

ORIGINAL ARTICLE

Bortezomib for rituximab-refractory immune-mediated thrombotic thrombocytopenic purpura in the caplacizumab era: an Italian multicenter study

Juri Alessandro Giannotta¹  | Andrea Artoni¹ | Ilaria Mancini¹ | Pasquale Agosti^{1,2} | Monica Carpenedo³ | Addolorata Truma² | Syna Miri¹ | Barbara Ferrari¹ | Pasqualina De Leo¹ | Prassede Salutari⁴ | Giorgia Mancini⁵ | Alfredo Molteni⁶ | Ermina Rinaldi⁷ | Monica Bocchia⁸ | Mariasanta Napolitano^{9,10} | Lucia Prezioso¹¹ | Annarosa Cuccaro¹² | Elisabetta Scarpa¹³ | Annalisa Condorelli¹⁴ | Daniele Grimaldi¹⁵ | Massimo Massaia¹⁵ | Flora Peyvandi^{1,2} 

¹Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy

²Department of Pathophysiology and Transplantation, Fondazione Luigi Villa, Università degli Studi di Milano, Milan, Italy

³Department of Hematology, Oncology and Molecular Medicine, Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

⁴Hematology Unit, Department of Oncology and Hematology, Spirito Santo Hospital, Pescara, Italy

⁵Hematological Unit, Polytechnic Marche University, Ancona, Italy

⁶UOC Ematologia e CTMO, ASST Cremona, Cremona, Italy

⁷Hematology Unit, A. Perrino Hospital, Brindisi, Italy

⁸Hematology Unit, Azienda Ospedaliero-Universitaria Senese, University of Siena, Siena, Italy

⁹Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy

¹⁰Haematology and Rare Diseases Unit, Hospital "V. Cervello," Palermo, Italy

¹¹Hematology and BMT Unit, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy

¹²Hematology Unit, Center for Translational Medicine, Azienda USL Toscana NordOvest, Livorno, Italy

¹³UOC Ematologia, Ospedale Cà Foncello, Treviso, Italy

¹⁴Hematology and Bone Marrow Transplant Unit, ASST Papa Giovanni XXIII, University of Milano-Bicocca, Bergamo, Italy

¹⁵Hematology Division, AO S. Croce e Carle, Cuneo, and Molecular Biotechnology Center "Guido Tarone," Torino, Italy

Correspondence

Flora Peyvandi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy.
Email: flora.peyvandi@unimi.it

Abstract

Background: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) patients are not responsive to standard rituximab in approximately 10% to 15% of cases, and oral immunosuppressants showed controversial results with significant toxicity. Targeting

Manuscript handled by: Karen Vanhoorelbeke

Final decision: Karen Vanhoorelbeke, 24 October 2024

© 2024 The Author(s). Published by Elsevier Inc. on behalf of International Society on Thrombosis and Haemostasis. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Funding information

The study was partially supported by the Italian Ministry of Health – Bando Ricerca Corrente. The Hemostasis & Thrombosis Unit of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico is a member of the European Reference Network on Rare Haematological Diseases EuroBloodNet – project ID number 101157011. European Reference Network-EuroBloodNet is partly cofunded by the European Union within the framework of the Fourth EU Health Programme.

plasma cells with bortezomib appears promising, but the available evidence is scarce and stems only from isolated reports in the caplacizumab era.

Objectives: To evaluate the safety and efficacy of bortezomib in rituximab-refractory iTTP patients.

Methods: We conducted a retrospective observational multicenter study among 13 Italian iTTP treating centers, collecting data from May 2017 to May 2023 (caplacizumab was licensed in Italy in January 2020).

Results: Bortezomib was effective in 10/17 patients (59%). Eleven were treated in the acute phase (9/11 responders, 82%, allowing discontinuation of caplacizumab in 5/6 treated patients), and 7 during clinical remission (2/7 responders, 28%). Responses occurred at a median time of 30 days, but 3 patients responded after 4 months. The median duration of response was 22 months (IQR, 10–38), still ongoing in 6 patients at the time of data cutoff. Responders had fewer previous acute iTTP episodes than nonresponders (median [IQR], 1 [1,2] vs 5.5 [2–7]; $P = .03$). Eight subjects (47%) reported toxicities, mostly in those treated with ≥ 2 cycles.

Conclusion: Durable responses to bortezomib were registered in about 60% of multirefractory iTTP patients with mild to moderate toxicities. The occurrence of late responses (ie, after 30 days) suggests a “watchful waiting” approach after bortezomib treatment.

KEYWORDS

ADAMTS-13, bortezomib, immune-mediated thrombotic thrombocytopenic purpura, immunosuppressants, rituximab

1 | INTRODUCTION

Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a rare autoimmune disease caused by autoantibodies directed against a disintegrin and metalloprotease with thrombospondin type 1 repeats member 13 (ADAMTS-13), a plasma metalloprotease that cleaves the von Willebrand factor (VWF) [1,2]. ADAMTS-13 deficiency leads to the accumulation of ultralarge VWF multimers in the circulation, resulting in increased platelet aggregation and microangiopathic hemolytic anemia [2]. The consequent microvascular organ damage, generally involving the central nervous system, heart, and kidney, can lead to death in patients in about 90% of cases if not promptly treated and accounts for important long-term sequelae in survivors [3–7]. The current treatment approach, including therapeutic plasma exchange (TPE), immunosuppression, and, more recently, the anti-VWF nanobody caplacizumab, warranted a dramatic fall in mortality rates below 10% [8–12]. Anti-CD20 therapy with rituximab improved response rates in TPE refractory cases, especially before the introduction of caplacizumab [13]. Up to 50% of iTTP survivors will eventually relapse, and rituximab proved effective in preventing clinical relapses when employed during the acute phase [14]. Moreover, it is used during remission in case of ADAMTS-13 relapse as a preemptive treatment to prevent clinical and ADAMTS-13 relapses (a fall in ADAMTS-13

activity to <20% after an ADAMTS-13 remission) [15], as recommended by international guidelines [10]. However, about 10% to 15% of patients do not achieve a sustained ADAMTS-13 remission (ie, ADAMTS-13 activity levels > lower limit of normal [LLN]) with the standard treatment, making the management of rituximab-refractory iTTP patients an important unmet clinical need because of the high risk of clinical relapse in case of low ADAMTS-13 levels [16]. In this scenario, different therapeutic strategies have been proposed, including intensified rituximab regimens [17]. Traditional oral immunosuppressants, such as azathioprine, cyclosporine A, and mycophenolate mofetil, have been employed with variable outcomes [18–21]. However, they can be burdened by significant toxicities, including gastrointestinal, hepatopancreatic, or hematological toxicities for azathioprine [21], as well as renal failure for cyclosporine, and the need for long-term administration makes oral immunosuppressants less appealing. In this setting, targeting the CD20-negative long-living plasma cells appears promising. In particular, the proteasome inhibitor bortezomib, widely employed in the treatment of multiple myeloma for 2 decades [22], exerts a proapoptotic effect on autoreactive short- and long-living plasma cells [23]. Therefore, it has been increasingly used in various refractory autoimmune diseases, such as systemic lupus erythematosus, myasthenia gravis, autoimmune cytopenias, and immunoglobulin (Ig)A pemphigus [24]. Bortezomib has also been used

in refractory iTTP patients, with a safe profile also in the pediatric age [25–28]. Nonetheless, available evidence consists only of isolated case reports and small case series, mostly registered before the advent of caplacizumab [29,30]. Therefore, we performed a multicenter survey to evaluate the safety and efficacy of bortezomib in rituximab-refractory iTTP patients among Italian reference centers in an updated clinical setting.

2 | METHODS

2.1 | Study design and patients

We conducted a retrospective cohort study via an electronic case report form sent to 31 Italian iTTP treating centers. The electronic case report form collected information about patients' demographics, clinical characteristics, ADAMTS-13 activity and anti-ADAMTS-13 antibody titer, concomitant/previous treatments, bortezomib schedule, response and adverse drug reactions to bortezomib, and clinical and ADAMTS-13 relapses after bortezomib treatment.

Patients were included if 1) aged ≥ 18 years; 2) diagnosed with iTTP (defined as clinical and laboratory features of thrombotic microangiopathy, ie, thrombocytopenia with microangiopathic hemolytic anemia in the absence of alternative causes with evidence of ADAMTS-13 activity $< 10\%$ and anti-ADAMTS-13 antibodies); 3) refractory to rituximab; 4) completed ≥ 1 bortezomib cycle, defined as the standard myeloma regimen of 1.3 mg/m^2 on days 1, 4, 8, and 11; 5) regular follow-up at the enrolling center for at least 6 months after bortezomib treatment. Patients treated with bortezomib between May 2017 and May 2023 were consecutively enrolled and followed up until December 2023. Bortezomib was administered during the acute phase of the disease to achieve clinical/ADAMTS-13 remission (as defined in the following paragraph) in TPE/caplacizumab-treated patients or during clinical remission as preemptive treatment in case of ADAMTS-13 relapse or if ADAMTS-13 activity was persistently $< 20\%$. Immunosuppressive therapies were considered concomitant if administered during bortezomib treatment or discontinued < 1 month before bortezomib exposure. Patients were considered refractory to rituximab if no clinical response or ADAMTS-13 remission was achieved after at least 15 days from the first rituximab administration.

ADAMTS-13 testing was performed with different assays across centers. ADAMTS-13 activity was measured using the Technozym ADAMTS-13 Activity ELISA assay (Technoclone; normal range, 40–130 IU/dL), the HemosIL AcuStar ADAMTS-13 Activity assay (Werfen; normal range, 67–129 IU/dL or 61–131 IU/dL, depending on the laboratory), or the FRETs-VWF73 assay (normal range, 45%–147%) [31]. Anti-ADAMTS-13 antibodies were measured using the Technozym ADAMTS-13 inhibitor ELISA assay (Technoclone), which measures the anti-ADAMTS-13 IgG titer (negative values $< 12 \text{ U/mL}$, borderline values: 12–15 U/mL, positive values $> 15 \text{ U/mL}$), or using a Bethesda-like mixing assay, which measures the neutralizing activity of anti-ADAMTS-13 antibodies (negative values $< 0.4 \text{ BU/mL}$).

Written informed consent was obtained from all subjects with the approval of the Ethics Committee of all institutions, in accordance with the Declaration of Helsinki.

2.2 | Response criteria and toxicity evaluation

The primary efficacy outcome was the cumulative incidence of an overall response to bortezomib, with no need for any subsequent/additional immunosuppressive treatment. Different definitions of response were used according to the treatment setting, all referring to the criteria in the revised International Working Group consensus report [32]. For TPE-treated patients during the acute phase, the response was defined as a clinical response (ie, sustained platelet count $> 150 \times 10^9/\text{L}$ and lactate dehydrogenase levels < 1.5 times the upper limit of normal and no evidence of ischemic organ injury), which allowed TPE discontinuation. For caplacizumab-treated patients during the acute phase, the response was defined as ADAMTS-13 remission, which allowed caplacizumab discontinuation. For patients treated preemptively during clinical remission, response was defined as ADAMTS-13 remission, including partial (ADAMTS-13 activity $> 20\%$ to $< \text{LLN}$) and complete remission (ADAMTS-13 activity $> \text{LLN}$).

Secondary outcomes were 1) the incidence of clinical exacerbation (when platelet count decreased to $< 150 \times 10^9/\text{L}$ within 30 days of stopping TPE/caplacizumab—other causes of thrombocytopenia ruled out), clinical relapse (when the latter occurred after a clinical remission with/without evidence of new organ injury), and ADAMTS-13 relapse in the follow-up period after bortezomib exposure, and 2) adverse reactions related to bortezomib, registered by the clinicians during the follow-up visits and graded according to the Common Terminology Criteria for Adverse Events version 5.0 [33].

2.3 | Statistical analysis

Categorical variables were expressed as counts and percentages, and continuous variables as medians and IQRs.

The differences in proportions and medians were evaluated with chi-square or Fisher's tests (where appropriate) and Mann-Whitney U-tests, respectively.

3 | RESULTS

3.1 | Baseline characteristics of patients at bortezomib treatment

Twenty-eight out of the 31 contacted centers replied to our survey, 15 out of 28 had not treated any patient with bortezomib, and the remaining 13 enrolled patients. Bortezomib was employed in 17 out of 392 (4.3%) consecutive iTTP patients. As shown in Table 1, 59% of enrolled subjects were female, with a median age of 43 years

TABLE 1 Baseline characteristics of patients with refractory immune-mediated thrombotic thrombocytopenic purpura at bortezomib administration.

Patient	Sex, age (y) ^a	Comorbidities ^a	No. of iTTP acute episodes prior to bortezomib ^a	Immunosuppressive treatments prior to bortezomib ^b
1	F, 53	Hashimoto's thyroiditis	1	RTX (A), IVIG (A)
2	M, 42	-	2	RTX (A)
3	M, 66	Ocular myasthenia gravis, arterial hypertension	2	RTX (A)
4	F, 65	Hashimoto's thyroiditis	1	RTX (A), CTX (A)
5	F, 30	-	1	RTX (A), CTX (A)
6	M, 52	-	2	RTX (A), CYA (P)
7	M, 34	-	1	RTX (A)
8	F, 61	Hashimoto's thyroiditis	2	RTX (A)
9	M, 27	Hyper IgE syndrome, Kallmann syndrome	7	RTX (A, P)
10	F, 38	Obesity, arterial hypertension, type 2 diabetes mellitus	7	RTX (A), MMF (P)
11	M, 77 ^a	Arterial hypertension ^a	2 ^a	RTX (A)
12	F, 26	Obesity	1	RTX (A, P), CTX (P)
13	F, 41	Obesity	3	RTX (A, P), CTX (P)
14	M, 43	Depression with anxious distress	6	RTX (A, P)
15	F, 24	Migraine	1	RTX (A, P), CYA (P), MMF (P)
16	F, 63	Hashimoto's thyroiditis	6	RTX (A, P), CYA (P), MMF (P), AZA (P)
17	F, 66	-	5	RTX (A), CYA (P), MMF (P), AZA (P)

AZA, azathioprine; CTX, cyclophosphamide; CYA, cyclosporine A; Ig, immunoglobulin; iTTP, immune-mediated thrombotic thrombocytopenic purpura; IVIG, intravenous immunoglobulins; MMF, mycophenolate mofetil; RTX, rituximab.

^a Age, comorbidities, and the number of iTTP acute episodes for patient 11 (who was treated with bortezomib on 2 separate occasions) are referred to the time of the first bortezomib administration.

^b A, drug used during an iTTP acute episode; P, drug used as a preemptive treatment.

(IQR, 32-64). Autoimmune comorbidities were present in 5 (29%) patients, mainly Hashimoto's thyroiditis. Patients had presented a median of 2 iTTP acute episodes (IQR, 1-5.5) before bortezomib exposure. Immunosuppressive treatments prior to bortezomib (Table 1) included rituximab for all and additional immunosuppressants for 10 subjects (59%): cyclophosphamide ($n = 4$), cyclosporine A ($n = 4$), mycophenolate mofetil ($n = 4$), azathioprine ($n = 2$), and intravenous immunoglobulins ($n = 1$).

3.2 | Efficacy of bortezomib

A total of 18 treatments with bortezomib were recorded in 17 patients; 11 received bortezomib during the acute phase, whereas 7 were during clinical remission (patient 11 was treated twice, once in the acute phase and once preemptively). Those treated in the acute phase had a shorter iTTP median duration at the time of bortezomib treatment (calculated as the date of bortezomib administration – date of iTTP diagnosis) than those treated preemptively (4 months; IQR,

1.8-6.2 vs 10.2 months; IQR, 5.2-16.5; $P = .04$). The treatment schedule was 1.3 mg/m² subcutaneously on days 1, 4, 8, and 11 (except for patient 13, treated on days 1, 8, and 15), repeated every 21 days when >1 cycle was administered. Nine treatments consisted of 1 cycle, 7 of 2 cycles, and 2 of 4 cycles, according to the clinician's indication and the patient's response.

The cumulative incidence of overall response was 59% (10/17 patients and 11/18 treatments): 9/11 treatments (82%) during the acute phase vs 2/7 treatments (28%) during clinical remission ($P = .05$). Responders had a lower median number of previous acute episodes (1 [IQR, 1-2] vs 5.5 [IQR, 2-7]; $P = .03$). This finding was also confirmed by analyzing responders vs nonresponders in acute and remission phases separately, although not statistically significant: 2 (IQR, 1-2) vs 4.5 (IQR, 3.25-5.75) in the acute treatment ($P = .2$); 1.5 (IQR, 1.25-1.75) vs 5 (IQR, 3-6) in the preemptive treatment ($P = .2$). No other associations with response were found among patients' baseline characteristics or treatment-related factors, in particular regarding the number of bortezomib cycles (7/9 responders, 78%, for 1 cycle vs 4/9 responders, 44%, for ≥ 2 cycles; $P = .33$) and the use of concomitant

TABLE 2 Outcomes of immune-mediated thrombotic thrombocytopenic purpura patients treated with bortezomib during/after the acute phase (ie, plasma exchange- and/or caplacizumab-treated patients).

Pt	Setting of treatment	Concomitant therapies (including IST administered <1 mo before)	Anti-ADAMTS-13 antibody titer prebortezomib	N of cycles	Response ^a	Time to response ^b	Anti-ADAMTS-13 antibody titer postbortezomib ^c	Duration of response (mo)	Toxicity (grade)	Relapse ^a	ADAMTS-13 activity at last follow-up
1	iTTP onset (3.5 mo after)	Caplacizumab, steroid, and RTX	13 U/mL	1	ADAMTS-13 CR	7 d	n.a.	24	-	ADAMTS-13 relapse responsive to steroid and RTX	46 IU/dL ^d
2	First relapse (2.3 mo after)	Caplacizumab, steroid, and RTX	2.2 BU/mL	1	NR	-	0.6 BU/mL	-	Paresthesia (1) Pneumonia (3)	Exacerbation 2 mo after bortezomib, responsive to CYA	60 IU/dL ^d
3	First relapse (1 mo after)	TPE, steroid, RTX, vincristine, CTX, and NAC	n.a.	1	Clinical response (with ADAMTS-13 CR)	1 mo	n.a.	38	Paresthesia (2), diarrhea (1)	2 ADAMTS-13 relapses, both responsive to RTX	58 IU/dL ^d
4	iTTP onset (4.5 mo after)	Caplacizumab	70 U/mL	4 ^e	ADAMTS-13 PR (20 IU/dL)	4 mo	18 U/mL	10, ongoing	Paresthesia (2), constipation (3)	No	30 IU/dL ^d
5	iTTP onset (15 d after)	TPE, steroid, RTX, CTX, and IVIG	1.6 BU/mL	1	Clinical response (ADAMTS-13 CR)	6 d	0.4 BU/mL	53, ongoing	-	No	90 IU/dL ^f
6	First relapse (5 mo after)	TPE every 2 d	>93 U/mL	4 ^g	Clinical response with reduction of TPE frequency (every 3 mo)	4 mo	<12 U/mL	50, ongoing	Neutropenia (2)	No	4 IU/dL ^f
7	iTTP onset (5.2 mo after)	Caplacizumab	702 U/mL	2	ADAMTS-13 PR (20.5 IU/dL)	4 mo	366 U/mL	8, ongoing	Paresthesia (1)	No	86 IU/dL ^f
8	First relapse (15 d after)	Caplacizumab, steroid, RTX, and CTX,	n.a.	1	ADAMTS-13 PR (44 IU/dL)	1 mo	n.a.	12	-	ADAMTS-13 relapse responsive to CYA	<0.2 IU/dL ^f
9	Sixth relapse (10 d after)	TPE and steroid	n.a.	2	NR	-	n.a.	-	-	No (treated with splenectomy and vincristine, retreated with steroid and RTX with response)	49 IU/dL ^d
10	Sixth relapse (15 d after)	Caplacizumab and steroid	35 U/mL	1	ADAMTS-13 PR (41 IU/dL)	17 d	<12 U/mL	1.5	-	ADAMTS-13 relapse, not treated	1 IU/dL ^d

TABLE 2 (Continued)

Pt	Setting of treatment	Concomitant therapies (including IST administered <1 mo before)	Anti-ADAMTS-13 antibody titer prebortezomib	N of cycles	Response ^a	Time to response ^b	Anti-ADAMTS-13 antibody titer postbortezomib ^c	Duration of response (mo)	Toxicity (grade)	Relapse ^a	ADAMTS-13 activity at last follow-up
11	First relapse (1.5 mo after)	TPE, steroid, and RTX	n.a.	1	Clinical response (with ADAMTS-13 PR, 28 IU/dL)	7 d	n.a.	24	-	ADAMTS-13 relapse treated with bortezomib (Table 3)	Table 3

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type 1 repeats member 13; CR, complete remission; CTX, cyclophosphamide; CYA, cyclosporine A; IST, immunosuppressive treatment; iTTP, immune-mediated thrombotic thrombocytopenic purpura; IVIG, intravenous immunoglobulins; n.a., not available; NAC, N-acetylcysteine; NR, no response; PR, partial remission; Pt, patient; RTX, rituximab; TPE, therapeutic plasma exchange.

^a Clinical response, ADAMTS-13 partial and complete remission, exacerbation, and clinical and ADAMTS-13 relapses were defined as per the International Working Group consensus report [27].

^b Counting from the date of the first dose of bortezomib.

^c Measured within 30 days after the completion of bortezomib treatment. When expressed in U/mL, anti-ADAMTS-13 antibodies were measured using the Technozym ADAMTS-13 inhibitor ELISA assay (Technoclone; negative values < 12 U/mL, borderline values: 12–15 U/mL, positive values > 15 U/mL, as per manufacturer's datasheet). When expressed in BU/mL, anti-ADAMTS-13 neutralizing antibodies were measured using a Bethesda-like assay (negative values < 0.4 BU/mL).

^d Measured with the Technozym ADAMTS-13 Activity ELISA assay (Technoclone; normal range, 40–130 IU/dL).

^e Bortezomib schedule reduced to 1 mg/m² on days 1 and 4 from cycle 4 due to grade 3 constipation.

^f Measured with the HemosIL AcuStar ADAMTS-13 Activity assay (Werfen; normal range, 67–129 IU/dL or 61–131 IU/dL, depending on the laboratory).

^g Bortezomib schedule reduced to 1.3 mg/m² on days 1 and 8 from cycle 3 due to grade 2 neutropenia.

immunosuppressive treatments (7/13 responders, 54%, with concomitant immunosuppression vs 3/4 responders, 75%, without; $P = .6$). Regarding the 11 patients treated in the acute phase (Table 2), 6 received caplacizumab (for >4 months in patients 4 and 7), and 5 TPE. Among the caplacizumab-treated patients, we registered 5/6 ADAMTS-13 remissions (4 partial and 1 complete remission), allowing a permanent discontinuation of caplacizumab. Specifically for patient 7 (Figure), 2 attempts of caplacizumab discontinuation before bortezomib, while ADAMTS-13 activity levels were still undetectable, resulted in 2 exacerbations. Bortezomib allowed a rapid and progressive decline of anti-ADAMTS-13 antibody titer, followed by ADAMTS-13 response (and caplacizumab discontinuation) 4 months after the first dose. Among the TPE-treated patients, we recorded 4/5 clinical responses, which consisted of 2 ADAMTS-13 complete remissions, 1 ADAMTS-13 partial remission, and 1 clinical response without ADAMTS-13 response but with a significant reduction of TPE frequency to 1 every 3 months (patient 6). Among the 9 responders, 6 were receiving concomitant immunosuppressants (steroid, vincristine, and/or cyclophosphamide) or had been recently (<1 month) treated with rituximab; all discontinued the concomitant medications. The median time to response in the acute phase was 30 days (IQR, 7–120); 4 patients responded in <30 days, 2 at day 30, and 3 after 4 months. Of note, patients 7 and 11 achieved ADAMTS-13 complete remission 1 month after their partial remission.

Regarding the 7 treatments during clinical remission (Table 3), 2 out of 4 patients treated for ADAMTS-13 relapse obtained ADAMTS-13 partial remission, while no responses were observed among the 3 patients treated in the case of persistently low ADAMTS-13 activity. However, patient 15, a 24-year-old girl who had not achieved any ADAMTS-13 response after clinical remission, notwithstanding immunosuppression with rituximab, cyclosporine A, and mycophenolate mofetil, experienced a progressive reduction until disappearance of anti-ADAMTS-13 antibodies after bortezomib. Eventually, her ADAMTS-13 activity was detectable for the first time at month +11 after treatment (0.4%, the last value available at the time of data cutoff). The median time to response for the treatments during remission was 31 days (range, 24–38). Complete ADAMTS-13 remission was achieved 3 months after partial remission in patient 11 and 1 month after partial remission in patient 12.

Patients were followed up for a median of 21 months (IQR, 7.2–35) after bortezomib treatment, with a longer median follow-up for responders (24 months; IQR, 10–45) than nonresponders (14 months; IQR, 7.5–22), $P = .2$. The median duration of ADAMTS-13 response was 22 months (IQR, 10–38), and 6 subjects were still responding at the time of data cutoff. Among the 10 responders, no clinical iTTP relapses occurred, while the cumulative incidence of ADAMTS-13 relapse was 5/10 patients. Patient 11 was successfully retreated with bortezomib. Among the 7 nonresponders, the incidence rate of clinical relapse was 3/6.9 patient-years. Notably, ADAMTS-13 relapsed in patients 1 and 3, and clinical relapse in patient 14 responded to rituximab, which was ineffective before bortezomib administration.

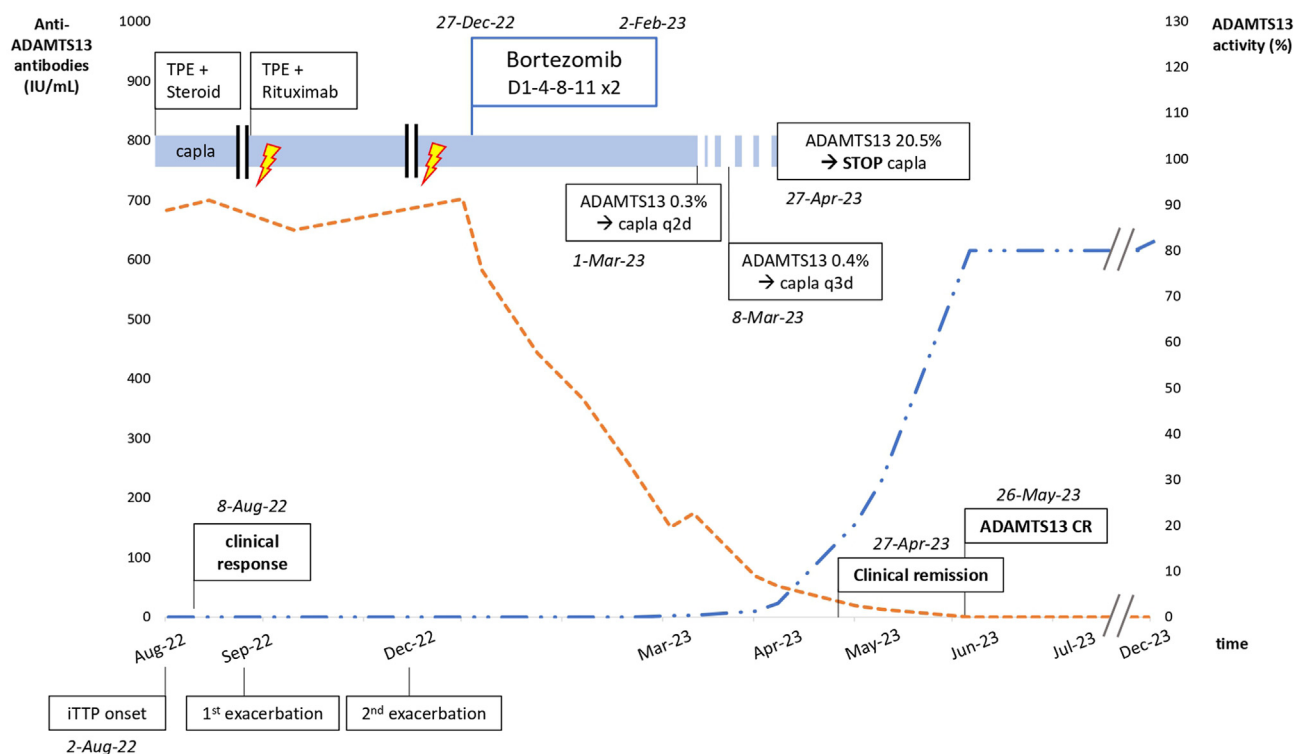


FIGURE Clinical history and laboratory values of patient 7. ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type 1 repeats member 13; capla, caplacizumab; CR, complete remission; iTTP, immune-mediated thrombotic thrombocytopenic purpura; q2d, every 2 days; q3d, every 3 days; TPE, therapeutic plasma exchange. Parallel vertical lines = period of capla discontinuation; yellow flash = iTTP exacerbation; dashed orange line = anti-ADAMTS-13 antibody titer; dotted-dashed blue line = ADAMTS-13 activity levels.

Anti-ADAMTS-13 antibody titer before and after treatment was available in 12 patients (Tables 2 and 3). All samples were collected within 30 days from the completion of bortezomib treatment. The specific antibody became negative in 3 out of 6 responders, decreased by 4 times in patients 4 and 5, and by 2 times in patient 7. In nonresponders, the titer decreased by 4 times in patient 2, by 2 times in patient 15, by 1.5 times in patient 17, remained unchanged in patients 13 and 16, and became positive in patient 14. Four patients had anti-ADAMTS-13 antibody tested at further time points; patients 4, 7, and 15 became negative at months 6, 3, and 12, respectively, while the titer of patient 17 decreased by 3 times from baseline at month 5.

3.3 | Safety of bortezomib

Eight subjects (47%) reported bortezomib-related adverse events, none requiring drug discontinuation. Six out of 9 patients receiving ≥ 2 cycles (67%) experienced an adverse event, while only 2 out of 9 (22%) with 1 cycle reported toxicity ($P = .15$). Paresthesia was the most common ($n = 6$, grade 1 in 2 patients and grade 2 in 4, lasting about 6 months in patient 17), followed by constipation ($n = 1$, grade 3, requiring dose reduction), diarrhea ($n = 1$, grade 1), neutropenia ($n = 1$, grade 2, leading to drug schedule reduction), and headache ($n = 1$, grade 2). Five patients received no antimicrobial prophylaxis during

bortezomib treatment, 2 received daily acyclovir 400 mg every 12 hours, and the remaining acyclovir + trimethoprim-sulfamethoxazole 160 to 800 mg 3 times a week. Patient 2 (receiving acyclovir) reported a grade 3 *Pneumocystis jirovecii* pneumonia 2 months after the end of bortezomib treatment and during concomitant therapy with rituximab and high-dose corticosteroids.

4 | DISCUSSION

In the present study, we showed a response rate of nearly 60% to bortezomib in rituximab-refractory and often multitreated iTTP patients, especially when employed in the acute phase. Responses occurred after a median of 30 days and were sustained for 2 years. Toxicity was reported in half of patients but never led to drug discontinuation. The pathogenesis of rituximab-refractoriness in iTTP is poorly investigated, accounting for a minority of difficult-to-treat subjects. One possible explanation is that rituximab-induced B cell depletion favors the emergence of CD20-negative long-lived plasma cells (LLPCs) that are resistant to anti-CD20 targeted therapies [34]. In immune thrombocytopenia, LLCs were found in the spleens of rituximab-refractory patients, who eventually responded to splenectomy [35]. In fact, splenectomy was successfully employed in the past for severe refractory iTTP patients, some of whom were not responsive to rituximab [36,37]. In antineutrophil cytoplasmic antibody-

TABLE 3 Outcomes of immune-mediated thrombotic thrombocytopenic purpura patients treated with bortezomib during clinical remission.

Pt	Setting of treatment	Concomitant therapies (including IST administered <1 mo before)	Anti-ADAMTS-13 antibody titer prebortezomib	N of cycles	Response ^a	Time to response ^b	Anti-ADAMTS-13 antibody titer postbortezomib ^c	Duration of response (mo)	Toxicity (grade)	Relapse ^a	ADAMTS-13 activity at last follow-up
11	ADAMTS-13 relapse	-	n.a.	1	ADAMTS-13 PR (28 IU/dL)	24 d	n.a.	20, ongoing	-	No	67% ^f
12	ADAMTS-13 relapse	CTX started 5 mo earlier (stopped at bortezomib start)	98 U/mL	2	ADAMTS-13 PR (20 IU/dL)	38 d	<12 U/mL	22, ongoing	-	No	54 IU/dL ^d
13	ADAMTS-13 relapse	CTX started 7 mo earlier (stopped at bortezomib start)	112 U/mL	2 ^g	NR	-	93 U/mL	-	Paresthesia (2)	No (preemptive daratumumab treatment 16 mo after bortezomib with PR)	6 IU/dL ^d
14	ADAMTS-13 relapse	Steroid	<0.4 BU/mL	1	NR	-	1 BU/mL	-	-	Clinical relapse 5 mo after bortezomib, responsive to plasma infusion, steroid, and RTX	89 IU/dL ^d
15	ADAMTS-13 persistently <10 IU/dL	MMF started 4 mo earlier (stopped at bortezomib start)	273 U/mL	2	NR	-	116 U/mL	-	Headache (2)	No	0.4 IU/dL ^e
16	ADAMTS-13 persistently <10 IU/dL	-	454 U/mL	2	NR	-	462 U/mL	-	-	Clinical relapse 1.5 mo after bortezomib with clinical response to steroid + caplacizumab, CYA started 1 mo later	<0.2 IU/dL ^e

(Continues)

TABLE 3 (Continued)

Pt	Setting of treatment	Concomitant therapies (including IST administered <1 mo before)	Anti-ADAMTS-13 antibody titer prebortezomib	N of cycles	Response ^a	Time to response ^b	Anti-ADAMTS-13 antibody titer postbortezomib ^c	Duration of response (mo)	Toxicity (grade)	Relapse ^a	ADAMTS-13 activity at last follow-up
17	ADAMTS-13 persistently <10 IU/dL	MMF started 3 mo earlier (stopped at bortezomib start)	145 U/mL	2	NR	-	91 U/mL	-	Paresthesia (2)	No	<0.2 IU/dL ^d

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type 1 repeats member 13; CTX, cyclophosphamide; CYA, cyclosporine A; IST, immunosuppressive treatment; MMF, mycophenolate mofetil; n.a., not available; NR, no response; PR, partial remission; Pt, patient; RTX, rituximab.

^a Clinical response, ADAMTS-13 partial and complete remission, exacerbation, and clinical and ADAMTS-13 relapses were defined as per the International Working Group consensus report [27].

^b Counting from the date of the first dose of bortezomib.

^c Measured within 30 days after the completion of bortezomib treatment. When expressed in U/mL, anti-ADAMTS-13 antibodies were measured using the Technozym ADAMTS-13 inhibitor ELISA assay (Technoclone; negative values < 12 U/mL, borderline values: 12–15 U/mL, positive values > 15 U/mL, as per manufacturer's datasheet). When expressed in BU/mL, anti-ADAMTS-13 neutralizing antibodies were measured using a Bethesda-like assay (negative values < 0.4 BU/mL).

^d Measured with the Technozym ADAMTS-13 Activity ELISA assay (Technoclone; normal range, 40–130 IU/dL).

^e Measured with the HemosIL AcuStar ADAMTS-13 Activity assay (Werfen; normal range, 67–129 IU/dL or 61–131 IU/dL, depending on the laboratory).

^f Measured with the FRETs-VWF73 assay [31] (normal range, 45%–147%).

^g Bortezomib schedule was 1.3 mg/m² on days 1, 8, and 15.

associated vasculitis, an increased risk of relapse was associated with the presence of circulating CD27+CD38+ LLPCs during disease remission [38]. Bortezomib represents the first plasma cell-directed treatment employed in autoantibody-mediated diseases [24,39]. By inhibiting the ubiquitin-proteasome pathway, it leads to protein engulfment and death of the pathogenic plasma cell, which may be responsible for persistent anti-ADAMTS-13 antibody production in rituximab-refractory iTTP. Bortezomib has been employed in refractory iTTP for 10 years, with good efficacy and an acceptable toxicity profile, as recently reviewed elsewhere [28,30]. The response rate to bortezomib in our study (59%) is slightly lower than what was previously reported (72% overall) [30]. However, most of the published articles are case reports, which may be subject to reporting bias. Moreover, we observed better responses when bortezomib was administered in the acute phase of disease onset or at first relapse. Therefore, the response rate we registered in the acute phase (82%) is consistent with that stemming from previous reports, where bortezomib was employed mostly at iTTP onset [28–30]. In this scenario, even though we did not find significantly different response rates between patients with/without concomitant immunosuppression, the latter could exert a confounding effect on the response. In particular, the increase of ADAMTS-13 activity even >3 months after rituximab has been reported [15], so delayed responses to anti-CD20 therapy for patients receiving bortezomib soon after cannot be excluded. In this regard, we may hypothesize a synergistic effect between bortezomib and rituximab. The association of the 2 drugs is successfully employed in mantle cell lymphoma [40,41], Waldenström's macroglobulinemia [42], and autoimmune hemolytic anemia [43]. Recently, a phase 2 prospective study exploring rituximab + bortezomib in acquired hemophilia A showed durable responses in 96% of patients [44]. Targeting both CD20-positive and CD20-negative cells at iTTP onset or first relapse may avoid the selection of resistant clones, which are responsible for further relapses. Notably, we observed that 3 rituximab-refractory patients (1, 3, and 14) became responsive to rituximab after the administration of bortezomib. We may speculate that bortezomib exerted a rituximab-sensitizing effect by eradicating LLPCs and reducing the autoimmune burden of the disease.

In the present study, we found lower response rates during clinical remission. The reasons why bortezomib was less effective when administered as a preemptive treatment are poorly understood. These patients had a longer history of iTTP at bortezomib administration and might have a more refractory disease requiring a longer time to respond. In the recent report by Lee et al. [30], bortezomib was effective in 3/5 ADAMTS-13 relapses, one of which after 7 months from treatment. Likewise, a significant decline of anti-ADAMTS-13 antibody titer was registered in some of our nonresponding patients, and the occurrence of responses 4 months after treatment, also in the acute phase (patients 4, 6, and 7), suggests that bortezomib can take a longer time than rituximab to work [13].

The long duration of ADAMTS-13 response to bortezomib (median, 22 months) appears impressive for the refractory setting represented here. Other reports showed responses lasting more than 5 years [26,45]. Repeating rituximab in previous responders is a

common strategy to treat ADAMTS-13 relapses without any decrease in relapse-free survival [16]. Given a median duration of response to rituximab of 17.5 months [46], patients are often retreated every 1.5 to 2 years, but long-term consequences of rituximab-related hypogammaglobulinemia and infections are of some concern, and almost unknown in iTTP [47]. Therefore, bortezomib might be considered in rituximab-dependent patients for its long-lasting effect, weighing the advantages of reducing the burden of repeated rituximab cycles with the seemingly lower response rates to bortezomib in the preemptive setting, as found in this study.

Regarding safety, bortezomib was associated with moderate adverse reactions in 47% of our patients, so more frequently than what was reported in the literature (5/36, 14% overall) [30]. Concomitant immunosuppressive treatments in 2 patients may have contributed to the toxicity, especially in the case of *P jirovecii* pneumonia. Peripheral neuropathy was the most common, while we did not register lung or cardiac toxicities [29,30,48,49]. No new adverse events were registered than those already known for the multiple myeloma population, where the most common toxicities are hematologic (thrombocytopenia, neutropenia, and/or anemia in about one-third of patients), fatigue, nausea, diarrhea, and peripheral neuropathy (15% of cases), often requiring drug reduction or discontinuation [50,51]. Considering the young age of our patients, this signal of toxicity deserves attention. We recorded fewer adverse events in patients treated with 1 cycle than those receiving ≥ 2 cycles without affecting bortezomib effectiveness. This finding confirms previous data in the literature, where reduced doses (eg, 1 mg/m²) and a single cycle of bortezomib have been employed in iTTP with good efficacy [28–30].

More than half of the patients in our study were receiving caplacizumab at the time of bortezomib treatment, representing a new clinical scenario. In fact, only 3 out of the 36 bortezomib-treated patients in the literature received caplacizumab, meaning that most of them received bortezomib because of disease refractoriness. Refractory iTTP is defined by the inability to achieve a clinical response after 5 daily TPE or a fall in platelet count after an initial improvement [52]. With the advent of caplacizumab, this situation has significantly improved [9,11,12,53]. Consequently, the current refractory patients are mostly those who attain clinical response with TPE + caplacizumab but are unable to obtain any ADAMTS-13 recovery with standard immunosuppression, including rituximab. In this scenario, the most represented in our study, caplacizumab discontinuation is a clinically significant goal, also considering the high cost of the drug and the lack of safety data in case of prolonged caplacizumab exposure [54], especially in elderly patients and those requiring antiplatelet/anticoagulant treatments. Thanks to its rapid efficacy, caplacizumab leads to a significant reduction of TPE duration [8,9,11,55], and TPE-free approaches are gaining attention in clinical practice [56,57]. However, a trend to a delayed normalization of ADAMTS-13 activity has been reported in the caplacizumab era, maybe due to reduced employment of TPE procedures [58,59]. ADAMTS-13 remission represents an important goal in iTTP treatment, not only to discontinue caplacizumab but mainly to reduce the risk of clinical relapse. Increasing

evidence supports an association between low ADAMTS-13 levels and a higher risk of ischemic stroke in iTTP patients during remission, as well as in the general population [60,61]. Given this scenario, the need for plasma cell-directed treatments in rituximab-refractory patients may become more and more significant in the near future.

The present study has limitations. The retrospective design of the study and the limited sample size, although the largest reported so far, should be mentioned. Nonetheless, our data suggest that bortezomib can be a valuable and generally safe option in rituximab-refractory iTTP, even when other traditional immunosuppressants have failed. It could be considered for refractory patients who do not have access to caplacizumab (eg, in low-resource settings) to obtain a clinical response or are “caplacizumab-dependent” because of a lack of ADAMTS-13 recovery. The latter scenario may be more and more common in high-resource healthcare systems, where a game-changing but costly drug like caplacizumab requires some pharmacoeconomic evaluations. Late-onset responses (ie, after 4 months) are possible, and treatment with 2 or more cycles was associated with higher toxicity without improving response. For these reasons, a “watchful waiting” approach may be considered after 1 cycle to avoid overtreatment and drug-related adverse events, especially if a declining trend of anti-ADAMTS-13 antibody titer is observed. Randomized trials on rituximab ± bortezomib, along with biological studies, would allow the identification of patients who could benefit from this association. Prospective controlled studies with bortezomib alone or in combination with less toxic anti-CD38 monoclonal antibodies, recently reported in iTTP [62–64], are warranted to establish the real contribution of this therapeutic strategy in refractory iTTP.

ACKNOWLEDGMENTS

The study was partially supported by the Italian Ministry of Health – Bando Ricerca Corrente. The Hemostasis & Thrombosis Unit of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico is a member of the European Reference Network on Rare Haematological Diseases EuroBloodNet – project ID number 101157011. European Reference Network-EuroBloodNet is partly cofunded by the European Union within the framework of the Fourth EU Health Programme.

AUTHOR CONTRIBUTIONS

J.A.G. and I.M. designed the study. All authors collected data. J.A.G. analyzed the data and wrote the first draft of the manuscript. All authors critically revised the manuscript and agreed with the final version.

DECLARATION OF COMPETING INTERESTS

A.A., P.A., M.C., and B.F. received honoraria for participating as a speaker at educational meetings organized by Sanofi; I.M. received honoraria for participating as a speaker at educational meetings organized by Instrumentation Laboratory and Sanofi; M.B. received conference fees from Novartis, Incyte, and AbbVie; M.N. acted as a consultant for Bayer, CSL Behring, and Novo Nordisk and received

speaker fees from Bayer, Takeda, Sobi, Novo Nordisk, CSL Behring, Sanofi, Amgen, Novartis, and Grifols; F.P. has received honoraria for participating as a speaker in education meetings organized by Sanofi, Spark, and Takeda and she is member of scientific advisory boards of CSL Behring, BioMarin, Roche, Sanofi, and Sobi. The other authors do not have any conflict of interest to disclose.

ORCID

Juri Alessandro Giannotta  <https://orcid.org/0000-0001-9349-1627>

Flora Peyvandi  <https://orcid.org/0000-0001-7423-9864>

REFERENCES

- [1] Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem*. 2001;276:41059–63.
- [2] Sukumar S, Lämmle B, Cataland SR. Thrombotic thrombocytopenic purpura: pathophysiology, diagnosis, and management. *J Clin Med*. 2021;10:536. <https://doi.org/10.3390/jcm10030536>
- [3] Scully M, Yarranton H, Liesner R, Cavenagh J, Hunt B, Benjamin S, Bevan D, Mackie I, Machin S. Regional UK TTP registry: correlation with laboratory ADAMTS 13 analysis and clinical features. *Br J Haematol*. 2008;142:819–26.
- [4] Lotta LA, Mariani M, Consonni D, Mancini I, Palla R, Maino A, Vucelic D, Pizzuti M, Mannucci PM, Peyvandi F. Different clinical severity of first episodes and recurrences of thrombotic thrombocytopenic purpura. *Br J Haematol*. 2010;151:488–94.
- [5] Deford CC, Reese JA, Schwartz LH, Perdue JJ, Kremer Hovinga JA, Lämmle B, Terrell DR, Vesely SK, George JN. Multiple major morbidities and increased mortality during long-term follow-up after recovery from thrombotic thrombocytopenic purpura. *Blood*. 2013;122:2023–9.
- [6] Riva S, Mancini I, Maino A, Ferrari B, Artoni A, Agosti P, Peyvandi F. Long-term neuropsychological sequelae, emotional wellbeing and quality of life in patients with acquired thrombotic thrombocytopenic purpura. *Haematologica*. 2020;105:1957–62.
- [7] Sukumar S, Brodsky M, Hussain S, Yanek L, Moliterno A, Brodsky R, Cataland SR, Chaturvedi S. Cardiovascular disease is a leading cause of mortality among TTP survivors in clinical remission. *Blood Adv*. 2022;6:1264–70.
- [8] Peyvandi F, Scully M, Kremer Hovinga JA, Cataland S, Knöbl P, Wu H, Artoni A, Westwood JP, Mansouri Taleghani M, Jilma B, Callewaert F, Ulrichs H, Duby C, Tersago D, Investigators TITAN. Caplacizumab for acquired thrombotic thrombocytopenic purpura. *N Engl J Med*. 2016;374:511–22.
- [9] Scully M, Cataland SR, Peyvandi F, Coppo P, Knöbl P, Kremer Hovinga JA, Metjian A, de la Rubia J, Pavenski K, Callewaert F, Biswas D, De Winter H, Zeldin RK, HERCULES Investigators. Caplacizumab treatment for acquired thrombotic thrombocytopenic purpura. *N Engl J Med*. 2019;380:335–46.
- [10] Zheng XL, Vesely SK, Cataland SR, Coppo P, Geldziler B, Iorio A, Matsumoto M, Mustafa RA, Pai M, Rock G, Russell L, Tarawneh R, Valdes J, Peyvandi F. ISTH guidelines for treatment of thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2020;18:2496–502.
- [11] Dutt T, Shaw RJ, Stubbs M, Yong J, Bailiff B, Cranfield T, Crowley MP, Desborough M, Eyre TA, Gooding R, Grainger J, Hanley J, Haughton J, Hermans J, Hill Q, Humphrey L, Lowe G, Lyall H, Mohsin M, Nicolson PLR, et al. Real-world experience with caplacizumab in the management of acute TTP. *Blood*. 2021;137:1731–40.
- [12] Coppo P, Bubenheim M, Azoulay E, Galicier L, Malot S, Bigé N, Poullin P, Provôt F, Martis N, Presne C, Moranne O, Benainou R, Dossier A, Seguin A, Hié M, Wynckel A, Delmas Y, Augusto JF, Perez P, Rieu V, et al. A regimen with caplacizumab, immunosuppression, and plasma exchange prevents unfavorable outcomes in immune-mediated TTP. *Blood*. 2021;137:733–42.
- [13] Scully M, McDonald V, Cavenagh J, Hunt BJ, Longair I, Cohen H, Machin SJ. A phase 2 study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. *Blood*. 2011;118:1746–53.
- [14] Westwood JP, Webster H, McGuckin S, McDonald V, Machin SJ, Scully M. Rituximab for thrombotic thrombocytopenic purpura: benefit of early administration during acute episodes and use of prophylaxis to prevent relapse. *J Thromb Haemost*. 2013;11:481–90.
- [15] Hie M, Gay J, Galicier L, Provôt F, Presne C, Poullin P, Bonmarchand G, Wynckel A, Benhamou Y, Vanhille P, Servais A, Bordessoule D, Coindre JP, Hamidou M, Vernant JP, Veyradier A, Coppo P, French Thrombotic Microangiopathies Reference Centre. Preemptive rituximab infusions after remission efficiently prevent relapses in acquired thrombotic thrombocytopenic purpura. *Blood*. 2014;124:204–10.
- [16] Westwood JP, Scully M. Management of acquired, immune thrombocytopenic purpura (iTTP): beyond the acute phase. *Ther Adv Hematol*. 2022;13:20406207221112217. <https://doi.org/10.1177/20406207221112217>
- [17] Barba C, Peyre M, Galicier L, Cathebras P, Ranta D, Malot S, Veyradier A, Coppo P. Intensive rituximab regimen in immune-mediated thrombotic thrombocytopenic purpura can circumvent unresponsiveness to standard rituximab treatment. *Br J Haematol*. 2021;192:e21–5. <https://doi.org/10.1111/bjh.17170>
- [18] Cataland SR, Jin M, Lin S, Kraut EH, George JN, Wu HM. Effect of prophylactic cyclosporine therapy on ADAMTS13 biomarkers in patients with idiopathic thrombotic thrombocytopenic purpura. *Am J Hematol*. 2008;83:911–5.
- [19] Changcharoen B, Bolger DT Jr. Thrombotic thrombocytopenic purpura as an initial presentation of systemic lupus erythematosus with acquired ADAMTS 13 antibody. *BMJ Case Rep*. 2015;2015:bcr2014208477. <https://doi.org/10.1136/bcr-2014-208477>
- [20] Cataland SR, Kourlas PJ, Yang S, Geyer S, Witkoff L, Wu H, Masias C, George JN, Wu HM. Cyclosporine or steroids as an adjunct to plasma exchange in the treatment of immune-mediated thrombotic thrombocytopenic purpura. *Blood Adv*. 2017;1:2075–82.
- [21] Bichard C, Mancini I, Agosti P, Capecchi M, De Leo P, Arcudi S, Ferrari B, Trisolini SM, Longu F, Fozza C, Artoni A, Peyvandi F. Efficacy and safety of azathioprine during remission of immune-mediated thrombotic thrombocytopenic purpura. *Blood Adv*. 2022;6:5463–6.
- [22] Kane RC, Bross PF, Farrell AT, Pazdur R. Velcade: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. *Oncologist*. 2003;8:508–13.
- [23] Neubert K, Meister S, Moser K, Weisel F, Maseda D, Amann K, Wiethe C, Winkler TH, Kalden JR, Manz RA, Voll RE. The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. *Nat Med*. 2008;14:748–55.
- [24] Pasquale R, Giannotta JA, Barcellini W, Fattizzo B. Bortezomib in autoimmune hemolytic anemia and beyond. *Ther Adv Hematol*. 2021;12:20406207211046428. <https://doi.org/10.1177/20406207211046428>
- [25] Shortt J, Oh DH, Opat SS. ADAMTS13 antibody depletion by bortezomib in thrombotic thrombocytopenic purpura. *N Engl J Med*. 2013;68:90–2.
- [26] Maschan A, Petrova U, Kalinina I, Kurnikova E, Fedorova D, Baidildina D, Kotskaya N, Avdonin P, Novichkova G. Bortezomib induces long-term remission in children with immune thrombotic thrombocytopenic purpura, refractory to plasma exchange,

- glucocorticoids, and rituximab: a report on two cases. *Pediatr Blood Cancer*. 2021;68:e28818. <https://doi.org/10.1002/pbc.28818>
- [27] Azapagasi E, Uysal Yazici M, Eroglu N, Albayrak M, Kucur Ö, Fettah A. Successful treatment with bortezomib for refractory and complicated acquired thrombotic thrombocytopenic purpura in an adolescent girl. *J Pediatr Hematol Oncol*. 2021;43:e587–91. <https://doi.org/10.1097/MPH.0000000000002026>
 - [28] Chen M, Shortt J. Plasma cell directed therapy for immune thrombotic thrombocytopenic purpura (iTTP). *Transfus Med Rev*. 2022;36:204–14.
 - [29] Patriquin CJ, Thomas MR, Dutt T, McGuckin S, Blombery PA, Cranfield T, Westwood JP, Scully M. Bortezomib in the treatment of refractory thrombotic thrombocytopenic purpura. *Br J Haematol*. 2016;173:779–85.
 - [30] Lee NCJ, Yates S, Rambally S, Sarode R, Ibrahim IF, Shen YM, Hofmann SL, Bavli NR. Bortezomib in relapsed/refractory immune thrombotic thrombocytopenic purpura: a single-centre retrospective cohort and systematic literature review. *Br J Haematol*. 2024;204:638–43.
 - [31] Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol*. 2005;129:93–100.
 - [32] Cuker A, Cataland SR, Coppo P, de la Rubia J, Friedman KD, George JN, Knoebl PN, Kremer Hovinga JA, Lämmle B, Matsumoto M, Pavenski K, Peyvandi F, Sakai K, Sarode R, Thomas MR, Tomiyama Y, Veyradier A, Westwood JP, Scully M. Redefining outcomes in immune TTP: an international working group consensus report. *Blood*. 2021;137:1855–61.
 - [33] National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). <https://ctep.cancer.gov/>. [accessed May 3, 2023].
 - [34] Mahévas M, Michel M, Weill JC, Reynaud CA. Long-lived plasma cells in autoimmunity: lessons from B-cell depleting therapy. *Front Immunol*. 2013;4:494. <https://doi.org/10.3389/fimmu.2013.00494>
 - [35] Mahévas M, Patin P, Huetz F, Descatoire M, Cagnard N, Bole-Feyssot C, Le Gallou S, Khellaf M, Fain O, Boutboul D, Galicier L, Ebbo M, Lambotte O, Hamidou M, Bierling P, Godeau B, Michel M, Weill JC, Reynaud CA. B cell depletion in immune thrombocytopenia reveals splenic long-lived plasma cells. *J Clin Invest*. 2013;123:432–42.
 - [36] Kappers-Klunne MC, Wijermans P, Fijnheer R, Croockewit AJ, van der Holt B, de Wolf JT, Löwenberg B, Brand A. Splenectomy for the treatment of thrombotic thrombocytopenic purpura. *Br J Haematol*. 2005;130:768–76.
 - [37] Dubois L, Gray DK. Case series: splenectomy: does it still play a role in the management of thrombotic thrombocytopenic purpura. *Can J Surg*. 2010;53:349–55.
 - [38] Treppo E, Binutti M, Agarinis R, De Vita S, Quartuccio L. Rituximab induction and maintenance in ANCA-associated vasculitis: state of the art and future perspectives. *J Clin Med*. 2021;10:3773. <https://doi.org/10.3390/jcm10173773>
 - [39] Khalesi N, Korani S, Korani M, Johnston TP, Sahebkar A. Bortezomib: a proteasome inhibitor for the treatment of autoimmune diseases. *Inflammopharmacology*. 2021;29:1291–306.
 - [40] Lamm W, Kaufmann H, Raderer M, Hoffmann M, Chott A, Zielinski C, Drach J. Bortezomib combined with rituximab and dexamethasone is an active regimen for patients with relapsed and chemotherapy-refractory mantle cell lymphoma. *Haematologica*. 2011;96:1008–14.
 - [41] Robak T, Jin J, Pylypenko H, Verhoef G, Siritanaratkul N, Drach J, Raderer M, Mayer J, Pereira J, Tumyan G, Okamoto R, Nakahara S, Hu P, Appiani C, Nemat S, Cavalli F. LYM-3002 investigators. Frontline bortezomib, rituximab, cyclophosphamide, doxorubicin, and prednisone (VR-CAP) versus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in transplantation-ineligible patients with newly diagnosed mantle cell lymphoma: final overall survival results of a randomised, open-label, phase 3 study. *Lancet Oncol*. 2018;19:1449–58.
 - [42] Buske C, Dimopoulos MA, Grunenberg A, Kastritis E, Tomowiak C, Mahé B, Troussard X, Hajek R, Viardot A, Tournilhac O, Aurran T, Lepretre S, Zerazhi H, Hivert B, Leblond V, de Guibert S, Brandefors L, Garcia-Sanz R, Gomes da Silva M, Kimby E, et al. Bortezomib-dexamethasone, rituximab, and cyclophosphamide as first-line treatment for Waldenström's macroglobulinemia: a prospectively randomized trial of the European Consortium for Waldenström's Macroglobulinemia. *J Clin Oncol*. 2023;41:2607–16.
 - [43] Yao M, Zhang J, Li Y, Lv L, Jia L, Yang C, Huang Y, Liu H, Wang J, Chen M, Zhang H. Combination of low-dose rituximab, bortezomib, and dexamethasone for the treatment of autoimmune hemolytic anemia. *Medicine (Baltimore)*. 2022;101:e28679. <https://doi.org/10.1097/MD.00000000000028679>
 - [44] Cai H, Cao X, Wang S, Zhao Y, Li J, Zhu TN. Rituximab and bortezomib for patients with newly diagnosed acquired haemophilia: single arm, single center, prospective phase 2 study. *Blood*. 2022;140:2715–6.
 - [45] Ratnasingam S, Walker PA, Tran H, Kaplan ZS, McFadyen JD, Tran H, Teh TC, Fleming S, Catalano JV, Chunilal SD, Johnston A, Opat SS, Shortt J. Bortezomib-based antibody depletion for refractory autoimmune hematological diseases. *Blood Adv*. 2016;1:31–5.
 - [46] Westwood JP, Thomas M, Alwan F, McDonald V, Benjamin S, Lester WA, Lowe GC, Dutt T, Hill QA, Scully M. Rituximab prophylaxis to prevent thrombotic thrombocytopenic purpura relapse: outcome and evaluation of dosing regimens. *Blood Adv*. 2017;1:1159–66.
 - [47] Tieu J, Smith RM, Gopaluni S, Kumararatne DS, McClure M, Manson A, Houghton S, Jayne DRW. Rituximab associated hypogammaglobulinemia in autoimmune disease. *Front Immunol*. 2021;12:671503. <https://doi.org/10.3389/fimmu.2021.671503>
 - [48] Yates S, Matevosyan K, Rutherford C, Shen YM, Sarode R. Bortezomib for chronic relapsing thrombotic thrombocytopenic purpura: a case report. *Transfusion*. 2014;54:2064–7.
 - [49] Acedillo RR, Govind M, Kashgary A, Clark WF. Treatment of severe, refractory and rapidly evolving thrombotic thrombocytopenic purpura. *BMJ Case Rep*. 2016;2016:bcr2016215491. <https://doi.org/10.1136/bcr-2016-215491>
 - [50] Pancheri E, Guglielmi V, Wilczynski GM, Malatesta M, Tonin P, Tomelleri G, Nowis D, Vattemi G. Non-hematologic toxicity of bortezomib in multiple myeloma: the neuromuscular and cardiovascular adverse effects. *Cancers (Basel)*. 2020;12:2540. <https://doi.org/10.3390/cancers12092540>
 - [51] Li T, Timmins HC, King T, Kiernan MC, Goldstein D, Park SB. Characteristics and risk factors of bortezomib induced peripheral neuropathy: a systematic review of phase III trials. *Hematol Oncol*. 2020;38:229–43.
 - [52] Scully M, Cataland S, Coppo P, de la Rubia J, Friedman KD, Kremer Hovinga J, Lämmle B, Matsumoto M, Pavenski K, Sadler E, Sarode R, Wu H, International Working Group for Thrombotic Thrombocytopenic Purpura. Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. *J Thromb Haemost*. 2017;15:312–22.
 - [53] Izquierdo CP, Mingot-Castellano ME, Fuentes AEK, García-Arroba Peinado J, Cid J, Jimenez MM, Valcarcel D, Gómez-Seguí I, de la Rubia J, Martin P, Goterris R, Hernández L, Tallón I, Varea S, Fernández M, García-Muñoz N, Vara M, Zarzoso MF, García-Candel F, Paciello ML, et al. Real-world effectiveness of caplacizumab vs the standard of care in immune thrombotic thrombocytopenic purpura. *Blood Adv*. 2022;6:6219–27.
 - [54] Rodgers GM, Berndt M, Fonteno M, Gilreath JA. Prolonged exposure to caplacizumab as rescue therapy in refractory immune

- thrombotic thrombocytopenic purpura. *J Blood Med.* 2023;14:209–11.
- [55] Agosti P, De Leo P, Capecchi M, Ferrari B, Mancini I, Gattillo S, Trisolini SM, Rinaldi E, Podda GM, Prezioso L, Salutari P, Facchini L, Caramazza D, Tolomelli G, Artoni A, Peyvandi F. Caplacizumab use for immune thrombotic thrombocytopenic purpura: the Milan thrombotic thrombocytopenic purpura registry. *Res Pract Thromb Haemost.* 2023;7:102185. <https://doi.org/10.1016/j.rpth.2023.102185>
- [56] Völker LA, Brinkkoetter PT, Knöbl PN, Krstic M, Kaufeld J, Menne J, Buxhofer-Ausch V, Miesbach W. Treatment of acquired thrombotic thrombocytopenic purpura without plasma exchange in selected patients under caplacizumab. *J Thromb Haemost.* 2020;18:3061–6.
- [57] Wang J, Cheng F, Niu Y, Yan L, Li J, Tan B, Qin L. Therapeutic plasma exchange-free treatment for first-episode TTP: a systematic review. *Transfus Apher Sci.* 2023;62:103661. <https://doi.org/10.1016/j.transci.2023.103661>
- [58] Prasannan N, Thomas M, Stubbs M, Westwood JP, de Groot R, Singh D, Scully M. Delayed normalization of ADAMTS13 activity in acute thrombotic thrombocytopenic purpura in the caplacizumab era. *Blood.* 2023;141:2206–13.
- [59] Mingot-Castellano ME, García-Candel F, Martínez-Nieto J, García-Arroba J, de la Rubia-Comos J, Gómez-Seguí I, Paciello-Coronel ML, Valcárcel-Ferreiras D, Jiménez M, Cid J, Lozano M, García-Gala JM, Angós-Vazquez S, Vara-Pampliega M, Guerra-Domínguez L, Ávila-Idrobo LF, Oliva-Hernandez A, Zalba-Marcos S, Tallón-Ruiz I, Ortega-Sánchez S, et al. ADAMTS13 recovery in acute thrombotic thrombocytopenic purpura after caplacizumab therapy. *Blood.* 2024;143:1807–15.
- [60] Sonneveld MA, de Maat MP, Portegies ML, Kavousi M, Hofman A, Turecek PL, Rottensteiner H, Scheiflinger F, Koudstaal PJ, Ikram MA, Leebeek FW. Low ADAMTS13 activity is associated with an increased risk of ischemic stroke. *Blood.* 2015;126:2739–46.
- [61] Upreti H, Kasmani J, Dane K, Braunstein EM, Streiff MB, Shanbhag S, Moliterno AR, Sperati CJ, Gottesman RF, Brodsky RA, Kickler TS, Chaturvedi S. Reduced ADAMTS13 activity during TTP remission is associated with stroke in TTP survivors. *Blood.* 2019;134:1037–45.
- [62] van den Berg J, Kremer Hovinga JA, Pflieger C, Hegemann I, Stehle G, Holbro A, Studt JD. Daratumumab for immune thrombotic thrombocytopenic purpura. *Blood Adv.* 2022;6:993–7.
- [63] Aggarwal A, White D, Pavord S, Thomas W, Desborough MJR. Daratumumab for refractory immune-mediated thrombotic thrombocytopenic purpura. *Br J Haematol.* 2023;202:429–33.
- [64] Xie XT, Xiao YY, Zhang Y, Luo ZM, Luo Y. Combination regimens containing daratumumab for initial diagnosed acquired thrombotic thrombocytopenic purpura. *J Thromb Thrombolysis.* 2023;55:399–405.