Serine Proteases and Pathophysiology of the neurovascular Unit (SP2U)

INSERM U919
Dir. Pr Denis Vivien (senior IUF)

University Caen Lower-Normandy
Caen, GIP Cyceron

How to improve stroke therapy: from bench to bedside
>1000 molecules reported with protective effects

>250 clinical trials performed

0 treatment for stroke

e except rtPA-induced reperfusion for acute treatment + thrombectomy

(in addition to the use of anticoagulants for prevention)

Can we do better?
Recanalization rate after i.v. thrombolysis (« Real World Experience »): 21.3%  
80% with thrombectomy

90-day Outcome

<table>
<thead>
<tr>
<th>Recanalization Status</th>
<th>mRS 0-2</th>
<th>mRS 3-6</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>acute recan IV tPA</td>
<td>77.8</td>
<td>14.6</td>
<td>7.4</td>
</tr>
<tr>
<td>later recan IA tPA</td>
<td>52.5</td>
<td>33.9</td>
<td>13.6</td>
</tr>
<tr>
<td>no recan</td>
<td>24.4</td>
<td>36.0</td>
<td>36.6</td>
</tr>
</tbody>
</table>

Occlusion site:

- MCA-M2: 30.4%
- MCA-M1: 32.8%
- ICA: 4.4%
- BA: 4.0%

Low HU
Platelet-rich: 5.9%

High HU
Erythrocyte and fibrin-rich: 29.4%

Size
> 8 mm: 0%

From Bathia et al., Stroke 2010

From Kim et al., Neurology 2006

33% of Ischemic Stroke Patients with potential access to thrombectomy
Is it possible to develop stroke models to assess thrombus subtype?

- Which treatment(s) for which thrombus subtype and localization?
- Embolism
  - α-thrombin
  - fibrinogen
  - FeCl₃

- In situ thrombosis
  - α-thrombin
  - fibrinogen
  - FeCl₃

Orset et al, Stroke 2007
Karatas et al, JCBFM 2011

VWF/C4D1/DAPI
Thrombus growth
50 µm
Lebehot, Gauberti et al., Blood, 2014
Wyseure et al., Blood, 2015
<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>IPA</th>
<th>Control</th>
<th>Mean</th>
<th>SD</th>
<th>Total</th>
<th>Mean</th>
<th>SD</th>
<th>Total</th>
<th>Weight</th>
<th>Mean Difference IV, Random, 95% CI</th>
<th>Mean Difference IV, Random, 95% CI</th>
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<tbody>
<tr>
<td>Ansar 2014</td>
<td>17</td>
<td>21.4</td>
<td>12</td>
<td>33.9</td>
<td>21.3</td>
<td>6</td>
<td>1.4%</td>
<td>18</td>
<td>-16.90 [15.92, 2.14]</td>
<td></td>
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<tr>
<td>Campos 2013</td>
<td>18.1</td>
<td>4</td>
<td>10</td>
<td>27.3</td>
<td>6.1</td>
<td>10</td>
<td>6.5%</td>
<td>15</td>
<td>-9.20 [13.72, -4.68]</td>
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<td>Duran 2012 (ii)</td>
<td>18.8</td>
<td>10.8</td>
<td>15</td>
<td>15.4</td>
<td>9</td>
<td>6</td>
<td>3.5%</td>
<td>14</td>
<td>3.20 [5.07, 1.27]</td>
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<td></td>
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<tr>
<td>El Amri 2012 (ii)</td>
<td>2.2</td>
<td>4.4</td>
<td>19</td>
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<td>6.1</td>
<td>10</td>
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<td>40</td>
<td>-16.30 [-20.57, -12.02]</td>
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<tr>
<td>Garcia-Velasco</td>
<td>16</td>
<td>2.6</td>
<td>10</td>
<td>27.2</td>
<td>4.7</td>
<td>13</td>
<td>7.3%</td>
<td>180</td>
<td>11.20 [14.22, -8.18]</td>
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<tr>
<td>Langhamer 2012</td>
<td>8.2</td>
<td>0.5</td>
<td>17</td>
<td>23.7</td>
<td>12</td>
<td>9</td>
<td>4.0%</td>
<td>30</td>
<td>-15.50 [24.32, -6.66]</td>
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<tr>
<td>Lemarchand 2015</td>
<td>4.7</td>
<td>1.1</td>
<td>2</td>
<td>6.5</td>
<td>1.4</td>
<td>3</td>
<td>7.8%</td>
<td>180</td>
<td>-1.00 [4.80, 6.40]</td>
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<tr>
<td>Mezine 2011 (ii)</td>
<td>18.9</td>
<td>4.6</td>
<td>10</td>
<td>29.7</td>
<td>5.5</td>
<td>16</td>
<td>7.2%</td>
<td>180</td>
<td>-9.89 [-13.17, -6.62]</td>
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<tr>
<td>Montagne 2012 (ii)</td>
<td>12.7</td>
<td>3.1</td>
<td>6</td>
<td>18.9</td>
<td>4.2</td>
<td>6</td>
<td>7.0%</td>
<td>180</td>
<td>-6.20 [-9.62, -2.56]</td>
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<tr>
<td>Study 1</td>
<td>17.5</td>
<td>9.2</td>
<td>16</td>
<td>25.9</td>
<td>6.4</td>
<td>8</td>
<td>9.6%</td>
<td>180</td>
<td>0.50 [4.65, -2.22]</td>
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<tr>
<td>Study 10</td>
<td>18.4</td>
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<td>26</td>
<td>20.9</td>
<td>6.1</td>
<td>20</td>
<td>5.6%</td>
<td>180</td>
<td>-2.20 [-8.68, 4.08]</td>
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<tr>
<td>Study 12 (ii)</td>
<td>15.5</td>
<td>10.9</td>
<td>16</td>
<td>20.1</td>
<td>6.8</td>
<td>11</td>
<td>4.1%</td>
<td>180</td>
<td>-4.50 [-13.05, 4.00]</td>
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<td></td>
</tr>
<tr>
<td>Study 14</td>
<td>14.9</td>
<td>8.6</td>
<td>16</td>
<td>16.6</td>
<td>8.5</td>
<td>12</td>
<td>6.3%</td>
<td>180</td>
<td>-1.70 [-8.15, 4.76]</td>
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<tr>
<td>Study 15 (i)</td>
<td>5.4</td>
<td>2.9</td>
<td>10</td>
<td>19.9</td>
<td>4.1</td>
<td>11</td>
<td>4.1%</td>
<td>180</td>
<td>-5.50 [8.86, -2.14]</td>
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<tr>
<td>Study 2</td>
<td>6.7</td>
<td>4.4</td>
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<td>14.9</td>
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<td>6.5%</td>
<td>180</td>
<td>-3.60 [15.08, -3.12]</td>
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<tr>
<td>Study 22</td>
<td>15.6</td>
<td>11.1</td>
<td>7</td>
<td>22.7</td>
<td>10.7</td>
<td>4</td>
<td>2.4%</td>
<td>180</td>
<td>-7.10 [20.43, 6.23]</td>
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<tr>
<td>Study 6</td>
<td>20.4</td>
<td>9.6</td>
<td>6</td>
<td>20.9</td>
<td>6.4</td>
<td>6</td>
<td>3.8%</td>
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<td>-0.50 [9.43, 8.73]</td>
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<tr>
<td>Study 7 (i)</td>
<td>20.7</td>
<td>11.4</td>
<td>16</td>
<td>18.4</td>
<td>10.2</td>
<td>17</td>
<td>4.6%</td>
<td>180</td>
<td>-2.30 [6.84, 6.84]</td>
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<tr>
<td>Study 9</td>
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<td>10.2</td>
<td>12</td>
<td>22.2</td>
<td>12.2</td>
<td>16</td>
<td>4.6%</td>
<td>180</td>
<td>-6.60 [-14.55, 1.36]</td>
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<tr>
<td>Subtotal (95% CI)</td>
<td>235</td>
<td>100.0%</td>
<td>207</td>
<td>90.0%</td>
<td>180</td>
<td>2.20</td>
<td>0.0001</td>
<td>137</td>
<td>20.70 [26.30, 15.10]</td>
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</tbody>
</table>

Heterogeneity: Tau² = 15.06; Ch² = 764.42, df = 18 (P < 0.0001), I² = 76%
Test for overall effect: Z = 5.20 (P < 0.0001)

### 1.1.2 Timing <3 hours

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>IPA</th>
<th>Control</th>
<th>Mean</th>
<th>SD</th>
<th>Total</th>
<th>Mean</th>
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<th>Mean Difference IV, Random, 95% CI</th>
<th>Mean Difference IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Amri 2012 (ii)</td>
<td>19</td>
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<td>16.5</td>
<td>6.1</td>
<td>10</td>
<td>12.5%</td>
<td>12</td>
<td>2.68 [2.41, 7.81]</td>
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<tr>
<td>Galizia 2011</td>
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<td>4</td>
<td>6</td>
<td>20.7</td>
<td>6.7</td>
<td>6</td>
<td>9.8%</td>
<td>18</td>
<td>2.65 [3.64, 5.65]</td>
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<td>30.7</td>
<td>1.3</td>
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<td>23.2</td>
<td>5.3</td>
<td>20</td>
<td>27.5%</td>
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<td>7.50 [5.85, 9.90]</td>
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<tr>
<td>Montague 2012 (ii)</td>
<td>28.9</td>
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<td>8</td>
<td>19.9</td>
<td>4.3</td>
<td>8</td>
<td>17.2%</td>
<td>18</td>
<td>8.00 [5.29, 17.21]</td>
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<tr>
<td>Study 12 (ii)</td>
<td>23</td>
<td>7.2</td>
<td>6</td>
<td>20.2</td>
<td>12.8</td>
<td>10</td>
<td>5.1%</td>
<td>18</td>
<td>2.90 [4.47, 12.07]</td>
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<tr>
<td>Study 13</td>
<td>18.4</td>
<td>7.6</td>
<td>7</td>
<td>20.1</td>
<td>9.1</td>
<td>6</td>
<td>6.2%</td>
<td>18</td>
<td>-1.70 [10.89, 7.48]</td>
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<tr>
<td>Study 15 (i)</td>
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<td>14.9</td>
<td>6.5</td>
<td>7</td>
<td>10.4%</td>
<td>18</td>
<td>4.50 [3.68, 10.56]</td>
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</tr>
<tr>
<td>Study 16</td>
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<td>15.1</td>
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<td>2.5%</td>
<td>18</td>
<td>0.60 [7.65, 0.04]</td>
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<tr>
<td>Study 7 (ii)</td>
<td>19.3</td>
<td>7.7</td>
<td>11</td>
<td>17.7</td>
<td>6.8</td>
<td>11</td>
<td>9.7%</td>
<td>18</td>
<td>1.60 [4.47, 7.78]</td>
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<tr>
<td>Subtotal (95% CI)</td>
<td>97</td>
<td>100.0%</td>
<td>84</td>
<td>94.0%</td>
<td>180</td>
<td>1.20</td>
<td>0.0001</td>
<td>137</td>
<td>20.70 [26.30, 15.10]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 3.28; Ch² = 13.68, df = 8 (P = 0.08), I² = 42%
Test for overall effect: Z = 4.36 (P < 0.0001)

Test for subgroup differences: Ch² = 46.78, df = 1 (P < 0.0001), I² = 97.9%

©2015 by American Society of Hematology
First step of thrombus formation:
- Physiological shear stress.
- VWF dependent platelet adhesion.
- Platelet-platelet interactions involving mainly GpIIb/IIIa.

Second step of thrombus formation:
- Very high, pathological, shear stress.
- Platelet-platelet interactions involving mainly VWF/Gplbα.
- Leads to vessel lumen closure.

Occlusive thrombus (early):
- Resistant to GpIIb/IIIa inhibitors, thrombin inhibition and tPA.

Complete Thrombus stabilization (late):
- Multi-resistant
Leo.H4: GpIIa/IIIb inhibitor; Xia.B2: Gp1b-alpha inhibitor

Lebehot, Gauberti et al., Blood 2014
State of the Art

Progressive Activation

Capture

Rolling

Slow Rolling

Firm Adhesion

Transmigration

Leukocyte

Endothelium

Selectins

Integrins

Chemoattractants on endothelial cell surface

Other chemoattractants in tissue
State of the Art
**State of the Art**

**PRE-CLINICAL DATA**

- **Anti-VLA-4** treatment inhibits cerebral leukocytes invasion, decreases ischemic injury volume and improves stroke outcome.

- **Effect of Natalizumab on Infarct Volume in Acute Ischemic Stroke (ACTION)**

  - This study has been completed. Sponsor: Biogen

  - ClinicalTrials.gov Identifier: NCT01955707

*Liesz et al., Brain, 2011*
Results of The First Multicenter Trial in Experimental Stroke Research: Anti-CD49d treatment in acute brain ischemia

Authors: Gemma Llovera1,2, Kerstin Hofmann1,2, Stefan Roth1,2, Angelica Salas-Pérdomo3,4, Maura Ferrer-Ferre3,4, Carlo Perego5, Elisa R. Zanier5, Uta Mamrack1,2, Andre Rex6, Hélène Party7, Véronique Agin7, Claudine Fauchon8, Cyrille Orset7,8, Benoît Haelewyn7,8, Ulrich Dirnagl6, Anna Planas3,4, Nikolaus Plesnila1,2, Maria-Grazia De Simoni5, Denis Vivien7,8, Arthur Liesz1,2*

Science Translational Medicine, 2015

.... Just, like in Human

Effect of Natalizumab on Infarct Volume in Acute Ischemic Stroke (ACTION) This study has been completed. Sponsor: Biogen ClinicalTrials.gov Identifier: NCT01955707
Results

2. SELECT THE BEST PHARMACOPHORE (CLONE M/K-2 VS A(429))

Anti-VCAM-1
(clone M/K-2 or A(429))

Previously published (McAteer et al., 2007)

Selected after IHC

IHC studies +24h following acute inflammation

Montagne*, Gauberti* et al., Neuroimage. 2012
Gauberti et al., Stroke, 2013
Bellière et al., Theranostics., 2015

ACUTE CEREBROVASCULAR INFLAMMATION

T2*W +24h

Montagne*, Gauberti* et al., Neuroimage. 2012
Gauberti et al., Stroke, 2013
Bellière et al., Theranostics., 2015

MPIO IV +24h

TNF (1µg)

IgG

M/K-2

A (429)

✓ MPIOs-αVCAM-1 (A429)

IgG

M/K2

A(429)

Signal void (%)
Results

4. TIME BETWEEN INJECTION/IMAGING

+24H FOLLOWING ACUTE INFLAMMATION

+2h following MPIOs (IHC)

![Images of MRI and fluorescence studies showing signal void area over time.](image)

- Imaging 20 minutes only after MPIOs injection
- Repeated imaging experiments and longitudinal assessment of cerebrovascular inflammation

---

Montagne*, Gauberti* et al., Neuroimage. 2012
Gauberti et al., Stroke, 2013
Bellière et al., Theranostics., 2015
Results

WHAT ABOUT STROKE?

![Graph showing changes in infarct volume and right/left turn ratio over time]

Liesz et al., 2011

Montagne*, Gauberti* et al., Neuroimage. 2012
Gauberti et al., Stroke, 2013
Bellière et al., Theranostic, 2015
Results

**ISCHEMIC STROKES**

A

**Permanent MCAo**

- **Transient MCAo**

- * +20%

- NS

B

Permanent MCAo

- T2w +24h
- T2w +72h

C

- MPIOs-αVCAM-1 IV +24h
- T2*w +24h

- Montagne*, Gauberti* et al., Neuroimage. 2012
- Gauberti et al., Stroke, 2013
- Bellière et al., Theranostic., 2015
Ischemic stroke
TIA

A

Sham
TIA
Ischemic stroke

T2 VCAM-1 P-Selectin

B

Signal void area (ipsilateral vs contralateral)

Sham
TIA
Ischemic stroke

VCAM-1 P-selectin

Molecular imaging
IHC
merged

Quénault et al., Submitted
Iron-Labeled Microparticles

BioPAL offers a 1 µm biodegradable superparamagnetic particle.

MicroTRACK™
tPA is a fibrinolytic agent

tPA is also a neuromodulator of NMDAR signaling
Parcq et al., Cell Death and Differ., 2012
Bertrand et al., Cell Death and Dis., 2015

**Fibrinolytic activity (% of tPA)**

- tPA
- d-tPA
- sc-tPA
- tc-tPA

**tPA versus tPAs**

**Video-calcium imaging**

**NMDA-evoked calcium influx (%)**

- sc-tPA 300nM
- tc-tPA 300nM

Parcq et al., Cell Death and Differ., 2012
Bertrand et al., Cell Death and Dis., 2015
OPHELIE is a multicenter study conducted in France in 25 centers where patients treated by iv rt-PA will be included.

700 patients are needed for the study assuming that a difference of 5% will be found in the primary outcome measure (modified Rankin scale 0-1 at 3 months) with alpha and beta risks respectively of 5% and 20%.

ClinicalTrials.gov Identifier: NCT01614080
control

Calcium video imaging

<table>
<thead>
<tr>
<th>Condition</th>
<th>% of responsiveness (post-incubation / pre-incubation)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td><img src="image1.png" alt="Graph" /></td>
</tr>
<tr>
<td>tPA</td>
<td><img src="image2.png" alt="Graph" /></td>
</tr>
<tr>
<td>tPA + 6C9B6</td>
<td><img src="image3.png" alt="Graph" /></td>
</tr>
<tr>
<td>tPA + Glunomab</td>
<td><img src="image4.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

Species | Gene bank numbers | Sequences (aa position) |
---------|-------------------|-------------------------|
Mus musculus | P35438 | 172EGRAAQKRLETLLEE186 |
Rattus norvegicus | P35439 | 172EGRAAQKRLETLLEE186 |
Homo sapiens | Q05586 | 172EGRAAQKRLETLLEE186 |

Lesept et al., submitted, R1
Calcium video imaging

[GlN1-1b / GluN2A]

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>tPA</th>
<th>tPA + Glunomab</th>
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</tr>
<tr>
<td>K178V</td>
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<td>#</td>
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<tr>
<td>K190V</td>
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<td>#</td>
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</tr>
</tbody>
</table>

% of responsiveness (post-incubation / pre-incubation)

Lesept et al., submitted, R1

Control Ab

GluN1

Glunomab

Extrasynaptic GluN1

Synaptic GluN1

Diffusion coefficient (µm²/s)

Calcium video imaging

Lesept et al., submitted, R1

GluN1

Ab

Imaging 10’

Imaging 20-30’

Glunomab

Homer 1c

tPA m

tPA

t0s

t25s

synapses
Preclinical evidence toward the use of ketamine for recombinant tissue-type plasminogen activator-mediated thrombolysis under anesthesia or sedation


Ketamine to Increase the Benefits of rtPA-induced thrombolysis: An Imaging-Based Efficacy Randomized Controlled Trial

ClinicalTrials.gov Identifier: NCT02258204

rt-PA + Ketamine + Thrombectomy
Serine proteases and Pathophysiology of the neurovascular Unit (SP2U)

INSERM U919