The enzyme and the substrate: Preclinical and clinical development of recombinant von Willebrand Factor and ADAMTS13

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Baxter’s Recombinant VWF (BAX 111) Program
Addressing the Medical Need for VWD

- Medical need: A recombinant VWF option
- Recombinant von Willebrand Factor (rVWF) is the largest and functionally most complex protein multimer ever produced by recombinant DNA technology
- rVWF and ADVATE are co-expressed in CHO cells
- ADVATE is purified and rVWF removed
- rVWF can be recovered, processed (pro-peptide cleavage) and purified
- rVWF multimers are preserved
rVWF: Multimeric Structure

→ rVWF contains the full spectrum of high molecular weight multimers making it hemostatically most active
The specific activity of rVWF is substantially higher than that of plasma derived VWF and VWF/FVIII products.

<table>
<thead>
<tr>
<th></th>
<th>VWF:Ag (IU/mg protein)</th>
<th>VWF:RCo (IU/mg protein)</th>
<th>VWF:CBA / VWF:Ag (IU/IU)</th>
<th>VWF:RCo / VWF:Ag (IU/IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rVWF</td>
<td>116 ± 7</td>
<td>134 ± 28</td>
<td>1.14 ± 0.16</td>
<td>1.16 ± 0.25</td>
</tr>
<tr>
<td>n=7</td>
<td>n=7</td>
<td>n=3</td>
<td>n=7</td>
<td></td>
</tr>
<tr>
<td>pasteurized pd VWF</td>
<td>17.9 ± 4.7</td>
<td>8.0 ± 1.7</td>
<td>0.84</td>
<td>0.51 ± 0.10</td>
</tr>
<tr>
<td>n = 12</td>
<td>n = 7</td>
<td>n = 1</td>
<td>n = 12</td>
<td></td>
</tr>
</tbody>
</table>

- The specific activity (VWF:RCo/protein) is substantially higher than that of pd VWF
  - pd VWF products contain other proteins including human albumin substantially lowering their specific activity
- rVWF is a highly concentrated product with a physiological VWF:RCo / VWF:Ag and VWF:CB / VWF:Ag ratio
  - Contains more active VWF than pd concentrates
Each VWF monomer contains one binding site for FVIII

- Theoretically, each monomer should be able to bind one FVIII molecule

Larger multimers can bind more FVIII than smaller ones

Pharmacokinetics of rVWF and pd VWF in von Willebrand-Deficient Dogs

- Von Willebrand-deficient dogs from colony at UNC Chapel Hill, NC (Principal Investigator: Dr. Timothy C. Nichols)
- VWD dogs have no detectable VWF:Ag and reduced FVIII levels compared to normal dogs

- Study drugs: Baxter’s rVWF or pd VWF
- Study design:
  - Infusion study of rVWF and pdVWF
  - Dog I 45: rVWF: #06001FC, 100 IU VWF:RCo/kg
  - Dog I 47: pd VWF, 100 IU VWF:RCo/kg (and 48 IU pdFVIII/kg)
Pharmacokinetics of rVWF and pd VWF in von Willebrand-Deficient Dogs

VWF:Ag ELISA (human plasma reference)

VWF:Ag plasma levels

<table>
<thead>
<tr>
<th>Test article</th>
<th>Dose adjusted AUC (0-inf)</th>
<th>Initial HL</th>
<th>Terminal HL</th>
<th>MRT</th>
<th>clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>rVWF</td>
<td>0.279 [(U/ml)/(U/kg)]</td>
<td>10.2 (h)</td>
<td>13.0 (h)</td>
<td>15.8 (h)</td>
<td>3.6 (ml/kg)</td>
</tr>
<tr>
<td>pd VWF</td>
<td>0.102 [(U/ml)/(U/kg)]</td>
<td>2.9 (h)</td>
<td>8.7 (h)</td>
<td>9.6 (h)</td>
<td>9.8 (ml/kg)</td>
</tr>
</tbody>
</table>

➤ rVWF has a longer half life than pdVWF in canine VWD
Pharmacokinetics of rVWF and pd VWF in von Willebrand-Deficient Dogs

FVIII chromogenic assay (human plasma reference)

• Both von Willebrand-deficient dogs have different pre-treatment levels of approx. 0.7 and 1 U FVIII/ml respectively
• Human rVWF stabilizes canine FVIII and induces the secondary rise in FVIII levels
• When normalized to starting levels FVIII remains elevated by approx. 30% between 20 and 30 hrs after rVWF treatment
Phase 1  Immediate safety & tolerability, dose escalation and comparative pharmacokinetics  
– COMPLETED –

Phase 3a  Efficacy & safety in the treatment of bleeding episodes  
– ONGOING –

Phase 3b  Efficacy & safety in prophylactic use for surgical procedures  
– ONGOING –

Phase 3c  Efficacy & safety in the treatment of bleeding episodes and surgery in children
rVWF Phase 1 Study
Endpoints and Design

- **Primary Endpoint**
  - Immediate tolerability and safety after single doses of rVWF:rFVIII at 2 IU/kg, 7.5 IU/kg, 20 IU/kg and 50 IU/kg VWF:RCo.

- **Secondary Endpoints**
  - PK for VWF:RCo, VWF:CB, VWF:Ag, FVIII and multimeric composition of the VWF.
  - PK comparison with pdVWF//pdFVIII [Cohort 4 (50 IU/kg VWF:RCo)].

[Diagram of study design with dose levels and randomization points.]
## Patient Demographics

<table>
<thead>
<tr>
<th>COHORT</th>
<th># of patients</th>
<th>Gender (Female/Male)</th>
<th>Age (years) Median (range)</th>
<th>Weight (kg) Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Type 3)</td>
<td>3</td>
<td>1/2</td>
<td>38 (26-44)</td>
<td>130</td>
</tr>
<tr>
<td>2 (Type 3)</td>
<td>5</td>
<td>2/3</td>
<td>26 (21-39)</td>
<td>86.2</td>
</tr>
<tr>
<td>3 (Type 3)</td>
<td>5</td>
<td>0/5</td>
<td>34 (19-55)</td>
<td>79</td>
</tr>
<tr>
<td>4A (Type 3)</td>
<td>22</td>
<td>11/11</td>
<td>33 (18-60)</td>
<td>74.8</td>
</tr>
<tr>
<td>4B (Severe Type 1)</td>
<td>3</td>
<td>2/1</td>
<td>25 (19-47)</td>
<td>82.1</td>
</tr>
<tr>
<td>Total</td>
<td>32*</td>
<td>15/17</td>
<td>33 (18-60)</td>
<td>81.1</td>
</tr>
</tbody>
</table>

*Six subjects from cohort 1-3 were re-enrolled in cohort 4A*
Pharmacokinetic Analysis
VWF:RCo / VWF:Ag (Cohort 4A [Type 3 VWD])

VWF:RCo

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>pd</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR [(U/dL)/(U VWF:RCo/kg)]</td>
<td>1.7 (0.62)</td>
<td>1.6 (0.62)</td>
</tr>
<tr>
<td>T_{1/2} [hours]</td>
<td>19.3 (10.9)</td>
<td>14.6 (6.4)</td>
</tr>
<tr>
<td>MRT [hours]</td>
<td>26.8 (13.6)</td>
<td>18.7 (5.5)</td>
</tr>
<tr>
<td>AUC_{0-∞} [h*U/dL]</td>
<td>1542 (554)</td>
<td>1180 (500)</td>
</tr>
</tbody>
</table>

All values are “means” (Standard Deviation)
Pharmacokinetic Analysis
VWF:RCo / VWF:Ag (Cohort 4A [Type 3 VWD])

VWF:RCo

<table>
<thead>
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<td>MRT [hours]</td>
<td>26.8 (13.6)</td>
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<tr>
<td>$AUC_{0-\infty}$ [h*U/dL]</td>
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VWF:Ag

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<td>25.3 (6.3)</td>
</tr>
<tr>
<td>MRT [hours]</td>
<td>33.5 (10.7)</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ [h*U/dL]</td>
<td>2245 (683)</td>
</tr>
</tbody>
</table>

All values are “means” () Standard Deviation

$r = r_{VWF}/r_{FVIII}$
$pd = pd_{VWF}/pd_{FVIII}$
Pharmacokinetics of FVIII:C
Type 3 VWD: rVWF/rFVIII vs pdVWF/pdFVIII

**Table:**

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td><strong>T&lt;sub&gt;1/2&lt;/sub&gt; [hours]</strong></td>
<td>24.3 (6.5)</td>
<td>19 (5.1)</td>
</tr>
<tr>
<td><strong>MRT [hours]</strong></td>
<td>38.9 (12.4)</td>
<td>32.7 (7.5)</td>
</tr>
<tr>
<td><strong>AUC&lt;sub&gt;0-∞&lt;/sub&gt; [h*U/dL]</strong></td>
<td>5376 (2380)</td>
<td>3361 (1350)</td>
</tr>
</tbody>
</table>

*All values are „means“, () Standard Deviation

**Diagram:**
- Blue line: rVWF/rFVIII (38.5 IU/kg FVIII:C)
- Red line: pdVWF/pdFVIII (21-25 IU/kg FVIII:C)

**Legend:**
- VWF:Ag

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BSTH, Antwerp, Nov. 22-23, 2012

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Pharmacokinetic of FVIII:C
Hemophila A (rFVIII)

<table>
<thead>
<tr>
<th>FVIII:C*</th>
<th>Advate**</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2}$ [hours]</td>
<td>12.03 (4.15)</td>
</tr>
<tr>
<td>MRT [hours]</td>
<td>15.81 (5.91)</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ [h*U/dL]</td>
<td>1644 (338)</td>
</tr>
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</table>

* All values are "means", ( ) Standard Deviation

** Advate prescribing information

BSIH, Antwerp, Nov. 22-23, 2012
Pharmacokinetic of FVIII:C
Type 3 VWD (rVWF/rFVIII) vs Hemophila A (rFVIII)

FVIII:C*

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*All values are “means“, () Standard Deviation

**Advate prescribing information

rVWF/rFVIII (38.5 IU/kg FVIII:C)
Advate Pivotal (50±5 IU/kg FVIII:C)

BSTH, Antwerp, Nov. 22-23, 2012
Pharmacokinetic of FVIII:C
Type 3 VWD (rVWF/rFVIII) vs Hemophila A (rFVIII)

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*All values are “means”, () Standard Deviation

**from Advate prescribing information

* rVWF/rFVIII (38.5 IU/kg FVIII:C)
* Advate Pivotal (50±5 IU/kg FVIII:C)
A) Shear flow, and transition to elongational flow in vessels at sites of constricted or ruptured vessels. Round orange spheres show the effect of elongational flow on the shape of apolymeric protein in the flow field.

C) Cartoon of VWF elongating, compressing, and tumbling in shear flow.
Conformational change of rVWF under shear stress: Susceptibility to physiological processing by ADAMTS13

Stretching was investigated by “Tapping Mode Atomic Force Microscopy” (AFM) at the Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria

- Broad size distribution for the individual molecules
- Substructure with sequential globular and stretched domains
- Similar overall structure for pdVWF and rVWF
- A fully extended molecule had an end-to-end length of 2770 nm (double arrow)
- Molecules with partially stretched regions (double arrows) might indicate the reversible nature of the shear-induced conformational change

VWF concatamer conformation, and mechanoenzymatic cleavage model. *Springer T A, JTH 2011, 9 (Suppl. 1): 130-143*

E) Schematic of VWF, with N-terminal end as triangle, A2 as spring, and C-terminal end as circle. Elongation results in stochastic unfolding of some A2 domains (ii), some of which are cleaved by ADAMTS13 (iii). The resulting fragments are shown (iv).
ADAMTS13 Induced Structure of VWF Multimers

Fragments resulting from ADAMTS13 proteolytic cleavage

140-260 → 260-140
176-260 → 260-176
260 → 260-140

1% 3%
VWF multimers

Proteolytic cleavage

ADAMTS13

Tyr_{1605}/Met_{1606}

satellite bands

BSTH, Antwerp, Nov. 22-23, 2012
No satellite bands are observed in the rVWF products
Not all pdVWF products show a satellite band structure similar to normal plasma
Time-dependent changes of rVWF activities and structure
Physiological concentrations of ADAMTS13 (denaturing conditions)

- VWF:RCo and VWF:CB activities rapidly decrease
- UHMW multimers disappear within 1 min at 1 U/mL ADAMTS13 concentration
- Satellite bands and specific cleavage products are clearly visible already after 1 minute
Susceptibility of rVWF to ADAMTS13 of Different Species

- rVWF is susceptible to rADAMTS13 and human plasma, but less to dog plasma
- rVWF is resistant to ADAMTS13 present in mouse plasma
- The prolonged half-life of rVWF in dogs may be caused by resistance of rVWF to ADAMTS13 specific multimer processing

**Collagen binding assay**

*Conditions*: 0.6 U/ml rVWF:Ag incubated with 0 – 33.3 mU/ml ADAMTS13 equivalent plasma activated with 8.5 mM BaCl₂ in 1.5 M urea, pH 8
The high molecular weight multimers in rVWF are susceptible to ADAMTS13 cleavage in VWD patients – as predicted by in vitro and animal studies

ADAMTS13 subunit cleavage products**

- ULMW multimers also found in the circulation after DDAVP treatment
- The ULMW multimers disappeared over time, similar to the disappearance in patients treated with DDAVP
- An ADAMTS13-specific cleavage product appeared already 15 min after administration
ADAMTS13: A disintegrin and metalloproteinase with thrombospondin type-1 motifs 13

- ADAMTS13 circulates as an active plasma enzyme with a plasma half-life of ~2-3 days
- ADAMTS13 cleaves unusually large VWF (ULVWF) at position M1605-Y1606 to yield smaller VWF, less reactive multimers
- ADAMTS13 activity is shear stress dependent - pivotal role in the regulation of VWF’s platelet-tethering function, i.e. its capacity to initiate thrombus formation
- Hereditary or acquired ADAMTS13 deficiency leads to TTP
Failure to control the size of VWF can lead to arterial thrombosis

ADAMTS13 is the only known regulator of VWF thrombogenicity
Recombinant ADAMTS13 Program (BAX 930)

- Developed initially for treatment of Thrombotic Thrombocytopenic Purpura (TTP)
  - Caused by a deficiency in ADAMTS13
  - Hereditary and acquired (auto-immune) TTP
    - Blood clots form suddenly throughout the body
    - Fatal if not treated immediately
    - Can cause serious sequelae
    - High recurrence rate
  - Current treatment options unsatisfactory
    - Plasma infusions (Hereditary)
    - Plasma exchange (Acquired)

- Status
  - Safety and efficacy of BAX 930 investigated in a comprehensive pre-clinical pharmacology / toxicology program
  - IMPD submission 2012
rADAMTS13 : Up & downstream process

- CHO DUKX-B11 cells
- Stirred tank fermenter technology in chemostat mode with continuous harvesting for up to 50 days
- Chemically defined cell growth media
- No additives of animal origin
- Successful up-scaling to a 1000-L fermenter size for the production of pre-clinical and clinical rADAMTS13 lots

- Downstream process based on conventional resins
- Two independent virus inactivation/removal steps
- High purity (no truncated variants)
- High specific activity (1500 U/mg)
- Molecular weight: 176 kDa (MALDI-MS)
- Approx. 80% sequence coverage by peptide mapping
- N-glycan profile similar to that of pdADAMTS13
- No phosphorylation or sulfation identified
- Low levels of CHO DNA (< 1pg/mL) and host cell protein (< 500 ppm)
# Structural and functional characterization of rADAMTS13

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetics of VWF cleavage under denaturing conditions</td>
<td>Urea-based cleavage analytics: VWF:Ristocetin cofactor assay (RCo), Collagen binding assay, Electrophoresis</td>
</tr>
<tr>
<td>VWF cleavage under shear stress</td>
<td>Flow-based Assays: Venaflux technology, Vortex method</td>
</tr>
<tr>
<td>Total mass determination</td>
<td>MALDI-TOF MS</td>
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<tr>
<td>N-terminal sequencing</td>
<td>Edman degradation</td>
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<tr>
<td>Peptide mapping</td>
<td>RP-HPLC and/or HPLC-MS</td>
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<tr>
<td>Monosaccharide composition (N-and-O-glycans)</td>
<td>HPLC method</td>
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<td>Isoforms and impurity pattern</td>
<td>2D gel electrophoresis</td>
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<td>Deamidation</td>
<td>Isoquant Kit (HPLC)</td>
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<td>Oxidation</td>
<td>HPLC-MS Peptide Mapping</td>
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<td>Aggregates</td>
<td>Analytical ultracentrifugation</td>
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<td>Dynamic Light Scattering (DLS)</td>
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<tr>
<td>Subvisible particles</td>
<td>Micro Flow Imaging</td>
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<td>Higher ordered structure</td>
<td>Fourier-transformed infrared spectroscopy (FTIR); DLS</td>
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<tr>
<td><strong>Pharmacokinetics</strong></td>
<td>ADAMTS13 ko mice, rats &amp; cynomolgus monkeys</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------</td>
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<tr>
<td><strong>Primary Pharmacodynamics</strong></td>
<td>Prophylactic efficacy in rVWF-induced TTP model (short-term prophylaxis &amp; prophylaxis over time)</td>
</tr>
<tr>
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<td>Therapeutic efficacy in rVWF-induced TTP model</td>
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<tr>
<td><strong>Safety Pharmacology</strong></td>
<td>General safety pharmacology in cynomolgus monkeys (included in repeated dose tox study)</td>
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<tr>
<td><strong>Toxicology</strong></td>
<td>Maximum tolerated dose in cynomolgus monkeys</td>
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<td>30 d repeated dose toxicity study in rats &amp; cynomolgus monkeys</td>
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<td></td>
<td>Local tolerance in rabbits</td>
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<td></td>
<td>6 m repeated dose toxicity study in rats *</td>
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<tr>
<td></td>
<td>DART studies in rats &amp; rabbits *</td>
</tr>
</tbody>
</table>

* prior to phIII
Mouse model mimicking human congenital TTP

- ADAMTS13 ko mice (B6.129-ADAMTS13\textsuperscript{tm1Dgi})
  - No spontaneous TTP
  - A trigger is needed

- Development of a rVWF-induced TTP model
  - Human rVWF is thrombogenic in ADAMTS13 ko mice, as these mice are virtually unable to cleave rVWF
  - Hypothesis: rVWF containing ULVWF multimers will trigger TTP-like symptoms in ADAMTS13 ko mice
Mice (n=20) challenged with rVWF (2000 VWF:RCoU/kg) developed TTP-like findings:

- Clinical symptoms (9/20), mortality (1/20)
- Schistocytosis
- Severe thrombocytopenia (~5% of baseline)
- Hemolytic anemia
- 2-5-fold LDH increase
- Acute myocardial necrosis, hemorrhage, neutrophilic inflammation
- Acute tubular necrosis
Efficacy of rADAMTS13 in a rVWF-induced TTP model in ADAMTS13 ko mice

Prophylaxis
1-200 U/kg rADAMTS13
5 mL/kg buffer

Day 0
200 U/kg rADAMTS13
5 mL/kg buffer

Day 1
Plt, Htc, schistocytes, LDH, organ damage

Therapy

Plt, rA13, rVWF

+15, +30, +180 min
Dose-dependent protection by rADAMTS13 in a prophylactic efficacy model

**Platelets:**
Prevention of thrombocytopenia ≥5U/kg

**LDH:**
Prevention of LDH increase ≥1U/kg
Therapy with rADAMTS13 prevents further development of thrombocytopenia

- Mice were already thrombocytopenic when treated
- Treatment interval-dependent therapeutic efficacy
- rADAMTS13 stabilized platelet count
- No clinical symptoms in mice treated with rADAMTS 13 15 and 30 min after rVWF challenge

Schiviz et al. 2012 Blood
Baxter currently develops the first recombinant therapies for hereditary deficiencies in both the substrate (VWF) and the enzyme (ADAMTS13)

- Both proteins are derived from recombinant CHO cells using Baxter’s established up- and down-stream processing performed under serum-free and protein-free conditions as previously established for the rFVIII product Advate.

rADAMTS13

- Robust production of rADAMTS13 with high potency in a CHO cell line
- rADAMTS13 is active under physiological conditions (shear stress)
- rADAMTS13 is efficacious in an animal model mimicking the situation in patients with hereditary TTP
- IND-enabling preclinical Pharmacology/Toxicology studies completed
rVWF

- rVWF manufacturing process yields in a product without signs of degradation
  - Well defined product
- The specific activity (VWF:RCo/protein) is substantially higher compared to plasma derived VWF/FVIII products
- The ratio VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag of rVWF is higher compared to plasma derived VWF/FVIII products
- rVWF contains the hemostatic most effective ultra-high molecular weight multimers
  - Susceptibility to ADAMTS13 cleavage allow physiological processing upon administration
- Improved PK profile for rVWF compared to pdVWF
- Improved FVIII stabilizing effect of rVWF compared to pdVWF
- rVWF was well tolerated in the clinical phase 1 trial
The Product Development and Preclinical Team @ Baxter:


+ the whole R&D team of Baxter BioScience
Flow-based ADAMTS13 activity assay (Venaflux Technology)

- Cell suspension with rVWF and ± rADAMTS13 is run over a microchannel coated with collagen and VWF under constant shear stress (2500 s⁻¹)
- Live fluorescence images are taken and the covered area is calculated by computerized image analysis

- ADAMTS13 causes a decrease in surface coverage of fluorescent-labeled platelets in a dose-dependent manner
- Excellent lot to lot consistency

Venaflux technology (Cellix Ltd.): commercial biochips containing microchannels

Image analysis of adhered labelled platelets

![Image analysis of adhered labelled platelets](image)

![Bar chart showing surface coverage](chart)
Acquired TTP
- Caused by inhibitory and non-inhibitory anti-ADAMTS13 autoantibodies

Current treatment
- Daily plasma exchange (PEX) until remission, immunosuppressive drugs
- PEX considered to remove circulating antibodies, antibody/ADAMTS13 complexes and to concurrently replenish the deficient protease

rADAMTS13 in acquired TTP
- Therapeutic efficacy compromised by free circulating anti-ADAMTS13 antibodies that bind and/or neutralize administered rADAMTS13
- Complex interplay between ADAMTS13 levels, inhibitory antibodies, and ADAMTS13-specific circulating immune complexes that is not completely understood
ADAMTS13-specific circulating immune complexes in a patient with acquired TTP

Co-immunoprecipitation of ADAMTS13 with total IgG

- Detection of ADAMTS13 by immunoblot

- Inverse correlation of ADAMTS13-specific immune complexes and free antibodies (inhibitor)
- Antibodies can be present even in the absence of measured free antibodies

Ferrari et al. 2011 J Thromb Haemost
In vitro recovery of rADAMTS13 in plasma from patients with acquired TTP

Calculation of the amount of rADAMTS13 required to restore activity (EC50) in TTP inhibitor plasma with different anti-ADAMTS13 inhibitor titers

Linear correlation between inhibitor titer and amount of rADAMTS13 needed to restore ADAMTS13 activity to a certain level

Plaimauer et al. 2011 J Thromb Haemost
Acquired TTP: Rat inhibitor model

Goal

- Investigate the recovery of rADAMTS13 administered to normal rats with a defined anti-ADAMTS13 inhibitor titer
  - Injection of anti-ADAMTS13 antibodies from goat
- Determine the amount of rADAMTS13 required to override circulating antibodies in vivo and to restore ADAMTS13 activity
- Monitor animals for occurrence of adverse events
Rat inhibitor model: rADAMTS13 shows inhibitor overriding efficacy in vivo

- **Anti-ADAMTS13 inhibitor 650 BU/kg**
- **Anti-ADAMTS13 inhibitor + 100 U/kg rADAMTS13**
- **Anti-ADAMTS13 inhibitor + 400 U/kg rADAMTS13**
- **Anti-ADAMTS13 inhibitor + 1600 U/kg rADAMTS13**
In acquired TTP patients, anti-ADAMTS13 antibodies can be present in a free and an immune-complexed form.

Main determinant for the required ADAMTS13 dose is the inhibitor titer.

In the rat inhibitor model, high doses of rADAMTS13 led to a 'typical' PK profile in the presence of inhibitor.

Most of the circulating antibodies were immediately complexed and neutralized upon injection of rADAMTS13.

Model is not appropriate to study a potential immune response in acquired TTP patients upon rADAMTS13 treatment.

Treatment with rADAMTS13 could be sufficient to achieve therapeutic ADAMTS13 levels in acquired TTP patients.