Conflict of Interest Statement

Within the past 12 months, I or my spouse/partner have had a financial interest/arrangement or affiliation with the organization(s) listed below.

☑ I have the following potential conflicts of interest to report:

☐ Consulting
☐ Employment in industry
☐ Stockholder of a healthcare company
☐ Owner of a healthcare company
☐ Other(s)

☒ I do not have any potential conflict of interest
Vulnerable Plaque: Investigation Plan

- Establish biological, pathological, and mechanical features of the vulnerable plaque: definition
- Look for the presence of vulnerable plaques elsewhere in the same patient
- Develop noninvasive and invasive tools to detect vulnerable plaque
- Test the performance of these tools to identify key features in plaques that have just caused ACS/Stroke/TIA
- Establish the natural history of these high-risk plaques
- Establish the potential impact of such finding on the procedural strategy and short-term outcome
- Test systemic and/or local therapies aiming at improving the natural history
**Distribution of various plaque types in patients affected by CAD**

<table>
<thead>
<tr>
<th>Plaque types</th>
<th>CS * of pts without SA who died for non-cardiac causes (CTRL group) (N=304)</th>
<th>CS * of pts with SA died for non-cardiac causes (SA group) (N=109)</th>
<th>CS * of pts died for AMI (AMI group) (N=544)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Culprit plaques “with thromb.”</td>
<td>0</td>
<td>0</td>
<td>16 (3.0%)</td>
</tr>
<tr>
<td>- associated to cap rupture</td>
<td>0</td>
<td>0</td>
<td>14 (2.6%)</td>
</tr>
<tr>
<td>- associated to cap erosion</td>
<td>0</td>
<td>0</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>“Vulnerable plaques”</td>
<td>13 (4.3%)</td>
<td>4 (3.7%)</td>
<td>109 (20.0%)</td>
</tr>
<tr>
<td>- thin-fibrous cap atheromata</td>
<td>13 (1.0%)</td>
<td>0</td>
<td>31 (5.7%)</td>
</tr>
<tr>
<td>- superficial calcified nodule</td>
<td>8 (2.6%)</td>
<td>4 (3.7%)</td>
<td>31 (5.7%)</td>
</tr>
<tr>
<td>- plaques with stenosis &gt;90%</td>
<td>2 (0.7%)</td>
<td>0</td>
<td>47 (8.6%)</td>
</tr>
<tr>
<td>- # vulnerable plaques/pts</td>
<td>1.4±0.3</td>
<td>0.8±0.3</td>
<td>6.8±0.5</td>
</tr>
<tr>
<td>“Stable plaques”</td>
<td>291 (95.7%)</td>
<td>105 (96.3%)</td>
<td>419 (77.0%)</td>
</tr>
</tbody>
</table>

* CS = coronary segments; Mauriello A et al. JACC 2005; 45:1585-1593
## Thrombotically Active Plaques, Cap Rupture and Cap Erosion by Disease State

<table>
<thead>
<tr>
<th></th>
<th>Patients with Major Ipsilateral Stroke (N=96)</th>
<th>Patients with TIA (N=91)</th>
<th>Asymptomatic (N=82)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Plaques</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiographic stenosis (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral carotid</td>
<td>86.1</td>
<td>79.5</td>
<td>84.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Controlateral Carotid</td>
<td>60.9</td>
<td>64.2</td>
<td>57.5</td>
<td>0.60</td>
</tr>
<tr>
<td>Thrombotically active plaque (n,%)</td>
<td>71 (74.0)</td>
<td>32 (35.2)</td>
<td>12 (14.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cap Rupture</td>
<td>64 (66.7)</td>
<td>21 (23.1)</td>
<td>11 (13.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cap Erosion</td>
<td>7 (7.3)</td>
<td>11 (12.1)</td>
<td>1 (1.2)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Spagnoli LG et al, JAMA 2004; 292:1845-1852
Frequency of acute ruptures by degree of luminal stenosis

CAROTID

<table>
<thead>
<tr>
<th>Luminal Stenosis %</th>
<th>% acute ruptures</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>0</td>
</tr>
<tr>
<td>25 - 50</td>
<td>5</td>
</tr>
<tr>
<td>50 - 75</td>
<td>10</td>
</tr>
<tr>
<td>75 - 100</td>
<td>25</td>
</tr>
</tbody>
</table>

CORONARY

<table>
<thead>
<tr>
<th>Luminal Stenosis %</th>
<th>% acute ruptures</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25%</td>
<td>1</td>
</tr>
<tr>
<td>25 - 50</td>
<td>2</td>
</tr>
<tr>
<td>50 - 75</td>
<td>4</td>
</tr>
<tr>
<td>75 - 100</td>
<td>6</td>
</tr>
</tbody>
</table>
### Thrombosis Related to Time Interval Between Symptom Onset and Surgery in pts with Stroke

<table>
<thead>
<tr>
<th>Time Interval between Acute Cerebrovascular Event and Endarterectomy, No %</th>
<th>0-2 mo (N=32)</th>
<th>3-6 mo (N=18)</th>
<th>7-12 mo (N=15)</th>
<th>13-24 mo (N=13)</th>
<th>25-30 mo (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombotically active plaques (TAPs)</td>
<td>32 (100)</td>
<td>13 (72.2)</td>
<td>11 (73.3)</td>
<td>7 (53.8)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>Organized thrombus</td>
<td>0</td>
<td>4 (22.2)</td>
<td>4 (26.7)</td>
<td>5 (38.5)</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td>No thrombosis</td>
<td>0</td>
<td>1 (5.6)</td>
<td>0</td>
<td>1 (7.7)</td>
<td>0</td>
</tr>
</tbody>
</table>

Spagnoli LG et al, JAMA 2004; 292:1845-1852
Rapid Tx of Symptomatic Patients

# of strokes prevented per 1,000 CEAs at 3 years

- 0-2 weeks: Approx. 340 strokes
- 2-4 weeks: Approx. 140 strokes
- 4-12 weeks: Approx. 80 strokes
- >12 weeks: Approx. 40 strokes

Time from last event to randomization

Adapted from Rotwell 2004
If we could identify potentially unstable atheroma before they are evident, clinically, we might even contemplate angioplasty of nonsignificant stenoses to induce smooth muscle cell proliferation and reinforce the plaque’s fibrous cap.
Strut Quantitative Measurements

- Neointima
- Old Fibrous Cap Area
- Lipid Core
- New Fibrous Cap Area
- Strut
Clinical presentation, plaque types and PAPP-A levels observed in 65 carotid plaques submitted to histologic examination

<table>
<thead>
<tr>
<th>Histological Definition</th>
<th>Stroke (N=19)</th>
<th>TIA (N=24)</th>
<th>Asymptomatic (N=29)</th>
<th>PAPP-A Serum Levels (mIU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruptured plaques (n=14)</td>
<td>7 (41.2%)</td>
<td>4 (20.0%)</td>
<td>3 (10.7%)</td>
<td>6.97±0.75</td>
</tr>
<tr>
<td>Vulnerable plaques (n=13)</td>
<td>5 (29.4%)</td>
<td>4 (20.0%)</td>
<td>4 (14.3%)</td>
<td>7.43±0.97</td>
</tr>
<tr>
<td>Stable plaques (N=38)</td>
<td>5 (29.4%)</td>
<td>12 (60.0%)</td>
<td>21 (75.0%)</td>
<td>4.02±0.18*</td>
</tr>
</tbody>
</table>

*p<0.02 Rupt/vuln vs. stable

Sangiorgi G et al, JACC 2006;47:2201-2211
Correlation PAPP-A / Cap Thickness

Correlation Coefficient = -0.6296  P<0.01

\[ y = -53.177x + 219.96 \]

\[ R^2 = 0.3964 \]
Correlation PAPP-A / Plaque Inflammation

Correlation Coefficient = 0.7041  P<0.01

\[ y = 12.736x + 13.84 \]

\[ R^2 = 0.4957 \]
PAPP-A Serologic Levels in Pts with Single Coronary Lesion after Stenting

PAPP-A serum levels (mIU/L)

Baseline
One-Month
Three months

AMI (n=20)
Stable (n=20)
Unstable (n=20)

P <.005 Baseline vs. 1 month vs. 3 months

Sangiorgi G. et al. Eur Heart J 2002
SUBMARINE Study Design
SERUM AND URINARY PLAQUE VULNERABILITY BIOMARKERS DETECTION BEFORE AND AFTER CAROTID STENT IMPLANTATION

Minor Stroke or First/Recurrent TIAs

Neurologic Assessment Duplex Examination

Early CAS with NP Filter
SUBMARINE Study Design

SERUM AND URINARY PLAQUE VULNERABILITY BIOMARKERS DETECTION BEFORE AND AFTER CAROTID STENT IMPLANTATION

Minor Stroke or First/Recurrent TIAs

Neurologic Assessment Duplex Examination

PAPP-A hs-CRP MMP-2/MMP-9 IL-6/IL-8 TNF alpha CD40L

Histologic evaluation of plaque debris

TIA 24/48 hrs Minor Stroke 14-30 days

Assessment of Vulnerability Biomarkers at Pre-Post- and FU
PAPP-A Serologic Levels at Different Time Intervals

- **Pre-procedure**
  - Overall: 8.8 (mIU/L)
  - TIA: 6.9 (mIU/L)
  - Minor Stroke: 15.1 (mIU/L)

- **Post-procedure**
  - Overall: 15.1 (mIU/L)

- **1-mo FU**
  - Overall: 6.9 (mIU/L)

**P < 0.01**

[IC –1,8 – 15,3]
hs-CRP Serologic Levels at Different Time Intervals

- Pre-procedure: Overall - 12.4
- Post-procedure: Overall - 23
- 1-mo FU: Overall - 9.7

Significance:
- Pre-procedure vs Post-procedure: P<0.01
- Post-procedure vs 1-mo FU: P<0.01

Intervals:
- [IC –6.7 –19.9]
- [IC –16.1 –5.0]
IL-6 Serologic Levels at Different Time Intervals

- Pre-procedure
  - Overall: 8
  - TIA: 10
  - Minor Stroke: 6

- Post-procedure
  - Overall: 13.5
  - TIA: 20
  - Minor Stroke: 10

- 1-mo FU
  - Overall: 7.4
  - TIA: 13
  - Minor Stroke: 15

Statistical significance:
- P<0.01
- [IC –3.6 – 9.1]
- [IC –8.1 – 2.7]
IL-8 Serologic Levels at Different Time Intervals

Pre-procedure
- 15.8

Post-procedure
- 19.86

1-mo FU
- 15.7

Overall
- TIA
- Minor Stroke
MMP-2 Serologic Levels at Different Time Intervals

- Pre-procedure
- Post-procedure
- 1-mo FU

- Overall
- TIA
- Minor Stroke

Pre-procedure:
- MMP-2 levels: 830.5
- Pre-procedure TIA: 830.5

Post-procedure:
- MMP-2 levels: 836.1
- Post-procedure TIA: 836.1

1-mo FU:
- MMP-2 levels: 960.2
- 1-mo FU TIA: 960.2

Statistical significance:
- P<0.001
- [IC -177.9, -77.6]
MMP-9 Serologic Levels at Different Time Intervals

- Pre-procedure
- Post-procedure
- 1-mo FU

Overall
TIA
Minor Stroke

Pre-procedure: 100.8
Post-procedure: 125.1
1-mo FU: 117.9

P < 0.01
[IC –43.5, –5.1]
Conclusions

- Plaque vulnerability biomarkers are elevated at symptoms onset both in coronary and carotids plaques due to the related complex plaque characteristics.

- Different biomarkers levels increase after stenting and at 1 month follow-up are significantly reduced to a level similar to the corresponding baseline time.

- Longer follow-up data are expected to demonstrate possible complete mechanical plaque stabilization.