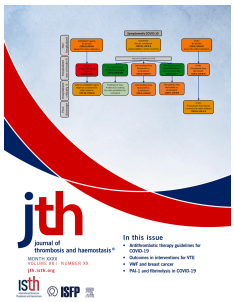

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Bortezomib for rituximab-refractory immune-mediated thrombotic thrombocytopenic purpura in the caplacizumab era: an Italian multicenter study

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Abstract

Background. Immune-mediated thrombotic thrombocytopenic purpura (iTTP) patients are not responsive to standard rituximab in approximately 10-15% of cases, and oral immunosuppressants showed controversial results with significant toxicity. Targeting plasma cells with bortezomib appears promising, but available evidence is scarce and stems only from isolated reports in the pre-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 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 cut-off. Responders had fewer previous acute iTTP episodes than non-responders [median (IQR) 1 (1-2) vs 5.5 (2-7), p=0.03]. Eight subjects (47%) reported toxicities, mostly in those treated with ≥2 cycles.

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

Keywords: immune-mediated thrombotic thrombocytopenic purpura, bortezomib, rituximab, ADAMTS13, immunosuppressants.

Introduction

Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a rare autoimmune disease caused by autoantibodies directed against ADAMTS13, a plasma metalloprotease that cleaves the von Willebrand Factor (VWF) [1, 2]. ADAMTS13 deficiency leads to the accumulation of ultra-large 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 (CNS), the heart and the kidney, can lead patients to death 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 to prevent clinical relapses, when employed during the acute phase [14]. Moreover, it is used during remission in case of ADAMTS13 relapse as pre-emptive treatment to prevent clinical and ADAMTS13 relapses (a fall in ADAMTS13 activity to <20% after an ADAMTS13 remission) [15], as recommended by international guidelines [10]. However, about 10-15% of patients do not achieve a sustained ADAMTS13 remission (i.e., ADAMTS13 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 ADAMTS13 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 of 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 two decades [22], exerts a pro-apoptotic 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 IgA pemphigus [24]. Bortezomib has been also 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.

Methods

Study design and patients

We conducted a retrospective cohort study via an electronic case report form (eCRF) sent to 31 Italian iTTP-treating centers. The eCRF collected information about patients' demographics, clinical characteristics, ADAMTS13 activity and anti-ADAMTS13 antibody titer, concomitant/previous treatments, bortezomib schedule, response and adverse drug reactions to bortezomib, and clinical and ADAMTS13 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, that is thrombocytopenia with microangiopathic hemolytic anemia, in absence of alternative causes, with evidence of ADAMTS13 activity <10% and anti-ADAMTS13 antibodies); 3) refractory to rituximab; 4) completed ≥1 bortezomib cycle, defined as the standard myeloma regimen of 1.3 mg/m^2 day 1, 4, 8 and 11; 5) on 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 disease to achieve clinical/ADAMTS13 remission (as defined in the following paragraph) in TPE/caplacizumab-treated patients, or during clinical remission as pre-emptive treatment in case of ADAMTS13 relapse or if ADAMTS13 activity was persistently <20%. Immunosuppressivetherapies 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 ADAMTS13 remission were achieved after at least 15 days from the first rituximab administration.

ADAMTS13 testing was performed with different assays across centers. ADAMTS13 activity was measured using the Technozym ADAMTS-13 Activity ELISA assay (Technoclone; normal range: 40 - 130 IU/dL) or the HemosIL AcuStar ADAMTS13 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-ADAMTS13 antibodies were measured using the TECHNOZYM ADAMTS-13 inhibitor ELISA assay, which measures the anti-ADAMTS13 IgG titer (Technoclone; negative values < 12 U/mL, borderline values: 12-15 U/mL, positive values > 15 U/mL) or using a Bethesda-like mixing assay, which measures the neutralizing activity of anti-ADAMTS13 antibodies (negative values < 0.4 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.

Response criteria and toxicity evaluation

The primary efficacy outcome was the cumulative incidence of overall response to bortezomib, with no need of any subsequent/additional immunosuppressive treatment. Different definitions of response were used according to the setting of treatment, all referring to the criteria in the revised International Working Group consensus report [32]. For TPE-treated patients during the acute phase, response was defined as a clinical response (i.e., sustained platelet count >150 x10^9/L and lactate dehydrogenase levels <1.5 times upper limit of normal (ULN) and no evidence of ischemic organ injury), which allowed TPE discontinuation. For caplacizumab-treated patients during the acute phase, response was defined as ADAMTS13 remission, which allowed caplacizumab discontinuation. For patients treated pre-emptively during clinical remission, response was defined as ADAMTS13 remission, including partial (ADAMTS13 activity >20% to <LLN) and complete remission (ADAMTS13 activity >LLN).

Secondary outcomes were 1) the incidence of clinical exacerbation (when platelet count decreased to <150 x10^9/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 ADAMTS13 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 (CTCAE) version 5.0 [33].

Statistical analysis

Categorical variables were expressed as counts and percentages, continuous variables as medians and interquartile ranges (IQR).

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

Results

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 (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).

Efficacy of bortezomib

A total of 18 treatments with bortezomib were recorded in 17 patients: 11 received bortezomib during acute phase, whereas 7 during clinical remission (patient 11 treated twice, once in acute phase and once pre-emptively). Those treated in the acute phase had a shorter iTTP median duration at the time of bortezomib treatment (calculated as date of bortezomib administration – date of iTTP diagnosis) than those treated pre-emptively (4 months, IQR 1.8-6.2, vs 10.2 months, IQR 5.2-16.5, p=0.04). The treatment schedule was 1.3 mg/m^2 subcutaneously on day 1, 4, 8 and 11 (except for patient 13, treated on day 1, 8 and 15), repeated every 21 days when >1 cycle was administered. Nine treatments consisted of one cycle, 7 of two cycles and 2 of four cycles, according to the clinician's indication and patient's response.

The cumulative incidence of overall response was 59% (10/17 patients, and 11/18 treatments): 9/11 treatments (82%) during acute phase vs 2/7 treatments (28%) during clinical remission (p=0.05). Responders had a lower median number of previous acute episodes [1 (IQR 1-2) vs 5.5 (IQR 2-7), p=0.03]. This finding was confirmed also analyzing responders vs non-responders in acute and remission phases separately, although not statistically significant: 2 (IQR 1-2) vs 4.5 (IQR 3.25-5.75) in acute (p=0.2); 1.5 (IQR 1.25-1.75) vs 5 (IQR 3-6) in pre-emptive treatments (p=0.2). 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 one cycle vs 4/9 responders, 44%, for ≥2 cycles, p=0.33) and the use of concomitant immunosuppressive treatments (7/13 responders, 54%, with concomitant immunosuppression vs 3/4 responders, 75%, without, p=0.6).As regards the 11 patients treated in acute phase (Table 2a), 6 were receiving caplacizumab (for >4 months in patients 4 and 7), and 5 TPE. Among the caplacizumab-treated patients, we registered 5/6 ADAMTS13 remissions (4 partial and 1 complete remission), allowing a permanent discontinuation of caplacizumab. More in detail for patient 7 (Figure 1), two attempts of caplacizumab discontinuation before bortezomib, while ADAMTS13 activity levels were still undetectable, resulted in two exacerbations. Bortezomib allowed a rapid and progressive decline of anti-ADAMTS13 antibody titer, followed by ADAMTS13 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 ADAMTS13 complete remissions, 1 ADAMTS13 partial remission, and 1 clinical response without ADAMTS13 response but with a significant reduction of TPE frequency to one 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, patient 7 and 11 achieved ADAMTS13 complete remission one month after their partial remission.

Regarding the 7 treatments during clinical remission (Table 2b), 2 out of 4 patients treated for ADAMTS13 relapse obtained ADAMTS13 partial remission, while no responses were observed among the 3 patients treated in case of persistently low ADAMTS13 activity. However, patient 15, a 24-year-old girl who had not achieved any ADAMTS13 response after clinical remission notwithstanding immunosuppression with rituximab, cyclosporine A and mycophenolate mofetil, experienced a progressive reduction until disappearance of anti-ADAMTS13 antibodies after bortezomib. Eventually, her ADAMTS13 activity was detectable for the first time at month +11 after treatment (0.4%, last value available at the time of data cut-off). The median time to response for the treatments during remission was 31 days (range 24-38). Complete ADAMTS13 remission was achieved three months after partial remission in patient 11, and one 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 non-responders (14 months, IQR 7.5-22), p=0.2. The median duration of ADAMTS13 response was 22 months (IQR 10-38), and 6 subjects were still responding at the time of data cut-off. Among the 10 responders, no clinical iTTP relapses occurred, while the cumulative incidence of ADAMTS13 relapse was 5/10 patients. Patient 11 was successfully retreated with bortezomib. Among the 7 non-responders, the incidence rate of clinical relapse was 3/6.9 patient-years. Notably, ADAMTS13 relapses in patients 1 and 3 and a clinical relapse in patient 14 responded to rituximab, which was ineffective before bortezomib administration.

Anti-ADAMTS13 antibody titer before and after treatment was available in 12 patients (Table 2a/b). 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 patient 4 and 5, and by 2 times in patient 7. In non-responders, 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 patient 13 and 16, and became positive in patient 14. Four patients had anti-ADAMTS13 antibody tested at further timepoints: patient 4, 7 and 15 became negative at month 6, 3 and 12, respectively, while the titer of patient 17 decreased by 3 times from baseline at month 5.

Safety of bortezomib

Eight subjects (47%) reported bortezomib-related adverse events, none requiring drug discontinuation. Six out of nine patients receiving ≥2 cycles (67%) experienced an adverse event, while only 2 out of 9 (22%) with one cycle reported toxicity (p=0.15). Paresthesia was the most common (n=6, grade 1 in two patients and grade 2 in four, 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 were receiving no antimicrobial prophylaxis during bortezomib treatment, 2 received daily acyclovir 400 mg every 12 hours, and the remaining acyclovir + trimethoprim-sulfamethoxazole 160-800 mg three times a week. Patient 2 (receiving acyclovir) reported a grade 3 Pneumocystis jirovecii pneumonia two months after the end of bortezomib treatment and during concomitant therapy with rituximab and high-dose corticosteroids.

Discussion

In the present study we showed a response rate of nearly 60% to bortezomib in rituximab-refractory and often multi-treated iTTP patients, especially when employed in acute phase. Responses occurred after a median of 30 days and were sustained for two years. Toxicity was reported in half of patients, but never lead 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, LLPCs 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 not responsive to rituximab [36, 37]. In antineutrophil cytoplasmatic antibody (ANCA)-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, that may be responsible for persistent anti-ADAMTS13 antibody production in rituximab-refractory iTTP. Bortezomib has been employed in refractory iTTP for ten 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 previously reported (72% overall) [30]. However, most of 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 ADAMTS13 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 two drugs is successfully employed in mantle cell lymphoma [40, 41], Waldenstrom'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, responsible for further relapses. Notably, we observed that three 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 disease.

In the present study, we found lower response rates during clinical remission. The reasons why bortezomib was less effective when administered as pre-emptive treatment are poorly understood. These patients had a longer history of iTTP at bortezomib administration, and might have a more refractory disease requiring longer time to respond. In the recent report by Lee et al., bortezomib was effective in 3/5 ADAMTS13 relapses, one of which after 7 months from treatment [30]. Likewise, a significant decline of anti-ADAMTS13 antibody titer was registered in some of our non-responding patients, andthe occurrence of responses 4 months after treatment also in the acute phase (patients 4, 6 and 7) suggests that bortezomib can take longer time than rituximab to work [13].

The long duration of ADAMTS13 response to bortezomib (median 22 months) appears impressive for the refractory setting here represented. Other reports showed responses lasting more than 5 years [26, 45]. Repeating rituximab in previous responders is a common strategy to treat ADAMTS13 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 1.5-2 years, but long-term consequences of rituximab-related hypogammaglobulinemia and infections are of some concerns 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 pre-emptive 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 reported in the literature (5/36, 14% overall) [30]. Concomitant immunosuppressive treatments in two patients may have contributed to the toxicity, especially for the case of P. jiroveciii 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 less adverse events in patients treated with one cycle than those receiving ≥2 cycles, without affecting bortezomib effectiveness. This finding confirms previous data in the literature, where reduced

doses (e.g., 1 mg/m^2) and a single cycle of bortezomib have been employed in iTTP with good efficacy [28-30].

More than half of patients in our study were receiving caplacizumab at the time of bortezomib treatment, this representing a new clinical scenario. In fact, only 3 out of the 36 bortezomib-treated patients in the literature were receiving 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 ADAMTS13 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 lacking safety data in case of prolonged caplacizumab exposure [54], especially in elderly patients and those requiring anti-platelet/anti-coagulant 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 ADAMTS13 activity has been reported in the caplacizumab era, maybe due to reduced employment of TPE procedures [58, 59]. ADAMTS13 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 ADAMTS13 levels and 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 (e.g., low-resource settings) to obtain a clinical response, or are "caplacizumab-dependent" because of lack of ADAMTS13 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 (i.e., after 4 months) are possible, and treatment with two or more cycles was associated with higher toxicity without improving response. For these reasons, a "watchful waiting" approach may be considered after one cycle to avoid over-treatment and drug-related adverse events, especially if a declining trend of anti-ADAMTS13 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.

Authors' Contributions

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

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Declaration of Competing Interests

AA, PA, MC and BF received honoraria for participating as a speaker at educational meetings organized by Sanofi; IM received honoraria for participating as a speaker at educational meetings organized by Instrumentation Laboratory and Sanofi; MB received conference fees from Novartis, Incyte, and AbbVie; MN acted as consultant for Bayer, CSL Behring, Novo Nordisk and received speaker fees from Bayer, Takeda, Sobi, Novo Nordisk, CSL Behring, Sanofi, Amgen, Novartis, and Grifols; FP 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, Sobi. The other authors do not have any conflict of interests to disclose.

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Figure legend

Figure 1. Clinical history and laboratory values of patient 7. TPE therapeutic plasma exchange, capla caplacizumab, q2d every 2 days, q3d every 3 days, iTTP immune-mediated thrombotic thrombocytopenic purpura, PR partial remission, CR complete remission.

Parallel vertical lines = period of caplacizumab discontinuation; yellow flash = iTTP exacerbation; dashed orange line = anti-ADAMTS13 antibody titer; dotted-dashed blue line = ADAMTS13 activity levels.

Table 1. Baseline characteristics of patients with refractory immune-mediated thrombotic thrombocytopenic purpura (iTTP) at bortezomib administration.

Patient	Sex, age (years)*	Comorbidities*	Number of iTTP acute episodes prior to bortezomib*	Immunosuppressive treatments prior to bortezomib†
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*	Arterial hypertension*	2*	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)

RTX rituximab, IVIG intravenous immunoglobulins, CTX cyclophosphamide, CYA cyclosporine A, MMF mycophenolate mofetil, AZA azathioprine.

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

† A, drug used during an iTTP acute episode; P, drug used as a pre-emptive treatment.

Table 2a. Outcomes of immune-mediated thro[Journal Pre-proof]uring/after the acute phase (i.e., plasma exchange- and/or caplacizumab-treated patients).

Pt	Setting of treatment	Concomitant therapies (including IST administered <1 month before)	Anti-ADAMTS13 antibody titer pre-bortezomib	N of cycles	Response*	Time to response†	Anti-ADAMTS13 antibody titer post-bortezomib‡	Duration of response (months)	Toxicity (grade)	Relapse*	ADAMTS13 activity at last follow-up
1	iTTP onset (3.5 months after)	Caplacizumab, steroid, RTX	13 U/mL	1	ADAMTS13 CR	7 days	n.a.	24	-	ADAMTS13 relapse responsive to steroid and RTX	46 IU/dL§
2	First relapse (2.3 months after)	Caplacizumab, steroid, RTX	2.2 BU/mL	1	NR	-	0.6 BU/mL	-	Paresthesia (1) Pneumonia (3)	Exacerbation 2 months after bortezomib, responsive to CYA	60 IU/dL§
3	First relapse (1 month after)	TPE, steroid, RTX, vincristine, CTX, NAC	n.a.	1	Clinical response (with ADAMTS13 CR)	1 month	n.a.	38	Paresthesia (2), diarrhea (1)	2 ADAMTS13 relapses, both responsive to RTX	58 IU/dL§
4	iTTP onset (4.5 months after)	Caplacizumab	70 U/mL	4 ¶	ADAMTS13 PR (20 IU/dL)	4 months	18 U/mL	10, ongoing	Paresthesia (2), constipation (3)	No	30 IU/dL§
5	iTTP onset (15 days after)	TPE, steroid, RTX, CTX, IVIG	1.6 BU/mL	1	Clinical response (ADAMTS13 CR)	6 days	0.4 BU/mL	53, ongoing	-	No	90 IU/dL **
6	First relapse (5 months after)	TPE every 2 days	>93 U/mL	4 ††	Clinical response with reduction of TPE frequency (every 3 months)	4 months	<12 U/mL	50, ongoing	Neutropenia (2)	No	4 IU/dL **
7	iTTP onset (5.2 months after)	Caplacizumab	702 U/mL	2	ADAMTS13 PR (20.5 IU/dL)	4 months	366 U/mL	8, ongoing	Paresthesia (1)	No	86 IU/dL**
8	First relapse (15 days after)	Caplacizumab, steroid, RTX, CTX,	n.a.	1	ADAMTS13 PR, (44 IU/dL)	1 month	n.a.	12	-	ADAMTS13 relapse responsive to CYA	<0.2 IU/dL**
9	Sixth relapse (10 days after)	TPE, steroid	n.a.	2	NR	-	n.a.	-	-	No (treated with splenectomy and vincristine, re-treated with steroid and RTX with response)	49 IU/dL§
10	Sixth relapse (15 days after)	Caplacizumab, steroid	35 U/mL	1	ADAMTS13 PR, (41 IU/dL)	17 days	<12 U/mL	1.5	-	ADAMTS13 relapse, not treated	1 IU/dL§
11	First relapse (1.5 months after)	TPE, steroid, RTX	n.a.	1	Clinical response (with ADAMTS13 PR, 28 IU/dL)	7 days	n.a.	24	-	ADAMTS13 relapse treated with bortezomib (*Table 2b*)	*Table 2b*

Table 2b. Outcomes of immune-mediated thro[Journal Pre-proof]uring clinical remission.

Pt	Setting of treatment	Concomitant therapies (including IST administered <1 month before)	Anti-ADAMTS13 antibody titer pre-bortezomib	N of cycles	Response*	Time to response†	Anti-ADAMTS13 antibody titer post-bortezomib‡	Duration of response (months)	Toxicity (grade)	Relapse*	ADAMTS13 activity at last follow-up
11	ADAMTS13 relapse	-	n.a.	1	ADAMTS13 PR (28 IU/dL)	24 days	n.a.	20, ongoing	-	No	67% ‡‡
12	ADAMTS13 relapse	CTX started 5 months earlier (stopped at bortezomib start)	98 U/mL	2	ADAMTS13 PR (20 IU/dL)	38 days	<12 U/mL	22, ongoing	-	No	54 IU/dL§
13	ADAMTS13 relapse	CTX started 7 months earlier (stopped at bortezomib start)	112 U/mL	2 §§	NR	-	93 U/mL	-	Paresthesia (2)	No (pre-emptive daratumumab treatment 16 months after bortezomib with PR)	6 IU/dL§
14	ADAMTS13 relapse	Steroid	<0.4 BU/mL	1	NR	-	1 BU/mL	-	-	Clinical relapse 5 months after bortezomib, responsive to plasma infusion, steroid and RTX	89 IU/dL§
15	ADAMTS13 persistently <10 IU/dL	MMF started 4 months earlier (stopped at bortezomib start)	273 U/mL	2	NR	-	116 U/mL	-	Headache (2)	No	0.4 IU/dL**
16	ADAMTS13 persistently <10 IU/dL	-	454 U/mL	2	NR	-	462 U/mL	-	-	Clinical relapse 1.5 months after bortezomib with clinical response to steroid + caplacizumab, CYA started one month later	<0.2 IU/dL**
17	ADAMTS13 persistently <10 IU/dL	MMF started 3 months earlier (stopped at bortezomib start)	145 U/mL	2	NR	-	91 U/mL	-	Paresthesia (2)	No	<0.2 IU/dL§

Pt patient, IST immunosuppressive treatment, ADAMTS13 a disintegrin and metalloprotease with thrombospondin type 1 repeats member 13, TPE therapeutic plasma exchange, RTX rituximab, CR complete remission, n.a. not available, BU/mL Bethesda units/mL, NR no response, CYA cyclosporine A, CTX cyclophosphamide, NAC N-acetylcysteine, PR partial remission, IVIG intravenous immunoglobulins, MMF mycophenolate mofetil.

* Clinical response, ADAMTS13 partial and complete remission, exacerbation, clinical and ADAMTS13 relapses were defined as per the International Working Group consensus report [27].
† Counting from the date of the first dose of bortezomib.
‡ Measured within 30 days after the completion of bortezomib treatment. When expressed in U/mL, anti-ADAMTS13 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-ADAMTS13 neutralizing antibodies were measured using a Bethesda-like assay (negative values < 0.4 BU/mL).
§ Measured with the Technozym ADAMTS-13 Activity ELISA assay (Technoclone: normal range: 40 - 130 IU/dL).
¶ Bortezomib schedule reduced to 1 mg/m^2 day 1 and 4 from cycle 4 due to grade 3 constipation.
** Measured with the HemosIL AcuStar ADAMTS13 Activity assay (Werfen; normal range: 67 - 129 IU/dL or 61 - 131 IU/dL, depending on the laboratory).
†† Bortezomib schedule reduced to 1.3 mg/m^2 day 1 and 8 from cycle 3 due to grade 2 neutropenia.
‡‡ Measured with the FRETS-VWF73 assay (ref. 31; normal range: 45 - 147%).
§§ Bortezomib schedule was 1.3 mg/m^2 day 1, 8 and 15.

Figure 1