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# Generation of anti-idiotypic antibodies to detect anti-spacer antibody idiotopes in acute thrombotic thrombocytopenic purpura patients

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**Abstract**

In autoantibody-mediated autoimmune diseases, autoantibody profiling allows to stratify patients and link autoantibodies with disease severity and outcome. However, in immune-mediated thrombotic thrombocytopenic purpura patients, stratification according to antibody profiles and their clinical relevance has not been fully explored. We aimed at developing a new type of autoantibody profiling assay for immune-mediated thrombotic thrombocytopenic purpura based on the use of anti-idiotypic antibodies. Anti-idiotypic antibodies against 3 anti-spacer autoantibodies were generated in mice and were used to capture the respective anti-spacer idiotopes from 151 acute immune-mediated thrombotic thrombocytopenic purpura plasma samples. We next deciphered these anti-spacer idiotope profiles in immune-mediated thrombotic thrombocytopenic purpura patients and investigated if these limited idiotope profiles could be linked with disease severity. We developed 3 anti-idiotypic antibodies that recognized particular idiotopes in the anti-spacer autoantibodies II-1, TTP73 or I-9, that are involved in ADAMTS13 binding. Thirty-five, 24 and 42% of patients were positive for antibodies with the II-1, TTP73 and I-9 idiotopes, respectively. Stratifying patients according to the corresponding 8 anti-spacer idiotope profiles revealed an until now unknown insight into the anti-spacer II-1, TTP73 and I-9 idiotope profiles in these patients. Finally, these limited idiotope profiles showed no association with disease severity. We successfully developed 3 anti-idiotypic antibodies that allowed us to determine the profiles of the anti-spacer II-1, TTP73 and I-9 idiotopes in immune-mediated thrombotic thrombocytopenic purpura patients. Increasing the number of patients and/or future development of additional anti-idiotypic antibodies against other anti-ADAMTS13 autoantibodies might allow to identify idiotope profiles of clinical, prognostic value.

**Keywords** autoimmune disease, antibody profiling, anti-idiotypic antibody, thrombotic thrombocytopenic purpura, ADAMTS13

## Introduction

In autoantibody-mediated autoimmune diseases, patients develop autoantibodies against self-antigens.[1] The autoantibody response can be directed to multiple self-antigens like in systemic sclerosis[2], Sjögren syndrome[3] and type 1 diabetes[4] or to a single self-antigen like myasthenia gravis[5] and Graves' disease.[6] Patients suffering from the autoimmune disorder immune-mediated thrombotic thrombocytopenic purpura (iTTP) present with an autoantibody response against one antigen, the von Willebrand factor (VWF) cleaving protease ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats, member 13).[7,8] Deficiency in ADAMTS13 leads to accumulation of hyper-active ultra-large VWF multimers that spontaneously interact with platelets. The resulting microthrombi block arterioles and capillaries, which leads to severe thrombocytopenia, hemolytic anemia and organ failure. The VWF cleaving protease ADAMTS13 consists of 14 domains: the metalloprotease (M), disintegrin-like (D), cysteine-rich (C) and spacer (S) domains, 8 thrombospondin type 1 repeats (T1-8) and 2 CUB domains.[9] It is known that the anti-ADAMTS13 autoimmune response in iTTP patients is polyclonal but 80-100% of patients possess autoantibodies targeting the cysteine-rich and spacer domain.[7,10–12] The standard treatment for iTTP is plasma exchange (PEX) often in combination with immunosuppressive agents (mainly steroids and rituximab).[8] Recently, the anti-VWF nanobody caplacizumab, used as a frontline therapy together with PEX hastened TTP recovery, opening promising perspectives to improve the prognosis of the disease.[13,14] Splenectomy is only performed in the most severe patients, when other measures have failed.[8,15]

Since autoimmune diseases manifest differently among patients and have a chronic course with recurring acute bouts, biomarkers are identified that allow patient stratification to predict disease outcome and prognosis and to adapt specific treatment.[16] Obviously, autoantibodies are useful biomarkers in autoimmune diseases and autoantibody profiling has been shown to be valuable in stratifying patients with autoimmune disorders.[17,18] On the one hand, autoantibody profiling approaches are based on the binding of the patient autoantibodies to the disease causing antigen (recombinant proteins, fragments thereof or peptides).[19,20] Whereas, on the other hand, autoantibody profiling can be done independent of the antigen using anti-idiotypic

antibodies that recognize autoantibodies that bind to the antigen (Figure 1).[21] Anti-idiotypic antibodies can be generated by immunizing mice with purified or cloned antigen-binding antibodies.[22–24] Antibodies that bind to particular idiotopes involved in antigen binding can next be used to detect specific autoantibodies in patient plasma or serum.[21] Finally, even if the disease-causing antigen is not known, antibody profiling can lead to the identification of disease-linked peptides using next generation sequencing[25] and mass spectrometry[26,27] of the total antibody response in autoimmune disease patients.

Also iTTP is a chronic disease with a variable disease outcome and risk for relapse.[28] Levels of ADAMTS13 activity, anti-ADAMTS13 autoantibody subtypes, ADAMTS13 antigen levels or a combination thereof have been used to identify patient groups with a worse disease outcome or a higher risk for relapse.[28–35] Although the outcome of the different studies is variable, it has been shown for example that an ADAMTS13 activity < 10% during acute disease is linked with an increased risk for relapse[35] and that presenting anti-ADAMTS13 autoantibody and ADAMTS13 antigen levels predict prognosis.[31] In addition, prognostic scoring systems based on clinical and or laboratory parameters have been set up to predict severe cases and patients at risk; from 1987 with the Rose index[36,37], to more recently the PLASMIC score[38] and the score by Benhamou *et al.*.[39] The predictive model set up by Benhamou and colleagues takes into account age, lactate dehydrogenase (LDH) levels and cerebral involvement and detects early death in acquired severe ADAMTS13 deficiency-associated idiopathic TTP.[39] However, in iTTP, autoantibody profiling has not been extensively explored yet to stratify patients.

In this project, we developed an autoantibody profiling assay for iTTP using anti-idiotypic antibodies that recognize particular idiotopes on anti-ADAMTS13 autoantibodies, idiotopes that are involved in ADAMTS13 binding (Figure 1). Since the ADAMTS13 spacer domain seems to be the main immunogenic region targeted in these patients[29], we generated an anti-idiotypic antibody against 3 available cloned human anti-spacer autoantibodies. The selected anti-idiotypic antibodies were then used to screen 151 iTTP plasmas for the presence of autoantibodies with the same idiotopes across patients, which resulted in stratification of iTTP

patients according to these anti-spacer idiotope profiles. We next investigated in a subgroup of 95 patients whether certain anti-spacer idiotope profiles could be linked with disease severity.

## Methods

### Immunization strategy and characterization of anti-II-1, anti-TTP73 and anti-I-9 antibodies

Anti-II-1, anti-TTP73 and anti-I-9 antibodies were developed by immunizing BALB/c mice (Janvier Labs, Le Genest-Saint-Isle, France) with the cloned human anti-spacer autoantibodies II-1[40], TTP73, or I-9[41], respectively (see immunization strategy in Supplemental methods). The binding of purified anti-II-1, anti-TTP73 or anti-I-9 antibodies to II-1, TTP73 and I-9 respectively and to the conserved regions (Figure 1, grey) in human immunoglobulin G (IgG) antibodies were identified using ELISA (see 'ELISA to study the binding of murine anti-II-1, anti-TTP73 and anti-I-9 antibodies to coated human anti-spacer autoantibodies II-1, TTP73 and I-9 and, to a pool of human IgG antibodies' in Supplemental material and methods).

### ELISA to identify anti-II-1, anti-TTP73 and anti-I-9 antibodies that inhibit the binding of respectively anti-spacer autoantibodies II-1, TTP73 or I-9 to ADAMTS13

Human anti-spacer autoantibodies II-1, TTP73 or I-9 (constant final EC50: 0.04, 0.85 and 0.04 µg/mL, respectively; see Supplemental methods), were pre-incubated with a 1 in 2 dilution series of murine anti-II-1, anti-TTP73 or anti-I-9 antibodies (final start concentration 10 µg/mL) respectively, in a pre-blocked plate. After 30 minutes, samples were transferred to a recombinant human (rh)ADAMTS13 (2.7 µg/mL in phosphate buffered saline (PBS)) coated plate. Bound human anti-spacer autoantibodies II-1, TTP73 or I-9 were detected using a mixture of HRP-labelled anti-human $IgG_{1-4}$ ($IgG_1$: 1/20,000 and $IgG_{2-4}$: 1/2,000; Sanquin, Amsterdam, The Netherlands) (see Supplemental methods for more details).

### ELISA to study the binding of the anti-idiotypic antibodies to the anti-spacer idiotopes of II-1, TTP73 and I-9

Murine anti-idiotypic antibodies 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) and 7D10 (anti-I-9 antibody) were coated at 5 µg/mL in carbonate/bicarbonate coating buffer (50mM $Na_2CO_3$/$NaHCO_3$, pH 9.6). After blocking, human anti-spacer autoantibodies II-1, TTP73 and I-9 were added at a start concentration of 1 µg/mL and further 1 in 2 diluted. Bound anti-spacer

autoantibodies were detected by adding a mixture of anti-human $IgG_{1-4}$-HRP antibodies (Sanquin) (see Supplemental methods for more details).

**Patient samples**

Detailed information about the 151 iTTP plasma samples can be found in Supplemental methods 'Patient samples'. The study protocol was approved by the Medical Ethical Committee of the University Medical Center Utrecht (Utrecht, The Netherlands), the Ethics Committee of Hospital Pitié-Salpêtrière and Hospital Saint-Antoine (Paris, France) and the Ethics Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy), and was in accordance with the Declaration of Helsinki.

**ELISA to identify the presence of anti-spacer idiotope profiles in plasmas of acute iTTP patients using the newly developed anti-idiotypic antibodies**

Murine anti-idiotypic antibody 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) or 7D10 (anti-I-9 antibody) were coated at 5 µg/mL. After blocking, patient plasma (start dilution 10%, v/v) was added and 1 in 2 diluted. Bound patient antibodies were detected with anti-human $IgG_{1-4}$-HRP (Sanquin) (see Supplemental methods for more details).

**Statistical analysis**

Graphpad Prism v5.03 software (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis (see Supplemental methods for more details).

## Results

### Development of anti-idiotypic antibodies against idiotopes in anti-spacer autoantibodies II-1, TTP73 or I-9 involved in ADAMTS13 binding

To generate anti-idiotypic antibodies recognizing particular idiotopes in anti-spacer autoantibodies involved in ADAMTS13 binding, three cloned human anti-spacer autoantibodies with different epitopes and inhibitory characteristics were available: II-1[40], TTP73[42] and I-9[41] (see supplemental material and methods) and were used to immunize BALB/c mice. As the injected anti-spacer autoantibodies are full IgG antibodies in which the variable regions are grafted on a human IgG1 constant region[40,41], the mice developed antibodies that either recognized conserved regions (e.g. constant regions: $C_H$ and $C_L$ and framework regions in $V_H$ and $V_L$; Figure 1, grey parts) or idiotopes in the complementarity determining regions (CDRs) of the $V_H$ and $V_L$ of II-1, TTP73 and I-9 (Figure 1, dark and light blue dots). We obtained 1 mouse monoclonal antibody that recognized anti-spacer autoantibody II-1, 2 that recognized anti-spacer autoantibody TTP73 and 10 that recognized anti-spacer autoantibody I-9 (Figure 2A) as the generated antibodies bound to the coated anti-spacer autoantibodies II-1, TTP73 or I-9, respectively. To identify which of the generated monoclonal antibodies recognized the conserved part of the human autoantibodies ($C_H$, $C_L$ and framework regions in $V_H$ and $V_L$; Figure 1, grey parts), their binding to a pool of purified human IgG antibodies was studied. Monoclonal antibody 17H9 recognizing anti-spacer autoantibody II-1 did not recognize the conserved part of the coated human IgG antibodies (Figure 2B), while 1 of the monoclonal antibodies (20H3) recognizing anti-spacer autoantibody TTP73 and 9 of the monoclonal antibodies (1E6, 5C8, 6C9, 7E8, 9F9, 9G9, 9H4, 11F7, and 14G6) recognizing anti-spacer autoantibody I-9 did bind to the conserved part of the coated human IgG antibodies (Figure 2B). Hence, monoclonal antibodies 17H9, 9G12 and 7D10 are anti-idiotypic antibodies that target idiotopes in the CDRs of $V_H$ and $V_L$ of respectively anti-spacer autoantibody II-1, TTP73 or I-9 (Figure 2B).

We next aimed to identify if the anti-idiotypic antibodies recognizing particular idiotopes in the anti-spacer autoantibodies II-1, TTP73 and I-9 are anti-idiotypic antibodies that are involved in ADAMTS13 binding (Figure 1, dark blue antibody). To do so, we used a competition ELISA where we studied if the binding of anti-spacer autoantibodies II-1, TTP73 and I-9 could be inhibited by

their respective anti-idiotypic antibody. The 3 developed anti-idiotypic antibodies (17H9, 9G12 and 7D10) inhibited the binding of their respective anti-spacer autoantibodies (II-1, TTP73 and I-9) to rhADAMTS13 (Figure 2C). ~~Figure 2D summarizes the developed murine anti-II-1, anti-TTP73 and anti-I-9 antibodies targeting the conserved region of the antibody (grey) or the anti-idiotypic antibodies specific for the idiotopes present in the CDRs (in red, green, orange) of anti-spacer autoantibody II-1, TTP73 and I-9 respectively.~~

In conclusion, we developed 3 anti-idiotypic antibodies that recognize particular idiotopes in the anti-spacer autoantibodies II-1, TTP73 and I-9 that are involved in ADAMTS13 binding, as they strongly inhibit the binding of anti-spacer autoantibodies II-1, TTP73 or I-9, respectively, to rhADAMTS13.

**Anti-idiotypic antibodies and their binding to idiotopes in II-1, TTP73 and I-9**

Since anti-spacer autoantibodies II-1 and I-9 have overlapping but different epitopes (see Supplemental methods)[43], they will have both shared and unique idiotopes. We therefore investigated whether anti-idiotypic antibodies developed against anti-spacer autoantibody II-1 (17H9) and I-9 (7D10) recognized shared or unique idiotopes in II-1 and I-9. As a control, we included the anti-idiotypic antibody 9G12 developed against the anti-spacer autoantibody TTP73, which does not have an overlapping epitope with II-1 and I-9.

The anti-idiotypic antibody against anti-spacer autoantibody II-1 (17H9) recognized a unique idiotope in II-1 as it only captured II-1 and not anti-spacer autoantibodies I-9 and TTP73 (Figure 3A). As expected, the anti-idiotypic antibody against TTP73 (9G12) also recognized a unique idiotope in anti-spacer TTP73 as it only captured TTP73 and not anti-spacer autoantibodies II-1 and I-9 (Figure 3B). In contrast, the anti-idiotypic antibody (7D10) against the anti-spacer I-9 idiotope captured both anti-spacer autoantibody I-9 and II-1 (Figure 3C) showing that anti-idiotypic antibody 7D10 recognizes a common idiotope in II-1 and I-9.

In conclusion, these data show that the anti-idiotypic antibodies against anti-spacer autoantibody II-1 (17H9) and TTP73 (9G12) recognize a unique idiotope in II-1 and TTP73 respectively, whereas the anti-idiotypic antibody developed against anti-spacer autoantibody I-9 (7D10) recognizes an idiotope present in both anti-spacer autoantibodies II-1 and I-9 (Figure 3).

**Identification of anti-spacer idiotope profiles in plasmas of acute iTTP patients using the newly developed anti-idiotypic antibodies**

In a first step, we screened the plasmas of 151 iTTP patients for the presence or absence of the anti-spacer II-1, TTP-73 and I-9 idiotopes using the 3 newly developed anti-idiotypic antibodies. In a second step, we stratified the patients according to their anti-spacer idiotope profile.

The 151 iTTP plasma samples were all collected during an acute iTTP episode (see detailed information in Supplemental methods). All patients presented with severe ADAMTS13 deficiency (< 10% activity) and detectable anti-ADAMTS13 IgG titers. Anti-ADAMTS13 IgG titers ranged from 16 to ≥100 IU/mL (median: 87 IU/mL, Figure 4). Of the 151 iTTP patients, 34% (52/151) were positive for antibodies with the anti-spacer II-1 idiotope (recognized by anti-idiotypic antibody 17H9) (Figure 4A, red dots) with median anti-spacer II-1 idiotope levels of 47 ng/mL (Figure 4A, red squares). Twenty-five percent (37/151) of the patients were positive for antibodies with anti-spacer TTP73 idiotope (recognized by anti-idiotypic antibody 9G12) (Figure 4B, green dots) with median anti-spacer TTP73 idiotope levels of 174 ng/mL (Figure 4B, green squares). Forty-two percent (63/151) of the patients were positive for antibodies with anti-spacer I-9 idiotope (recognized by anti-idiotypic antibody 7D10) (Figure 4C, orange dots) with median anti-spacer I-9 idiotope levels of 57 ng/mL (Figure 4C, orange squares).

We next stratified the acute iTTP patients according to their anti-spacer idiotope profile (Figure 5). The 8 possible profiles correspond to the presence of either 1, 2, 3 or none of the 3 anti-spacer idiotopes. All 8 anti-spacer idiotope profiles were identified in the iTTP patient cohort (n=151) (Figure 5). In 28% (42/151) of the patients, only one particular idiotope could be detected in the plasma, with 8% (12/151) having the II-1 idiotope (profile 1), 4% (6/151) having the TTP73 idiotope (profile 2) and 16% (24/151) having the I-9 idiotope (profile 3). In 19% (28/151) of the patients, 2 idiotopes were identified in their antibody repertoire, with 5% (7/151) having II-1 and TTP73 idiotopes (profile 4), 10% (15/151) having II-1 and I-9 idiotopes (profile 5) and 4% (6/151) having I-9 and TTP73 idiotopes (profile 6). In 12% (18/151) of the patients all 3 idiotopes were present in their antibody repertoire (profile 7). In 42% (63/151) of the patients none of the 3 idiotopes were detected (profile 8).

In conclusion, using the 3 developed anti-idiotypic antibodies, we here for the first time unraveled the specific II-1, TTP73 and I-9 idiotope profiles in iTTP patients and showed that 58% of the patients had antibodies with II-1, TTP73 and I-9 idiotopes in their plasma and this in different combinations while 42% of the patients were negative for these idiotopes.

**Anti-spacer idiotope profiles and their possible link with disease severity**

We next investigated whether the identified anti-spacer idiotope profiles (Figure 5) could be linked with disease severity, although the number of patients per profile group was rather low and we only screened for the presence or absence of 3 anti-spacer idiotopes. As a measure of disease severity, we studied disease outcome and applied treatment strategy. This part of the study was performed on the 95 patients of the French Reference Center for TMA, as detailed information on laboratory, clinical and outcome parameters were available for these patients (Supplemental Table 2).

We first analyzed whether the anti-spacer idiotope profiles could be linked with disease outcome. Disease outcome was previously identified in the patients at time of diagnosis by determining a score defined by Benhamou *et al.*.[39] This score (either 1, 2, 3 or 4) is a risk score for early death in TTP based on 3 factors related to clinical and biological presentation (age, high LDH levels and cerebral involvement). A score of ≥ 3 has a positive predictive value for mortality (patients at risk for 30-day mortality after treatment initiation) and a score < 3 has a negative predictive value.[39] To check whether the disease outcome parameter could be linked with specific anti-spacer idiotope profiles, we performed chi-square-based analysis. However, none of the anti-spacer idiotope profiles could be linked with a score of ≥ 3 (chi square, non-significant) (Figure 6A). In line with this, there was no link between the anti-spacer idiotope profiles and the individual factors related to the score by Benhamou *et al.*.[39] (age: ANOVA, non-significant; cerebral involvement and high LDH levels: chi square, non-significant) (Supplemental Figure 1).

We next used the same approach to investigate whether anti-spacer idiotope profiles could be linked with the applied treatment strategy. We therefore compared the anti-spacer idiotope profiles in patients treated with PEX with/without rituximab and patients treated with PEX

with/without rituximab and additional treatment(s) (either steroids or other immunosuppressive drugs, e.g. cyclophosphamide, bortezomib; or/and caplacizumab or/and splenectomy; Supplemental Table 2). However, also treatment could not be linked with anti-spacer idiotope profiles (chi square, non-significant) (Figure 6B).

## Discussion

In this paper, we successfully generated 3 anti-idiotypic antibodies that specifically recognized the idiotopes of anti-spacer autoantibodies II-1, TTP73 and I-9. With this anti-idiotypic assay, we could for the first time identify the presence or absence of anti-spacer II-1, TTP73 and I-9 idiotopes in iTTP patients. In addition, grouping the patients according to the absence or presence of one, two or three of the anti-spacer idiotopes, revealed an until now unknown insight into the anti-spacer II-1, TTP73 and I-9 idiotopes in these patients. Although the resulting idiotope profiles could not be linked with disease severity, our data show that anti-idiotypic antibodies are interesting tools to determine an antibody profile in patients with any autoimmune disease.

Many studies have used groups of ADAMTS13 domains to identify which ADAMTS13 domains (e.g. MDTCS, MDT, CS, T2-C2, T2-T8, C1-C2) are targeted by anti-ADAMTS13 autoantibodies in individual iTTP patients. All these studies concluded that the immune response in iTTP patients is polyclonal with an immuno-dominant epitope in the cysteine-spacer domain.[8,10–12,29,43–45] Antibody profiling based on these data, stratifies patients according to either the presence or absence of anti-ADAMTS13 antibodies against (a) certain domain(s). Only 2 studies investigated the link between domain specificity of anti-ADAMTS13 antibodies and disease severity or platelet counts. Thomas *et al.*[29] stratified iTTP patients according to having either anti-MDTCS or anti-T2-C2 autoantibodies but could not identify a link with disease severity while Zheng *et al.*[10] reported an inverse correlation between the presence of IgG antibodies against the T2-T8 and/or C1-C2 domains and platelet counts on admission. In our study, we used anti-idiotypic antibodies to stratify iTTP patients according to the presence or absence of anti-ADAMTS13 antibodies with specific idiotopes. By using an anti-idiotypic antibody, we can hence investigate whether a specific anti-ADAMTS13 idiotope is present or absent in an iTTP patient. Indeed, with our 3 anti-idiotypic antibodies, we determined the until now unknown anti-spacer II-1, TTP73 and I-9 idiotope profiles in 151 iTTP patients in acute phase. Eighteen of the 151 iTTP patients had all 3 anti-spacer idiotopes in their plasma, 63 patients had none of the anti-spacer idiotopes and 70 patients had either one or a combination of 2 of the anti-spacer idiotopes in their

plasma, showing that the presence of these 3 anti-spacer idiotopes is not a common feature in iTTP patients. In addition, anti-spacer autoantibody II-1[40] used in this study, is a well characterized iTTP patient autoantibody that targets the R568-F592-R660-Y661-Y665 epitope in the ADAMTS13 spacer domain[43] and is a strong inhibitor of ADAMTS13 activity[40]. Although about 50% of the iTTP patients have inhibitory anti-ADAMTS13 autoantibodies[29,46], it is currently not known if all these patients have a II-1 idiotope in their plasma. Using our anti-idiotypic antibody against the anti-spacer II-1 idiotope, we now provided insight into the incidence of this anti-spacer II-1 idiotope in iTTP patients. Indeed, our study showed that only 34% of the patients had this anti-spacer idiotope in their plasma. Insight into the diversity of inhibitory anti-ADAMTS13 autoantibodies that target the R568-F592-R660-Y661-Y665 epitope is important in view of the development of a targeted antibody therapy. In addition, anti-idiotypic antibodies allow to study epitope spreading observed in iTTP patients by following the presence of specific idiotopes in function of time. An additional advantage of using anti-idiotypic antibodies for antibody profiling is that the antigen itself is not needed for the profiling assay.[21,47] Production of recombinant ADAMTS13 and its fragments in the case of iTTP is more expensive and complex than producing and purifying murine anti-idiotypic antibodies.

Finally, we investigated whether we could establish a link between these anti-spacer idiotope profiles and disease severity (disease outcome and applied treatment strategy). However, the current idiotope profiles did not allow to identify specific profiles that are linked with disease severity. On the one hand, this can be due to the relative low number of patients per idiotope profile. Hence, increasing the number of patients in each idiotope profile could result in a link between certain profiles and disease severity. On the other hand, although that majority of iTTP patients do have autoantibodies against the cysteine-spacer domain, autoantibodies targeting other regions within or outside the cysteine-spacer domain could be of relevance, as the immune response is polyclonal. Hence, multiple anti-idiotypic antibodies recognizing a large number of anti-ADAMTS13 autoantibodies might be needed to identify autoantibody profiles in iTTP that predict disease outcome or that are linked with treatment. We are therefore currently expanding our panel of anti-idiotypic antibodies with anti-idiotypic antibodies recognizing anti-

ADAMTS13 autoantibodies outside the spacer domain to identify autoantibody profiles of clinical, prognostic value.

The strength of autoantibody profiling to predict disease severity and outcome in an autoimmune disorder where autoantibodies are developed against a single self-antigen has been clearly demonstrated for example in myasthenia gravis. Indeed, it has been shown that the presence of autoantibodies against a specific epitope in AChR is linked with disease severity in these patients.[48,49] Hence, future development of anti-idiotypic antibodies against anti-ADAMTS13 autoantibodies that are linked with disease severity, outcome and relapse remains a promising approach to personalize treatment of iTTP patients.

In conclusion, we have shown that anti-idiotypic antibodies are useful to unravel anti-spacer autoantibody specificity in iTTP patients. Moreover, this approach is broadly applicable and can therefore be translated to perform autoantibody profiling in any antibody-mediated autoimmune disease.

## Authors contributions

ASS designed and performed experiments, analyzed and interpreted the data and wrote the manuscript; JV delivered antibodies II-1 and I-9, ER, IP and MB performed experiments; IM, BJ, JV, RF, AV, PC and FP provided the patient plasma samples and critically reviewed the manuscript; ER, HD, SFDM, BJ and AV, analyzed data and critically reviewed the manuscript; KV designed the experiments, interpreted the data, wrote the manuscript and provided funding.


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## Conflict of interest

FP has received honoraria for participating as a speaker at satellite symposia and educational meetings organized by Ablynx, Grifols, Novo Nordisk, Roche, Shire and Sobi. She has received consulting fees from Kedrion and she is a member of the scientific advisory board of Ablynx. PC is member of the Clinical Advisory Board for Alexion, Ablynx, Shire and Octapharma. AV and KV are members of the Clinical Advisory Board for Ablynx. All other authors declare no competing financial interests.

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## Figure Legends

**Figure 1: Anti-idiotypic antibodies directed against different idiotopes in autoantibodies** A representative autoantibody is illustrated with the variable regions of heavy ($V_H$) and light ($V_L$) chains and the constant regions of heavy ($C_H$) and light ($C_L$) chains. Variable regions consist of framework regions and complementarity determining regions (CDRs). The CDRs are unique among antibodies and consist of idiotopes that are involved in binding to the (self-) antigen (dark blue dots) and idiotopes that are not involved in binding to the (self-) antigen (light blue dots). All other regions are conserved regions (grey) between different antibodies and comprise the framework regions of the $V_H$ and $V_L$ and the constant regions of $C_H$ en $C_L$ chains. Anti-idiotypic antibodies (Abs) bind to idiotopes involved in (self-) antigen binding and hence inhibit the binding of the autoantibody to the (self-) antigen are depicted in dark blue. Anti-idiotypic antibodies that bind to idiotopes not involved in binding to the (self-) antigen are depicted in light blue. Anti-conserved region antibodies are depicted in grey.

**Figure 2: Development and characterization of anti-idiotypic antibodies that inhibit the binding of respectively anti-spacer autoantibody II-1, TTP73 or I-9 to rhADAMTS13** (A) Binding of purified murine anti-II-1 (red), anti-TTP73 (green) and anti-I-9 (orange) antibodies to coated human anti-spacer autoantibodies II-1, TTP73 or I-9. Bound murine anti-II-1, anti-TTP73 and anti-I-9 antibodies were detected using GAM-HRP. Murine anti-II-1, anti-TTP73 or anti-I-9 antibody binding was expressed as relative absorbance values (mean ± SD, n=3) with absorbance of the respective positive controls (sera of mice immunized with either II-1, TTP73 or I-9) set as 1. (B) Binding of purified murine anti-II-1 (red), anti-TTP73 (green) and anti-I-9 (orange) antibodies to a coated human IgG pool. Bound murine anti-II-1, anti-TTP73 and anti-I-9 antibodies were detected using GAM-HRP. Murine anti-II-1, anti-TTP73 or anti-I-9 antibody binding was expressed as relative absorbance values (mean ± SD, n=3) with absorbance of the respective positive controls (sera of mice immunized with either II-1, TTP73 or I-9) set as 1. Binding to coated human IgG pool indicates that the murine antibodies bind to the conserved regions of antibodies. (C) Inhibition of anti-spacer autoantibody binding to rhADAMTS13 by anti-idiotypic antibodies. A 1 in 2 dilution of murine anti-II-1 (red), anti-TTP73 (green) or anti-I-9

antibodies (orange) were pre-incubated with constant amounts of respectively anti-spacer autoantibody II-1, TTP73 and I-9 before addition to a rhADAMTS13 coated 96-well microtiter plate. Bound II-1 (red), TTP73 (green) and I-9 (orange) antibodies were detected using a mixture of anti-human $IgG_{1-4}$-HRP. Data are expressed as % binding (mean ± SD, n=3) of anti-spacer autoantibodies II-1 (red), TTP73 (green) or I-9 (orange) to rhADAMTS13 in the presence of the competing murine anti-II-1 (17H9), anti-TTP73 (9G12) or anti-I-9 (7D10) antibody relative to the binding of II-1, TTP73 or I-9 in the absence of anti-idiotypic antibodies (dotted lines). ~~(D) Overview of the binding sites of the generated anti-II-1, anti-TTP73 and anti-I-9 antibodies in II-1 (red), TTP73 (green) and I-9 (orange) respectively.~~

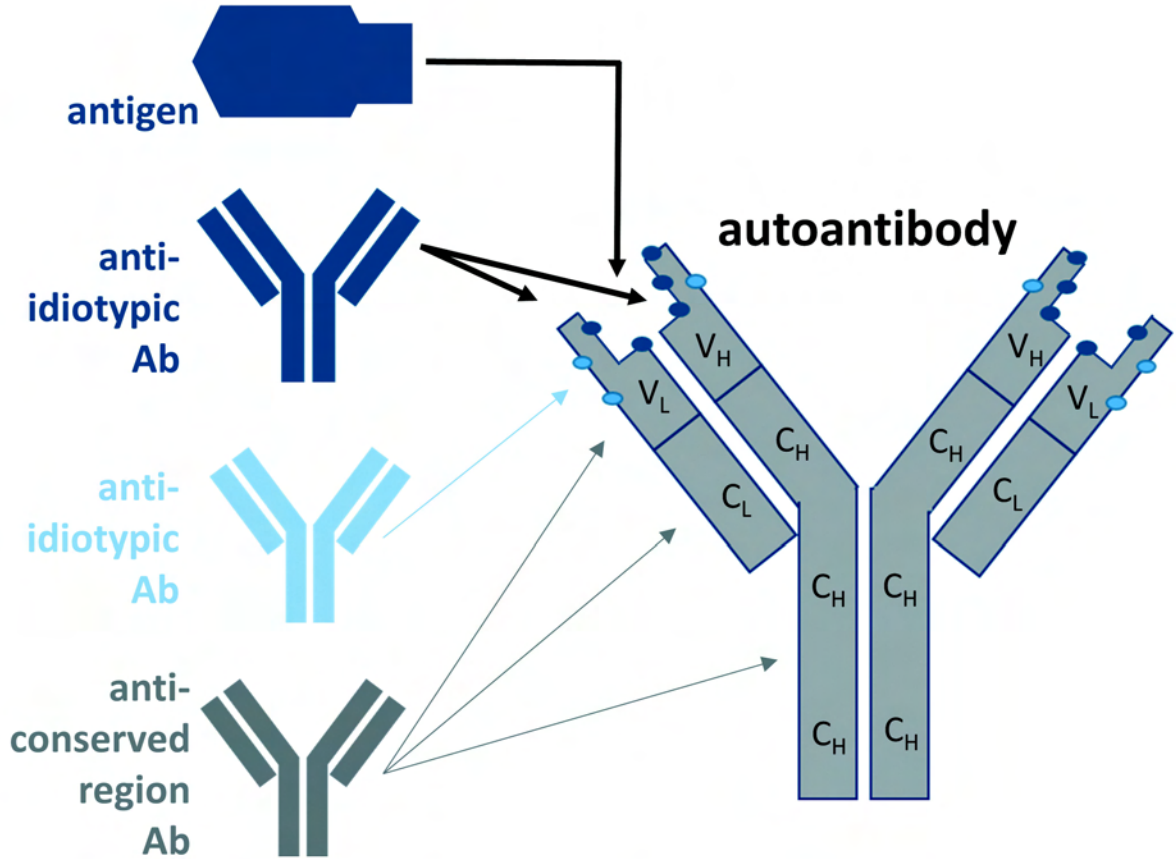
**Figure 3: Anti-idiotypic antibodies and their binding to the anti-spacer idiotopes of II-1, TTP73 and I-9** Binding of human anti-spacer autoantibodies (autoAbs) II-1 (red), TTP73 (green), I-9 (orange) and of a pool of human IgG antibodies (negative control, black) to coated murine anti-idiotypic antibody (Ab) 17H9 developed against II-1 (A), 9G12 developed against TTP73 (B) and 7D10 developed against I-9 (C). Bound human anti-spacer autoantibodies II-1, TTP73 and I-9 were detected using a mixture of anti-human $IgG_{1-4}$-HRP. Data are expressed as relative absorbance values (mean ± SD, n=3) with absorbance of binding of II-1, TTP73 and I-9 at 1 µg/mL to their respective anti-idiotypic antibodies set as 1.

**Figure 4: Total anti-ADAMTS13 IgG titers and anti-spacer II-1, TTP73 and I-9 idiotope concentrations in acute iTTP patients** (A-C) Total anti-ADAMTS13 IgG titers (IU/mL) were determined via TECHNOZYM® with exception of 3 samples (ID 124, 128 and 130) which were determined via an in house developed anti-ADAMTS13 IgG ELISA (Supplemental Table 1, indicated by §). Anti-spacer II-1 (red square) (A), TTP73 (green square) (B) and I-9 (orange square) (C) idiotope concentrations were determined by adding patient plasma to coated murine anti-idiotypic antibody 17H9 (A), 9G12 (B) or 7D10 (C). Bound human autoantibodies were detected using a mixture of anti-human $IgG_{1-4}$-HRP. A dilution series of human anti-spacer autoantibodies II-1 (A), TTP73 (B) or I-9 (C) was used as a calibration curve to determine

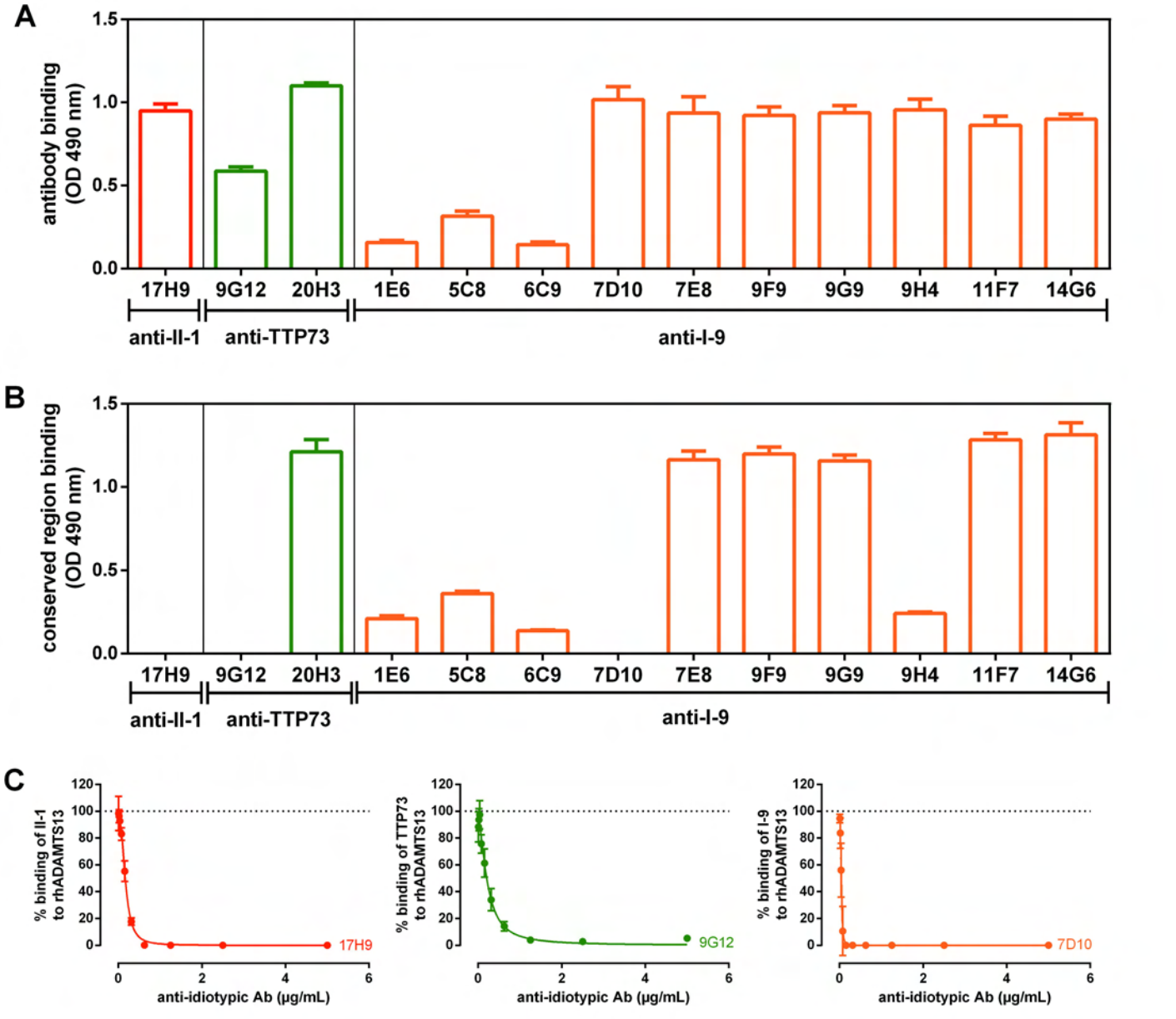
idiotope concentrations (ng/mL). Median is represented for total anti-ADAMTS13 IgG titers and II-1, TTP73 and I-9 idiotope concentrations.

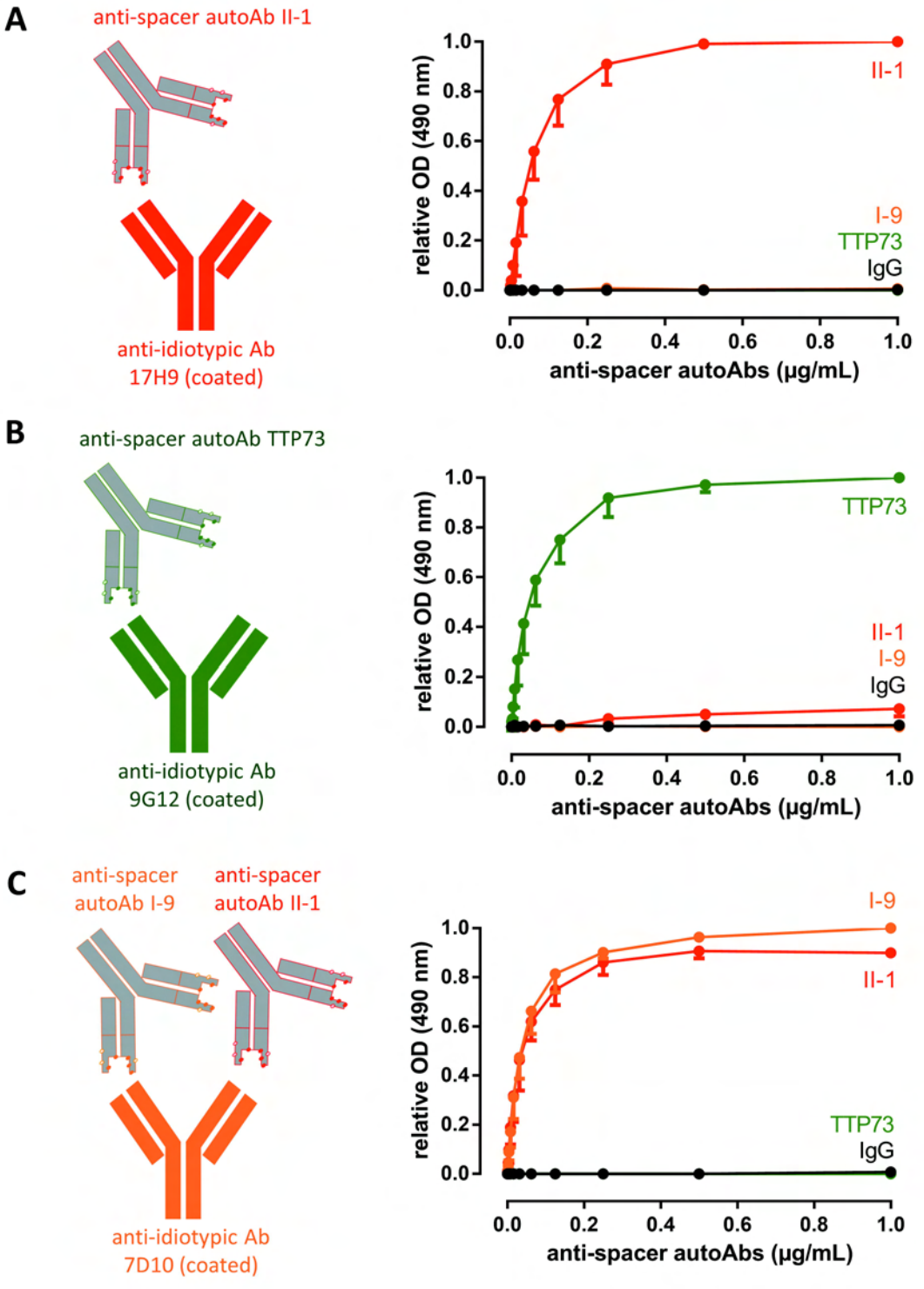
**Figure 5: Anti-spacer idiotope profiles in acute iTTP patients** Acute iTTP patients (n=151) were stratified according to the presence (+) or absence (-) of II-1, TTP73 and I-9 idiotopes as determined in Figure 4.

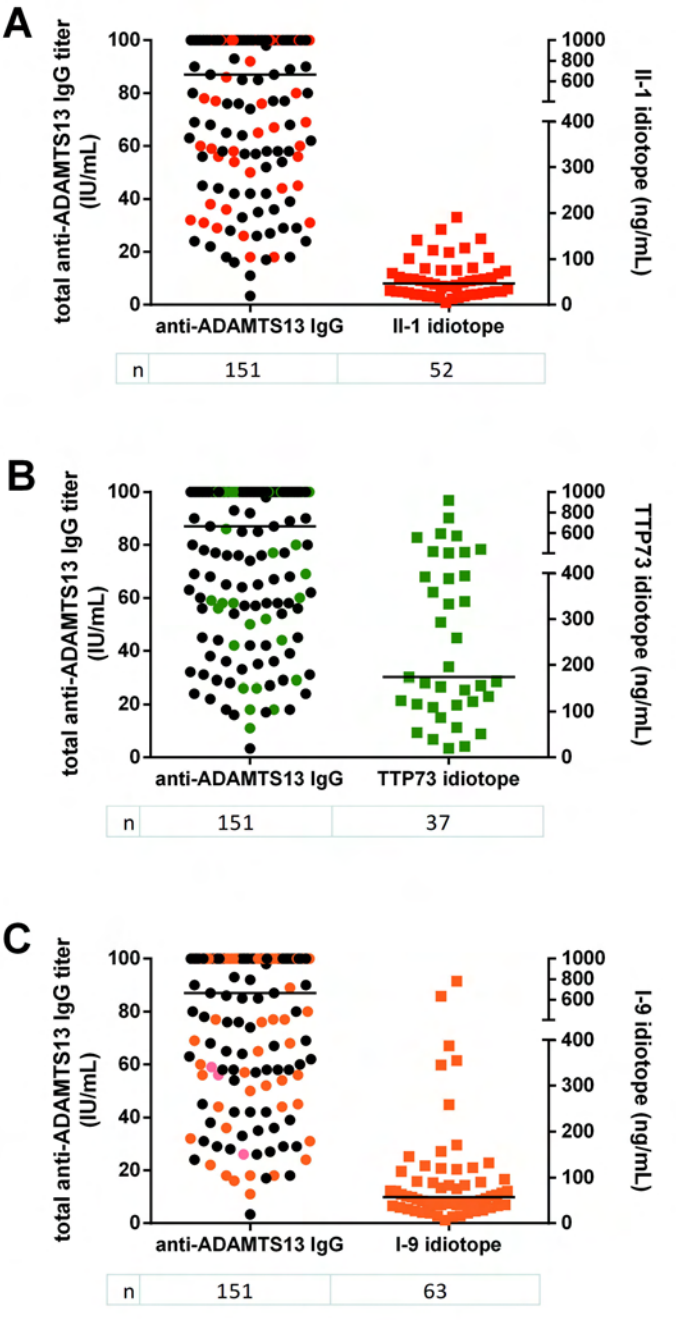
**Figure 6: Disease outcome and treatment according to the anti-spacer idiotope profiles** Stratification of the 95 acute iTTP patients of the French Reference Center for TMA according to the 8 idiotope profiles for (A) the scoring system of Benhamou *et al.*[39] (score < 3, white bars; score ≥ 3, black bars) and for (B) treatment with plasma exchange (PEX) with/without rituximab (white bars) or PEX with/without rituximab and additional treatments(s) (black bars). Three patients were excluded as they deceased before treatment initiation (Supplemental Table 2, indicated by †).

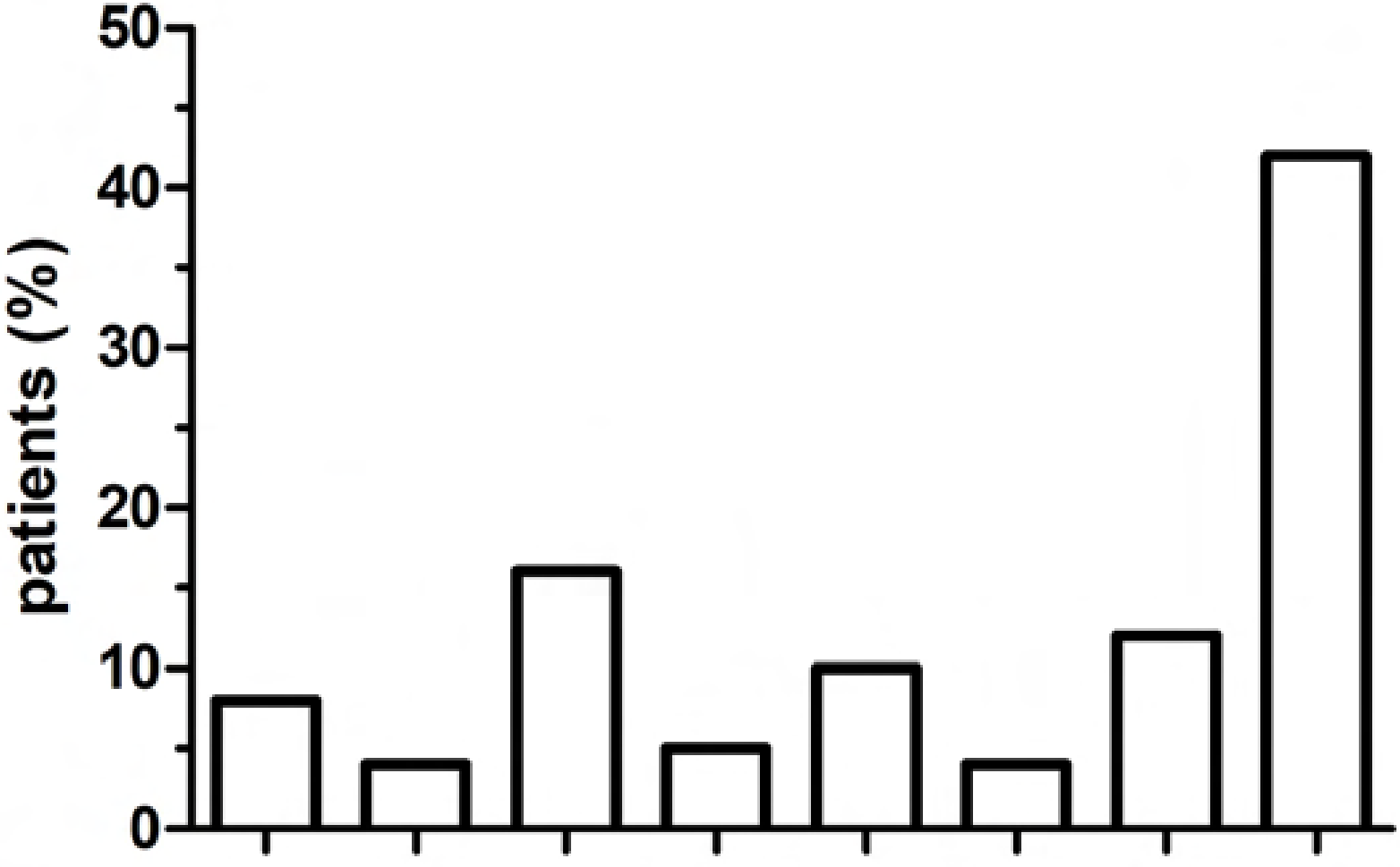



- Idiotopes involved in antigen binding
- Idiotopes not involved in antigen binding


A
antibody binding (OD 490 nm)
1.5
1.0
0.5
0.0
17H9
9G12
20H3
1E6
5C8
6C9
7D10
7E8
9F9
9G9
9H4
11F7
14G6
anti-II-1
anti-TTP73
anti-I-9
B
conserved region binding (OD 490 nm)
1.5
1.0
0.5
0.0
17H9
9G12
20H3
1E6
5C8
6C9
7D10
7E8
9F9
9G9
9H4
11F7
14G6
anti-II-1
anti-TTP73
anti-I-9
C
% binding of II-1 to rhADAMTS13
120
100
80
60
40
20
0
0
2
4
6
anti-idiotypic Ab (μg/mL)
17H9
% binding of TTP73 to rhADAMTS13
120
100
80
60
40
20
0
0
2
4
6
anti-idiotypic Ab (μg/mL)
9G12
% binding of I-9 to rhADAMTS13
120
100
80
60
40
20
0
0
2
4
6
anti-idiotypic Ab (μg/mL)
7D10

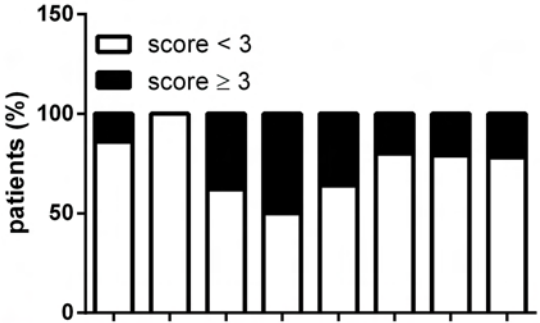

A
anti-spacer autoAb II-1
anti-idiotypic Ab
17H9 (coated)
relative OD (490 nm)
1.0
0.8
0.6
0.4
0.2
0.0
II-1
I-9
TTP73
IgG
0.0
0.2
0.4
0.6
0.8
1.0
anti-spacer autoAbs (µg/mL)
B
anti-spacer autoAb TTP73
anti-idiotypic Ab
9G12 (coated)
relative OD (490 nm)
TTP73
II-1
I-9
IgG
anti-spacer autoAbs (µg/mL)
C
anti-spacer
autoAb I-9
anti-spacer
autoAb II-1
anti-idiotypic Ab
7D10 (coated)
relative OD (490 nm)
I-9
II-1
TTP73
IgG
anti-spacer autoAbs (µg/mL)


A
total anti-ADAMTS13 IgG titer (IU/mL)
100
80
60
40
20
0
1000
800
600
400
300
200
100
0
Il-1 idiotope (ng/mL)
anti-ADAMTS13 IgG
Il-1 idiotope
n
151
52
B
total anti-ADAMTS13 IgG titer (IU/mL)
TTP73 idiotope (ng/mL)
anti-ADAMTS13 IgG
TTP73 idiotope
n
151
37
C
total anti-ADAMTS13 IgG titer (IU/mL)
I-9 idiotope (ng/mL)
anti-ADAMTS13 IgG
I-9 idiotope
n
151
63



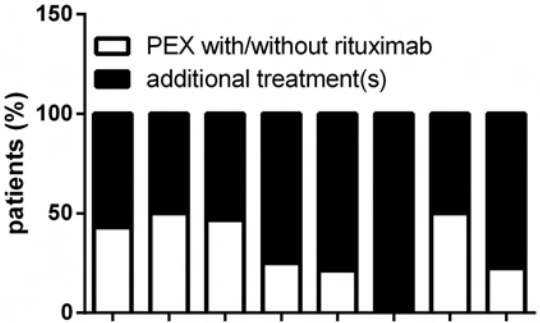

| idiotope profile | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| II-1 idiotope | + | - | - | + | + | - | + | - |
| TTP73 idiotope | - | + | - | + | - | + | + | - |
| I-9 idiotope | - | - | + | - | + | + | + | - |
| n | 12 | 6 | 24 | 7 | 15 | 6 | 18 | 63 |

A




| idiotope profile | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| II-1 idiotope | + | - | - | + | + | - | + | - |
| TTP73 idiotope | - | + | - | + | - | + | + | - |
| I-9 idiotope | - | - | + | - | + | + | + | - |
| n | 7 | 3 | 16 | 4 | 14 | 5 | 14 | 32 |

B




| idiotope profile | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| II-1 idiotope | + | - | - | + | + | - | + | - |
| TTP73 idiotope | - | + | - | + | - | + | + | - |
| I-9 idiotope | - | - | + | - | + | + | + | - |
| n | 7 | 2 | 15 | 4 | 14 | 5 | 14 | 31 |

# Generation of anti-idiotypic antibodies to detect anti-spacer antibody idiotopes in acute thrombotic thrombocytopenic purpura patients

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## Supplemental methods

### Animals

Animal experiments were approved by the Institutional animal Care and Use Committee of KU Leuven, Belgium (project number P055/2015). Mice were anesthetized using isoflurane/$O_2$ before subcutaneous or intra-peritoneal injections and retro-orbital venipuncture. Serum was obtained from blood samples by 1 hour incubation at 37°C and centrifugation at 13,400 rpm for 10 minutes. Serum samples were stored at -20°C.

### Cloned human anti-spacer autoantibodies

Three cloned human anti-spacer autoantibodies with different epitopes and different inhibitory characteristics were used for the development of anti-idiotypic antibodies: anti-spacer autoantibody II-1[1], TTP73[2] and I-9[3]. Anti-spacer autoantibody II-1 targets the R568-F592-R660-Y661-Y665 epitope in ADAMTS13's spacer domain[4] and is a strong inhibitor of ADAMTS13 activity[1]. Anti-spacer autoantibody I-9 targets the R568-R660-Y661-Y665 epitope[4] and is a weak inhibitor of ADAMTS13 activity[1]. Anti-spacer autoantibody TTP73[2] recognizes an epitope in ADAMTS13 that does not overlap with the epitope of anti-spacer autoantibodies II-1 and I-9 (epitope at the amino acid level not known) and does not inhibit ADAMTS13 activity.

**Immunization strategy and characterization of anti-II-1, anti-TTP73 and anti-I-9 antibodies**

*Immunization strategy*

BALB/c mice (Janvier Labs, Le Genest-Saint-Isle, France) were immunized with cloned human anti-spacer autoantibodies II-1[1], TTP73[2], or I-9[3]. Briefly, for each antibody, mice were injected subcutaneously with 10 µg antibody (either II-1, TTP73, or I-9) in complete Freund's adjuvant (BD, Franklin Lakes, NJ, USA) at day 1 and intraperitoneally with 10 µg of antibody in incomplete Freund's adjuvant (BD) at day 14. Mice immunized with vehicle only were used as a negative control. Twenty-one days after the first immunization, blood samples were taken to detect the presence of murine anti-human II-1, TTP73 and I-9 antibodies in ELISA (see below). The immune response in mice was boosted at day 56 and 58 by injection of each antibody (either II-1, TTP73, or I-9). At day 59, the presence of murine anti-human II-1, TTP73 and I-9 antibodies in mouse sera was confirmed using ELISA (see below). At day 60, mice were sacrificed and their spleens were isolated. Spleen cells were fused with SP2/0 myeloma cells to generate hybridoma cells according to the method of Köhler and Milstein.[5] Fourteen days after fusion, media of the cultured hybridoma cells was screened for the presence of either anti-II-1, anti-TTP73 or anti-I-9 antibodies using ELISA (see below). Hybridoma cells positive for anti-II-1, anti-TTP73 or anti-I-9 antibodies were further cultured and anti-II-1, anti-TTP73 or anti-I-9 antibodies were purified from the culture medium using protein G sepharose affinity chromatography (ÄKTA, GE Healthcare, Waukesha, WI, USA). Antibody concentration was determined by measuring absorbance at 280 nm and antibody purity was checked via sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein blue staining (Westburg, Leusden, The Netherlands). The binding of the purified anti-II-1, anti-TTP73 or anti-I-9 antibodies to II-1, I-9 and TTP73 respectively was confirmed in ELISA (see below). ELISAs to identify the anti-idiotypic antibodies amongst the anti-II-1, anti-TTP73 or anti-I-9 antibodies are described below. The finally selected anti-idiotypic antibodies 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) and 7D10 (anti-I-9 antibody) were subcloned as described elsewhere[6] and purified as described above.

*ELISA to study the binding of murine anti-II-1, anti-TTP73 and anti-I-9 antibodies to coated human anti-spacer autoantibodies II-1, TTP73 and I-9*

A 96-well microtiter plate was coated with either anti-spacer autoantibody II-1 (1.5 µg/mL), TTP73 (5 µg/mL) or I-9 (1.5 µg/mL in carbonate/bicarbonate coating buffer; 50mM $Na_2CO_3$/$NaHCO_3$, pH 9.6) and incubated overnight at 4°C. Next, the plate was washed and blocked with 3% milk powder in phosphate buffered saline (PBS) (blocking buffer). Either sera (start dilution 10%, v/v) from mice injected with anti-

spacer autoantibody II-1, TTP73 or I-9 or media (start dilution, 17%, v/v) from the anti-II-1, anti-TTP73 and anti-I-9 producing hybridoma cells or purified murine anti-II-1, anti-TTP73 and anti-I-9 antibodies (start concentration 1 µg/mL) were added to the respectively II-1, TTP73 or I-9 coated 96-well microtiter plates and a 1 in 2 dilution series was made. For each plate, a serum sample (21 days post first immunization, 1/500 start dilution) of mice immunized with either II-1, TTP73 or I-9 respectively was used as a positive control. Sera samples taken before immunization, naïve culture media or anti-glycoprotein Ib antibody 6B4[7] were used as a negative control respectively. Bound anti-II-1, anti-TTP73, or anti-I-9 antibodies were detected using horse radish peroxidase (HRP)-labelled goat anti-mouse (GAM) antibodies (1/10,000; Jackson ImmunoResearch, West Grove, PA, USA). Colouring reaction was performed using ortho-phenylenediamine dihydrochloride (OPD) and $H_2O_2$ and stopped with 4M sulfuric acid. Absorbance was measured at 490 nm. Data for the II-1, TTP73 or I-9 ELISA's were expressed as relative absorbance values (mean ± SD, n=3) with absorbance of the respective positive controls (sera of mice immunized with either II-1, TTP73 or I-9) set as 1.

*ELISA to study the binding of murine anti-II-1, anti-TTP73 and anti-I-9 antibodies to a pool of human IgG antibodies*

Murine anti-II-1, anti-TTP73 and anti-I-9 antibodies targeting the conserved regions (Figure 1, grey) in human immunoglobulin G (IgG) antibodies were identified using ELISA. A pool of human IgG antibodies (5 µg/mL in carbonate/bicarbonate coating buffer; Sigma-Aldrich, Saint-Louis, MO, USA) was coated on a 96-well microtiter plate. Plates were blocked with blocking buffer and murine anti-II-1, anti-TTP73 and anti-I-9 antibodies were added (5 µg/mL) and a 1 in 2 dilution series was made. For each plate, a serum sample (21 days post first immunization, 1/500 start dilution) of mice immunized with either II-1, TTP73 or I-9 respectively was used as a positive control. The anti-glycoprotein Ib antibody 6B4[7] was used as a negative control. Bound murine anti-II-1, anti-TTP73 and anti-I-9 antibodies were detected with GAM-HRP (1/10,000; Jackson ImmunoResearch). Colouring reaction was performed as described above and absorbance was measured at 490 nm. Data for the II-1, TTP73 or I-9 ELISA's were expressed as relative absorbance values (mean ± SD, n=3) with absorbance of the respective positive controls (sera of mice immunized with either II-1, TTP73 or I-9) set as 1.

**ELISA to identify anti-II-1, anti-TTP73 and anti-I-9 antibodies that inhibit the binding of respectively anti-spacer autoantibodies II-1, TTP73 or I-9 to ADAMTS13**

*ELISA to determine the effective concentration at half maximal binding (EC50) of each anti-spacer autoantibody to ADAMTS13*

The EC50 of the anti-spacer autoantibodies II-1, TTP73 and I-9 was determined via ELISA. A 96-well microtiter plate was coated with recombinant human (rh)ADAMTS13 (15nM in PBS) and incubated overnight at 4°C. After blocking, the anti-spacer autoantibodies II-1, TTP73 or I-9 were added (10 µg/mL) and a 1 in 2 dilution series was made. Bound anti-spacer autoantibodies II-1, TTP73 or I-9 were detected using HRP-labelled rabbit anti-human IgG and IgM antibodies (1/10,000; Jackson ImmunoResearch). Colouring reaction was performed as described above and absorbance was measured at 490 nm. Binding curves were fitted using specific binding with Hill slope to determine EC50 for each anti-spacer autoantibody (Graphpad Prism v5.03 software Inc., San Diego, CA, USA). The determined EC50 for anti-spacer autoantibody II-1, TTP73 and I-9 are respectively 0.04, 0.85 and 0.04 µg/mL.

*ELISA to identify inhibiting anti-II-1, anti-TTP73 and anti-I-9 antibodies*

Human anti-spacer autoantibodies II-1, TTP73 or I-9 (constant final EC50) were pre-incubated with a 1 in 2 dilution of murine anti-II-1, anti-TTP73 or anti-I-9 antibodies (final start concentration 10 µg/mL) respectively, in a pre-blocked plate. After 30 minutes, samples were transferred to a rhADAMTS13 (15nM in PBS) coated 96-well microtiter plate. Bound human anti-spacer autoantibodies II-1, TTP73 or I-9 were detected using a mixture of HRP-labelled anti-human $IgG_{1\text{-}4}$ ($IgG_1$: 1/20,000 and $IgG_{2\text{-}4}$: 1/2,000; Sanquin, Amsterdam, The Netherlands). Colouring reaction was performed as described above and absorbance was measured at 490 nm. Binding (%) of anti-spacer autoantibodies II-1, TTP73 or I-9 to rhADAMTS13 in the presence of the competing murine anti-II-1 (17H9), anti-TTP73 (9G12) or anti-I-9 (7D10) antibody was expressed relative to binding of respectively II-1, TTP73 or I-9 with no competing antibody (buffer only) to rhADAMTS13 (set at 100% binding). Data were expressed as mean ± SD (n=3).

**ELISA to study the binding of the anti-idiotypic antibodies to the anti-spacer idiotopes of II-1, TTP73 and I-9**

Murine anti-idiotypic antibodies 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) and 7D10 (anti-I-9 antibody) (5 µg/mL in carbonate/bicarbonate coating buffer) were coated on 96-well microtiter plates. After blocking, human anti-spacer autoantibodies II-1, TTP73 and I-9 were added at a start concentration of 1 µg/mL and 1 in 2 serial diluted. Addition of a pool of human Immunoglobulin G (IgG) antibodies (Sigma-Aldrich Saint-Louis, MO, USA) was included as a negative control. Bound anti-spacer autoantibodies were detected by adding a mixture of HRP-labelled anti-human $IgG_{1\text{-}4}$ antibodies ($IgG_1$: 1/20,000 and $IgG_{2\text{-}4}$: 1/2,000; Sanquin). Colouring reaction was performed as described above and absorbance was measured

at 490 nm. Data were expressed as relative absorbance values (mean ± SD, n=3) with absorbance of binding of II-1, TTP73 and I-9 at 1 µg/mL to anti-idiotypic antibodies 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) and 7D10 (anti-I-9 antibody) respectively set as 1.

**Patient samples**

Plasma samples were collected from 151 iTTP patients during acute phase before treatment. All patients presented with TTP-related clinical signs (thrombocytopenia, microangiopathic haemolytic anaemia), ADAMTS13 activity <10% measured via FRETS-VWF73 assay (Peptides International, Louisville, KY, USA)[8] with exception of 2 samples that were measured using the collagen binding assay (CBA, patient's ID 128 and 131, Supplemental Table 1 indicated by *)[9] and anti-ADAMTS13 IgG titer >15 IU/mL measured via the TECHNOZYM ADAMTS13-INH ELISA® kit (Technoclone, Vienna, Austria) with exception of 3 samples that were measured using an in house anti-ADAMTS13 IgG ELISA (patient's ID 124, 128 and 129, Supplemental Table 1 indicated by §). Patients were diagnosed with idiopathic iTTP (without any underlying cause). Twenty-one plasma samples were derived from the University Medical Center Utrecht (The Netherlands), 35 samples from the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center of Milan (Italy) and 95 plasma samples from the French Reference Center for Thrombotic MicroAngiopathies (TMA) (France).
Besides age, sex, total anti-ADAMTS13 IgG titer and ADAMTS13 activity, detailed information on laboratory, clinical and outcome parameters was available for the 95 iTTP patients enrolled in the French Reference Center for TMA (Supplemental Table 2). Laboratory parameters such as platelet count and lactate dehydrogenase (LDH) levels (except for 10 patients) were available (Supplemental Table 2). Assessment of cerebral involvement at time of diagnosis included clinical signs including headaches, confusion, aphasia, transient focal defects, convulsion, seizure, stroke and/or coma. Treatment consisted of either plasma exchange (PEX) with/without rituximab, or PEX with/without rituximab supplemented with additional treatment(s); steroids, other immunosuppressive drugs (e.g. cyclophosphamide, bortezomib) and/or caplacizumab and/or splenectomy. Patient's outcome was investigated in terms of the pre-defined scoring system established by Benhamou *et al*. which includes age (years), LDH level (elevated LDH level: ≥ 2500 IU/L) and cerebral involvement.[10]

**ELISA to identify the presence of anti-spacer idiotope profiles in plasmas of acute iTTP patients using the newly developed anti-idiotypic antibodies**

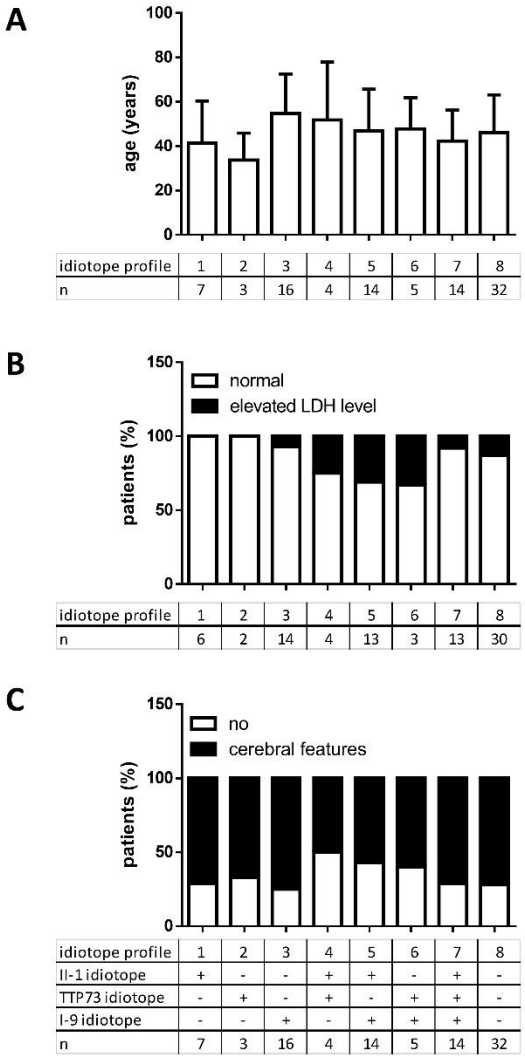
Ninety-six-well microtiter plates were coated with either murine anti-idiotypic antibody 17H9 (anti-II-1 antibody), 9G12 (anti-TTP73 antibody) or 7D10 (anti-I-9 antibody) (5 µg/mL in carbonate/bicarbonate

coating buffer) and incubated overnight at 4°C. After blocking, patient plasma (start dilution 10%, v/v) was added and 1 in 2 serial diluted. Bound patient antibodies (antibodies with the same idiotopes as the anti-spacer autoantibodies II-1, TTP73, or I-9), were detected with HRP-labelled anti-human $IgG_{1-4}$ ($IgG_1$: 1/20,000 and $IgG_{2-4}$: 1/2,000; Sanquin). Colouring reaction was performed as described above and absorbance was measured at 490 nm. The human anti-spacer autoantibodies II-1, TTP73 or I-9 were used to set up a calibration curve. Anti-spacer autoantibody II-1 (0.25 µg/mL), TTP73 (1.25 µg/mL) or I-9 (0.25 µg/mL) were spiked in a normal human plasma pool of 10 healthy donors (NHP; start dilution 10%, v/v) and 1 in 2 serial diluted. The equation derived after linear regression was used to determine respectively II-1, TTP73 or I-9 idiotope levels (ng/mL) in patient samples. As a negative control, NHP was added in each assay in triplicate and used to define the Lower Limit of Detection (LLoD, mean of negative control + 3*SD) for the II-1, TTP73 and I-9 idiotope screening ELISA ($LLoD_{II\text{-}1\ idiotope}$ = 0.8 ng/mL, $LLoD_{TT773\ idiotope}$= 3.9 ng/mL and $LLoD_{I\text{-}9\ idiotope}$ = 0.8 ng/mL).

**Statistical analysis**

Graphpad Prism v5.03 software (GraphPad Software Inc.) was used for statistical analysis. Continuous variables were described as mean and standard deviation (SD) and categorical variables as counts and percentages. Continuous (age) and categorical (categorized LDH levels, cerebral involvement, score system by Benhamou *et al*.[10] and treatment) data were compared using ANOVA and chi square tests, respectively.

**Supplemental Figure + Figure Legends**




| idiotope profile | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| n | 7 | 3 | 16 | 4 | 14 | 5 | 14 | 32 |

| idiotope profile | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| n | 6 | 2 | 14 | 4 | 13 | 3 | 13 | 30 |

| idiotope profile | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| II-1 idiotope | + | - | - | + | + | - | + | - |
| TTP73 idiotope | - | + | - | + | - | + | + | - |
| I-9 idiotope | - | - | + | - | + | + | + | - |
| n | 7 | 3 | 16 | 4 | 14 | 5 | 14 | 32 |

**Supplemental Figure 1: Separate parameters included in the score by Benhamou *et al.*[10] according to the anti-spacer idiotope profiles** Stratification of the 95 acute iTTP patients of the French Reference Center for TMA according to the 8 idiotope profiles for (A) age, (B) lactate dehydrogenase (LDH) levels (normal levels, white bars; elevated levels: ≥ 2500 IU/L, black bars) and (C) cerebral involvement (no cerebral involvement, black bars; cerebral involvement, white bars). Ten patients were excluded since no LDH measurement was performed (Supplemental Table 2, indicated by N/A).

**Supplemental Tables + Table Legends**

**Supplemental Table 1: ADAMTS13 activity, anti-ADAMTS13 IgG, anti-spacer II-1, anti-spacer TTP73 and anti-spacer I-9 idiotopes of iTTP patients during acute TTP** Identity (ID), ADAMTS13 activity (%) using FRETS-VWF73 assay or collagen binding assay (*), anti-ADAMTS13 IgG titer (IU/mL) via TECHNOZYM® or in-house anti-ADAMTS13 IgG ELISA (§), anti-spacer II-1 idiotope, anti-spacer TTP73 idiotope and anti-spacer I-9 idiotope levels (ng/mL) of 151 acute iTTP patients. 'x' indicates no detectable anti-spacer idiotopes.

| ID | ADAMTS13 activity (%) | anti-ADAMTS13 IgG (IU/mL) | anti-spacer II-1 idiotope (ng/mL) | anti-spacer TTP73 idiotope (ng/mL) | anti-spacer I-9 idiotope (ng/mL) |
|---|---|---|---|---|---|
| **1** | < 5 | 56 | 75 | x | 70 |
| **2** | < 5 | 78 | 29 | x | x |
| **3** | < 5 | 22 | x | x | 121 |
| **4** | < 10 | 18 | 49 | 556 | 70 |
| **5** | < 5 | 100 | 49 | x | 91 |
| **6** | < 5 | 100 | x | x | x |
| **7** | < 5 | 100 | x | x | x |
| **8** | < 5 | 64 | x | x | x |
| **9** | < 5 | 100 | x | x | x |
| **10** | < 5 | 100 | 144 | 412 | 146 |
| **11** | < 5 | 100 | x | x | x |
| **12** | < 5 | 31 | 19 | x | 120 |
| **13** | < 5 | 100 | x | x | 44 |
| **14** | < 5 | 57 | x | x | x |
| **15** | < 5 | 85 | x | x | x |
| **16** | < 5 | 100 | 141 | x | x |
| **17** | < 5 | 100 | x | x | x |
| **18** | < 5 | 98 | x | x | x |
| **19** | < 5 | 29 | x | x | x |
| **20** | < 5 | 100 | x | x | x |
| **21** | < 5 | 100 | x | x | x |
| **22** | < 5 | 100 | 38 | x | 26 |
| **23** | < 5 | 57 | x | x | 24 |
| **24** | < 5 | 29 | x | 116 | x |
| **25** | < 5 | 100 | 75 | 165 | 71 |
| **26** | < 5 | 90 | x | x | x |
| **27** | < 5 | 26 | 26 | 147 | 88 |
| **28** | < 5 | 59 | 37 | 392 | 38 |
| **29** | < 5 | 56 | 53 | 122 | 62 |

| | | | | | |
|---|---|---|---|---|---|
| **30** | < 5 | 100 | x | x | 125 |
| **31** | < 5 | 100 | 52 | 133 | x |
| **32** | < 5 | 100 | 124 | 439 | 83 |
| **33** | < 5 | 100 | 19 | 916 | x |
| **34** | < 5 | 100 | 23 | x | x |
| **35** | < 5 | 100 | x | 394 | x |
| **36** | < 5 | 68 | x | x | x |
| **37** | < 5 | 58 | 191 | 333 | x |
| **38** | < 5 | 69 | 35 | 124 | x |
| **39** | < 5 | 69 | x | x | 355 |
| **40** | < 5 | 32 | 5 | x | 22 |
| **41** | < 5 | 100 | x | x | x |
| **42** | < 5 | 45 | 31 | x | 34 |
| **43** | < 5 | 77 | x | 162 | 28 |
| **44** | < 5 | 100 | x | x | 31 |
| **45** | < 5 | 52 | x | 156 | 16 |
| **46** | < 5 | 100 | x | x | x |
| **47** | < 5 | 100 | x | x | 14 |
| **48** | < 5 | 100 | 69 | 87 | 96 |
| **49** | < 5 | 27 | x | x | x |
| **50** | < 5 | 67 | 29 | x | x |
| **51** | < 5 | 38 | 22 | x | x |
| **52** | < 5 | 80 | x | x | x |
| **53** | < 5 | 87 | x | x | x |
| **54** | < 5 | 100 | x | x | x |
| **55** | < 5 | 100 | x | x | 7 |
| **56** | < 5 | 87 | x | x | x |
| **57** | < 5 | 100 | 74 | 109 | 635 |
| **58** | < 5 | 100 | 102 | x | 77 |
| **59** | < 5 | 100 | 18 | x | 59 |
| **60** | < 5 | 100 | x | x | 63 |
| **61** | < 5 | 100 | 164 | 359 | 345 |
| **62** | < 5 | 44 | 28 | 196 | 118 |
| **63** | < 5 | 76 | 43 | x | 157 |
| **64** | < 5 | 100 | 119 | 174 | 132 |
| **65** | < 5 | 60 | 27 | x | 45 |
| **66** | < 10 | 24 | x | x | x |
| **67** | < 5 | 58 | x | x | x |
| **68** | < 5 | 18 | x | x | 259 |
| **69** | < 5 | 100 | x | 746 | 38 |
| **70** | < 5 | 89 | x | x | 83 |
| **71** | < 5 | 65 | 16 | x | 90 |
| **72** | < 5 | 36 | 23 | x | 45 |

| | | | | | |
|---|---|---|---|---|---|
| **73** | < 5 | 44 | x | x | 59 |
| **74** | < 5 | 100 | x | 114 | 35 |
| **75** | < 10 | 100 | x | x | 40 |
| **76** | < 5 | 100 | 16 | 54 | 47 |
| **77** | < 5 | 24 | x | x | 45 |
| **78** | < 10 | 18 | 29 | 51 | 75 |
| **79** | < 5 | 100 | 57 | x | 52 |
| **80** | < 5 | 100 | x | x | x |
| **81** | < 5 | 80 | x | x | 113 |
| **82** | < 5 | 100 | x | 65 | 47 |
| **83** | < 5 | 100 | x | x | x |
| **84** | < 10 | 28 | x | x | x |
| **85** | < 5 | 100 | x | x | 49 |
| **86** | < 5 | 100 | x | x | x |
| **87** | < 5 | 100 | x | x | x |
| **88** | < 5 | 100 | x | x | x |
| **89** | < 10 | 42 | 25 | x | x |
| **90** | < 5 | 100 | x | x | x |
| **91** | < 5 | 90 | x | x | x |
| **92** | < 5 | 42 | x | 416 | x |
| **93** | < 5 | 100 | 56 | x | 66 |
| **94** | < 5 | 17 | x | x | x |
| **95** | < 5 | 45 | 68 | x | x |
| **96** | < 10 | 100 | x | x | x |
| **97** | < 5 | 100 | 81 | 39 | 57 |
| **98** | < 5 | 100 | x | 20 | x |
| **99** | < 10 | 100 | x | x | 46 |
| **100** | < 5 | 100 | x | x | x |
| **101** | < 5 | 100 | x | x | 16 |
| **102** | < 5 | 100 | 79 | 259 | 387 |
| **103** | < 10 | 100 | x | x | x |
| **104** | < 5 | 77 | 45 | x | 40 |
| **105** | < 10 | 77 | x | x | 22 |
| **106** | <10 | 76 | x | x | x |
| **107** | < 5 | 74 | x | x | x |
| **108** | < 10 | 65 | x | x | x |
| **109** | < 5 | 62 | x | x | x |
| **110** | < 5 | 60 | 40 | 572 | x |
| **111** | < 10 | 58 | x | x | x |
| **112** | < 5 | 58 | x | 338 | x |
| **113** | < 10 | 58 | x | x | x |
| **114** | < 5 | 54 | x | x | 27 |
| **115** | < 5 | 50 | 13 | 293 | 43 |

| | | | | | |
|---|---|---|---|---|---|
| **116** | < 5 | 100 | 59 | 403 | 50 |
| **117** | < 5 | 18 | x | x | x |
| **118** | < 5 | 100 | x | x | x |
| **119** | < 5 | 33 | x | x | x |
| **120** | < 5 | 16 | x | x | 54 |
| **121** | < 5 | 100 | x | x | x |
| **122** | < 10 | 26 | x | 388 | x |
| **123** | < 5 | 100 | x | x | x |
| **124** | < 5 | 39[§] | x | x | x |
| **125** | < 5 | 100 | x | x | x |
| **126** | < 5 | 42 | x | x | x |
| **127** | < 5 | 92 | 41 | x | x |
| **128** | < 6* | 11[§] | x | 593 | 781 |
| **129** | < 5 | 3.4[§] | x | x | x |
| **130** | < 5 | 86 | 22 | 153 | x |
| **131** | < 6* | 100 | x | x | x |
| **132** | < 5 | 68 | x | x | 47 |
| **133** | < 5 | 80 | 54 | 24 | x |
| **134** | < 5 | 36 | x | x | x |
| **135** | < 5 | 100 | x | x | x |
| **136** | < 5 | 100 | x | x | x |
| **137** | < 5 | 100 | x | x | 40 |
| **138** | < 5 | 93 | x | x | x |
| **139** | < 5 | 100 | 61 | x | x |
| **140** | < 5 | 35 | x | x | x |
| **141** | < 5 | 100 | x | x | x |
| **142** | < 5 | 100 | x | x | x |
| **143** | < 5 | 85 | x | x | x |
| **144** | < 5 | 76 | x | x | x |
| **145** | < 5 | 56 | x | x | 171 |
| **146** | < 5 | 100 | x | x | x |
| **147** | < 5 | 100 | x | x | x |
| **148** | < 5 | 29 | 56 | x | x |
| **149** | < 5 | 63 | x | x | x |
| **150** | < 5 | 54 | 114 | x | x |
| **151** | < 5 | 31 | 101 | x | x |

**Supplemental Table 2: Detailed information on laboratory, clinical and outcome parameters of the 95 iTTP patients of the French reference center.** Identity (ID; patients 1-95 depicted here, are the same patients 1-95 in Table 1), sex (M: male, F: female), age (years), platelet count (x$10^9$/L), idiotope profile (1-8), Benhamou score (< 3 (1-2) or ≥ 3 (3-4)), lactate dehydrogenase levels (LDH) level (IU/L), cerebral involvement and treatment. N/A: not assessed, † deceased, PEX: plasma exchange

| ID | sex (M/F) | age (years) | platelet count (x$10^9$/L) | idiotope profile | Score by Benhamou *et al.*[40] | LDH level (IU/L) | cerebral involvement | additional treatment to PEX |
|---|---|---|---|---|---|---|---|---|
| **1** | F | 66 | 24 | 5 | ≥ 3 (3) | < 2500 | yes (seizure, headaches, confusion) | + rituximab + steroids |
| **2** | F | 41 | 8 | 1 | < 3 (2) | N/A | yes (confusion) | PEX only |
| **3** | M | 56 | 17 | 3 | < 3 (2) | < 2500 | yes (stroke) | + steroids |
| **4** | F | 65 | 132 | 7 | ≥ 3 (3) | < 2500 | yes (confusion, transient focal defect) | + rituximab + steroids |
| **5** | F | 74 | 16 | 5 | ≥ 3 (3) | < 2500 | yes (stroke) | PEX only |
| **6** | M | 55 | 52 | 8 | < 3 (1) | < 2500 | no | + rituximab + steroids + cyclophosphamide |
| **7** | M | 74 | 41 | 8 | ≥ 3 (3) | < 2500 | yes (confusion) | + rituximab + steroids |
| **8** | M | 33 | 6 | 8 | < 3 (1) | < 2500 | yes (headaches, convulsion and seizure) | + rituximab + steroids |
| **9** | F | 45 | 4 | 8 | < 3 (2) | ≥ 2500 | no | steroids only |
| **10** | F | 27 | 16 | 7 | < 3 (1) | < 2500 | yes | + steroids |
| **11** | M | 55 | 24 | 8 | < 3 (1) | N/A | no | no treatment† |
| **12** | F | 27 | 7 | 5 | < 3 (0) | < 2500 | no | + steroids |
| **13** | M | 68 | 23 | 3 | ≥ 3 (3) | < 2500 | yes | + rituximab + steroids |
| **14** | F | 25 | 13 | 8 | < 3 (1) | < 2500 | yes (headaches) | + rituximab + steroids |
| **15** | F | 48 | 9 | 8 | < 3 (2) | < 2500 | yes (headaches) | + rituximab + steroids |
| **16** | F | 31 | 18 | 1 | < 3 (1) | < 2500 | yes (confusion) | PEX only |
| **17** | F | 37 | 32 | 8 | < 3 (1) | < 2500 | yes (headaches) | PEX only |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **18** | M | 30 | 21 | 8 | < 3 (0) | < 2500 | no | + rituximab<br>+ steroids<br>+ cyclophosphamide<br>+ bortezomib |
| **19** | F | 65 | 14 | 8 | ≥ 3 (3) | < 2500 | yes (transient focal defect) | + steroids |
| **20** | F | 60 | 18 | 8 | < 3 (2) | ≥ 2500 | no | + rituximab |
| **21** | M | 48 | 26 | 8 | < 3 (2) | < 2500 | yes (confusion) | + rituximab |
| **22** | F | 56 | 27 | 5 | < 3 (2) | < 2500 | yes (stroke, blindness) | + steroids |
| **23** | F | 32 | 13 | 3 | < 3 (1) | < 2500 | yes (aphasia) | + rituximab<br>+ steroids |
| **24** | F | 23 | 12 | 2 | < 3 (0) | < 2500 | no | + rituximab<br>+ steroids |
| **25** | M | 38 | 16 | 7 | < 3 (1) | < 2500 | yes (headaches) | + caplacizumab |
| **26** | F | 26 | 7 | 8 | < 3 (1) | < 2500 | yes (headaches) | + rituximab<br>+ steroids |
| **27** | M | 34 | 44 | 7 | < 3 (1) | < 2500 | yes | PEX only |
| **28** | F | 62 | 9 | 7 | ≥ 3 (3) | ≥ 2500 | yes (aphasia) | PEX only |
| **29** | M | 24 | 18 | 7 | < 3 (0) | < 2500 | no | PEX only |
| **30** | F | 38 | 17 | 3 | < 3 (0) | < 2500 | no | PEX only |
| **31** | F | 80 | 14 | 4 | ≥ 3 (3) | < 2500 | yes (confusion, convulsion) | + rituximab |
| **32** | M | 44 | 20 | 7 | < 3 (2) | < 2500 | yes (confusion, headaches) | PEX only |
| **33** | F | 31 | 5 | 4 | < 3 (0) | < 2500 | no | + steroids |
| **34** | F | 71 | 20 | 1 | ≥ 3 (3) | < 2500 | yes (convulsion) | PEX only |
| **35** | F | 31 | 9 | 2 | < 3 (1) | < 2500 | yes (headaches) | + rituximab |
| **36** | F | 27 | 20 | 8 | < 3 (1) | N/A | yes (headaches) | + steroids |
| **37** | F | 28 | 5 | 4 | < 3 (1) | ≥ 2500 | no | + steroids |
| **38** | F | 68 | 5 | 4 | ≥ 3 (3) | < 2500 | yes (seizure) | + rituximab<br>+ steroids |
| **39** | F | 45 | 39 | 3 | < 3 (2) | < 2500 | yes (transient focal defect) | + rituximab<br>+ steroids |
| **40** | F | 21 | 14 | 5 | < 3 (2) | ≥ 2500 | yes (confusion, headaches) | + steroids |
| **41** | F | 63 | 12 | 8 | < 3 (2) | < 2500 | no | steroids only |
| **42** | M | 35 | 7 | 5 | < 3 (0) | < 2500 | no | + rituximab |

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|---|---|---|---|---|---|---|---|---|
| **43** | F | 41 | 12 | 6 | < 3 (2) | N/A | yes (headaches) | + steroids |
| **44** | F | 53 | 31 | 3 | < 3 (1) | N/A | no | + rituximab |
| **45** | F | 35 | 12 | 6 | < 3 (2) | ≥ 2500 | yes (headaches, visual disorders) | + steroids |
| **46** | F | 54 | 10 | 8 | < 3 (2) | < 2500 | yes (transient focal defect) | + steroids |
| **47** | M | 58 | 13 | 3 | < 3 (2) | < 2500 | yes (confusion) | + rituximab |
| **48** | F | 24 | | 7 | < 3 (1) | < 2500 | yes (aphasia) | + rituximab + steroids |
| **49** | F | 40 | 10 | 8 | < 3 (1) | < 2500 | yes (confusion) | + rituximab + steroids |
| **50** | F | 65 | 12 | 1 | < 3 (2) | < 2500 | no | + rituximab + steroids |
| **51** | F | 23 | 6 | 1 | < 3 (1) | < 2500 | yes (headaches) | + steroids |
| **52** | M | 20 | 9 | 8 | < 3 (1) | < 2500 | yes (headaches, convulsion) | + rituximab + steroids |
| **53** | M | 52 | 4 | 8 | < 3 (1) | < 2500 | no | + rituximab + steroids + vincristin + splenectomy (anti-vWF Abs) |
| **54** | F | 76 | 41 | 8 | ≥ 3 (3) | < 2500 | yes (stroke) | + rituximab |
| **55** | F | 53 | 12 | 3 | ≥ 3 (3) | ≥ 2500 | yes (confusion, convulsion) | + rituximab + steroids |
| **56** | F | 70 | 30 | 8 | ≥ 3 (3) | < 2500 | yes (stroke) | PEX only |
| **57** | M | 41 | 18 | 7 | < 3 (1) | < 2500 | no | PEX only |
| **58** | F | 66 | 26 | 5 | ≥ 3 (4) | ≥ 2500 | yes (confusion, stroke) | + rituximab + steroids |
| **59** | F | 25 | 12 | 5 | < 3 (1) | ≥ 2500 | no | + steroids |
| **60** | M | 32 | 10 | 3 | < 3 (1) | < 2500 | yes | + rituximab + steroids |
| **61** | F | 53 | 5 | 7 | < 3 (2) | < 2500 | yes (disorder of language) | + steroids |
| **62** | F | 41 | 13 | 7 | < 3 (1) | < 2500 | no | PEX only |
| **63** | M | 36 | 9 | 5 | < 3 (0) | N/A | no | PEX only |
| **64** | F | 34 | 8 | 7 | < 3 (1) | < 2500 | yes | + rituximab + steroids |
| **65** | F | 50 | 18 | 5 | < 3 (2) | < 2500 | yes | + rituximab + steroids |

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|---|---|---|---|---|---|---|---|---|
| **66** | F | 45 | 11 | 8 | < 3 (2) | < 2500 | yes (headaches, visual disorders) | PEX only |
| **67** | M | 60 | 6 | 8 | ≥ 3 (3) | ≥ 2500 | yes (headaches) | + steroids |
| **68** | F | 79 | 82 | 3 | ≥ 3 (3) | < 2500 | yes (confusion, coma) | + rituximab |
| **69** | M | 65 | 35 | 6 | ≥ 3 (3) | < 2500 | yes (aphasia, confusion) | + steroids |
| **70** | M | 27 | 29 | 3 | < 3 (1) | < 2500 | yes (headaches) | + steroids |
| **71** | F | 45 | 6 | 5 | < 3 (1) | < 2500 | no | + steroids |
| **72** | F | 67 | 8 | 5 | ≥ 3 (3) | ≥ 2500 | no | + rituximab<br>+ steroids |
| **73** | M | 44 | 32 | 3 | < 3 (1) | N/A | no | PEX only |
| **74** | F | 37 | 13 | 6 | < 3 (0) | < 2500 | no | + steroids |
| **75** | M | 61 | 13 | 3 | < 3 (2) | < 2500 | no | PEX only |
| **76** | M | 42 | 10 | 7 | ≥ 3 (3) | < 2500 | yes (stroke) | + rituximab<br>+ steroids |
| **77** | F | 83 | 12 | 3 | ≥ 3 (3) | < 2500 | yes (stroke) | PEX only |
| **78** | M | 63 | 30 | 7 | < 3 (2) | N/A | no | PEX only |
| **79** | F | 64 | 4 | 5 | ≥ 3 (3) | < 2500 | yes (headaches, transient focal defect) | + rituximab<br>+ steroids |
| **80** | F | 85 | 9 | 8 | ≥ 3 (3) | < 2500 | yes (transient focal defect) | + steroids |
| **81** | M | 83 | 9 | 3 | ≥ 3 (3) | < 2500 | yes (confusion) | + rituximab<br>+ steroids |
| **82** | M | 61 | 11 | 6 | < 3 (2) | N/A | no | + rituximab<br>+ steroids<br>+ cyclophosphamide<br>+ splenectomy |
| **83** | F | 33 | 63 | 8 | < 3 (1) | < 2500 | yes (transient focal defect) | steroids only |
| **84** | M | 40 | 34 | 8 | < 3 (1) | < 2500 | yes (cerebral lesions) | + steroids |
| **85** | F | 63 | 7 | 3 | ≥ 3 (3) | < 2500 | yes (stroke) | no treatment† |
| **86** | F | 40 | 8 | 8 | < 3 (1) | < 2500 | yes (headaches) | + steroids |
| **87** | F | 27 | 11 | 8 | < 3 (0) | < 2500 | no | + steroids |
| **88** | F | 21 | 11 | 8 | < 3 (1) | < 2500 | yes (headaches, transient focal defect) | + rituximab |

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|---|---|---|---|---|---|---|---|---|
| **89** | F | 31 | 55 | 1 | < 3 (1) | < 2500 | yes (headaches, seizure) | + rituximab + steroids |
| **90** | F | 33 | 6 | 8 | < 3 (1) | < 2500 | yes (headaches) | + rituximab + steroids |
| **91** | F | 46 | 5 | 8 | ≥ 3 (3) | ≥ 2500 | yes (headaches) | + rituximab + steroids |
| **92** | M | 47 | 10 | 2 | < 3 (2) | N/A | yes (seizure, aphasia, coma) | no treatment† |
| **93** | M | 24 | 7 | 5 | < 3 (1) | < 2500 | yes (headaches) | + rituximab + steroids |
| **94** | M | 44 | 7 | 8 | < 3 (1) | < 2500 | no | + rituximab + steroids |
| **95** | F | 27 | 8 | 1 | < 3 (0) | < 2500 | no | + rituximab + steroids |

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