

Workshop Siset

Bergamo, 29-30 Gennaio 2016

**POST-ISTH:
NOVITA' DAL MEETING DI TORONTO**

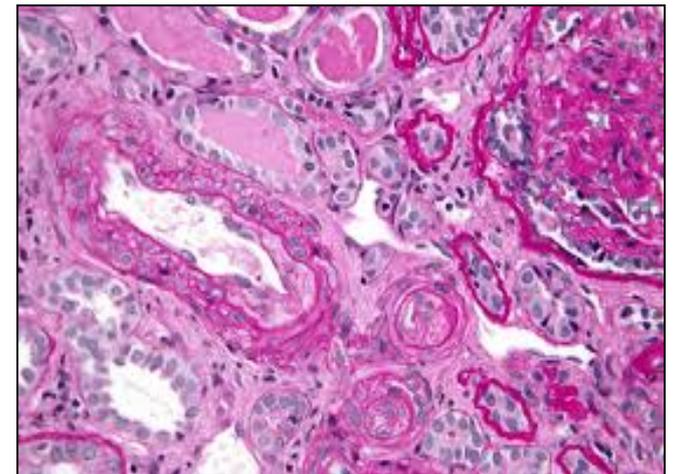
**MICROANGIOPATIE TROMBOTICHE:
focus su TTP**

Flora Peyvandi

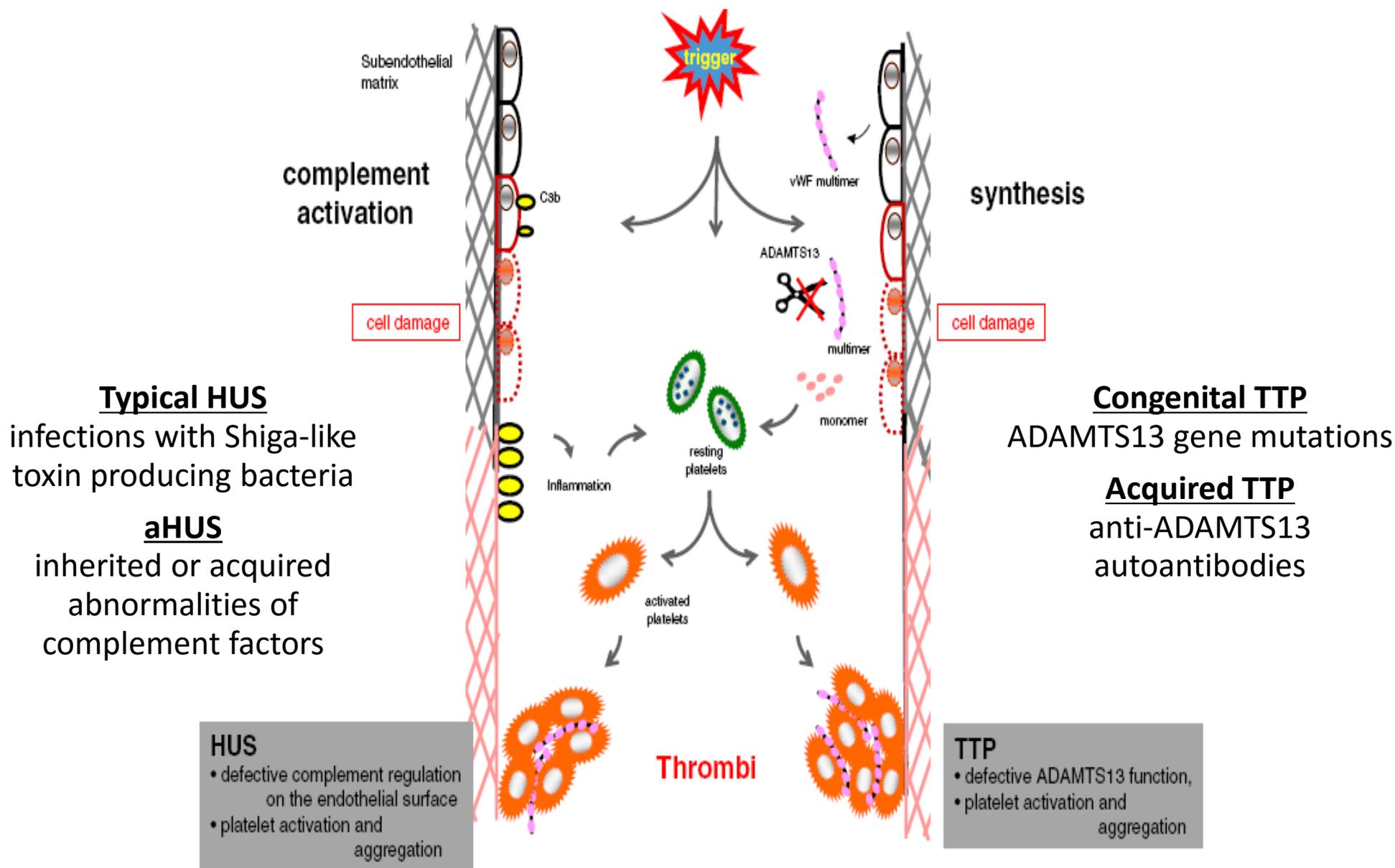
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Thrombotic microangiopathies (TMAs)

- Nowadays defined as a group of disorders characterized by:
 - ✓ Widespread ischemic damage
(due to microthrombosis in arterioles)
 - ✓ Thrombocytopenia
(due to platelet trapping)
 - ✓ Microangiopathic hemolytic anemia
(due to red blood cell fragmentation)
- Main TMAs are thrombotic thrombocytopenic purpura (TTP) and haemolytic-uraemic syndrome (HUS)



Pathogenesis



TTP: Hot topics from the Toronto ISTH meeting

- **Etiology**

- Pathogenic mechanisms other than ADAMTS13 deficiency could contribute to TTP development

- **Animal models**

- Animal models are crucial to investigate TTP pathogenesis and test new treatment options

- **Prognostic markers**

- About one-third of patients recur, but predictive markers are still scarce

TMA: Hot topics from the Toronto ISTH meeting

- **Novel treatments**

- Despite advances in the treatment of acute TTP, mortality is still 10% in the acute phase
- Eculizumab dramatically improved therapeutic response in aHUS patients, but patients with specific C5 polymorphisms are resistant to this treatment

TTP etiology

(OR153) Endogenous plasmin levels control the development of acute episodes of thrombotic thrombocytopenic purpura in mice.

Tersteeg C¹ et al. ¹*Laboratory for Thrombosis Research, KU Leuven Kulak, Kortrijk, Belgium.*

Background:

- Exogenous plasmin cleaves platelet-decorated VWF strings and is able to rescue *Adamts13*^{-/-} mice from TTP (*Tersteeg et al., Circulation 2014*).

Aims:

- Elucidation of the role of endogenous plasmin in a TTP mouse model.

Methods:

- ADAMTS13 or PAI-1 activity was blocked using monoclonal antibodies in either *uPAR*^{-/-}, *α2-antiplasmin*^{-/-} or wild type (WT) mice.
- TTP was triggered using recombinant human (rh)VWF (*Schiviz et al., Blood 2012*).
- *In vivo* cleavage of fluorescently-labeled platelet-decorated VWF strings was assessed in FeCl₃ injured mesenteric venules.

TTP etiology

Results:

- **WT mice WITH inhibited ADAMTS13** → 500 U/kg rhVWF induced TTP, while 250 U/kg rhVWF did not.
- ***uPAR*^{-/-} mice WITH inhibited ADAMTS13** → 250 U/kg rhVWF induced TTP. VWF string cleavage was strongly delayed compared with WT.
- ***α2-antiplasmin*^{-/-} mice WITH inhibited ADAMTS13 and PAI-1** → 500 U/kg rhVWF did not induce TTP. VWF string cleavage was accelerated compared with WT.

Conclusions:

- Endogenous plasmin is able to cleave VWF.
 - Blocking plasmin generation via uPAR render mice more susceptible to TTP, whereas unrestrained endogenous plasmin is able to prevent acute TTP episodes.
- Could interventions to increase plasmin generation be beneficial during acute episodes?

TTP: animal models

(OR151) Recombinant ADAMTS13 as an effective therapy for acquired thrombotic thrombocytopenic purpura in rats.

Tersteeg C¹ et al. ¹*Laboratory for Thrombosis Research, KU Leuven Kulak, Kortrijk, Belgium.*

Subsequently published in: Tersteeg et al., ATVB 2015.

Aims:

- To establish a rat model for acquired TTP.
- To investigate the therapeutic efficacy of recombinant human (rh)ADAMTS13.

Methods:

- Rats were injected with polyclonal goat anti-ADAMTS13 IgG to inhibit endogenous rat ADAMTS13 activity.
- Rats were subsequently challenged with recombinant human VWF (rhVWF) to trigger TTP.
- Rats were subsequently injected with rhADAMTS13 to evaluate its efficacy in the treatment of acquired TTP.

TTP: animal models

Results:

- Antibody-induced ADAMTS13 deficiency alone did not trigger TTP, as in ADAMTS13^{-/-} mice.
- After being triggered with rhVWF, these rats developed TTP (thrombocytopenia, hemolytic anemia and VWF-rich thrombi in kidney and brain).
- After rhADAMTS13 injection, circulating anti-ADAMTS13/rhADAMTS13 immune complexes were observed in plasma.
- Despite the formation of circulating immune complexes, rhADAMTS13 was able to restore ADAMTS13 activity and resolve acquired TTP.

Conclusion:

- A new laboratory animal model mimicking various aspects of acquired TTP was established.
- rhADAMTS13 was demonstrated to be an effective therapy for acquired TTP in this model.

TTP: prognostic markers

(OR362) Timely formation of ULVWF multimers and reduction of ADAMTS13 activity precede clinical events of TTP relapse

Wu H¹ et al. ¹*Ohio State University, Columbus, USA*

Aims:

- To study ADAMTS13 and VWF as predictors of TTP relapse.

Methods:

- Analysis of ULVWF multimers and ADAMTS13 activity on blood samples collected quarterly up during remission until relapse.
- Comparison of these variables among TTP patients experiencing a relapse or not:
 - 43 samples from patients experiencing TTP relapse < 3 months after collection (Pre-relapse)
 - 33 samples from patients experiencing TTP relapse > 3 months after collection (Relapse)
 - 21 samples from non-relapsing patients (Non-relapse)

TTP prognostic markers

Results:

- **ULVWF** was present in 74% of Pre-relapse, 52% of Relapse and 14% of Non-relapse samples.
- **ADAMTS13 activity** was significantly lower in both Pre-relapse (5%) and Relapse (19%) groups than Non-relapse group (70%).
- For patients with ADAMTS13 activity < 20%, ROC analysis showed that a presence of ULVWF in Pre-relapse samples would predict a relapse in > 60% of cases.
- When data were analyzed as a time-course to relapse (8–12 weeks, 4–8 weeks, 2–4 weeks, and < 2 weeks), Pre-relapse samples showed an increasing trend in ULVWF toward the time point of relapse, with ULVWF present in > 90% of the samples collected within < 2 weeks prior to relapse.

Conclusions:

- Patients with relapsing TTP exhibit a baseline increase in ULVWF and a decrease in ADAMTS13 throughout remission to relapse.
- A substantial increase in ULVWF along with a large reduction of AD13 is a significant risk factor predicting TTP relapse.

TTP: novel treatments

(LB006) Additional data from the TITAN trial with the anti-VWF nanobody caplacizumab in the treatment of acquired TTP

Flora Peyvandi¹ et al. ¹*Coordinating Investigator TITAN trial, University of Milan, Italy*

Background:

- Caplacizumab (CAP) is an anti-VWF Nanobody developed for the treatment of acquired TTP.
- Mode of action: CAP binds to the A1 domain of VWF preventing VWF-mediated platelet aggregation.
- The **TITAN trial** is a phase II single blind, randomised, placebo controlled trial to study the efficacy and safety of CAP administered in acute acquired TTP, in conjunction with standard of care (plasma exchange and immunosuppressants).

TTP: novel treatments

Aims:

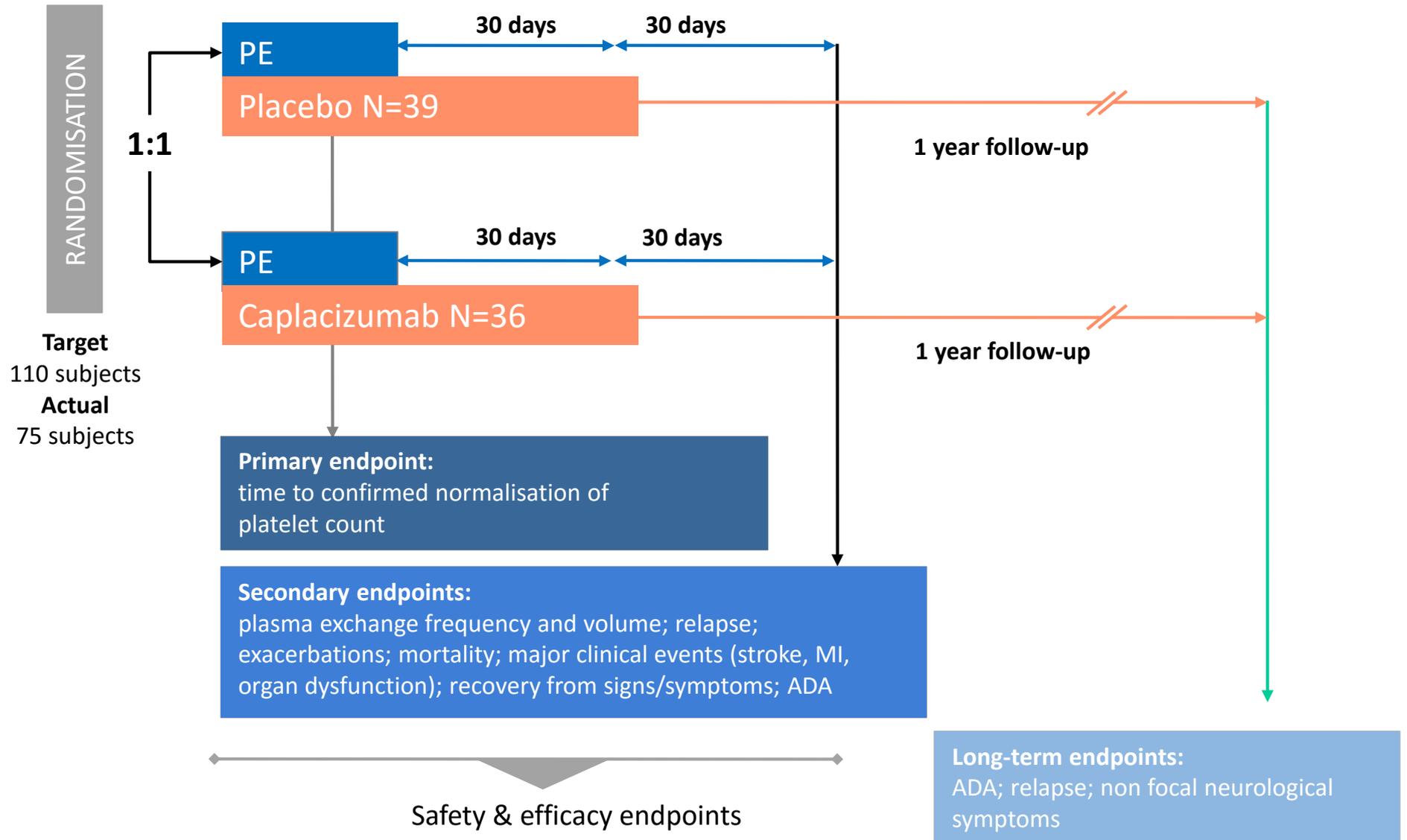
- **Primary endpoint:** time to confirmed platelet response (platelets $\geq 150,000 \mu\text{L}$, confirmed after 48 h).
- **Secondary endpoints:** plasma exchange frequency and volume; relapse; exacerbations; mortality; major clinical events (stroke, MI, organ dysfunction); recovery from signs/symptoms; ADA.
- **Long-term endpoints:** Anti-drug-antibodies (ADA), relapse, non-focal neurological symptoms.

Methods:

- Treatment consisted of one intravenous bolus injection of CAP or placebo (PLC) prior to first on-study PE, followed by daily subcutaneous administrations of 10 mg CAP or PLC after each PE session and for 30 days following the last PE.

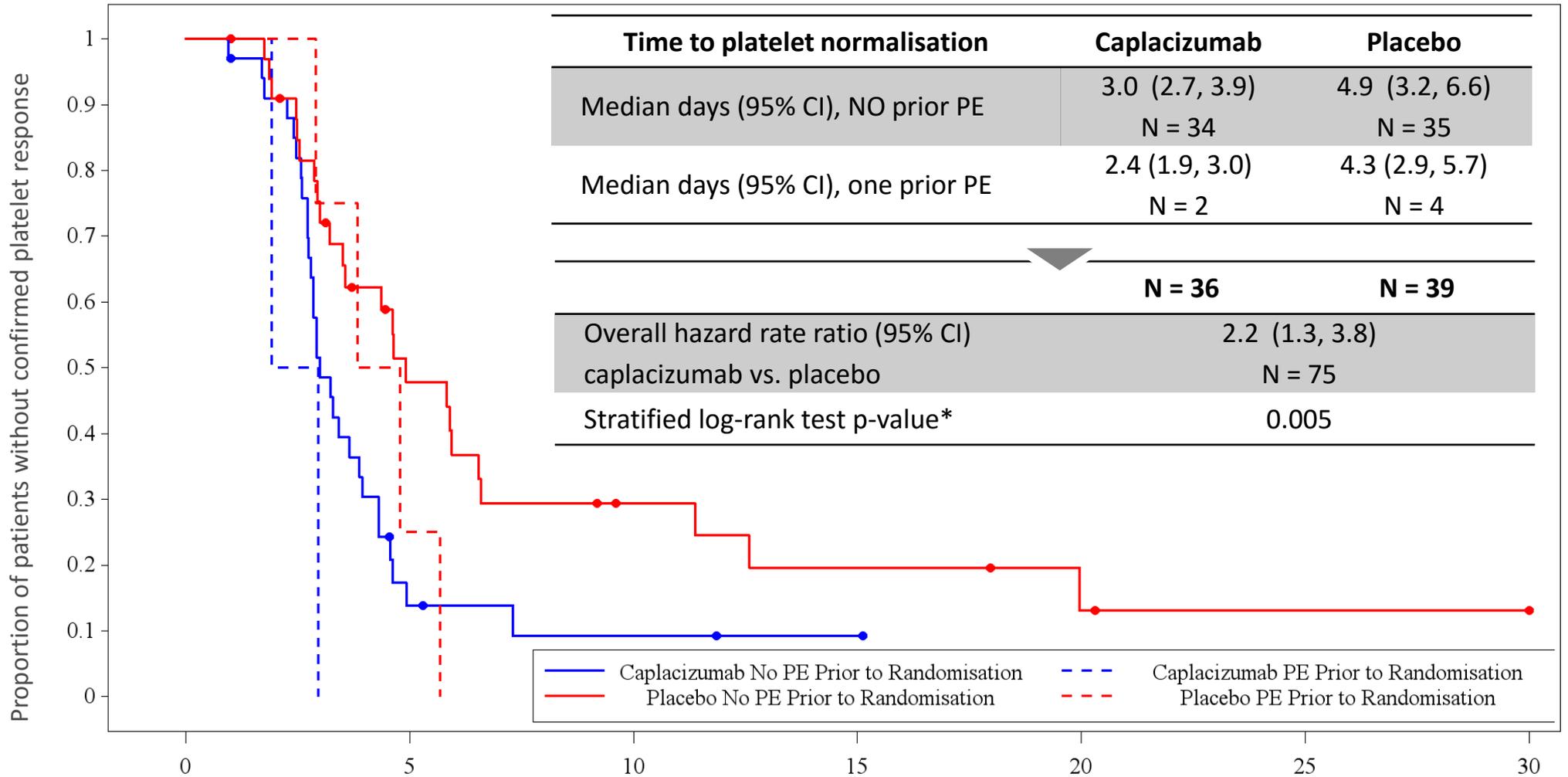
Caplacizumab Phase II TITAN trial

Design and schedule



Caplacizumab Phase II TITAN trial

Primary endpoint – time to platelet normalisation



* log-rank test p-value = 0.013 evaluated time to confirmed platelet response between the 4 groups presented above

The group of patients treated with caplacizumab achieved confirmed platelet normalisation at more than twice the rate of the group receiving placebo

Caplacizumab Phase II TITAN trial

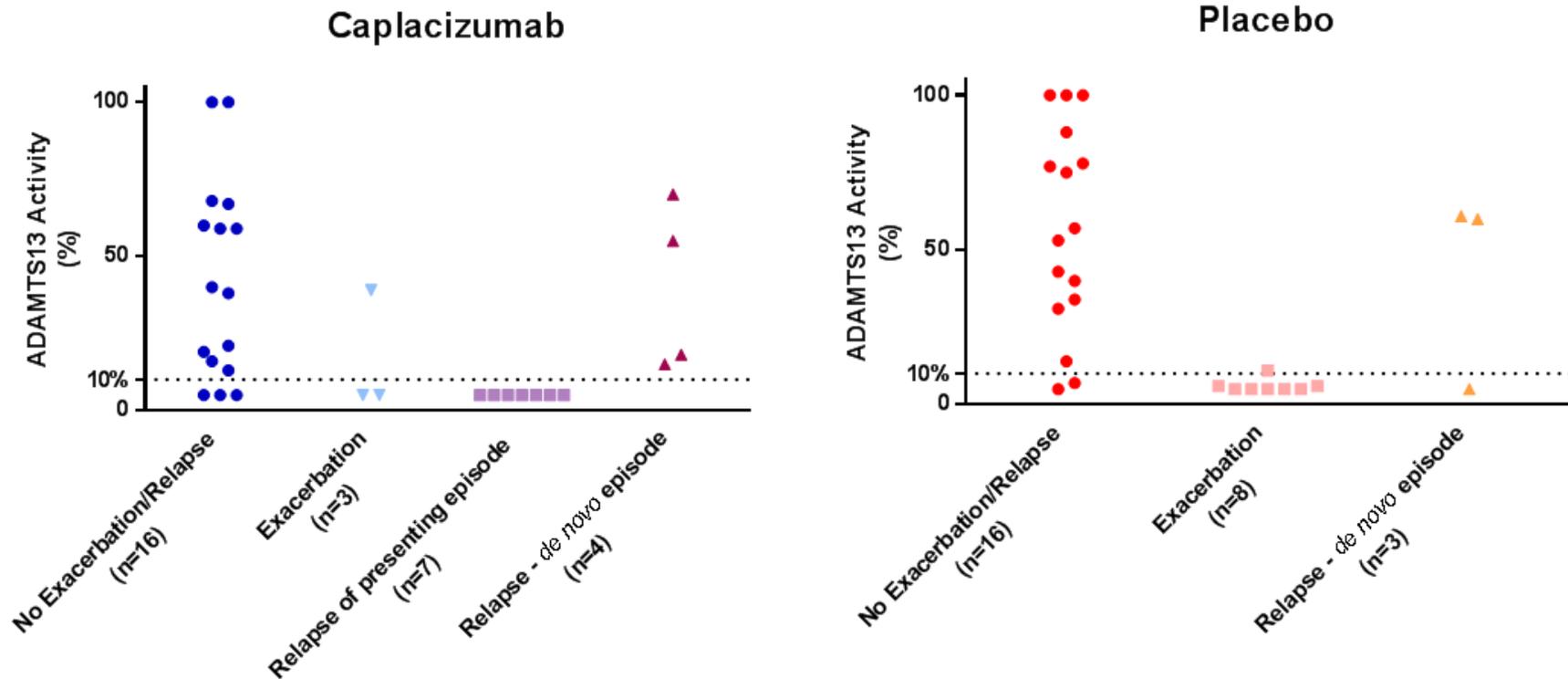
Key secondary endpoints

Proportion (number) of subjects (ITT population)	Caplacizumab N = 36	Placebo N = 39
Complete remission	81% (29)	46% (18)
Exacerbation	8% (3)	28% (11)
Exacerbation and/or relapse up to 1 month follow-up	28% (10)	28% (11)
Deaths, n	0	2

In the caplacizumab treatment group a higher proportion of subjects achieved complete remission and fewer patients had exacerbations of TTP

Caplacizumab Phase II TITAN trial

Post-hoc analysis: relapses, exacerbations and ADAMTS13 activity



Data plotted: ADAMTS13 activity data available closest to treatment stop or data close to the day of exacerbation

- 7 patients relapsed within 10 days after stopping caplacizumab
 - All had continuous low ADAMTS13 activity (<10%) during and near treatment stop
 - Continue caplacizumab treatment in case underlying disease activity is not resolved

Caplacizumab Phase II TITAN trial

Safety profile

Proportion of subjects (safety population)	Caplacizumab N = 35	Placebo N = 37
Subjects with any TEAE	97%	100%
- with bleeding event	54%	38%
Subjects with any TE Serious AEs	57%	51%
- with serious bleeding event	6%	5%
Subjects discontinued due to TEAE	8%	0%

Increased bleeding tendency in caplacizumab treatment group

80% of reported events were mild

only 3 subjects required drug treatment; no requirement for VWF/FVIII substitution

Caplacizumab treatment resulted in an increased tendency for mild/moderate bleeding events but which were readily managed

Caplacizumab Phase II TITAN trial

Summary of results (1):

- The intent-to-treat population consisted of 36 CAP and 39 PLC subjects.
- The median time to platelet response was significantly faster in the CAP than in the PLC group (2.97 vs 4.79 days; hazard ratio 2.2).
- CAP treatment reduced the number of exacerbations during treatment.
- The higher number of relapses observed in the CAP group was explained by the underlying still active disease, as demonstrated by ADAMTS13 activity levels.
- CAP treatment resulted in an increased tendency for mild/moderate bleeding events but which were readily managed.

Caplacizumab Phase II TITAN trial

Summary of results (2):

- Further analysis demonstrated that caplacizumab reduces the number of PE days in the treatment of acquired TTP in line with the faster platelet normalisation.
- Exploratory analysis showed a trend towards more rapid normalization of organ damage markers (troponin, creatinine, LDH).

Conclusions:

- Caplacizumab seems promising for the treatment of acute TTP, in addition to standard therapy.

aHUS: novel therapeutic approaches

(OR154) Coversin, a novel complement C5 inhibitor and potential therapeutic agent, prevents C5 activation in patients with C5 polymorphisms

Mackie IJ¹ et al. ¹*University College, London, UK*

Background:

- The monoclonal antibody eculizumab prevents cleavage of complement C5 by C5 convertase, and is currently used to treat PNH and aHUS patients.
- Patients with p.R885H and, probably, p.R885C heterozygous C5 polymorphisms are resistant to treatment with eculizumab, causing an increased thromboembolic risk (Nishimura et al, NEJM 2014).
- Coversin, a small protein inhibitor of C5, binds to a different site on C5 than eculizumab and is a potent inhibitor of C5 activation in a wide range of mammals.

Aim:

- To compare the ability of Coversin and eculizumab to prevent activation of human C5 in patients with C5 p.R885 polymorphisms.

aHUS: novel therapeutic approaches

Methods:

- Serum from 6 normal controls and 2 Caucasian patients with C5 p.R885 polymorphisms were tested for complement activation in a CH50 ELISA.
- Serial therapeutic concentrations of eculizumab and Coversin were used to spike patient and normal control sera before testing.

Results and conclusions:

- As expected, Eculizumab completely inhibited normal samples at concentrations $>35 \mu\text{g/mL}$, but it only inhibited CH50 activity by 75% in patients with the p.R885 polymorphisms, even at concentrations up to $100 \mu\text{g/mL}$.
 - In contrast, Coversin inhibited complement activity equally well in resistant patients and normal controls.
- Coversin might provide a useful alternative to eculizumab in patients where no other prophylaxis option is available.

Conclusions

- Despite advances, TTP still remains a largely unpredictable disease

- Many opened questions on TTP:

[?] One third of patient show mild-to-moderate ADAMTS13 deficiency or even normal levels in acute phase: *other pathogenic mechanisms?*

[?] *Which is the optimal treatment in acute phase?*

[?] *Which patients will relapse?*

[?] *Which is the best treatment in remission phase?*

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