American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

A regimen with caplacizumab, immunosuppression and plasma exchange prevents unfavorable outcomes in immune-mediated TTP

Tracking no: BLD-2020-008021R1

Paul Coppo (AP-HP.SU, France) Michael BUBENHEIM (CHU Rouen, France) Elie Azoulay (Hôpital Saint Louis APHP, France) Lionel Galicier (Hôpital Saint-Louis, France) Sandrine Malot (AP-HP, France) Naïke Bigé (AP-HP, France) Pascale Poullin (AP-HM, France) François Provôt (CHRU, France) Nihal Martis (Université Côte d'Azur, France) Claire Presne (Amiens Picardie Universitary Hospital, France) Olivier Moranne (CHU de Nimes, France) Ruben Benainous (AP-HP, France) Antoine Dossier (Paris Diderot University, France) Amélie Seguin (CHU de Nantes, France) Miguel Hié (APHP, CHU la Pitié-Salpêtrière, France) Alain Wynckel (CHU, France) Yahsou Delmas (University Hospital Pellegrin, France) Jean-François Augusto (Angers University Hospital, France) Pierre Perez (CHU Nancy, Algeria) Virginie Rieu (CHU de Clermont-Ferrand, France) christelle barbet (centre hospitalier universitaire Bretonneau, France) François Lhote (Hôpital Delafontaine,) Marc Ulrich (Centre Hospitalier de Valenciennes, France) Anne Charvet Rumpler (CHRU Besancon, France) Sten De Witte (Centre Hospitalier de Libourne, France) Thierry Krummel (University Hospital of Strasbourg, France) Agnès Veyradier (Hôpital Lariboisière, France) Ygal Benhamou (Service de médecine interne, Normandie université, UniRouen, U1096, 76000 Rouen, France., France)

Abstract:
The anti-von Willebrand factor nanobody caplacizumab was licensed for adults with immune-mediated thrombotic thrombocytopenic purpura (iTTP) based on prospective controlled trials. However, few data are available on post-marketing surveillance. We treated 90 iTTP patients with a compassionate frontline "triplet regimen" associating therapeutic plasma exchange (TPE), immunosuppression with corticosteroids and rituximab, and caplacizumab. Outcomes were compared to 180 historical patients treated with the standard frontline treatment (TPE and corticosteroids, with rituximab as salvage therapy). The primary outcome was a composite of refractoriness and death within 30 days since diagnosis. Key secondary outcomes were exacerbations, time to platelet count recovery, the number of TPE and the volume of plasma required to achieve durable remission. The percentage of patients in the triplet regimen with the composite primary outcome was 2.2% vs. 12.2% in historical patients (p=0.01). One elderly patient in the triplet regimen died of pulmonary embolism. Patients from this cohort experienced less exacerbations (3.4% vs. 44%, p<0.01); they recovered durable platelet count 1.8 times faster than historical patients (95% confidence interval, 1.41-2.36, p<0.01), with fewer TPE sessions and lower plasma volumes (p<0.01 both). The number of days in hospital was 41% lower in the triplet regimen than in the historical cohort (13 days vs. 22 days, p <0.01). Caplacizumab-related adverse events occurred in 46 patients (51%), including 13 major or clinically relevant non-major hemorrhagic events. Associating caplacizumab to TPE and immunosuppression, by addressing the three processes of iTTP pathophysiology, prevents unfavorable outcomes and alleviates the burden of care.

Conflict of interest: COI declared - see note

COI notes: M. Bubenheim, E. Azoulay, S. Malot, Naïke Bigé, L. Galicier, F. Provôt, M. Nihal, O. Moranne, R. Benainous, A. Dossier, A. Seguin, M. Hié, J-F. Augusto, P. Perez, V. Rieu, C. Barbet, F. Lhote, M. Ulrich, A. Charvet Rumpler, S. de Witte and T. Krummel do not have any conflict of interest to declare. P. Coppo is member of the Clinical Advisory Board for Alexion, Sanofi, Shire and Octapharma. Y. Benhamou, P. Poullin, A. Wynckel, Y. Delmas, C. Presne and A. Veyradier have participated to Advisory boards for Sanofi.

Preprint server: No;

Author contributions and disclosures: Paul Coppo and Ygal Benhamou designed the study, interpreted the results, and wrote the manuscript. Michael Bubenheim performed the statistical analysis of the French Registry for Thrombotic Microangiopathies. Elie Azoulay, Naïke Bigé, Lionel Galicier, Pascale Poullin, François Provôt, Martis Nihal, Claire Presne, Olivier Moranne, Ruben Benainous, Antoine Dossier, Amélie Seguin, Miguel Hié, Alain Wynckel, Yahsou Delmas, Jean-François Augusto, Pierre Perez, Virginie Rieu, Christelle Barbet, François Lhote, Marc Ulrich, Anne Charvet Rumpler, Sten de Witte, Thierry Krummel, Agnès Veyradier, Ygal Benhamou and Paul Coppo enrolled patients and collected clinical and laboratory information. Sandrine Malot collected the data from all patients. All of the authors critically reviewed and substantially improved the manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Emails to the corresponding author

Clinical trial registration information (if any):

A regimen with caplacizumab, immunosuppression and plasma exchange prevents unfavorable outcomes in immune-mediated TTP

Paul Coppo (1,2,3), Michael Bubenheim (4), Elie Azoulay (1,5), Lionel Galicier (1,6), Sandrine Malot (1), Naïke Bigé (1,7), Pascale Poullin (1,8), François Provôt (1,9), Nihal Martis (10), Claire Presne (1,11), Olivier Moranne (12), Ruben Benainous (13), Antoine Dossier (14), Amélie Seguin (1,15), Miguel Hié (1,16), Alain Wynckel (1,17), Yahsou Delmas (1,18), Jean-François Augusto (1,19), Pierre Perez (1,20), Virginie Rieu (1,21), Christelle Barbet (1,22), François Lhote (23), Marc Ulrich (24), Anne Charvet Rumpler (1,25), Sten de Witte (26), Thierry Krummel (1,27), Agnès Veyradier (1,28,29), Ygal Benhamou (1,31,31), for the French Reference Center for Thrombotic Microangiopathies

(1) Centre de Référence des Microangiopathies Thrombotiques (CNR-MAT), AP-HP, Paris ;

(2) Service d'Hématologie, APHP.SU, Paris ;

(3) INSERM UMRS1138, Centre de Recherche des Cordeliers, Paris;

(4) CHU Rouen, Department of Clinical Research and Innovation, F-76000 Rouen;

(5) Médecine intensive réanimation, Hôpital Saint Louis, AP-HP, Paris ;

(6) Service d'immunologie Clinique, Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris and Univ Paris Diderot, Sorbonne Paris Cité, Paris ;

(7) Service de Médecine intensive et Réanimation, Hôpital Saint-Antoine, AP-HP, Paris ;

(8) Service d'Hémaphérèse, CHU La Timone, Marseille;

(9) Service de Néphrologie, Hôpital Albert Calmette, Lille ;

(10) Service de Médecine Interne, CHU de Nice ;

(11) Service de Néphrologie, Hôpital Sud, CHU Amiens ;

(12) Service de Néphrologie-Dialyse-Aphérèse, hôpital Universitaire de Nîmes ;

(13) Service de Médecine Interne, CHU Avicenne, Bobigny ;

(14) Service de Médecine Interne, CHU Bichat, Paris ;

(15) Service de Réanimation Médicale, CHU de Nantes ;

(16) Service de Médecine Interne, CHU La Pitié Salpétrière, Paris ;

(17) Service de Néphrologie, Hôpital Maison Blanche, Reims ;

(18) Service de Néphrologie, CHU Bordeaux, Bordeaux ;

(19) Service de néphrologie - dialyse - transplantation, CHU d'Angers ;

(20) Service de Médecine intensive réanimation, Hôpital Brabois, Nancy ;

(21) Service de Médecine Interne, CHU de Clermont-Ferrand ;

(22) Service de Néphrologie - Immunologie clinique, CHRU de Tours ;

(23) Service de Médecine Interne, CH Delafontaine, Saint-Denis ;

(24) Service de Néphrologie, CH de Valenciennes ;

(25) Service d'Hématologie, CHRU de Besançon ;

(26) Service d'Hématologie, CH de Libourne;

(27) Service de Néphrologie, CHRU de Strasbourg ;

(28) Hématologie biologique, Hôpital Lariboisière, AP-HP ; Paris ;

(29) EA-3518, Institut de recherche Saint Louis, Université de Paris;

(30) Département de Médecine Interne, CHU Charles Nicolle, Rouen ;

(31) Normandie Univ, UNIROUEN, INSERM U1096 EnVI, Rouen ; France.

Corresponding author

Prof Paul Coppo,

Service d'Hématologie, Centre de Référence des Microangiopathies Thrombotiques (CNR-MAT),

Hôpital Saint-Antoine, AP-HP, 75571Paris

Phone: +33 1 49 28 26 21

Fax: +33 1 49 28 33 03

paul.coppo@aphp.fr

Running title: A triplet regimen for immune-mediated TTP

Financial support: This work was partly funded by a grant of the French Ministry of Health (Programme Hospitalier de Recherche Clinique; P120118; AOM12259)

Keywords: thrombotic thrombocytopenic purpura; caplacizumab; ADAMTS13; rituximab; prognosis.

The members of the Reference Center for Thrombotic Microangiopathies are cited in appendix.

KEYPOINTS

- A triplet regimen associating plasma exchange, immunosuppression and caplacizumab reduce prevent unfavorable outcomes in immune-mediated TTP.
- The triplet regimen alleviates the burden of care.

Abstract

The anti-von Willebrand factor nanobody caplacizumab was licensed for adults with immune-mediated thrombotic thrombocytopenic purpura (iTTP) based on prospective controlled trials. However, **few** data are available on post-marketing surveillance. We treated 90 iTTP patients with a compassionate frontline "triplet regimen" associating therapeutic plasma exchange (TPE), immunosuppression with corticosteroids and rituximab, and caplacizumab. Outcomes were compared to 180 historical patients treated with the standard frontline treatment (TPE and corticosteroids, with rituximab as salvage therapy). The primary outcome was a composite of refractoriness and death within 30 days since diagnosis. Key secondary outcomes were exacerbations, time to platelet count recovery, the number of TPE and the volume of plasma required to achieve durable remission. The percentage of patients in the triplet regimen with the composite primary outcome was 2.2% vs. 12.2% in historical patients (p=0.01). One elderly patient in the triplet regimen died of pulmonary embolism. Patients from this cohort experienced less exacerbations (3.4% vs. 44%, p<0.01); they recovered durable platelet count 1.8 times faster than historical patients (95% confidence interval, 1.41-2.36, p<0.01), with fewer TPE sessions and lower plasma volumes (p<0.01 both). The number of days in hospital was 41% lower in the triplet regimen than in the historical cohort (13 days vs. 22 days, p <0.01). Caplacizumab-related adverse events occurred in 46 patients (51%), including 13 major or clinically relevant non-major hemorrhagic events. Associating caplacizumab to TPE and immunosuppression, by addressing the three processes of iTTP pathophysiology, prevents unfavorable outcomes and alleviates the burden of care.

Introduction

Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a devastating disease characterized by the association of a microangiopathic haemolytic anemia, a profound thrombocytopenia, organ involvement of variable severity and a severe (< 10% of normal activity) deficiency in the von-Willebrand factor (vWF) cleaving protease ADAMTS13 (A Disintegrin And Metalloproteinase with ThromboSpondin-1 Motifs, 13th member). Left untreated, iTTP is almost always fatal. However, prompt diagnosis and treatment allow survival rates of up to 85% [1, 2]. Historically, the standard iTTP treatment consisted in the association of daily therapeutic plasma exchange (TPE) and steroids [3, 4]. Rituximab, a B-cell depleting therapy, progressively evolved from a second-line agent in patients with unfavorable outcomes under standard care to become a frontline strategy [5-8]. Rituximab was shown to shorten the duration of daily TPE, especially in long-term responders, and led to durable platelet responses within 30 days as a result of a faster and more durable improvement in ADAMTS13 activity [5, 6, 9, 10]. However, rituximab efficacy is only observed after a mean time of 2 weeks following its first infusion; during this period patients are consequently exposed to unfavorable outcomes, especially death, typically heralded by refractoriness, and exacerbations [5, 6, 11, 12]. Recently, caplacizumab (Cablivi™, Ablynx, a Sanofi Company), a nanobody directed against the A1 domain of ultra-large vWF multimers, has been evaluated in iTTP through 2 pivotal trials with the aim to prevent the formation of microthrombi and subsequent microcirculation occlusion and ischemic organ damage. In both trials, iTTP patients were treated with caplacizumab in association with the standard treatment (TPE/corticosteroids, and rituximab as per clinician's practice). Patients who received caplacizumab recovered platelet counts more rapidly and more durably when compared to patients in the placebo group, despite the early use of rituximab [13, 14,15]. Neither death nor refractoriness was observed with caplacizumab, although the difference with the placebo arm could not reach the statistical significance level. [13, 14] Caplacizumab treatment appeared to be safe, with side effects mostly consisting in minor mucocutaneous bleeding [13, 14]. Although caplacizumab was approved for the treatment of iTTP in conjunction with TPE and

immunosuppression by the European Medicines Agency in Europe (September 2018) and the Food and Drug Agency in the United States (February 2019), uncertainties remain regarding the role of caplacizumab in the therapeutic arsenal of iTTP and post-marketing studies are urgently needed. Recently the very first report of real world experience, although uncontrolled and unplanned, confirmed that caplacizumab allows a rapid recovery of the disease, whether used frontline or as a salvage therapy in patients experiencing a refractory disease or an exacerbation [16].

France has been one of the first countries to allow a caplacizumab compassionate-use program for iTTP patients, starting in September 2018. We hereby report findings from the follow-up of patients treated by caplacizumab and reported to the French Reference Center for thrombotic microangiopathies (CNR-MAT) since the onset of this program.

Methods

Study design

The use of caplacizumab for iTTP patients was allowed in France in a compassionate-use program in September 2018; from February 2019, caplacizumab is authorized in France for initial treatment of iTTP. To rapidly improve our experience in its use, the CNR-MAT (www.cnr-mat.fr) recommended, based on a consensus of all participating centers, a "triplet regimen" comprising of (i) daily TPE, (ii) immunosuppression with corticosteroids and rituximab, and (iii) caplacizumab [8]. In France, rituximab obtained in 2011 a transient recommendation of use as a salvage therapy in iTTP patients with a suboptimal response to TPE/corticosteroids; in 2019, this recommendation was obtained for iTTP patients as a frontline therapy in an order to prevent long-term responses to TPE/corticosteroids treatment, but also one to 2-year relapses [5, 6, 9, 10] (https://ansm.sante.fr/var/ansm_site/storage/original/application/d793fd3d291b2f91af34e2

7cfea51786.pdf; page 11) (document in French; translated in Supplemental Table 1). Therefore, the use of rituximab as frontline therapy in iTTP has become part of the standard of care in our country.

The primary outcome was the prevalence of a composite of two crucial outcomes within 30 days since diagnosis: death, as well as refractoriness, usually heralding death [11, 12] (see definition in Supplemental Table 2). Key secondary outcomes were refractoriness, death, exacerbations, the time to durable platelet count recovery, the number of TPE and the volume of plasma required to achieve durable platelet count recovery, the length of hospitalization, and caplacizumab-related adverse events. We considered that the triplet regimen would represent an improvement in iTTP management if the primary outcome was at least three times lower than historically observed (10%) [5, 6, 9, 10]. To achieve this goal, we estimated that at least 65 patients were needed for the present study. The study was conducted according to the STROBE methodology.

Patients and data collection

All data from French patients with a clinical diagnosis of iTTP and treated according to our "triplet" regimen were analyzed from September 2018. The clinical diagnosis of iTTP was considered in patients with features of thrombotic microangiopathy (TMA) and a French score of 1 or 2 [8, 17]. The French score was calculated in patients with features of TMA and no associated condition (cancer, chemotherapy, pregnancy, transplantation, severe disseminated intravascular coagulopathy). Patients with a French score of 0 (platelet count ≥ 30 x 10^3/mm^3 and serum creatinine ≥ 200 µmol/L [2.27 mg/dL]) were considered as having an alternative diagnosis, mostly the hemolytic and uremic syndrome (HUS) and were not considered here. A French score of 2 (platelet count < 30 x 10^3/mm^3 and serum creatinine < 200 µmol/L [2.27 mg/dL]) was highly suggestive of iTTP. Patients with only one of these 2 items were considered as having a probable iTTP and daily TPE with corticosteroids and caplacizumab were immediately started;

in this scenario however, rituximab was started subsequently, only after iTTP diagnosis was confirmed (*i.e.*, if ADAMTS13 activity **was < 10%**). The final diagnosis of iTTP was confirmed in patients with a severe acquired ADAMTS-13 deficiency (< 10% of activity with anti-ADAMTS13 antibodies ≥15 U/mL) [18]. After TPE cessation, ADAMTS13 activity was assessed weekly until normalization (activity ≥50%).

Severity of iTTP at baseline was assessed using cerebral involvement (including confusion, stupor, coma or focal deficiency), age, as well as lactate dehydrogenase (LDH) level (reflecting mostly organ injury). Patients were classified into 2 groups: low-intermediate and high risk of early death, according to age, cerebral involvement and very high LDH levels [12]. Cardiac involvement was defined as an increase of troponin and/or electrocardiographic abnormalities. However, troponin assessment was not performed homogeneously through all centers; consequently, troponin was used for initial prognostic evaluation but detailed prognostic analyses were not performed.

In order to more accurately address improvements in the disease burden due to caplacizumab, the outcome of patients treated with the triplet regimen (triplet regimen cohort) was compared to a historical cohort of iTTP patients (historical cohort) managed with the standard regimen (i.e., daily TPE and steroids in association with salvage rituximab in patients experiencing refractoriness or an exacerbation of the disease). Patients from the triplet regimen were compared on a 1-to-2 ratio with the more recent patients of the historical cohort. The choice of a 1-to-2 ratio with the historical cohort was driven by the rarity of iTTP and the desire to include patients with standardized management.

Treatment

In all centers, daily TPE, corticosteroids (prednisone 1.0 mg/kg per day [maximal dose 100 mg/day]) and caplacizumab (10 mg intravenous loading dose followed by daily 10 mg subcutaneous doses) were started as soon as the clinical diagnosis of iTTP was suspected based

on the French score. Rituximab (375 mg/m^2) was administered intravenously on a day1-4-8-15 schedule [5,]. It could be started from day 1 if the French score was highly suggestive for the diagnosis of iTTP (French score = 2) [8, 14, 17], or alternatively by day 4 of the management once severe ADAMTS13 deficiency was ascertained (French score = 1) (Figure 1). Caplacizumab was continued for 30 days after TPE cessation and could be extended until ADAMTS13 improvement (i.e., activity ≥20%) [19, 20]. TPE were performed daily until 2 days of a normal platelet count (≥150x10^3/mm^3) and interrupted with no maintenance. Corticosteroids were pursued for 3 weeks, as previously described [5, 6, 9, 10].

Outcomes

Assessment of response to treatment was performed as previously described [1] (Supplemental Table 2). Adverse events related to caplacizumab were reported. The definition of major bleeding and clinically relevant non-major bleeding event is adapted from the International Society on Thrombosis and Hemostasis (ISTH). [21]

Ethics

This study was part of the Thrombotic Microangiopathy program study approved by our institutional review board (CPP04807) in accordance with the Declaration of Helsinki, and the French Data Protection Authority.

Statistics

Cohorts are described by absolute numbers with percentages for nominal variables and otherwise by the median with 1st and 3rd quartiles [Q1–Q3]. To assess whether cohorts differed at treatment start, the Mann-Whitney-Wilcoxon test was used for ordinal variables and Freeman-Halton's test otherwise. As follow-up time varied between patients, Poisson regression was used to reach the end-points. Patients' time under risk was estimated using the actuarial

method. A p-value <0.05 was considered as statistically significant. Analyses were performed using the SAS 9.4 statistical software (SAS institute Inc, Cary, NC).

Results

Population characteristics and iTTP presentation

Between September 2018 (date of caplacizumab availability for iTTP in France) and December 2019, 139 patients were diagnosed with iTTP. Twenty-two patients who had a confirmed diagnosis of iTTP were managed without caplacizumab, mostly because of unawareness of practitioners about the availability of the compound (18 cases) or because patients were considered at risk of bleeding (1 case of recent surgery and 3 cases of active bleeding). Three other patients with a retrospectively confirmed iTTP died at the time of diagnosis before any therapeutic measure could be enacted. Caplacizumab was therefore administered in a total of 114 patients with a clinical diagnosis of iTTP. In 6 patients who had a French score of 1, caplacizumab was stopped by day 3 because ADAMTS13 activity was suggestive of an alternative diagnosis (i.e., activity ≥20%). Eighteen additional patients with a confirmed iTTP (ADAMTS13 activity <10%) received caplacizumab on a different schedule (mainly as salvage therapy, *i.e.*, not frontline) and were not further considered here. Finally, 90 patients with a confirmed iTTP were treated according to the triplet regimen; they were compared to a historical cohort of 180 patients, registered in the French cohort between September 2018 and June 2015 (Flowchart in Figure 2). During this period, patients of the historical cohort were managed consensually and homogeneously; particularly, rituximab was systematically performed in patients experiencing refractoriness or exacerbation based on national recommendations. [22, 23] Patients treated according to the triplet regimen were consecutively recruited from 32 centers, including 8 centers in Ile-de-France (Paris and suburbs). Centers included a median of 2 patients (IQR, 1-3)(extremes: 1-12).

Clinical presentation of patients of the triplet regimen cohort was comparable to this of patients in the historical cohort, except for the LDH level (p=0.01), providing evidence that our cohort of studied patients is fully representative of the whole iTTP population. Especially, 19% of patients from the triplet regimen and 14% from the historical cohort (p=0.37) were considered to be at higher risk of death (Table 1). Moreover, the clinical presentation of patients who were not treated with caplacizumab was comparable to this of patients of the triplet regimen cohort, except for a higher percentage of previous iTTP episodes, suggesting that these patients did not have a more severe disease (Supplemental Table 3).

iTTP treatment

Daily TPE and corticosteroids were started immediately after the clinical diagnosis of iTTP. Patients received caplacizumab mostly within 3 days following TPE/corticosteroids initiation (median time, 0 days [0-1][extremes, 0-4]). In 47 patients, caplacizumab was started on the same day as TPE (day +0); in the others it was started at day +1 (24 patients), day +2 (8 patients), day +3 (6 patients) or day +4 (5 patients). The total duration of caplacizumab treatment was 33 days (29-38); caplacizumab was pursued for 32 days (28-37) post-TPE. In 12 patients, caplacizumab was interrupted empirically when ADAMTS13 activity reached ≥20%, without further event. Most patients (80%) had a French score of 2 and started rituximab within 3 days (before ADAMTS13 activity was available), whereas the 18 others had a French score of 1 and started rituximab by day-4, after severe ADAMTS13 deficiency was ascertained. In the historical cohort, 68% of patients were treated with rituximab (Table 1). Prophylactic anticoagulation and/or prophylactic antiplatelet aggregation was performed in 33 patients (37%) when platelet count increased above $50x10^3/mm^3$.

Primary outcome

The percentage of patients in the triplet regimen with the composite primary outcome was 2.2%, versus 12.2% in patients with the historical treatment (p=0.01). Therefore, patients treated with the triplet regimen were 6.2 times less likely to have refractory iTTP or iTTP-related death than patients from the historical cohort (95% CI, 1.4 to 26.3, p=0.013). As expected, the death rate was 6.7% (n=12) in the historical cohort, whilst, in the triplet regimen cohort only one patient died (1.1%, p=0.06). This 83-yo died at day 9; the cause of death reported by the practitioner was a massive pulmonary embolism. She had cardiac involvement (cTn 0.51 µg/L) without cerebral involvement at the time of diagnosis and her treatment consisted in the triplet regimen frontline from day 1. After an initial improvement, she subsequently presented an exacerbation of her disease at day-5 and died of cardiogenic shock despite salvage thrombolysis. She did not receive thromboprophylaxis when platelet count improved. Whether this complication resulted from a complication of the central venous catheter insertion could not be ascertained. Only one patient was classified as having a refractory disease as platelet count did not double after 4 days of treatment; however, response to treatment occurred slowly thereafter while no intensification was needed. On the opposite, 16 patients experienced this outcome (18%, p=0.01) in the historical treatment. Of note, death was preceded by refractoriness in 6 patients (50% of cases).

Key secondary outcomes

A significant decrease in exacerbations was noted in the triplet regimen cohort when compared to the historical cohort (3.4% vs. 44%, p<0.01). Therefore, exacerbations were 16.4 times more frequent in the historical cohort than in the triplet regimen cohort (95% CI, 5.2 to 52.1, p<0.01). The cause of the 3 exacerbations of the triplet regimen cohort remains unclear; especially, no triggering factor (*i.e.*, infectious event) could be identified. None of the 3 patients interrupted caplacizumab therapy; all had a favorable outcome in the following days with no organ involvement and no treatment intensification was required [24]. As a result, patients in this

cohort recovered durable platelet count 1.8 more rapidly than those of the historical cohort (5 [4-6] days *vs* 12 [6-17] days; [95% CI, 1.41 to 2.36, p<0.01]) (Figure 3). Patients in the triplet regimen cohort needed a median of 5 days (4-7) of TPE as compared to 10 days (6-16) of TPE in the historical cohort (p<0.01). Consequently, the corresponding median volume of plasma was 24.2 liters (18.3-30.2) for patients in the triplet regimen cohort as compared to 44.4 liters (26.3-74.3) for patients in the historical cohort (p<0.01), corresponding to a 45% reduction. In addition, the length of hospitalization was reduced by 41% in the triplet regimen cohort (Table 2). After a median follow-up of 127 days (47-200 days), only one clinical relapse occurred in the triplet regimen cohort. In this patient, caplacizumab was interrupted 33 days post-TPE while ADAMTS13 activity was still <10%. Relapse occurred at day 6 after caplacizumab interruption. The outcome was rapidly favorable after TPE and caplacizumab were resumed, and ADAMTS13 activity improved at day 49 post-TPE of the first episode. One additional patient experienced a severe ADAMTS13 deficiency at 14 months of follow-up that resolved after a single infusion of rituximab.

Other outcomes

ADAMTS13 activity was assessed weekly post-TPE. In the triplet regimen, there was a great disparity in the time to ADAMTS13 activity improvement (activity ≥20%) (median 28 days, IQR 14-42; extremes 7-164), but >50% patients recovered ADAMTS13 activity at day 28, although in 10 cases, this threshold was reached after day 56. Those patients with a slow ADAMTS13 response improved all their activity ≥20% within day 64 and day 164 post-TPE. In 2 of them, an additional course of 4-infusion rituximab was performed during this period, while the others improved with no further action.

One patient relapsed clinically after caplacizumab was stopped, whereas the 9 others could pursue caplacizumab until ADAMTS13 improvement, without relapse. In the historical cohort, where rituximab was not systematically performed, >50% of patients improved

ADAMTS13 activity at day 42 (Figure 4); moreover, up to 40% improved ADAMTS13 activity after day 56. As a result, patients in the triplet regimen recovered ADAMTS13 activity >20% four times faster than those in the historical cohort (95% CI, 3.03 to 5.26, p<0.01).

Outcome according to severity cohorts

Consistent with our previous findings, the percentage of patients with the composite primary outcome in the historical cohort was 8.3% for iTTP patients with a severity score of 0-2 whereas in patients with a more severe disease (severity score ≥3), the composite primary outcome was significantly higher (33%, p<0.01). However, only two of the patients treated with the triplet regimen (both with a severity score of 0-2) reached the composite primary outcome, whereas all the 17 patients with a severity score ≥3 recovered from their disease (Table 2).

Safety

Ultimately, 46 (51%) patients experienced at least one drug-related adverse event in the triplet regimen cohort (Table 3). Hemorrhagic events were mostly drug-related (30 patients, 33%), with epistaxis and gingival bleeding being the most prevalent. All these events resolved without specific intervention. Major bleeding events and clinically relevant non-major bleeding events were observed in 2 and 11 patients, respectively. One major bleeding event from the digestive tract was complicated by a hemorrhagic shock of favorable outcome with symptomatic measures and the interruption of caplacizumab. No patient required infusions of vWF concentrates or of any coagulation concentrate. No temporal relationship between the occurrence of bleeding and the duration of exposure to caplacizumab was observed. Six patients (6.7%) developed an inflammatory reaction consisting in a swelling at the injection sites of caplacizumab that occurred typically by the end of the treatment course; however, no premature interruption of the treatment was needed. Nineteen patients (21%) developed a mild to severe

transient thrombocytosis (platelet count >450 x 10^3/mm^3) along with platelet count recovery (Table 3 and Supplemental Table 4), although the association with the use of caplacizumab remains questionable.

Thromboembolic events

Eleven patients (12%) of the triplet regimen cohort experienced a thromboembolic event (TEE), consisting in PE without apparent deep venous thrombosis or central venous catheter thrombosis (5 cases), lower limb deep venous thrombosis (3 cases) and central venous catheter-related thrombosis (4 cases). One patient had both a central venous catheter-related thrombosis associated with a more distal deep venous thrombosis. None of them had received thromboprophylaxis when platelet count increased above 50 x 10^3/mm^3. On the other hand, we found no clear association between the occurrence of TEE and thrombocytosis (thrombocytosis was present in 3 (27%) patients with TEE *vs* 16 (21%) patients without TEE). Moreover, the prevalence of TEE was similar in both cohorts (p=0.79).

Discussion

We hereby report the outcome of iTTP patients managed with a triplet strategy associating caplacizumab to frontline rituximab and the historical TPE/corticosteroids treatment. This regimen, established from the HERCULES trial and submitted to patients with a clinical diagnosis of iTTP[14], is aimed at addressing all three aspects of iTTP pathophysiology: to replenish the missing ADAMTS13 enzyme, to suppress the production of anti-ADAMTS13 antibodies, and to inhibit the excessive vWF-platelet interaction leading to microthrombi formation, organ failure and death [8]. In this prospective study performed in "real-life" conditions, we confirm previous findings from both pivotal trials and uncontrolled observational reports in unselected patients homogeneously treated with a standardized approach, the "triplet

regimen" [13, 14, 16]. We have shown that early treatment with caplacizumab allows significantly preventing unfavorable outcomes during the very acute phase of iTTP as only one case of death and one slow response were identified, whereas very few cases of exacerbations were observed. Although the rarity of events for death led to a trend to less death in the triplet regimen, a difference may have emerged with a larger cohort of patients. By contrast, in our historical cohort of patients, these events were more prevalent and consistent with previous reports [5, 17]. Importantly, the prevention of such events also alleviated the burden of the disease that translates into a dramatic reduction of plasma volumes and TPE sessions and a shortened hospital stay. From these results, the triplet regiment is now considered the best standard of care in France.

One limitation of our study lies on the different modalities of use of rituximab, which was used frontline in the triplet regimen but as a salvage therapy in the historical cohort. However, our work was not only aimed at demonstrating the superiority of the addition of caplacizumab to the standard treatment; instead, we wished to show here that a strategy addressing the three aspects of iTTP pathophysiology simultaneously from diagnosis is superior to more sequential/escalating strategies [5, 6, 9, 10], while the risk/benefit effect remains acceptable.

While frontline rituximab was reported to prevent slow responders to TPE and prevent 1- to 2-year relapses, it scarcely improved survival and other early unfavorable outcomes in acute iTTP [6, 22]. Therefore, the substantial improvement of iTTP prognosis we observed here, reflected by a rapid and durable platelet count recovery, is likely due to the early administration of caplacizumab. Consequently, caplacizumab and rituximab should be considered as complementary, non-redundant agents in the management of iTTP. By preventing the neoformation of microthrombi, caplacizumab allows a rapid and stable platelet count recovery with organ protection, especially during very early management of iTTP during which most deaths occur and during which rituximab is not efficient yet [6, 12]. On the other hand, by suppressing the autoimmune response against ADAMTS13, rituximab improves and stabilizes

durably ADAMTS13 activity after usually a 2- to 5-week period [14]. The combination of both agents (*i.e.*, a standard course of 4 infusions of rituximab to improve ADAMTS13 activity and daily caplacizumab to prevent additional microthrombi formation until ADAMTS13 improvement) should therefore prevent clinical relapses occurring when caplacizumab is interrupted while ADAMTS13 activity is still undetectable [13, 14]. Here, the systematic use of rituximab frontline in the triplet cohort accounts for the faster improvement of ADAMTS13 activity than with the historical regimen where rituximab was only introduced later as a salvage therapy.

We provide evidence that patients treated with the triplet regimen have a favorable outcome irrespective of the disease severity on diagnosis [12], suggesting that the negative impact of cerebral involvement and very high LDH level is suppressed by caplacizumab. However, the benefit of caplacizumab on prognosis needs further evaluation in the elderly, as the single case of fatal outcome and the most serious adverse events were observed in this population. Moreover, the usual prevalence of TEE was not apparently reduced with the use of caplacizumab [25]. One explanation would be a more prevalent omission of thromboprophylaxis (in 63% of patients) with the use of caplacizumab, as clinicians considered here that the association of caplacizumab and anticoagulation could have exposed patients to an increased risk of bleeding. Consequently, iTTP patients should be considered at risk of TEE despite the use of caplacizumab and thromboprophylaxis should be more systematically offered, especially when platelet count increases above 50 x 10^3/mm^3.

A main obstacle to the use of caplacizumab may be its high cost. In this way, medico-economic studies are still pending to address whether the outcomes realized with caplacizumab-containing regimens, including the dramatic decrease in the burden of care, are sufficiently superior to be cost-effective. On the other hand, the price of caplacizumab recognizing its innovative nature will have to be balanced with the improvement of survival with usually no sequelae in a disease involving young patients.

This post-marketing surveillance study confirms the results of caplacizumab trials in unselected iTTP patients. A triplet strategy systematically associating TPE, immunosuppression with corticosteroids and rituximab, and caplacizumab prevents unfavorable outcomes of the disease and alleviates substantially the burden of care in these patients.

Table 1. Clinical features and concomitant treatment of patients on diagnosis according to the treatment regimen.

Characteristic	**Triplet regimen (N=90)**	**Historical cohort (N=180)**	**P-value**
Age (y)	45 (34-57)	43 (30-57)	1.00
Female sex	63 (70%)	127 (70%)	0.30
Weight (kg)	71 (60-91)	71 (60-86)	0.83
Body mass index	27.2 (22.7-32.2)	26.6 (23-31.7)	0.68
Ethnicity			
White	74	149	0.39
African-West Indies	10	25	
Asian	6	6	
Ongoing antiplatelet agent/anticoagulation	9 (10%)	16 (8.9%)	0.77
Antiplatelet agent	7	11	
Anticoagulant	2	5	
Relapse	12 (13.3%)	21 (11.7%)	0.70
Cerebral involvement	55 (61%)	111 (62%)	0.91
Headache	19	58	
Confusion	22	36	
Seizure	10	15	
Coma	2	5	
Focal deficiency	20	26	
Cardiac involvement	51 (56%)	86 (47%)	0.15
Hemoglobin (g/dL)	8.9 (7.5-10.2)	8.6 (7.3-10.1)	0.54
Platelet count (x 10^3/mm^3)	12 (10-20)	12 (8-23)	0.88
LDH level xN (U/L)	5.1 (4.0-6.5)	3.7 (2.4-5.6)	0.01
Serum creatinine level (µmol/L)	92 (71-120)	86 (68-133)	0.17
GFR (mL/min/1.73m^2) (MDRD)	74 (51-108)	80 (46-120)	0.85
ADAMTS13 activity (%)	**< 10%**	**< 10%**	**-**
Anti-ADAMTS13 antibodies (U/mL)	78 (39-91)	80 (36-100)	0.44
French Severity score:			
0-2	72 (81%)*	145 (87%)$	0.37
3-4	17 (19%)	21 (13%)	
Immunosuppressive therapy			
-Corticosteroids	88 (98%)	166 (92%)	0.10
-Rituximab	90 (100%)	123 (68%)	<0.01
Time between 1st infusion and 1st TPE	2 (1-3)	7 (4-10)	<0.01
Other therapies	0	25 (13.9%)	< 0.01
Twice-daily TPE		20	
Cyclophosphamide		4	
Splenectomy		2	
Vincristine		3	
Bortezomib		1	
> 1 salvage therapy		4	

Abbreviations: LDH: lactate dehydrogenase. GFR: Glomerular Filtration Rate. MDRD Modification of Diet in Renal Disease. TPE: therapeutic plasma exchange. ADAMTS13: A Disintegrin And Metalloproteinase with ThromboSpondin-1 motifs, 13rd member. Data are given as median (25th-75th percentile) for quantitative variables and as n (%) for qualitative

variables. Severe ADAMTS13 activity was defined as an activity <10% (normal range for ADAMTS13 activity: 50%-100%). The positivity threshold for anti-ADAMTS13 immunoglobulin G (IgG) was 12 U/mL, according to the manufacturer's instructions (Technoclone®). Cardiac involvement was defined as an increase of troponin and/or electrocardiographic abnormalities. Patients at high risk of early death of iTTP were defined by a French severity score ≥3 (cerebral involvement: yes=1 / no=0, LDH: >10xU.LN=1 / ≤10xULN=0, age: >60 years=2 / >40 and ≤60 years=1 / ≤40 years=0) [12]. Data *from 89 patients and $from 166 patients.

Table 2. Primary and secondary outcomes according to the treatment regimen.

Outcome	Triplet regimen (N=90)	Historical cohort (N=180)	P-value
Primary outcome			
Composite of death and refractoriness			
All patients	**2 (2.2%)**	22 (12.2%)*	0.01
According to French Severity score:			
0-2	**2 (2.8%)**	15 (8.3%)	<0.01
3-4	0	7 (33%)	
Secondary outcomes			
Death	**1 (1.1%)**	**12 (6.7%)**	**0.06**
Refractoriness	**1 (1.1%)**	**16 (18%)£**	**0.01**
Exacerbations	3 (3.4%)	70 (44%)	<0.01
Time to durable platelet count recovery	5 (4-6)	12 (6-17)	<0.01
Number of daily TPE until remission	5 (4-7)	10 (6-16)	<0.01
Volume of plasma (Liter) until remission	24.2 (18.3-30.2)	44.4 (26.3-74.3)	<0.01
Time to ADAMTS13 activity > 20% (days)	28 (14-42)	48(24-83)	<0.01
Length of hospitalization (days)	13 (9-19)	22 (15-30)	0.01
Thromboembolic events	11 (12%)	20 (11.1%)	0.79

Abbreviations: TPE: therapeutic plasma exchange. Data are given as median (25th-75th percentile) for quantitative variables and as n (%) for qualitative variables. Patients at high risk of early death of iTTP were defined by a French severity score ≥3 (cerebral involvement: yes=1 / no=0, LDH: >10xU.LN=1 / ≤10xULN=0, age: >60 years=2 / >40 and ≤60 years=1 / ≤40 years=0) [12]. *Includes 10 refractory patients who survived (only one event per patient was considered). £Includes 6 deaths.

Table 3. Caplacizumab-related adverse events.

	Number of adverse events	**Description**
Major bleeding	2	1 hemorrhagic shock with lower digestive bleeding 1 abundant menorrhagia with a decrease in hemoglobin level of 2.5 g/dL
Clinically relevant non major bleeding	11	3 Macroscopic gastrointestinal hemorrhage 7 epistaxis 1 Subcutaneous hematoma larger than 25 cm2
Non clinically relevant non major bleeding	17	9 ecchymosis or small hematoma 6 Gingival bleedings 2 Catheter site hemorrhage
Inflammatory reaction	6	Inflammatory **swelling** at the injection site, especially at the end of the treatment course
Thrombocytosis	19	Platelet count (x $10^3/mm^3$): >450 - 600: 11 cases >600 – 900: 7 cases >900: 1 case

Figure 1. The CAPLAVIE regimen. iTTP: immune-mediated Thrombotic Thrombocytopenic Purpura. A13: ADAMTS13. TPE: therapeutic plasma exchange. ADAMTS13 activity was assessed weekly until normalization or day 56.

Figure 2. Flow Chart of the study. iTTP: immune-mediated Thrombotic Thrombocytopenic Purpura. *Mostly because of unawareness of practitioners about the availability of the compound (18 cases) or because patients were considered at risk of bleeding (1 case of recent surgery and 3 cases of active bleeding).

Figure 3. Cumulative daily rate of platelet count recovery after 1st therapeutic plasma exchange within 35 days by cohort.

Figure 4. Cumulative percentage of patients who achieved ADAMTS13 activity ≥20% following the last therapeutic plasma exchange in the triplet regimen (red columns) and the historical cohort (blue columns). ADAMTS13 activity was assessed weekly until normalization or day 56.

Acknowledgments

Patients were recruited with the help of the members of the Reference Center for Thrombotic Microangiopathies (CNR-MAT) (listed in the appendix). We thank S. Thouzeau, S. Capdenat, S. Savigny (Laboratoire d'Hématologie, Hôpital Lariboisière, AP-HP, Paris), and Raïda Bouzid (Centre de Référence des Microangiopathies Thrombotiques, Hôpital Saint-Antoine, AP-HP, Paris) for technical assistance. This work was partly funded by a grant from the French Ministry of Health (Projet Hospitalier de Recherche Clinique; P120118; AOM12259). This work was also supported by the National Plan for Rare Diseases of the French Ministry of Health (Direction Générale de l'Offre de Soin (DGOS)).

Authorship contributions

Paul Coppo and Ygal Benhamou designed the study, interpreted the results, and wrote the manuscript. Michael Bubenheim performed the statistical analysis of the French Registry for Thrombotic Microangiopathies. Elie Azoulay, Naïke Bigé, Lionel Galicier, Pascale Poullin, François Provôt, Martis Nihal, Claire Presne, Olivier Moranne, Ruben Benainous, Antoine Dossier, Amélie Seguin, Miguel Hié, Alain Wynckel, Yahsou Delmas, Jean-François Augusto, Pierre Perez, Virginie Rieu, Christelle Barbet, François Lhote, Marc Ulrich, Anne Charvet Rumpler, Sten de Witte, Thierry Krummel, Agnès Veyradier, Ygal Benhamou and Paul Coppo enrolled patients and collected clinical and laboratory information. Sandrine Malot collected the data from all patients. All of the authors critically reviewed and substantially improved the manuscript.

Disclosure of Conflict of Interests

M. Bubenheim, E. Azoulay, S. Malot, Naïke Bigé, L. Galicier, F. Provôt, M. Nihal, O. Moranne, R. Benainous, A. Dossier, A. Seguin, M. Hié, J-F. Augusto, P. Perez, V. Rieu, C. Barbet, F. Lhote, M. Ulrich, A. Charvet Rumpler, S. de Witte and T. Krummel do not have any conflict of interest to declare. P. Coppo is member of the Clinical Advisory Board for Alexion, Sanofi, Shire and

Octapharma. Y. Benhamou, P. Poullin, A. Wynckel, Y. Delmas, C. Presne and A. Veyradier have participated to Advisory boards for Sanofi.

Data sharing

For original data, please email the corresponding author.

References

1. Scully M, Cataland S, Coppo P, et al. Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. J Thromb Haemost 2017;15(2):312-22.
2. Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. Blood 2017;129(21):2836-46.
3. Bell WR, Braine HG, Ness PM, Kickler TS. Improved survival in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Clinical experience in 108 patients. The New England journal of medicine 1991;325(6):398-403.
4. 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. The New England journal of medicine 1991;325(6):393-7.
5. Froissart A, Buffet M, Veyradier A, et al. Efficacy and safety of first-line rituximab in severe, acquired thrombotic thrombocytopenic purpura with a suboptimal response to plasma exchange. Experience of the French Thrombotic Microangiopathies Reference Center. Critical care medicine 2012;40(1):104-11.
6. Scully M, McDonald V, Cavenagh J, et al. A phase 2 study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. Blood 2011;118(7):1746-53.
7. Scully M, Cohen H, Cavenagh J, et al. Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction in IgG antibodies to ADAMTS-13. British journal of haematology 2007;136(3):451-61.
8. Coppo P, Cuker A, George JN. Thrombotic thrombocytopenic purpura: Toward targeted therapy and precision medicine. Res Pract Thromb Haemost 2018;3(1):26-37.
9. Page EE, Kremer Hovinga JA, Terrell DR, Vesely SK, George JN. Rituximab reduces risk for relapse in patients with thrombotic thrombocytopenic purpura. Blood 2016.
10. Mazepa AM, Evans M, Davis E, et al. Differential effect of rituximab on relapse-free survival in de novo and relapse immune thrombotic thrombocytopenic purpura in African-American and Caucasian populations. Blood 2019;134 Suppl (190) DOI: https://doi.org/10.1182/blood-2019-129383.
11. Patton JF, Manning KR, Case D, Owen J. Serum lactate dehydrogenase and platelet count predict survival in thrombotic thrombocytopenic purpura. American journal of hematology 1994;47(2):94-9.
12. Benhamou Y, Assie C, Boelle PY, et al. Development and validation of a predictive model for death in acquired severe ADAMTS13 deficiency-associated idiopathic thrombotic thrombocytopenic purpura: the French TMA Reference Center experience. Haematologica 2012;97(8):1181-6.
13. Peyvandi F, Scully M, Kremer Hovinga JA, et al. Caplacizumab for Acquired Thrombotic Thrombocytopenic Purpura. The New England journal of medicine 2016;374(6):511-22.
14. Scully M, Cataland SR, Peyvandi F, et al. Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. The New England journal of medicine 2019;380(4):335-46.
15. Knoebl P, Cataland S, Peyvandi F, et al. Efficacy and safety of open-label caplacizumab in patients with exacerbations of acquired thrombotic thrombocytopenic purpura in the HERCULES study. J Thromb Haemost 2020;18(2):479-84.
16. Volker LA, Kaufeld J, Miesbach W, et al. Real-world data confirm the effectiveness of caplacizumab in acquired thrombotic thrombocytopenic purpura. Blood Adv 2020;4(13):3085-92.
17. Coppo P, Schwarzinger M, Buffet M, et al. Predictive features of severe acquired ADAMTS13 deficiency in idiopathic thrombotic microangiopathies: the French TMA reference center experience. PloS one 2010;5(4):e10208.
18. Prevel R, Roubaud-Baudron C, Gourlain S, et al. Immune thrombotic thrombocytopenic purpura in older patients: prognosis and long-term survival. Blood 2019;134(24):2209-17.

19. Hie M, Gay J, Galicier L, et al. Preemptive rituximab infusions after remission efficiently prevent relapses in acquired thrombotic thrombocytopenic purpura: experience of the French Thrombotic Microangiopathies Reference Center. Blood 2014.
20. Jestin M, Benhamou Y, Schelpe AS, et al. Preemptive rituximab prevents long-term relapses in immune-mediated thrombotic thrombocytopenic purpura. Blood 2018;132(20):2143-53.
21. Schulman S, Kearon C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost 2005;3(4):692-4.
22. Froissart A, Veyradier A, Hie M, Benhamou Y, Coppo P. Rituximab in autoimmune thrombotic thrombocytopenic purpura: A success story. Eur J Intern Med 2015;26(9):659-65.
23. Benhamou Y, Paintaud G, Azoulay E, et al. Efficacy of a rituximab regimen based on B cell depletion in thrombotic thrombocytopenic purpura with suboptimal response to standard treatment: Results of a phase II, multicenter noncomparative study. American journal of hematology 2016;91(12):1246-51.
24. Soucemarianadin M, Benhamou Y, Delmas Y, et al. Twice-daily therapeutical plasma exchange-based salvage therapy in severe autoimmune thrombotic thrombocytopenic purpura: the French TMA Reference Center experience. European journal of haematology 2016;97(2):183-91.
25. Vendramin C, McGuckin S, Alwan F, Westwood JP, Thomas M, Scully M. A single-center prospective study on the safety of plasma exchange procedures using a double-viral-inactivated and prion-reduced solvent/detergent fresh-frozen plasma as the replacement fluid in the treatment of thrombotic microangiopathy. Transfusion 2017;57(1):131-6.

Figure 1

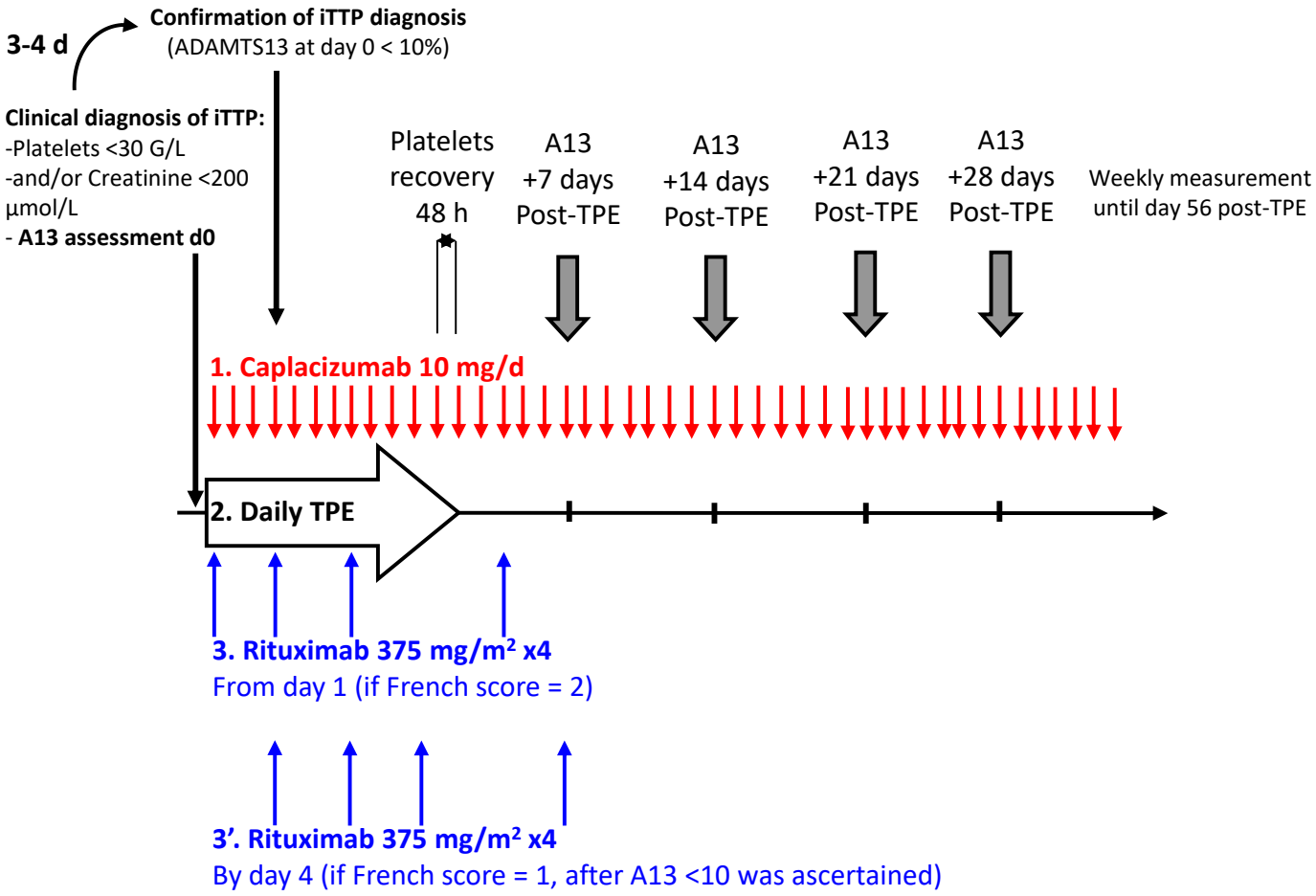

Figure 2

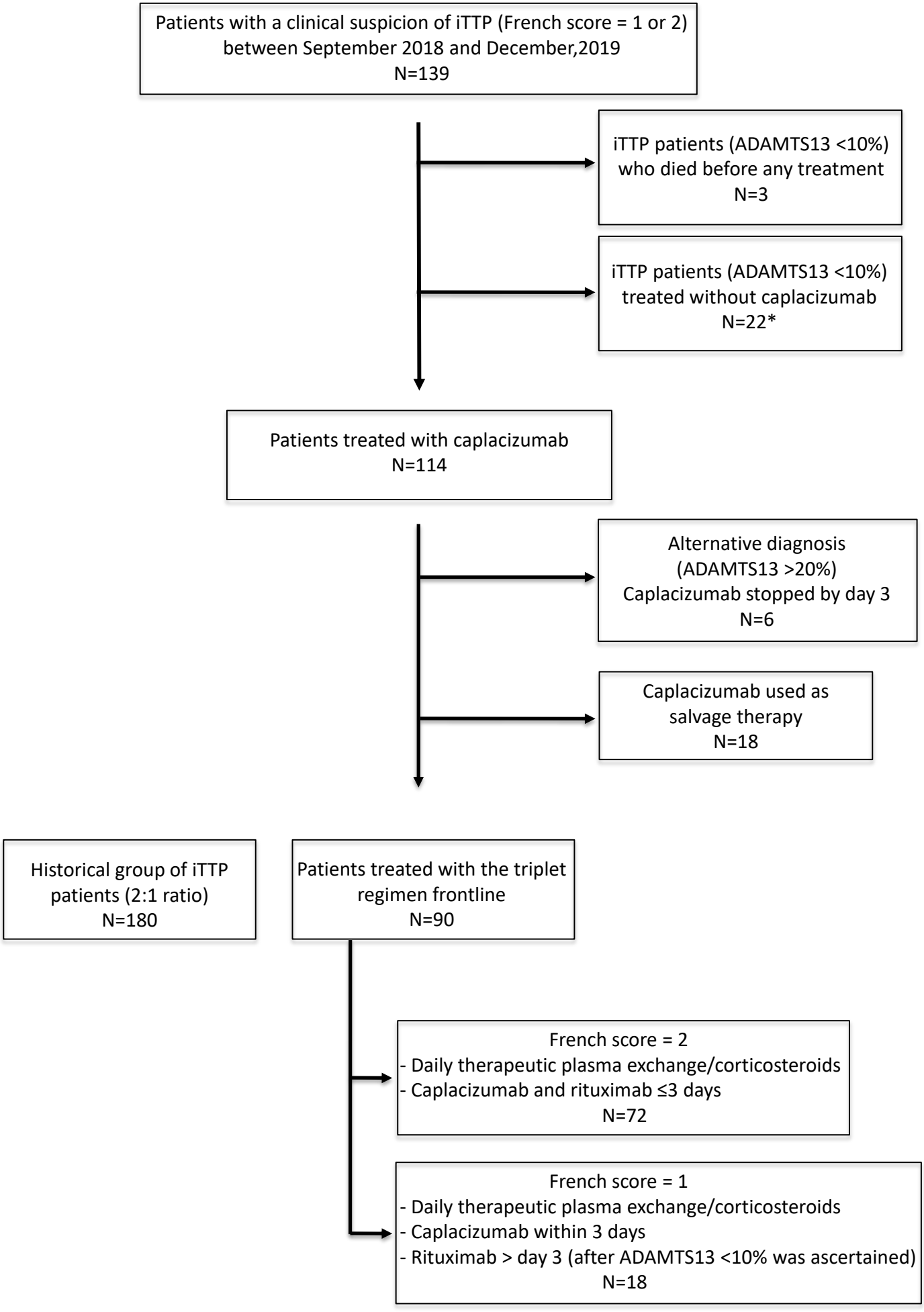

Figure 3

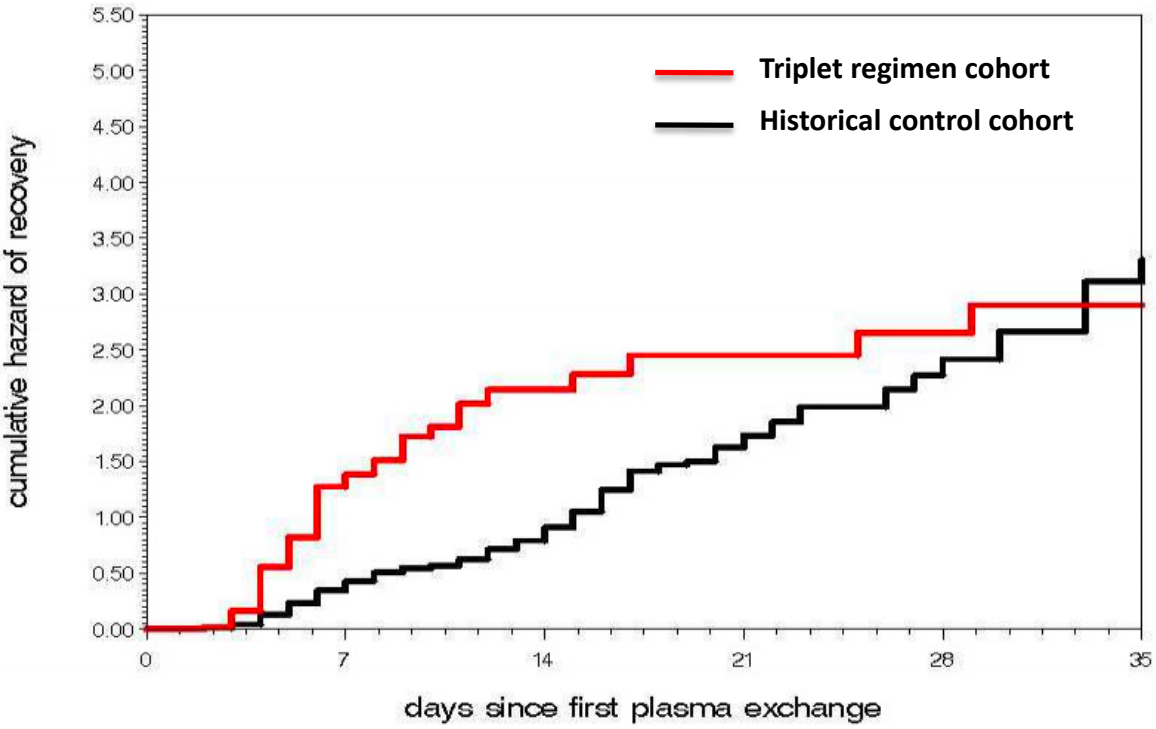

Figure 4