

Webinars

Thrombotic Microangiopathies

Thrombotic thrombocytopenic purpura

EuroBloodNet  Topic on Focus

Animal models of Thrombotic Thrombocytopenic Purpura

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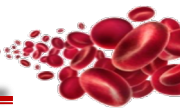
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European
Reference
Network
for rare or low prevalence
complex diseases
 Network
Hematological
Diseases (ERN EuroBloodNet)



Member of the Advisory Board of Takeda

Research grant Takeda

Consultant for GC Pharma



1. Different types of animal models for TTP

1. Signs and symptoms


2. Triggers

2. Use of animal models in drug discovery



Thrombotic thrombocytopenic purpura

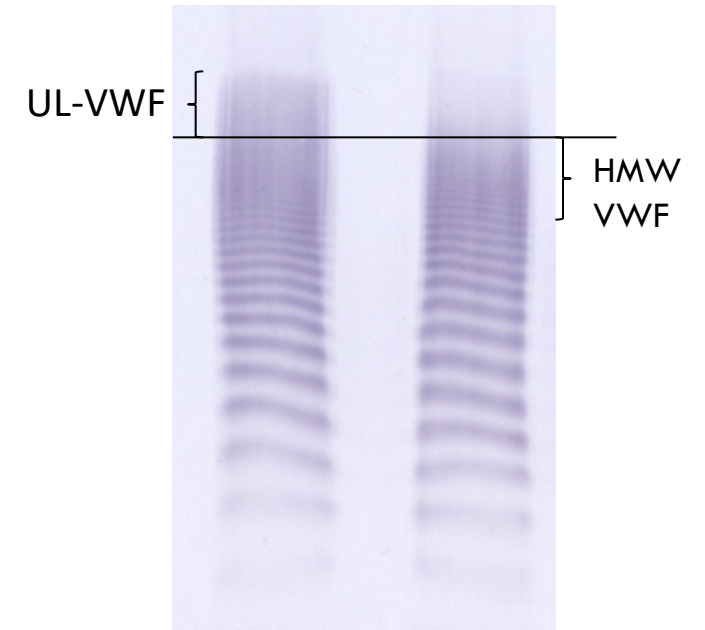
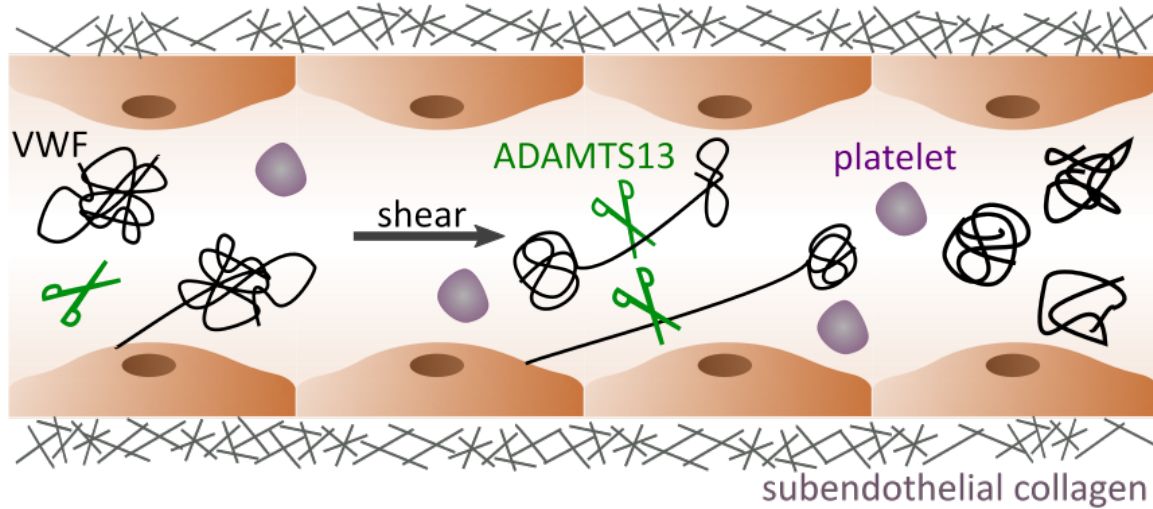


- Rare disease
- Devastating disease
 - Intensive care
 - Lethal when left untreated
 - Life-long
- Types
 - Congenital TTP (5%) 
 - Immune-mediated TTP (95%)





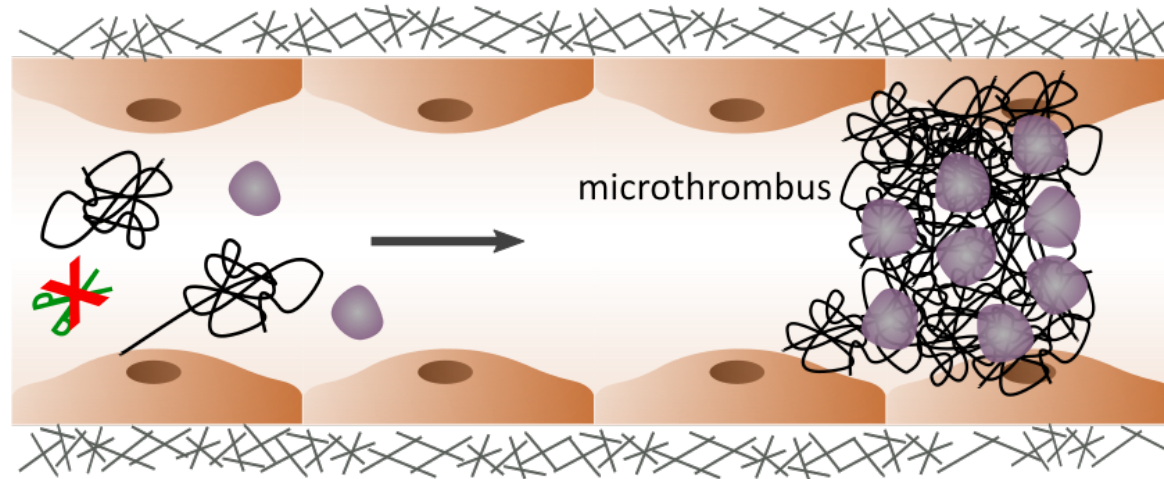
- Healthy individuals



→ VWF cleaving protease ADAMTS13 digests prothrombotic UL-VWF multimers into more quiescent HMW VWF multimers

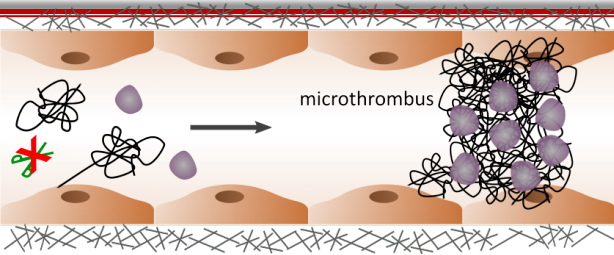


- Thrombotic thrombocytopenic purpura (TTP)

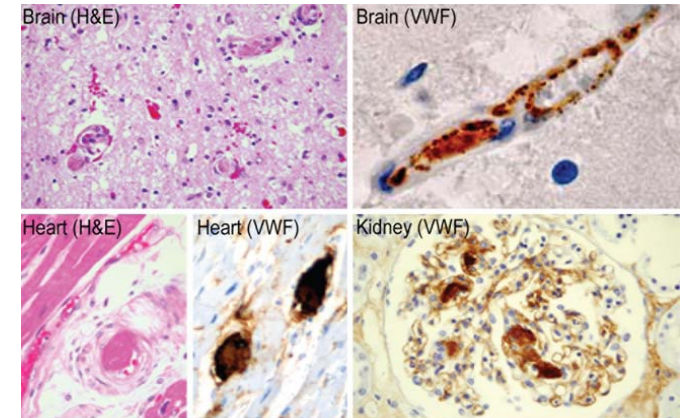
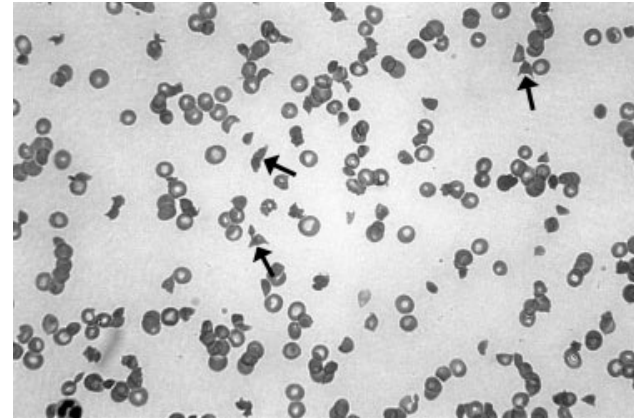


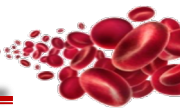
Deficiency in the VWF cleaving protease ADAMTS13 → prothrombotic UL-VWF multimers
→ microthrombi

Signs and symptoms

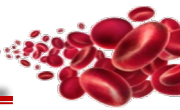


- Severe thrombocytopenia
- Haemolytic anemia
- Disseminated microthrombi
- Organ damage/failure





- Patients can live for years with ADAMTS13 deficiency: no TTP
- External trigger
 - Pregnancy
 - Stress
 - Surgery
 - Infection
 - ...





Animal models for TTP

Animal models for TTP

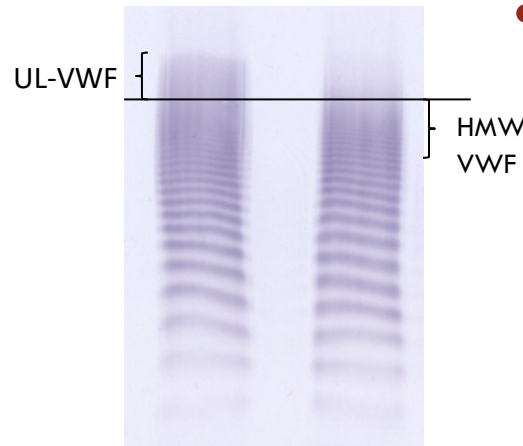
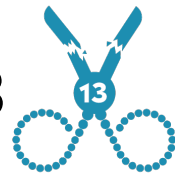


- 2 types of animal models

- Congenital TTP 
- Immune-mediated TTP 

- **ADAMTS13/VWF**

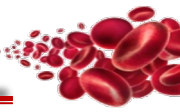
- Deficient ADAMTS13
- UL-VWF



- Signs and symptoms

- Severe thrombocytopenia
- Haemolytic anemia
- Disseminated microthrombi
- Organ damage/failure

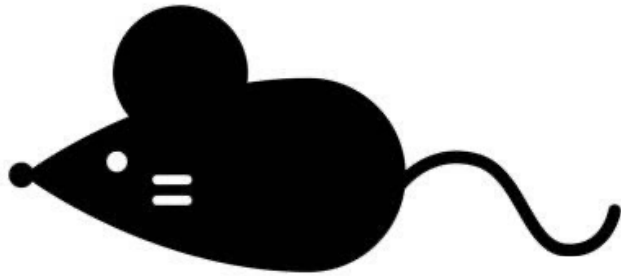
- **Trigger?**



Animal models for congenital TTP



- Mouse models



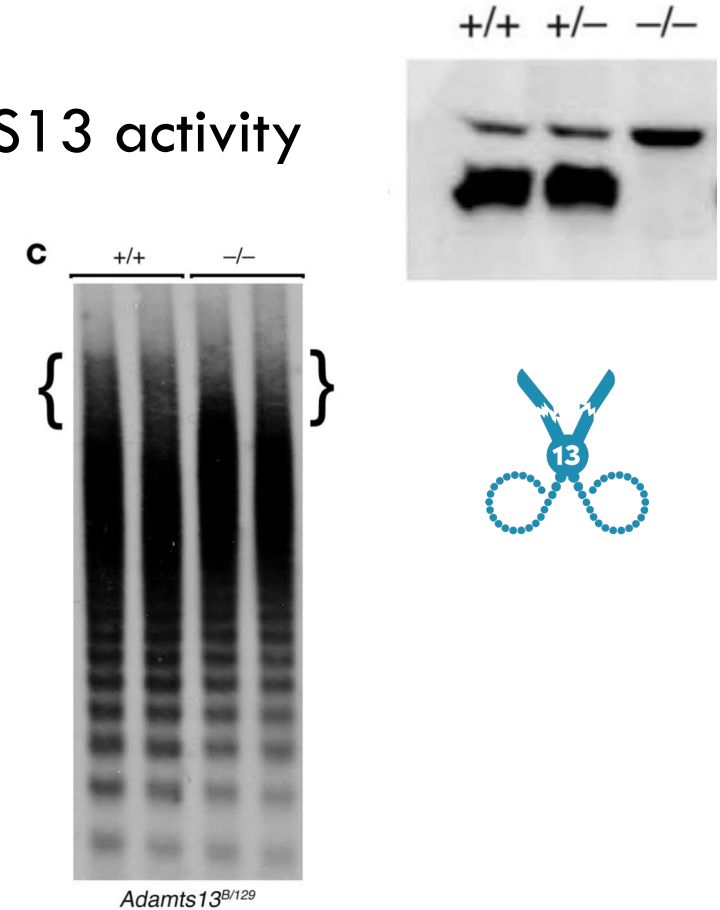
- Zebrafish model

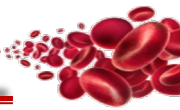


Animal models for congenital TTP

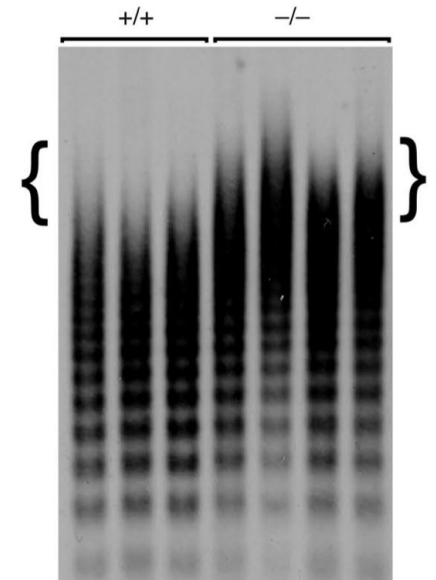


- Mouse models: genetic background: C57BL/6J and 129X1/SvJ (*Adamts13^{B/129}*)
 - Disruption of the ADAMTS13 gene (exon 1-6) → no ADAMTS13 activity
 - UL-VWF multimers: no difference between +/+ and -/-
 - Normal development and survival
 - No spontaneous TTP signs and symptoms!
 - Pregnancy did not trigger TTP

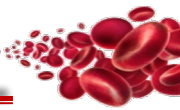




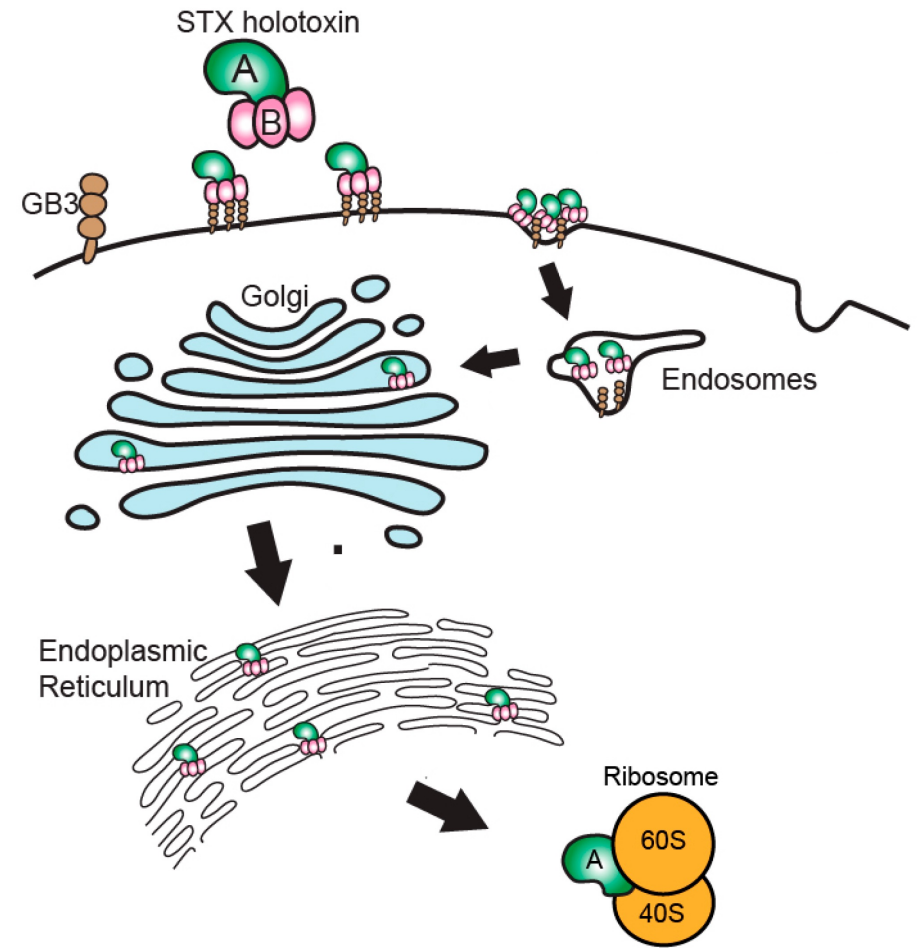
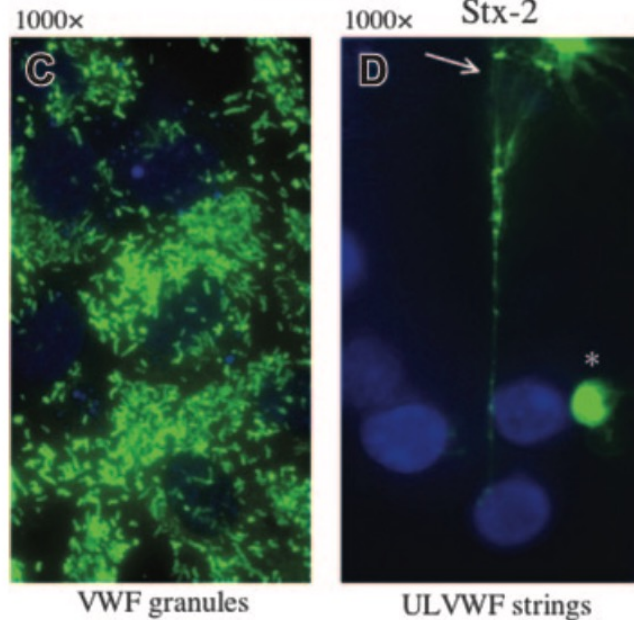
- **Mouse models:** genetic background back crossed on CASA/Rk (*Adamts13*^{B/CN2-/-})
 - 5- to 10-fold increase in VWF levels
 - Disruption of the ADAMTS13 gene (exon 1-6) → no ADAMTS13 activity
 - UL-VWF multimers: difference between +/+ and -/-
 - Normal development and survival
 - Pregnancy not a trigger for TTP
 - Sporadic mice with spontaneous TTP signs and symptoms



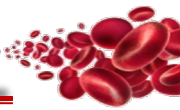
Animal models for congenital TTP



- **Mouse models: trigger needed to induce TTP**
 - Shigatoxin (STX) to trigger TTP?
 - STX B subunit activates endothelial cells
 - release of UL-VWF



Animal models for congenital TTP



- Shigatoxin as a trigger for TTP:

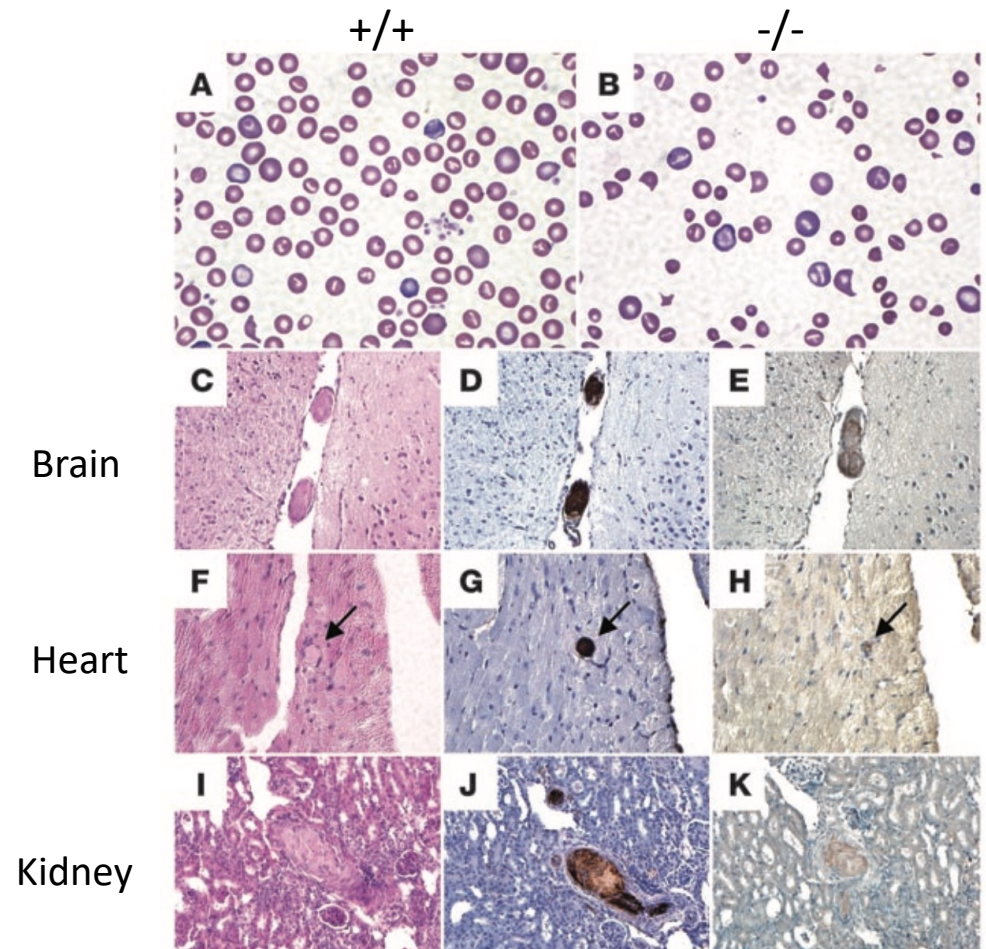
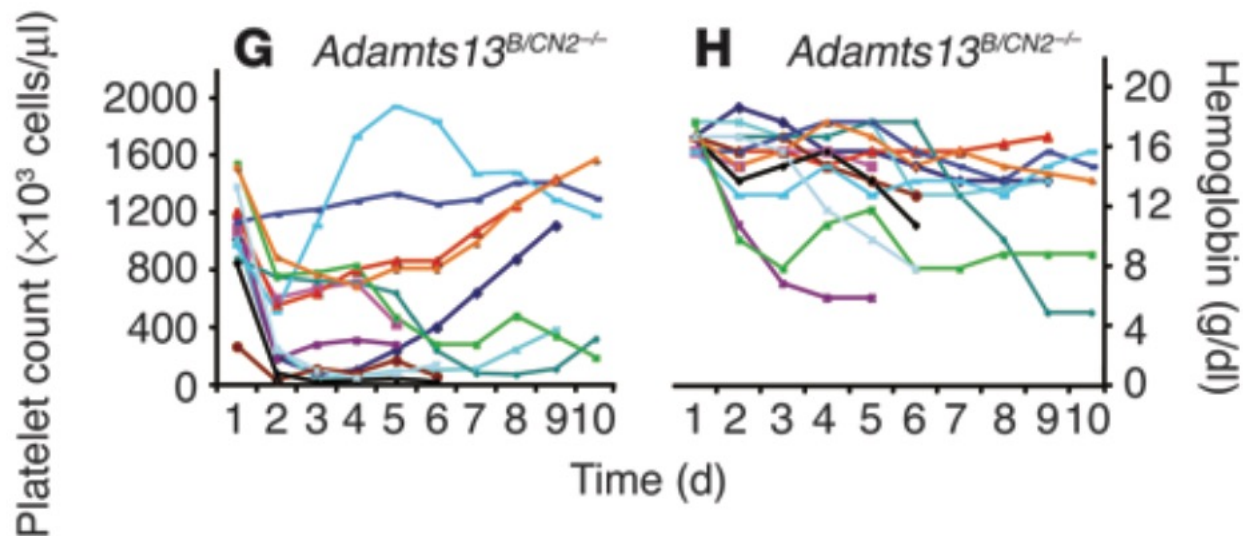
- Injection of Shigatoxin in *Adamts13^{B/129-/-}*: no TTP

- Injection of Shigatoxin in *Adamts13^{B/CN2-/-}*: TTP but not in all animals

- Severe thrombocytopenia (5/13)

- Hemolytic anemia

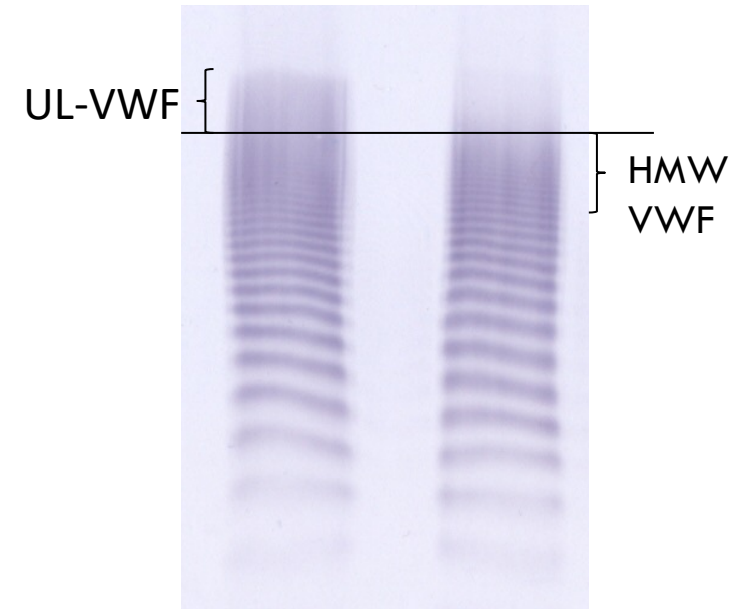
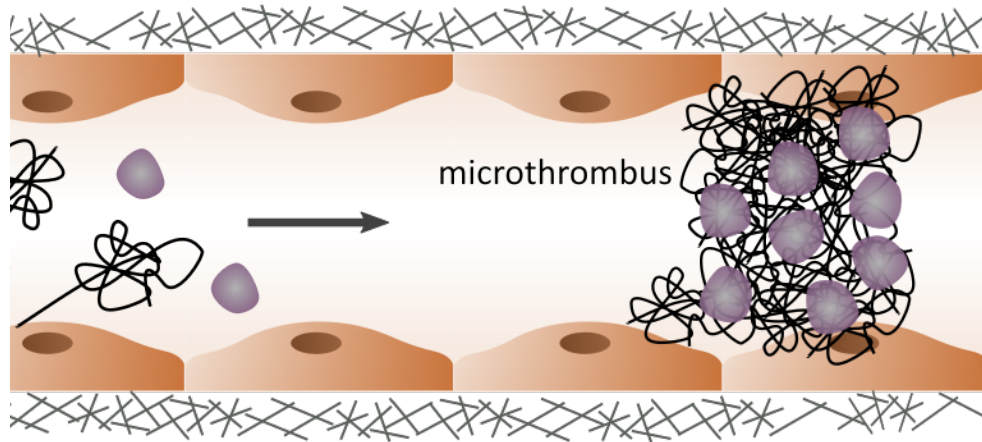
- Few microthrombi



Animal models for congenital TTP



- rVWF as a trigger for TTP:



Animal models for congenital TTP



- **rVWF as a trigger for TTP:**

- Injection of rVWF in *Adamts13^{B/129-/-}* :TTP

- rVWF contains UL-VWF multimers

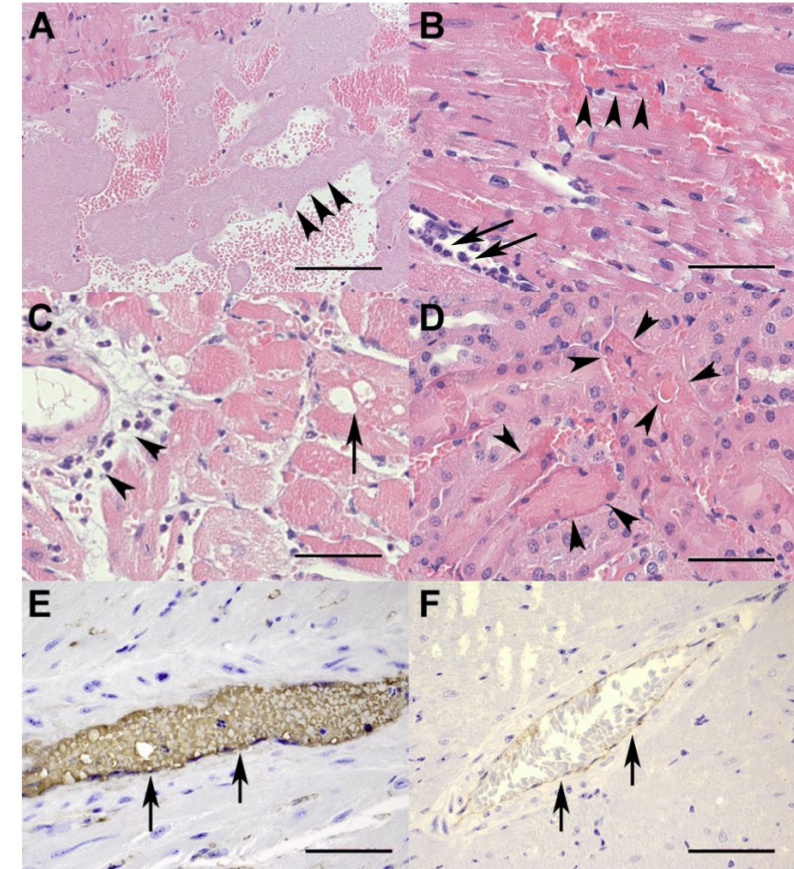
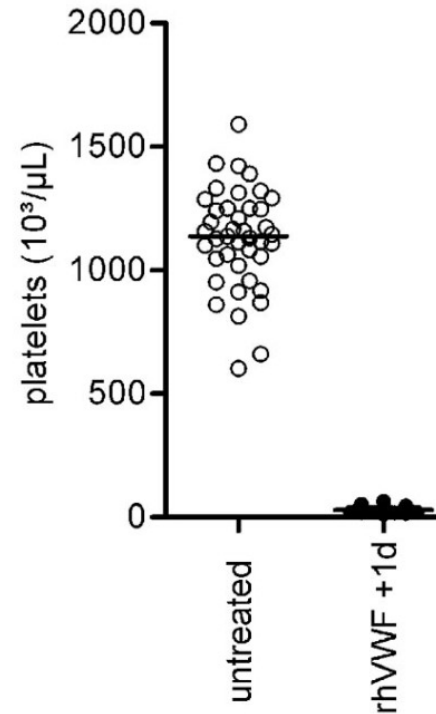
- Severe thrombocytopenia (all mice)

- Hemolytic anemia

- Decreased hematocrit
- schistocytes

- Organ damage

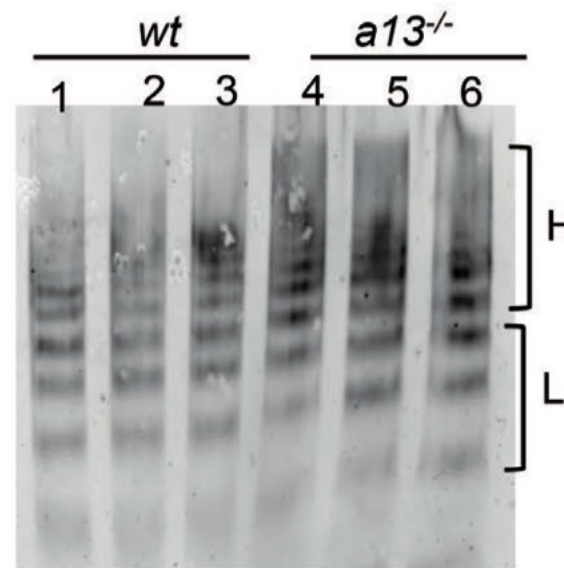
- Macroscopic lesions in the heart
- Platelet-VWF aggregates
- Increased LDH





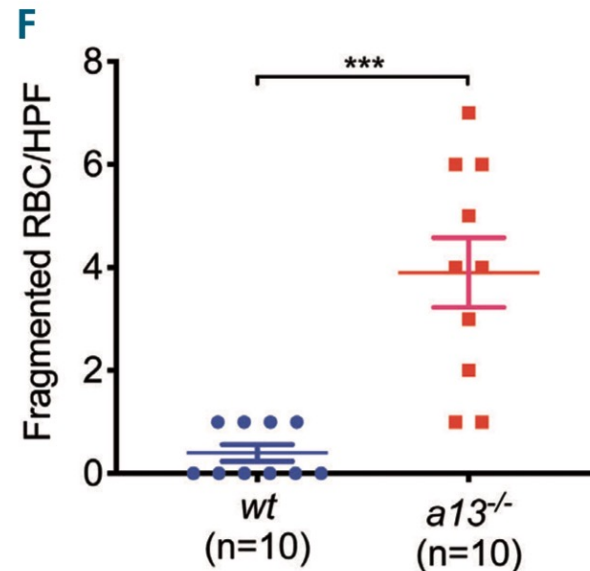
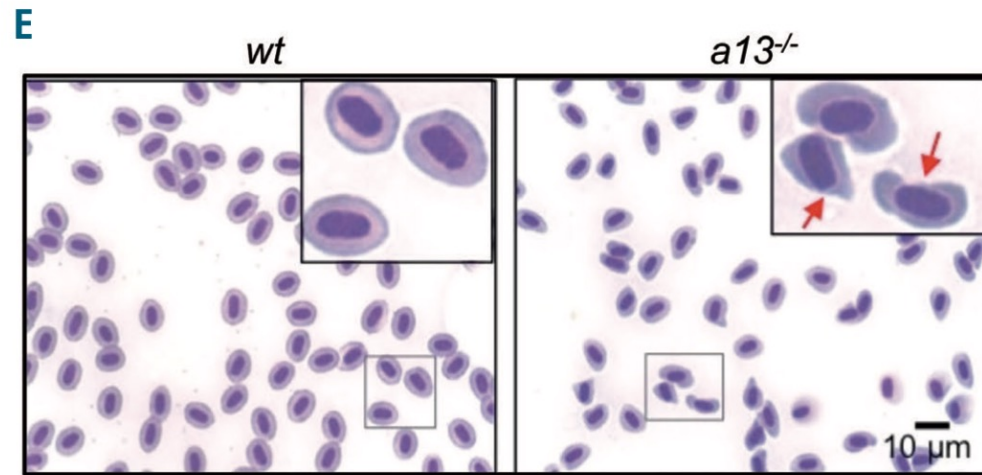
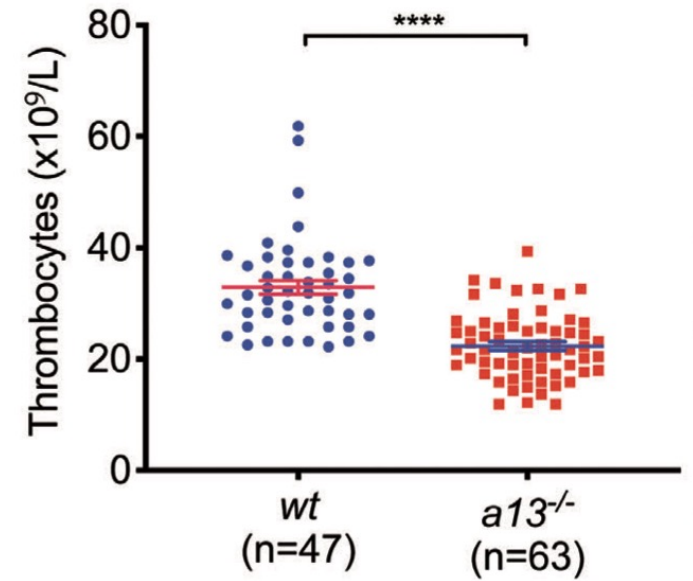
- Zebrafish model

- Disruption of the ADAMTS13 gene (CRISPR/Cas9, 8 nt deletion is signal peptide) → no expression of the ADAMTS13 protein (WB), no ADAMTS13 activity
- UL-VWF multimers: difference between +/+ and -/-



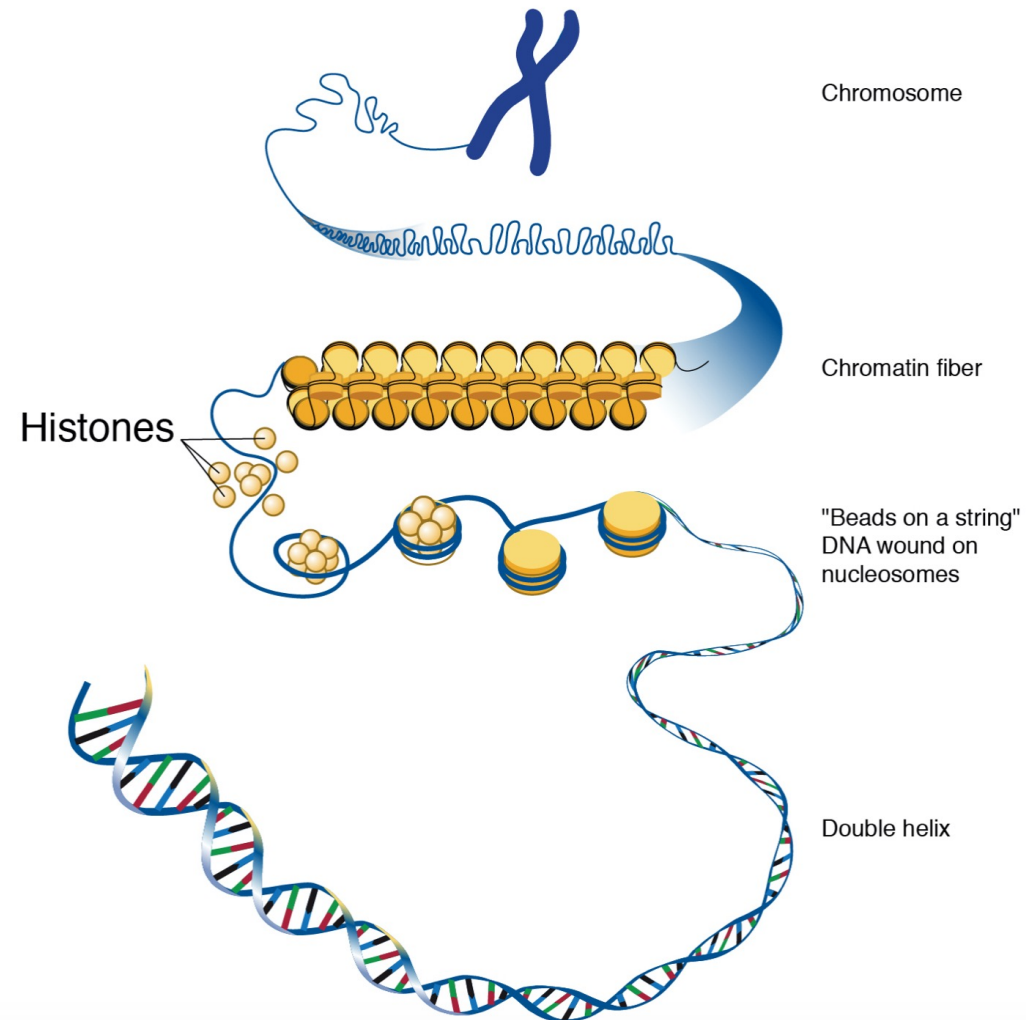


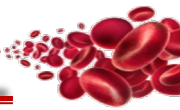
- No spontaneous TTP
 - Mild thrombocytopenia
 - Mild hemolytic anemia



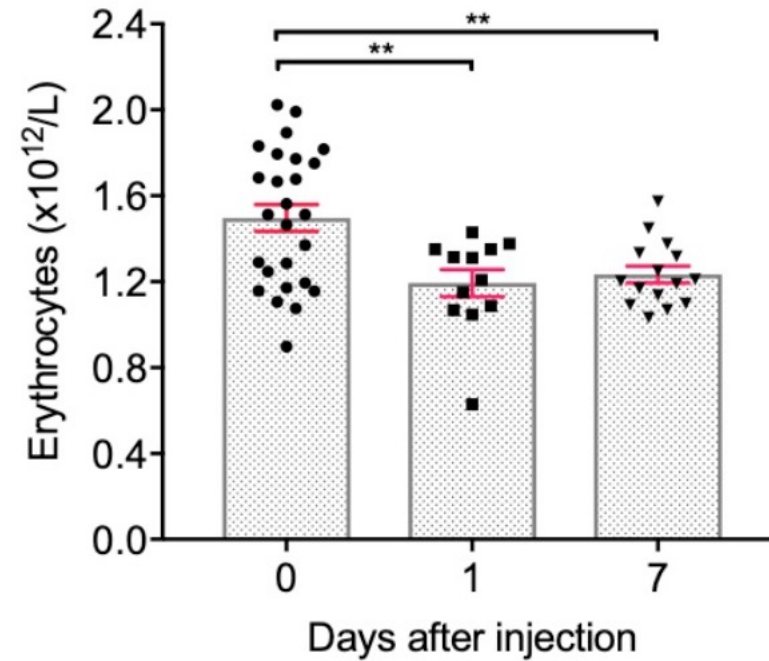
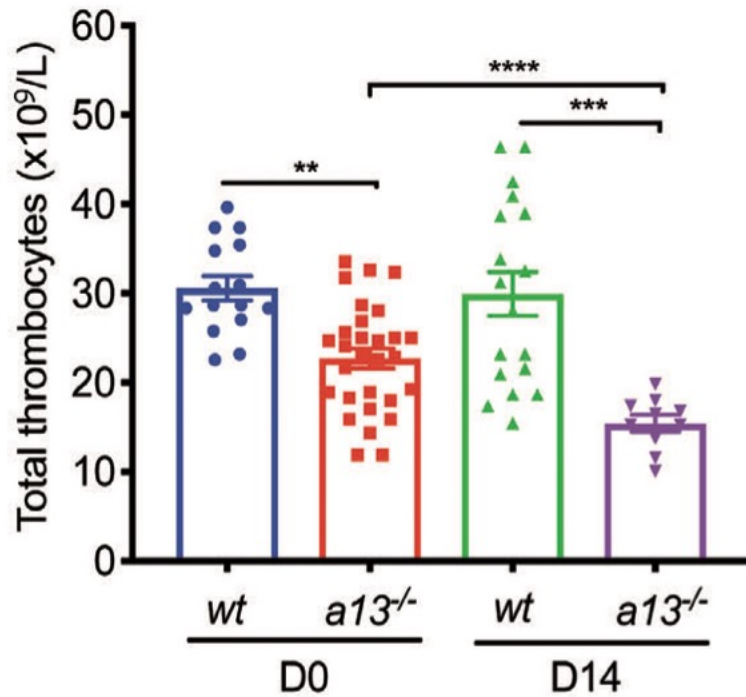


- Lysine-rich histones as a trigger for TTP



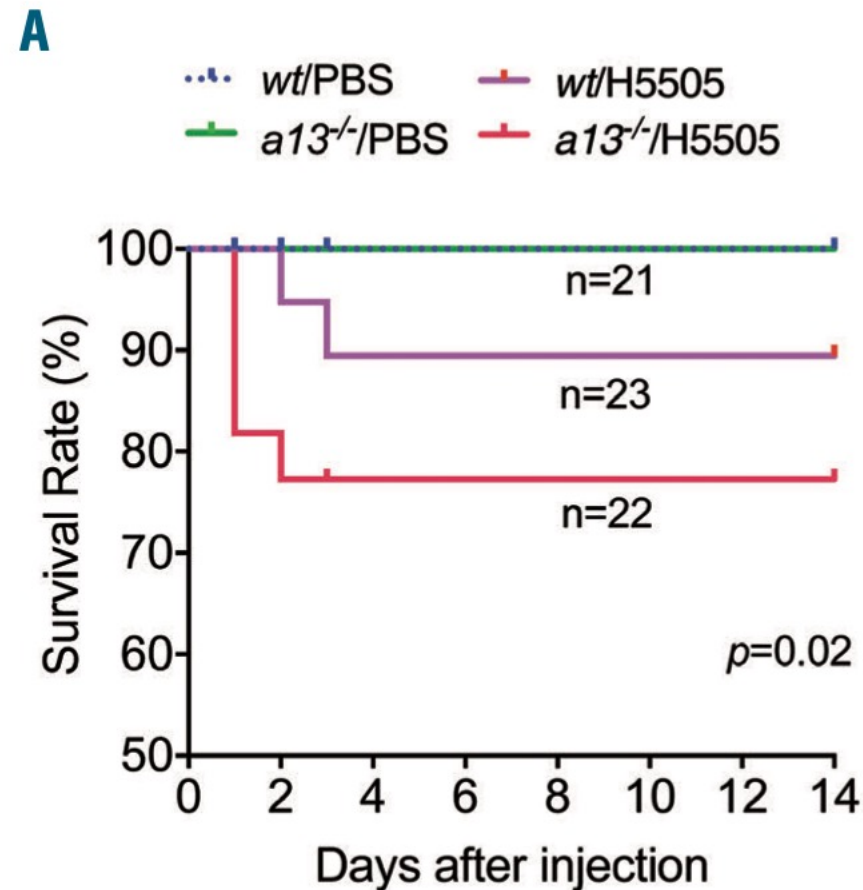


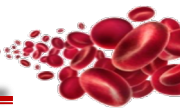
- Lysine-rich histones as a trigger for TTP
 - Significant Thrombocytopenia
 - Mild hemolytic anemia



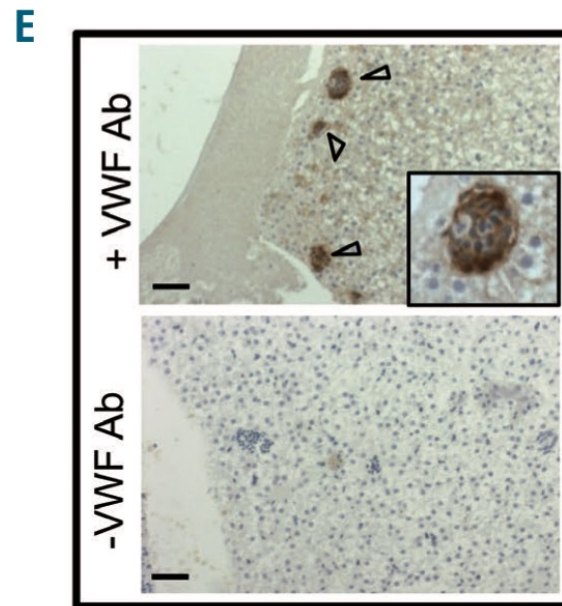
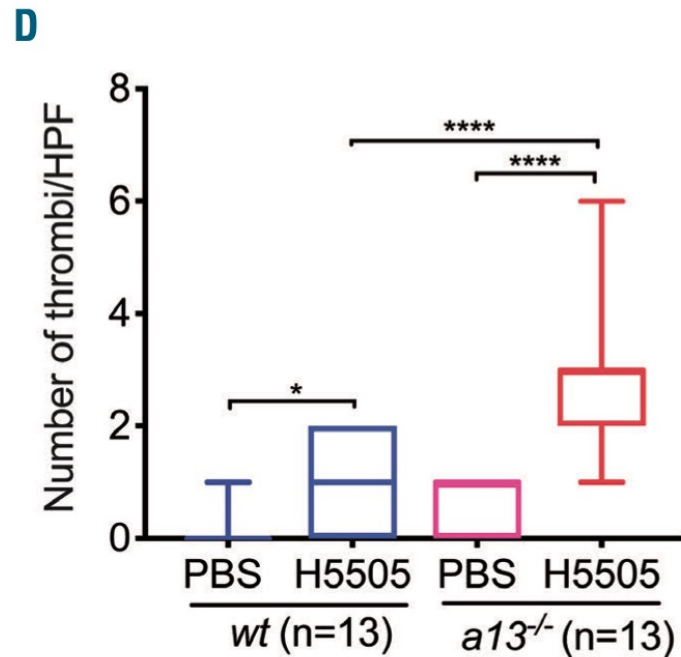


- Lysine-rich histones as a trigger for TTP
 - Decreased survival of *Adamt13*^{-/-} zebrafish challenged with Lysine-rich histones (H5505)





- Lysine-rich histones as a trigger for TTP
 - Multiple large microvascular thrombi in liver and mesenteric vessels in *Adamt13*^{-/-} zebrafish challenged with H5505





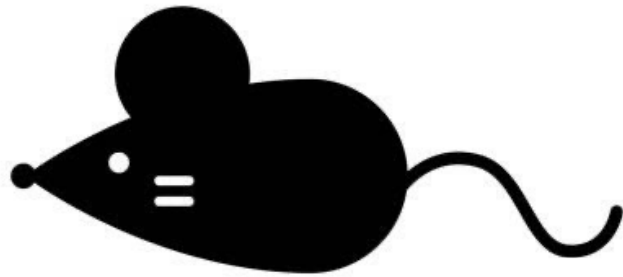
- Mouse and zebrafish models:
- ADAMTS13 deficiency not lethal and well tolerated
 - Humans: cTTP patients can remain free of TTP until early adulthood
- Signs and symptoms: to various extent
 - Thrombocytopenia, hemolytic anemia, microthrombi (organ damage, LDH)
- Triggers: Shigatoxin, rVWF, Lysine-rich histones
 - Stimulation of UL-VWF release
 - Other genetic factors: variations in platelet or vessel wall function?
 - Humans: pregnancy, stress, infection, surgery, ...



Animal models for immune-mediated TTP



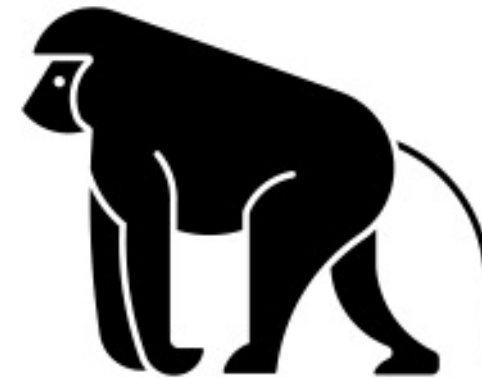
- Mouse models



- Rat model



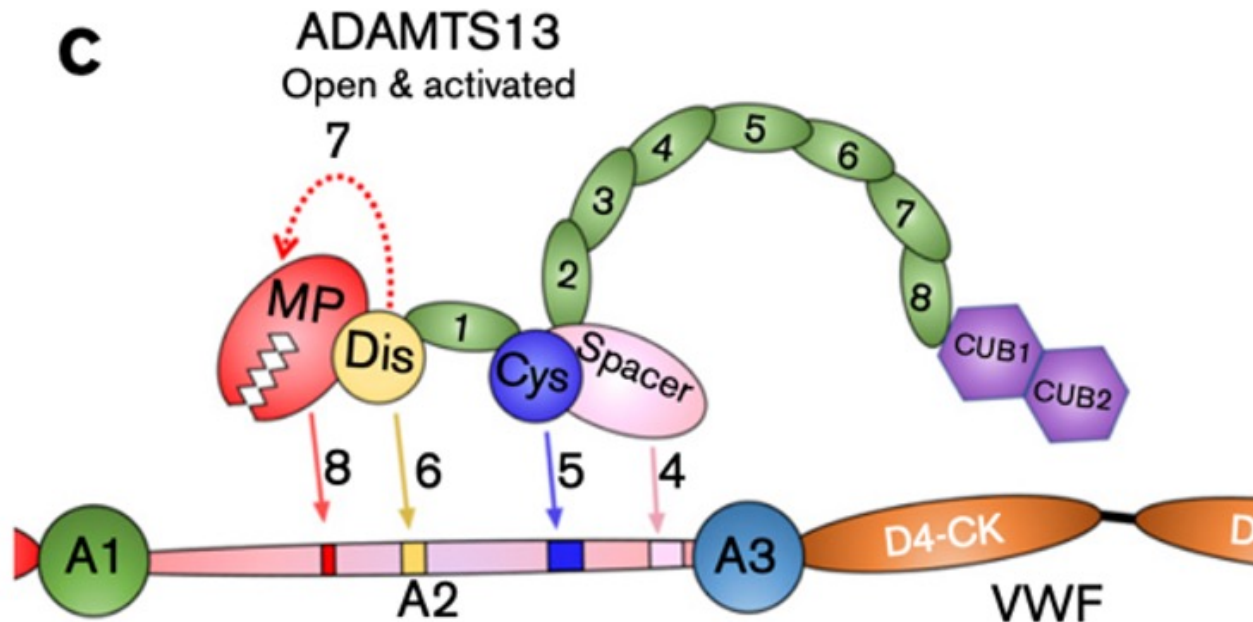
- baboon model





- Mouse models: WT mice

- Injection of murine inhibiting anti-ADAMTS13 antibodies
- Injection of human inhibiting anti-ADAMTS13 autoantibody

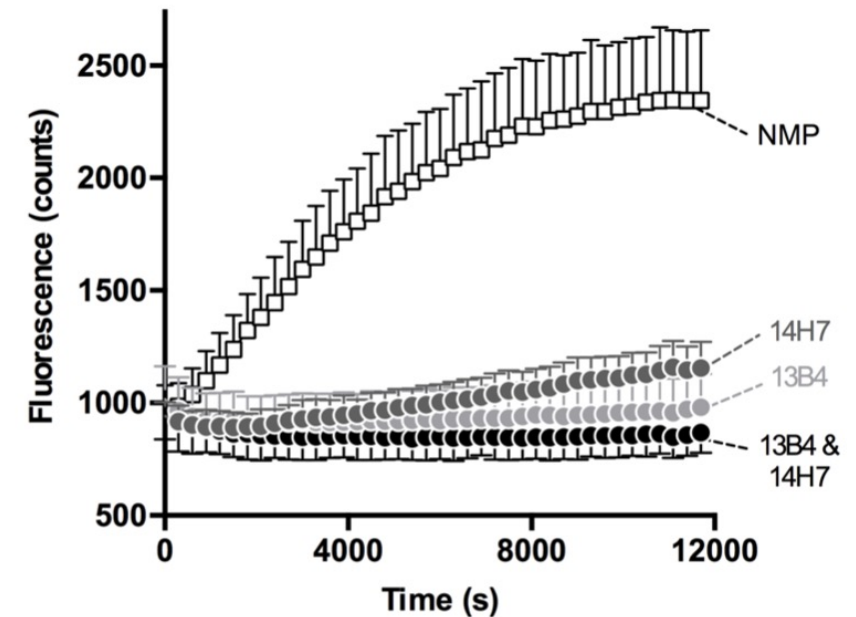
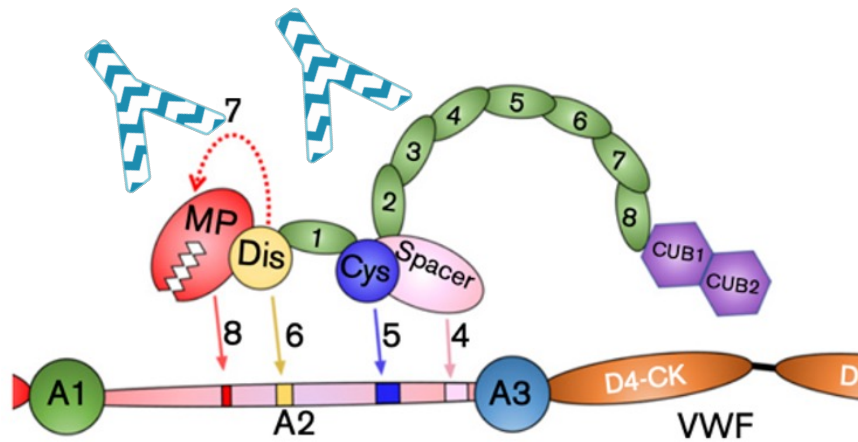


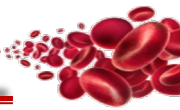


- Mouse models: WT mice

- Injection of murine inhibiting anti-ADAMTS13 antibodies

- Anti-MDTCS: 13B4 & 14H7
- No ADAMTS13 activity

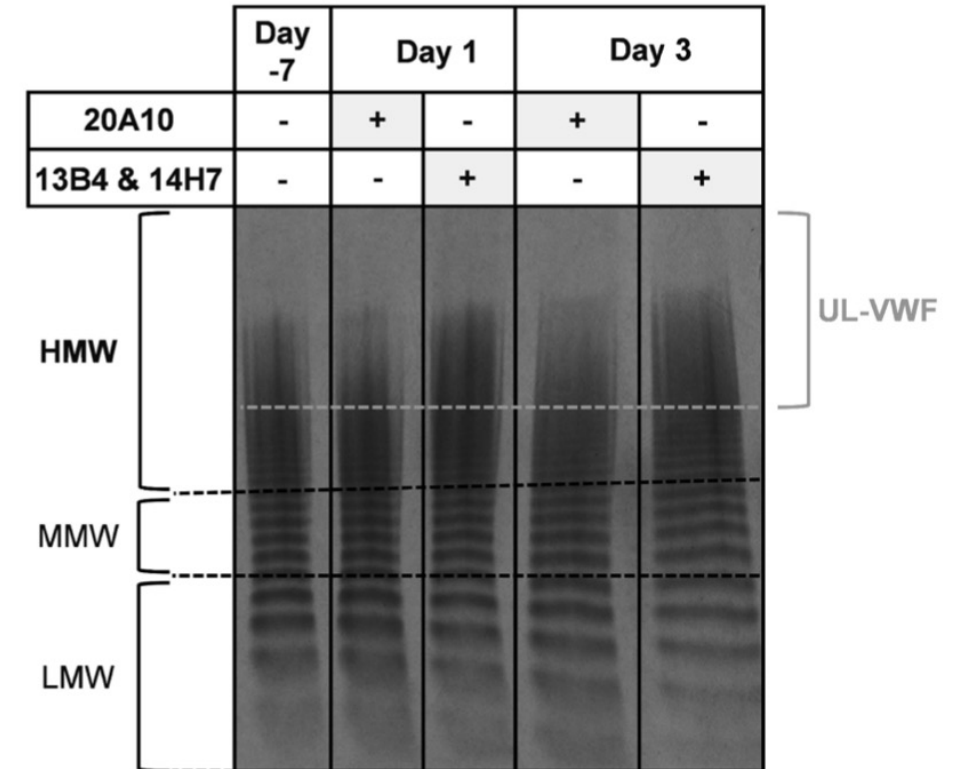
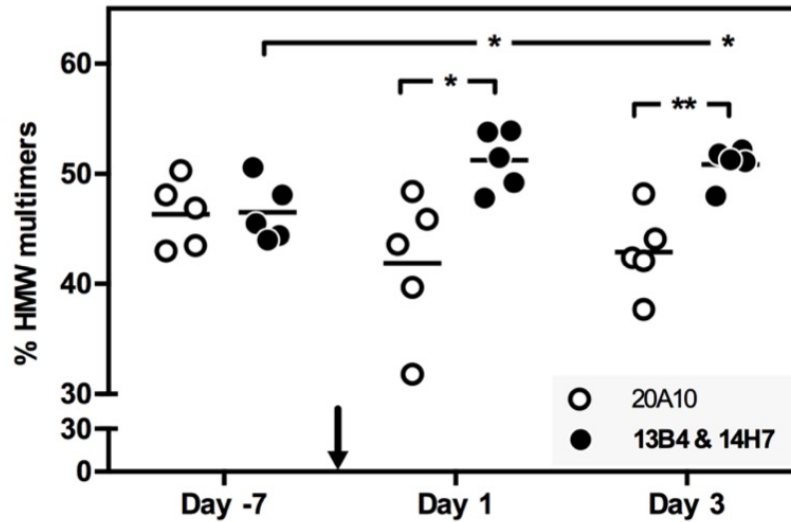




- Mouse models: WT mice

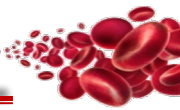
- Injection of murine inhibiting anti-ADAMTS13 antibodies

- UL-VWF

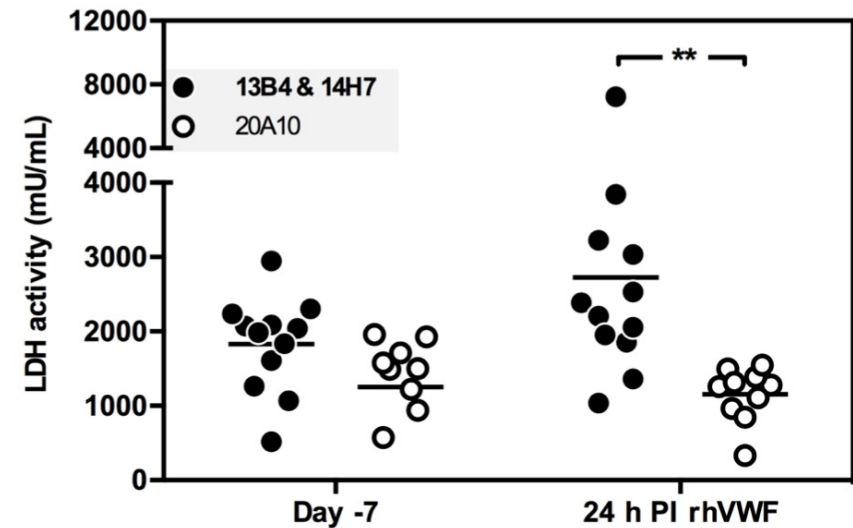
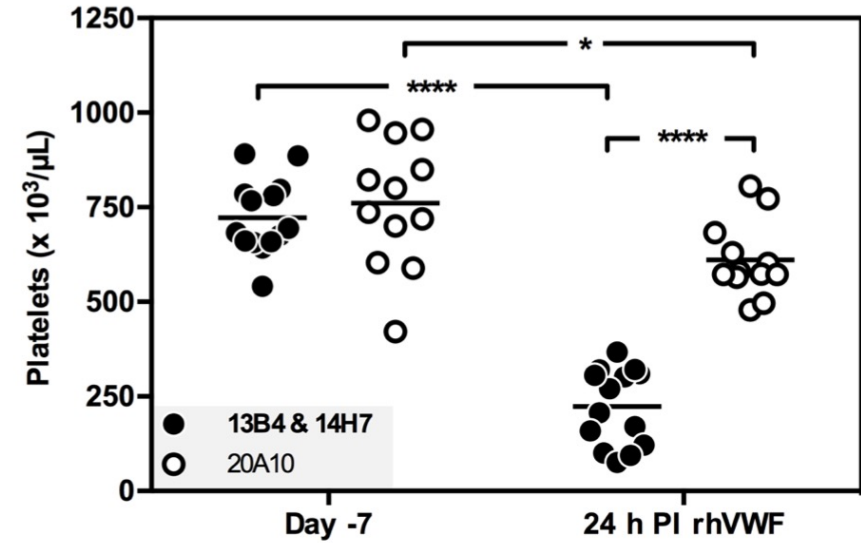


- No spontaneous TTP: no thrombocytopenia, no increase in LDH

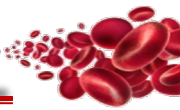
Animal models for Immune-mediated TTP



- rVWF as a trigger for TTP:
 - Injection of rVWF
 - rVWF contains UL-VWF multimers
 - Severe thrombocytopenia (all mice)
 - Organ damage
 - Few microthrombi
 - Increased LDH

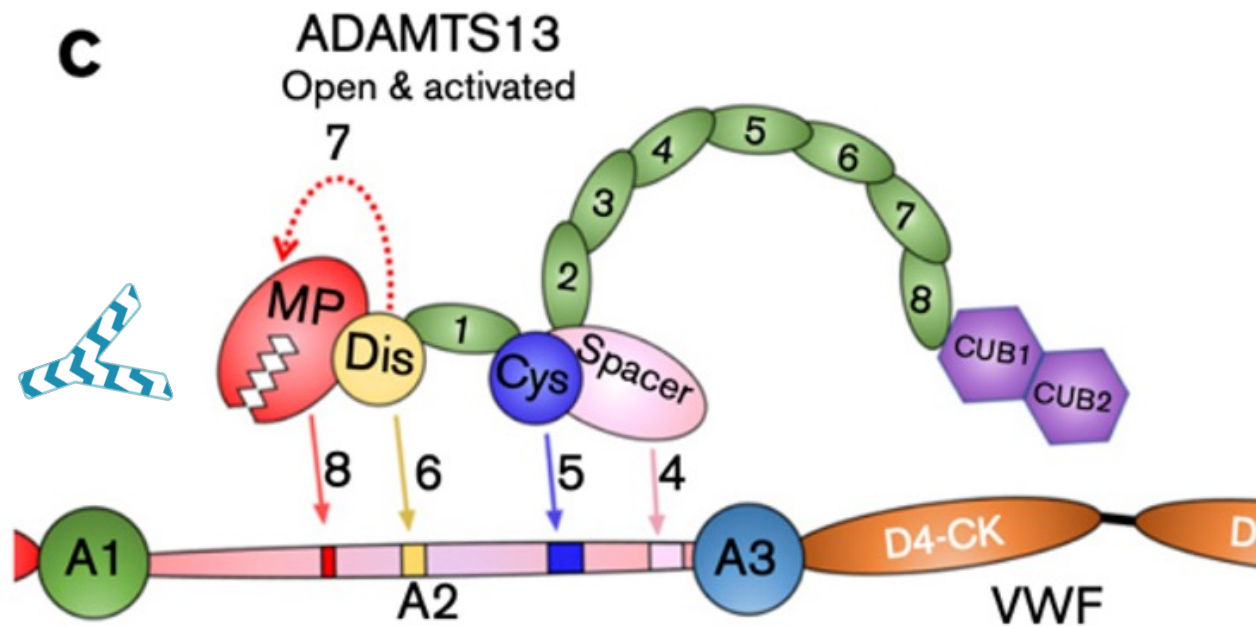


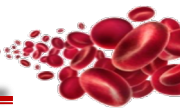
Animal models for Immune-mediated TTP



- Baboon model: WT baboon

– Injection of murine inhibiting anti-ADAMTS13 antibody

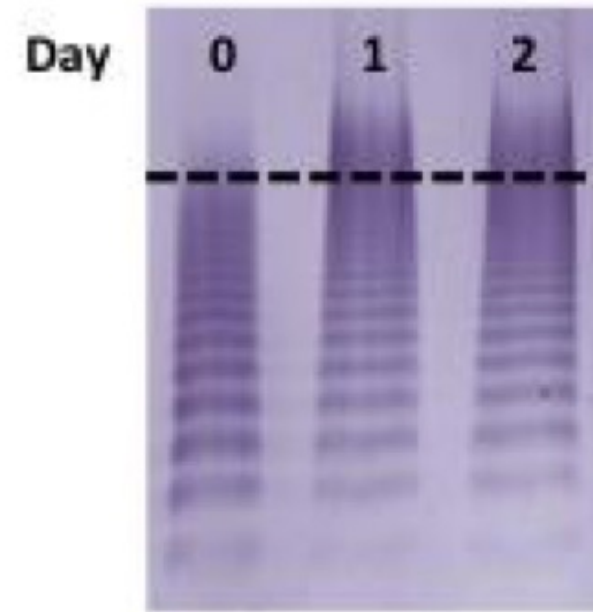
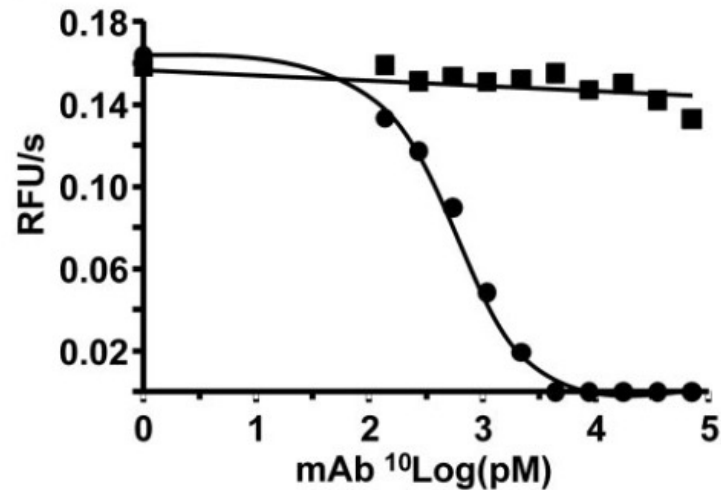
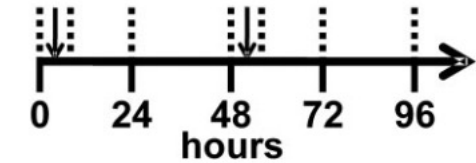




- Baboon model: WT baboon

- Injection of murine inhibiting anti-ADAMTS13 antibody

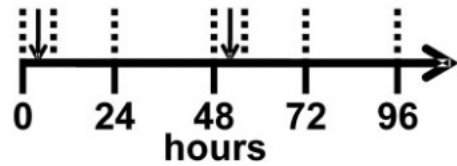
- Inhibition of baboon ADAMTS13 activity
- Appearance of UL-VWF



Animal models for Immune-mediated TTP

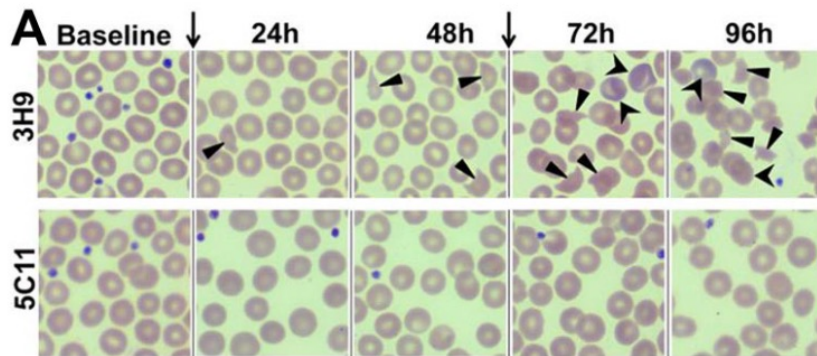
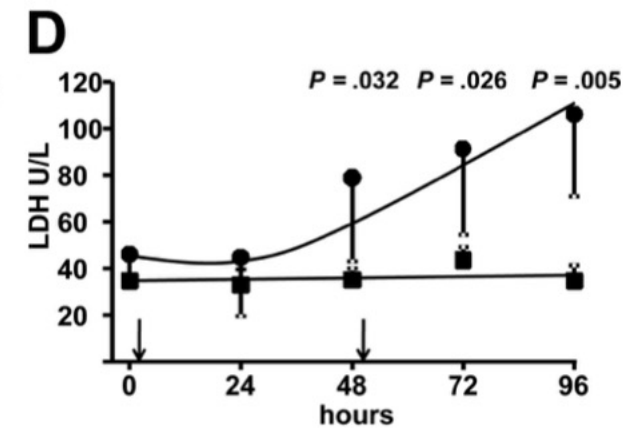
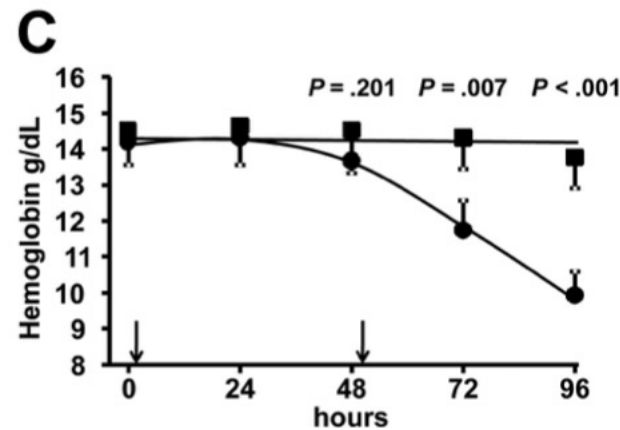
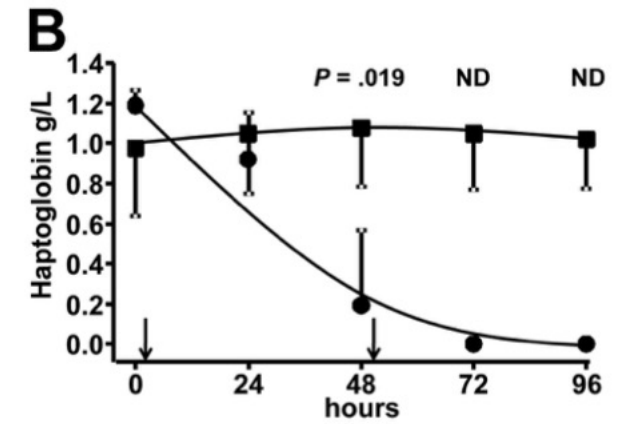
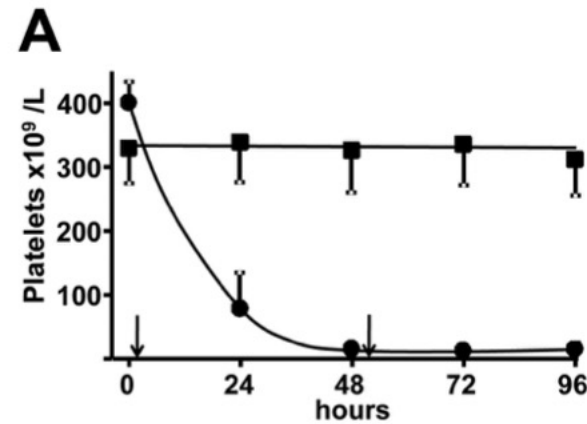


- Baboon model: WT baboon



– Spontaneous TTP in all baboons

- Severe thrombocytopenia
- Hemolytic anemia
- Increase in LDH



Animal models for Immune-mediated TTP

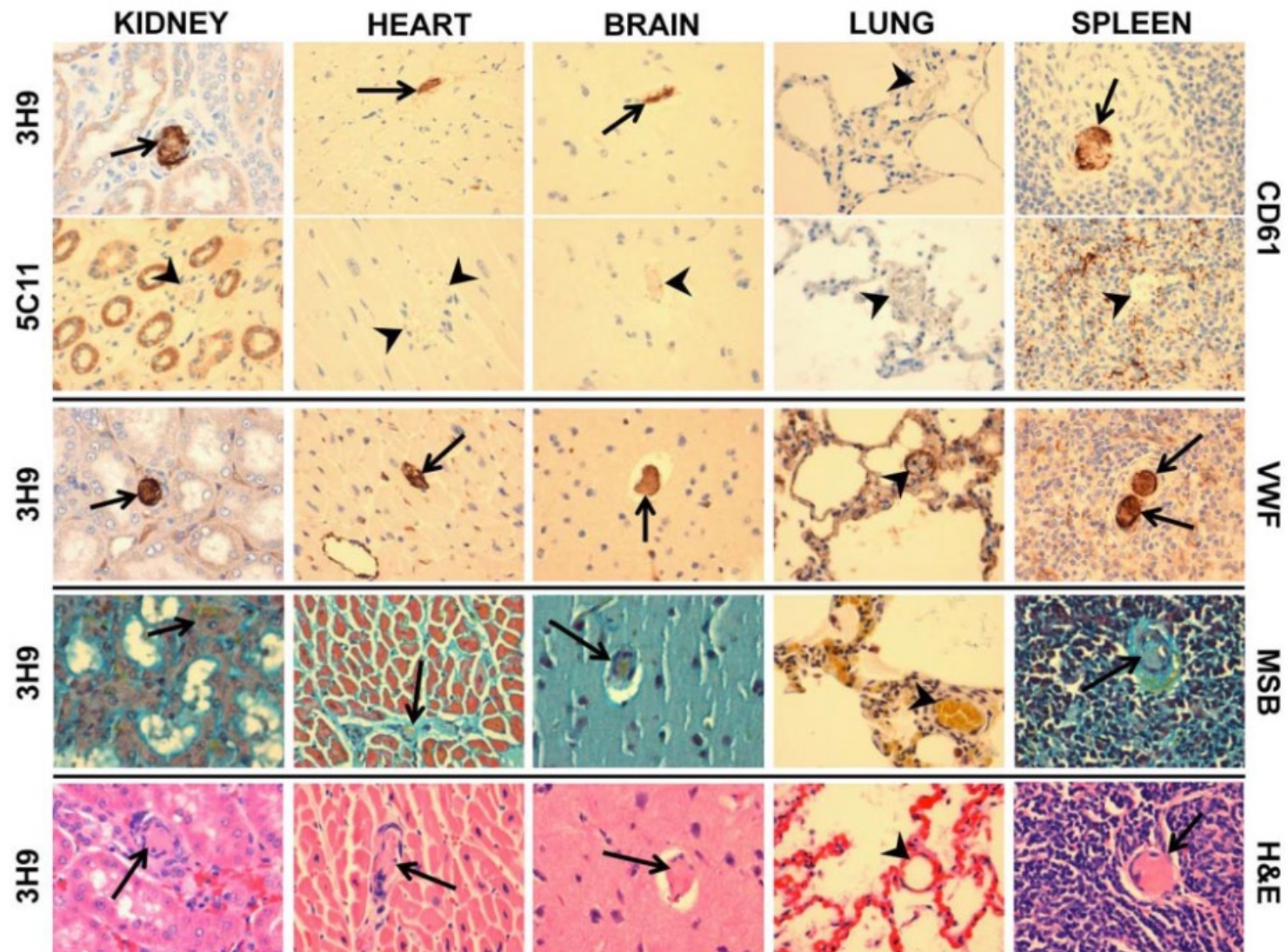


- Baboon model: WT baboon

- Spontaneous TTP in all baboon

- Microthrombi

→ Early stages of TTP





- Mouse, rat and baboon models:
- Antibodies to inhibit ADAMTS13
 - Mice: mouse or human anti-MDTCS or anti-S antibodies
 - Rat: polyclonal anti-human antibodies
 - Baboon: mouse anti-M antibody
- Mice and rats: ADAMTS13 deficiency not lethal and well tolerated
- Baboon: ADAMTS13 deficiency leads to early stages of TTP

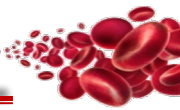


- Mice and rats: signs and symptoms: to various extent
 - Thrombocytopenia, hemolytic anemia, microthrombi (organ damage, LDH)
- Baboons: clear signs and symptoms

- Mice and rats: triggers: Shigatoxin, rVWF
- Baboons: no trigger for early phase of TTP, trigger for end stage TTP?



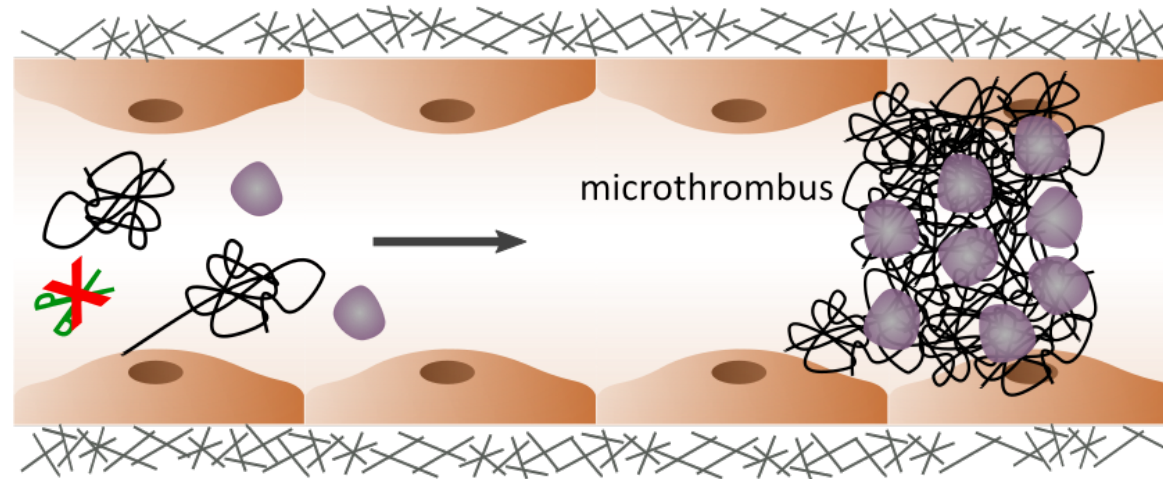
Animal models for TTP: developing novel therapies

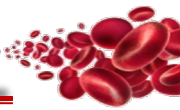


- Recombinant ADAMTS13
 - Replace

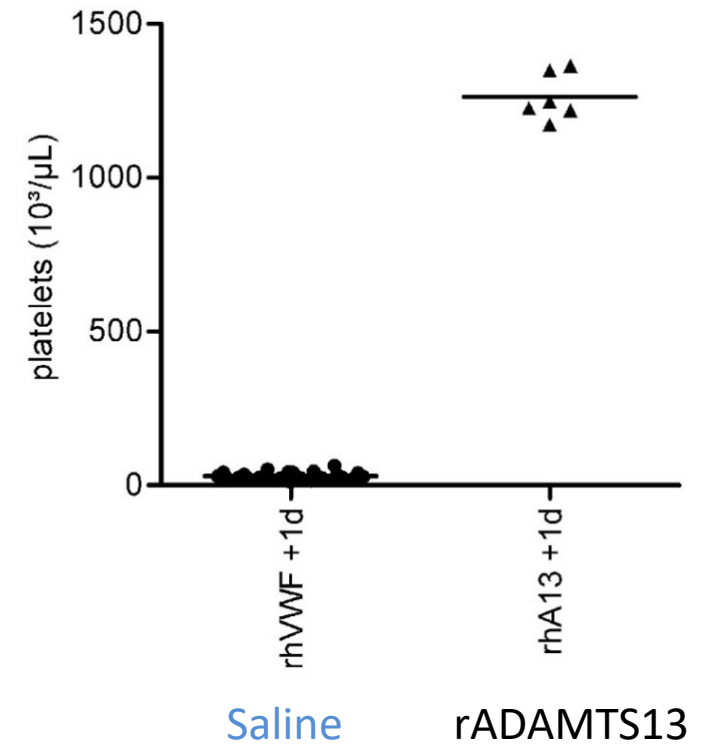
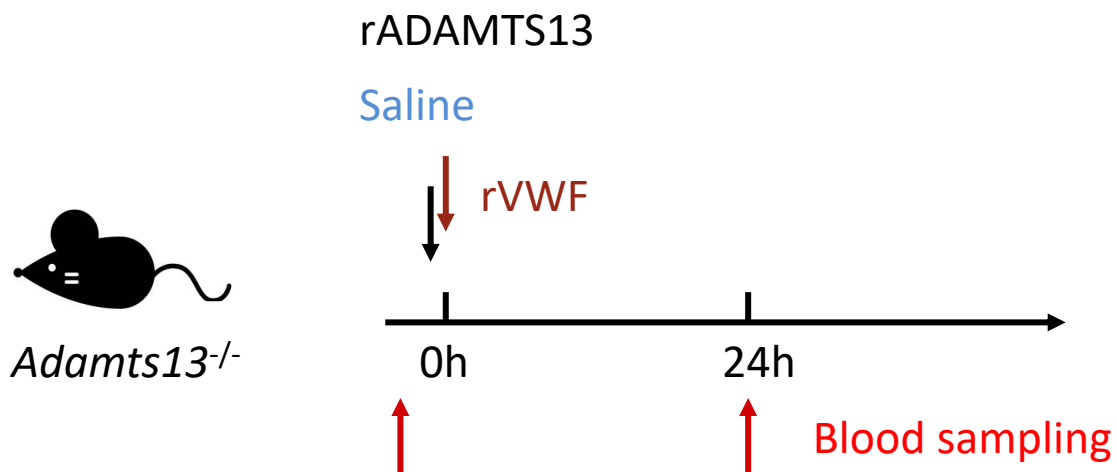


- As treatment for congenital TTP





- Recombinant ADAMTS13
- As treatment for congenital TTP
 - Prophylactic



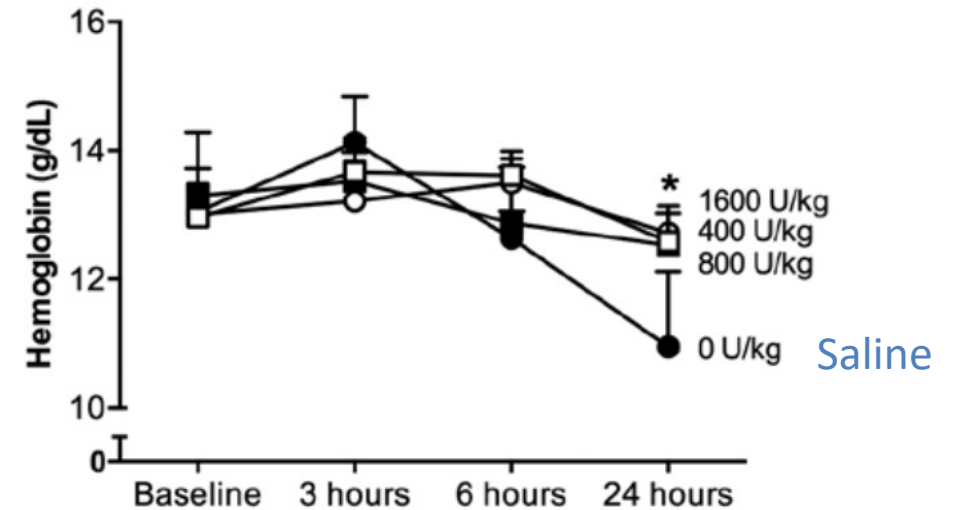
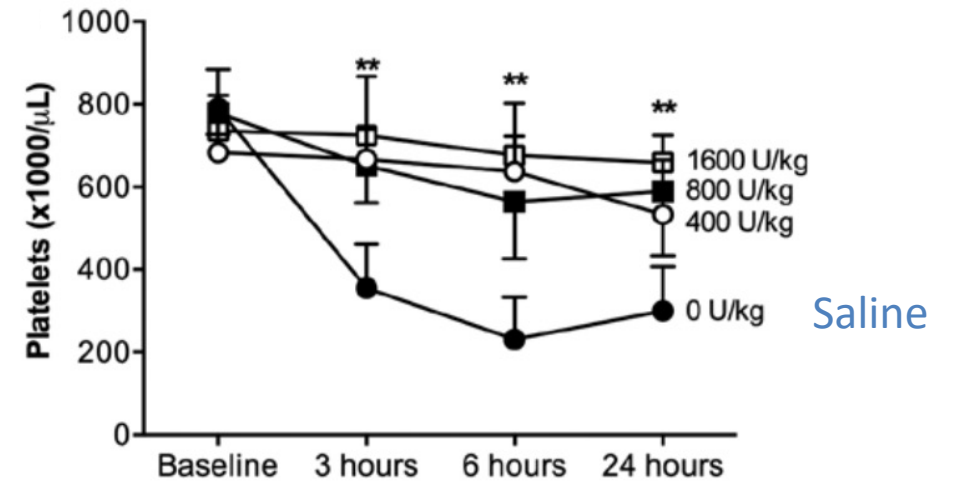
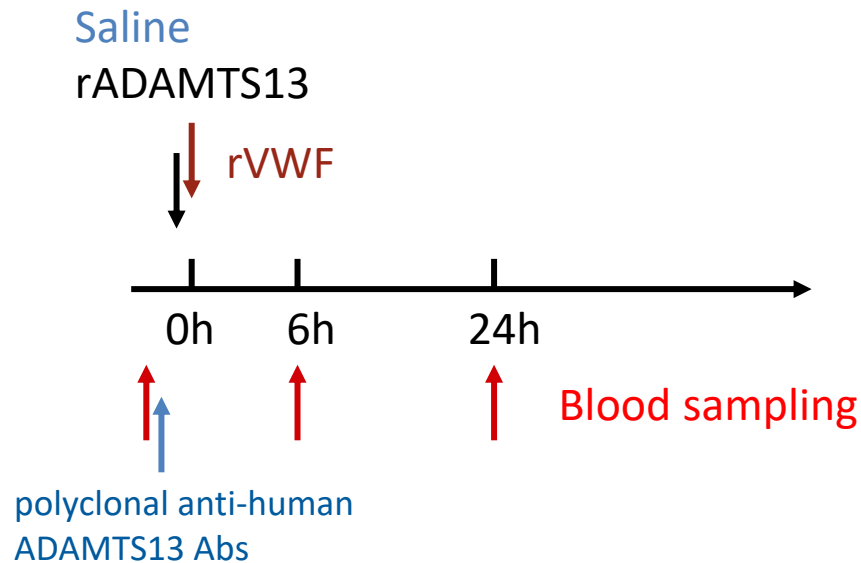
Novel therapies for TTP



- Recombinant ADAMTS13
- As treatment for immune-mediated TTP
 - Prophylactic
 - Prevents: thrombocytopenia, hemolytic anemia, increase in LDH

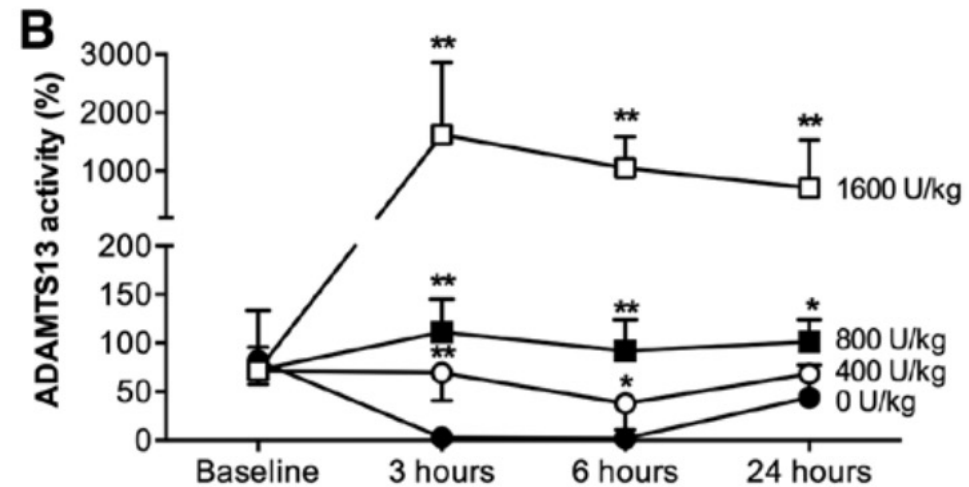
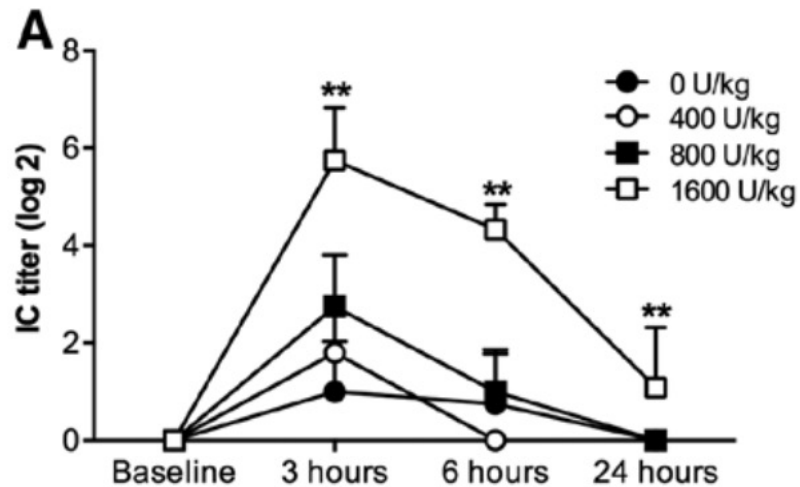


Rat injected with polyclonal anti-human ADAMTS13 Abs: ADAMTS13 deficient



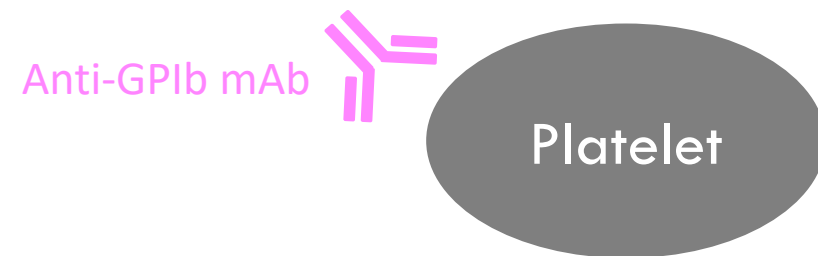
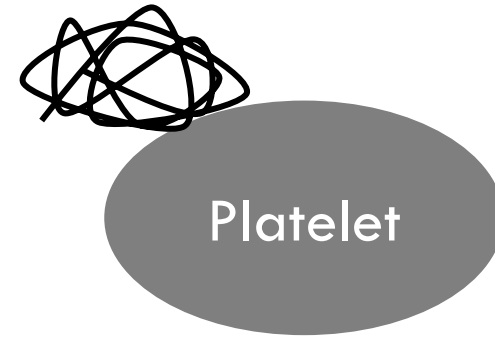
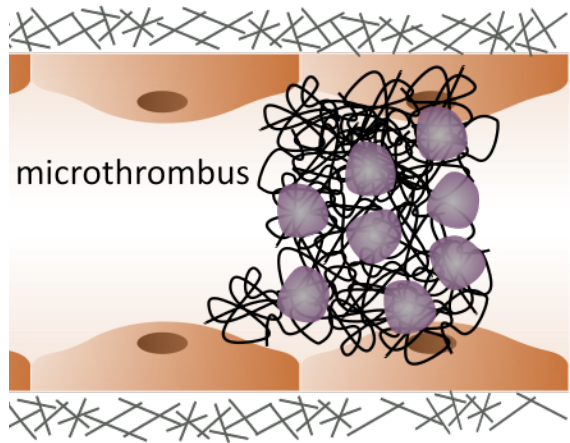


- Recombinant ADAMTS13
- As treatment for immune-mediated TTP
 - Overrides inhibitory/clearing autoantibodies, restores ADAMTS13 activity: immune complexes are formed





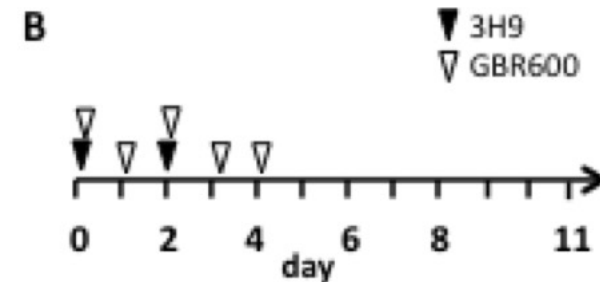
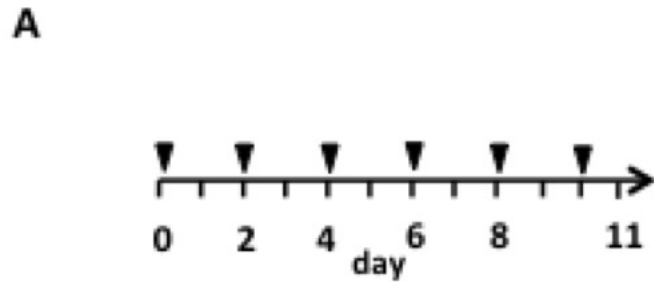
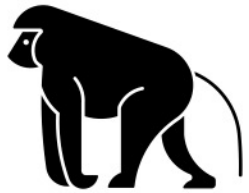
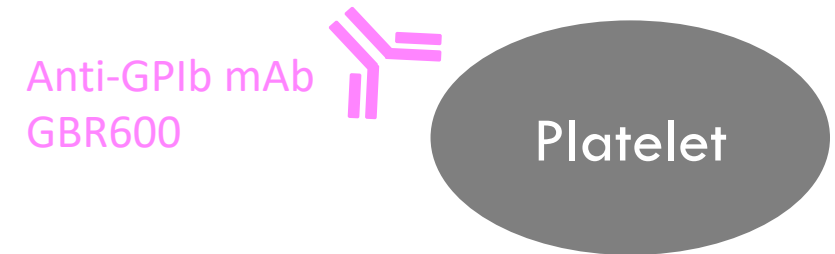
- Inhibiting microthrombi formation



Novel therapies for TTP

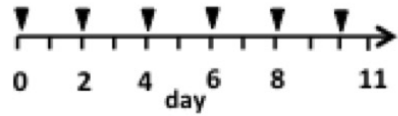
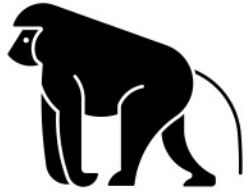


- Inhibiting microthrombi formation
- As treatment for immune-mediated TTP
 - Prophylactic

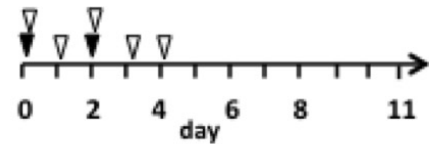
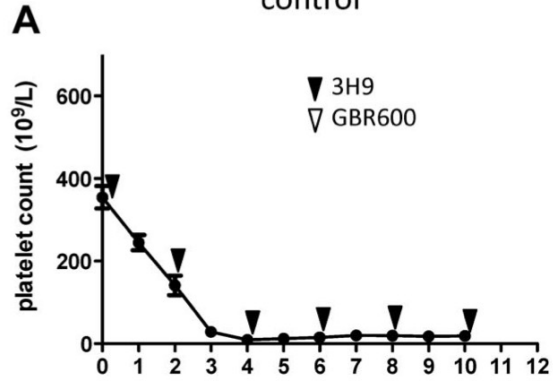




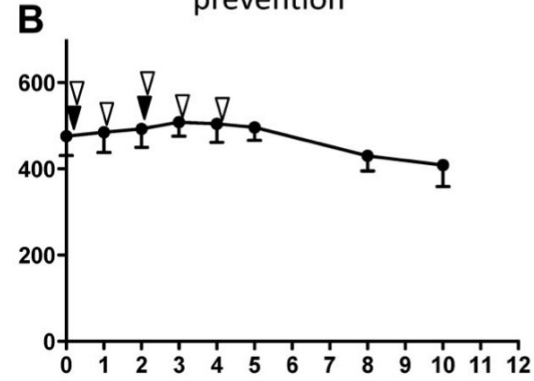
Anti-ADAMTS13
mAb 3H9



control



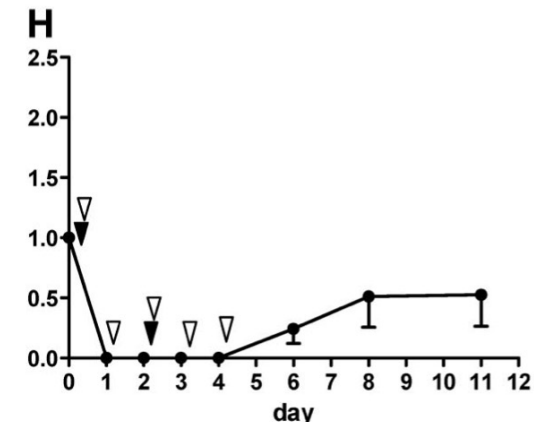
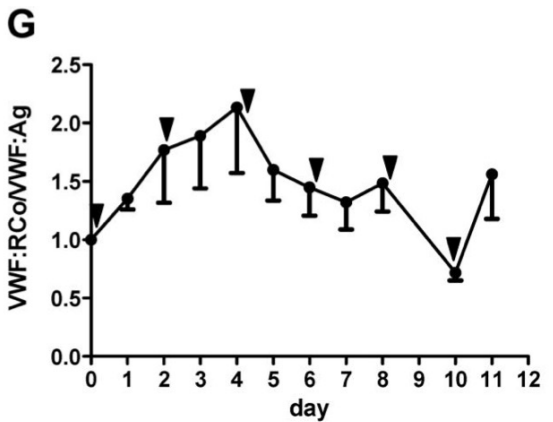
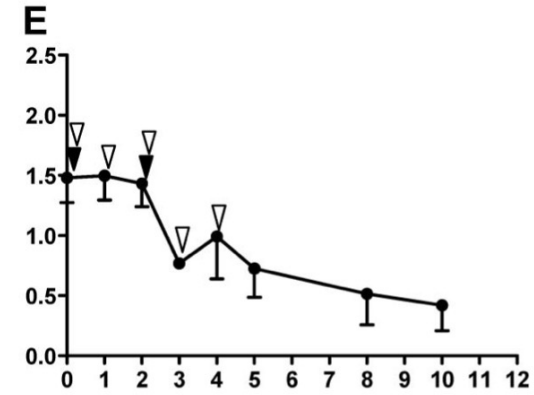
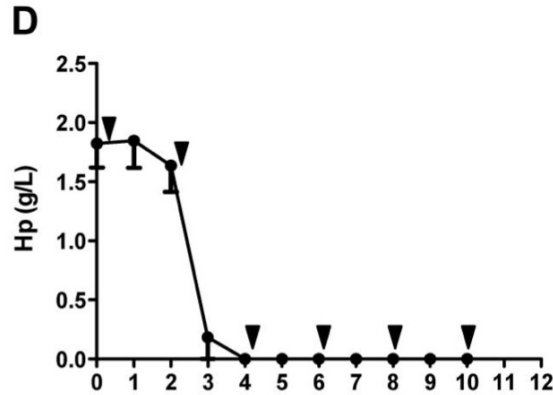
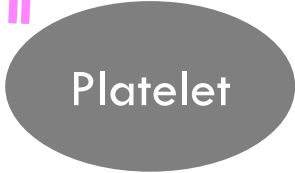
prevention



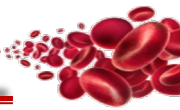
Anti-ADAMTS13
mAb 3H9



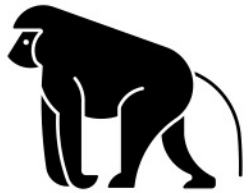
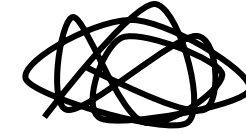
Anti-GPIb mAb
GBR600



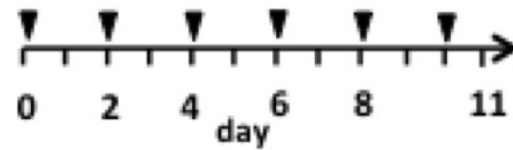
Novel therapies for TTP



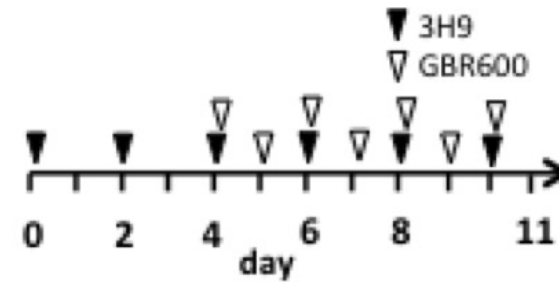
- Inhibiting microthrombi formation
- As treatment for immune-mediated TTP
 - As treatment



A

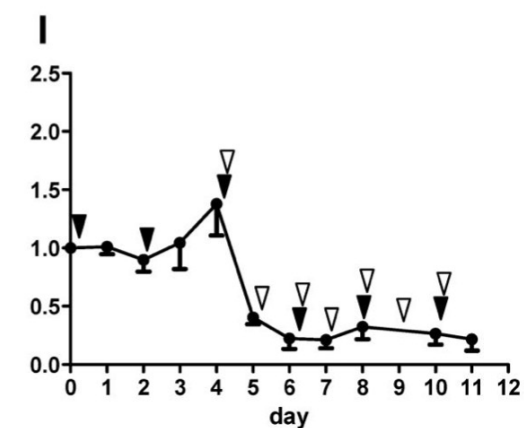
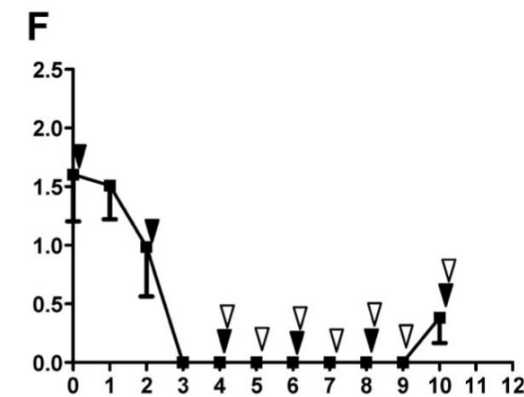
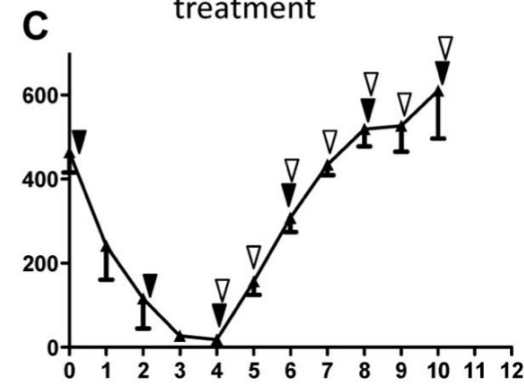
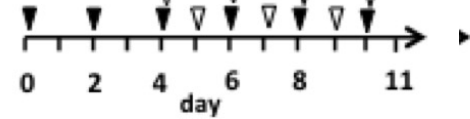
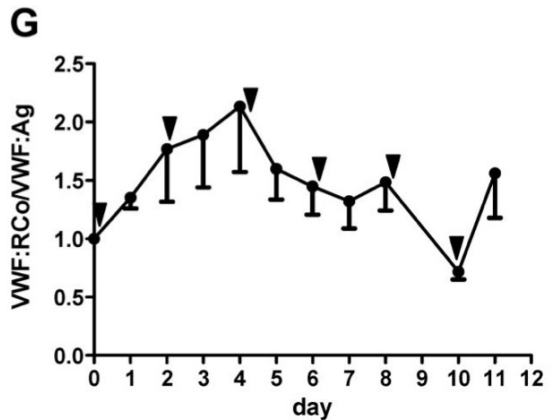
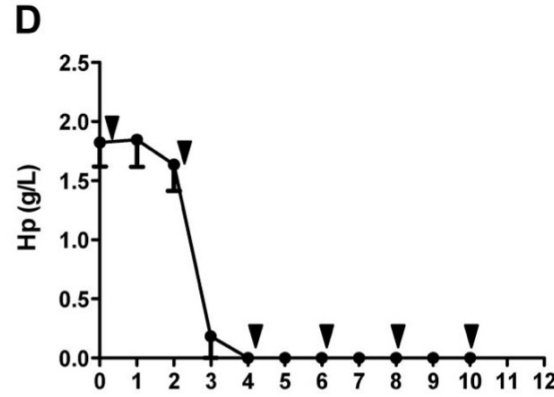
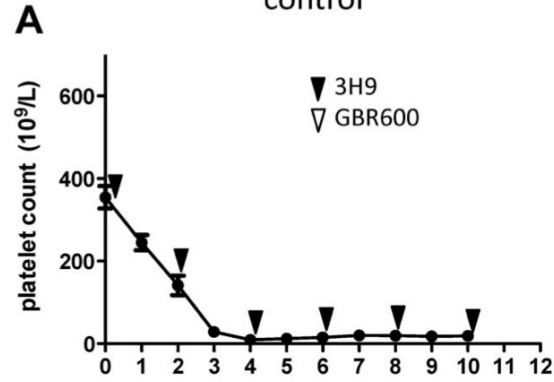
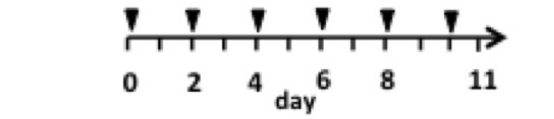
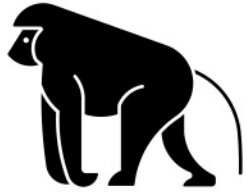


B





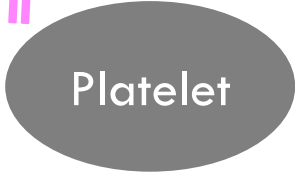
Anti-ADAMTS13
mAb 3H9



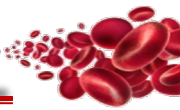
Anti-ADAMTS13
mAb 3H9



Anti-GPIb mAb
GBR600



Platelet



Conclusion

- rADAMTS13
 - Effective to treat congenital and immune mediated TTP in mice and rat TTP models
 - Clinical trials for cTTP and iTTP



Conclusion

- Anti-VWF and anti-GPIIb antibodies to inhibit microthrombi formation
 - Effective to treat immune mediated TTP in baboon TTP model
 - Caplacizumab on the market



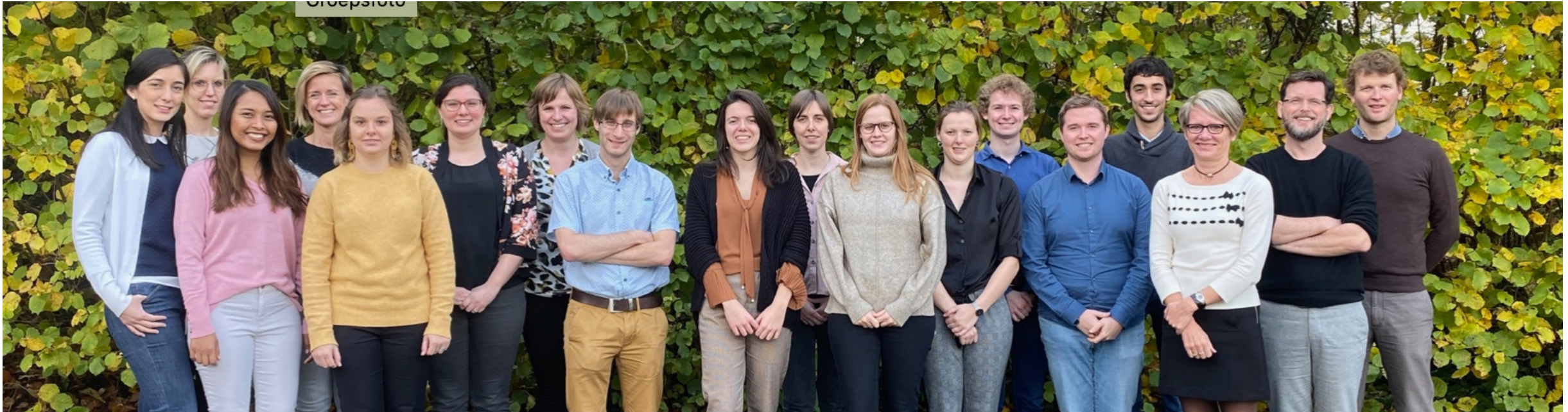
- 1. Animal models of TTP represent main characteristics of human disease**
- 2. Animal models of TTP aid in understanding the pathophysiology of TTP**
- 3. Animal models of TTP are valuable in drug discovery**

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Seb Lamprecht



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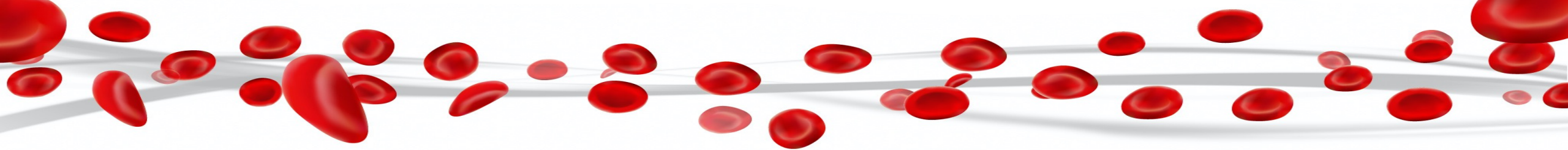
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Funding agencies



Webinars
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Discussion