A Multi-Center, Randomized Study of Cyclosporine or Corticosteroids as an Adjunct to Plasma Exchange in Initial Therapy of Thrombotic Thrombocytopenic Purpura

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1.0 OBJECTIVES

This will be a multi-center, open-label, randomized study that directly compares the use of cyclosporine and plasma exchange to corticosteroids and plasma exchange as the initial treatment of patients with thrombotic thrombocytopenic purpura. This study has the following objectives:

Primary Objective

1.1 To determine if cyclosporine given individually as an adjunct to plasma exchange decreases the rate of exacerbation in patients with TTP compared to corticosteroids given as an adjunct to plasma exchange.

Secondary Objectives

1.2 To compare the CSA and PE treatment arm to the corticosteroid and PE treatment arm with respect to:

- exacerbation rate within 30 days post-PE
- clinical response rate
- number of exchange procedures to achieve clinical response
- relapse rate (recurrent disease >30 days after last PE)
- safety data for CSA as an adjunct to PE.
- the effects of corticosteroids vs. cyclosporine on ADAMTS13 antigen, activity, and ADAMTS13 antibody concentration and inhibitor titers using serial measurements both during and after discontinuation of study drug
- the VWF multimeric patterns after initial therapy and throughout longitudinal follow-up between the two treatment arms
- the laboratory biomarker(s) that are prognostic for TTP exacerbation and the risk of relapse

2.0 BACKGROUND

2.1 Clinical Presentation and Therapy of TTP

There have been tremendous advances in our knowledge and understanding of the pathophysiology of TTP since Drs. Amorosi and Ultmann characterized the pentad of findings (fever, microangiopathic hemolytic anemia, thrombocytopenia, neurologic, and renal findings) in a 1966 review of all 271 previously reported cases.(1) Despite early attempts at therapy which included corticosteroids, splenectomy, and heparin, TTP was almost uniformly fatal with 90% of patients dying from the disease. Following observations of clinical improvement with plasma-based therapy, clinical trials soon demonstrated an 80-90% remission rate. (2) Rock et al reported one of the only randomized clinical trials in TTP and were able to demonstrate the superiority of

therapeutic PE over simple plasma infusion in the acute treatment of TTP.(3) Based upon this study, PE is considered the standard of care of acute TTP.

Despite the dramatic improvement in outcome with PE, complications related to the central venous catheter (infection, hemorrhage, and pneumothorax) and the plasma infusion (hypersensitivity, volume depletion, and transmission of infection) may be associated with significant morbidity and mortality. Howard et al recently updated their experience with PE for TTP at the Oklahoma Blood Institute, reporting roughly a third of patients experienced significant complications from PE and a 2.4% mortality rate.(4) More importantly, approximately 50% of patients with idiopathic or acquired TTP will suffer a recurrence of the disease after tapering plasma exchange, requiring repeated exposure to the risks of PE therapy.(5) These historical data were confirmed by our cohort study of corticosteroids and PE in which 60% of patients required the re-initiation of plasma-based therapy within 30 days of discontinuing PE therapy.(6)

2.2 Advances in the Pathophysiology of TTP

The earliest observations by Amorosi and Ultmann from post-mortem examinations described microthrombi in multiple organs of the body, including the heart, kidneys, adrenal glands, and the brain.(1) The etiology of the microthrombi was not known, but the findings were fitting with the clinical presentation and course of the patients. In 1982, Moake et al were able to demonstrate that some patients with TTP had unusually large multimeric forms of vWF, similar to those stored in vascular endothelial cells and platelets, but typically absent from normal plasma.(7, 8) Given the finding that these forms disappeared with the acute presentation of TTP and reappeared in patients after remission, it was hypothesized that these highly active multimers of vWF were central to the pathogenesis of the microthrombi seen in TTP. It was postulated that a vWF "depolymerase" was responsible for the conversion of unusually large multimers of vWF to the smaller multimers that are usually found in the circulation.(9) In this scenario, the unusually large multimers of vWF would spontaneously react with platelets resulting in the platelet thrombi in the microvasculature. It was also hypothesized that an antibody inhibitor of the "depolymerase" could account for the unusually large multimers seen in the plasma based upon reports of patients with chronic relapsing TTP being controlled with immunosuppressive therapy.(10) The subsequent identification of a specific protease(11, 12) that was shown to cleave purified human vWF as well as the finding that patients with TTP had deficiencies of protease function further strengthened the hypothesis. Furlan et al went on to describe both a familial form of TTP who had a constitutional deficiency, as well as a non-familial form due to an inhibitor of the vWF-cleaving protease.(13) This protease, now known as ADAMTS13, was recently purified and the gene localized to the long arm of chromosome 9.(14, 15)

2.3 ADAMTS13 Activity and Inhibitors in Idiopathic TTP

Initial reports correlating ADAMTS13 activity with the clinical diagnosis of acquired TTP showed greater than 70% of patients with idiopathic TTP to have a severe deficiency of protease activity (< 5%) with the majority having demonstrable antibody inhibitors of the

protease.(13, 16, 17) Autoantibody inhibitors of ADAMTS13 are now recognized to be central to the pathophysiology of idiopathic TTP, as well as patients with pregnancy associated TTP and TTP secondary to an autoimmune disease (SLE) (18, 19). Additionally, persistent or recurrent antibody inhibitors of ADAMTS13 have been associated with recurrences of TTP as well as a chronic relapsing form of the disease, giving more validity to the hypothesis of targeting the antibody inhibitor of ADAMTS13 (20-22). In their review of 142 consecutive patients with TTP from the Oklahoma TTP-HUS Registry, Vesely et al described an inverse relationship between ADAMTS13 activity and both exacerbations (recurrent thrombocytopenia requiring the resumption of PE less than 30 days from the cessation of PE) and relapses of TTP that was most striking in patients with a severe ADAMTS13 deficiency. (21) Of the 18 patients with a severe deficiency of the protease, all but one had a demonstrable antibody inhibitor. Therefore, although patients are able to achieve a clinical remission with PE, a persistent antibody inhibitor of ADAMTS13 likely is a factor contributing to higher rates of exacerbation and relapse in patients with idiopathic TTP. Similar data was reported by Zheng et al, showing that 3 of 4 patients with severe ADAMTS13 deficiency and high titer inhibitory antibodies had a chronic relapsing course of their disease.(23) In these 4 patients PE did not result in any detectable plasma ADAMTS13 activity or a consistent reduction in the inhibitor titer. It is important to note that the risk of relapse in patients with persistent inhibitors is not uniform, as not all patients with persistent inhibitors are destined to relapse.(24) Recent data has also suggested that a severe deficiency of ADAMTS13 protease function alone may not always be sufficient to trigger an acute episode of TTP.(25)

2.4 Immunotherapy of Idiopathic TTP

There is an obvious rationale for the role of immunosuppressive therapy in patients with idiopathic TTP, both as an adjunct to PE therapy as well as a prophylactic therapy preventing the recurrence of disease. In theory, PE would serve as a “supportive therapy”, physically removing the inhibitor and inducing remission, while at the same time allowing CSA, a “disease-modifying therapy”, time to exert its effect. An effective immunosuppressive therapy could inhibit the production of the antibody inhibitor of ADAMTS13, resulting in shorter courses of PE, decreased exacerbations and relapse rates, and decreased exposure to the potential risks associated with plasma based therapy. Many cytotoxic and immunosuppressive agents have been reported to be effective in relapsed and refractory TTP, including: corticosteroids, cyclophosphamide, vincristine, cyclosporine, and rituximab.(22, 23, 26-38) Unfortunately, there has been very little good, prospective data with which to accurately judge the effectiveness of a particular therapy without confounding factors. Rituximab has been reported to be effective in refractory TTP, as well as being effective as prophylactic therapy in patients with recurrent inhibitors and a chronic relapsing course of TTP.(22) Rituximab though may have significant drawbacks including the need for intravenous administration, the potential for infusional toxicity and infectious complications. The issue of the active removal of rituximab when combined with PE therapy also remains to be addressed. Advantages with both corticosteroids and cyclosporine include their ability to be

administered orally, and in the case of cyclosporine, the ability to be administered concurrently with PE without significantly affecting cyclosporine concentration.(39)

2.5 Corticosteroids

Corticosteroids are routinely been used and recommended(40) as an adjunct to PE, but until recently no prospective studies have addressed their efficacy as an adjunct to PE. Among the series of patients reported by Zheng et al, four patients with undetectable ADAMTS13 activity and high titer inhibitors of the protease were followed serially. Two were treated with corticosteroids as an adjunct to plasma exchange. Both showed no sustained improvement in ADAMTS13 activity or inhibitor titers during the follow-up period. Data from our prospective cohort study of corticosteroids as an adjunct to PE also showed that the addition of corticosteroids to PE did not reduce the exacerbation rate compared to historical data from the Oklahoma TTP registry where corticosteroids are not routinely used.(6, 21, 41)

2.6 Clinical Outcome- Concurrent Corticosteroids and PE as Initial Therapy

A total of 12 patients with idiopathic TTP were treated with corticosteroids and PE. Patients received prednisone at a dose of 1 mg/kg/day concurrently with daily plasma exchange therapy which was continued until remission was achieved (platelet count >150 x 10^9/ul). PE was then tapered and discontinued with the prednisone tapered over the next 4 weeks. Ten of 12 patients achieved remission after a median of 6.5 daily exchanges, but exacerbations of TTP occurred in 6/10 (60%), comparable to the 50% exacerbation rate from the Oklahoma Blood Institute where steroids were not routinely used. (Table 1) More importantly, there was no significant improvement in ADAMTS13 activity at remission nor suppression of the inhibitor measured within a week of tapering PE. Given these data, we then began to study alternative forms of immunosuppressive therapy as an adjunct to PE.

Table 1.

Clinical and Laboratory Data From Steroid and Plasma Exchange-Treated Patients

Clinical Data Remission	10/12
Med. Days to Remission	6.5 (range, 4 to 10)
Exacerbation Rate	6/10 (60%)
Laboratory Data Med. Pre-Treatment ADAMTS13	<1% (range, <1 to 5)
Med. Remission ADAMTS13	4% (range, <1 to 72)
Med. Pre-Treatment Inhibitor (ug/ml)	632 (range 188 to 3,574)
Med. Remission Inhibitor (ug/ml)	788 (range 144 to 8,113)

3.0 Rationale for Concurrent Cyclosporine and Plasma Exchange in TTP

Cyclosporine has been reported to be effective in cases of relapsed or refractory TTP.(26, 30, 31, 33) Our group has published a series of 3 patients with early

recurrences of TTP treated with cyclosporine alone, without the need for plasma exchange or infusion.(42) In addition to the clinical improvement seen, all 3 patients had significant increases in ADAMTS13 activity and suppression of the autoantibody inhibitor that correlated with a clinical remission of their disease. The therapeutic effect of CSA is thought to be related to its ability to inhibit the activation of genes necessary for the B-cell "helper" function (IL-4 and CD 40 ligand) of T-cells and T-cell proliferation (IL-2) required for the sustained production of high-affinity antibodies and memory B-cells to perpetuate a significant and sustained antibody response.(43) There is also increasing evidence that $CD4^+/CD25^+$ T-regulatory cells may be important in the control of pathologic immune responses in autoimmune hematologic disorders such as autoimmune hemolytic anemia and immune thrombocytopenic purpura (44-47). Low dose therapy with cyclosporine has also been shown to promote the development of $CD24^+/CD25^-$ T-regulatory cells in an animal model, providing an additional rationale for the use of CSA concurrent with PE.

3.1 Concurrent Cyclosporine and PE for Exacerbations and Refractory TTP

Ten patients with exacerbations or refractory TTP after their initial therapy received CSA concurrent with the resumption of PE.(48) All 10 patients were treated with concurrent CSA (2-3 mg/kg in a twice daily divided dose) and PE. CSA was continued for a total of 6 months and then discontinued. Nine of the 10 patients achieved remission after a median of 11 daily exchanges (range, 5-45), with only 1/9(9%) suffering an exacerbation after tapering PE. Median follow-up for the entire cohort is 31 months (range, 12 to 41). Eight of the 10 patients maintained a continuous remission throughout the planned 6 months of CSA and then discontinued therapy.

3.2 Concurrent Cyclosporine and PE as Upfront Therapy

Building on the findings of our initial study that suggested a clinical benefit for the addition of CSA to PE, we initiated a study of concurrent CSA and PE as the upfront therapy of idiopathic TTP. Eleven of 12 patients achieved remission after a median of 5 daily exchanges. Median follow-up for the entire cohort was 16 months (range, 1 to 25). None of the 10 evaluable patients achieving remission have suffered an exacerbation, a statistically significant difference compared to the corticosteroid treated patients (0% v. 60%, p=<0.05).{Cataland, 2006 #375} Seven of the 10 patients maintained a continuous remission throughout the planned 6 month course of CSA, with one recently enrolled patient having only completed 5 months of CSA to date. Two patients suffered a recurrence of TTP while still taking CSA after 3 and 4 months of CSA therapy respectively, with one of the patients relapsing 2 weeks after a 50% dose reduction of CSA as mandated by the study for an increase in the serum creatinine to 1.5 mg/dl (range, 0.9-1.4 mg/dl). Further analysis of this patient revealed his CSA concentration to be sub-therapeutic, arguing that his mild renal insufficiency was not related to CSA toxicity, but rather may have been related to his underlying thrombotic microangiopathy. These data from both cohort studies suggest that the use of CSA as an adjunct to PE resulted in a clinically and statistically significant reduction in the exacerbation rate

compared to concurrent corticosteroids and PE, preventing the need for re-exposure to the potential risks associated with PE therapy.

3.3 Combined Analysis: ADAMTS13 Activity, Antigen and Inhibitor After Concurrent CSA and PE

Patients on both cohort studies (exacerbation/refractory study and upfront study) of CSA and PE were followed serially to study the effect of CSA on ADAMTS13 activity, antigen, and inhibitor concentration and correlated with clinical outcome data. Complete ADAMTS13 and inhibitor data obtained serially for all CSA and PE treated patients during and after the 6 month period of CSA therapy is shown graphically in Figure 1. Although not randomized, these data suggest that cyclosporine improved ADAMTS13 activity via the suppression of the inhibitory IgG antibody. Although it could be argued that the improvement in ADAMTS13 activity could have resulted from PE therapy, the significant delay before the improvement in the ADAMTS13 activity argue that the CSA resulted in the suppression of the inhibitory antibody and the improvement in ADAMTS13 activity. Furthermore, the gradual decline seen after the discontinuation of CSA also support the hypothesis that it was the CSA and not the delayed effect of PE that resulted in the steady improvement in ADAMTS13 activity seen in this cohort of patients.

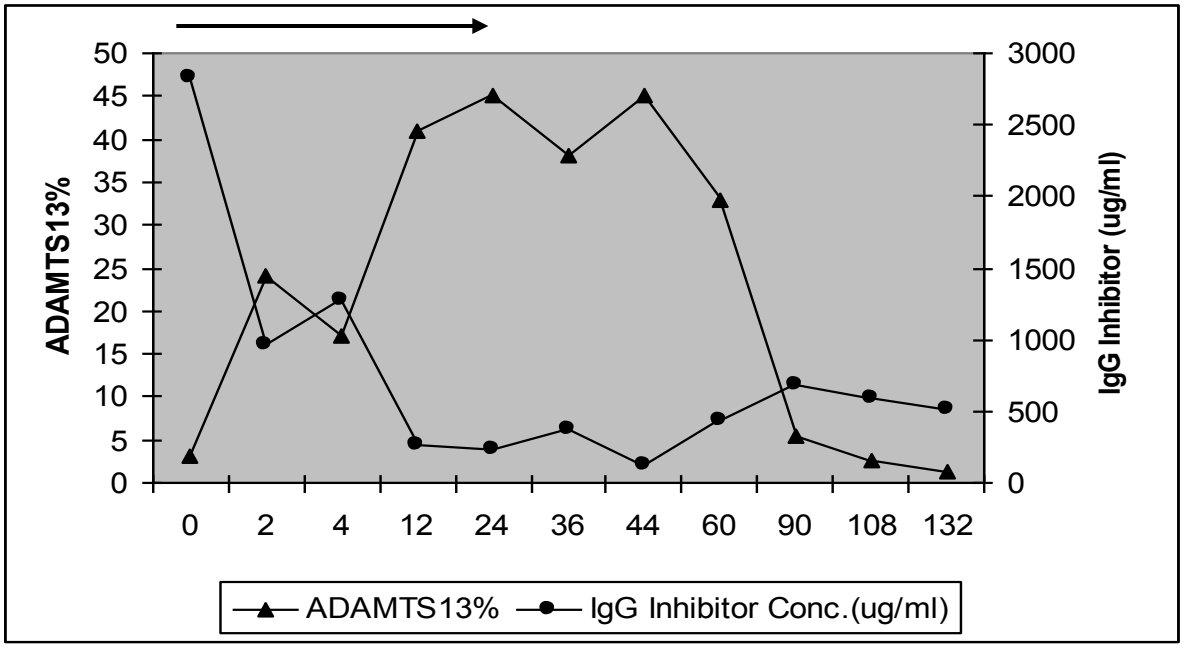

Figure 1. Serial determinations of the mean ADAMTS13% activity and inhibitor concentration throughout clinical follow-up both during and after therapy with cyclosporine. The arrow at the top of each graphs represent the duration of therapy with cyclosporine.

4.0 Toxicity of Combined Analysis of CSA and PE

Overall therapy with concurrent CSA and PE was well tolerated. Two patients treated with concurrent cyclosporine and PE had increases of the serum creatinine to 1.5 mg/dl

(normal < 1.4 mg/dl) after two and 4 weeks of cyclosporine therapy; both patients had their dose of cyclosporine decreased by 50% with subsequent normalization of the serum creatinine. Although this was presumed to be related to the cyclosporine, the concentrations of the drug obtained at the same time were not above the therapeutic range, raising the possibility that this mild increase in the serum creatinine may be related to the renal effects of TTP. Mild gastrointestinal toxicity was transiently experienced by 2 patients as well but did not require a decrease in the dose or frequency of the CSA therapy

5.0 Patient Selection

5.1 Inclusion of Women and Minorities.

Entry to this study is open to both men and women ages 18 and older, of all racial and ethnic subgroups. This study will be conducted in accordance with the International Conference on Harmonisation and the Helsinki Guidelines.

5.2 Eligibility Criteria.

5.2.1 Patients with a clinical diagnosis of idiopathic TTP as defined by a microangiopathic hemolytic anemia and thrombocytopenia ($<100 \times 10^3$)

5.2.2 Additional components of the pentad (fever, renal and neurologic abnormalities) need not be present.

5.2.3 Additional explanations for the microangiopathic changes including DIC and malignancy should be excluded.

5.2.4 Patients with pregnancy associated TTP will be permitted on this therapeutic trial if the child is delivered prior to the initiation of therapy for TTP. However, female patients that are breastfeeding and are unwilling to discontinue breastfeeding at the time of enrollment will be excluded from this study (see section 5.3.5)

5.2.5 Patients with a previous diagnosis of TTP are eligible to be enrolled provided they meet eligibility criteria and have not been treated for TTP in the past 30 days.

5.2.6 Given the potential for nephrotoxicity with CSA, all patients must have a serum creatinine of $\leq$ 2.5 mg/dl prior to enrollment

5.3 Exclusion Criteria.

5.3.1 In light of concern for the prompt initiation of PE, all patients with suspected TTP may be enrolled on this trial. If it is subsequently found that the patient does not meet enrollment criteria, they will be removed and their spot replaced for study purposes. Patients removed from the

study after enrollment will continue to be followed longitudinally for 6 months to be monitored for safety and will be included in the safety database.

5.3.2 Patients with TTP clinically categorized as secondary to stem cell transplant and solid organ, bloody diarrhea associated, malignancy associated, and drug associated will not be enrolled on this therapeutic study.

5.3.3 Incarcerated patients will be excluded from the study due to the inherent difficulties in maintaining close follow-up for study purposes in patients who are incarcerated.

5.3.4 Any patients already being treated chronically with corticosteroids or cyclosporine and taking these at the time of their presentation will be excluded from this study.

5.3.5 Female patients that are pregnant or breastfeeding and are unwilling to discontinue breastfeeding at the time of enrollment will be excluded from this study

5.3.6 Patients taking any medications contraindicated in combination with CSA that cannot be safely discontinued will be excluded from this study. A complete list of contraindicated medications is provided in Appendix 4.

5.4 ENROLLMENT/REGISTRATION OF PATIENTS

5.4.1 Patients must give written, informed consent prior to enrollment on this study. Informed consent may be obtained by the protocol chairman, the principal investigator at one of the additional sites, or one of their designees.

It is not uncommon for patients to have mental status changes related to their diagnosis of TTP (typically reversible with therapy) that may prevent them from being able to provide informed consent. If the principal investigator, co-investigator, or research study nurse do not feel the patient is able to provide informed consent, the study consent may be signed by the patient's legally authorized representative to enroll the patient in the study.

If the patient's legally authorized representative signs the consent, the patient must be then re-consented with the "re-consent form" if/when the patient regains the ability to provide informed consent.

5.4.2 Given concerns for the need to rapidly initiate, PE therapy should not be withheld while awaiting enrollment to this study. To allow sufficient time to evaluate for eligibility and obtain informed consent, patients will be permitted to be enrolled within 48 hours after the first PE procedure.

5.4.3 At the time of enrollment on this protocol, patients must be registered by phone with the study coordinating office at Ohio State University, the lead institution for this study. Contact information for all study personnel, protocol chairman, and co-investigators is detailed in section 15.0 of the protocol.

6.0 Randomization of Patients

6.1 Patients will be randomized at the time of registration/enrollment on this clinical trial via contact with the study nurse/coordinator at the time of the initial registration of the subject. The study statistician (Dr. Susan Geyer) will conduct the randomization and relay this information to the study nurse who will be in contact with the treating/enrolling physician so that the appropriate medication (prednisone or cyclosporine) may be immediately started with PE.

7.0 Treatment Plan: Initial Therapy

7.1 Therapeutic Plasma Exchange-Both Study Arms

7.1.1 Plasma exchange will be started as soon as the diagnosis of TTP has been made. (section 5.2.1). As it is important to institute therapy as soon as possible, patients will not have therapy delayed while obtaining informed consent.

7.1.2 Plasma exchange will be performed daily with one plasma volume exchanged using plasma as the replacement fluid until a complete remission is obtained (see response criteria 8.0).

7.1.3 Patients will receive either prednisone or cyclosporine orally as an adjunct to PE depending on the outcome of randomization (see sections 7.2-7.3 below). Therapy with these adjuncts will be initiated concurrent with and continue throughout plasma exchange therapy as described below.

7.1.4 After a complete remission (see response criteria 8.0) has been attained patients will have one exchange performed every other day for a total of 2 more exchanges, with the first day that the platelet count and LDH are normal counting as the first of the two exchanges.

7.2 Corticosteroid Study Arm

7.2.1 At the time of diagnosis, patients will be started on daily prednisone therapy at a dose of 1 mg/kg/day (rounded off to the nearest 20 mg multiple) concurrently with daily PE. Patients unable to take oral corticosteroids will be switched to an equivalent intravenous corticosteroid until they are able to take oral medications. Patients may receive intravenous corticosteroids at any time to urgently treat reactions to the

infused plasma and for prophylaxis against future reactions prior to the next TPE.

7.2.2 Prednisone should be continued at full dose for 30 days after the last PE procedure. Subsequently, patients should decrease the dose by 50% each week over 4 consecutive weeks, starting on day 31. All corticosteroids should be discontinued completely by the end of the 4 week taper (approximately 8 weeks total of corticosteroid therapy).

7.3 Cyclosporine (Neoral) Study Arm

7.3.1 As not all brands of cyclosporine can be used interchangeably due to the potential for inconsistencies in cyclosporine drug concentrations, physicians will use the Neoral brand of cylcosporine whenever possible. If an additional brand must be used, the patient must stay on that same brand of cyclosporine throughout the 6 month course as the differing brands cannot be used interchangeably.

7.3.2 At the time of diagnosis, patients will be started on daily cyclosporine (Neoral) therapy at a dose of 2-3 mg/kg/day (rounded to the nearest 50 mg increment) concurrent with daily plasma exchange. Doses will be administered in the capsule form. The total dose will be divided into twice daily dosing. For consistency and standardization of cyclosporine levels throughout the study, doses of Neoral (cyclosporine) should be given at 8 AM and 8 PM each day. Neoral (cyclosporine) should be taken on an empty stomach, and patients should not eat for at least 30 minutes after their dose of Neoral (cyclosporine). Cyclosporine will be continued for a total of six months of therapy and then discontinued.

7.3.3 Patients with renal insufficiency at presentation will have the dose of CSA modified to minimize any potential added nephrotoxicity of CSA. Patients with a serum creatinine of 2.5 mg/dl or greater will not begin therapy with CSA until the serum creatinine has fallen below this threshold. Patients with a serum creatinine of 1.5-2.5 mg/dl will have the CSA dose decreased by 50% until the serum creatinine has normalized (< 1.5 mg/dl). After renal function as measured by the serum creatinine has normalized patients will have the dose increased to the intended dose of 2-3 mg/kg/day.

7.4 Management of Cyclosporine Dosing and Concentrations

7.4.1 The optimal therapeutic concentration of CSA in TTP is not known. For the purpose of this study, the target CSA concentration will be identical to the accepted therapeutic CSA concentration at each institution(100-400 ng/ml at Ohio State University) when used as immunosuppressive therapy. As described below, dose adjustments of CSA will not be made to reach this goal, but rather the dosing will only be changed for patients with supratherapeutic concentrations or a decline in renal function. Only

trough concentrations should be used to adjust the CSA dose. If patients have already taken their CSA on the morning of the CSA concentration measurements, dose adjustments should only be made in clinical toxicity is present (hypertension. anxiety, diarrhea, emesis).

7.4.2 Cyclosporine levels (trough) will be monitored after the third day of therapy, weekly for the first 4 weeks of therapy and then monthly while they are taking CSA to monitor drug levels and minimize any potential nephrotoxic effects (see section 9.2, Laboratory Studies During Plasma Exchange) Patients should not take their CSA prior to having the CSA levels drawn, but should take their scheduled dose after their blood is drawn that day in order to check trough CSA levels. Trough levels of CSA should be obtained at 8 AM, at the same time the morning dose of CSA is to be given for hospitalized patients. CSA levels will then be obtained at each follow-up visit to monitor drug concentrations to minimize the potential for CSA toxicity. The CSA dose will not be increased based on these levels, only decreased or held as a response to potentially toxic levels of CSA.

7.4.3 Patients with trough CSA concentrations above the upper limit of normal will have the dose altered as described below and levels checked again in 3 days (see Appendix 2). Subjects with trough cyclosporine concentration of 401-500 ng/ml should decrease the dose of cyclosporine by 25% and recheck the trough concentration in 3 days. Subjects with cyclosporine concentrations of 501-600 ng/ml should decrease the dose by 50% and recheck the concentration in 3 days. Subjects with cyclosporine concentrations greater than 600 ng/ml should hold any further cyclosporine doses until the levels are rechecked in 3 days. If the cyclosporine concentration is now less than 600 ng/ml, a 50% dose reduction should be performed with future dose adjustments made depending on future cyclosporine concentration determinations as described above. Although there is a theoretical concern regarding the effect of daily plasma exchange on CSA levels, it has been reported that plasma exchange had no significant effect on measured CSA levels.(39)

7.5 Dose Adjustments During Cyclosporine Therapy and Renal Insufficiency

7.5.1 Patients who subsequently develop renal insufficiency during the 6 month course of CSA (serum creatinine >1.5, but $\leq$ 2.5 mg/dl) will have the dose decreased by 50% (see Appendix 2). The dose will be returned to the same planned dose (2-3 mg/kg) after the serum creatinine has returned to normal (<1.5 mg/dl). If patients do not tolerate the resumption of CSA to the full dose (recurrent increase in the serum creatinine, high CSA levels), patients should be maintained on the dose level that was able to achieve a therapeutic CSA levels without renal toxicity. Patients with a serum creatinine greater than 2.5 mg/dl will have the CSA held, and restarted only when the serum creatinine is $\leq$ 2.5 mg/dl, with the change in dosing schedule the same as described above.

8.0 Clinical Response Criteria

8.1 Complete Remission

A patient will be deemed to be in remission if all of the following criteria have been met within 4 weeks from enrollment and maintained after the tapering of plasma exchange:

- platelet count ≥150,000
- normalization of LDH
- a decreased or unchanged serum creatinine compared to the creatinine measurement obtained at the time of enrollment.
- clinical improvement in neurologic findings/deficits without the development of new neurologic findings/deficits by exam or radiographic studies.

8.2 Partial Response

8.2.1 Patients showing improvement (increase in platelet count and a decrease in the LDH, but not meeting the full criteria for complete remission) should continue concurrent CSA or prednisone and plasma exchange therapy for up to 4 weeks. If at that time patients still have not met the criteria for remission, patients will be considered to have been unable to achieve remission but will remain on this protocol for the purposes of data and clinical sample collection and for safety monitoring, but may be treated at the discretion of their treating physician.

8.3 Refractory Disease

8.3.1 Patients not showing signs of improvement (partial or complete response) or benefit with daily plasma exchange and CSA therapy after 1 week of continuous therapy should be considered to have refractory disease. These patients will remain on this protocol for the purposes of data and clinical sample collection and for safety monitoring, but may be treated at the discretion of their treating physician and will be considered to have failed therapy**.**

8.4 Exacerbation/Relapse Determination

8.4.1 Criteria to determine recurrences of disease and the need for re-initiation of therapy with plasma exchange will be the same as those to diagnose TTP (section 5.2.1). Patients requiring the reinstitution of plasma-based therapy (plasma exchange or infusion) within the first 30 days after discontinuing PE will be considered to have an “exacerbation” of their TTP.

8.4.2 Patients with a recurrence of TTP at a time point greater than 30 days from the end of plasma exchange therapy will be considered to have a "relapse" for the purposes of this study.

8.5 Treatment after Recurrences of TTP

8.5.1 Patients suffering exacerbations of TTP as described in the protocol (Section 6.4) after treatment on the corticosteroid arm of this study may then be treated with CSA concurrent with the resumption of plasma exchange at the same dose and schedule as described in the CSA arm of this randomized trial, assuming that they still meet enrollment criteria (including the serum creatinine). Patients will also discontinue the corticosteroids on the same day that CSA is initiated. Patients treated with CSA and PE for exacerbations of TTP will then be prospectively followed as described below (Section 9.3 – 9.5), but will be analyzed independently from the patients treated with CSA and PE as the upfront therapy.

8.5.2 Patients suffering a relapse (recurrence greater than 30 days after the last exchange procedure) whether during CSA or after completing 6 months of CSA will continue to be followed on this study for the purposes of gathering follow-up data, but may be treated at the discretion of the treating physician.

9.0 Clinical Studies

9.1 Laboratory Studies at Enrollment

9.1.1 The initial laboratory tests will be obtained at the time of enrollment on this study. Laboratory tests will include (see Appendix 1):

·CBC (complete blood count), platelet count and evaluation of the peripheral smear
·chemistry panel (Na, K, Cl,CO2, Cr, BUN, Ca, and glucose), total and direct bilirubin
·LDH (lactate dehydrogenase), haptoglobin
·PT /PTT /Fibrinogen
·Direct antiglobulin test (DAT)
·liver function tests (AST,ALT, GGT, Alkaline Phosphatase)

9.1.2 We will collect at least 100 cc plasma and 10 cc/2 teaspoons of whole blood prior to beginning of the plasma exchange for following laboratory investigations:

·ADAMTS13 activity

·ADAMTS13 inhibitor assay

·ADAMTS13 antigen level

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9.2 Laboratory Studies During Plasma Exchange

9.2.1 During plasma exchange therapy, the following labs will be obtained daily (see Appendix 1):

·CBC with a differential and platelet count

·chemistry panel (Na, K, Cl, CO2, Cr, BUN, Ca, and glucose) with LDH

9.3 Laboratory Studies During Plasma Exchange-Cyclosporine Monitoring

9.3.1 For patients randomized to the CSA arm of the study, CSA concentrations should be obtained on the 3rd day of PE, and then weekly thereafter as long as they remain in the hospital concurrent CSA and PE (see Appendix 1).

9.3.2 Samples for CSA levels should be obtained in the morning, prior to the first dose of CSA in order to obtain trough levels of CSA (section 7.4.2).

9.4 Outpatient/Remission Follow-up and Correlative Laboratory Studies

9.4.1 After discharge from the hospital, patients will be seen weekly in the outpatient clinic for the first month. Patients will then be seen monthly for the next 6 months, regardless of which arm of the study they were randomized to (see Appendix 1).

9.4.2 The following laboratory studies will be obtained at each follow-up visit:

·CBC with a platelet count
·chemistry panel (Na, K, Cl,CO2, Cr, BUN, and glucose), total and direct bilirubin
·LDH
·cyclosporine concentration (patient should be told not take their CSA dose until after the blood has been drawn to ensure that we are accurately measuring trough drug levels

9.4.3 In addition patients will have peripheral blood samples (10cc/2 teaspoons) obtained at the same intervals for:

·ADAMTS13 activity

·ADAMTS13 inhibitor assay

·ADAMTS13 antigen level

.

-ADAMTS13 antibody (IgG) concentration

-VWF multimeric analysis

9.5 Outpatient Visits

9.5.1 After discharge from the hospital, patients will be seen weekly in the outpatient clinic for the first month. Patients will then be seen monthly for the next 6 months, regardless of which arm of the study they were randomized to. After six months of continuous follow-up patients will then be seen every 3 months for the next 3 years to monitor the course of their disease and obtain laboratory follow-up studies (see Appendix 1)

10.0 Safety Monitoring During Study

10.0.1 To ensure the safety of all patients enrolled on this study careful monitoring and follow-up for all patients will be an important aspect of this study. At the time patients begin therapy with plasma exchange and either CSA or prednisone, all patients will be hospitalized and monitored for both response to therapy as well as any potential toxicities. Laboratory studies performed daily will monitor the clinical response to therapy, but will also monitor for the earliest signs any other end-organ toxicities (Section 9.1 – 9.4). In the CSA-treated patients the potential for renal toxicity is the primary concern, but patients on both arms will be monitored carefully for this as well as other potential toxicities through daily laboratory as well as daily clinical evaluations. After discharge, patients will continue to be monitored closely in the same manner with laboratory and clinical evaluations at regular intervals as described in Section 9.5 and Appendix 1 of the study protocol.

10.1 Definition of Adverse Events/Severe Adverse Events

10.1.1 Adverse events as defined in the International Council on Harmonisation Guideline for Good Clinical Practice are described as: “an untoward medical occurrence in a patient or clinical investigation administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment” (ICH E6:1.2).

10.1.2 A serious adverse event is an adverse event that:

-is fatal
-is life threatening (places the subject at immediate risk of death)
-results in inpatient hospitalization or prolonged existing hospitalization
-results in persistent or significant disability/incapacity

-is a congenital anomaly/birth defect
-other significant hazard

10.2 Reporting of Adverse Events

10.2.1 The principal investigator at each site is responsible for reporting each adverse event to the Protocol Chairman or the study nurse/coordinator coordinating site (Ohio State University Hospital). Contact information is provided in section 16.0 of the study protocol. Adverse events must be reported verbally and faxed to the coordinating site within 7 days of the occurrence using the supplied "Adverse Event" form (see Appendix 3). All serious adverse events must be communicated to the coordinating site verbally within the first 24 hours of the occurrence of the event. The "Adverse Event" form (see Appendix 3) must also be completed and returned to the coordinating site within 7 days of the occurrence of the event. Using these mechanisms of rapid reporting we will be able to rapidly evaluate and minimize the potential risks to patients from the occurrence of these events. Any significant adverse events that potentially would affect other enrolled patients and those yet to be enrolled will be communicating to all participating sites and investigators in a written format and via teleconference if warranted.

10.3 Follow-up of Adverse Events

10.3.1 Patients experiencing an adverse event will continue to be followed and carefully monitored until the resolution of the adverse event. This applies whether the patient continues on study or is removed from the study for any reason.

11.0 Sample Procurement

11.0.1 As stated in section 9.0**,** both plasma and blood samples will be taken as a part of this protocol. Theses samples will be obtained at the same time that standard clinical samples or procedures are being performed so patients will not be exposed to additional venipunctures or procedures by their participation in this study. The plasma to be studied is the byproduct of the plasma exchange procedure and would otherwise be discarded. Instead of discarding the entire plasma sample, 100 cc will be collected for study as is described in the protocol.

11.1 Sample Storage and Handling

11.1.1 At the time of enrollment and registration with the coordinating institution, arrangements will be made for the biologic samples to be delivered to the laboratory of Dr. Haifeng Wu for processing and analysis. Samples will be

labeled with a unique identification number so that the biological data obtained may be correlated with the patient's clinical course by the investigators. This will help ensure patient confidentiality, as the investigators will be the only people to know the patient name and medical record number that correspond with the identification number. Samples to be used for future study will be frozen at -80°C and also be stored in the laboratory of Dr. Wu. Laboratory studies unique to this protocol, including: ADAMTS13 activity, antigen level, and inhibitor assays will be performed in Dr. Wu's laboratory.

11.2 Sample Utilization

11.2.1 As our understanding of the pathophysiology of TTP increases, we will likely discover additional proteins or molecules that play a role in the initiation or the progression of TTP. It is for this reason that we will bank samples for future study. The patient samples held in storage will be kept indefinitely or until the sample is completely utilized for correlative studies. The patient samples will only be studied at the Ohio State University and will not be shared with any other researcher for study without prior approval of the IRB. The subject may withdraw consent at any time and the sample remaining and any data will be destroyed to the extent that is possible. Patients also will not receive any financial gain from the research associated with the tissue sample research or its applications.

12.0 Risks Associated with Testing of Patient Samples

12.1 A breach of confidentiality is the most serious risk which patients may be exposed to regarding to the testing of biologic samples. Confidentiality will be maintained by labeling the patient samples with only an identification number and not their name or medical record number. By using this approach, patient samples may be tied to clinical information by the investigators but will preserve the confidentiality of patients. Only the principal investigator, co-investigator, and the study nurse/coordinator will have access to both the study number and the corresponding patient identification information. Clinical information or laboratory data used for publication will not identify patients in any way that could potentially breach patient confidentiality.

13.0 ADAMTS13 Assays and ADAMTS13 Inhibitor Assays

13.1 Novel SELDI-Based ADAMTS13 Assay

Dr. Wu's laboratory has invested tremendous efforts to validate and standardize the tests for ADAMTS13 activity and inhibitory titer to meet the requirements set forth by the College of American Pathologists (CAP) for clinical testing. Both tests are done using a SELDI-TOF mass spectrometer. Validation studies were performed to evaluate test accuracy, test precision, reportable range, and test linearity. For example,

test accuracy was partly evaluated by testing a set of unknowns and correlating the results with those from the Coagulation Reference Laboratory at the BloodCenter of Wisconsin (kindly supported by Dr. Ken Friedman). Excellent correlation was achieved between SELDI-TOF and the FRET assays for ADAMTS13 (activity and inhibitor titer). ADAMTS13 activity and inhibitor titer assayts are now offered as clinical tests at OSUMC and are performed under SOPs with QC reagents (normal and abnormal) included in each run to ensure test accuracy. Longitudinal evaluation of QC samples is performed to monitor the test reproducibility and analytical performance of the instrument.

In late 2005, Dr. Wu's laboratory started using the SELDI-TOF mass spectrometry-based method to determine ADAMTS13 activity in patient samples (49). This new test offers a rapid result within 4 hours and has excellent test reproducibility (6, 42, 49, 50). In 2008, Dr. Wu laboratory modified the SELDI-TOF assay to now detect as low as 0.5% of ADAMTS13 activity in clinical samples (51). With this improvement we now can more precisely measure ADAMTS13 activity in most samples with extremely low activity. This in turn provides a dataset with quantifiable values in >95% of TTP samples to help for statistical analysis.

13.2 Laboratory Method for ADAMTS13 Antigen and Antibody (IgG) Concentrations

Quantification of ADAMTS13 antigen and ADAMTS13 autoantibody (IgG) will be measured using commercially available ELISA kits from American Diagnostica Inc. (IMUBIND® ADAMTS13 ELISA and ADAMTS13 Autoantibody ELISA). These two kits have been validated in our laboratory and used for several studies (6, 42, 49, 50). The reference ranges for both kits were determined using healthy donors (n=41) recruited from the local population. The results for the autoantibody ELISA are reported as micrograms of IgG antibody and for ADAMTS13 antigen reported as nanograms of ADAMTS13 protein per milliliter of patient plasma respectively.

13.3 Laboratory Method for ADAMTS13 Inhibitor Titer Determination

In order to evaluate ADAMTS13 inhibitory titer, we set up a SELDI-TOF based test for the determination of inhibitory titer as Bethesda units (BU). BU in each sample is defined as the dilution of patient plasma that neutralizes 50% of ADAMTS13 activity in an equal volume of pooled normal plasma (PNP). This method is similar to the standard laboratory procedure for the determination of BU for clotting factors that requires 1 hour incubation after mixing patient plasma (or dilute) with PNP(52).**13.4 Laboratory Method for VWF multimeric analysis**:

VWF multimeric analysis will be performed in Biomarker Reference Laboratory at the Ohio State University Medical Center. Dr. Wu is the

director of this clinical laboratory. The cost for this test will be paid from research fund under this clinical trial study.

14.0 Statistical Analysis

14.1 Statistical Considerations-Sample Size Determination

For the study of the primary endpoint (treatment success rate), the sample size required is calculated based on the data from our previous publications. In these previous studies that looked separately at CSA/PE in 10 patients and at corticosteroids/PE in another 10 patients, we saw promising results that led to our hypothesis that CSA/PE treatment for TTP patients results in greater treatment response. In those studies, the primary endpoint was the proportion of patients who had TTP exacerbation, which is a more stringent endpoint that does not include other types of treatment failures such as refractory patients and/or death within 30 days post-PE. In the previous published pilot studies, we showed that 6/10 corticosteroid-treated patients exacerbated versus 0/10 CSA-treated patients. Combined with experience with subsequently treated patients, we observed an overall treatment success rate of 80% and 83% in refractory vs. de novo TTP patients, respectively, treated with CSA+PE versus 33% in those treated with corticosteroids+PE.

In terms of our primary endpoint of treatment success rate, we have designed a phase III trial that compares the proportion of treatment successes in each arm. Specifically, this study design will have at least 80% power to determine a significant difference in the treatment success rates between the arms if the true rates are 50% vs. 80%, assuming a significance level of 0.05 and a two-sided test. This reflects a clinically meaningful difference, and the data from our previous studies and experience support it as a feasible difference to detect. We plan to enroll a total of 72 patients to this trial (36 to each arm) to test the null hypothesis that there is no difference in treatment success rate between these treatments.

14.2 Analysis Plan

Patients will be followed for 30 days after their last PE procedure unless a treatment failure has been documented prior to that timepoint. The treatment success rates will be calculated for each of the treatment arms along with their 95% confidence intervals. These proportions will be compared between treatment arms using Mantel-Haenszel chi-square test to accommodate the

stratification factor (recurrent vs. de novo TTP event). Statistical significance will be concluded based on a significance level of 0.05.

This study includes an interim analysis and formal “look” at the data after 24 patients have been accrued (12 to each arm), where the null hypothesis will be rejected if the calculated p-value comparing the two observed success rates for the treatment arms is <0.0002 (based on the Lan-DeMets alpha spending function and O’Brien-Fleming boundary calculation). We will also evaluate safety and tolerability data for each of the arms at the interim analysis, and these data will be reviewed not only by the study team but by the DSMB.

Through this study we also planned an internal pilot study to be completed after 12 patients have been accrued (6 to each arm) in an effort to obtain information on the feasibility of accrual and treatment follow-up for the endpoints; as an internal pilot study, all patients will be included in the overall trial and thus included in the interim and final analyses unless serious logistical issues were identified that would introduce bias by their inclusion. We reached the accrual point for this internal pilot study and no such logistical issues were identified; therefore, all patients will be included in the overall study. With so few patients, this internal pilot was used for assessment of feasibility; the early data were not formally evaluated and did not require a re-evaluation of our assumptions for the study design described above.

Additionally, we will evaluate several secondary clinical endpoints through this trial, including: 1) exacerbation rate within 30 days post-PE; 2) clinical response rate; 3) the number of PE procedures required to achieve a clinical response; 4) relapse rate; and 5) safety and tolerability of each of the adjuvant treatments to PE. Each of the rates will be calculated assuming that these endpoints are binomially distributed, and 95% confidence intervals generated. For the exacerbation rate endpoint, we will only include those patients who have achieved clinical response (i.e. excluding refractory patients) and compare these rates between the two arms. We will have at least 80% power to detect a difference if the true exacerbation rates are 55% vs. 90% for this subset analysis (not adjusting for multiple comparisons); however, this comparison is distinctly ancillary to our primary clinical interest of achieving treatment success in all patients treated versus only keeping responding patients from experiencing TTP exacerbation. We will also evaluate relapse as a time-to-event variable, where Kaplan-Meier methods will be used to assess time to relapse from last plasma exchange in responding patients. The number of exchange procedures required to achieve clinical response will be assessed between arms using Poisson regression. Safety and tolerability data will be summarized by type of adverse event with frequencies calculated and compared between arms. In addition to these planned quantitative analyses, we will also use graphical

analyses to assess patterns and differences in these factors between the treatment arms.

14.3 Statistical Considerations-Correlative Laboratory Studies

The correlative biomarker outcomes described above will be descriptively summarized across and within treatment arms, and patterns in these changes in biomarkers and how they relate to treatment success, relapse, and TTP exacerbation will be explored graphically. For the study to determine the effects of corticosteroids and cyclosporine given individually on serial measurements of ADAMTS13 biomarker profiles and VWF multimeric patterns after their initial therapy and throughout longitudinal follow-up, we will evaluate these biomarkers in relation to the clinical outcomes of interest. For treatment success rates, we will evaluate baseline levels of these biomarkers to identify if there are any associations between these biomarkers and how these patients respond to treatment. These will first be explored graphically, also denoting those patients in the two different treatment arms. Logistic regression for whether or not the patient had a treatment success will be modeled with these baseline biomarkers as well as treatment arm.

In addition, we will compare the changes for each biomarker between the two groups. TTP exacerbations and relapses will be modeled separately. Both of these events are preceded by a period of clinical response where patients exhibit resolution of clinical signs and normal laboratory values. However, as stated above, there are important differences that warrant modeling TTP exacerbation and TTP relapses independently. First, the frequencies of specimen collection are different between the observation periods that lead to the exacerbation and relapses. The patients in the period of initial clinical response are monitored weekly, while the patients in the sustained clinical remission are followed quarterly. Secondly, the underlying pathophysiology for exacerbation and relapse are likely to be different. The disease exacerbation probably represents a continuation of an incompletely resolved disease while relapses of TTP most likely represent a de novo episode of TTP. As a result, the biomarkers that predict TTP exacerbation and relapse are likely to be different.

To examine the potential of the 4 indices (ADAMTS13 activities, ADAMTS13 antigen levels, ADAMTS13 (IgG) autoantibody levels, and antibody inhibitor titers) to predict the recurrences of TTP, we will use the longitudinal data in two ways by separating indices obtained during the sustained clinical remission when they are measured every three months (quarterly) from those indices measured during the initial clinical response when patients are monitored weekly. The data for the relapse analysis will come from all quarterly samples collected from qualified study subjects during sustained remission. The outcome of interest will be whether or not the

individual experiences a relapse of TTP during the three months following the date the measurements are taken. We anticipate obtaining a total of >400 observations that will correspond to approximately >16 events of relapse and other quarterly measurements (n ~ 400) without relapses. For analysis of exacerbation in initial clinical remission, the outcome of interest will be whether or not the individual experiences an event of exacerbation in the week following the measurement. The weekly samples in initial clinical remission from the same group of study subjects will be analyzed. We anticipate a total of about 200 weekly data points, corresponding to about >16 measurements with events of exacerbation and 200 measurements without events in the week following measurement. We will use the logistic regression model to analyze all 4 indices independently as well as to evaluate their interacting effects.

Since each individual has several observations, possibly with and without relapse or exacerbation, this would induce a special correlation structure. For this reason, we will introduce random effects into the model (53-55). The covariates corresponding to gender, age, race, and treatment modalities (e.g. CSA vs. corticosteroids) will also be evaluated in the model. Additionally, various variance-covariance structures will be investigated. The Generalized Estimation Equation will also be used if necessary if our specific covariance structures lead to un-interpretable results. Since the number of predictor variables is relatively small, a standard traditional model selection procedure will likely be feasible to study the reach of the predictors as well as their interacting effects. Various model selection criteria such as Ra2, Mallows Cp, AIC, and BIC will be used to evaluate the putative models. In the case where samples are not available on all subjects at consistent timepoints, we will utilize the more flexible growth curve modeling approach to identify changes over time in the biomarker on the incidence and time to relapse as well as TTP exacerbation.

VWF multimer patterns form a relatively complex dataset. We will dissect VWF patterns into several parameters that will all give rise to a continuous measurement. This will be the density of VWF multimers in bands 1 through 15 and the area above band 15. All data will be normalized by the reference plasma included in each gel. For investigating the relationship between ULVWF (area above band 15) and relapse in the quarter immediately following, a logistic regression analysis as described will be performed. Specifically, the response variable will be 1 or 0, depending on whether relapse has occurred or not. The explanatory variable will be the ULVWF value at the beginning of the quarter-interval, with a random effect also included to account for correlations among the different response variable observations over time in a given patient. The effects of other covariates will be included in the model and adjusted for as necessary.

15.0 Costs of the Study

15.1 Given the fact that patients all patients will be receiving standard therapy for TTP (plasma exchange therapy), either the patient or their insurance carrier will be responsible for all of the medical expenses associated with the treatment of their TTP. The costs associated with the experimental laboratory studies will be paid by the study researchers and will not be passed on to patients or their insurance carriers. Patients enrolled on this study will not incur any additional expenses as a result of enrolling on this protocol.

16.0 Contact Information for Study Personnel:

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Columbus, OH 43210

8/30/2007 ed.

Appendix 1. Research Study Calendar

Time (weeks)	0	Day 3		1***	2	3	4	8	12	16	20	24	CSA Stop	28
Inpatient Treatment*	→	→*												
Clinic Visit**				X	X	X	X	X	X	X	X	X		X
Clinical Studies														
CBC	→	→*		X	X	X	X	X	X	X	X	X		X
Platelets	→	→*		X	X	X	X	X	X	X	X	X		X
Smear Review														
Chemistry Panel	→	→*		X	X	X	X	X	X	X	X	X		X
Total bilirubin	X													
Indirect bilirubin	X													
LDH	→	→*		X	X	X	X	X	X	X	X	X		X
Haptoglobin	X													
PT	X													
PTT	X													
Fibrinogen	X													
Direct antibody test	X													
Liver function tests	X													
Cyclosporine Level		X**		X	X	X	X	X	X	X	X	X		
Biologic Samples****	X			X	X	X	X	X	X	X	X	X		X

*Continue daily until remission and plasma exchange tapered and stopped.

**Cyclosporine levels start on day 3, then weekly until discharge.

*** Weekly visits begin after discharge from hospital.

**** Research samples:10cc blood, 100c plasma only at diagnosis at the outset of exchange procedure

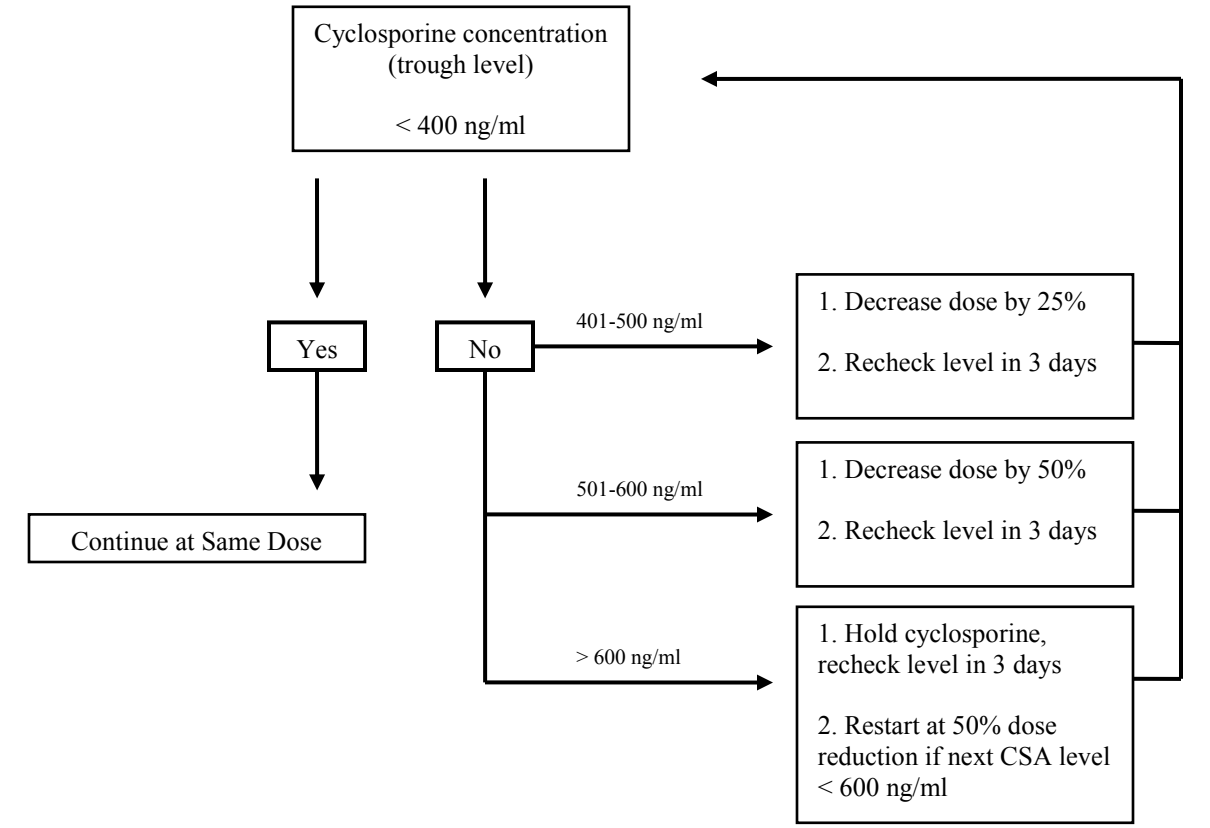
Time (weeks)	40	52	64	76	88	100	112	124	136	148	160
Inpatient Treatment*											
Clinic Visit**	X	X	X	X	X	X	X	X	X	X	X
Clinical Studies											
CBC	X	X	X	X	X	X	X	X	X	X	
Platelets	X	X	X	X	X	X	X	X	X	X	
Smear Review											
Chemistry Panel	X	X	X	X	X	X	X	X	X	X	
Total bilirubin											
Indirect bilirubin											
LDH	X	X	X	X	X	X	X	X	X	X	
Haptoglobin											
PT											
PTT											
Fibrinogen											
Direct antibody test											
Liver function tests											
Cyclosporine Level											
Biologic Samples****	X	X	X	X	X	X	X	X	X	X	X

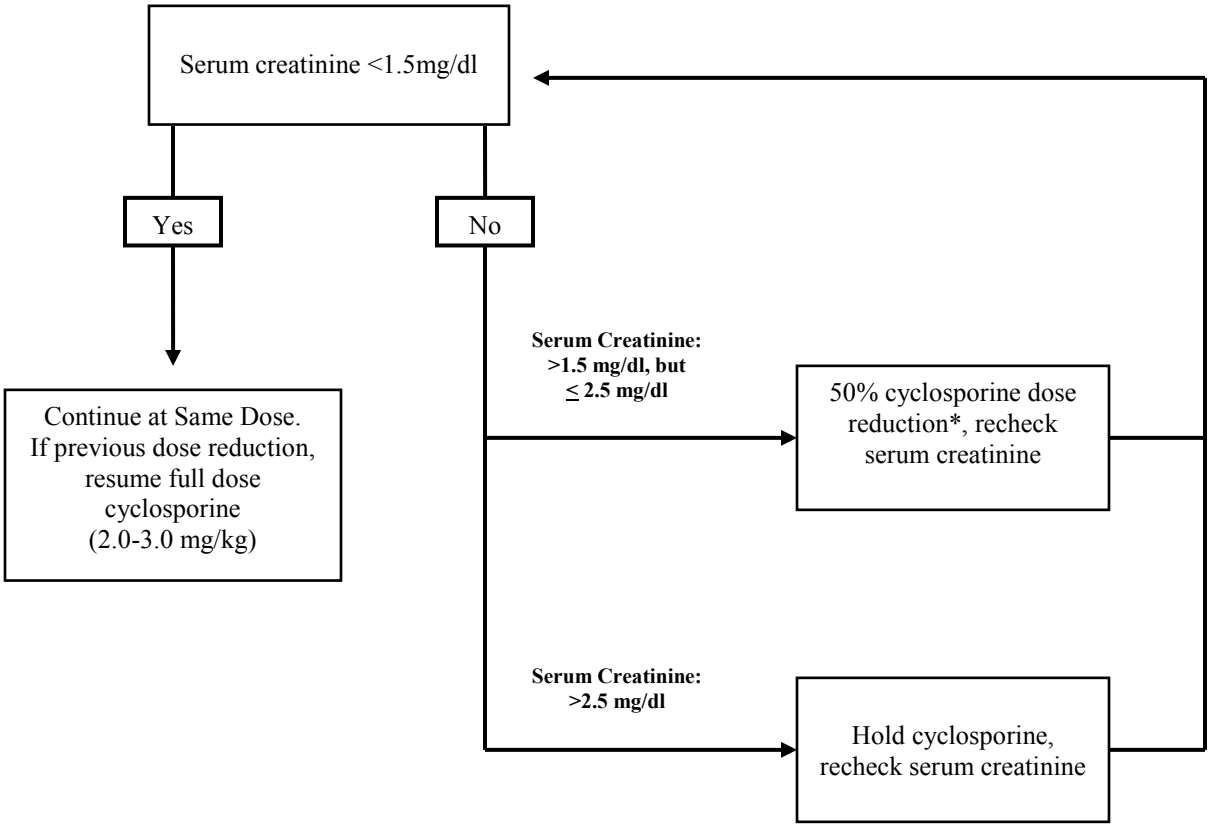
*Continue daily until remission and plasma exchange tapered and stopped.

**Cyclosporine levels start on day 3, then weekly until discharge.

*** Weekly visits begin after discharge from hospital.

**** Research samples:10cc blood, 100c plasma only at diagnosis at the outset of exchange procedure





Appendix 2. Algorithm for dose adjustment of cyclosporine for both elevated drug levels and renal insufficiency

*reduce cyclosporine dose if not already done. If previously dose reduced and stable serum creatinine, continue at same dose. If cyclosporine was previously held and the serum creatinine is now > 1.5 mg/dl but ≤ 2.5 mg/dl , restart at 50% of planned dose (1.0-1.5 mg/kg).

8.30.07 version

Appendix 3. Adverse Event Reporting Form

Patient #	Initials	DOB	Age	Sex		Study Arm (√) CSA	Steroids

Check all that apply	
Patient Died	☐
Life Threatening	☐
Prolonged Hosp.	☐
Congenital Anomaly	☐
Other Med. Important	☐

Describe Event

Suspected Drug		Dose	Route	Improvement After Drug Stopped

Drug Started	
Concurrent Meds.	

Other Relevant History

All serious adverse events need to be communicated by phone within 24 hours to 614-293-8183
Adverse event form must be faxed within 7 days of the adverse event to 614-293-7529

Appendix 4. Drugs Contraindicated in Combination with Cyclosporine (Neoral)

- Bosentan (probable)
- Alfalfa (probable)
- Atorvastatin (probable)
- Black Cohosh (probable)
- Caspofungin (probable)
- Cerivastatin (probable)
- Colchicine (probable)
- Cyclophosphamide (probable)
- Etoposide (probable)
- Felodipine (probable)
- Itraconazole (probable)
- Lovastatin (probable)
- Nafcillin (probable)
- Octreotide (probable)
- Orlistat (probable)
- Posaconazole (probable)
- Pyrazinamide (theoretical)
- Red Yeast Rice (probable)
- Rifabutin (theoretical)
- Rifampin (probable)
- Rosuvastatin (probable)
- Simvastatin (established)
- St John's Wort (established)
- Sulfinpyrazone (established)
- Tacrolimus (theoretical)
- Voriconazole (established)

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