Post-ischemic neovascularization: basic concept and new treatment options

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Index of this Lecture

• Introduction to ischemia and post-ischemic neovascularisation

• Research Models

• Therapeutic angiogenesis research: past, present and future

• microRNAs in post-ischaemic vascular regeneration

• Novel stem cell products: Empowered EPCs, Pericyte progenitor cells
Ischemic Disease

- Tissue ischaemia is caused by poor blood flow supply, which impairs the delivery of oxygen (creating hypoxia) and nutrients (creating tissue starvation)

- Ischaemic disease is favoured by atherosclerosis, diabetes, aging and a series of cardiovascular risk factors

- Ischaemic disease is one of the biggest medical epidemic worldwide and its treatment is still an unmet clinical need.
Post-Ischaemic Vascular Regeneration: the Medical Need

LIMB ISCHAEMIA

CRITICAL LIMB ISCHAEMIA

MYOCARDIAL INFARCT

ISCHAEMIC STROKE
Vascular Regeneration: what can we try?

Substituting the diseased (large) vessel

By-pass grafting with pieces of patient own vessels (vena saphena and/or mammary artery)

By-pass grafting with “artificial” vessels created in the laboratory
Therapeutic Neovascularization

SDF-1, VEGF, IL-8, ....
Strategies for Post-Izchemic Neovascularisation

• Increase the expression/activity of endogenous “pro-angiogenic” factors in ischemic tissues
• Contrast “anti-angiogenic” molecular signatures induced by pathology and/or risks factors
• Stimulate endogenous stem and progenitor cells with various pro-angiogenic capacities
• Transplantation of vascular stem and progenitor cells with pro-angiogenic capacities
In Vivo Research Models
Occlusion of LAD

Experimental models of myocardial infarction (MI) are routinely performed in Rodents.
- Permanent ligation of LAD
- Ischemia/reperfusion

Animal Models of Ischemia: MI

Mouse model of myocardial infarct
Animal Models of Ischemia: LI

Experimental models of limb ischemia (LI) is used in mice to mimic a situation comparable to peripheral artery disease. Since peripheral artery disease is often associated with diabetes, this model is also performed in diabetic mice. LI is performed by permanent ligation of one femoral artery.

Ligation of the (left) femoral artery

Mouse ventral view
Intravenous infusion of stem cells/genes:
- through the femoral vein
- through the tail vein
- through the left ventricular cavity

Limits of systemic delivery:
- Not possible to target a specific organ
- (Loss of cell number)
- (Loss of cell viability)
Local Gene/Cell Delivery

Cardiac:
1. Endomyocardial needle injection
2. Catheter delivery (large animals)
3. Intracoronary infusion (large animals/humans)
4. Intrapericardial (large animals)

Lower limb
1. Direct intramuscular injection
2. Intra-arterial infusion

Cells/Genes are directly injected into the infarcted border zone. Injection is often performed immediately after MI.

Cells/genes are directly injected into the adductor muscle often immediately after ischemia.
Transgene/Cell Tracking after Local Delivery

In vivo optical bioluminescence imaging (by using luminescence protein)

Body distribution of cells labelled with D-luciferin after intracardiac injection of different types of stem cells

- BM-derived mononuclear cells
- Skeletal myoblasts
- Mesenchymal stem cells
- Fibroblasts

Types of injected stem cells

Van der Bogt et al, Circulation 2008

Time after cell injection
New 3D bioluminescent IVIS system (Caliper)
Non Invasive Colour Laser Doppler

Blood Flow Recovery
Blood flow ratio (ischemic/contralateral foot)

Group A

Group B
Histology /IHC for vessel analyzes

**Capillary density**

![Graph showing capillary density over time and treatment conditions.](image)

**Small Arteriole density**

![Graph showing small arteriole density over time and treatment conditions.](image)

**Myocardial Blood Flow**

![Graph showing myocardial blood flow over time and treatment conditions.](image)
Absolute Blood Flow Measurement in Tissues

Artificial Respiration

Reference Blood Flow Collection (170 μl/min)

Microsphere injection in LV.
After 2 minutes, collect tissues

BF (mL/min/gr) = \frac{R \times (fl \text{ tissues/ fl ref blood})}{\text{gr of tissue}}

R = rate (mL/min) of ref blood withdrawal

Meloni et al. Circ Res, 2010
Mouse Echocardiography

**Diagram A**
- IVS: Interventricular Septum
- AV: Aortic Valve
- Ao: Aorta
- LV: Left Ventricle
- AMVL: Anterior Mitral Valve Leaflet
- PMVL: Posterior Mitral Valve Leaflet
- LA: Left Atrium
- Papillary muscle
- Chordae

**Parasternal long-axis view**

**Diagram B**

Source: JACC © 2010 American College of Cardiology Foundation
Randomization will be based on baseline LVEF by MRI.
Spontaneous post-ischemic neovascularization process
Aging impairs post-ischemic collateral formation and healing in limbs

Experimental limb ischemia

Rivard & Isner, Circulation, 1999
Hypercholesterolemia attenuates post-ischemic angiogenesis in limbs

Van Belle, & Isner
Circulation 1997
Diabetes impairs post-ischemic angiogenesis and blood flow recovery

Rivard & Isner, Am J Pathol, 1999
High blood pressure impairs post-ischemic angiogenesis and blood flow recovery in rats.

**Capillary Density**

- No-ischemia
- Ischemia

**Foot Blood Flow Recovery**

- WKY
- SHR

Emanueli & Madeddu. Hypertension 2001
Ischemic patients have at least one of these aforementioned conditions and often all of them plus additional risk factors (obesity, “wrong” genetic and epigenetic backgrounds, smoke, etc) to prevent spontaneous healing. They need some help to fight ischaemia!
Therapeutic Neovascularisation of Cardiac and Peripheral Ischaemia

First Strategic Mind to use Angiogenesis as a Therapy

- **First attempt**
  To increase the expression of the prototypical pro-angiogenic factor VEGF-A in ischaemic tissues using a supply-side approach, mainly by *gene therapy via* plasmids and first generation adenoviruses

- **Second attempt**
  Transplantation of *endothelial progenitor cells (EPCs)* in ischaemic tissues. Done in collaboration with Prof Asahara then a fellow of Isner. This approach was then *clinically translated* by others (mainly in Frankfurt and Japan)
“Classical” pro-angiogenic factors in mono-therapies

FGFR  PDGFR  VEGFR
Vascular stabilisation vs vascular regression

Fig. 2

Blood vessel

Combination
FGF-2 + PDGF-B

Single factor
FGF-2, PDGF-B or VEGF

Angiogenic Vessel

Withdrawal of stimulus

Maturation and stability
Stable vessel

Regression

Withdrawal of stimulus
Effect of fibroblast growth factor NV1FGF on amputation and death: a randomised placebo-controlled trial of gene therapy in critical limb ischaemia

Jill Belch, William R Hiatt, Iris Baumgartner, I Vickie Driver, Sigrid Nikol, Lars Norgren, Eric Van Belle, on behalf of the TAMARIS Committees and Investigators

Summary
Background Patients with critical limb ischaemia have a high rate of amputation and mortality. We tested the hypothesis that non-viral 1 fibroblast growth factor (NV1FGF) would improve amputation-free survival.

Methods In this phase 3 trial (EFC6145/TAMARIS), 525 patients with critical limb ischaemia unsuitable for revascularisation were enrolled from 171 sites in 30 countries. All had ischaemic ulcer in legs or minor skin gangrene and met haemodynamic criteria (ankle pressure <70 mm Hg or a toe pressure <50 mm Hg, or both, or a transcutaneous oxygen pressure <30 mm Hg on the treated leg). Patients were randomly assigned to either NV1FGF at 0.2 mg/mL or matching placebo (visually identical) in a 1:1 ratio. Randomisation was done with a central interactive voice response system by block size 4 and was stratified by diabetes status and country. Investigators, patients, and study teams were masked to treatment. Patients received eight intramuscular injections of their assigned treatment in the index leg on days 1, 15, 29, and 43. The primary endpoint was time to major amputation or death at 1 year analysed by intention to treat with a log-rank test using a multivariate Cox proportional hazard model. This trial is registered with ClinicalTrials.gov, number NCT00566657.

Findings 259 patients were assigned to NV1FGF and 266 to placebo. All 525 patients were analysed. The mean age was 70 years (range 50–92), 365 (70%) were men, 280 (53%) had diabetes, and 248 (47%) had a history of coronary artery disease. The primary endpoint or components of the primary did not differ between treatment groups, with major amputation or death in 86 patients (33%) in the placebo group, and 96 (36%) in the active group (hazard ratio 1.11, 95% CI 0.83–1.49; p=0.48). No significant safety issues were recorded.

Interpretation TAMARIS provided no evidence that NV1FGF is effective in reduction of amputation or death in patients with critical limb ischaemia. Thus, this group of patients remains a major therapeutic challenge for the clinician.
Options for Improving Traditional Post-Ischaemic Neovascularisation Therapeutics

• More basic science to understand all the players in the angiogenesis and anti-angiogenesis orchestras
• Test different angiogenesis modulator factors (growth factors and more)
• Combinatory approaches
• More severity in preclinical testing (large animal models, risk factors associated to experimental ischemia, etc)
• Work at gene vectors
• Success in *in vivo* small vessel imaging
• Design better clinical trials

• Do not give up!
microRNAs (miRs)

Thomas Thum
Break-out session
Tuesday 2PM
Dicer generates short interfering RNAs (including miRNAs) from longer double-stranded RNAs.

Mice homozygous for a hypomorphic allele of Dicer showed impaired developmental angiogenesis and died at days 12.5 - 14.5 of gestation.


First evidence for a regulatory role of miRs in vascular development

Dicer generates short interfering RNAs (including miRNAs) from longer double-stranded RNAs.
Spontaneous post-ischemic neovascularization process

Bone marrow-derived endothelial progenitor cells (EPCs)/proangiogenic circulating cells (PACs)

Scientific Questions:
Do miRs modulate it? What miRs? At what levels?
Therapeutic exploitation of miRs?
miR expression is regulated by hypoxia and ischemia

Hypoxia

Table 1: Compilation of microRNAs associated with the hypoxia response by recent publications

<table>
<thead>
<tr>
<th>MicroRNAs upregulated by hypoxia</th>
<th>MicroRNAs downregulated by hypoxia</th>
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<tbody>
<tr>
<td>Mir-7</td>
<td>Mir-15a</td>
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<td>Mir-21</td>
<td>Mir-16</td>
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<tr>
<td>mir-23a</td>
<td>Mir-15b</td>
</tr>
<tr>
<td>Mir-24</td>
<td>Mir-17</td>
</tr>
<tr>
<td>Mir-26a</td>
<td>Mir-20a</td>
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<tr>
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<tr>
<td>Mir-28b</td>
<td>Mir-30a</td>
</tr>
<tr>
<td>Mir-28c</td>
<td>Mir-30b</td>
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<tr>
<td>Mir-30a</td>
<td>Mir-40a</td>
</tr>
<tr>
<td>Mir-30b</td>
<td>Mir-101</td>
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<tr>
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<td>Mir-108</td>
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<td>Mir-120a</td>
<td>Mir-109</td>
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<td>Let-7-g</td>
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<td>Let-7-i</td>
<td>Mir-145</td>
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Table 2: Top 15 Downregulated MicroRNAs

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<tr>
<th>miRNA</th>
<th>FC change</th>
<th>Expression Baseline</th>
<th>6 h</th>
<th>24 h</th>
<th>3 d</th>
<th>7 d</th>
<th>14 d</th>
<th>Function</th>
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<tr>
<td>Mmu-miR-223</td>
<td>-7.79</td>
<td>1392</td>
<td>3606</td>
<td>5208</td>
<td>10841</td>
<td>2663</td>
<td>2143</td>
<td>Hematopoietic lineage differentiation</td>
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<tr>
<td>Mmu-miR-342</td>
<td>5.49</td>
<td>1608</td>
<td>1728</td>
<td>2112</td>
<td>8825</td>
<td>2954</td>
<td>2463</td>
<td>Unknown</td>
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<tr>
<td>Mmu-miR-720</td>
<td>5.07</td>
<td>868</td>
<td>1237</td>
<td>1338</td>
<td>4405</td>
<td>895</td>
<td>1410</td>
<td>Unknown</td>
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<tr>
<td>Mmu-miR-21</td>
<td>3.54</td>
<td>16 240</td>
<td>17365</td>
<td>23 199</td>
<td>57 514</td>
<td>29 067</td>
<td>31 587</td>
<td>VSMC proliferation</td>
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<tr>
<td>Mmu-miR-26a</td>
<td>2.99</td>
<td>1440</td>
<td>1106</td>
<td>1442</td>
<td>4299</td>
<td>1231</td>
<td>1646</td>
<td>Part of the miR-17-92 cluster</td>
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<tr>
<td>Mmu-miR-211</td>
<td>2.89</td>
<td>954</td>
<td>665</td>
<td>995</td>
<td>2757</td>
<td>1281</td>
<td>1837</td>
<td>Inhibits angiogenesis</td>
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<tr>
<td>Mmu-miR-158</td>
<td>2.79</td>
<td>3127</td>
<td>5258</td>
<td>6446</td>
<td>8738</td>
<td>4786</td>
<td>3933</td>
<td>Potential inhibitor of VEGF</td>
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<td>Mmu-miR-172</td>
<td>2.46</td>
<td>1890</td>
<td>15718</td>
<td>3582</td>
<td>6468</td>
<td>4572</td>
<td>2578</td>
<td>Potential inhibitor of VEGF</td>
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<td>Mmu-miR-17-5p</td>
<td>2.33</td>
<td>905</td>
<td>5875</td>
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<td>700</td>
<td>908</td>
<td>3244</td>
<td>Potential inhibitor of VEGF</td>
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<td>Mmu-miR-214</td>
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<td>3034</td>
<td>3047</td>
<td>4231</td>
<td>7219</td>
<td>5277</td>
<td>5974</td>
<td>Potential inhibitor of VEGF</td>
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<td>Mmu-miR-181a</td>
<td>1.57</td>
<td>3195</td>
<td>3113</td>
<td>3277</td>
<td>5870</td>
<td>4006</td>
<td>3040</td>
<td>Regulates myoblast differentiation</td>
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<tr>
<td>Mmu-miR-92</td>
<td>1.79</td>
<td>3914</td>
<td>3544</td>
<td>4567</td>
<td>700</td>
<td>3821</td>
<td>2729</td>
<td>Strong inhibitor of angiogenesis</td>
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<tr>
<td>Mmu-miR-26a</td>
<td>1.50</td>
<td>3497</td>
<td>5093</td>
<td>5232</td>
<td>3773</td>
<td>3540</td>
<td>Induced by VEGF in endothelial cells</td>
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<tr>
<td>Mmu-miR-25</td>
<td>1.40</td>
<td>16 240</td>
<td>15658</td>
<td>20 359</td>
<td>35 051</td>
<td>21 583</td>
<td>25 448</td>
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<tr>
<td>Mmu-miR-191</td>
<td>1.46</td>
<td>10 000</td>
<td>11 265</td>
<td>15 030</td>
<td>11 026</td>
<td>10 404</td>
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</table>

Kulshreshtha & Ivan, CDD, 2008.

Mouse limb ischaemia model

Grundmann & Moser, Circ , 2011

Study cited, and corresponding cell types and conditions: (1) Kulshreshtha et
miR-92a: first example of miR targeting for post-ischemic angiogenesis

Systemic miR-92a inhibition stimulates recovery after hind limb ischaemia

Blood flow
Microvessel density

Day 0, 2, 4, 7, and 9
Antagomir 92a or Co

Bonauer & Dimmeler, Science, 2009

Slide kindly provided by Stefanie Dimmeler
Example for miR targeting and contrasting anti-angiogenic factors
miR-503: how we joined the miR club!

Deregulation of microRNA-503 Contributes to Diabetes Mellitus–Induced Impairment of Endothelial Function and Reparative Angiogenesis After Limb Ischemia

Andrea Caporali, PhD; Marco Meloni, PhD; Christine Völlenkle, PhD; Desiree Bonci, PhD; Graciela B. Sala-Newby, PhD; Roberta Addis, BSc; Gaia Spinetti, PhD; Sergio Losa, MD; Rachel Masson, PhD; Andrew H. Baker, PhD; Reuven Agami, PhD; Carlos le Sage, PhD; Gianluigi Condorelli, MD, PhD; Paolo Madeeddu, MD; Fabio Martelli, PhD; Costanza Emanuel, PhD
Member of miR-15/107 group: AGCAGC seed sequence

Examination of the mir-424 and mir-503 loci showed that they are separated by 383 bases on the genome and derived from the same primary transcript.

Finnerty JR et al. JMB 2010
miR-503 expression in ECs is increased by culture conditions mimicking hyperglycaemia and ischemia.

**miR-503 expression in HUVECs**

- **NG** = normal glucose, 5 mM
- **HG** = high glucose, 25 mM
- **LGF** = reduced growth factors

* = statistically significant difference.
Consequences of miR-503 overexpression in endothelial cells

Impaired EC cycle

% cells gated

G0/G1
S
G2/M

GFP
miR503

Impaired EC migration

GFP
miR503

Impaired EC network formation

GFP
miR503

miR-503 target genes

675-882 cdc25A 3'UTR
5'...CGCUGUGGUAACUGGGGCUGCUUGCUAU...

has-miR-503
3'GACGUCUUGACAAGGGCGACGAU

248-254 CCNE1 3'UTR
5'...GUGCGUGCUCCCGAUGUCUGCUAU...

has-miR-503
3'GACGUCUUGACAAGGGCGACGAU

486-492 CCNE1 3'UTR
5'...AACUGUUUUUGUAAGUCUGCUAU...

has-miR-503
3'GACGUCUUGACAAGGGCGACGAU
Local gene therapy with a decoy for miR-503 improves post-ischaemic angiogenesis in diabetic mice

### miR-503 expression

<table>
<thead>
<tr>
<th>Condition</th>
<th>miR-503 Expression</th>
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<tbody>
<tr>
<td>Non diabetic, No Isch</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Non diabetic, Isch</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Diabetic, No Isch</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Diabetic, Isch</td>
<td>1.5 ± 0.5</td>
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### CD146pos ECs

<table>
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<th>Condition</th>
<th>CD146pos ECs</th>
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<tbody>
<tr>
<td>Isch Non Diab</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Isch Diab</td>
<td>3.0 ± 0.2</td>
</tr>
</tbody>
</table>

### Limb microvessels

- Non Diab Isch + Null
- Diab Isch + Null
- Diab Isch + decoy503

### Blood flow recovery

- Null
- decoy503

<table>
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<tr>
<th>Time post-ischemia</th>
<th>Foot BF Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>Non Diab Isch + Null</td>
</tr>
<tr>
<td>7d</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>14d</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>21d</td>
<td>0.6 ± 0.3</td>
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*Significant differences: *p < 0.05, **p < 0.01, ***p < 0.001.
New cell therapy products
**Human circulating pro-angiogenic cells: PACs**

**PACs**

**Isolation and culture**

- HISTOPOAQUE MNC SEPARATION
- ADHERENCE ENRICHMENT ON FIBRONECTIN
- 4 DAYS CULTURE IN EC MEDIUM

**Phenotypic characterization**

- **UEAI**
  - 98%
- **AcLDL**
  - 99%
- **Negative control**
- **Specific marker**

**Fluorescence intensity**

- **CD34**
  - 11±2%
- **CXCR4**
  - 68±8%
- **KDR**
  - 59±7%
- **CD45**
  - 82±5%
- **CD14**
  - 88±8%
- **CD11b**
  - 90±6%

**Plasma MNCs**

**Adherence enrichment on fibronectin**

**Floating adherent cells**

**Histopaque MNC separation**

**Erythrocytes**

**Phenotypic characterization with flow cytometry**

**4 Days culture in EC medium**

**Negative control**

**Specific marker**

**Fluorescence intensity**

**CD45**

**KDR**

**AcLDL**

**UEAI**

**P-eNOS**

**FBS**

**p-eNOS/eNOS**

**β-act**
MicroRNA-15a and MicroRNA-16 Impair Human Circulating Proangiogenic Cell Functions and Are Increased in the Proangiogenic Cells and Serum of Patients With Critical Limb Ischemia

Gaia Spinetti, Orazio Fortunato, Andrea Caporali, Saran Shantikumar, Micol Marchetti, Marco Meloni, Betty Descamps, Ilaria Floris, Elena Sangalli, Rosa Vono, Ezio Faglia, Claudia Specchia, Gianfranco Pintus, Paolo Madeddu, Costanza Emanueli
miR screening in blood PACs of CLI Patients with/out Diabetes

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**PAC miR-15a**

<table>
<thead>
<tr>
<th></th>
<th>Relative Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.01</td>
</tr>
<tr>
<td>CLI</td>
<td>0.02</td>
</tr>
<tr>
<td>T2D+CLI</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**PAC miR-16**

<table>
<thead>
<tr>
<th></th>
<th>Relative Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.00</td>
</tr>
<tr>
<td>CLI</td>
<td>0.20</td>
</tr>
<tr>
<td>T2D+CLI</td>
<td>0.30</td>
</tr>
</tbody>
</table>

---

Table showing relative concentrations of various miRs.
PAC miR-15a and -16 expression can be manipulated ex vivo

**Controls PACs**

- **miR-15a**
  - SCR: [Data Point]
  - pre-miR-15a: [Data Point]
  - pre-miR-16: [Data Point]
  - pre-miR-15a/16: [Data Point]

- **miR-16**
  - SCR: [Data Point]
  - pre-miR-15a: [Data Point]
  - pre-miR-16: [Data Point]
  - pre-miR-15a/16: [Data Point]

**T2D+CLI PACs**

- **miR-15a**
  - SCR: [Data Point]
  - anti-miR-15a: [Data Point]
  - anti-miR-16: [Data Point]
  - anti-miR-15a/16: [Data Point]

- **miR-16**
  - SCR: [Data Point]
  - anti-miR-15a: [Data Point]
  - anti-miR-16: [Data Point]
  - anti-miR-15a/16: [Data Point]

**Legend**

- Pre-miR
- Anti-miR

*Significant differences
miR-15a and -16 overexpression increases apoptosis of “healthy” PACs
miR-15a and -16 inhibition improves survival of patient-derived PACs
Overexpressing miR-15a and miR-16 inhibits the migratory capacity of healthy PACs.
Inhibition of miR-15a together with miR-16 improves the migratory capacity of diseased PACs
miR-15a and miR-16 target VEGF-A and AKT-3

3’UTR luciferase activity assays
Ex-vivo miR-15a and miR-16 inhibition in PACs increases their regenerative potential:

Foot Blood flow....

Medium

PACs+SCR

PACs+pre-miR15a/16

PACs+anti-miR15a/16

Blood flow ratio [ischemic/contralateral foot]

30 min  day 7  day 14
.... Microvascular Density
Blood circulating miR-15a and -16

Online Table III. Age, gender and clinical characteristics of type-2 diabetic patients at the moment they underwent angioplasty for critical limb ischemia

<table>
<thead>
<tr>
<th>Factor</th>
<th>T2D+CLI (n=122)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.2 (9.3)</td>
</tr>
<tr>
<td>Gender (% males)</td>
<td>67</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>48</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>65</td>
</tr>
<tr>
<td>Neuropathy (%)</td>
<td>21</td>
</tr>
<tr>
<td>Retinopathy (%)</td>
<td>20</td>
</tr>
<tr>
<td>Ictus (%)</td>
<td>12</td>
</tr>
<tr>
<td>Active smoker (%)</td>
<td>13</td>
</tr>
<tr>
<td>HbA1c (%Hb)</td>
<td>7.8±1.5</td>
</tr>
<tr>
<td>Oral anti-diabetic drugs (%)</td>
<td>36</td>
</tr>
<tr>
<td>Insulin therapy (%)</td>
<td>64</td>
</tr>
<tr>
<td>Diet (%)</td>
<td>22</td>
</tr>
<tr>
<td>Aspirin therapy (%)</td>
<td>67</td>
</tr>
<tr>
<td>Clopidogrel therapy (%)</td>
<td>7</td>
</tr>
<tr>
<td>Anticoagulant therapy (%)</td>
<td>20</td>
</tr>
<tr>
<td>Statin therapy (%)</td>
<td>41</td>
</tr>
</tbody>
</table>

Quantitative data are expressed as mean and standard deviation (SD).
T2D = type 2 diabetes, CLI = critical limb ischemia, CAD = coronary artery disease.
Association between serum miR-15 or miR-16 at revascularization and restenosis/amputation at follow up (1 year)

### Online Table IV. Incidence of adverse events at one year follow up after angioplasty in type 2 diabetic patients described in Online Table IV (Total patients: N=122).

<table>
<thead>
<tr>
<th>Event</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any event</td>
<td>61 (50%)</td>
</tr>
<tr>
<td>Death (only)</td>
<td>17</td>
</tr>
<tr>
<td>Restenosis (only)</td>
<td>20</td>
</tr>
<tr>
<td>Amputation (only)</td>
<td>2</td>
</tr>
<tr>
<td>Restenosis and death</td>
<td>8</td>
</tr>
<tr>
<td>Restenosis and amputation</td>
<td>13</td>
</tr>
<tr>
<td>Restenosis and amputation and death</td>
<td>1</td>
</tr>
<tr>
<td>No event</td>
<td>61 (50%)</td>
</tr>
</tbody>
</table>

### Online Table V. Association between miR expression and adverse events (restenosis and amputation)

<table>
<thead>
<tr>
<th>Event</th>
<th>miR (2-ddCT)</th>
<th>OR*</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restenosis (first event)</td>
<td>circulating miR-15a</td>
<td>1.28</td>
<td>1.01-1.61</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>circulating miR-16</td>
<td>0.96</td>
<td>0.73-1.26</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>PAC miR-15a</td>
<td>1.26</td>
<td>0.72-2.19</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>PAC miR-16</td>
<td>0.79</td>
<td>0.51-1.23</td>
<td>0.30</td>
</tr>
<tr>
<td>Restenosis plus amputation</td>
<td>circulating miR-15a</td>
<td>2.10</td>
<td>1.32-3.36</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>circulating miR-16</td>
<td>2.07</td>
<td>1.17-3.63</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>PAC miR-15a</td>
<td>1.74</td>
<td>0.70-4.30</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>PAC miR-16</td>
<td>0.70</td>
<td>0.35-1.41</td>
<td>0.315</td>
</tr>
</tbody>
</table>

*for 1 unit increase in log2
miRNA Summary and Perspective

• miRs modulate the post-ischemic neovascularization responses at multiple levels

• miR therapeutic can directly target ischemic tissues in vivo and be used for ex-vivo enhancement of the proangiogenic capacities of stem and progenitor cells

• Circulating miRs might be novel predictive and pognostic markers in ischemic patients

• There is a huge potential for miR translational research
Bristol Vena Saphena derived-Pericyte Progenitor Cells (SVPs)

Paolo Madeddu-Wednesday AM
Pericyte coverage of is essential for microvessel maturation and stabilisation, including during post-ischemic vascular repair.
The saphenous vein: a convenient source of pericyte progenitor cells

Campagnolo et al, Circulation, 2010
Identification of pericytes in human saphenous vein vasa vasorum

Tunica media
Adventitia

Vasa vasorum

CD34 vWF DAPI

CD34 vWF

CD34

CD31

NG2

DAPI

CD34

CD31

NG2

DAPI

CD34 vWF

CD34

CD31

PDGF-R β

DAPI
Conclusions

- Myocardial and limb ischaemia are still unmet medical needs

- Novel options and revisited approaches hold therapeutic potential to treat limb and myocardial ischaemia

- This area of research can deliver disappointments as well as huge satisfaction

- Basic science and translational approaches must progress together to the ultimate clinical goal.
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Saran Shantikumar
Ilaria Floris
Micol Marchetti
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Andrea Caporali
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Frankfurt
Stefanie Dimmeler
Carlo Gaetano

Yale
Bill Sessa
Carlos Fernandez

Umass
Nathan Lawson

Madrid
Integromics

Vienna
Klemens Verlienger
We are currently recruiting and always happy to host visiting PhD students and sponsor excellent candidates for fellowship applications.

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