled through conservative, non-immunosuppressive treatment. If proteinuria can be brought below 5g/24h after 3 months or more of conservative therapy, the individual will <u>not</u> be randomized to one of the study's treatment arms. In the rare case where a patient is intolerant to even a very low dose of ACEior ARB therapy, participation in the randomized treatment component of the trial may still be possible | A brief paragraph to clarify the reason for the run-in phase has been added. |

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| | (provided the patient still fulfills the other entry criteria), but will require review and approval by the study PI(s) prior to randomization. . | |
| Patients whose blood pressure control is not at target will receive additional medications in the following order: a loop diuretic, a cardioselective β -blocker, a non-dihydropyridine calcium channel blocker (CCB), and clonidine. | For patients whose blood pressure control is still not at target, it is recommended that they receive additional medications in the following order: a loop diuretic, a cardioselective β -blocker, a non-dihydropyridine calcium channel blocker (CCB), and clonidine. | These are meant as guidelines only so the language has been made softer. These guidelines are to be applied at the treating physician's discretion. |
| Concomitant Medications | | |
| At the start of the run-in/conservative phase of the study and as part of the standard of care for patients with nephrotic syndrome and significant hyperlipidemia, patients will be started on atorvastatin 10 mg a day | At the start of the run-in/conservative phase of the study (or at the start of the treatment phase of the study for those patients who are run-in exempt) and as part of the standard of care for patients with nephrotic syndrome and significant hyperlipidemia, patients will be started on atorvastatin 10 mg a day | Clarification regarding the timing of statin initiation for those who are run-in exempt. |
| Stopping Points for Medications | | |
| <p>1. For Cyclosporine: At 6 months, patients who failed therapy (as previously defined) will have CSA dose tapered and discontinued.</p> <p>After 12 months of therapy, regardless of the degree of proteinuria, Cyclosporine will be tapered and discontinued as described above.</p> <p>2. Patients in the RTX arm who have failed therapy at 6 months (as previously defined) will not have a repeat treatment course.</p> <p>After the second course of RTX therapy at six months they will have no further RTX therapy regardless of their proteinuria level</p> | <p>1. For Cyclosporine: At 6 months, patients in complete remission (as previously defined) will have CSA dose tapered and discontinued while those who fail therapy will have CSA discontinued immediately (no need to taper the dose).</p> <p>After 12 months of therapy, regardless of the degree of proteinuria, Cyclosporine will be tapered and discontinued as described above.</p> <p>2. Patients in the RTX arm who have either failed therapy or are in complete remission at 6 months (as previously defined) will not have a repeat treatment course.</p> <p>After the second course of RTX therapy at six months they will have no further RTX therapy regardless of their proteinuria level</p> | Stopping points #1 and #2 have been edited for consistency with what is described elsewhere in the protocol. |

| Staining original renal biopsy for CD20+ B cells | | |
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| We will obtain each patient's permission to review renal biopsy slides and to obtain the renal biopsy paraffin block in order to obtain biopsy tissue for staining with antibody against CD20 positive cells and anti-APL2R (3 slides, 3µ thick). | We will obtain each patient's permission to review renal biopsy slides and to obtain 3 unstained slides, 3 microns thick for staining with antibody against CD20 positive cells and anti-APL2R. | It is unlikely that center's will be in a position to send the renal biopsy paraffin block and 3 unstained slides are really all that is required. |
| Protection of Human Subjects: 3. Adverse Event Reporting | | |
| Adverse events will be reviewed [how often] by the research team. | Adverse events will be reviewed at least every 6 months by the research team. | This was an oversight in the earlier version of the protocol. The time frame for review of adverse events has now been added. |
| <p>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.</p> <p>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.</p> | <p>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 or product monograph.</p> <p>Expected adverse events are those that are identified in the research protocol or product monograph as having been previously associated with or having the potential to arise as a consequence of participation in the study.</p> | Clarification of what defines an unexpected versus expected adverse event. |
| [Text addition] | All adverse events that occur between Time 0 and study termination will be reported. | The time line for AE reporting has been added. The focus is on AEs that occur while a patient is on study drug and during the subsequent wash-out and observation phase. |
| 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 | All other AEs must be reported to the DMCC within 20 working days of the notification of the event or of the site becoming aware of the event. | Centers will report AEs to the Data Management and Coordinating Center (DMCC). The DMCC will in turn share AE data with the RDCRN. |

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| aware of the event. | | |
| As part of the reporting structure, SAEs will be reported to the FDA, Health Canada, Genentech Drug Safety and the Genentech medical science liaison as stipulated below. An adverse event summary form will be completed and will be summarized for each IRB, the FDA and Health Canada annually. | As part of the reporting structure, SAEs will be reported to Health Canada, Genentech Drug Safety and the Genentech medical science liaison. An adverse event summary form will be completed and will be summarized for each IRB and Health Canada annually. | This is an IND exempt study with no requirement to report SAEs to the FDA. |
| Upon entry of a serious adverse event, the system will notify the study team, study PIs, the designated Medical Review Officer (MRO) and any other designated personnel by email. The MRO will then review the SAE data to determine causality | Upon entry of a serious adverse event, the system will notify the DMCC by email. The DMCC will in turn notify the study PIs, the designated medical monitor and any other designated personnel by email. The medical monitor will then review the SAE data to determine causality | Clarification of notification process. |

Protocol: MEmbrous Nephropathy Trial Of Rituximab (MENTOR)

Version: 10.0, 17-Jun-2014

Summary of Changes

An amendment has been drafted to the MENTOR study protocol. The following is a summary of key changes to the previous version of the protocol (9.0, Jul 30, 2013) including the rationale for each change. Formatting changes and minor administrative changes (typos, grammatical changes, minor clarifications in wording) are not included in this table.

| Original Text | Modified Text | Rationale | | | | | | | | | | | | | | | | | | | | | | | | |
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| COVER PAGE | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phone: 507-266-7083 | Phone: 507-266-1045 | Dr. Fervenza’s contact number has changed. | | | | | | | | | | | | | | | | | | | | | | | | |
| OVERALL RESEARCH DESIGN AND METHODS | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table><tr><th colspan="2">Table 1. Definition of remission status at 24 months</th></tr><tr><th>Remission status</th><th>Proteinuria (UP g/24 hours) after treatment</th></tr><tr><td>Complete remission (CR)</td><td>UP ≤ 0.3 g and serum albumin > 3.5g/dl</td></tr><tr><td>Partial remission (PR)</td><td>Reduction in baseline UP of > 50% plus final UP ≤ 3.5 g but >0.3g</td></tr><tr><td>Non-response (NR)</td><td>Reduction in baseline UP of < 25% (includes increase in UP)</td></tr><tr><td>Relapse</td><td>Development of nephrotic range proteinuria following CR or PR, i.e. >3.5g/d</td></tr></table> | Table 1. Definition of remission status at 24 months | | Remission status | Proteinuria (UP g/24 hours) after treatment | Complete remission (CR) | UP ≤ 0.3 g and serum albumin > 3.5g/dl | Partial remission (PR) | Reduction in baseline UP of > 50% plus final UP ≤ 3.5 g but >0.3g | Non-response (NR) | Reduction in baseline UP of < 25% (includes increase in UP) | Relapse | Development of nephrotic range proteinuria following CR or PR, i.e. >3.5g/d | <table><tr><th colspan="2">Table 1. Definition of remission status</th></tr><tr><th>Remission status</th><th>Proteinuria (UP g/24 hours) after treatment</th></tr><tr><td>Complete remission (CR)</td><td>UP ≤ 0.3 g/24h and serum albumin ≥ 3.5g/dl</td></tr><tr><td>Partial remission (PR)</td><td>Reduction in baseline UP of ≥ 50% plus final UP ≤ 3.5 g/24h but > 0.3 g/24h</td></tr><tr><td>Non-response (NR)</td><td>Reduction in baseline UP of < 25% (includes increase in UP)</td></tr><tr><td>Relapse</td><td>Development of nephrotic range proteinuria following CR or PR, i.e. > 3.5g/24h</td></tr></table> | Table 1. Definition of remission status | | Remission status | Proteinuria (UP g/24 hours) after treatment | Complete remission (CR) | UP ≤ 0.3 g/24h and serum albumin ≥ 3.5g/dl | Partial remission (PR) | Reduction in baseline UP of ≥ 50% plus final UP ≤ 3.5 g/24h but > 0.3 g/24h | Non-response (NR) | Reduction in baseline UP of < 25% (includes increase in UP) | Relapse | Development of nephrotic range proteinuria following CR or PR, i.e. > 3.5g/24h | Title has been changed to reflect the fact that definitions of remission remain constant throughout the study, although our primary end point is still remission status at 24 months. |
| Table 1. Definition of remission status at 24 months | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Remission status | Proteinuria (UP g/24 hours) after treatment | | | | | | | | | | | | | | | | | | | | | | | | | |
| Complete remission (CR) | UP ≤ 0.3 g and serum albumin > 3.5g/dl | | | | | | | | | | | | | | | | | | | | | | | | | |
| Partial remission (PR) | Reduction in baseline UP of > 50% plus final UP ≤ 3.5 g but >0.3g | | | | | | | | | | | | | | | | | | | | | | | | | |
| Non-response (NR) | Reduction in baseline UP of < 25% (includes increase in UP) | | | | | | | | | | | | | | | | | | | | | | | | | |
| Relapse | Development of nephrotic range proteinuria following CR or PR, i.e. >3.5g/d | | | | | | | | | | | | | | | | | | | | | | | | | |
| Table 1. Definition of remission status | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Remission status | Proteinuria (UP g/24 hours) after treatment | | | | | | | | | | | | | | | | | | | | | | | | | |
| Complete remission (CR) | UP ≤ 0.3 g/24h and serum albumin ≥ 3.5g/dl | | | | | | | | | | | | | | | | | | | | | | | | | |
| Partial remission (PR) | Reduction in baseline UP of ≥ 50% plus final UP ≤ 3.5 g/24h but > 0.3 g/24h | | | | | | | | | | | | | | | | | | | | | | | | | |
| Non-response (NR) | Reduction in baseline UP of < 25% (includes increase in UP) | | | | | | | | | | | | | | | | | | | | | | | | | |
| Relapse | Development of nephrotic range proteinuria following CR or PR, i.e. > 3.5g/24h | | | | | | | | | | | | | | | | | | | | | | | | | |
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| Cyclosporine: | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Patients will have their doses adjusted according to their blood levels of CSA as monitored every 2 weeks +/- 3 days until the target trough level is reached. | Patients will have their doses adjusted according to their blood levels of CSA as monitored every 2 weeks +/- 3 days until the target trough level is reached. After reaching target, CSA trough levels will continue to be checked as per the visit schedule (Table 2a). | Serum potassium, creatinine and CSA trough levels are important safety metrics for subjects in the CSA arm. The language has been revised to provide clarity as to the testing requirements and to ensure that subjects in the CSA arm are properly monitored. | | | | | | | | | | | | | | | | | | | | | | | | |
| If Cyclosporine dose is changed during treatment, potassium, creatinine and CSA levels will be rechecked two weeks +/- 3 days post | If Cyclosporine dose is changed during treatment, potassium, creatinine and CSA levels will be rechecked every two weeks +/- 3 | | | | | | | | | | | | | | | | | | | | | | | | | |

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| increase/decrease in CSA dosage. | days post increase/decrease in CSA dosage until levels are stable and at target. | |
| Figure 4. Schematic representation of the study design | | |
| <p>* Here defined as equal to or > 25% reduction in baseline proteinuria</p> | <p>NR = non-responder; PR = partial remission; CR = complete remission * Here defined as >25% reduction in baseline proteinuria (for determining whether treatment to continue at 6 months)</p> | Study design schematic has been updated to reflect the optional extension of the follow-up period for the remission cohort. |
| Screening Visit | | |
| Candidates who have already been on conservative therapy for 3 months or more without a documented decrease in proteinuria of >50% as compared to a value from 12 months ago (or a pro-rated percent reduction if the time period is less than a year, for instance a decrease in proteinuria of >25% as compared to a value from 6 months ago – see MOP for further details) do not need to fulfill the run-in requirement as long as their local labs indicate that proteinuria remains $\geq 5g/24h$. | Candidates who have already been on conservative therapy for 3 months or more without a documented decrease in proteinuria of >50% as compared to a value from within the last 3 to 12 months do not need to fulfill the run-in requirement as long as their local labs indicate that proteinuria remains $\geq 5g/24h$. | For clarity, we have simplified the guidance for determining if someone is responding or not to conservative therapy. It is important that all candidates be exposed to conservative therapy prior to being randomized to study drug. Patients who do respond to conservative therapy should not be unnecessarily exposed to immunosuppression. |
| Patient monitoring and evaluation | | |
| [Text addition] | In addition, as described later in the protocol (see ancillary studies) we will seek permission to follow the remission cohort (i.e., those in PR or CR at 12 months or CR at 6 months as well as those who are not in remission but have a $\geq 50\%$ reduction in proteinuria from baseline by month 12) for an additional 12 months of observation beyond the previous end point of observation of 24 months to monitor for relapses and changes in PLA2R. | The remission cohort, with an extended follow-up to a maximum of 3 years from the time of starting study drug for periodic sampling of anti-PLA2R titer and urinary protein/creatinine ratios, will provide a unique opportunity in which to study biomarkers of the immunological mechanism as predictors of clinical relapse. |

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| <p>Cyclosporine trough level measurements will be done every 2 weeks +/- 3 days until levels are stable and at target. Potassium and creatinine will also be checked 2 weeks +/- 3 days after initiation of Cyclosporine, in conjunction with CSA level. If Cyclosporine dose is changed during treatment, potassium and creatinine will be rechecked at the 2 week +/- 3 days time point.</p> | <p>For those patients in the Cyclosporine arm, Cyclosporine trough level measurements will be done every 2 weeks +/- 3 days after initiation of Cyclosporine until levels are stable and at target. Potassium and creatinine will also be checked in conjunction with CSA level. If Cyclosporine dose is changed during treatment, potassium, creatinine and CSA level will be rechecked every 2 weeks +/- 3 days until levels are stable and at target.</p> | <p>Serum potassium, creatinine and CSA trough levels are important safety metrics for subjects in the CSA arm. The language has been revised to provide clarity as to the lab requirements and ensure that subjects in the CSA arm are properly monitored.</p> |
| <p>Patients who cannot tolerate the medications or who are treatment failures at 6 months will exit the study.</p> | <p>Patients who cannot tolerate the medications and/or who are treatment failures at 6 months will exit the study at 6 months (i.e., the 6 month visit and accompanying data collection should still occur).</p> | <p>Clarification on the study exit point at 6 months – all subjects regardless of their 6m outcome will have the 6m visit and their data collected</p> |
| <p>Patients who go in to complete remission will discontinue study drug but will continue with full visit follow-ups until month 24 as scheduled. However, the therapeutic/management plan for these patients will be solely at the discretion of the managing nephrologist. Likewise, patients who relapse from either a partial or complete remission will continue with the visit schedule, but the therapeutic/management plan will be solely at the discretion of the managing nephrologist.</p> | <p>Patients who go in to complete remission will discontinue study drug but will continue with full visit follow-ups until month 24 as scheduled. However, the therapeutic/management plan for these patients will be solely at the discretion of the managing nephrologist although while in CR no further immunosuppressive therapy is recommended. Likewise, patients who relapse from either a partial or complete remission or who are not in partial or complete remission by the end of the treatment period (i.e., 12 months) will continue with the visit schedule, but the therapeutic/management plan will be solely at the discretion of the managing nephrologist.</p> | <p>The use of additional immunosuppressants during the observation period should be discouraged as long as subjects remain in remission. This is to prevent excessive immunosuppression and will also enable us to accurately assess study endpoints.</p> <p>This section also clarifies that all subjects who reach 12m will continue to have follow-up visits as scheduled during the observation period to ensure the robustness of the study data regardless of the subsequent course of treatment.</p> |
| <p>Laboratory testing</p> | | |
| <p>In all patients, CBC, serum creatinine, electrolytes, albumin, urinalysis, 24-hour Uprot/Ucrea ratio, and creatinine clearance will be evaluated as noted in Tables 2a and 2b.</p> | <p>In all patients, CBC with differential, serum creatinine, electrolytes, albumin, urinalysis, 24-hour Uprot/Ucrea ratio, and creatinine clearance will be evaluated as noted in Tables 2a and 2b.</p> | <p>Clarification</p> |

| Anti-PLA2R Assay | | |
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| We will collect serum samples (5 mL), at Screening, Time 0, and months 3, 6, 9, 12, 18, and 24 for the measurement of anti-PLA2R antibodies. | [text deletion] | The schedule for sample collection is described elsewhere in this section where it has been updated to include optional additional collections for those in the remission cohort |
| In those patients that have no remission from their disease in response to RTX, we will determine if circulating anti-PLA2R is still present which might suggest the need for a second course of RTX or alternate immunosuppressive therapy. | <p>In those patients that have no remission from their disease in response to RTX/CSA, we will determine if circulating anti-PLA2R is still present which might suggest the need for a second course of RTX, continued treatment with CSA, or alternate immunosuppressive therapy. Furthermore, in those patients that are in complete remission at 6 months, partial or complete remission at 12 months, or have a $\geq 50\%$ reduction in proteinuria from baseline by 12 months (i.e., the MENTOR remission cohort), we intend to explore the following critically important questions:</p> <ol style="list-style-type: none"> 1. The relationship between the rate of increase of anti-PLA2R titer and patient relapse 2. The relationship between the absolute change in anti-PLA2R titer (from baseline/remission point) and clinical phenotype (i.e., response/relapse time) 3. The predictive capacity of changes in anti-PLA2R titer with regard to relapse and/or durability of remission 4. The timing between changes in anti-PLA2R titer and any subsequent clinical relapse | It is expected that 75% of the IMN cases will initially be anti-PLA2R positive, which has been confirmed in our initial assessment of PLA2R in 25% (N= 28) of the sample size of randomized patients. At the completion of the immunosuppression treatment component of the protocol, the expectation is that 70% of all randomized IMN cases will experience both a clinical and immunological remission. The immunologic remission will be documented by anti-PLA2R levels significantly reduced from baseline with most levels in the normal range. Our anticipated/expected rate of proteinuria relapse will be between 30% and 50% between months 12 and month 36. The remission cohort will provide a unique and probably one-time only opportunity in which to study biomarkers of the immunological mechanism as predictors of clinical relapse. |
| The sera will be tested in batches for reactivity toward immobilized recombinant PLA2R by western blot (the assay currently in use) or ELISA (expected to be in use at the time | The sera will be tested in batches for reactivity toward immobilized recombinant PLA2R by ELISA, with appropriate positive and negative controls. | ELISA is currently in use for testing anti-PLA2R levels. It has higher sensitivity and stronger specificity than western blot assay. |

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| this proposal is in effect), with appropriate positive and negative controls. | | |
| [text addition] | <p>For those in the remission cohort (see ancillary studies), additional serum samples will be collected from those that achieve partial or complete remission or $\geq 50\%$ reduction in proteinuria by 12 months at additional time points as follows:</p> <ul style="list-style-type: none"> • For patients in the CSA arm that are in partial or complete remission or $\geq 50\%$ reduction in proteinuria at 12 months additional samples will be collected at months 13, 14, 15, 16, 17, 20, 22, 28, 32, and 36; for those who achieve complete remission at 6 months additional samples will be collected at months 7, 8, 10, and 11 then at 14, 16, 20, 22, 28, 32, and 36. • For patients in the RTX arm that achieve partial or complete remission or $\geq 50\%$ reduction in proteinuria at 12 months additional samples will be collected at months 14, 16, 20, 22, 26, 28, 30, 32, 34, and 36; for those in the RTX arm that have achieved complete remission at 6 months, additional samples will be collected at months 10, 14, 16, 20, 22, 26, 28, 30, 32, 34, and 36. <p>Whole blood for these particular serum samples may be collected at a peripheral lab and shipped to the individual study site within 48 hours for processing and storage in a -20°</p> | <p>This section specifies the schedule for additional serum samples collected from those participating in the optional remission cohort.</p> <p>Immunologic remission will be documented by anti-PLA2R levels significantly reduced from baseline with most levels in the normal range. Our anticipated/expected rate of proteinuria relapse will be between 30% and 50% between months 12 and month 36. The remission cohort will provide a unique and probably one-time only opportunity in which to study biomarkers of the immunological mechanism (anti-PLA2R, sPLA2R) as predictors of clinical relapse.</p> <p>The same number of additional samples will be collected from remission cohort participants in each arm. However, the schedule is slightly different given that our pilot studies suggest that the time to relapse will be shorter in the CSA arm.</p> |

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| | freezer as described in the ancillary studies section later in this protocol. | |
| Table 2a: Test Schedule and Monitoring for Cyclosporine Treatment Arm | | |
| [Text addition] | Uprot/Ucrea (spot urine) row added | To accommodate the additional sample collections scheduled for the remission cohort (optional) |
| [Text addition] | 7, 8, 10, 11, 13, 14, 15, 16, 17, 20, 22, 28, 32, 36 months column added | |
| [Text addition – footnotes added] | ^c For participants in the MENTOR remission cohort who are in PR or CR at 12 months or have a ≥50% reduction in proteinuria from baseline by 12 months ^d For participants in the MENTOR remission cohort who are in CR at 6 months | |
| Table 2b: Test Schedule and Monitoring for Rituximab Treatment Arm | | |
| [Text addition] | Uprot/Ucrea (spot urine) row added | To accommodate the additional sample collections scheduled for the remission cohort (optional) |
| [Text addition] | 10, 14, 16, 20, 22, 26, 28, 30, 32, 34, and 36 months column added | |
| [Text addition – footnotes added] | ^c For participants in the MENTOR remission cohort who are in PR or CR at 12 months or have a ≥50% reduction in proteinuria from baseline by 12 months ^d For participants in the MENTOR remission cohort who are in CR at 6 months | |
| Concomitant Treatment | | |
| Patients randomized to receive Rituximab should be started on single strength Bactrim one a day (or its equivalent) for pneumocystis pneumonia prophylaxis. This treatment will continue until study medication is stopped and B cells (CD19/20+) have been replete (>15 cells/microliter on peripheral blood flow cytometry). B cells (CD19/20+) are expected to be replete by Month 12. In the rare event that B cells (CD19/20+) are not replete by Month 12, flow cytometry should be repeated at Month 18 (see table 2b). | Patients randomized to receive Rituximab should be started on single strength Bactrim one a day (or its equivalent) for pneumocystis pneumonia prophylaxis. This treatment will continue until the end of the study treatment phase (12 months) and the B cells (CD19/20+) have been repleted (>15 cells/microliter on peripheral blood flow cytometry). B cells (CD19/20+) are expected to be replete by Month 12. In the rare event that B cells (CD19/20+) are not replete by Month 12, flow cytometry should be repeated at Month 18 (see table 2b) and Bactrim (or its equivalent) continued to that point of repletion. | To clarify the duration of required Bactrim treatment as patients remain immunosuppressed for several months following the RTX infusion. |

| Ancillary studies: | | |
|--------------------|---|---|
| [Text addition] | <p>1. <u>The MENTOR Remission Cohort</u></p> <p>Participation in this ancillary study will be restricted to patients in the MENTOR cohort who respond to immunosuppression with partial or complete remission at month 12 (as defined in Table 1), complete remission at 6 months, or $\geq 50\%$ reduction in proteinuria from baseline by month 12. At the completion of the immunosuppression treatment component of the protocol, the expectation is that 70% of all randomized IMN cases ($N=126 \times 70\% = 88$ patients) will experience both a clinical (complete or partial proteinuria remission) and immunological remission. The immunologic remission is expected to be documented by anti-PLA2R levels significantly reduced from baseline with most levels in the normal range (less than 40U). This population is estimated to be 75% of the initial PLA2R + cohort ($75\% \times 88 = 66$ patients). The great percentage of these patients will reach this remission at the 12 month study point. There will be rare cases of CR by 6 months. Our anticipated/expected rate of proteinuria relapse will be between 30% and 50% between month 12 and month 36 (20-30% in those with CR at 6 months). Although the clinical proteinuria remission status at 24 months remains the main determinant of the RCT, the remission cohort (with an extension up to month 36 for periodic sampling of anti-PLA2R titer and urinary protein/creatinine ratios only) will provide a unique and probably one-time only opportunity in which to study a number of biomarkers of the immunological mechanism as</p> | <p>The benefit of this study will be the improved capacity to predict those patients at high risk of clinical relapse and the potential to institute therapy potentially in advance of clinical relapse. It may also allow us to determine if the changing immunologic profile can predict the severity of relapse and provide the opportunity to consider, in the future, matching this profile to preventive strategies and/or early and less aggressive immunosuppressive interventions and/or gauge when risk-benefit favours reducing immunosuppressive therapy.</p> |

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| | <p>predictors of clinical relapse. Patients will be followed as per protocol for at least 12 months of observation (i.e., to month 24) after achieving proteinuria remission or $\geq 50\%$ proteinuria reduction, but for those in the remission cohort we will extend this up to month 36 (a maximum observation time of 24 months post treatment phase) in those that remain in CR or PR. Those who relapse between months 24 and 36 will only be followed until their relapse which is defined as development of nephrotic range proteinuria ($> 3.5\text{g/day}$) following CR or PR.</p> <p>In addition, we are now able to measure other components of the immunological elements of primary/idiopathic MN activity and are now better able to more precisely define the remission state when combining these additional biomarkers of the immune remission state with PLA2R titres. These additional biomarkers include soluble PLA2R. A detectable level of soluble PLA2R antigen (sPLA2R) is present in normal serum as a result of specific secretion of PLA2R and likely cleavage of the extracellular membrane receptor, and is likely to be a characteristic of the normal, remission state of patients with idiopathic MN. We hypothesize that as patients experience immunological relapse, their biomarker status will change from sPLA2R +ve, anti-PLA2R -ve to sPLA2R -ve , anti-PLA2R+ve. The timeframe of this change needs to be established and our remission cohort provides a unique opportunity to study this hypothesis. This change could vary over weeks dependent on the level of sPLA2R</p> | |
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| | <p>and the rate of production of anti-PLA2R. In addition, there may be a distinct phase of soluble circulating immune complexes where the patient is apparently seronegative for both free sPLA2R and free anti-PLA2R until excess of anti-PLA2R antibodies dominate. This MENTOR Remission cohort offers a unique opportunity to investigate:</p> <ul style="list-style-type: none"> a) the transition from immunological remission to relapse b) the link between immunological relapse and clinical relapse (proteinuria) c) additional biomarkers of relapse. <p>The benefit of this study will be the improved capacity to predict those patients at high risk of clinical relapse and the potential to institute therapy potentially in advance of clinical relapse. It may also allow us to determine if the changing immunologic profile can predict the severity of relapse and provide the opportunity to consider, in the future, matching this profile to preventive strategies and/or early and less aggressive immunosuppressive interventions and/or gauge when risk-benefit favours reducing immunosuppressive therapy.</p> <p>We will obtain each patient's permission to obtain 5mL of blood at designated time points (up to a maximum of 11 extra samples in total) up until month 36 or until relapse (see Tables 2a and 2b for visit schedule) to be used for PLA2R and sPLA2R testing. In addition, a urine sample will be collected to determine relapse by measuring urine protein/creatinine ratios. Samples may be collected at a</p> | |
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| | <p>peripheral lab where possible in order to minimize the demands on patient travel time, but blood will need to be sent to study sites for processing and storage (-20 degrees C) within 48 hours of being drawn. Serum samples will be kept in a dedicated freezer until a point when they can be shipped in bulk to Dr. Fervenza at Mayo Clinic, Rochester, Minnesota. Mayo Clinic will then send the samples in bulk to Dr. Paul Brenchley at the Manchester Royal Infirmary in the UK.</p> | |
| ESTIMATED DURATION OF THE STUDY | | |
| <p>The estimated duration of the study, given a 24-month enrollment period, a 2-year follow-up for each patient, and 1 year for data analysis is approximately 60 months.</p> | <p>The estimated duration of the study, given a 24-month enrollment period, a 2-year follow-up for each patient, and 1 year for data analysis is approximately 60 months. For those patients in the MENTOR remission cohort, their participation will be extended up to 3-years follow-up from the first day of study drug.</p> | <p>To accommodate the optional extension of the follow-up period for the remission cohort</p> |

MENTOR

MEmbranous Nephropathy Trial Of Rituximab

Statistical Analysis Plan (SAP)

Version: 1.0

Authors: Peter Jüni and Bruno da Costa

Date: Aug 3, 2017

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1. Objectives

The specific objectives of this trial are to test the following hypotheses:

1. Rituximab is non-inferior to Cyclosporine in inducing long-term complete remission (CR) or partial remission (PR) of proteinuria in patients with idiopathic membranous nephropathy (primary hypothesis; non-inferiority analysis at 24 months).
2. Rituximab is non-inferior to Cyclosporine in inducing CR or PR of proteinuria in patients with idiopathic membranous nephropathy during the active treatment phase at 12 months (secondary hypothesis; non-inferiority analysis at 12 months).
3. Rituximab reduces the number of relapses (efficacy in sustaining remission) and increases the time to relapse when compared with Cyclosporine (secondary hypothesis; superiority analysis at 24 months).
4. Rituximab has a better side effect profile when compared with Cyclosporine in patients with idiopathic membranous nephropathy (secondary hypothesis; superiority analysis at 24 months).
5. Rituximab is associated with improved quality of life when compared with Cyclosporine in patients with idiopathic membranous nephropathy (secondary hypothesis; superiority analysis at 6, 12 and 24 months).

2. Study populations

2.1. Patient flow

A CONSORT patient flow diagram will be drawn following the CONSORT 2010 standards (<http://www.consort-statement.org/consort-2010>).

2.2. Intention-to-treat (ITT) population

The full analysis set consists of all randomized patients. Patients will be analysed regardless of whether they actually received the allocated intervention or not or any other protocol deviations in the group they were originally allocated to.

2.3. Per-protocol (PP) population

The per-protocol population consists of all subjects who received a full course of study medications according to protocol.

2.4. Safety population

The safety population consists of all randomised patients who received the allocated study medication at least once.

2.5. Responder population

The responder population consist of all randomised patients who received the allocated study medication at least once and reached CR or PR at 12 months.

2.6. Anti-PLA2R+ population

The Anti-PLA2R+ population consists of all randomised patients who had a positive Anti-Phospholipase A2 Receptor (Anti-PLA2R) Antibody test at baseline.

3. Data management

3.1. Data export

Clinical data will be provided by the Applied Health Research Centre and will be imported from the electronic data capturing system into R and Stata by the trial statistician for data preparation, validation and analysis.

3.2. Data validation

All variables used in the analysis, including the derived variables, will be checked for missing values, outliers, and inconsistencies and queried.

3.3. Data preparation

We will derive the composite primary outcome of CR or PR at 24 months and the secondary composite outcomes of CR or PR at 6, 12 and 18 months based on the following definitions specified in the protocol:

- CR: proteinuria ≤ 0.3 g/24h and serum albumin ≥ 3.5 g/dl

- PR: Reduction in baseline proteinuria of $\geq 50\%$ plus final proteinuria ≤ 3.5 g/24h but >0.3 g/24h
- Non-response (NR): Reduction in baseline proteinuria of $<25\%$ (this also includes increases in proteinuria)
- Relapse: Development of nephrotic range proteinuria of >3.5 g/24h following CR or PR

Creatinine and proteinuria levels obtained in duplicate urine measurements at baseline and 6, 12, 18 and 24 months will be averaged at each timepoint.

Data on laboratory variables such as creatinine clearance, proteinuria, Anti-PLA2R levels and quality of life data collected after initiation of a medication other than the study medications for the treatment of membranous nephropathy will be omitted from the analysis.

Scores for the Kidney Disease Quality of Life 36 item (KDQOL-36) short form will be derived as specified in the User Manual

(https://www.rand.org/health/surveys_tools/kdqol.html).

The body mass index (BMI) will be derived based on height and weight data: the weight in kilograms divided by the square of the height in meters.

4. Variables

4.1. Baseline characteristics

The following baseline characteristics and lab data will be summarized descriptively by group:

- Age
- Sex
- Blood pressure (systolic and diastolic; mmHg)
- Height (cm)
- Weight (kg)
- BMI (kg/m²)
- History of immunosuppressive therapy (yes/no)
- Lipid – total cholesterol (mg/dL)
- Lipid – LDL cholesterol (mg/dL)

- Anti-PLA2R (u/mL)
- Serum albumin (g/dL)
- Serum creatinine (mg/dL)
- 24 hour urine protein (mg/24h)
- 24 hour urine creatinine (mg/24h)
- Creatinine Clearance (mL/min/BSA)
- Protein/creatinine ratio (mg/mg)

4.2. Primary outcome

The primary outcome is the composite of CR or PR of proteinuria.

Patients who reached less than 25% reduction of proteinuria from baseline at 6 months, patients who relapsed, patients who deviated from the protocol before 24 months, including those who received a medication other than the study medications for the treatment of their membranous nephropathy, and patients who did not meet criteria of CR or PR at 24 months will be considered failures. Patients who were lost to follow-up will be considered failures unless they were found to have achieved CR or PR at their 18 month visit.

The primary analysis will be performed in the ITT population. A secondary analysis will be done in the PP population.

4.3. Secondary outcomes

The secondary outcomes and the principal population analysed for these outcomes are as follows:

- Composite of CR or PR
 - ITT population at 6, 12 and 18 months

CR is defined as proteinuria ≤ 0.3 g/24h and serum albumin ≥ 3.5 g/dl; PR is defined as a reduction in baseline proteinuria of $\geq 50\%$ plus final proteinuria ≤ 3.5 g/24h but > 0.3 g/24h.
- Time to composite of CR or PR
 - ITT population
- Proteinuria
 - ITT population at 6 and 12 months

- Responder population at 18 and 24 months
- Relapse
 - Responder population at 24 months

Relapse is defined as development of nephrotic range proteinuria of >3.5g/24h following CR or PR.
- Time to relapse
 - Responder population
- Anti-PLA2R levels
 - Anti-PLA2R+ population at 6 and 12 months
 - All randomised patients of the Anti-PLA2R+ population who received the allocated study medication at least once and reached CR or PR at 12 months (Anti-PLA2R+ patients of the responder population) at 18 and 24 months
- Quality of life as assessed by the Kidney Disease Quality of Life 36 item (KDQOL-36) short form
 - ITT population at 6 and 12 months
 - Responder population at 24 months

The KDQOL-36 short form includes the SF-12 as generic core plus the burden of kidney disease, symptoms/problems of kidney disease, and effects of kidney disease scales from the KDQOL-SF v1.3: items 1-12: SF-12; items 13-16: burden of kidney disease; items 17-28: symptoms/problems; items 29-36: effects of kidney disease.
- End stage renal disease
 - ITT population up to 24 months

End stage renal disease is defined as stage 5 chronic kidney disease with an estimated or calculated GFR <15 mL/min or initiation of permanent renal replacement therapy (dialysis or transplant).
- Creatinine Clearance
 - ITT population at 6 and 12 months
 - Responder population at 18 and 24 months

4.4. Safety outcomes

The safety outcomes, which are also considered secondary outcomes, and the principal population analysed for these safety outcomes are as follows:

- All adverse events
 - Safety population up to 24 months
- Adverse events are defined as any untoward medical occurrence and their severity graded as follows:
- Grade 1: mild
 - Grade 2: moderate
 - Grade 3: severe or medically significant
 - Grade 4: life-threatening
 - Grade 5: fatal
- Adverse events grade ≥ 3
 - Safety population up to 24 months
 - Adverse events grade < 3
 - Safety population up to 24 months
 - Serious adverse events
 - Safety population up to 24 months

5. Sample size

The primary objective of this trial is to determine whether Rituximab is non-inferior to Cyclosporine in inducing long-term CR or PR of proteinuria in patients with idiopathic membranous nephropathy at 24 months after randomization. Assuming that 55% of patients on Rituximab, and 45% on Cyclosporine have a CR or PR of proteinuria at 24 months after randomization, 63 patients per group would result in 80% power to detect non-inferiority at a one-sided alpha of 0.025 (corresponding to a two-sided alpha of 0.05) and a non-inferiority margin of 15% on an absolute risk difference scale.

6. Statistical analyses

6.1. Baseline characteristics

No statistical comparisons of patient characteristics at baseline will be performed between groups.

6.2. Primary stepwise analysis of primary outcome

The primary analysis of the primary outcome of the composite of CR or PR at 24 months will be performed in the ITT population.

We will perform stepwise testing. First, we will perform a confirmatory test of non-inferiority of Rituximab as compared with Cyclosporine. Using the non-inferiority test as a gatekeeper to control the family-wise type I error, we will only continue to perform a confirmatory test of superiority of Rituximab if the non-inferiority test was significant.

Non-inferiority will be declared if the upper limit of the two-sided 95% confidence interval of the absolute risk difference of proteinuria at 24 months is not greater than 15%. One-sided p values for non-inferiority will be calculated from Z tests comparing differences between groups with the non-inferiority margin, calculating the standard error for the Z test for the primary outcome using a general linear model. The test will be considered significant if the one-sided p value is <0.025 (corresponding to a two-sided p-value of <0.05).

If the one-sided p-value of non-inferiority is significant at $p<0.025$, we will perform a confirmatory test of superiority of Rituximab at a two-sided $p<0.05$. If the two-sided p-value for superiority is significant at $p<0.05$, we will declare superiority.

6.3. Secondary non-inferiority analysis of primary outcome

A secondary non-inferiority analysis of the primary outcome at 24 months will be performed in the PP population.

Non-inferiority will be declared if the upper limit of the two-sided 95% confidence interval of the absolute risk difference of the primary outcome of CR or PR of proteinuria at 24 months is not greater than 15%. One-sided p values for non-inferiority will be calculated as described above. Again, the test will be considered significant if the one-sided p value is <0.025 .

6.4. Non-inferiority analysis of secondary outcomes

A non-inferiority analysis of the composite of CR or PR at 12 months will in addition be performed in the ITT population.

Non-inferiority will be declared if the upper limit of the two-sided 95% confidence interval of the absolute risk difference of the secondary outcome of CR or PR of proteinuria at 12 months is not greater than 15%. One-sided p values for non-inferiority will be calculated as described above. Again, the test will be considered significant if the one-sided p value is <0.025 .

6.5. Analyses of secondary outcomes

Any other comparison of outcomes between randomised groups will be done in the ITT population using two-sided 95% confidence intervals and two-sided p values for superiority with p values considered significant if <0.05 . No adjustment for multiple comparisons will be done for secondary outcomes, as all comparisons will be considered exploratory.

Binary outcomes will be compared between groups based on risk differences with 95% confidence intervals, using chi-squared tests to derive two-sided p-values. Time-to-event outcomes will be analysed using Kaplan Meier curves and hazard ratios from Cox regression models with 95% confidence intervals and two-sided p-values.

Continuous outcomes will be analysed using analysis of covariance adjusted for baseline values of the analysed continuous outcome. If data are strongly positively skewed, the distribution of data per group will be summarised as medians with interquartile ranges instead, and differences in medians with bootstrapped 95% confidence intervals and two-sided p-values from Wilcoxon's rank-sum test will be presented. Complete-case analyses will be performed for the analysis of continuous outcomes throughout, including patients with available data at each timepoint, analysing patients in the group they were originally allocated to.

6.6. Analyses of safety outcomes

Safety outcomes will be tabulated by investigator-assessed relationship to study drug, by body system and by type of adverse event if a type of event, for example headache, occurs in at least 4 patients during the trial. Comparisons between groups will be done for both, the number of patients experiencing an event and for the number of events. The number of patients will be compared between groups with a chi-squared test or Fisher's exact test of the number of expected events per group is

below 5. The number of events, with the possibility of multiple events per patient, will be compared between groups with a negative binomial model.

6.7. Sensitivity analyses

Pre-specified sensitivity analyses of the primary outcome and secondary outcomes of CR or PR at 6, 12 and 18 months will include the following:

- Generalized estimating equation (GEE) to derive risk differences with two-sided 95% confidence intervals and two-sided p-values
- Logistic regression, crude and adjusted for center and proteinuria at baseline (<8g/24h vs ≥8g/24h), to derive odds ratios with two-sided 95% confidence intervals and two-sided p-values
- Ordinal logistic regression to compare the ordered outcome of CR, PR or no response, crude and adjusted for center and proteinuria at baseline (<8g/24h vs ≥8g/24h)
- Wilcoxon rank-sum test to derive a two-sided p-value for differences between groups in ordered outcome of CR, PR or no response

Pre-specified sensitivity analyses of continuous outcomes at 6, 12, 18 and 24 months will include the following:

- Mixed repeated measures linear regression with random intercepts for patients to derive differences in arithmetic means with two-sided 95% confidence intervals and two-sided p-values
- Mixed repeated measures linear regression with nested random intercepts for patients and centers to derive differences in arithmetic means with two-sided 95% confidence intervals and two-sided p-values
- Analysis of covariance of log transformed outcome data adjusted for log transformed baseline values of the outcome to derive ratios of geometric means with two-sided 95% confidence intervals and two-sided p-values in case of strongly positively skewed data

6.8. Subgroup analyses

We will conduct subgroup analyses on the risk difference scale according to the following characteristics at baseline:

Statistical Analysis Plan (MENTOR Trial)

- Sex
- Age (≤ 50 versus > 50 years of age)
- Proteinuria ($< 8\text{g}/24\text{h}$ vs $\geq 8\text{g}/24\text{h}$)
- Anti-PLA2R status (positive, defined as > 40 U/mL, versus negative)

MENTOR

MEmbranous Nephropathy Trial Of Rituximab

Statistical Analysis Plan (SAP)

Version: 1.4

Authors: Peter Jüni and Bruno da Costa

Date: Sept 16, 2017

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1. Objectives

The specific objectives of this trial are to test the following hypotheses:

1. Rituximab is non-inferior to Cyclosporine in inducing long-term complete remission (CR) or partial remission (PR) of proteinuria in patients with idiopathic membranous nephropathy (primary hypothesis; non-inferiority analysis at 24 months).
2. Rituximab is non-inferior to Cyclosporine in inducing CR or PR of proteinuria in patients with idiopathic membranous nephropathy during the active treatment phase at 12 months (secondary hypothesis; non-inferiority analysis at 12 months).
3. Rituximab reduces the number of relapses (efficacy in sustaining remission) and increases the time to relapse when compared with Cyclosporine (secondary hypothesis; superiority analysis at 24 months).
4. Rituximab has a better side effect profile when compared with Cyclosporine in patients with idiopathic membranous nephropathy (secondary hypothesis; superiority analysis at 24 months).
5. Rituximab is associated with improved quality of life when compared with Cyclosporine in patients with idiopathic membranous nephropathy (secondary hypothesis; superiority analysis at 6, 12 and 24 months).

2. Study populations

2.1. Patient flow

A CONSORT patient flow diagram will be drawn following the CONSORT 2010 standards (<http://www.consort-statement.org/consort-2010>).

The flow chart will consider specifically:

- N assessed for eligibility
- N not included in trial (with reasons)
- N randomized
- N allocated to intervention/control
 - N receiving allocated intervention
 - N not receiving allocated intervention (with reasons)

- N follow-up to month 6, 12, 18 and 24 (with reasons for termination)
- N discontinued intervention before month 6 and 12 (with reasons)
- N analyzed
 - N excluded from primary analysis (with reasons)

2.2. Intention-to-treat (ITT) population

The intention-to-treat population consists of all randomized patients. Patients will be analysed regardless of whether they actually received the allocated intervention or not or any other protocol deviations in the group they were originally allocated to.

2.3. Per-protocol (PP) population

The per-protocol population consists of all subjects who received a full course of study medications, defined as at least 1 completed 6-month treatment cycle, according to protocol. Patients who stopped treatment prematurely because of an adverse event or because of disease activity will be included in the PP population and be considered treatment failures.

2.4. Safety population

The safety population consists of all randomised patients who received the allocated study medication at least once.

2.5. Anti-PLA2R+ population

The Anti-PLA2R+ population consists of all randomised patients who had a positive Anti-Phospholipase A2 Receptor (Anti-PLA2R) Antibody test at baseline, defined as > 40 U/mL using ELISA (corresponds to Anti-PLA2R+ patients of the ITT population).

3. Data management

3.1. Data export

Clinical data will be provided by the Applied Health Research Centre and will be imported from the electronic data capturing system into R and Stata by the trial statistician for data preparation, validation and analysis.

3.2. Data validation

All variables used in the analysis, including the derived variables, will be checked for missing values, outliers, and inconsistencies and queried.

3.3. Data preparation

We will derive the composite primary outcome of complete remission (CR) or partial remission (PR) at 24 months and the secondary composite outcomes of CR or PR at 6, 12 and 18 months based on the following definitions specified in the protocol:

- CR: proteinuria ≤ 0.3 g/24h and serum albumin ≥ 3.5 g/dl
- PR: Reduction in baseline proteinuria of $\geq 50\%$ plus final proteinuria ≤ 3.5 g/24h but >0.3 g/24h
- Non-response (NR): Reduction in baseline proteinuria of $<25\%$ (this also includes increases in proteinuria)
- Relapse: Development of nephrotic range proteinuria of >3.5 g/24h following CR or PR

Creatinine and proteinuria levels obtained in duplicate urine measurements at baseline and 6, 12, 18 and 24 months will be averaged at each timepoint.

Data on laboratory variables such as creatinine clearance, proteinuria, Anti-PLA2R levels and quality of life data collected after initiation of an immunosuppressive medication other than the study medication for the treatment of membranous nephropathy will be omitted from the analysis.

By design, patients did not undergo systematic assessment of continuous outcomes (laboratory outcomes, quality of life scores) once they satisfied criteria for treatment failure.

Therefore, continuous outcomes will be analyzed in a cross-sectional manner at each time point only in patients with complete or partial remission to allow exploratory comparisons of the quality of remission between groups at each time point; this approach will not allow longitudinal comparisons between time points.

Scores for the Kidney Disease Quality of Life 36 item (KDQOL-36) short form will be derived as specified in the User Manual

(https://www.rand.org/health/surveys_tools/kdqol.html)

The body mass index (BMI) will be derived based on height and weight data: the weight in kilograms divided by the square of the height in meters.

4. Variables

4.1. Baseline characteristics

The following baseline characteristics and lab data will be summarized descriptively by group:

- Age
- Sex
- Blood pressure (systolic and diastolic; mmHg)
- Height (m)
- Weight (kg)
- BMI (kg/m²)
- History of immunosuppressive therapy (yes/no)
- Lipid – total cholesterol (mg/dL)
- Lipid – LDL cholesterol (mg/dL)
- Anti-PLA2R+ status
- Anti-PLA2R levels (u/mL)
- Serum albumin (g/dL)
- Serum creatinine (mg/dL)
- 24-hour urine protein (g/24h)
- 24-hour urine creatinine (g/24h)

- Creatinine Clearance (mL/min/BSA)
- Protein/creatinine ratio (mg/mg)

4.2. Primary outcome

The primary outcome is the composite of CR or PR of proteinuria at 24 months after randomization as defined in section 3.3 and the protocol.

Patients who reached less than 25% reduction of proteinuria from baseline at 6 months, patients who relapsed, patients who had a premature termination of the protocol-specified treatment schedule before 12 months due to disease activity or adverse event, patients who used an immunosuppressive medication other than the study medication for the treatment of membranous nephropathy before 12 months, patients who used any immunosuppressive medication for the treatment of membranous nephropathy after 12 months and before 24 months and patients who did not meet criteria of CR or PR at 24 months will be considered failures. Patients who were lost to follow-up at 24 months will be considered failures unless they were found to have achieved CR or PR at their 18-month visit.

The primary analysis will be performed in the ITT population; all randomised patients will contribute to the analysis in the group they were originally allocated to. A secondary analysis will be done in the PP population.

4.3. Secondary outcomes

The secondary outcomes and the principal population analysed for these outcomes are as follows:

- Composite of CR or PR
 - ITT population, at 6, 12 and 18 months

CR is defined as proteinuria ≤ 0.3 g/24h and serum albumin ≥ 3.5 g/dl; PR is defined as a reduction in baseline proteinuria of $\geq 50\%$ plus final proteinuria ≤ 3.5 g/24h but >0.3 g/24h.
- Time to composite of CR or PR
 - ITT population, up to 12 months

- CR
 - ITT population, at 6, 12 and 18 months
- Proteinuria
 - Patients with complete or partial remission at each time point, at months 6, 12, 18 and 24
 - Anti-PLA2R+ patients with complete or partial remission at each time point, at months 6, 12, 18 and 24
- Relapse
 - Patients with CR or PR at end of 12-month treatment period, at 24 months

Relapse is defined as development of nephrotic range proteinuria of >3.5g/24h.
- Treatment failure
 - ITT population, at 6, 12, 18 and 24 months

Treatment failure (also see section 4.2. for definition) is defined as less than 25% reduction of proteinuria from baseline at 6 months, relapse (see above for definition), premature termination of the protocol-specified treatment schedule before 12 months due to disease activity or adverse event, use of a immunosuppressive medication other than the study medication for the treatment of membranous nephropathy before 12 months, use of any immunosuppressive medication for the treatment of membranous nephropathy after 12 months and before 24 months, loss to follow-up before 24 months, or not meeting criteria of CR or PR at 24 months (see section 3.3. for definition).

- Time to treatment failure
 - ITT population
 - Subgroup of patients with CR or PR at the end of treatment period, from 12 to 24 months
- Anti-PLA2R levels
 - Anti-PLA2R+ patients with complete or partial remission at each time point, at months 6, 12, 18 and 24
 - Patients with complete or partial remission at each time point, at months 6, 12, 18 and 24

- Quality of life as assessed by the Kidney Disease Quality of Life Short Form (KDQOL-SF), Version 1.3
 - Patients with complete or partial remission at each time point, at months 6, 12 and 24

The KDQOL-SF includes the SF-36 as a generic core plus various subscales related to kidney disease.

The following subscales will be given precedence: physical health composite and mental health composite of SF-36; burden of kidney disease; symptom/problems; effects of kidney disease on daily life.

- $\geq 50\%$ decrease in creatinine clearance from baseline
 - ITT population, at 24 months
- End stage renal disease
 - ITT population, at 24 months

End stage renal disease is defined as stage 5 chronic kidney disease with an estimated or measured GFR <15 mL/min or initiation of permanent renal replacement therapy (dialysis or transplant).

- Creatinine Clearance
 - Patients with complete or partial remission at each time point, at months 6, 12, 18 and 24

4.4. Safety outcomes

The safety outcomes, which are also considered secondary outcomes, and the principal population analysed for these safety outcomes are as follows:

- All adverse events
 - Safety population, up to 24 months

Adverse events are defined as any untoward medical occurrence and their severity graded as follows:

- Grade 1: mild
- Grade 2: moderate
- Grade 3: severe or medically significant
- Grade 4: life-threatening

- Grade 5: fatal
- Adverse events grade ≥ 3
 - Safety population, up to 24 months
- Adverse events grade < 3
 - Safety population, up to 24 months
- Serious adverse events
 - Safety population, up to 24 months

Serious adverse events are defined as any untoward medical occurrence that results in death, is life-threatening requires inpatient hospitalization or causes prolongation of an existing hospitalization, results in disability or permanent damage, or in a congenital anomaly/birth defect in the offspring.

5. Sample size

The primary objective of this trial is to determine whether Rituximab is non-inferior to Cyclosporine in inducing long-term CR or PR of proteinuria in patients with idiopathic membranous nephropathy at 24 months after randomization. Assuming that 55% of patients on Rituximab, and 45% on Cyclosporine have a CR or PR of proteinuria at 24 months after randomization, 63 patients per group would result in 80% power to detect non-inferiority at a one-sided alpha of 0.025 (corresponding to a two-sided alpha of 0.05) and a non-inferiority margin of 15% on an absolute risk difference scale.

6. Statistical analyses

6.1. Baseline characteristics

Patient characteristics and lab data at baseline will be summarized by treatment group. Continuous variables will be summarized using mean and standard deviation, or median and inter-quartile range if data are non-normally distributed. Categorical variables will be summarized with counts and percentages. No statistical comparisons of patient characteristics at baseline will be performed between groups.

6.2. Primary stepwise analysis of primary outcome

The primary analysis of the primary outcome of the composite of CR or PR at 24 months will be performed in the ITT population.

We will perform stepwise testing. First, we will perform a confirmatory test of non-inferiority of Rituximab as compared with Cyclosporine. Using the non-inferiority test as a gatekeeper to control the family-wise type I error, we will only continue to perform a confirmatory test of superiority of Rituximab if the non-inferiority test was significant.

Non-inferiority will be declared if the lower limit of the two-sided 95% confidence interval of the absolute risk difference of CR or PR at 24 months (risk of CR or PR in the Rituximab group minus the risk of CR or PR in the Cyclosporine group) is not lower than -15%. One-sided p values for non-inferiority will be calculated from Z tests comparing differences between groups with the non-inferiority margin, calculating the standard error for the Z test for the primary outcome using a general linear model. The test will be considered significant if the one-sided p value is <0.025 (corresponding to a two-sided p-value of <0.05).

If the one-sided p-value of non-inferiority is significant at $p < 0.025$, we will perform a confirmatory test of superiority of Rituximab at a two-sided $p < 0.05$. If the two-sided p-value for superiority is significant at $p < 0.05$, we will declare superiority.

6.3. Secondary non-inferiority analysis of primary outcome

A secondary non-inferiority analysis of the primary outcome at 24 months will be performed in the PP population.

Non-inferiority will be declared if the lower limit of the two-sided 95% confidence interval of the absolute risk difference of the primary outcome of CR or PR at 24 months is not lower than -15%. One-sided p values for non-inferiority will be calculated as described above. Again, the test will be considered significant if the one-sided p value is <0.025 .

6.4. Non-inferiority analysis of secondary outcomes

A non-inferiority analysis of the composite of CR or PR at 12 months will in addition be performed in the ITT population.

One-sided p values for non-inferiority will be calculated as described above. Since this is a secondary outcome, we will adopt a Bonferroni correction, which allows for this outcome to be tested in addition to the primary outcome at an alpha level of 0.0125 (0.025/2). The test will therefore be considered significant if the one-sided p value is <0.0125 .

6.5. Analyses of secondary outcomes

Any other comparison of outcomes between randomised groups will be done using two-sided 95% confidence intervals and two-sided p values for superiority with p values considered significant if <0.05 . No adjustment for multiple comparisons will be done for secondary outcomes, as all comparisons will be considered exploratory.

Binary outcomes will be compared between groups based on risk differences with 95% confidence intervals, using chi-squared tests to derive two-sided p-values. Time-to-event outcomes will be analysed using Kaplan Meier curves and hazard ratios from Cox regression models with 95% confidence intervals and two-sided p-values.

Continuous outcomes will be analysed using analysis of covariance adjusted for baseline values of the analysed continuous outcome. If data are strongly positively skewed, as is expected for proteinuria and anti-PLA2R levels, data will be log transformed, geometric means with 95% confidence intervals will be calculated per group, and ratios of geometric means will be derived using analysis of covariance adjusted for log transformed baseline values; to achieve better interpretability, differences in geometric means will be estimated from the difference between geometric mean in control group and product of geometric mean in control group and geometric mean ratio, with appropriate 95% confidence intervals estimated from the difference between geometric mean in control group and product of geometric mean in control group and upper and lower limits of the geometric mean ratio. Box and Whisker plots of proteinuria and anti-PLA2R levels will be done after log transformation of the data. Complete-case analyses will be performed for the analysis of continuous outcomes throughout, including patients with available data at each timepoint, analysing patients in the group they were originally allocated to.

6.6. Analyses of safety outcomes

Safety outcomes will be tabulated by investigator-assessed relationship to study drug, by body system and by type of adverse event if a type of event, for example headache, occurs in at least 4 patients during the trial. Comparisons between groups will be done for both, the number of patients experiencing an event and for the number of events. The number of patients will be compared between groups with a two-sided chi-squared test, or a two-sided Fisher's exact test if the number of expected events per group is below 5. The number of events, with the possibility of multiple events per patient, will be compared between groups with a negative binomial model.

6.7. Sensitivity analyses

Pre-specified sensitivity analyses of the primary outcome of CR or PR at 24 months and secondary outcomes of CR or PR at 6, 12 and 18 months will include the following:

- Generalized estimating equation (GEE) to derive risk differences with two-sided 95% confidence intervals and two-sided p-values
- Logistic regression, crude and adjusted for center and proteinuria at baseline (<8g/24h vs ≥8g/24h), to derive odds ratios with two-sided 95% confidence intervals and two-sided p-values
- Ordinal logistic regression to compare the ordered outcome of CR, PR or no response, crude and adjusted for center and proteinuria at baseline (<8g/24h vs ≥8g/24h)
- Wilcoxon rank-sum test to derive a two-sided p-value for differences between groups in ordered outcome of CR, PR or no response

Pre-specified sensitivity analyses of continuous outcomes at 6, 12, 18 and 24 months will include the following:

- Mixed repeated measures linear regression with random intercepts for patients to derive differences in arithmetic means with two-sided 95% confidence intervals and two-sided p-values to compare creatinine clearance and quality of life scores between groups

- Mixed repeated measures linear regression with nested random intercepts for patients and centers to derive differences in arithmetic means with two-sided 95% confidence intervals and two-sided p-values to compare creatinine clearance and quality of life scores between groups

6.8. Subgroup analyses

We will conduct subgroup analyses of the primary outcome on the risk difference scale according to the following characteristics at baseline:

- Sex
- Age (≤ 50 versus > 50 years of age)
- Proteinuria ($< 8\text{g}/24\text{h}$ vs $\geq 8\text{g}/24\text{h}$)
- Anti-PLA2R status (positive, defined as $> 40\text{ U/mL}$, versus negative)
- Previous immunosuppressive therapy (naïve versus history of previous immunosuppressive therapy)

Subgroup analyses will be accompanied by tests for interaction between treatment and subgroup.

Protocol: Membranous Nephropathy Trial Of Rituximab (MENTOR)

Current SAP Version: 1.4, 16SEP2017

Summary of SAP Changes

The following is a summary of key changes made to the previous versions of the Statistical Analysis Plan. Formatting changes and minor administrative changes (typos, grammatical changes, minor clarifications in wording) are not included in this table.

Version 1.0 to 1.1:

| Section | Changes |
|--|---|
| 2.1 Patient flow | Added CONSORT flow diagram specifications: <ul style="list-style-type: none">• N assessed for eligibility• N not included in trial (with reasons)• N randomized• N allocated to intervention/control<ul style="list-style-type: none">○ N receiving allocated intervention○ N not receiving allocated intervention (with reasons)• N follow-up to month 6, 12, 18 and 24 (with reasons for termination)• N discontinued intervention before month 6 and 12 (with reasons)• N analyzed<ul style="list-style-type: none">○ N excluded from primary analysis (with reasons) |
| 4.2 Primary outcome | Added a clarification that the primary outcome is at 24 months after randomization as defined in the protocol |
| 6.2 Primary stepwise analysis of primary outcome | Clarified the absolute risk difference of the primary outcome as the risk of CR/PR in rituximab group minus the risk of CR/PR in cyclosporine group |
| 6.7 Sensitivity analyses | Clarified primary outcome as CR/PR status at 24 months |
| 6.8 Subgroup analyses | Added a statement that subgroup analyses will be accompanied by tests for interaction between treatment and subgroup |

Version 1.1 to 1.2:

| Section | Changes |
|---------------------|---|
| 4.2 Primary outcome | Added a clarification that all randomized patients will contribute to the analysis in the group they were originally allocated to |

| | |
|---------------------|-------------------------|
| 4.4 Safety outcomes | Added definition of SAE |
|---------------------|-------------------------|

Version 1.2 to 1.3:

| Section | Changes |
|------------------------------------|--|
| 2.6 Anti-PLA2R+ population | Added definition of anti-PLA2R+ (>40 U/mL) |
| 6.1 Baseline characteristics | Added baseline characteristic analyses specifications: <i>Patient characteristics and lab data at baseline will be summarized by treatment group. Continuous variables will be summarized using mean and standard deviation, or median and inter-quartile range if data are non-normally distributed. Categorical variables will be summarized with counts and percentages.</i> |
| 6.5 Analyses of secondary outcomes | Added proteinuria as an anticipated example of a strongly positively skewed data |

Version 1.3 to 1.4:

| Section | Changes |
|---|---|
| 2.2 Intention-to-treat (ITT) population | Corrected “full analysis set” to “intention-to-treat population” |
| 2.3 Per-protocol (PP) population | Added further clarification on PP population: <i>At least 1 completed 6-month treatment cycle, according to protocol. Patients who stopped treatment prematurely because of an adverse event or because of disease activity will be included in the PP population and be considered treatment failures</i> |
| 2.5 Responder population | Responder population was removed from analysis as continuous secondary outcomes such as proteinuria, anti-PLA2R, creatinine clearance and KDQoL will be examined cross-sectionally in patients who experienced complete or partial remission at each time point. |
| 2.5 Anti-PLA2R+ population | Specified assay used for anti-PLA2R (ELISA) and added clarification that anti-PLA2R+ population corresponds to anti-PLA2R+ patients of the ITT population |
| 3.3 Data preparation | Added a clarification that data will be omitted from analysis after initiation of a non-study <u>immunosuppressive</u> medication |
| | Added a clarification that continuous outcomes will be analyzed in a cross-sectional manner to allow comparisons of the quality of remission between groups: <i>By design, patients did not undergo systematic assessment of continuous outcomes (laboratory outcomes, quality of life scores) once they satisfied criteria for treatment failure. Therefore, continuous outcomes will be analyzed in a cross-sectional manner at each time point only in patients with complete or partial remission to</i> |

| | |
|--|--|
| | <i>allow exploratory comparisons of the quality of remission between groups at each time point; this approach will not allow longitudinal comparisons between time points.</i> |
| 4.1 Baseline characteristics | Updated units |
| | Added anti-PLA2R positive status |
| 4.2 Primary outcome | Expanded the definition of treatment failure for clarity: <i>Patients who reached less than 25% reduction of proteinuria from baseline at 6 months, patients who relapsed, patients who had a premature termination of the protocol-specified treatment schedule before 12 months due to disease activity or adverse event, patients who used an immunosuppressive medication other than the study medication for the treatment of membranous nephropathy before 12 months, patients who used any immunosuppressive medication for the treatment of membranous nephropathy after 12 months and before 24 months and patients who did not meet criteria of CR or PR at 24 months will be considered failures. Patients who were lost to follow-up at 24 months will be considered failures unless they were found to have achieved CR or PR at their 18 month visit.</i> |
| 4.3 Secondary outcomes | Specified time to composite of CR/PR as up to 12 months (end of treatment period) |
| | Added assessment of CR alone in the ITT population at 6, 12 and 18 months |
| | Revised analysis of proteinuria to be done cross-sectionally in patients with CR/PR at each time point |
| | Added proteinuria analysis for anti-PLA2R positive subgroup |
| | Analysis of relapse will be performed on patients with remission at the end of treatment period |
| | Time to relapse was replaced with Time to treatment failure in ITT population and in subgroup of patients with remission at the end of treatment period |
| | Added analysis of treatment failure at each time point for ITT population. Reiterated the definition of treatment failure |
| | Clarified that anti-PLA2R level analyses will be performed cross-sectionally in anti-PLA2R positive patients who are CR/PR at each time point and in all patients with CR/PR at each time point |
| | Corrected KDQOL version and scales names |
| | Added that analysis of physical health composite, mental health composite, burden of kidney disease, symptom/problems and effects of kidney disease subscales of KDQOL will be given precedence |
| | Added analysis of $\geq 50\%$ decrease in creatinine clearance for ITT population at 24 months |
| | Revised analysis of creatinine clearance to be done cross-sectionally in patients with CR/PR at each time point |
| 6. Statistical analyses | Corrections made to the non-inferiority condition: <i>Non-inferiority will be declared if the lower limit of the two-sided 95% confidence interval of the absolute risk difference of CR or PR at 24 months (risk of CR or PR in the Rituximab group minus the risk of CR or PR in the Cyclosporine group) is not lower than -15%</i> |
| 6.4 Non-inferiority analysis of secondary outcomes | Clarification added: <i>Since this is a secondary outcome, we will adopt a Bonferroni correction, which allows for this outcome to be tested in addition to the primary outcome at an alpha level of 0.0125 (0.025/2). The test will therefore be</i> |

| | |
|------------------------------------|--|
| | <i>considered significant if the one-sided p value is <0.0125.</i> |
| 6.5 Analyses of secondary outcomes | Clarified analysis method for difference in medians for strongly positively skewed continuous outcomes |
| | Added log transformed box and whisker plots for proteinuria and anti-PLA2R levels |
| 6.6 Analyses of safety outcomes | Clarified that chi-squared test and Fisher's exact test will be two-sided |
| 6.7 Sensitivity analyses | Clarified mixed repeated measures linear regression with random intercepts and nested random intercepts will be done to compare creatinine clearance and QoL scores between groups |
| | Removed analysis of covariance of log transformed outcome data in case of strongly positively skewed data, as is anticipated for proteinuria |
| 6.8 Subgroup analyses | Clarified that subgroup analyses will be done on the primary outcome |
| | Added history of previous immunosuppressive therapy for subgroup analysis |