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ORIGINAL ARTICLE

Complex immunogenicity assessment in caplacizumab-treated patients with immune-mediated thrombotic thrombocytopenic purpura who have received plasma exchange

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Abstract

Background: International Society on Thrombosis and Haemostasis guidelines for immune-mediated thrombotic thrombocytopenic purpura (iTTP) treatment recommend concurrent therapeutic plasma exchange (TPE), immunosuppressive therapy (IST), and caplacizumab. TPE can complicate antidrug antibody (ADA) measurements by transferring pre-existing antibodies (pre-Abs) into patients via donor plasma and/or diluting treatment-emergent (TE) ADAs.

Objectives: To assess the presence of ADAs in patients with iTTP who received caplacizumab.

Methods: Immunogenicity data from patients with iTTP receiving caplacizumab once daily plus TPE/immunosuppressive therapy in 4 clinical trials (TITAN, HERCULES, Post-HERCULES, and a trial conducted in Japanese patients) in the clinical development program were analyzed. ADA and modified ADA assays differentiated pre-Abs from TE ADAs. A functional neutralizing antibody (NAb) assay and a neutralizing epitope characterization assay (NECA) assessed the presence of ADAs with neutralizing potential. The impact of ADAs on efficacy, pharmacokinetics/pharmacodynamics, and safety was evaluated.

Results: Among 228 patients in 4 studies, prevalence of pre-Abs ranged from 17.1% (TITAN) to 56.7% (HERCULES), while TE ADA prevalence ranged from 3.1% (HERCULES) to 14.3% (Japanese study). The TE NAb-positive rate ranged from 0% (Japanese study) to 12% (Post-HERCULES) using the functional NAb assay and from 2.7% (Post- HERCULES) to 14.3% (Japanese study) using the NECA. The presence of these antibodies did not impact treatment efficacy or safety.

Conclusion: A complex immunogenicity assay strategy was required to define the pre-Ab/TE ADA status of patients with iTTP treated with caplacizumab in a clinical trial setting. In addition to the wide range of pre-Abs observed, few patients had detectable TE ADAs or NAbs, neither of which affected efficacy/safety.

KEYWORDS
ADAMTS13 protein, autoantibodies, biological assay, immune-mediated thrombotic thrombocytopenic purpura, single-domain antibodies

Essentials

- Therapeutic plasma exchange (TPE) complicates antidrug antibody (ADA) measurements.
- ADAs were measured in 4 clinical trials in patients with immune-mediated thrombotic thrombocytopenic purpura receiving caplacizumab.
- Pre-existing antibodies, treatment-emergent ADAs, and drug-neutralizing antibodies were assessed.
- None of the antibody types impacted clinical efficacy or safety with caplacizumab plus TPE.

1 | INTRODUCTION

Immune-mediated thrombotic thrombocytopenic purpura (iTTP), also known as acquired thrombotic thrombocytopenic purpura, is a rare, life-threatening hematologic disorder marked by the formation of systemic microthrombi in small blood vessels [1]. iTTP develops due to the formation of autoantibodies against ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), an enzyme that cleaves von Willebrand factor (VWF). Severe deficiency in ADAMTS13 activity leads to the accumulation of ultralarge VWF multimers, which results in unregulated platelet adhesion and aggregation and microthrombi formation [1]. These microthrombi may restrict blood flow to vital organs and lead to tissue ischemia, severe thrombocytopenia, and microangiopathic hemolytic anemia [1,2]. Each episode of iTTP puts patients at risk for significant morbidity and mortality, with complications such as cerebral vascular events, acute coronary events, acute renal injury, and bowel ischemia. Additionally, 30% to 40% of patients suffer at least one relapse requiring treatment reinitiation [2], and long-term consequences include a higher risk of stroke, hypertension, and depression [3,4].

The current standard of care for patients with iTTP includes daily therapeutic plasma exchange (TPE), which replenishes ADAMTS13 and removes ultralarge VWF multimers and anti-ADAMTS13 autoantibodies [1,5,6], in combination with immunosuppressive therapy (IST), which includes corticosteroids and biologic therapies such as the anti-CD20 monoclonal antibody rituximab to suppress the underlying autoimmune response [1,7]. In addition, for patients with iTTP experiencing an acute event (a first event or a relapse), the International Society on Thrombosis and Haemostasis 2020 TTP guidelines recommend the use of caplacizumab in combination with TPE and IST [7]. Caplacizumab is a bivalent anti-VWF humanized single-variable domain immunoglobulin (Nanobody®) molecule that binds to the A1 domain of VWF, blocking the interaction of VWF with platelets. Platelets are consequently prevented from binding to ultralarge VWF, thus inhibiting microthrombi formation [8,9].

As part of its clinical development program, caplacizumab was administered to patients with iTTP in the phase 2 TITAN trial, phase 3 HERCULES and Post-HERCULES trials, and a phase 2/3 trial in Japanese patients (referred to hereafter as the Japanese trial). Time to platelet count response and platelet count normalization were shown to improve with caplacizumab treatment, as were long-term outcomes and incidence of iTTP recurrence [10–13]. In all 4 trials, drug-induced antidrug antibodies (ADAs) were measured and reported [10–13]. Induction of humoral immune responses by any protein-based biological therapy can trigger the formation of ADAs, which can lead to drug inactivation, disruption of its intended targeting, and/or increased clearance of drug–ADA complexes, thus compromising drug efficacy [14]. Furthermore, the use of TPE alongside caplacizumab treatment may complicate the measurement of ADAs by transferring pre-existing antibodies (pre-Abs) via donor plasma or by dilution of treatment-emergent ADAs (TE ADAs).

This analysis compiles the immunogenicity results from 4 clinical trials in the caplacizumab clinical development program. Presence of pre-Abs and TE ADAs was assessed, neutralizing potential of ADAs was measured, and the effect of ADAs on caplacizumab efficacy, pharmacokinetics, pharmacodynamics, and safety was evaluated.

2 | METHODS

2.1 | Study design and eligibility criteria

Immunogenicity data from all 4 iTTP clinical trials in the caplacizumab development program were compiled and evaluated. Study design and eligibility criteria for each of the 4 trials have been published previously [10–13].

TITAN (NCT01151423), a phase 2 trial, evaluated the impact of caplacizumab vs. placebo in adults ≥18 years old who were experiencing an acute episode of iTTP, had a platelet count of $<100 \times 10^9$/L, did not have active bleeding, and required plasma exchange [11]. Patients were randomized 1:1 to receive caplacizumab or placebo, in addition to daily TPE and IST. An intravenous loading dose of caplacizumab 10 mg or placebo was administered from 6 hours to 15 minutes prior to the first TPE done on study (ie, after randomization). This first on-study TPE was followed by subcutaneous (SC) administration of 10 mg study drug. Throughout the TPE treatment period, including tapering and TPE administered for exacerbations, study drug was administered as a SC dose daily within 30 minutes after the end of

each TPE session, and daily SC dosing was continued for 30 days after the last TPE session. Patients received treatment for up to 90 days and were followed up until 1 year after the last dose of study drug.

HERCULES (NCT02553317) was a phase 3 trial that assessed the impact of caplacizumab vs. placebo in adults ≥18 years old with clinical diagnosis of iTTP (defined as the presence of both thrombocytopenia and microangiopathic hemolytic anemia with schistocytes seen on blood smear) who had undergone exactly 1 TPE treatment [12]. Patients were randomized 1:1 to receive caplacizumab or placebo, in addition to standard of care, which included daily TPE and corticosteroids. Patients received an intravenous loading dose of caplacizumab 10 mg or placebo from 6 hours to 15 minutes prior to the first on-study TPE. Throughout the TPE treatment period, study drug was administered as a SC dose daily within 2 hours of completing TPE; daily SC dosing was continued for 30 days after the last TPE session. Administration of caplacizumab or placebo could be extended up to 28 days beyond the 30 days based on risk factors for TTP recurrence, such as persistent severe ADAMTS13 deficiency, and was accompanied by optimization of the IST (adjusted as needed). In cases of disease recurrence (ie, where a new decrease in platelets required reinitiating daily TPE at any time during the treatment period), patients switched to open-label caplacizumab, although their initial trial-group assignment remained blinded. There was a 28-day follow-up period after the end of the treatment period. Any recurrences during this follow-up period were managed with standard of care, without restarting the trial regimen.

Post-HERCULES (NCT02878603) was a 3-year follow-up study for patients who completed the HERCULES trial [13]. Following completion of HERCULES, patients were invited to attend twice-yearly visits for 3 years, starting with a baseline visit coinciding with or scheduled within 1 month after the final 28-day follow-up visit in HERCULES. Patients experiencing an iTTP recurrence during the trial could receive open-label caplacizumab along with concurrent TPE and IST until 30 days after the last daily TPE. Up to one TPE could be given before initiating caplacizumab, as long as it was considered to be part of the TPE for the treatment of the presenting iTTP episode. In case of persistent signs and symptoms of underlying disease activity (eg, if ADAMTS13 activity levels remained severely reduced), caplacizumab treatment could be extended for up to a further 28 days. Patients with contraindications to caplacizumab or those with other exclusion criteria, such as pregnancy, were treated with TPE and IST. iTTP recurrence was defined as recurrent thrombocytopenia requiring TPE initiation in TITAN, HERCULES, and Post-HERCULES [11–13].

The phase 2/3 Japanese trial (NCT04074187) evaluated the impact of caplacizumab in adults ≥18 years old with clinical iTTP (initial or recurrent) that required initiation of daily TPE [10]. Clinical iTTP was defined in this study as thrombocytopenia (platelet count $<100 \times 10^9$/L), microangiopathic hemolytic anemia, and increased lactate dehydrogenase levels. Patients were eligible for inclusion if they had received no more than one TPE treatment within the preceding 24 hours. The first caplacizumab dose (10 mg) was administered IV any time from 6 hours to 15 minutes prior to the first on-study TPE, followed by a 10 mg subcutaneous SC injection after the first on-study TPE. Subsequently, patients received caplacizumab 10 mg s.c. once daily (within 2 hours of completing TPE on days when TPE was administered), in addition to standard care, throughout the TPE treatment period and for 30 days thereafter. The minimum study period was the duration of daily TPE treatment plus 30 days of post-TPE treatment and 30 days of follow-up period. Caplacizumab treatment was continued for additional 1-week periods in patients whose ADAMTS13 activity profile remained <10% or those who had other clinical signs of underlying immunologic disease. The maximum extension period was 8 weeks; thus, the study period range was approximately 2 to 6 months. The decision to reinitiate TPE was based on patients' risk of relapse during optimized immunosuppression, assessed by ADAMTS13 activity measured weekly. iTTP recurrence was defined as recurrent thrombocytopenia after initial recovery of platelet count (confirmed platelet count $\geq 150 \times 10^9$/L) with discontinuation of daily TPE, requiring reinitiation of daily TPE [10].

All studies received approval from the ethics committee or institutional review board at each site, and all patients provided written informed consent. In addition, the studies adhered to international ethics guidelines such as the Declaration of Helsinki and the International Council for Harmonization Good Clinical Practice Guidelines [10–13]. For TITAN and HERCULES, an independent data and safety monitoring board reviewed safety data [11,12].

2.2 | Sample collection and laboratory methods

Four assay techniques, including a conventional ADA assay, a modified ADA (mADA) assay, a functional neutralizing antibody (NAb) assay, and a neutralizing epitope characterization assay (NECA), were used to test patient samples from the 4 clinical trials for the presence of ADAs (Figure 1). Full details of the ADA, mADA, NECA, and functional NAb assays are provided in the Supplementary Methods. These different methods were validated based on applicable health authority regulatory guidelines and were all conducted at the same location [15,16].

ADA and mADA assay data were available for all 4 trials, and NAb assay and NECA data were available for all trials except TITAN. ADAs were assessed at multiple time points (Supplementary Table 1). In TITAN, ADAs were assessed by the central laboratory at baseline (ie, <1 hour prior to first study drug administration), at day 14 of the treatment phase, on the day of last daily TPE (if after day 14 of treatment phase), weekly during post–daily TPE, and at 1, 2, 3, 6, and 12 months of follow-up (once daily ± 2 hours) using the validated assays described below. In HERCULES, ADAs were evaluated at baseline during day 1 of the daily TPE period, at day 1 (ie, week 1) of the 30-day post–daily TPE period, and at the end of the 30-day post–daily TPE period (ie, week 5, ±1 day), and at the first (7 days after last dosing, ±1 day) and last (28 days, ±1 day) follow-up visits. In patients who participated in the treatment extension period of HERCULES, ADAs were additionally assessed at week 9 (ie, 28 days after end of the 30-day post–daily TPE period) and at any recurrence visit. In Post-HERCULES, ADAs were assessed at baseline and every 6 months (±1 month) up to 36 months. In the Japanese trial, ADAs were evaluated at baseline (day 1 of the daily TPE period), at day 1 (ie, week 1) of the

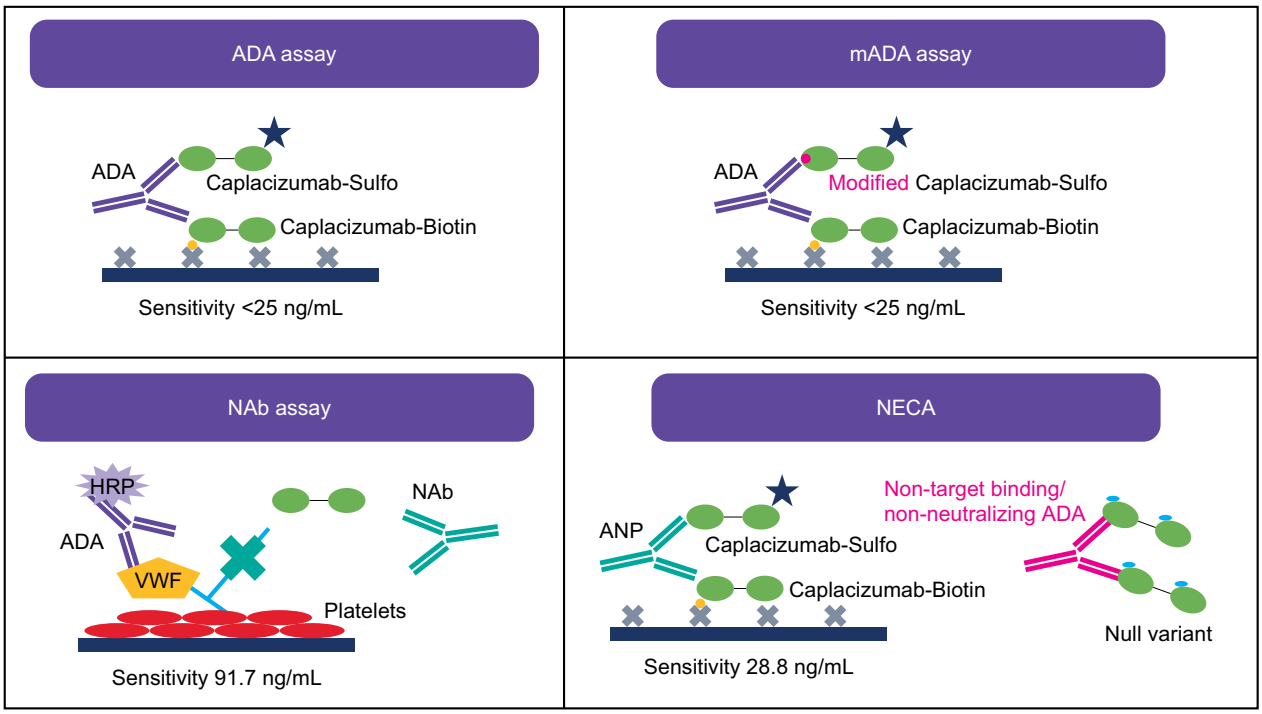

FIGURE 1 Overview of assay types used to detect ADAs. Sensitivity data for all assays were derived from the pooled serum of patients with immune-mediated thrombotic thrombocytopenic purpura using an affinity-purified pAb Rb pool. These sensitivity thresholds align with Food and Drug Administration guidance that screening and confirmatory IgG and IgM ADA assays achieve a sensitivity of at least 100 ng/mL [15]. With respect to drug tolerance characteristics: in the presence of 10.0 μg/mL of drug, 257.7 ng/mL, 87.5 ng/mL, and <100.0 ng/mL affinity-purified pAb Rb pool can be detected in the ADA, mADA, and NECA assays, respectively. The functional NAb assay was not found to be sufficiently drug tolerant to measure NAbs under treatment. ADA, antidrug antibody; ANP, antibody with neutralizing potential; HRP, horseradish peroxidase; mADA, modified antidrug antibody; NAb, neutralizing antibody; NECA, neutralizing epitope characterization assay; pAb Rb, rabbit polyclonal antibody; VWF, von Willebrand factor.

30-day post–daily TPE period, and at the end of the 30-day post–daily TPE period (ie, week 5, ±1 day) or early termination visit, and at the first (7 days after last dosing, ±1 day) and last (28 days after last dosing, ±1 day) follow-up visits [10]. In patients who participated in the treatment extension period of the Japanese trial, ADAs were additionally assessed at week 9 (ie, 28 days after end of the 30-day post–daily TPE period) and week 13 (ie, 56 days after end of the 30-day post–daily TPE period). For all trials, ADAs were evaluated prior to the start of TPE or caplacizumab administration; ADAs were also assessed when adverse events (AEs) and unscheduled visits occurred [10-13].

Serum samples were assessed for the presence of pre-Abs, TE ADAs, NAbs, and ADAs with neutralizing potential (ANPs) by a tiered approach. First, overall ADAs, including pre-Abs and TE ADAs, were measured using a Meso Scale Discovery electrochemiluminescence-based bridging ADA assay; this assay was first used in TITAN and optimized for subsequent studies. Briefly, a mixture of biotinylated and sulfo-tagged caplacizumab was preincubated with patient serum, allowing ADA-containing complexes to form. Samples were captured on streptavidin-coated plates before detection. Samples from patients who had detectable ADAs were then assessed for presence of TE ADAs with a mADA assay. To differentiate pre-Abs from TE ADAs, a modified version of caplacizumab that prevents binding of ADAs to the C-terminus of caplacizumab (the predominant site of pre-Ab binding) was used in detection, allowing measurement of TE ADAs. Drug concentrations in the ADA samples were below the drug tolerance levels of the ADA and mADA assays. Although drug concentrations were not expected to interfere with ADA detection, titers could have been underestimated if drug was present in the sample. Further details of the ADA/mADA assays are described in the Supplementary Methods.

Finally, a functional NAb assay and a NECA (previously known as an alternative NAb assay) were used to determine the neutralizing potential of ADA-positive samples from the HERCULES, Post-HERCULES, and Japanese studies. Functional NAb assays determine if the detected ADAs are capable of functionally blocking caplacizumab activity. Specifically, this ELISA-based assay assesses the inhibition of the VWF–platelet interaction by caplacizumab, given that the presence of NAbs against caplacizumab restores the binding of VWF to coated blood platelets, leading to an increase in assay signal. The functional NAb assay is drug sensitive; sensitivity may be reduced by residual caplacizumab in patient samples. Taking the limited drug tolerance of the functional NAb assay into consideration, the use of this method was restricted to baseline and drug wash-out samples.

The NECA, described previously [17], detects ANPs and is similar to the conventional ADA assay described above, except where an excess of null-variant caplacizumab is added to the mixture of biotinylated and sulfo-tagged caplacizumab. The null variant of caplacizumab contains altered complementarity determining regions, making the variant unable to bind to VWF in addition to ANPs (ie, while retaining binding of ADAs directed to the framework regions, which are mostly nonneutralizing). Details of the functional NAb and NECA assays are described in the Supplementary Methods.

2.3 | Immunogenicity analyses

Immunogenicity data, specifically relating to pre-Abs, TE ADAs, and NAbs, were analyzed to determine whether these antibodies impacted efficacy, pharmacokinetic (PK), pharmacodynamic (PD), and safety outcomes. In TITAN, the impact of pre-Abs and TE ADAs on PK/PD profiles was evaluated by visual analysis of plots that correlated ADA levels with PK and PD outcomes among patients in the caplacizumab group. Also, the relationship between pre-Abs and TE ADAs and safety measures was evaluated. In the HERCULES trial, time to platelet count responses and drug concentrations were evaluated based on patient classifications (ie, pre-Abs and/or TE Abs present/absent) from the results of the different types of ADA assays (ie, conventional ADA, mADA, functional NAb, and NECA). The impact of pre-Ab and TE ADAs on time to platelet count responses was also assessed through visual analysis of a Kaplan–Meier plot and inspection of tabulations outlining descriptive statistics for the responses. A mean plot of PK concentrations based on patient classification (ie, pre-Abs or TE Abs present/absent) and a scatter plot of PK concentrations based on ADA, mADA, NAb, and NECA assay categories were evaluated. Ristocetin cofactor (RICO) activity and percentage change from baseline VWF antigen (VWF:Ag) concentration were evaluated based on ADA, mADA, NAb, and NECA categories; a mean plot of these outcomes based on patient classification and scatter plots of these outcomes based on ADA, mADA, NAb, and NECA categories were also evaluated. The Post-HERCULES trial evaluated box plots comparing the duration of iTTP between ADA, mADA, NAb, and NECA categories to assess the impact of immunogenicity on efficacy. The trial also analyzed scatter plots of drug concentrations and RICO activity based on ADA, mADA, NAb, and NECA categories. For the Japanese trial, the impact of pre-Ab and TE ADAs on time to platelet count responses was assessed through visual analysis of a Kaplan–Meier plot. Scatter plots of drug concentrations, RICO activity, and percentage change from baseline VWF:Ag concentration based on ADA, mADA, and NECA categories were evaluated. The association between hypersensitivity reactions and the presence of pre-Abs and/or drug-induced TE ADAs was evaluated in the HERCULES, Post-HERCULES, and Japanese trials.

3 | RESULTS

3.1 | Immunogenicity outcomes

Immunogenicity data from patients with iTTP from the 4 clinical trials were assessed. Table 1 shows the demographics for the intention-to-treat populations (double-blind period only) across the 4 trials. TITAN included 72 patients (of whom 71 received a treatment; caplacizumab, n = 35), HERCULES included 145 patients (of whom 144 received a treatment; caplacizumab, n = 71), Post-HERCULES included 104 patients (caplacizumab, n = 75), and the Japanese trial involved 21 patients (caplacizumab, n = 21). Twenty-six patients who received placebo in the double-blind treatment period of HERCULES went on to receive caplacizumab during the open-label treatment period, bringing the total number of caplacizumab-treated patients in that study to 97 (Table 2). In the Post-HERCULES study, 9 of 75 patients required retreatment with caplacizumab. Data are presented here for patients who only received caplacizumab in all 4 studies (n = 228).

Incidence of pre-Ab, drug-induced TE ADA, and TE NAb/ANP were assessed as percentages of patients treated with caplacizumab in each trial population. Across all 4 studies, 17.1% to 56.7% of patients were positive for pre-Abs (TITAN, 17.1%; HERCULES, 56.7%; Post-HERCULES, 26.7%; Japanese study, 38.1%; Table 2). Drug-induced TE ADAs were detected in 3.1% to 14.3% of patients (TITAN, 8.6%; HERCULES, 3.1%; Post-HERCULES, 10.7%; Japanese study, 14.3%). TE functional neutralizing ADA (NAb) and TE ANP were measured in 3 of the 4 trials. TE functional NAbs were present in 2.1% of patients in HERCULES and in 12.0% of patients in Post-HERCULES; no patients in the Japanese study had detectable functional NAb. TE ANPs were detected in 4.1% of patients in HERCULES, in 2.7% of patients in Post-HERCULES, and in 14.3% of patients in the Japanese study. Overall, titers of TE ADA-positive subjects were low, and no differences in magnitude of response were observed between cohorts within a given study.

The following sections will focus on results from HERCULES and the Japanese study, as the results from these studies are representative of all; TITAN and Post-HERCULES are described in more detail where relevant.

3.2 | Efficacy outcomes

In the largest cohort analyzed (HERCULES, N = 97), comparable time to platelet count response was observed in patients who were negative for pre-Abs or TE ADAs, positive for pre-Abs only, or positive for TE ADAs only (Figure 2A). Similarly, no effect of pre-Ab or TE ADA on platelet count response was observed in the TITAN, Post-HERCULES, or Japanese study populations when assessed by patients categorized by their ADA, mADA, and NAb assay results (data not shown).

TABLE 1 Patient demographics in the intention-to-treat population for all trials.

	TITAN [11] NCT01151423		HERCULES [12][a] NCT02553317		Post-HERCULES [13] NCT02878603		Japanese study [10] NCT04074187
Characteristic	**Caplacizumab**	**Placebo**	**Caplacizumab**	**Placebo**	**Caplacizumab**	**Placebo**	**Caplacizumab**
Number of patients, N	36	39	72	73	75	29	21
Age, y (mean)	41 (19-72)	43 (21-67)	45 (18-77)	47 (21-79)	46 (12)	52 (14.8)	58 (21)
Female sex, n (%)	24 (67)	20 (51)	49 (68)	51 (70)	51 (68)	23 (79)	10 (48)
Race, n (%)							
White	32 (89)	34 (87)	47 (65)	50 (68)	52 (69)	21 (72)	Japanese: 21 (100)
Black	4 (11)	5 (13)	15 (21)	13 (18)	13 (17)	6 (21)	
Asian	-	-	4 (6)	0	3 (4)	0	
Other	-	-	3 (4)	1 (1)	2 (2.7)	0	
Data missing	-	-	3 (4)	9 (12)	5 (7)	2 (7)	

[a]Double-blind period only.

3.3 | PK/PD outcomes

In the HERCULES trial, an increase in mean caplacizumab concentration over time was seen in 2 patients with TE ADAs, and mean caplacizumab concentrations appeared slightly lower in patients with pre-Abs compared to those without pre-Abs (Figure 2B). While pre-Abs did not influence the percentage change from baseline in VWF:Ag concentrations, patients who were positive for TE ADAs did show an increase in VWF:Ag over time (Figure 2C). No apparent influence of pre-Abs, TE ADAs, or NAbs/ANPs on RICO activity was observed (Figure 2D). In the Japanese trial, the caplacizumab concentration was slightly higher in patients with pre-Abs vs. those without pre-Abs; there was no clear impact of TE ADAs or NAbs/ANPs on caplacizumab concentration over time. Pre-abs, TE ADAs, or NAbs/ANPs did not appear to impact RICO activity or percentage change from baseline in VWF:Ag concentrations (data not shown). Additionally, ADAs did not have an effect on RICO or VWF:Ag in the TITAN (pre-Ab and TE ADA) or Post-HERCULES (pre-Ab, TE ADA, and NAb/ANPs) trials.

3.4 | Safety outcomes

The rates of AEs and serious AEs were comparable in the caplacizumab and placebo groups across the 4 trials in safety-evaluable patients (Table 3). AEs were reported in 91% to 100% of patients who received caplacizumab and 90% to 100% of patients who received placebo. Serious AEs were reported in 32% to 37% and 16% to 55% of patients in the caplacizumab and placebo groups, respectively. Across the 4 trials, no serious AEs were reported among patients with TE ADAs.

No association was found between caplacizumab-binding antibodies/ADAs and safety outcomes. In TITAN, no relationship was found between pre-Abs or TE ADAs and AEs. In subgroup analyses

TABLE 2 Immunogenicity results in patients with iTTP treated with caplacizumab.

	TITAN [11] NCT01151423	HERCULES [12] NCT02553317	Post-HERCULES [13] NCT02878603	Japanese study [10] NCT04074187
Antibody type	**N = 35**	**N = 97[a]**	**N = 75[b]**	**N = 21**
Patients pre-Ab positive, n (%)	6 (17.1)	55 (56.7)	20 (26.7)	8 (38.1)
Patients drug-induced TE ADA positive, n (%)	3 (8.6)	3 (3.1)	8 (10.7)	3 (14.3)
Patients TE NAb positive, n (%) (NAb assay)	N/A	2 (2.1)	9 (12.0)	0 (0)
Patients TE NAb positive, n (%) (NECA)	N/A	4 (4.1)	2 (2.7)	3 (14.3)

ADA, antidrug antibody; iTTP, immune-mediated thrombotic thrombocytopenic purpura; NAb, neutralizing antibody; NECA, neutralizing epitope characterization assay; pre-Ab, pre-existing antibody; TE, treatment-emergent.
[a]All patients who were treated with caplacizumab (either during the double-blind period only and/or during the open-label period; n = 97).
[b]A total of 9 patients were re-treated with caplacizumab.

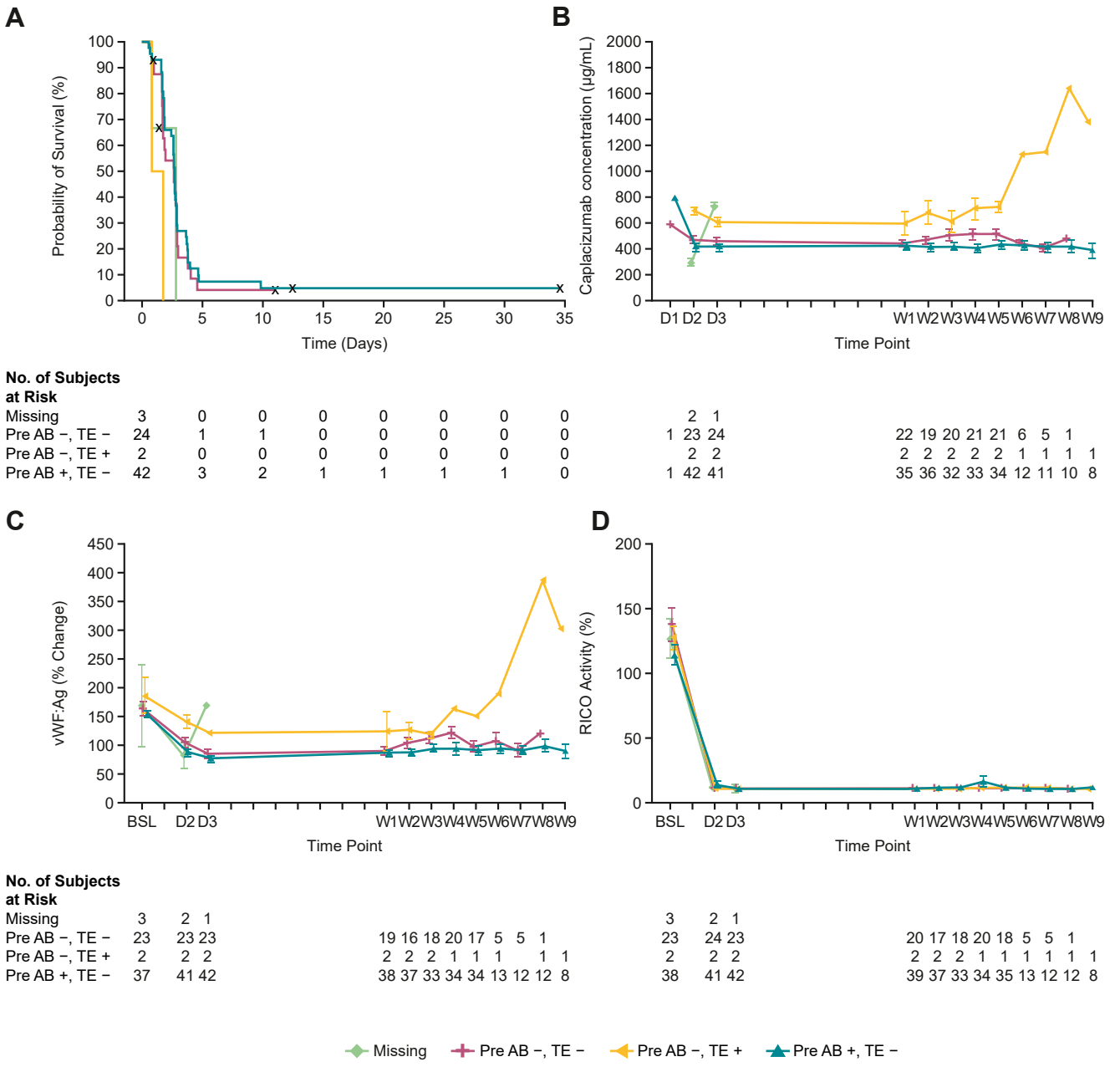

FIGURE 2 (A) Time to platelet count response, (B) pharmacokinetic concentration, (C) percent change from baseline in von Willebrand factor antigen concentration, and (D) mean RICO activity by ADA/mADA status (HERCULES study). −, negative; +, positive; BSL, baseline; D, day; Pre-AB, pre-existing antibodies PK, pharmacokinetic; RICO, ristocetin cofactor activity; TE, treatment-emergent; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; W, week.

performed in the HERCULES, Post-HERCULES, and Japanese studies, there was no association between hypersensitivity reactions and the presence of pre-Abs and/or drug-induced TE ADAs.

4 | DISCUSSION

This analysis of data from the 4 completed trials in the caplacizumab clinical development program demonstrates that caplacizumab treatment is associated with a low incidence of TE ADAs and TE ADAs with neutralizing potential in patients with iTTP. The proportion of patients with pre-Abs varied across studies, with the highest proportion in HERCULES and the lowest in TITAN. The incidence of drug-induced TE ADAs did not exceed 15% in any trial. Furthermore, presence of antibodies did not appear to impact the primary efficacy endpoints in the HERCULES (time to platelet count response) or Japanese (recurrence of iTTP) studies. For PK/PD outcomes, although caplacizumab concentration varied slightly according to presence of pre-Abs, there was no apparent impact of any antibody type on RICO activity or change from baseline in VWF:Ag concentrations. It is important to

TABLE 3 Overall safety outcomes.

	TITAN [11] NCT01151423 Safety population		HERCULES [12] NCT02553317 ITT population		Post-HERCULES [13] NCT02878603 ITO population		Japanese study [10] NCT04074187 mITT population
AEs	Caplacizumab	Placebo	Caplacizumab	Placebo	Caplacizumab	Placebo	Caplacizumab
Number of patients, N	35	37	72	73	75	29	21
AEs	34 (97)	37 (100)	68 (96)	66 (90)	68 (91)	26 (90)	21 (100)
SAEs	13 (37)	12 (32)	23 (32)	12 (16)	28 (37)	16 (55)	5 (24)
Bleeding-related AEs	19 (54)	14 (38)	46 (65)	35 (48)	16 (21)	9 (31)	7 (33)
Death	0	2 (5)	1 (1)	3 (4)	0	1 (3)	0

AE, adverse event; DB, double-blind; ITO, intention to observe (all enrolled patients); ITT, intention to treat (all randomized patients); mITT, modified intention to treat (enrolled patients who received ≥1 dose of study drug); OL, open-label; SAE, serious adverse event.

note that the overall numbers of TE ADA-positive subjects in the studies to date are relatively small, and conclusions regarding variations between studies cannot be drawn. No association was noted between antibody presence and safety outcomes in any of the studies.

The results of this analysis showed that pre-Abs were more common than TE ADAs in caplacizumab-treated populations in TITAN, HERCULES, Post-HERCULES, and the Japanese study. TPE can complicate the measurement of ADAs by transfer of pre-Abs via donor plasma or by dilution of TE ADAs, potentially skewing assessment of caplacizumab immunogenicity or efficacy. The use of the mADA assay in caplacizumab clinical studies reduced the signal from pre-Abs and enabled differentiation between pre-Abs and TE ADAs, which provided a more accurate assessment of the immune responses to caplacizumab. However, while the signal is reduced via this bioanalytical strategy, full exclusion of pre-Abs in the assay cannot be guaranteed.

The process of measuring pre-Abs accurately could be simplified if patients did not also receive TPE; on this note, the MAYARI study should be mentioned. The MAYARI study is an ongoing phase 3, open-label, single-arm trial that aims to evaluate the efficacy and safety of caplacizumab and IST without first-line TPE in adults with iTTP [16]. Without the potential interference of TPE, the therapeutic approach adopted in MAYARI will hopefully allow for a more straightforward evaluation of the immunogenicity of caplacizumab. The MAYARI study will utilize only the NECA assay for ANP detection because this method is considered to have good sensitivity properties and also meets drug tolerance requirements; the latter criterion is not fulfilled with the functional NAb assay [17].

Notably, caplacizumab is the first approved Nanobody®-based medicine for the treatment of iTTP [18]. Ozoralizumab, used for inflammatory diseases such as rheumatoid arthritis, is another approved Nanobody®-based medicine that showed no impact of immunogenicity on safety and efficacy in clinical trials, which should be encouraging for both patients and physicians [19,20].

To the best of our knowledge, this is the first comprehensive report of the immunogenicity response in patients with iTTP who received caplacizumab. Strengths of this study include the robust bioanalytical strategy that used 4 types of assays, allowing differentiation of pre-Abs from TE ADAs and characterization of the immune response for the neutralizing potential of detected ADAs. The wide geographic range across the 4 separate trials allowed for assessment of ADAs in diverse patient populations.

In conclusion, a robust assay strategy was able to demonstrate the levels of pre-Ab and TE ADA in patients with iTTP receiving caplacizumab after TPE. This study found that pre-Abs were common in some populations, but TE ADAs and NAbs/ANPs were not commonly observed in any of the tested populations, signifying that de novo development of ADAs to caplacizumab does not occur frequently. Moreover, pre-Abs, TE ADAs, and NAbs/ANPs do not appear to impact caplacizumab efficacy or safety, supporting the use of caplacizumab as an ongoing, reliable treatment option for patients with iTTP.

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AUTHOR CONTRIBUTIONS

B.V.B., M.L.S.-M., A.P.M., Y.V., G.M., S.G., and S.P. wrote/reviewed the draft manuscript and approved the final version for publication.

RELATIONSHIP DISCLOSURE

B.V.B., M.L.S.-M., A.P.M., Y.V., G.M., S.G., and S.P. are employees of Sanofi and may hold shares and/or stock options in the company.

DATA AVAILABILITY

Qualified researchers may request access to patient-level data and related study documents, which may include clinical study report,

study protocol with any amendments, statistical analysis plan, and dataset specifications. Patient-level data are anonymized. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at: https://www.vivli.org/.

REFERENCES

[1] Sukumar S, Lämmle B, Cataland SR. Thrombotic thrombocytopenic purpura: pathophysiology, diagnosis, and management. *J Clin Med*. 2021;10:536.

[2] Hanlon A, Metjian A. Caplacizumab in adult patients with acquired thrombotic thrombocytopenic purpura. *Ther Adv Hematol*. 2020;11:2040620720902904.

[3] Chaturvedi S, Abbas H, McCrae KR. Increased morbidity during long-term follow-up of survivors of thrombotic thrombocytopenic purpura. *Am J Hematol*. 2015;90:E208.

[4] Upreti H, Kasmani J, Dane K, Braunstein EM, Streiff MB, Shanbhag S, et al. Reduced ADAMTS13 activity during TTP remission is associated with stroke in TTP survivors. *Blood*. 2019;134:1037–45.

[5] Joly BS, Coppo P, Veyradier A. An update on pathogenesis and diagnosis of thrombotic thrombocytopenic purpura. *Expert Rev Hematol*. 2019;12:383–95.

[6] Kremer Hovinga JA, Coppo P, Lämmle B, Moake JL, Miyata T, Vanhoorelbeke K. Thrombotic thrombocytopenic purpura. *Nat Rev Dis Primers*. 2017;3:17020.

[7] Zheng XL, Vesely SK, Cataland SR, Coppo P, Geldziler B, Iorio A, et al. ISTH guidelines for treatment of thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2020;18:2496–502. https://doi.org/10.1111/jth.15010

[8] Duggan S. Caplacizumab: first global approval. *Drugs*. 2018;78:1639–42.

[9] Sanofi. Cablivi - Highlights of prescribing information. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/761112s000lbl.pdf; 2019 [accessed August 20, 2024].

[10] Miyakawa Y, Imada K, Ichikawa S, Uchiyama H, Ueda Y, Yonezawa A, et al. The efficacy and safety of caplacizumab in Japanese patients with immune-mediated thrombotic thrombocytopenic purpura: an open-label phase 2/3 study. *Int J Hematol*. 2023;117:366–77.

[11] Peyvandi F, Scully M, Kremer Hovinga JA, Cataland S, Knöbl P, Wu H, et al. Caplacizumab for acquired thrombotic thrombocytopenic purpura. *N Engl J Med*. 2016;374:511–22. https://doi.org/10.1056/NEJMoa1505533

[12] Scully M, Cataland SR, Peyvandi F, Coppo P, Knöbl P, Kremer Hovinga JA, et al. Caplacizumab treatment for acquired thrombotic thrombocytopenic purpura. *N Engl J Med*. 2019;380:335–46.

[13] Scully M, de la Rubia J, Pavenski K, Metjian A, Knöbl P, Peyvandi F, et al. Long-term follow-up of patients treated with caplacizumab and safety and efficacy of repeat caplacizumab use: Post-HERCULES study. *J Thromb Haemost*. 2022;20:2810–22.

[14] van Brummelen EMJ, Ros W, Wolbink G, Beijnen JH, Schellens JHM. Antidrug antibody formation in oncology: clinical relevance and challenges. *Oncologist*. 2016;21:1260–8.

[15] Food and Drug Association (FDA). Guidance Document: Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/immunogenicity-testing-therapeutic-protein-products-developing-and-validating-assays-anti-drug; 2019 [accessed August 20, 2024].

[16] European Medicines Agency (EMA). Guideline on Immunogenicity assessment of therapeutic proteins. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-immunogenicity-assessment-therapeutic-proteins-revision-1_en.pdf; 2017 [accessed August 20, 2024].

[17] Coddens A, Snoeck V, Bontinck L, Buyse MA, Pine SO. An innovative method for characterizing neutralizing antibodies against antibody-derived therapeutics. *J Immunol Methods*. 2020;487:112896.

[18] Jin BK, Odongo S, Radwanska M, Magez S. NANOBODIES: a review of diagnostic and therapeutic applications. *Int J Mol Sci*. 2023;24:5994.

[19] Ishiwatari-Ogata C, Kyuuma M, Ogata H, Yamakawa M, Iwata K, Ochi M, et al. Ozoralizumab, a humanized anti-TNFα NANOBODY compound, exhibits efficacy not only at the onset of arthritis in a human TNF transgenic mouse but also during secondary failure of administration of an anti-TNFα IgG. *Front Immunol*. 2022;13:853008.

[20] Kyuuma M, Kaku A, Mishima-Tsumagari C, Ogawa B, Endo M, Tamura Y, et al. Unique structure of ozoralizumab, a trivalent anti-TNFα NANOBODY compound, offers the potential advantage of mitigating the risk of immune complex-induced inflammation. *Front Immunol*. 2023;14:1149874.

SUPPLEMENTARY MATERIAL

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