STUDY PROTOCOL

Adjuvant Low Dose Rituximab for Acquired TTP with Severe ADAMTS13 Deficiency

(The ART Study)

STUDY DRUG: **RITUXAN (rituximab)**

IND NUMBER: **114955**

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ABSTRACT

The ART study (Adjuvant Rituximab in TTP) is a pilot safety/efficacy study of adjuvant low dose rituximab (100 mg/week x 4 doses) plus standard plasma exchange and corticosteroids for the treatment of thrombotic thrombocytopenic purpura (TTP) with severe ADAMTS13 deficiency. Results for study subjects will be compared to historical controls treated initially with plasma exchange and corticosteroids. This study proposes to test the hypothesis that adjuvant low dose rituximab may decrease the incidence of composite primary endpoint (exacerbations or refractory disease) in acquired TTP with severe ADAMTS13 deficiency. A novel ADAMTS13 assay will be used to identify patients with TTP and severe ADAMTS13 deficiency for enrollment, and to assess the utility of ADAMST13 as a biomarker for response to therapy and prognosis.

DEFINITIONS

Day 1: The first day a subject receives plasma exchange for treatment of TTP.

Treatment Response: 2 consecutive days with platelet count ≥150, 000/μL

Durable Treatment Response: Treatment Response that persists for ≥30 days after discontinuation of plasma exchange.

Exacerbation: Recurring TTP ≤30 days after a Treatment Response and discontinuation of plasma exchange.

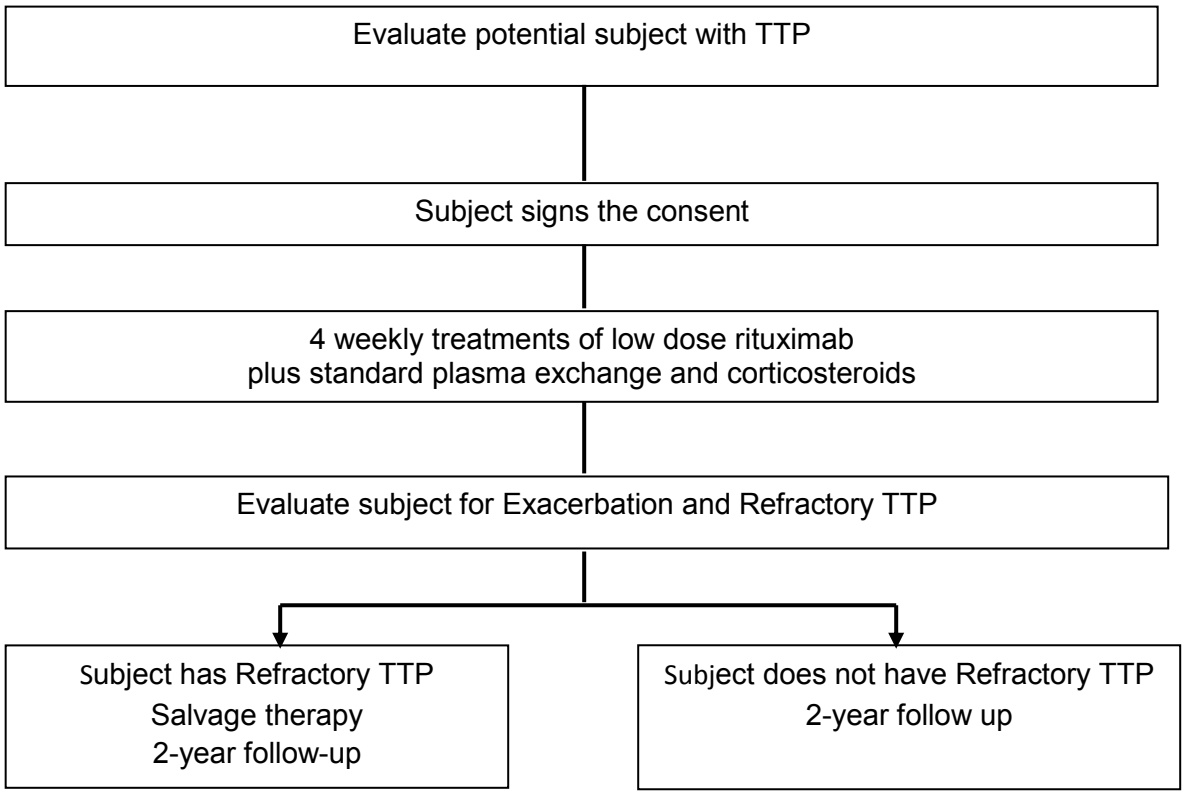
Refractory TTP: Failure to achieve a Treatment Response by day 28, or failure to achieve a Durable Treatment Response by day 60.

Composite Primary Endpoint: Exacerbation or Refractory TTP.

Relapse: Recurring TTP >30 days after Treatment Response.

Observation Period: The interval of 2 years after a subject first achieves a Durable Treatment Response.

Study Schemata:



1. OBJECTIVES

This study will evaluate the effect of low dose adjuvant rituximab combined with plasma exchange and corticosteroids on the course of TTP and severe ADAMTS13 deficiency (<10% activity).

1.1 Primary Endpoint

- Incidence of composite primary endpoint, Exacerbation or Refractory TTP

1.2 Secondary Endpoints

1.2.1 Clinical and Safety Endpoints

- Incidence of Durable Treatment Response
- Number of days to Durable Treatment Response
- Number of plasma exchanges to Durable Treatment Response
- Incidence of relapse
- Numbers of days to relapse
- 4 week, 1 year and 2 year incidence of death
- Incidence, type and severity of treatment-related adverse events

1.2.2 Laboratory Objectives

- Assess the relationship of ADAMTS13 enzyme activity and ADAMTS13 autoantibody titer to indicators of disease activity including:
    - Primary and secondary clinical endpoints
    - Platelet count
    - LDH
    - B cell depletion and recovery
- Assess the relationship of rituximab levels to the extent and duration of B cell depletion
- Describe the effect of plasma exchange on rituximab levels

2. BACKGROUND AND SIGNIFICANCE

2.1. Utility of ADAMTS13 data in TTP

The introduction of plasmapheresis with plasma exchange has increased survival in patients with TTP from less than 10 percent to 80 percent and is the only therapy demonstrated to be effective in a randomized clinical trial (Rock 1991). Plasma exchange rapidly restores circulating levels of the von Willebrand factor cleaving protease, ADAMTS13, and removes inhibitors to the enzyme. Although clearly beneficial, plasma exchange does not correct the primary cause of the disorder. More than 30% of patients with TTP have at least one relapse within 2 years (Kremer 2008). In addition plasma exchange may itself cause significant morbidity. One study documents a 31% risk for major complications, including a 3% fatality rate (McMinn 2003).

An autoimmune etiology of acquired TTP in adults is strongly supported by the finding of circulating ADAMTS13 inhibitors due to IgG autoantibodies. These inhibitors are found in 48 to 80% of individuals with idiopathic TTP (Moake 2002, Tsai 2003, Veyradier 2001). ADAMTS13, a von Willebrand factor-cleaving protease, regulates the size of von Willebrand factor (VWF) multimers. Deficiency of ADAMTS13 activity is associated with persistence of ultra large molecular weight VWF (ULVWF) multimers. ULVWF avidly binds to platelets, leading to

microvascular platelet deposition, the hallmark of TTP. Subsequent series identified severe ADAMTS13 activity deficiency in 30% to 80% of cases of TTP (Lammle 2005).

Although severe deficiency of ADAMTS13 activity (<5%) appears to be specific for TTP, the syndrome can also be seen in subjects with less severe deficiency and even normal levels of ADAMTS13 making diagnostic decisions difficult (George 2004). However, the risk of relapse is higher (~45%) for patients with persistent severe ADAMTS13 deficiency during remission, and essentially all of these patients have severe ADAMTS13 deficiency when they relapse (Zheng 2004). ADAMTS 13 inhibitors also correlate with relapses, delayed response to plasma exchange, refractory disease, and early death (Zheng 2004, Bohm 2005, Coppo 2006, Kremer Hovinga 2010).

Based on these studies, measurement of ADAMST13 activity and inhibitor level may be useful to identify patients with TTP who can benefit from immunosuppressive therapy in addition to plasma exchange. However, current ADAMTS13 assays have limited sensitivity, especially for detecting inhibitors. At BJH, ADAMTS13 assays have a turnaround time of 3 to 7 days, which is too slow to be useful for therapeutic decisions. To address these points, Dr. Evan Sadler's laboratory has developed a new fluorogenic ADAMTS13 substrate that has substantially increased sensitivity for measuring ADAMTS 13 activity and inhibitor titer. Results can be available in <24 hours, increasing the practical use of this assay in patient management.

2.2 Efficacy of Rituximab in TTP

Refractory or relapsing TTP has been treated with a variety of immunosuppressive agents (Ziman 2005, Bell 1991, Alford 2003). The most consistently effective of them is rituximab, a chimeric monoclonal antibody directed at the CD20 antigen expressed on the surface of B lymphocytes. Rituximab has been used successfully in the therapy of B-cell malignancies (O'Brien 2001, Plosker 2003, Cartron 2004). Efficacy has also been demonstrated in autoimmune disorders. Stasi et al., observed a 52% response rate of immune thrombocytopenic purpura with rituximab (Stasi 2001) and an 80% response rate of acquired anti-factor VIII antibodies (Stasi 2004). Rituximab-mediated B cell depletion occurs by several mechanisms including complement mediated cell lysis and antibody-dependent cellular cytotoxicity (Cartron 2004). In animal experiments, peripheral blood B-cells are reduced to undetectable levels (>95% depletion) within 24 hours after infusion (Reff 1994) and lymphopenia persists for as long as 8 days. The majority of lymph node B-cells are depleted within 15 days after weekly antibody treatment, requiring 60 to 90 days to reach normal levels. Based on the recovery of peripheral blood B-cells approximately 8 days after single dosing, a weekly infusion schedule has been adopted in the treatment of B-cell malignancies.

The use of rituximab in the treatment of TTP has recently been reviewed. Of 73 evaluable patients with TTP due to immune-mediated ADAMTS13 deficiency, the majority (95%) achieved a complete remission within 2-3 weeks of the first dose of ritixumab. Relapses were reported in 10 of 69 responding patients (14%), with a median time to relapse of 22 months. For patients not treated with rituximab, the expected relapse rate is as high as 60%. However, the favorable results with rituximab should be interpreted cautiously given the relatively short median duration of follow-up of approximately 10 months. Rituximab was generally well tolerated, with few serious adverse events reported. Three severe infectious complications were identified, including viral reactivation in keeping with black box warnings for this agent (Elliot 2009).

Experience with rituximab in TTP at Washington University was reviewed by Ling et al (2009). Twelve of 13 patients (92%) with severe ADAMTS13 deficiency (<10%) achieved a complete response; no relapses occurred with a median follow-up of 24 months (range, 13–84

months). Of eight patients with available ADAMTS13 data after rituximab treatment, seven had recovery of normal ADAMTS13 activity and disappearance of a detectable inhibitor (Ling 2009).

A prospective study in France (Froissart 2012) compared 22 adults with refractory TTP treated with rituximab and plasma exchange to 53 historical controls who received plasma exchange with or without vincristine. The time to a durable remission was significantly shorter for patients receiving rituximab (P = 0.03), although the plasma volume required to achieve a durable remission was not significantly different compared to controls. Of the rituximab-treated patients, none relapsed within the first year although three relapsed up to 3 years later. Treatment with rituximab produced a rapid and profound depletion of peripheral B-cells that lasted 9 months and correlated with a higher level of ADAMST13 activity and lower antibody titers. These differences were no longer significant after 12 months. No severe side effects occurred.

Thus, treatment with rituximab normalizes ADAMTS13 and induces durable remissions in >90% of patients with refractory or relapsing TTP. Late relapses have occurred in ~10% of these patients after 9 months to 4 years; all but one had a prolonged remission when retreated with rituximab (Sadler 2008). These results suggest that adjuvant rituximab might increase the rate of remission and reduce relapses for patients with severe ADAMTS13 deficiency at diagnosis. In fact, a recent study of adjuvant full dose rituximab (375 mg/m^2 weekly x 4) observed a 10% relapse rate compared to 57% in historical controls (Scully 2011).

2.3 Standard Dose versus Low Dose Rituximab in ITP

Barriers to the use of adjuvant rituximab include cost and side effects. A standard course of rituximab (375 mg/m^2 weekly x 4) costs ~$14,000. Complications of standard dosing include mild infusion reactions, and less commonly serum sickness or skin rash. Rare serious complications include (1 each) bronchospasm, anaphylaxis (non-fatal), transient cardiogenic shock, CMV reactivation, HZV myelitis, and fatal pneumonia among 306 patients with ITP (Arnold 2007)) and 73 with TTP (Elliot 2009). Hepatitis B or C reactivation and progressive multifocal leukoencephalopathy are potential risks, but the incidence appears to be very low in autoimmune disorders (Stasi 2010).

The optimal dose for TTP is unknown and the current standard dose is based on regimens used to treat B cell lymphoma. In TTP and other autoimmune diseases the total B cell mass is much less than in patients with lymphoma and therefore a reduced dosage of rituximab might be sufficient.

For example, in a study of 11 patients with various autoimmune cytopenias, sustained complete responses to low dose rituximab (100 mg per week for 4 weeks) occurred in 4 of 7 patients with ITP and 1 patient with autoimmune pancytopenia. A partial response was observed in a patient with autoimmune hemolytic anemia, whereas no effect was observed in 1 patient each with pure red cell aplasia and autoimmune neutropenia (Provan 2007).

Several studies of ITP also suggest that low dose rituximab may be effective. In 48 patients with immune thrombocytopenia (ITP), low dose rituximab (100 mg per week for 4 weeks) induced overall and complete responses in 60.5 % and 39.5% of patients respectively. A review of 19 studies using full dose rituximab in ITP calculated overall and complete response rates of 62% and 46% (Arnold 2007), which is comparable to the results reported for low dose rituximab (Stasi 2010). In responders to low dose rituximab the median time to response was 35 days. 16 of 29 responders relapsed and 14 of them needed further treatment. The 12-month and 24-month cumulative relapse-free survival was 61% and 45%, respectively (Zafa 2010). A direct comparison of low-dose and standard-dose rituximab will be required to determine whether the low dose regimen is equivalent with respect to time to response and relapse rate.

2.4. Proposed Study of Low-Dose Adjuvant Rituximab for TTP

The substitution of low dose for standard dose rituximab would reduce the drug cost 7-fold to approximately $2,000 for a 4 week course, which approximates the cost of one plasma exchange procedure. Low dose rituximab (100 mg) may also have a decreased risk of complications. Two studies of low dose rituximab for ITP reported mainly mild infusional reactions. The rate of serious complications is probably low but not known precisely: one of 59 patients with ITP treated with low dose rituximab developed interstitial pneumonia that responded to steroids (Provan 2007, Zaja 2010).

Based on the currently available data we propose a pilot study to determine whether adjuvant low dose rituximab, in addition to standard therapy with plasma exchange and corticosteroids, can shorten the time to treatment response and reduce the risk of exacerbation for patients with TTP caused by ADAMTS 13 deficiency.

We will use a novel assay to evaluate the utility of ADAMTS 13 as a biomarker to assess the response to therapy and predict the risk of relapse. If successful this therapy would not only improve patient outcomes but also would be cost saving compared to standard dose, salvage rituximab, which is the current standard of care.

3. STUDY POPULATION

3.1 Inclusion criteria

1) Age ≥18 years
2) Diagnosis of suspected TTP
    a. Platelet count of <80,000/μL for newly diagnosed patients and <120,000/μL for relapsed patients
    b. Microangiopathic hemolytic anemia (MHA) with RBC fragmentation
    c. LDH >1 x ULN
3) Subjects who will receive treatment for TTP with plasma exchange
4) Subjects who have not started the 5^{TH} plasma exchange.
5) Plasma ADAMTS13 activity <10%

3.2 Exclusion criteria

1) Treatment for TTP within the past 2 months
2) Severe active infection indicated by sepsis (requirement for pressors with or without positive blood cultures) or clinical evidence of enteric infection with *E. coli* O157:H7 or related organism.
3) Currently under treatment for cancer or with a current diagnosis of cancer (subjects with localized skin carcinoma will be accepted)
4) Microangiopathic hemolytic anemia due to a mechanical heart valve
5) Severe hypertension, as defined by systolic BP >180 AND diastolic BP >120, or papilledema
6) Organ or stem cell transplant

7) Use of calcineurin inhibitors (e. g., sirolimus, tacrolimus, cyclosporin A) within 6 months prior to diagnosis
8) Disseminated intravascular coagulation, as defined by:
    a. INR >2.0 (unrelated to anticoagulation, unresponsive to vitamin K) ***or***
    b. Fibrinogen <100 mg/dL
9) Pregnancy
10) Known congenital TTP.
11) Rituximab within the previous year.
12) HIV history or positive serology
13) History of hepatitis B or positive serology for HBsAg or Anti-HBc
14) Persistent or unexplained platelet count below 150,000/μL within 3 months of current TTP presentation
15) Hypersensitivities or allergies to murine and/or humanized antibodies
16) Current participation in trials of investigational therapies or devices, except that investigational central catheters are not exclusions

4. STUDY ENROLLMENT

4.1 Recruitment and screening

Subjects will be recruited through the Transfusion Medicine or Hematology Consult services at Washington University and up to 5 additional sites. A potentially eligible subject will be identified by his or her physician. Once a potentially eligible subject is identified, and after the treating staff has obtained permission from the subject to be contacted, a member of the study staff will contact the subject, describe the study, and obtain consent.

After signing the consent form the subject will be assigned a study ID number and study staff will determine if the subject is eligible based on his or her medical history and laboratory results. A subject is considered enrolled in the study after the informed consent document is signed by the subject and the subject meets all eligibility criteria. Within one day (Monday-Friday) after enrollment, the site should submit a copy of the Inclusion/Exclusion Criteria Checklist that has been signed by a site investigator to the Washington University Clinical Coordinating Center.

5. STUDY INTERVENTION AND CONCOMITANT THERAPIES

5.1 Rituximab

5.1.1 Description

The RITUXAN® (rituximab) antibody is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. The antibody is an IgG1 kappa immunoglobulin containing murine light- and heavy-chain variable region sequences and human constant region sequences. Rituximab is composed of two heavy chains of 451 amino acids and two light chains of 213amino acids (based on cDNA analysis) and has an approximate molecular weight of 145 kD. Rituximab has a binding affinity for the CD20 antigen of approximately 8.0 nM.

The chimeric anti-CD20 antibody is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product. The anti-CD20 antibody is purified by affinity and ion exchange chromatography. The purification process includes specific viral inactivation and removal procedures. Rituximab drug product is manufactured from bulk drug substance manufactured by Genentech, Inc. (US License No. 1048).

Rituximab is a sterile, clear, colorless, preservative-free liquid concentrate for intravenous (IV) administration. Rituximab is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for IV administration in 9 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Water for Injection. The pH is adjusted to 6.5.

5.1.2 Administration

Each study site will utilize its own supply of rituximab, and will store under conditions consistent with the drug's package insert. The prescribed dose of rituximab will be reconstituted in normal saline to a concentration no more than 4 mg/mL. Rituximab dose is fixed dose of 100 mg, given intravenously, repeated at one-week intervals for a total of four doses. Rituximab is to be administered only by slow intravenous infusion; it is not to be administered as an IV push or bolus. The infusion may be administered via a peripheral venous catheter; no central catheter is required. Treatment may be on an out-patient basis; in-patient hospitalization is not required. Each rituximab infusion bag will be labeled by the pharmacy as "Investigational Drug – ART Study". An Investigational Drug Accountability Log will be provided to each site by the sponsor-investigator and will be maintained by personnel at each study site. This Log will be updated by study personnel each time rituximab is dispensed by the pharmacy and a copy will be provided to the site research coordinator and the Washington University liaison. All expired or unused drug will be destroyed in accordance with local institutional practice.

Rituximab is to be infused every 7 days with a window of 5-9 days considered acceptable. If a dose is administered outside the 5-9 day window, then the schedule will be altered such that the next dose will be given 7 days later and any subsequent doses will be given at 7 day intervals.

The first rituximab infusion will be given as soon as possible after study eligibility is established but before the 5th plasma exchange.

The initial rituximab solution for infusion will be administered intravenously at an initial rate of 50 mg/hr. Subsequent rituximab infusion can be administered at an initial rate of 100 mg/hr.

5.1.3 Pre-medication

Pre-medication with oral acetaminophen, diphenhydramine for prevention of reactions to rituximab is strongly recommended. If the patient has started or completed the taper of prednisone consider a single dose of 100 mg methyl prednisone before rituximab infusion.

5.1.4 Administration While on Plasma Exchange

The rituximab infusion is to be given immediately after plasma exchange. The next plasma exchange is to be performed as late as possible in order to maximize exposure to rituximab. At least a 20-hour time interval between rituximab and plasma exchange is the goal. Plasma exchange is not to be performed sooner than 12 hours after the end of a rituximab infusion.

5.1.5 Administration After Plasma Exchange

Subjects who are no longer receiving plasma exchange may continue to receive the remainder of the four weekly rituximab infusions on an outpatient basis. Each subject receiving rituximab as an outpatient will be contacted by study personnel by telephone the day after each infusion to assess medical status.

5.1.6 Interrupting Infusions

Rituximab infusion will be interrupted for severe reactions. In most cases, the infusion can be resumed at a 50% reduction in rate (e.g. from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Treatment of infusion related symptoms with diphenhydramine and acetaminophen is recommended. Additional treatment with bronchodilators or IV saline may be indicated. Most subjects who have experienced non-life-threatening infusion-related reactions have been able to complete the full course of rituximab therapy. Epinephrine, antihistamines and corticosteroids will be available for immediate use according to hospital procedures for an emergent situation such as hypersensitivity reaction to rituximab (e.g. anaphylaxis).

5.2 Plasma Exchange and Corticosteroids

5.2.1 Initial Treatment with Plasma Exchange and Corticosteroids

The target for plasma replacement is 1.5 plasma volumes. Fresh frozen plasma (FFP) is the preferred replacement fluid. Frozen plasma 24 (plasma frozen with 24 hours of draw) or cryoprecipitate-poor plasma (CPP) may be used if FFP is not available. Some clinicians may prefer to switch to CPP replacement and this is acceptable. Prophylaxis for reactions to plasma during plasma exchange is at the investigator's discretion.

Each subject will also be given 1 mg/kg of prednisone (or equivalent) (within a margin of 10%) per day. Subjects with medical contraindications for steroid therapy (e. g. history of psychosis, brittle or uncontrolled diabetes, peptic ulcer disease, poorly controlled hypertension, or severe osteoporosis) may have corticosteroids withheld. The following prophylaxis will be used for corticosteroids:

- Gastric protection is strongly encouraged, but the method is at the investigator's discretion.
- Thrush and pneumocystis prophylaxis will be at the investigator's discretion.

5.2.2 Stopping Initial Treatment with Plasma Exchange and Corticosteroids.

When the criteria for Treatment Response are met (platelet count ≥150,000/μL for 2 consecutive days), plasma exchange is to be discontinued and tapering of corticosteroid initiated. Steroid tapering may be accelerated at the discretion of the clinician.

Table 1: Suggested **Prednisone Taper Schedule**

Week after stopping PE	Dose of Prednisone (mg/kg)
1	0.5 per day
2	0.25 per day
3	0.375 every other day
4	0.25 every other day
5	0.125 every other day
6	0.0625 every other day
7	stop

5.2.3 Treatment of Exacerbation with Plasma Exchange and Corticosteroids.

If a subject achieves a Treatment Response by day 28 and subsequently has a platelet count (confirmed by a repeat count) ≤120,000/μL within 30 days after stopping plasma exchange, then plasma exchange and corticosteroid treatments are to be restarted as described under **section 5.2.1.** The confirmatory platelet count must be a discrete collection drawn 1 to 24 hours after the first collection.

5.2.4 Stopping Treatment of Exacerbation with Plasma Exchange and Corticosteroids

After an early exacerbation plasma exchange is to be stopped and corticosteroids tapered when the subject has a platelet count ≥150,000/μL on 3 consecutive days.

5.2.5 Treatment of Refractory TTP

The subject will be considered to have Refractory TTP if they do not have a Treatment Response by day 28, or do not have a Durable Treatment Response by day 60. The treatment of Refractory TTP will include resuming plasma exchange and corticosteroids. Additional salvage therapy is at the physician's discretion and can include full dose rituximab 375 mg/m^2 weekly for 4 doses. Patient with Refractory TTP will be followed for 2 years with laboratory monitoring.

5.2.6 Treatment of Relapsed TTP

Recurring TTP >30 days after Treatment Response is considered to be Relapsed TTP. The treatment of Relapsed TTP will include resuming plasma exchange and corticosteroids. Additional salvage therapy is at the physician's discretion and can include full dose rituximab 375 mg /m^2 weekly for 4 doses. Patients with Relapsed TTP will be followed for 2 years with laboratory monitoring.

5.3 Non-Protocol Treatments

The following medications and other treatments, which can influence the course of TTP, will be considered protocol violations and secondary endpoints if they are given after enrollment.

1) Immunosuppresses/cytotoxic agents, including:

a. Cyclophosphamide
b. Azathioprine
c. Vincristine
d. High dose corticosteroids (>1.5 mg/kg)

2) Calcineurin inhibitors, including:

a. Cyclosporine A (brands - Sandimmune, Gengraf, Neoral)
b. Tacrolimus (FK506) (brand - Prograf)
c. Sirolimus (brand - Rapamune)

3) Anti-platelet agents:

Aspirin, ticlopidine, dipyridamole, NSAIDs, clopidogrel, and other anti-platelet agents may not be given for treatment of TTP per se, but may be administered when indicated for other conditions.

4) Cytokines/growth factors and megakaryocyte simulating drugs, including:

a. Oprelvekin (IL-11) (Brand- Neumega)

5) Splenectomy

6) Staphylococcal protein A column treatment

7) Intravenous immunoglobulin (IVIG)

6. ASSESSMENT

6.1 Schedule of Measurements: Refer to Table 2 for Panel descriptions

6.1.1. Pre-Screening

Screening logs will be maintained to track the enrollment status of all patients for participation.

6.1.2 Screening

1) Infectious disease testing

a. Anti-HIV
b. HBsAg, Anti-HBc

2) Pregnancy test (serum β-HCG)

6.1.3 Baseline (Table 3)

After evaluation for eligibility, the following measurements will be taken at baseline. Data collected for baseline measurements are to be abstracted from the subject's medical record using the earliest information after admission for TTP. If a laboratory test is not included in the subject's record, it is to be obtained either by using extra serum or plasma collected prior to the first plasma exchange and retained in the clinical laboratory for possible clinical needs, or from initial waste plasma from the first plasma exchange.

1) Demographic and Medical History

- Weight and height
- Gender
- Ethnic origin
- Race
- History of TTP

- Date of onset of symptoms
- Date of first plasma exchange
- Previous plasma infusion prior to enrollment in study during this admission
- Past Medical History including: (see case report form for complete list)
    - Autoimmune disease (SLE, other)
    - Prior thrombotic disease (CVA, MI, DVT, Other)
    - Diabetes (Diet Controlled, Oral Antigylcemics, Insulin)
    - Hypertension (Medication Yes/No)

2) Surveillance panel
3) Peripheral smear
4) Coagulation panel
5) Reticulocyte count
6) Chemistry panel
7) Expanded chemistry panel
8) Other Laboratory
    - DAT
    - Serum IgG, IgM, and IgA
    - Urinalysis with microscopic examination
9) ADAMTS13 activity and antibody (plasma samples will be sent to the Blood Center of Wisconsin and Dr. Sadler's Laboratory)
10) Samples for repository (drawn prior to any plasma exchange or rituximab treatment)
    - Serum
    - Citrated plasma
    - Lithium-heparin plasma
    - Packed cells
11) Assessment
    - Documentation of clinical status on Baseline Visit CRF
    - Documentation of medications on Concomitant Medications CRF
    - Plasma Exchange log – for all plasma exchanges since admission

6.1.4 During Plasma Exchange (Table 3)

1) Surveillance panel – a minimum of one per day
2) Chemistry Panel- one per day
3) ADAMTS13 activity and antibody- collect at least once between 9-13 days after the day of the first plasma exchange, and at least weekly thereafter if the patient is receiving plasma exchange
4) Assessment
    - Documentation of clinical status on Follow-Up During Plasma Exchange CRF
    - Concomitant Medication Log, if changes have occurred
    - Plasma Exchange log

6.1.5 After Plasma Exchange is stopped (Table 4)

1) First month
   - Surveillance panel – one per week ± 2 days
   - Chemistry panel – one per week ± 2 days
   - ADAMTS13 activity and antibody – on day 7 ± 2 days and day 30 ± 7 days after plasma exchange is stopped
   - Peripheral smear – on day 7 ± 2 days after plasma exchange is stopped
   - Clinical status (Follow-Up Post-PE CRF) and Concomitant Medication Log – one per week ± 2 days
2) Months 1 to 3
   - Surveillance panel – one per month ± 7 days
   - Chemistry panel – one per month ± 7 days
   - ADAMTS 13 activity and antibody – one at 3 months ± 7 days after plasma exchange is stopped
   - Clinical status (Follow-Up Post-PE CRF) and Concomitant Medication Log – one per month ± 7 days
3) Months 4 to 24
   - Surveillance panel – every 3 months for one year after plasma exchange is stopped ± 14 days
   - Chemistry Panel – one year after plasma exchange is stopped ± 14 days
   - ADAMTS13 activity and antibody – every 3 months ± 14 days
   - Clinical status (Follow-Up Post-PE CRF) and Concomitant Medication Log – every 3 months ± 14 days

6.1.6 After Exacerbation and until plasma exchange is stopped (Table 3)

1) Surveillance Panel – one per day
2) Chemistry Panel – one per day
3) Coagulation – one during the first day after exacerbation is diagnosed
4) Direct Antiglobulin Test – one during the first day after exacerbation is diagnosed
5) Peripheral smear– one during first day after exacerbation is diagnosed
6) ADAMTS 13 activity and antibody – one during the first day after exacerbation is diagnosed, one between 9-13 days later, and then weekly ± 2 days if the patient is still on plasma exchange.
7) Documentation of clinical status (Follow-Up During Plasma Exchange CRF) and Concomitant Medication log (if changes have occurred)
8) Plasma exchange log – one per day

6.1.7 Refractory TTP (Table 5)

1) ADAMTS13 activity and antibody – during the first day after Refractory TTP is diagnosed and then at least every 3 months for 2 years thereafter ± 14 days
2) Serum IgG, IgM, IgA – 1 year after Refractory TTP is diagnosed ± 14 days
3) Clinical status and medication log – every 3 months for 2 years ± 14 days
4) If patient receives standard dose of rituximab as a salvage therapy, the B lymphocyte and rituximab level with be measured based on schedules described in section 6.1.9 and table 6.

6.1.8 Relapsed TTP until the plasma exchange is stopped (Table 3)

1) Surveillance Panel – every day
2) Chemistry Panel – every day
3) ADAMTS13 activity and antibody – one during the first day after relapse is diagnosed, one 9-13 days later, and then weekly ± 2 days if the patient is still on plasma exchange.
4) Direct Antiglobulin Test – one during the first day after relapse is diagnosed
5) Peripheral Smear – one during the first day after relapse is diagnosed
6) Clinical Status and Medication Log – every day

6.1.9 B Lymphocyte Levels

Collect B lymphocyte samples at the following time points. The samples will be collected prior to subject receiving that day's plasma exchange and rituximab, if applicable. Samples will be sent to a central lab for testing.

- Prior to any rituximab infusion
- 3 months (± 7 days) after the first plasma exchange
- Six months (± 14 days) after the first plasma exchange
- Nine months (± 14 days) after the first plasma exchange
- Twelve months (± 14 days) after the first plasma exchange

6.1.10 Schedule for measurements of rituximab level

- As described in Table 6

Table 2: Panel Descriptions

Panel Name	Tests in the panel	Amount of sample and tube
Surveillance panel	CBC LDH	5.0 ml purple-top EDTA 0.3 plasma, green top
Peripheral smear	Description of smear by hematologist or hematopathologist.	Use EDTA from surveillance panel
Coagulation lab	INR, PTT, fibrinogen, D-Dimer	5.0 ml blue-top citrate
Chemistry panel	BUN, Creatinine	0.5 ml plasma, green top
Expanded chemistry panel	CPK, ALT, AST, Bilirubin (total and direct)	0.5 ml plasma, green top
Infectious disease testing	Anti-HIV, HBsAg, HBc-Antibody	HIV:10 ml I ml serum, red top
Pregnancy test	Serum β-HCG	0.5 ml plasma, green top
ADAMTS13 activity and antibody; plasma Li-heparin		5 ml green top
Direct Antiglobubin Test		5 ml purple-top EDTA
Serum IgG, IgM and IgA		3 ml serum, red top
B lymphocyte		5 ml EDTA tube
Rituximab Level (PK)		5 ml red/gray top
Reticulocyte count		5.0 ml purple-top EDTA

Table 3: Schedule of Measurements Until Plasma Exchange (PE) is Stopped for Treatment Response

	Baseline	During PE
Assessment		
Demographic and Medical History	X	
Clinical Status Log	X	One per day
Medication log	X	One per day
PE log		Every PE
Laboratory		
Surveillance Panel	X	One per day
Coagulation panel	X	
Chemistry Panel	X	One per day
Pregnancy Test (screening)		
Infectious disease testing (screening)		
ADAMTS13 activity and ADAMTS13 antibody	X	Day 10 -14,and weekly thereafter if the patient is receiving plasma exchange
Direct Antiglobulin Test	X	
Peripheral smear	X	On first day of PE
Serum IgG, IgM and IgA	X	

Table 4: Schedule of Measurements After Plasma Exchange (PE) is Stopped for Treatment Response

	First 30 days of no PE	Months 1 to 3	4 Months to 2 years
Assessment			
Clinical status	1 per week ± 2 days	1 per month ± 7 days	Every 3 months for 2 years ± 14 days
Medication log	1 per week ± 2 days	1 per month ± 7 days	Every 3 months for 2 years ± 14 days
Laboratory			
Surveillance Panel	1 per week ± 2 days	1 per month ± 7 days	Every 3 months for 1 year after first PE ± 14 days
Chemistry Panel	1 per week ± 2 days	1 per month ± 7 days	1 year after first PE ± 14 days
ADAMTS13 activity and antibody	Day 7 ± 2 days and day 30 ± 7 days	1 at month 3 ± 14 days	Every 3 months for 2 years after first PE ± 14 days
Peripheral smear	Day 7± 2 days		
Serum IgG, IgM and IgA			One year after first PE ± 14 days

Table 5: Schedule of Measurements When Refractory TTP is diagnosed

Assessment	Frequency
Clinical status	4 times per year (quarterly) after the first PE for 2 years ± 14 days
Medication log	After the first PE for 2 years ± 14 days
Laboratory	
Serum IgG, IgM, IgA	1 year after the first PE ± 14 days
ADAMTS13 activity and antibody	On the day Refractory TTP is diagnosed Then every 3 months up for 2 years ± 14 days

Table 6: Schedule for Measurements of Rituximab Level

Event	Samples
First rituximab infusion	Within 30 minutes before starting the infusion
	Within 30 minutes after the end of the infusion
	13-25 hours after the end of infusion, and before the next PE
	Within 30 minutes after the first PE following the rituximab infusion
	Within 30 minutes before the second PE following the rituximab infusion
Second rituximab infusion	Within 30 minutes before starting the infusion
Third rituximab infusion	Within 30 minutes before starting the infusion
Fourth rituximab infusion	Within 30 minutes before starting the infusion
	Within 30 minutes after the end of the infusion
Follow up	3 months after the last infusion of rituximab ± 14 days
	6 months after the last infusion of rituximab± 14 days
	12 months after the last infusion of rituximab± 14 days

6.2 Specimen collection procedures

To avoid dilution effects on screening and eligibility tests (especially HBsAg, Anti-HBc antibody, β-HCG and ADAMTS13 antibody measurements) we will screen subjects and obtain samples prior to the first plasma exchange when possible.

If enrollment occurs after plasma exchange has begun, the following alternative sequence of specimen acquisition is to be followed:

We will attempt to utilize extra serum or plasma held for possible clinical needs in the routine clinical laboratory for these tests – provided it meets the laboratory's criteria for HBsAg, Anti HBc, and β-HCG testing.

Otherwise properly procured peripheral blood sample will be obtained for HBsAg, Anti-HBc and β-HCG to confirm clinical eligibility despite plasma exchange(s).

Measurement of HBsAg, Anti-HBc, and β-HCG on undiluted waste plasma (see below) is to be run in parallel.

Directions for obtaining and storing this waste plasma are as follows: After the first 50 mL of plasmapheresis plasma is collected into the waste bag, 50 mL will be diverted into a transfer bag and retained for special studies (pending informed consent of the subject if not already obtained). The remainder of the plasmapheresis plasma will then be run into the waste bag. The transfer bag will be sent to the blood bank or other location and stored at 1-6ºC until disposition is determined. Material in this bag will be aliquoted into at least six 5 mL freezer-safe tubes (two of these may be used for HBsAg and β-HCG screening, one may be used for ADAMTS13 activity and antibody, and two may be used for sample repository, if needed), and one 15 mL aliquot. Samples are to be frozen at -80ºC. Samples may be initially stored at -20ºC in a non-frost-free freezer for a maximum of 30 days. Unless used for screening purposes, the tubes and aliquot will be batched and forwarded to the repository for storage.

1) Peripheral Blood smears will be collected for confirmation of microangiopathic changes.
2) ADAMTS13 activity and antibody samples
   - 5 ml green top tube (or saved plasma) to yield 2.5 mL of lithium-heparinized plasma. The leftover plasma will be sent to the repository for storage.
3) B lymphocyte levels:
   - 5 ml EDTA tube

6.3 Sample repository

Samples will be prepared, aliquoted, and stored as below:

- Serum – draw 5 mL in red- or tiger-toped tube or equivalent. Aliquot into four 0.6 mL capped tubes and freeze at -80ºC.
- Plasma, citrated – draw 10 mL into two 5 mL in blue-topped tubes. After processing, aliquot plasma into eight 0. 6 mL capped tubes and freeze at -80ºC.
- Plasma lithium heparin – draw 10 mL into two 5 ml green-topped tubes.
- Packed Cells – packed cells remaining from citrated plasma, aliquoted into two 2.0 mL capped tubes for later batched shipment. Samples are to be frozen at -80ºC.

Samples may be initially stored at -20ºC in a non-frost-free freezer for a maximum of 30 days. Repository samples will be labeled with a repository ID number and will carry no direct identifiers of the subject. The sample repository will be maintained indefinitely.

7. ADVERSE EVENT CRITERIA AND REPORTING

We will use the descriptive terminology developed by the National Cancer Institute for reporting adverse events (AEs): Common Toxicology Criteria for Adverse Events (CTC) version 4. 0 dated May 28 2009. The CTC includes a grading (severity) scale for each adverse event term. Grades were developed using the following guidelines:

- Grade 0 - No adverse event or within normal limits
- Grade 1 - Mild adverse event
- Grade 2 - Moderate adverse event
- Grade 3 - Severe adverse event
- Grade 4 - Life threatening or disabling adverse event
- Grade 5 - Death related to adverse event

In general, investigators are to report adverse events as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities, and sequel. The following types of occurrences are not considered adverse events and do not need to be reported: 1) pre-existing medical conditions that are present at the start of the study; and 2) previously scheduled hospitalizations and hospitalizations needed for diagnostic or elective surgical procedures for the management of pre-existing medical conditions.

7.1 Expectedness of Adverse Events

7.1.1 Criteria for Expectedness

Each AE should be evaluated by the site investigator as to whether it was expected or unexpected. An unexpected AE is defined as any AE the nature, severity, or frequency of which is not consistent with either:

1) the known or forseeable risk of AEs associated with the procedures involved in the research that are described in the IRB-approved study protocol, any applicable investigator brochure, the current IRB-approved informed consent document, or other relevant sources of information such as product labeling or package inserts; or

2) the expected natural progression of any underlying disease, disorder, or condition of the subject and his/her predisposing risk factor profile for the AE.

AEs that do not meet the above criteria for an unexpected AE should be graded as expected.

7.1.2 Toxicities associated with rituximab

Non-Infectious pulmonary toxicity

Non-infectious pulmonary toxicity is a rare but potentially fatal complication of rituximab therapy (Hadjinicolaou et al, 2012). A total of 121 cases of potential rituximab-induced lung toxicity were identified from 61 clinical studies and case reports. In most cases, rituximab was part of combination chemotherapy for treatment of cancers. The mean time of onset from the last rituximab infusion until symptom development is 30 days (range 0-158 days). Most patients with this side effect improved completely with corticosteroids. The disease was fatal in 18 cases.

The following Adverse Events and other safety information are described in the Rituxan® Package Insert (2011).

Severe Infusion Reactions

Rituximab can cause severe, including fatal, infusion reactions. These severe reactions typically occurred during the first infusion with time to onset of 30 to 120 minutes. Monitor closely during infusion; discontinue with grade 3 or 4 infusion reactions. Signs and symptoms of

severe infusion reactions may include urticaria, hypotension, angioedema, hypoxia, or bronchospasm, and may require interruption of rituximab administration. The most severe manifestations and sequelae include pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, cardiogenic shock, and anaphylactic and anaphylactoid events. In the reported cases, the following factors were more frequently associated with fatal outcomes: female gender, pulmonary infiltrates, and chronic lymphocytic leukemia or mantle cell lymphoma.

Hypersensitivity reactions

Rituximab has been associated with hypersensitivity reactions (non-IgE-mediated reactions), which may respond to adjustments in the infusion rate and in medical management. Hypotension, bronchospasm, and angioedema have occurred in association with rituximab infusion (see Severe Infusion Reactions). Therefore, subjects require close monitoring during first and all subsequent infusions. Premedication with diphenhydramine and acetaminophen is recommended.

Rituximab infusion will be interrupted for severe hypersensitivity reactions and can be resumed at a 50% reduction in rate (e. g., from 100 mg/hour to 50 mg/hour) when symptoms have completely resolved. Treatment of these symptoms with diphenhydramine and acetaminophen is recommended; additional treatment with bronchodilators or IV saline may be indicated. In most cases, patients who have experienced non-life-threatening hypersensitivity reactions have been able to complete the full course of therapy. Medications for the treatment of hypersensitivity reactions, e. g., epinephrine, antihistamines, and glucocorticoids, should be available for immediate use in the event of a reaction during administration.

Other infusion reactions

Other reported adverse events, including fever, chills, headache, nausea, vomiting, rhinitis, and mild hypotension, occurred primarily during rituximab infusions and typically responded to an interruption of the infusion and resumption at a slower rate. More rare but serious events include tumor lysis Syndromes (in subjects with malignant lymphoma and chronic lymphocytic leukemia), mucocutaneous reactions, cardiac arrhythmias and angina in subjects with preexisting cardiac disease, and renal failure (also associated with tumor lysis).

Infections

Serious including fatal, bacterial fungal and new or reactivated viral infections can occur during and up to one year following the completion of rituximab – based therapy .New or reactivated viral infectious included cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus and hepatitis B and C. Discontinue rituximab for serious infections and institute appropriate ant -infective therapy.

Cardiovascular Reactions

Infusions should be discontinued in the event of serious or life-threatening cardiac arrhythmias. Patients who develop clinically significant arrhythmias should undergo cardiac monitoring during and after subsequent infusions of rituximab. Patients with pre-existing cardiac conditions including angina have had recurrences of these events during rituximab therapy and should be monitored throughout the infusion and immediate post-infusion period.

Renal

Rituximab administration has been associated with severe renal toxicity, including acute renal failure requiring dialysis, and in some cases has led to a fatal outcome in subjects treated for lymphoma who have tumor lysis syndrome, often associated with concomitant cisplatin therapy.

Severe mucocutaneous reactions

Mucocutaneous reactions, some with fatal outcome, have been reported in subjects treated with rituximab. These reports include paraneoplastic pemphigus (an uncommon disorder which is a manifestation of the subject's underlying malignancy), Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. The onset of the reaction in the reported cases has varied from 1-13 weeks following rituximab exposure. Subjects experiencing a severe mucocutaneous reaction will not receive any further infusions and will receive prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and guide subsequent treatment. The safety of re-administration of rituximab to subjects with any of these mucocutaneous reactions has not been determined.

Hematologic events

In clinical trials, Grade 3 and 4 cytopenias were reported in 48% of subjects treated with rituximab; these include: lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). The median duration of lymphopenia was 14 days (range, 1 to 588 days) and of neutropenia was 13 days (range, 2 to 116 days). A single occurrence of transient aplastic anemia (pure red cell aplasia) and two occurrences of hemolytic anemia following rituximab therapy were reported.

In addition, there have been a limited number of post-marketing reports of prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia (defined as occurring 40 days after the last dose of rituximab) in subjects with hematologic malignancies. In reported cases of late onset neutropenia (NCI-CTCAE Grade 3 and 4), the median duration of neutropenia was 10 days (range 3 to 148 days). Documented resolution of the neutropenia was described in approximately half of the reported cases; of those with documented recovery, approximately half received growth factor support. In the remaining cases, information on resolution was not provided. More than half of the reported cases of delayed onset neutropenia occurred in subjects who had undergone prior autologous bone marrow transplantation. In an adequately designed, controlled, clinical trial, the reported incidence of NCI-CTCAE Grade 3 and 4 neutropenia was higher in subjects receiving rituximab in combination with fludarabine as compared to those receiving fludarabine alone (76% [39/51] vs. 39% [21/53]).

Carcinogenesis, mutagenesis, and impairment of fertility

No long-term animal studies have been performed to establish the carcinogenic or mutagenic potential of rituximab, or to determine its effects on fertility in males or females. Male and female subjects will be advised to use effective contraceptive methods during treatment and for 12 months following rituximab therapy.

Hepatitis B Reactivation with Related Fulminant Hepatitis and Other Viral Infections

Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some subjects with hematologic malignancies treated with rituximab. The majority of subjects received rituximab in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately 4 months after the initiation of rituximab and approximately one month after the last dose. A direct causal relationship between rituximab and HBV viral reactivation has not been established.

The following additional serious viral infections, either new, reactivated or exacerbated, have been identified in clinical studies or post-marketing reports. The majority of patients received Rituxan in combination with chemotherapy or as part of a hematopoietic stem cell transplant. These viral infections included JC virus [progressive multifocal leukoencephalopathy (PML)], cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus,

and hepatitis C. In some cases, the viral infections occurred up to one year following discontinuation of Rituxan and have resulted in death.

Vaccinations

The safety of immunization with live viral vaccines following rituximab therapy has not been studied and vaccination with live virus vaccines is not recommended. The ability to generate a primary or anamnestic humoral response to vaccination is currently being studied. If vaccination using non-live vaccines is being considered, physicians should consult the Centers for Disease Control and Prevention (CDC) guidelines.

Post Marketing Experience

Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. Decisions to include these reactions in labeling are typically based on one or more of the following factors: (1) seriousness of the reaction, (2) frequency of reporting, or (3) strength of causal connection to rituximab.

Hematologic: prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia, hyperviscosity syndrome in Waldenstrom's macroglobulinemia.

Cardiac: fatal cardiac failure.

Immune/Autoimmune Events: uveitis, optic neuritis, systemic vasculitis, pleuritis, lupus-like syndrome, serum sickness, polyarticular arthritis and vasculitis with rash.

Infection: increased in fatal infections in HIV-associated lymphoma.

Skin: severe mucocutaneous reactions.

Gastrointestinal: bowel obstruction and perforation.

7.1.3 Toxicities associated with plasma exchange

Subjects undergoing plasma exchange may experience the following side effects/complications:

- Complications secondary to vascular access including: hematoma, venous sclerosis, thrombosis and embolism, inadvertent arterial puncture, pneumothorax and/or hemothorax
- Vasovagal reaction
- Citrate toxicity including: circumoral paresthesias, nausea, vomiting, chills, muscular twitching, tetany, and syncope. Severe citrate toxicity may manifest as cardiac arrhythmia.
- Transfusion reaction associated with plasma, including: fever, chills, urticaria,
- Transfusion-related acute lung injury (TRALI)

7.1.4 Toxicities associated with corticosteroids

The following adverse side effects may be seen in subjects on steroid therapy:

- Cardiovascular: arrhythmia, edema, hypertension
- Central nervous system: insomnia, delirium, psychoses, and seizures
- Dermatologic: hirsutism, skin atrophy, bruising, hyperpigmentation
- Endocrine and metabolic: glucose intolerance, Cushing's syndrome, pituitary adrenal axis suppression (adrenal insufficiency)

- Hematologic: transient leukocytosis
- Ocular: cataracts, glaucoma
- Miscellaneous: infections, hypersensitivity reactions, buffalo hump, moon face

7.2 Relatedness to Participation in the Research Study

Each AE should be evaluated as to whether it was related or possibly related to participation in the research study (meaning that there is a reasonable possibility that the AE may have been caused by the procedures involved in the research). AEs determined to be solely caused by an underlying disease, disorder, or condition of the subject; or other circumstances unrelated to the research should be categorized as being not related to participation in the research.

7.3 Relatedness to Use of the Study Drug (Rituximab)

Each AE should also be evaluated as to whether it was related to the use of rituximab:

Definite	An AE is clearly related to use of rituximab
Probable	An AE has a strong temporal relationship to use of rituximab, and another etiology is significantly less likely.
Possible	An AE has a strong temporal relationship to use of rituximab, and an alternative etiology is equally or less likely.
Unlikely	An AE has little or no temporal relationship to use of rituximab, and/or a more likely alternative etiology exists.
Not Related	An AE is not related to use of rituximab (no temporal relationship, or a much more likely alternative etiology exists).

7.4 Reporting and Monitoring Adverse Events

Information about all adverse events that occur within 1 year after enrollment in subjects will be collected. The clinical course of AEs will be followed until a medical outcome is determined. Additional information that becomes available may be added to previously submitted AE reports at any time. Site investigators should follow their local IRB's guidelines in terms of reporting AEs to them. All AEs must be reported to Washington University's Clinical Coordinating Center (CCC) on designated case report forms (CRFs) to be provided by the sponsor-investigator.

7.4.1 Expedited Reporting of Serious Adverse Events

Serious adverse events (SAEs) are defined as AEs that cause death, a life-threatening adverse experience, or a persistent or significant disability/incapacity (i.e. Grade 4 and 5 events); inpatient hospitalization or prolongation of existing hospitalization; pregnancy abortion, or a congenital anomaly, birth defect, or cancer in a neonate/infant born to a female subject.

Serious adverse events must be reported by Clinical Center personnel to the Washington University CCC within 24 hours of the Clinical Center's awareness (Monday-Friday) by electronically mailing the completed Serious Adverse Event form and de-identified source documentation to Dr. Elaine Majerus (emajerus@wustl.edu) and Patricia Nieters, RN, BSN (nietersp@mir.wustl.edu). Each Clinical Center will follow their local IRB reporting policy of AEs and SAEs. If a SAE is determined to be unexpected and possibly, probably, or definitively related to rituximab or to participation in the research study, it must be reported to the local IRB within 24 hours of the Clinical Center's awareness of the event. Data on all serious adverse events that occur in subjects will be collected through the end of their study participation (i.e. the full 2-year follow-up period).

7.4.2 Monitoring of Serious and Unexpected Adverse Events

Serious adverse events will be reported by the CCC to the Medical Monitor in an expedited manner, irrespective of the attribution of the event to the study drug/device/procedure/treatment. Serious adverse events will be promptly reviewed by the Medical Monitor. The Medical Monitor will review the categorizations of the SAE to verify consistency with protocol reporting criteria, and communicate with the site investigator if there are discrepancies or questions. The Medical Monitor has medical expertise relevant to the study protocol and may request the subject's treatment assignment when reviewing the adverse event. The Medical Monitor shall review the data with particular attention to the complexities of assessing toxicity due to rituximab, corticosteroids and PE in TTP subjects. The Medical Monitor is responsible for notifying the Principal Investigator (IND Holder) of any changes to the categorizations of serious adverse events and of any concerns regarding the frequency or type of adverse event(s). The NHLBI Project Officer will be notified immediately if a SAE is determined to be unexpected and possibly, probably, or definitively related to rituximab; additionally, information about the event will be included in the monthly summary report. If an event is determined to be unrelated or unlikely related to rituximab, it will still be included in the monthly summary report. The CCC will prepare monthly summary reports of all serious adverse events for review by the Medical Monitor, the Principal Investigator, and the NHLBI.

7.4.3 Reporting Adverse Events to NHLBI

Reports of accrual information, outstanding queries, serious adverse events, and protocol violations will be distributed to NHLBI monthly. Federal guidelines for reporting of Unanticipated Problems will be followed (see http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

7.4.4 Reporting Adverse Events to FDA

All serious adverse events that are unexpected and possibly, probably, or definitely related to the use of rituximab will be reported to FDA no later than 7 days from the sponsor-investigator's original receipt of the information. FDA will receive annual summaries of all adverse events.

8. CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY

8.1. Criteria for removal from Protocol therapy with adjuvant low dose rituximab

1) Salvage Therapy before all four doses of rituximab are administered
2) Two or more exacerbations in the first 30 days after enrollment
3) Plasma Exchange is performed sooner than 12 hours after the end of rituximab infusion

8.2. Off study criteria

1) Death
2) Lost to follow up
3) Participation in other trials of investigational therapies

9. STATISTICAL CONSIDERATIONS

9.1 Sample Size and Power

Review of 40 patients with TTP at BJH who would have met the inclusion criteria indicates that the composite primary endpoint rate was 55% in this historical group. Assuming a 10 percent of composite primary endpoint rate in the treated group and 40 historical controls, 14 treated patients are needed to have a 90% power to detect a 45 % difference in the incidence of exacerbation at the 5% significance level. We will enroll 20 patients total and expect to enroll 12 patients per year. Therefore, recruitment may be complete within less than 2 years. The time would be reduced by participation of other regional hospitals.

9.2 Data analysis

Fisher Exact tests will be used to compare the incidence of the composite primary endpoint, Durable Treatment Response, Relapse, treatment-related adverse events and 4-week, 1-year and 2-year incidence of death between the treated group and historical controls. We will consider Chi-Square test to examine differences in the type and severity of adverse events between the treated group and historical controls.

The time to Durable Treatment Response and Relapse will be described by Kaplan-Meier estimators and log rank tests. The effects of patient characteristics will be evaluated with Cox proportional hazards models. Numbers of plasma exchanges to Durable Treatment Response will be compared with non-parametric tests (e.g. Kruskal-Wallis analysis of variance).

The value of ADAMTS13 biomarker data for predicting Exacerbation, Durable Treatment Response or Relapse will be analyzed by logistic regression. The relationship between ADAMTS13 biomarker parameters and platelet count, LDH or B cell levels will be investigated with linear regression models.

Rituximab levels and the extent of B cell depletion will be analyzed as stratified categorical data with Cochran-Mantel-Haenszel statistics.

9.3 Data Confidentiality

Data will be captured on CRFs that will be submitted to the WU-CC by fax or electronic mail. Personal identifiers will not be sent to the WU-CC. CRFs and supporting materials will be stored in a secure location at the study site (e.g. locked office, password-protected and fire-wall protected computer systems) with access restricted to the study team. Data and supporting materials will be de-identified by site personnel prior to submission to the WU-CC. Subjects will be provided with a unique subject identification number upon enrollment that will be used to label data and supporting materials that are submitted to the WU-CC. The study will be conducted in accordance with the Health Insurance Portability and Accountability Act (HIPAA).

10. INVESTIGATOR RESPONSIBILITIES

10.1 Study Initiation

Before enrollment of the first subject at each study site, the following documents must be on file at the ART Study Coordinating Center at Washington University:

- Original U.S. FDA Form 1572, signed by the site principal investigator. The names of all co-investigators at the site must also appear on this form.

- Current curriculum vitae of the principal investigator and all co-investigators.

- Current, dated Institutional Review Board (IRB) membership list

- Written documentation of IRB approval of the protocol and informed consent document
- A copy of the IRB-approved authorization for Protected Health Information. This may be integrated into the informed consent document.
- Current laboratory certification (Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP)) of the institution listed on the Form 1572
- Current board certification and medical license of all physician investigators listed on the Form 1572
- Site Initiation Authorization Letter signed by the sponsor-investigator (Dr. Sadler)

10.2 Study Completion

The following data and materials must be on file at the WU-CC before the study can be considered complete or terminated: laboratory findings, clinical data, and test results from screening through end of follow-up for each enrolled patient; properly-completed CRFs for each enrolled patient; copies of protocol amendments and IRB approvals; review of final checklist by sponsor-investigator or a designee; copy of study termination letter sent to the local IRB; and all regulatory documents.

10.3 Institutional Review Board Approval

The protocol, informed consent document, and supporting information must be approved by the local IRB before study initiation. The site principal investigator is responsible for keeping the local IRB apprised of study progress and protocol changes, at a minimum of once a year. The site principal investigator is responsible for following the local IRB's AE reporting policies, and for forwarding to the IRB any safety updates provided by the sponsor-investigator.

10.4 Informed Consent

Template informed consent documents will be provided to each site. The final IRB-approved document must be provided to the sponsor-investigator. The informed consent document must be signed by the subject or his/her legally authorized representative before study participation.

10.5 Study Monitoring Requirements

Site monitoring visits will be routinely conducted by authorized representatives of the Washington University Principal Investigator to inspect study data, informed consent forms, subjects' medical records, and Case Report Forms. The site principal investigator will permit authorized representatives of the FDA, NHLBI, Washington University, and other local health authorities to inspect relevant facilities and records.

10.6 Disclosure of Data

Subject information obtained by this study is confidential, and disclosure to parties other than those noted here is prohibited. Upon the subject's permission, medical information may be given to his/her physician or other medical personnel responsible for his/her welfare.

10.7 Retention of Records

U.S. Department of Health and Human Services (DHHS) Regulations (45 CFR 46.115) mandate that IRB records of the study must be retained for at least 3 years after study completion. In addition, FDA regulations require that records and documents pertaining to the conduct of this study, including CRFs, signed informed consent forms, supporting source documentation for values or responses in the CRFs, supporting documentation for AEs, laboratory test results, and medication inventory records, must be retained by the site investigator for at least 2 years after

the study ends. Washington University reserves the right to secure data clarification and additional medical documentation on patients enrolled in this trial. The following records should be maintained by the site investigator:

- Signed Confidentiality Agreement
- Study Protocol and Protocol Amendments
- Signed Clinical Trial Agreement
- FDA Form 1572 and Investigational Drug Accountability Logs
- IRB Approval Letter, Continuing Review Approval Letters, and Correspondence
- IRB Membership List
- Curriculum vitae and licenses for all investigators
- Site personnel signature list
- Financial disclosure forms
- Patient screening & enrollment log
- Laboratory certifications
- NIH training certifications for Reponsible Conduct of Research
- Signed Study Informed Consent Forms for each patient
- Copies of all completed CRFs and supporting source documentation
- Supporting source documentation for adverse events

11. REFERENCES

Allford SL, Hunt BJ, Rose P, et al. Guidelines on the diagnosis and management of the thrombotic microangiopathic haemolytic anaemias. Br J Haematol 2003; 120:556-73.

Arnold DM, Dentali F, Crowther MA, et al. Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. Ann Intern Med. 2007; 146:25-33.

Bell WR, Braine HG, Ness PM, et al: Improved survival in thrombotic thrombocytopenic purpura hemolytic uremic syndrome. N Engl J Med 1991; 325:398-400.

Biogen Idec, Inc. and Genentech Inc. Rituxan® Package Insert. Available <http://www. gene. com/gene/products/information/pdf/rituxan-prescribing. pdf>. 2011.

Bohm M, Betz C, Miesbach W, et al. The course of ADAMTS-13 activity and inhibitor titer in the treatment of thrombotic thrombocytopenic purpura with plasma exchange and vincristine. Br J Haematol. 2005; 129:644-652.

Cartron G, Watier H, Golay J, et al. From the bench to the bedside: ways to improve rituximab efficacy. Blood 2004; 104:9:2635-42.

Coppo P, Wolf M, Veyradier A, et al. Prognostic value of inhibitory anti-ADAMTS13 antibodies in adult-acquired thrombotic thrombocytopenic purpura. Br J Haematol. 2006; 132:66-74.

Elliott MA, Heit JA, Pruthi RK, et al. Rituximab for refractory and or relapsing thrombotic thrombocytopenic purpura related to immune-mediated severe ADAMTS13 deficiency: a report of four cases and a systematic review of the literature. Eur J Haematol. 2009;83:365-372.

Froissart A, Buffet M, Veyradier A, et al. Efficacy and safety of first-line rituximab in severe acquired thrombotic thrombocytopenic purpura with suboptimal response to plasma exchange. Experience of the French Thrombotic Microangiopathies Reference Center. Crit Care Med 2012;40:104-111.

Hadjinicolaou AV,Nisar MK,Parfrey H, et al. Non- infectious pulmonary toxicity of rituximab :a systemic review .Rheumatology 2012;51 :653-662.

Kremer Hovinga JA, Vesely SK, Terrell DR, et al. Survival and relapse in patients with thrombotic thrombocytopenic purpura. Blood 2010; 115:1500-1511.

Kremer Hovinga JA, Mottini M, Lammle B. Measurement of ADAMTS-13 activity in plasma by the FRETS-VWF73 assay: comparison with other assay methods. J Thromb Haemost 2006; 4:1146-1148.

Lammle B, Kremer JA, Alberio L. Thrombotic thrombocytopenic purpura. J Thromb Haemostas 2005:3:1663-1675.

Ling H, Field J, Blinder, M. Sustained response with rituximab in patients with thrombotic thrombocytopenic purpura: A report of 13 cases and review of the literature. Am J Hematol 2009;84:418-421.

McMinn JR, Thomas IA Terrel DR, et al. Complications of plasma exchange in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: a study of 78 additional subjects. Transfusion 2003;43:415-6.

Moake JL. Thrombotic microangiopathies. NEJM 2002; 347:8:589-600.

O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 19(8):2165-2170, 2001.

Plosker GL, Figgit DP. Rituximab: a review of its use in non-hodgkin's lymphoma and chronic lymphocytic leukaemia. Drugs 63(8):803-843, 2003.

Provan D, Butler T, Evangelista ML, et al. Activity and safety profile of lowdose rituximab for the treatment of autoimmune cytopenias in adults. Haematologica 2007;92:1695-1698.

Reff ME, Carner K, Chambers KS, et al. Depletion of B-cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994; 83(2):435-45.

Rock GA, Shumak KH, Buskard NA, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group. N Engl J Med 1991; 325:393-397

Sadler JE, Moake JL, Miyata T, et al. Recent advances in thrombotic thrombocytopenic purpura. Hematology (Am Soc Hematol Educ Program) 2004; 407-423.

Sadler JE. von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. Blood 2008;112:11-18.

Scully M, McDonald V, Cavenagh J, et al. A phase II study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. Blood 2011;118:1746-1753.

Stasi R, Brunetti M, Stipa E, et al. Selective B-cell depletion with rituximab for the treatment of subjects with acquired hemophilia. Blood 2004; 103:4424-8.

Stasi R, Pagano A, Stipa E, et al. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001; 98:952-7.

Stasi R. Rituximab in autoimmune hematologic diseases: not just a matter of B cells. Semin Hematol 2010; 47:170-179.

Tsai HM, Shulman K. Rituximab induces remission of cerebral ischemia caused by thrombotic thrombocytopenic purpura. Eur J Haematol 70:183-185, 2003.

Tsai HM. Advances in the pathogenesis, diagnosis, and treatment of thrombotic thrombocytopenicpurpura. J Amer Soc of Nephrol 2003; 14:1072-81

Veyradier A, Obert B, Hollier A, et al. Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. Blood 2001; 98:6:1765-72. .

Zaja F, Vianelli N, Volpetti S, et al. Low-dose rituximab in adult patients with primary immune thrombocytopenia. Eur J Haematol 2010;85:329-334.

Zheng XL, Kaufman RM, Goodnough LT, et al. Effect of plasma exchange on plasma ADAMTS13 metalloprotease activity, inhibitor level, and clinical outcome in patients with idiopathic and nonidiopathic thrombotic thrombocytopenic purpura. Blood 2004:103: 4043-4049.

Ziman A, Mitri M, Klapper E, et al. Combination Vincristine and plasma exchange as initial therapy in subjects with thrombotic thrombocytopenic purpura: one institutions' experience and review of the literature. Transfusion 2005; 45:41-9.