This study was funded by the Heinz-Nixdorf-Stiftung.

The Ludwig Maximilians University is the holder of the patents “Use of G-CSF for Treating Ischemia” (EP 03 02 4526.0 and US 60/514,474) and „ Remedies for Ischemia“ (EP2007/003272 and US 60/792,943).

Consulting fee: Recardio

Speaker honorarium in the last 2 years: MSD, Astra Zeneca
Sitagliptin plus Granulocyte-Colony Stimulating Factor in Acute Myocardial Infarction

November 18th 2013, AHA Dallas, Texas

Wolfgang-Michael Franz
DPP-IV Inhibition and G-CSF improves survival after myocardial infarction

Increased cardiac homing

Improved ventricular wall thickness

Improved cardiac function

Decreased mortality

Zaruba M, Theiss H et al. & Franz WM, Cell Stem Cell 2009
Transfer „from bench to bedside“: SITAGRAMI-Study in patients with acute STEMI

Safety and efficacy of Sitagliptin plus GRanulocyte-colony-stimulating factor in patients suffering from Acute Myocardial Infarction – EudraCT-Nummer: 2007-003941-34

- Multi-center, randomised, double-blind, placebo-controlled study

Hypothesis

- A mean individual increase of EF from baseline to 6-months follow-up, which would be by 3.5% higher in the G-CSF/Sitagliptin group than in the placebo group would be considered relevant. Standard deviation of the individual EF change can be assumed to be 5.5% or less.

- After 70 patients a blinded interim analysis by an external statistician revealed a higher standard deviation of 8%.

- Therefore the number of patients included was increased from 100 to 174 to achieve a power of 80%.
Inclusion criteria

- >18 years, male or female
- Acute ST segment elevation myocardial infarction (typical chest pain of more than 30 minutes duration, presence of ST-segment elevation in at least two contiguous leads or left bundle-branch block) and/or occluded coronary artery
- PCI/Stenting within 2-24 hours after onset of acute myocardial infarction
- Creatinin kinase > 540 U/l
- Regional wall motion abnormality (comprising hypo-, a- or dyskinesia) demonstrated with MRI
- Given written informed consent
Exclusion criteria
(selection of most relevant)

- Contraindications against magnetic resonance imaging (e.g. implants, claustrophobia)
- Other severe concurrent illness (e.g. active infection, malignancy)
- Life expectancy of less than one year
- Crea level >1.7 mg/dL
- Diabetes type 1 patients
- Concomitant medications known to cause hypoglycemia, such as sulfonylureas
- Severe liver dysfunction
- Malignant haematological diseases, i.e. chronic myeloic leukemia (CML) or myelodysplatic syndromes (MDS)
- Acute cardiogenic shock, EF > 25%
- Prior thrombolysis
- Left ventricular thrombus
- Severe cardiac arrhythmias (i.e. malignant sustained or non-sustained ventricular tachycardia or ventricular fibrillation)
- Acute massive pulmonary infiltrations
Primary Endpoint

The primary efficacy endpoint hierarchically combined global left and right ventricular ejection fraction change from baseline to 6 months follow-up ($\Delta_{\text{LVEF}}, \Delta_{\text{RVEF}}$) using magnetic resonance imaging.
Secondary Endpoints

- Several MRI-measurements: Delayed enhancement, myocardial perfusion, regional contractile reserve, end-diastolic and end-systolic volumes, stroke volume, and cardiac output etc.
- Major adverse cardiac events (death, myocardial infarction, CABG, or re-intervention) up to 12 months
- Peripheral blood stem cell populations: CD34, CD34/KDR and CD34/CD26 positive cells prior to and 5 days after therapy initiation
- NT-pro-BNP, glucose, complete blood count, platelets, CK and cTnI prior to and 5 and 28 days, and 6 and 12 months after therapy initiation
- Assessment of in stent restenosis using angiography 6 months after facultative PCI
Enrollment and Outcomes

340 pts assessed for eligibility

- 156 pts excluded
  - 48 pts inclusion criteria
  - 30 pts declined
  - 78 pts exclusion criteria
- 184 pts gave written informed consent
- 10 pts excluded
  - 1 pt inclusion criteria
  - 2 pts declined
  - 7 pts exclusion criteria
- 174 pts underwent randomisation

Study medication after MRI

- 86 pts placebo
  - 1 ventricle thrombus
- 87 pts verum

6 month MRI

- 81 pts
  - (1 died, 2 refused, 1 drop out, 1 technical problem)

12 month safety

- 78 pts
  - (1 died, 1 refused, 1 follow-up, 4 telefone only)
- 81 pts
  - (3 lost to follow-up, 3 technical)

86 pts (1 died, 2 refused, 1 drop out, 1 technical problem)

81 pts

87 pts

86 pts

81 pts

81 pts

81 pts

86 pts

87 pts
### Patient Characteristics - I

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Placebo (n=86)</th>
<th>GS (n=87)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age - yr</td>
<td>60.1 ±11.5</td>
<td>61.3 ± 11.0</td>
<td>0.474</td>
</tr>
<tr>
<td>Male sex – no (%)</td>
<td>70 (81.4)</td>
<td>69 (79.3)</td>
<td>0.878</td>
</tr>
<tr>
<td>Arterial Hypertension – no (%)</td>
<td>62 (72.1)</td>
<td>66 (75.9)</td>
<td>0.695</td>
</tr>
<tr>
<td>Hypercholesterinemia</td>
<td>48 (55.8)</td>
<td>44 (50.6)</td>
<td>0.591</td>
</tr>
<tr>
<td>Diabetes – no (%)</td>
<td>14 (16.3)</td>
<td>9 (10.3)</td>
<td>0.272</td>
</tr>
<tr>
<td>Smoking – no (%)</td>
<td>51 (59.3)</td>
<td>58 (66.7)</td>
<td>0.398</td>
</tr>
<tr>
<td>Family history of MI – no (%)</td>
<td>33 (38.4)</td>
<td>28 (32.1)</td>
<td>0.488</td>
</tr>
<tr>
<td>Infarct treatment</td>
<td>Placebo (n=86)</td>
<td>GS (n=87)</td>
<td>P value</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>----------------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Infarct related vessel – no (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>40 (46.5)</td>
<td>38 (43.7)</td>
<td></td>
</tr>
<tr>
<td>LCX</td>
<td>30 (34.9)</td>
<td>32 (36.8)</td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>16 (18.6)</td>
<td>17 (19.5)</td>
<td></td>
</tr>
<tr>
<td>Time from angina to PCI (hrs)</td>
<td>60.1 ±11.5</td>
<td>61.3 ±11.0</td>
<td>0.474</td>
</tr>
<tr>
<td>Drug eluting stent – no (%)</td>
<td>70 (81.4)</td>
<td>69 (79.3)</td>
<td>0.878</td>
</tr>
<tr>
<td>Bare metal stent – no (%)</td>
<td>17 (19.8)</td>
<td>25 (28.7)</td>
<td>0.231</td>
</tr>
<tr>
<td>Peak Creatinekinase (U/l)</td>
<td>3080±2120</td>
<td>3095±2113</td>
<td>0.688</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stem cell population (/µl blood)</th>
<th>Placebo (n=78)</th>
<th>GS (n=77)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+CD45−-cells at baseline</td>
<td>8.7±13.7</td>
<td>7.1±13.7</td>
<td>0.282</td>
</tr>
<tr>
<td>CD34+CD45−-cells at day 5</td>
<td>8.6±13.9</td>
<td>58.0±89.4</td>
<td>0.011</td>
</tr>
<tr>
<td>Medication at discharge – no (%)</td>
<td>Placebo (n=86)</td>
<td>GS (n=87)</td>
<td>P value</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Aspirin</td>
<td>86 (100)</td>
<td>87 (100)</td>
<td>1.000</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>54 (62.8)</td>
<td>52 (59.8)</td>
<td>0.801</td>
</tr>
<tr>
<td>Prasugrel</td>
<td>14 (16.3)</td>
<td>19 (21.8)</td>
<td>0.461</td>
</tr>
<tr>
<td>Ticagrelor</td>
<td>18 (20.9)</td>
<td>16 (18.4)</td>
<td>0.819</td>
</tr>
<tr>
<td>Statins</td>
<td>86 (100)</td>
<td>87 (100)</td>
<td>1.000</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>79 (91.9)</td>
<td>74 (85.1)</td>
<td>0.245</td>
</tr>
<tr>
<td>AT1-blocker</td>
<td>5 (5.8)</td>
<td>11 (12.6)</td>
<td>0.198</td>
</tr>
<tr>
<td>Betablockers</td>
<td>85 (98.8)</td>
<td>86 (98.9)</td>
<td>1.000</td>
</tr>
<tr>
<td>Aldosteron antagonists</td>
<td>10 (98.8)</td>
<td>12 (13.8)</td>
<td>0.842</td>
</tr>
</tbody>
</table>
Primary efficacy endpoints
LVEF and RVEF

**Figure:** Primary efficacy endpoints LVEF and RVEF at screening and after 6 months follow-up. Red line: mean
The estimated treatment effect is displayed before and after adjusting for additional pre-treatment covariates using analysis of covariance (ANCOVA). Red bar at the top: pre-specified confirmatory primary efficacy analysis; black bar: ANCOVA with an additional covariate as main effect. The position of the diamonds represents the point estimates of the treatment effect, i.e. the absolute change of LVEF and RVEF, respectively; the horizontal lines represent the 95% confidence intervals.

Figure B): Adjusting for gender revealed that there is a significant interaction between gender and treatment group (p-value = 0.037); dashed black bar: corresponding ANCOVA model without interaction term.
MACE during 12 month

HR 0.785 (95% CI 0.414 to 1.488)
p = 0.458

Number of events: Placebo (n=21) vs. GS (n=17)
- Sudden cardiac death: 2 vs. 0
- In-stent restenosis: 8 vs. 9
- De-novo stenosis: 15 vs. 7
- Severe MI: 0 vs. 1
- Bypass surgery: 0 vs. 1
- In-stent + de-novo stenosis: 4 vs. 0
## MACE during 12 month

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (n=22)</th>
<th>GS (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudden cardiac death</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Severe myocardial infarction</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Bypass surgery</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>In-stent restenosis</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>De-novo Stenosis</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>In-stent and de-novo stenosis</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
Summary and Conclusions

- Dual stem cell therapy comprising the use of GCSF and Sitagliptin after successfully revascularized AMI is safe but fails to show a beneficial effect on cardiac function.

- In an unplanned subgroup analysis, female patients showed a significant increase in RVEF after GS treatment compared to placebo which we cannot explain from a clinical point of view.

- The secondary endpoint: de novo-stenosis after 6 months showed a trend for reduction in the GS group (15 vs 7).

- There are limitations of the SITAGRAMI trial:
  - Only 14% of our patients had a LVEF below 40% (21% below 50%),
  - Study medication was applied after randomization, 2 to 6 days after MI (dosage?)
  - All STEMI were treated with heparin

- The strengths of the trial are:
  - It is the largest trial on stem cell mobilization in the cardiovascular field so far
  - MRI is used to analyse cardiac function, which represents the diagnostic gold standard today
  - The fraction of missing data for the primary efficacy endpoint and the dropout rate were very small
  - In the primary intention-to-treat analysis, the placebo group showed a $\Delta_{LVEF}$ of 4.6% (SD 7.9%), which realistically reflects the observations in previous publications like the REPAIR-AMI trial and is in contrast to other stem cell studies which reported only a small improvement of LVEF after 6 months.
Thanks to...

Medical Department I
University of Munich – Grosshadern

PD Dr. Hans D. Theiss
Dr. Christoph Brenner
Dr. Ulrich Grabmeier
Lisa Gross

Institute for Informatics, Biometry, Epidemiology – IBE (LMU)

Prof. Dr. Ulrich Mansmann
Dipl. Stat. Christine Adrion

Study Nurse: Britta Halter

Institute of Radiology (LMU)
Prof. Dr. M. Reiser,
Dr. Daniel Theisen

Medical Department – Innenstadt (LMU)
PD Dr. Sohn

Division of Cardiology - Bogenhausen
Prof. Dr. E. Hoffmann
PD Dr. Alexander Leber