Stem Cell Therapy for Acute MI: Are we Making Progress?

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Regenerative Medicine and Cardiovascular Disease
Regenerative Medicine?

Cell Therapy-Regenerative
Where are we in 2013?

• Focus on Autologous Marrow-Derived selected cell and Allogenic Mesenchymal Cell Populations
• Phase II/III clinical trials
  Acute MI
  CLI
  Ischemic Cardiomyopathy

Incidence, Prevalence and Cost of Cardiovascular Disease in the United States

<table>
<thead>
<tr>
<th>Condition</th>
<th>Incidence/Prevalence</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial Infarction</td>
<td>1,255,000/year</td>
<td>$96 Billion Total (Hosp, Phys, Drug, Nursing home care)</td>
</tr>
<tr>
<td>First Event</td>
<td>785,000/year</td>
<td></td>
</tr>
<tr>
<td>Recurrent Event</td>
<td>470,000/year</td>
<td></td>
</tr>
<tr>
<td>At Risk for MACE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>8,000,000</td>
<td>$35 Billion Total (22 Billion related to ischemia)</td>
</tr>
<tr>
<td>Myocardial Ischemia</td>
<td>37,000,000</td>
<td></td>
</tr>
</tbody>
</table>

*NHLBI Oct 2009 Report Morbidity and Mortality
*Projected Direct cost for 2010
Cell Therapy Types Under Investigation

- Bone Marrow Derived Mononuclear Cells (EPC)
- Hematopoietic (Autologous)
  - Un-manipulated MNC
  - Purified CD34+ or CD33+ cells
- Mesenchymal (Autologous and Allogeneic)
- Blood Derived Mononuclear Cells
  - G-CSF Mobilized CD34+ cells
- Muscle Derived Cells
  - Skeletal myoblast
  - Atrial appendage (CSC and CDC)
- Fat Derived Cells
- Embryonic
- Induced Pluripotent Stem Cells

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Post STEMI Complications Are a Function of Left Ventricular Ejection Fraction

1-year survival declines dramatically when left ventricular ejection fraction is <45%. The increase in mortality is driven by sudden cardiac death and progressive pump failure.

Solomon, 2006
NEJM, 2003
Once Lost, Cardiomyocytes are Unable to Significantly Regenerate to Restore Cardiac Function

- $10^8$ – $10^9$ cardiomyocytes may be lost after sub-lethal AMI in humans (1)
- Bermann, et al (2), measured the integration of carbon-14, into DNA of cardiomyocytes in humans.
- They report that cardiomyocytes regenerate at 1% each year up to the age of 25 and then annual regeneration rates gradually fall to 0.45% at the age of 75.

Cardiomyocyte Renewal is Very Limited Over the Course of a Normal Life Span, and Decreases with Age

1. van Laake et al., Heart repair and stem cells, J Physiol. 2006 December 1; 577(Pt 2): 467–478.

Problem: Preservation of Cardiac Function Post AMI

- Despite the standard of care, 20% of MI patients (Approximately 160,000 annually in US alone) experience progressive deterioration in heart muscle function ($↓$ LVEF, $↑$ LVESV, $↓$ LVWM) and an increase in Major Adverse Cardiac Events (MACE), including:
  - Premature Death
  - Recurrent Myocardial Infarction
  - Congestive Heart Failure
  - This deterioration is caused by inadequate perfusion (microvascular insufficiency) leading to hibernating cardiomyocytes and progressive cardiomyocyte loss due to apoptosis.
Cell Type: Circulating CD34⁺ Cell Levels and Migratory Capacity Correlate with Cardiac Function

- Circulating CD34⁺ cell quantity 1 year post MI significantly correlates (positive) to left ventricular ejection fraction (LVEF), wall motion score index, end diastolic volume and end systolic volume.
- The number of circulating stem cells mobilized early (<12 hours) in AMI was significantly correlated with LVEF for CD34⁺ cells, for CXCR4⁺ cells, for CD117⁺ cells and c-met+ cells (P value < 0.004). (1)
- In patients with LVEF less than or equal to 40%, the peak circulating number of CD34⁺, CXCR4⁺, CD117⁺ and c-met⁺ cells was significantly lower when compared to patients with LVEF greater than 40% (p=0.02). (2,3)
- The only cytokine independently associated with significant increases in circulating CD34⁺ cells is SDF (not VEGF). (4)
- In the TOPCARE-AMI study, the migratory capacity of infused CXCR4⁺ progenitors induced by SDF-1 was the strongest independent predictor of the reduction of the infarct size assessed by contrast MR. (5)

Cell Type: CD34⁺CXCR4⁺ Cells are Involved in a Natural Repair Mechanism Post AMI to Improve Perfusion

The body attempts to rescue damaged tissue to prevent ventricular remodeling:

- A distress signal (HIF) is induced by hypoxia in the peri-infarct zone
- HIF induces synthesis of SDF and VEGF, which mobilize CD34⁺CXCR4⁺ cells
- The mobilized cells are trophic to the peri-infarct zone, preventing apoptosis and effecting neoangiogenesis

2. Wojciech Wojakowski et al. European Heart Journal 2006; 27: 283-289. Mobilization of CD34⁺, CXCR4⁺, CD117⁺, c-met⁺ stem cells is correlated with left ventricular ejection fraction and plasma NT-proBNP levels in patients with acute myocardial infarction
4. Tomoda et al Clin Cardiol 2003; 26: 455-457 Bone Marrow stimulation and left ventricular function in acute myocardial infarction
Homing Differs by Cell Type

- Bone Marrow Derived CD34+ Cells
  - CXCR-4 receptor-SDF-1 Ligand
- Blood Derived CD34+ Cells
  - Down Regulation of CXCR-4 due to G-CSF
- Ex Vivo expanded Mesenchymal Stem Cells
  - Low Expression of CXCR-4
  - Increased Integrins (CD 29) and Ligands (tenascin –c, fibronectin, VCAM-1 and laminin)

2. Dlubek et al. Bone Marrow Transplantation 2006; 37: 19-23

IRA Infusion of Bone Marrow-MNCs Preserve Cardiac Function and Reduce MACE in a Dose-Dependent Fashion in Patients Early and Late Post AMI. The Benefits Persist Out to Five Years

- Over 1000 AMI patients have received IRA Infusion of Bone Marrow MNC (BMNC) Post AMI and have had significant improvements in:
  - LVEF (absolute increase by 3-7%)
  - LVESV (decrease by 5-8 ml)
  - Infarct size (absolute decrease by 4-6%)
  - MACE (decreased incidence of recurrent AMI, new onset CHF and death)
- Significant Improvement in cardiac function and reduction in MACE dependant on:
  - IRA infusion of B-MNC 5 or more days post STEMI (avoid hot phase)
  - IRA infusion of more than 109 and ideally more than 1010 BMNC
  - IRA infusion of BMNC with migratory potential in an SDF-1 gradient.
- Durability of significant effect is long term (4-5 years) whether BMNC are administered acutely (4-21) or late (median 8 years) after a STEMI:
  - Acute BMNC administration preserves cardiac function for up to 4 years and reduces MACE at two years
  - Late BMNC administration restores cardiac function and reduces mortality four fold at 5 years (15.6% versus 3.7% p<0.001)

5. Strauer B.E. Eur J of Heart Failure 2010: 12
Solution: Effective Product to Fill Current Therapeutic Gap

- A therapy that can improve microvascular density (perfusion) to rescue at-risk cardiomyocytes from hibernation and apoptosis.
- This in turn will preserve heart muscle function and prevent downstream Major Adverse Cardiac Events (MACE).

Pre-clinical and clinical studies indicate that Bone Marrow Mononuclear Cells have the potential to fill this post-AMI therapeutic gap, but efficacy* depends on four key variables:

1. **Cell Type:** CD34+ expressing CXCR4+ (the SDF-1 receptor ligand) are best able to improve function
2. **Cell Dose:** Patients receiving >10^9 mononuclear cells had greatest effect
3. **Timing:** Infusion during the Repair Phase after STEMI produced better results
4. **Migratory Capacity:** Biologic potency depends on activity as measured by cell’s migratory capacity in an SDF-1 gradient

*Significant but modest improvement in LVEF and reduction is MACE

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Cell Type: Isolated CD34⁺ Cells Most Able to Improve Perfusion, Prevent Apoptosis and Rescue Hibernating Cardiomyocytes

CD34⁺ Cells Exhibit Increased Potency and Safety for Therapeutic Neovascularization after AMI Compared with Total Mononuclear Cells in Nude Rats:

- **PBS** = Phosphate-buffered saline
- loMNCs = 5x10^5 MNC
- hiMNCs = contains 5x10^5 CD34⁺ cells within MNCs
- CD34⁺ = 5x10^5 CD34⁺ cells

*Cappillary Density (perfusion) is greatest in CD34⁺ cell cohort, and this correlates with decreased incidence of fibrosis. Effect increases with dose.*

Kawamoto et al., Circulation 2006;114;2163–2169
The Superior Improvement in Capillary Density and Decrease in Fibrosis seen with purified CD34+ Cells Infusion Correlates with Superior Improvement in Cardiac Muscle Function:

Cell Type: Isolated CD34+ Cells Best Able to Maintain Cardiac Function

**Fractional Shortening**

- PBS = Phosphate-buffered saline
- LoMNCs = 5x10⁵ MNC
- hiMNCs = contains 5x10⁵ CD34+ cells within MNCs
- CD34+ = 5x10⁵ CD34+ cells

Kawamoto et al., Circulation 2006;114;2163–2169

**Regional Wall Motion Score**

<table>
<thead>
<tr>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>&lt;0.01</td>
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<tr>
<td>&lt;0.05</td>
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</table>

CD34+ Cells Localize in the Peri-infarct Zone

Localization of transplanted CD34 cells in the peri-infarct area of the heart is revealed by coregistration of MRI, micro-CT and micro-PET.

A1. Three-dimension rendering of micro-CT to show anatomy and viewing angle for (A2 and A3) micro-PET maximum intensity projections after registration. PET maximum intensity projections demonstrate graft-related uptake and other nonspecific (ie, normal) uptake in various organs. A3. Localizer for the slice shown in B.

Tissue slices using CT (B1), MRI (B3), and PET (B5) after registration. Coregistration of CT/MRI and MRI/PET are shown in (B2) and (B4), respectively.
CD34+ cells survive in the heart for over 12 months

Long-term BLI of TGL-CD34 in SCID mice:

A. The bioluminescent signal in the heart was superimposed on a photograph of a SCID mouse for the indicated time points after CD34 cell injection (representative mouse).

B. BLI intensity in SCID mice injected with CD34 cells is significantly higher than the mice received PBS injection over a 52-week time period. BLI intensity was assessed by measuring the photon flux from region of interest drawn over the precordium. Data are expressed in mean ± SE (n7/group). **P0.01; *P0.05.

Wang et al., Circ. Res. 2010:1904-1911

LVEF significantly improved in treated mice compared to control mice for up to 52 weeks

Evaluation of cardiac function using MRI:

A. Representative sequential images of the ES and ED volumes from a CD34+ cell-transplanted mouse and a control mouse over 25 weeks.

B. Dot graph of the LVEF in control mice vs CD34+ cell-transplanted mice over a 52-week time period. There is a significant difference between groups for LVEF at each time point. Data are expressed in mean ± SE (n7/group, except for week 52). NS: PNS; **P0.01; *P0.05.

Wang et al., Circ. Res. 2010:1904-1911
In vivo antibody treatments inhibit myogenesis/angiogenesis and affect cardiac function induced by injection of CD34+ cells into mice after MI.

B. Anti-α4B1, but not anti-VEGF, antibodies inhibited the formation of human-derived cardiomyocytes (HLA/troponin T), as determined by FACS analysis.

C. Only anti-VEGF inhibited the formation of human-derived endothelial cells (HLA/VE-cadherin).

D. Anti-VEGF, but not anti-α4B1, antibodies diminished the effect on the improvement in the LVEF caused by the injection of human CD34+ cells.

E. Treatment with anti-α4B1 or anti-VEGF antibodies did not affect LVEF following MI without cell therapy (Data are expressed in mean ± SE (n4/group). **P<0.01; *P<0.05.

Wang et al., Circ. Res. 2010: 106:1904-1911

Mechanism of Improved Cardiac Function After Bone Marrow Mononuclear Cell Therapy

- Induced AMI in (nu/nu) mice treated by direct IM injection of 1 million human MNC
- Lentiviral delivery of TK, which converts pro-drug gancyclovir to a cytotoxic, selectively inserted into endothelium, smooth muscle and cardiac committed cells within MNC
- Elimination of endothelial cells (two weeks post infarct) abrogated the salutary effect of MNC with significant reduction in capillary and arteriole density and animal death rates similar to PBS
Mechanism of Improved Cardiac Function After Bone Marrow Mononuclear Cell Therapy

- Selective elimination of endothelial cells (eNOS) had the greatest adverse effect on LVEF.
- Selective elimination of smooth muscle cells (SM22ap) had less of an adverse effect on LVEF despite a 4 times greater prevalence than endothelial cells.
- Selective elimination of cardiac muscle cells had no adverse effect on LVEF.


Phase 1 Clinical Summary

<table>
<thead>
<tr>
<th>Indication</th>
<th>Post-AMI with LVEF ≤50% and Wall Motion Abnormality in IRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Endpoint</td>
<td>Safety in post-AMI Patients</td>
</tr>
<tr>
<td>Key Inclusion Criteria</td>
<td>Confirmation of ST Elevation MI; Ejection fraction ≤ 50%</td>
</tr>
<tr>
<td>Dosing Frequency</td>
<td>Single dose</td>
</tr>
<tr>
<td>4 Groups and Randomization</td>
<td>3 dose cohorts (5,10,15 Million) (randomized 1:1)</td>
</tr>
<tr>
<td>Number of Subjects</td>
<td>31</td>
</tr>
<tr>
<td>Other Endpoints</td>
<td>RTSS (Perfusion); LVEF; ESV; SDF Mobility</td>
</tr>
<tr>
<td>Number of Sites</td>
<td>4</td>
</tr>
<tr>
<td>Geography</td>
<td>United States</td>
</tr>
<tr>
<td>Trial Duration</td>
<td>6 months</td>
</tr>
</tbody>
</table>
### Phase 1 Clinical Trial Process

1. Patient presents with chest pain + STEMI, and is assessed via ECHO

2. Patient receives stenting and usual medical Rx

3. Patient screened, and deemed eligible if Ejection Fraction (EF) ≤ 50%

4. Baseline SPECT and MRI
   - Patient Bone Marrow Harvested

5. CD34⁺/CXCR4⁺ cells isolated using patented technology Isolex

6. Intracoronary AMR-001 infusion

7. Assess for Effect
   - ↓ RTSS
   - ↑ LVEF
   - ↓ LVESV

### AMR-001 Preparation was Feasible and Safely Administered

<table>
<thead>
<tr>
<th>Phase I Adverse Events</th>
<th>Treated (5 Million Cells) (N=5)</th>
<th>Treated (10 Million Cells) (N=5)</th>
<th>Treated (15 Million Cells) (N=6)</th>
<th>All Treated (N=15)</th>
<th>Control Group (N=15)</th>
<th>P-Value All Treated vs. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse Events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Chest Pain</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal Pain</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0.33</td>
</tr>
<tr>
<td>Upper Respiratory Infection</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td>Fever</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serious Adverse Events</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Sum of Adverse Events*:
- Arrhythmia: 1
- Chest Pain: 1
- Musculoskeletal Pain: 1
- Upper Respiratory Infection: 1
- Rash: 1
- Dyspnea: 2
- Fever: 1
- Serious Adverse Events: 7

Treated subjects experienced no increased frequency of atrial or ventricular arrhythmias compared to controls. $P$ value (all treated vs. controls) = 0.46
AMR-001 Showed No Excess Serious Adverse Events

<table>
<thead>
<tr>
<th></th>
<th>All Treated (N=15)</th>
<th>Control Group (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAEs before hospital discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute stent thrombosis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SAEs at 1 year follow up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re-hospitalization for heart failure</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chest pain requiring admission</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>In-stent restenosis resulting in revascularization</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Septic thrombophlebitis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total SAEs</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

None of the SAEs were judged by investigators to be treatment related.

AMR-001 Shows Dose Response

There is a correlation between increasing doses of AMR-001 and reduction in infarct region.

There is a correlation between increasing doses of AMR-001 and reduction in hypoperfusion.
Higher Doses of AMR-001 Reduce Hypoperfusion

RTSS (Hypo-Perfusion)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Base Line</th>
<th>6 months</th>
<th>Delta</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>259</td>
<td>273.5</td>
<td>+14.5</td>
<td>+5.6</td>
</tr>
<tr>
<td>5 M</td>
<td>714.2</td>
<td>722.0</td>
<td>+7.8</td>
<td>+1.1</td>
</tr>
<tr>
<td>10 M</td>
<td>998.6</td>
<td>635.8</td>
<td>-362.8</td>
<td>-36.4</td>
</tr>
<tr>
<td>15 M</td>
<td>584.0</td>
<td>462.0</td>
<td>-122.0</td>
<td>-20.9</td>
</tr>
</tbody>
</table>

RTSS results show promising changes consistent with hypoperfusion mechanism of action and establish a threshold dose at 10 Million Cells.

Above Threshold Dose vs. Below Threshold Dose

RTSS (Hypo-Perfusion)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>6 month</th>
<th>Delta</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below Threshold</td>
<td>385.4</td>
<td>398.1</td>
<td>+12.6</td>
</tr>
<tr>
<td>Above Threshold</td>
<td>814.3</td>
<td>558.6</td>
<td>-255.8</td>
</tr>
</tbody>
</table>

Ejection Fraction

<table>
<thead>
<tr>
<th>Cohort</th>
<th>6 month</th>
<th>Δ%</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below Threshold</td>
<td>51.0</td>
<td>51.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Above Threshold</td>
<td>48.2</td>
<td>52.7</td>
<td>+4.5</td>
</tr>
</tbody>
</table>

End Systolic Volume

<table>
<thead>
<tr>
<th>Cohort</th>
<th>6 month</th>
<th>Δml</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below Threshold</td>
<td>77.7</td>
<td>81.3</td>
<td>+3.6</td>
</tr>
<tr>
<td>Above Threshold</td>
<td>94.1</td>
<td>88.4</td>
<td>+5.7</td>
</tr>
</tbody>
</table>

Patients dosed at or above Threshold Dose show significant improvement in perfusion and positive trends in other tests of cardiac function.

* change in 10/15 group significant compared to 5M/Control
Phase 2 Clinical Plan

<table>
<thead>
<tr>
<th>Indication</th>
<th>Post-AMI Preservation of Cardiac Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Endpoints</td>
<td>Increased Cardiac Perfusion (RTSS) measured by SPECT, preservation of LVEF by CMR, and Safety</td>
</tr>
<tr>
<td>Other Endpoints</td>
<td>Reduction in cumulative MACE and adverse vascular events at 12 months and 18, 24 and 36 months (recurrent AMI, hospitalization for CHF, cardiac-related death and other vascular events)</td>
</tr>
<tr>
<td>Dosing Frequency</td>
<td>Single dose</td>
</tr>
<tr>
<td>Dosing and Randomization</td>
<td>Minimum dose for release ≥10 M cells Randomized 1:1 treatment to sham placebo control</td>
</tr>
<tr>
<td>Number of Subjects</td>
<td>160</td>
</tr>
<tr>
<td>Number of Sites</td>
<td>34</td>
</tr>
<tr>
<td>Geography</td>
<td>United States</td>
</tr>
<tr>
<td>Trial Duration</td>
<td>18 months (Perfusion, Cardiac Function, QOL) 24, 30, and 36 months (MACE and other vascular events)</td>
</tr>
</tbody>
</table>

Phase 2 Clinical Trial Process

1. Patient presents with chest pain + STEMI, and is assessed via Ventriculography (EF <45%)  
   Ventriculography

   Day 1

2. Patient receives stenting and usual medical Rx  
   Day 1 - 3

3. Patient screened, and enrolled in trial if Ejection Fraction (EF) ≤ 48%  
   CMR

   Day 4

4. Patient randomized into Treatment or Control

   Day 6-8

5. Patient Bone Marrow Harvested

6. CD34<sup>+</sup>CXCR4<sup>+</sup> isolated using patented technology

   Isolate

   Day 5-8

7. Intra coronary CD34<sup>+</sup>CXCR4<sup>+</sup> cell product infusion or media

   Day 6-10

8. Cardiac function measures by SPECT MPI and MRI

   + RTSS  
   + EF  
   + ESV  
   + EDV  

   6 Months

9. Major Adverse Cardiac Events

   + Mortality  
   + AMI  
   + Admission for CHF  
   + Vascular events

   12, 18, 24, 36 Months
PreSERVE AMI Trial Endpoints

- Primary Endpoint: Perfusion (SPECT–RTSS)

- Secondary Endpoint: Cardiac Function and Remodeling (CMR), Quality of Life at 6 months: MACE and other clinical vascular endpoints (reperfusion, coronary syndrome) at 6, 12, 18, 24 and 36 months

- Tertiary endpoints: Quality and Quantity of SDF-1 mobile CD34 cells infused and effect