University of BRISTOL

Post-ischaemic neovascularisation:

basic concept and new treatment options

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Chair of Vascular Pathology and Regeneration

ESC Summer School, Nice-13 June 2011

Index of this Lecture

- Introduction to ischemia and post-ischemic neovascularisation
- Therapeutic angiogenesis: past, present and future
- Novel pro-angiogenic growth factors
- microRNAs in post-ischaemic angiogensis
- Novel stem cell products: ESC-derived “very young” endothelial cells

Ischaemic Disease

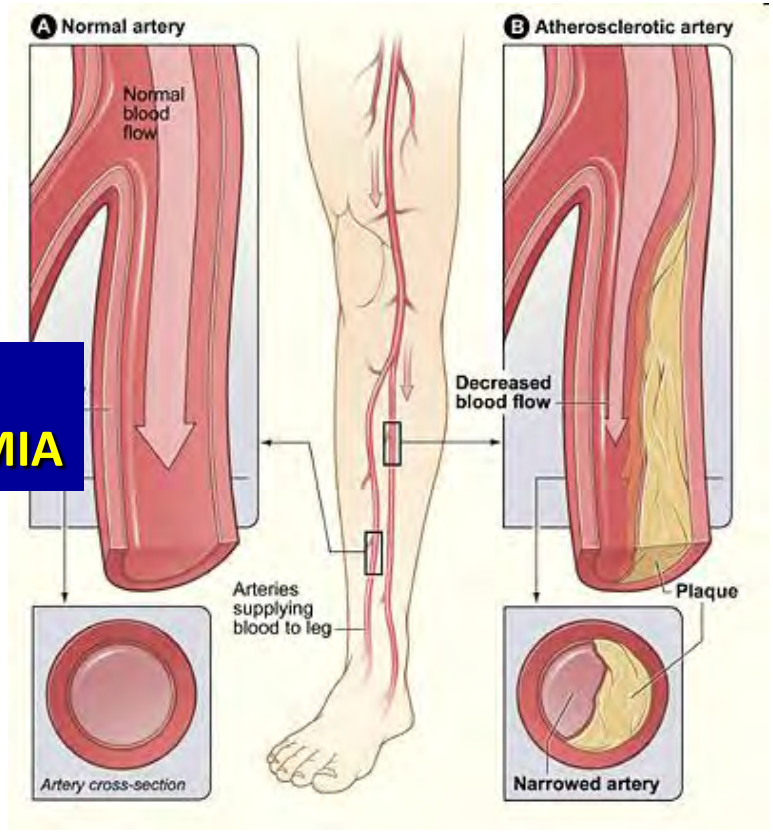
- **Tissue ischaemia is caused by poor blood flow supply, which impairs the delivery of oxygen (creating hypoxia) and nutrients (creating tissue starvation)**

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

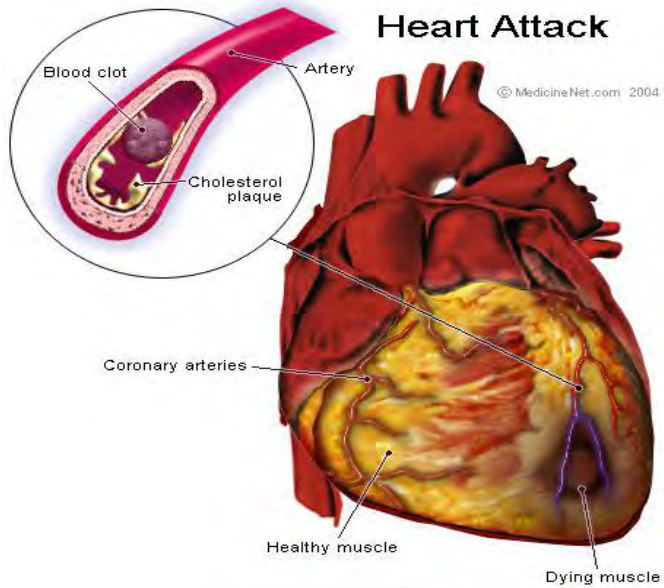
- **Ischaemic disease is one of the biggest medical epidemic worldwide and its treatment is still an umet clinical need.**

Post-Ischaemic Vascular Regeneration: the Medical Need

LIMB ISCHAEMIA

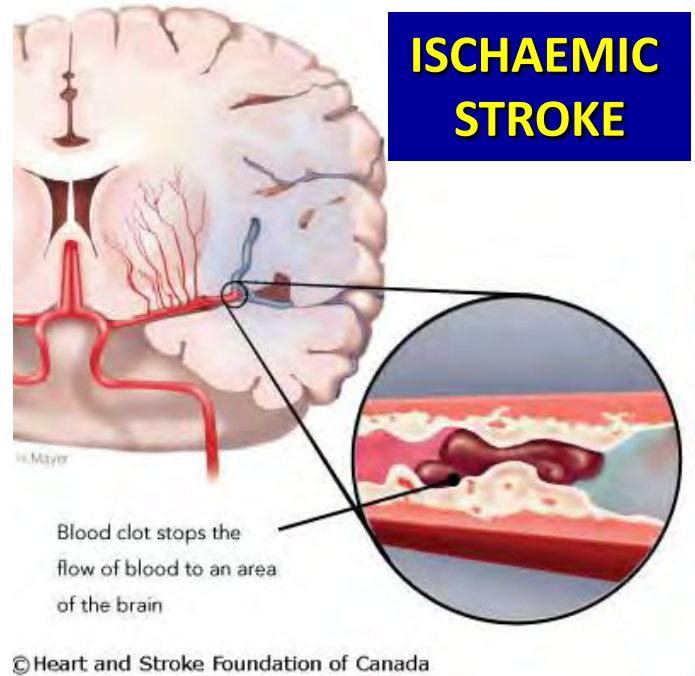


MYOCARDIAL INFARCT

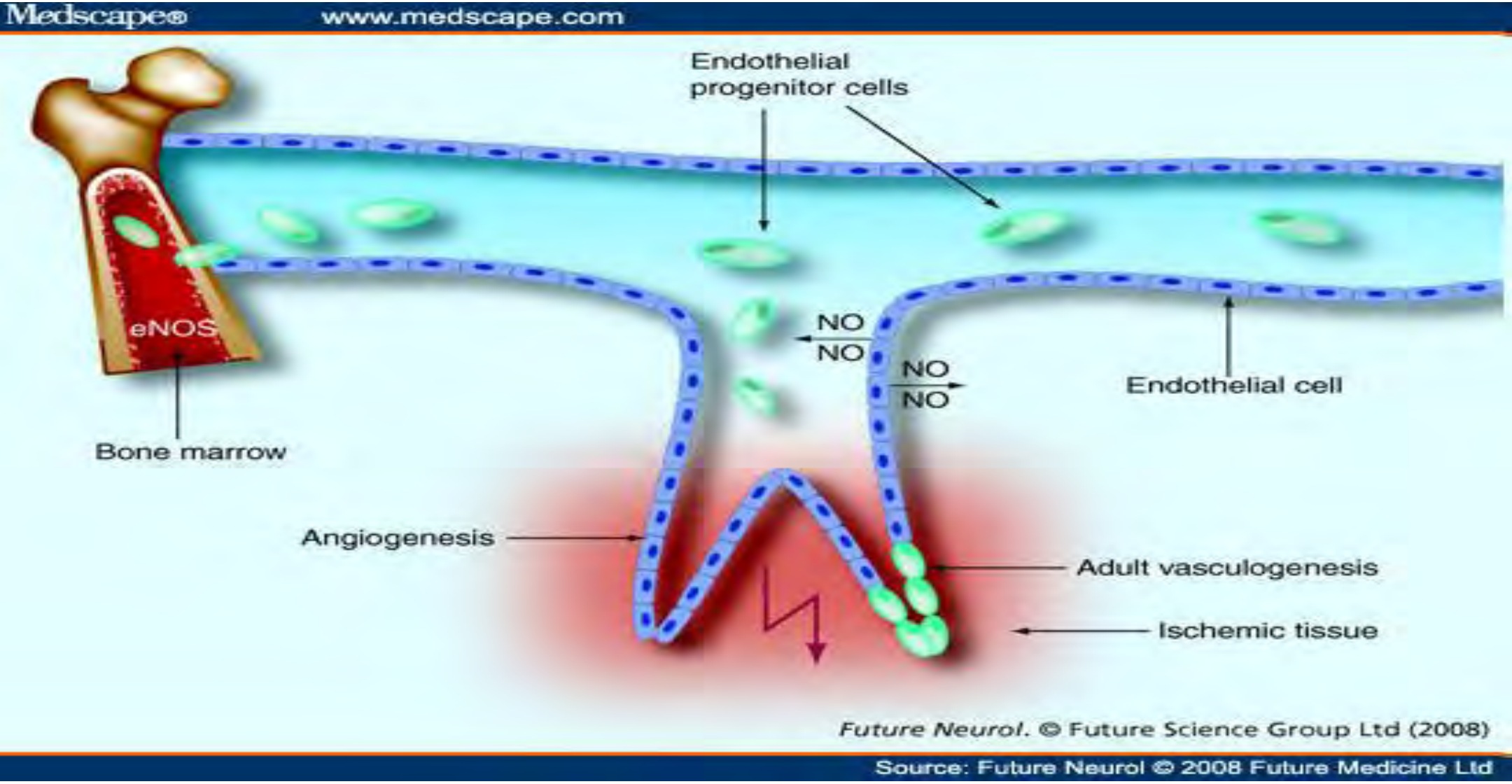


CRITICAL LIMB ISCHAEMIA

ISCHAEMIC STROKE

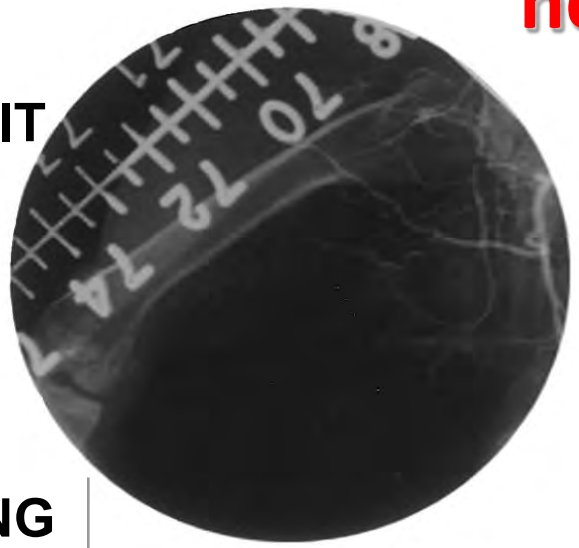


Spontaneous post-ischaemic neovascularisation process

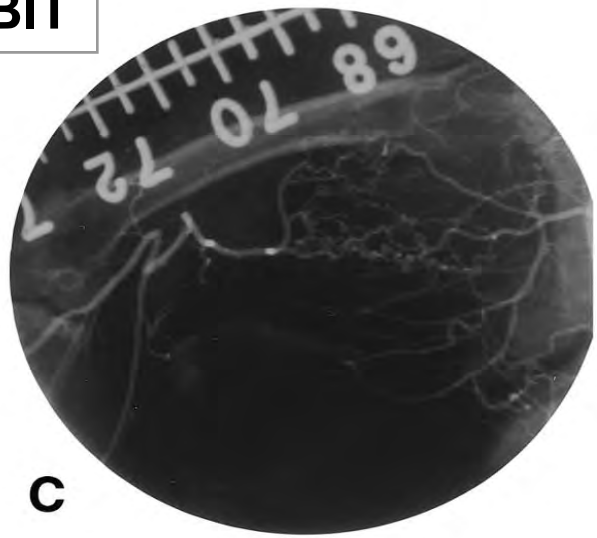


Aging impairs post-ischaemic collateral formation and healing in limbs

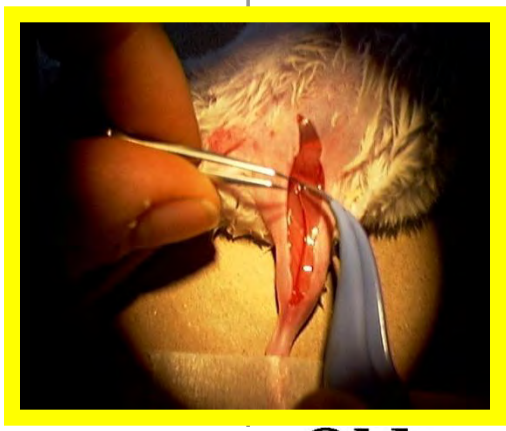
OLD RABBIT



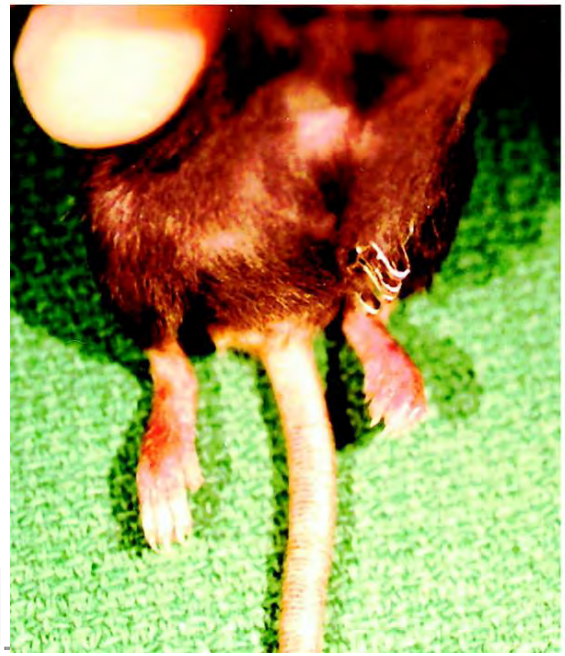
YOUNG RABBIT



Experimental limb ischemia



Young



Old

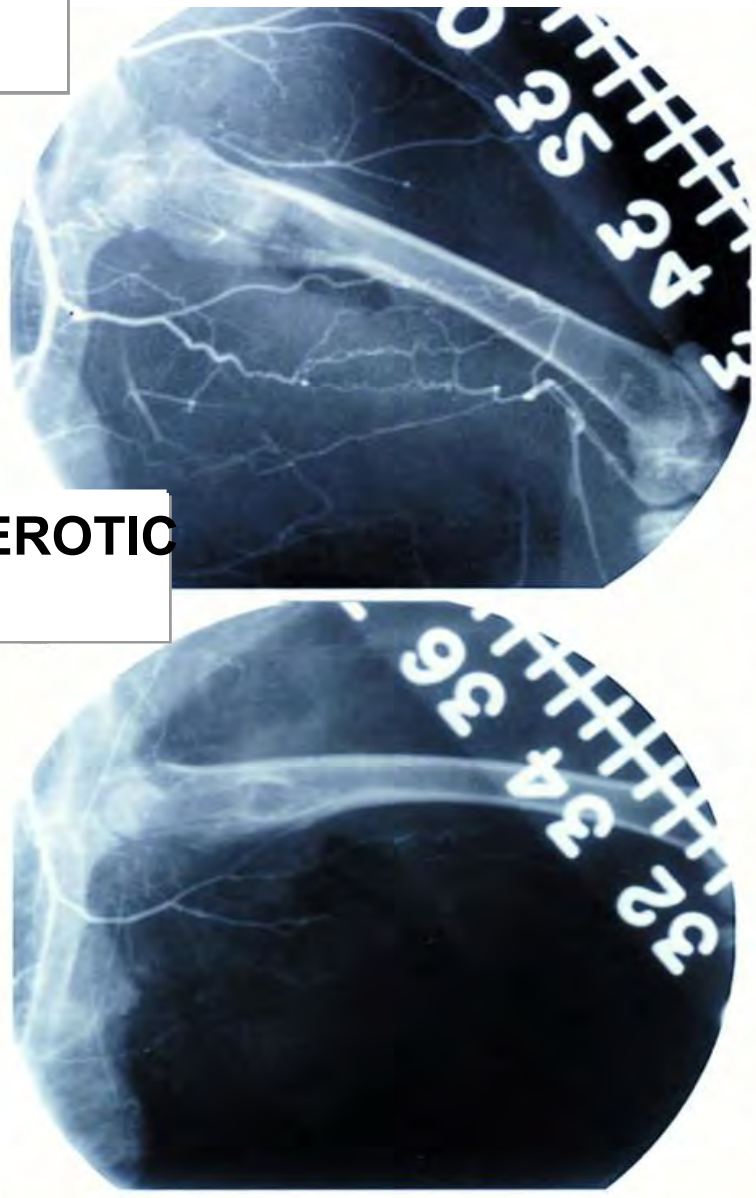
MICE

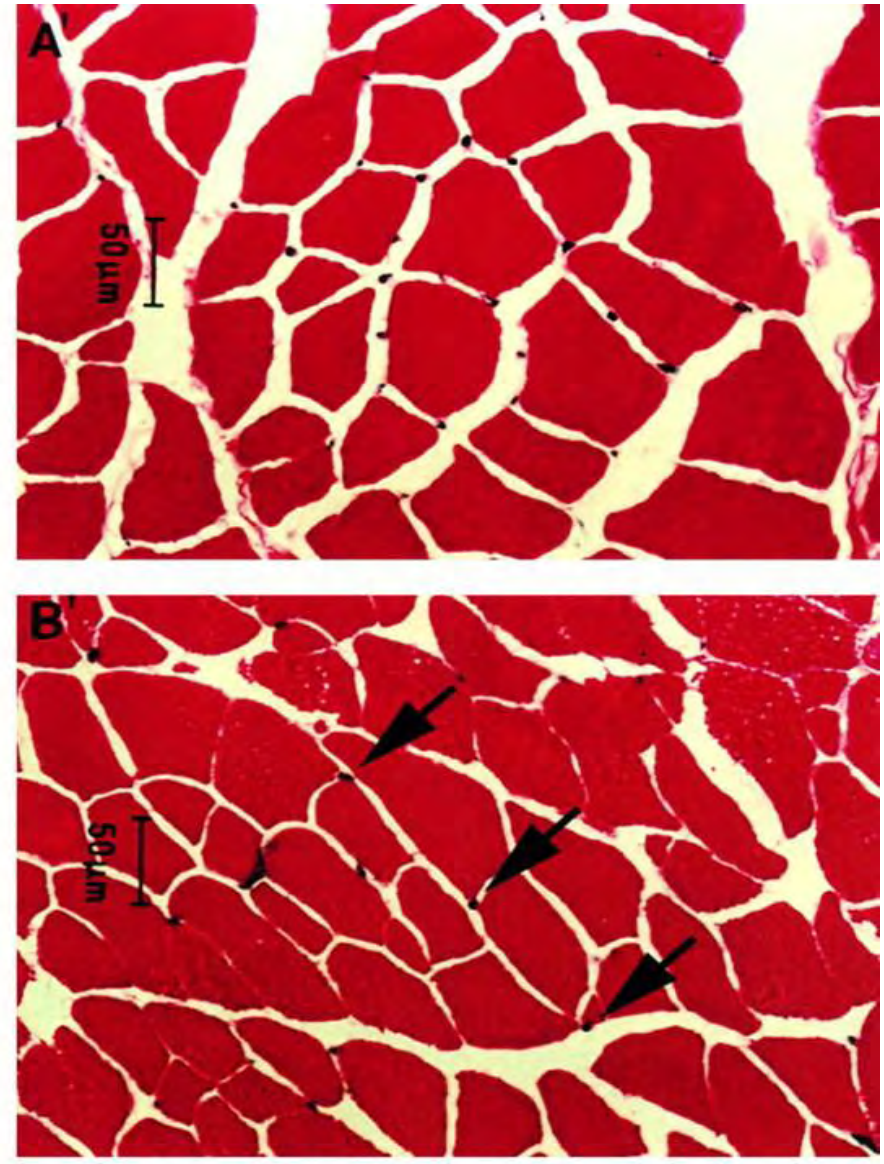
Rivard & Isner, Circulation, 1999

Hypercholesterolemia attenuates post-ischaemic angiogenesis in limbs

NORMAL RABBIT

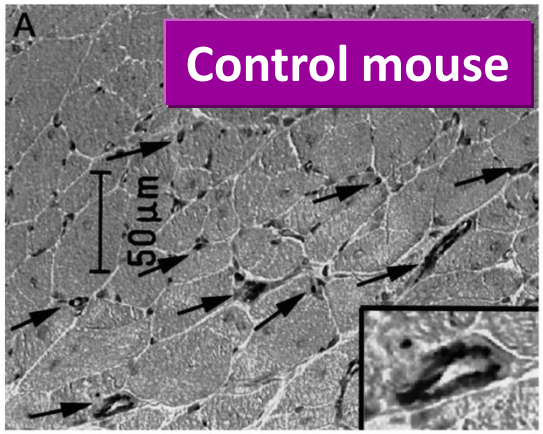
ATHEROSCLEROTIC RABBIT

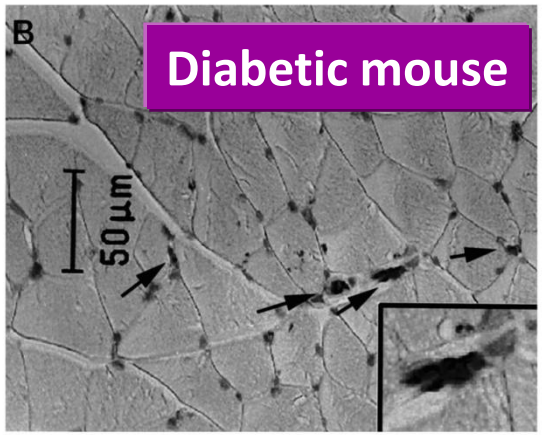


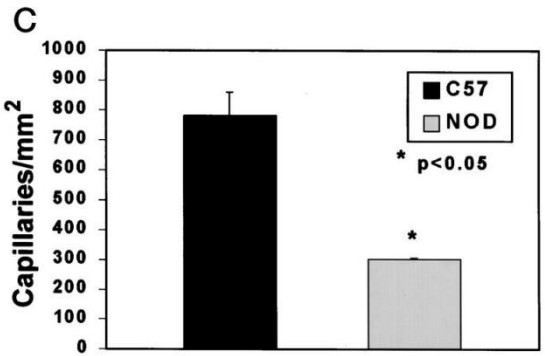


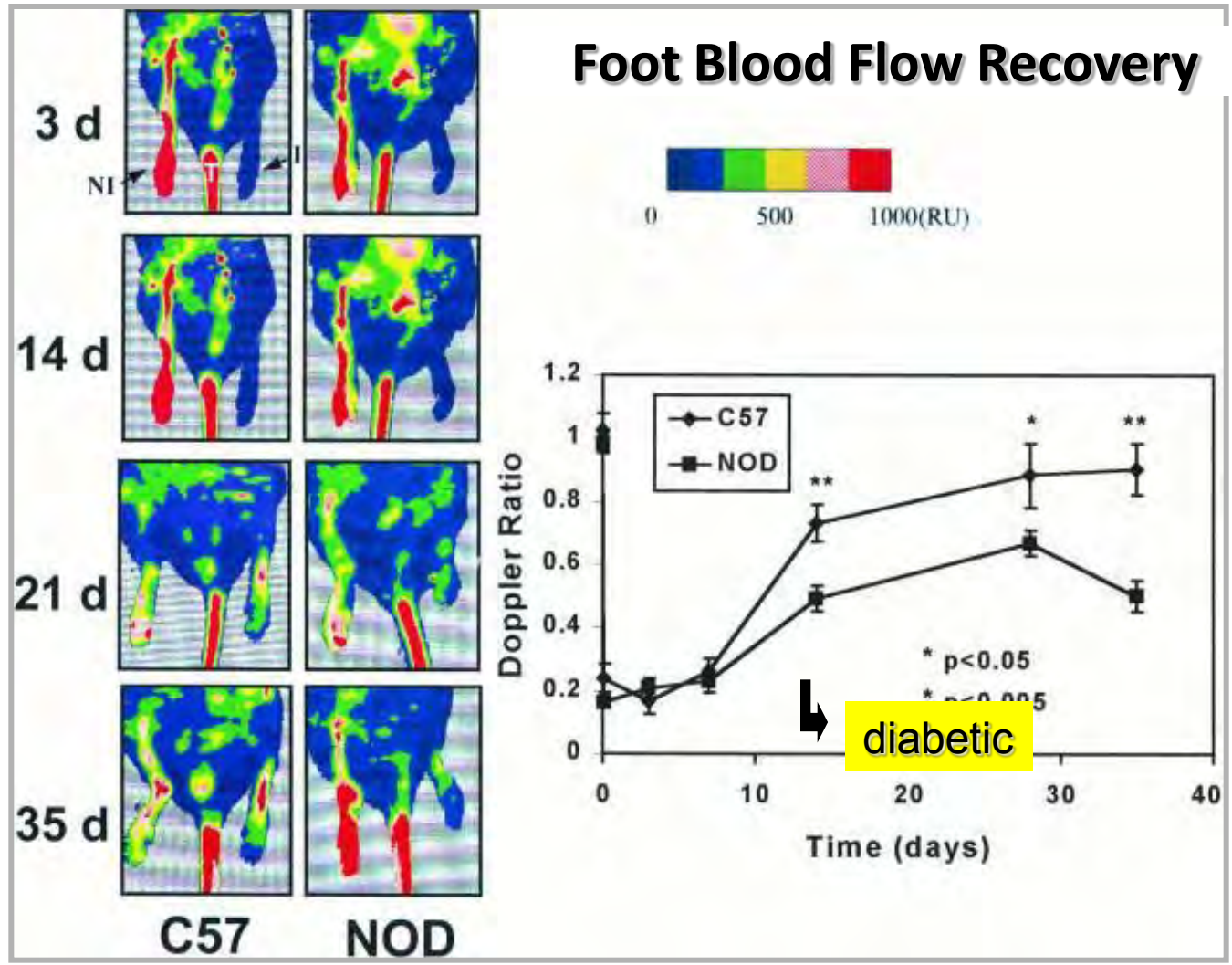
Van Belle, & Isner
Circulation 1997

Diabetes impairs post-ischaemic angiogenesis and blood flow recovery





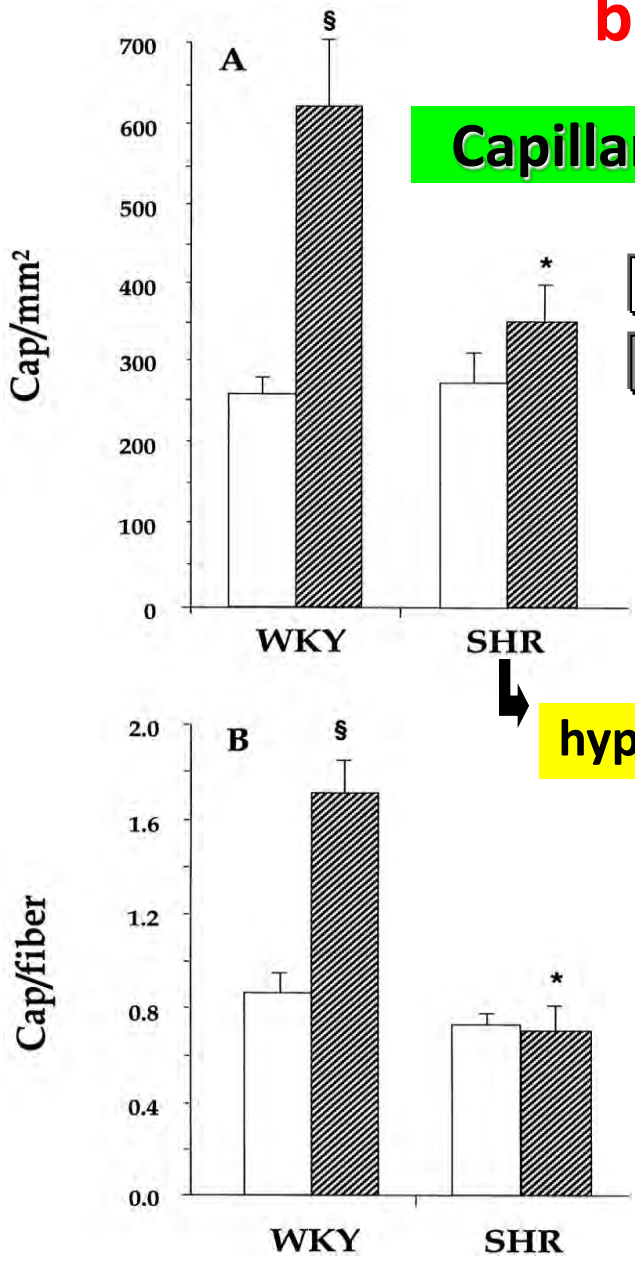




diabetic

Rivard & Isner, Am J Pathol, 1999

High blood pressure impairs post-ischaemic angiogenesis and blood flow recovery in rats

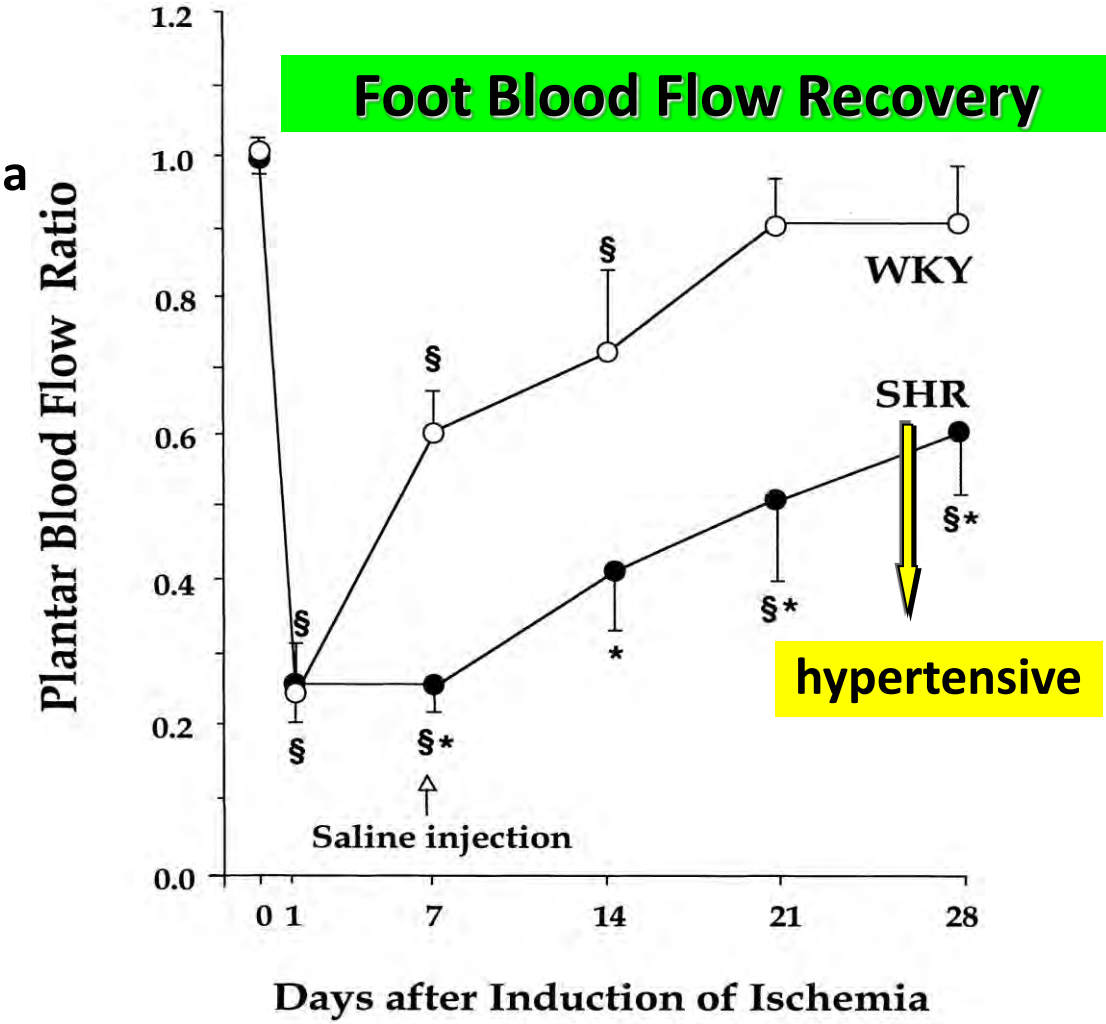


Capillary Density

No-ischemia

Ischemia

hypertensive



Foot Blood Flow Recovery

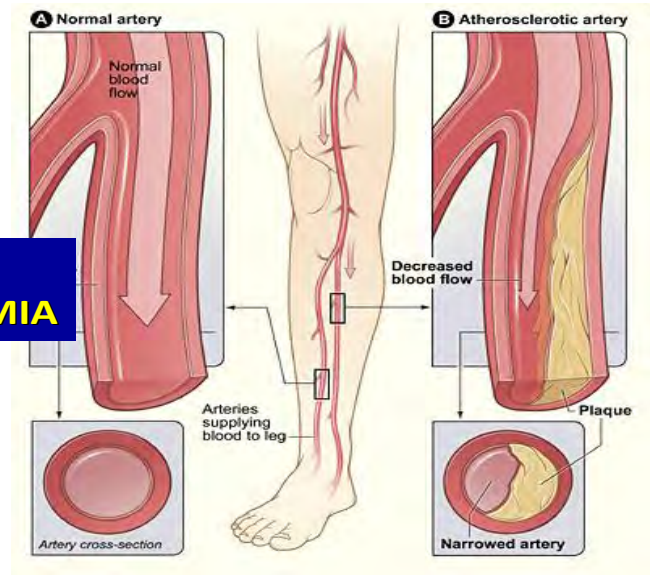
hypertensive

Emanueli & Madeddu. Hypertension 2001

Ischaemic 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!

LIMB ISCHAEMIA



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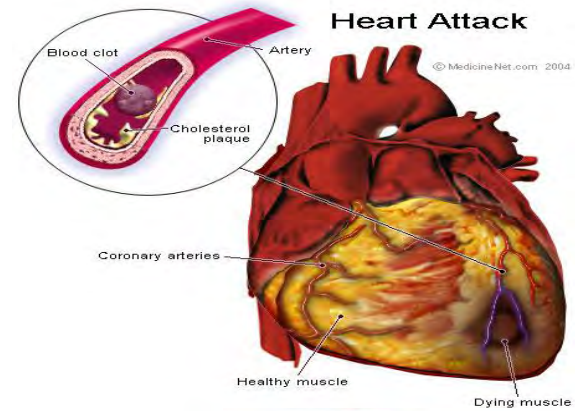

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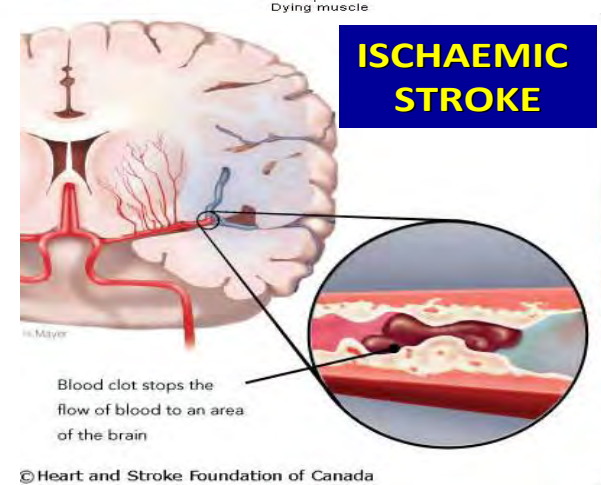


Therapeutic Neovascularisation of Cardiac and Peripheral Ischaemia

Jeff Isner

First Stretegic Mind to use Angiogenesis as a Therapy

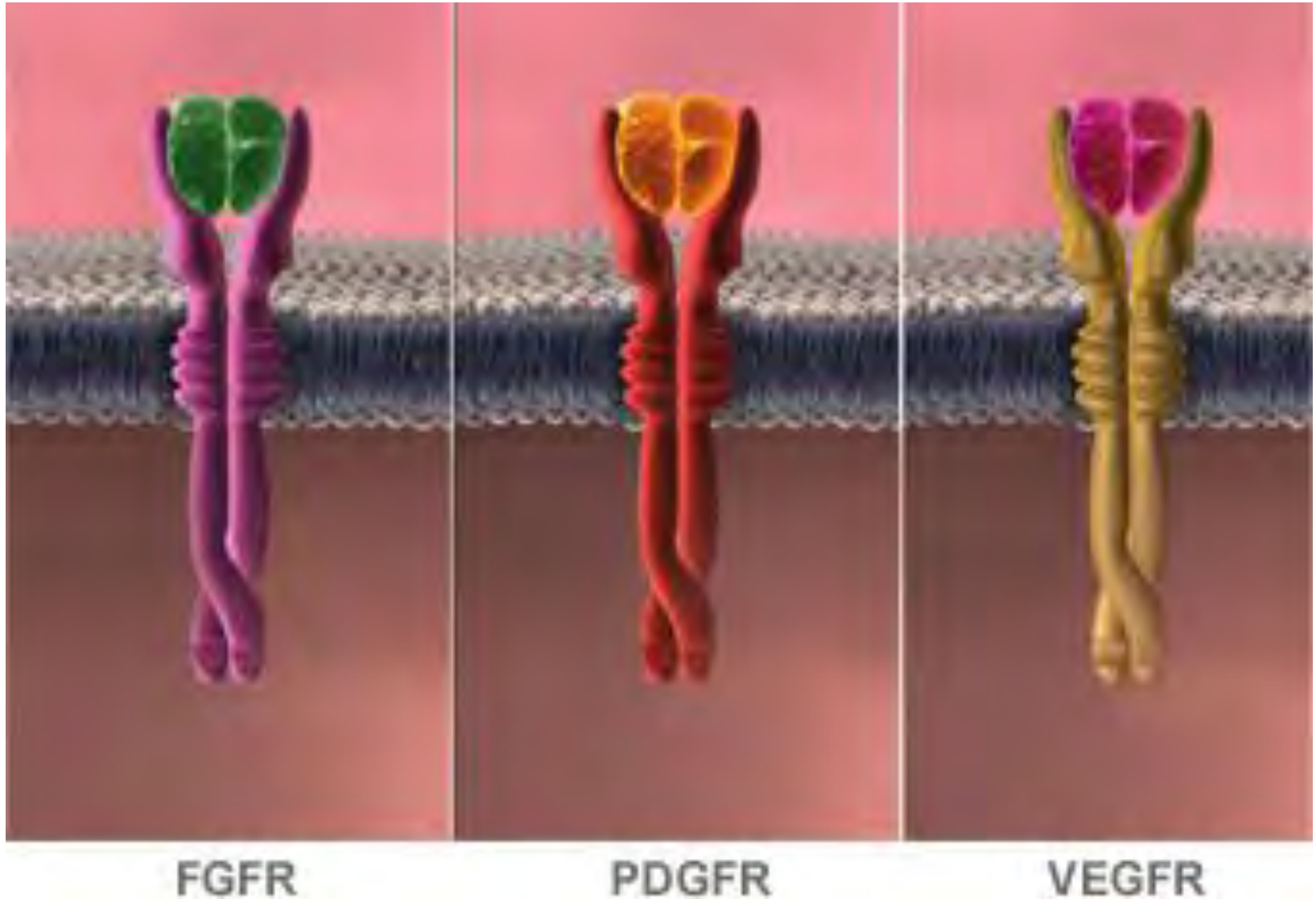
- First attempt

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**
(Prof Baker's lecture on Gene Therapy this Wednesday)

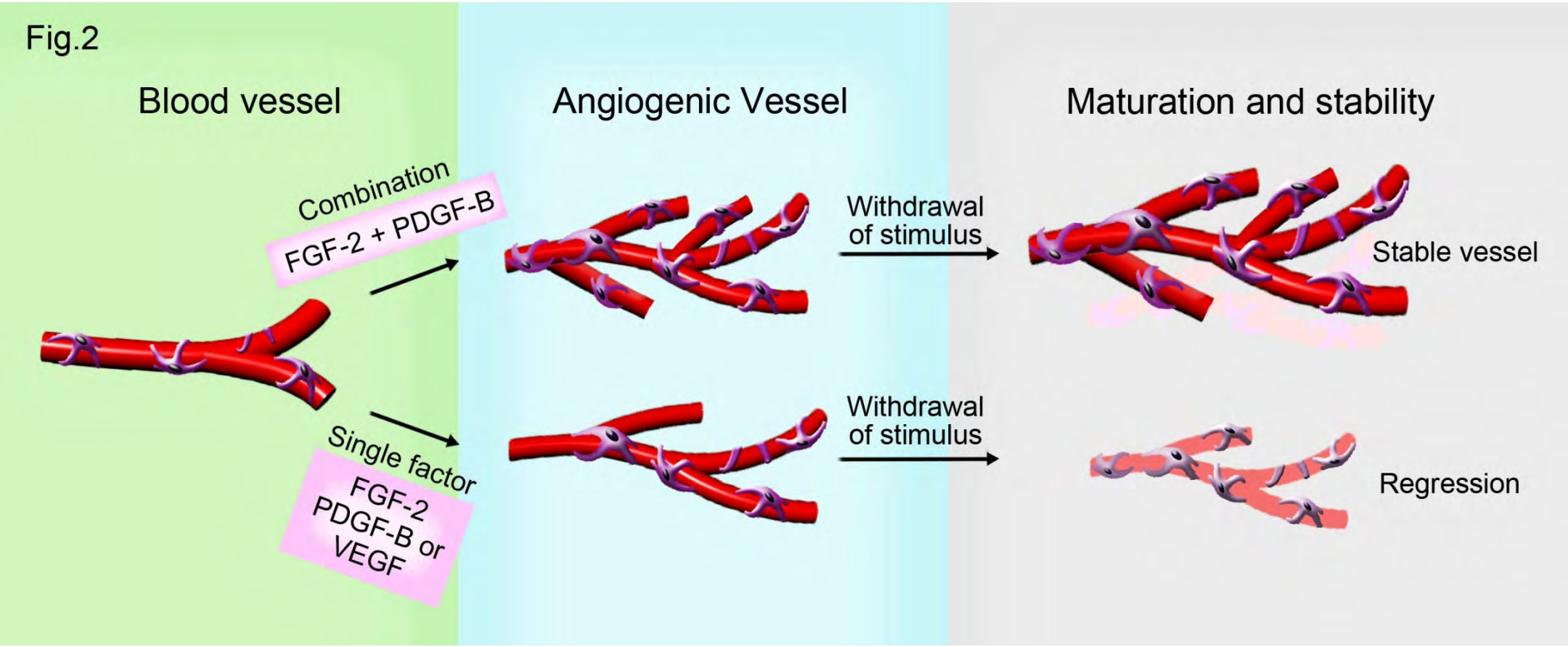
- Second attempt

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 momotherapies



Vascular stabilisation vs vascular regression



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

Lancet 2011; 377: 1929–37

Published Online
May 31, 2011
DOI:10.1016/S0140-6736(11)60394-2

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.

Possibility for Improving Traditional Post-Ischaemic Neovascularisation Therapeutics

- Design better clinical trials

- Work at gene vectors

- Combine pro-angiogenic growth factors able to initiate (VEGF-A?) and complete (???) the angiogenesis process

- More basic science to understand all the players in the angiogenesis and anti-angiogensis orchestras

- Do not give up!

Alternative/Complementary Pathways to Therapeutic Post-Ischaemic Neovascularisation

- Increase the expression/activity of "novel" pro-angiogenic factors in ischaemic tissues (example: NGF, NT-3, FGF9)

- Combinatory approaches

- Contrast "anti-angiogenic" molecular signatures induced by pathology and/or risks factors (examples: miRNA-92a and -503)

- "Play" with microRNAs

- Use other novel sources of adult stem cells and progenitor cells for autologous transplantation (see Wednesday plenary session on Stem cells), inluding hESC-derived ECs

“novel” pro-angiogenic factors: focus on NGF, NT-3 and FGF9

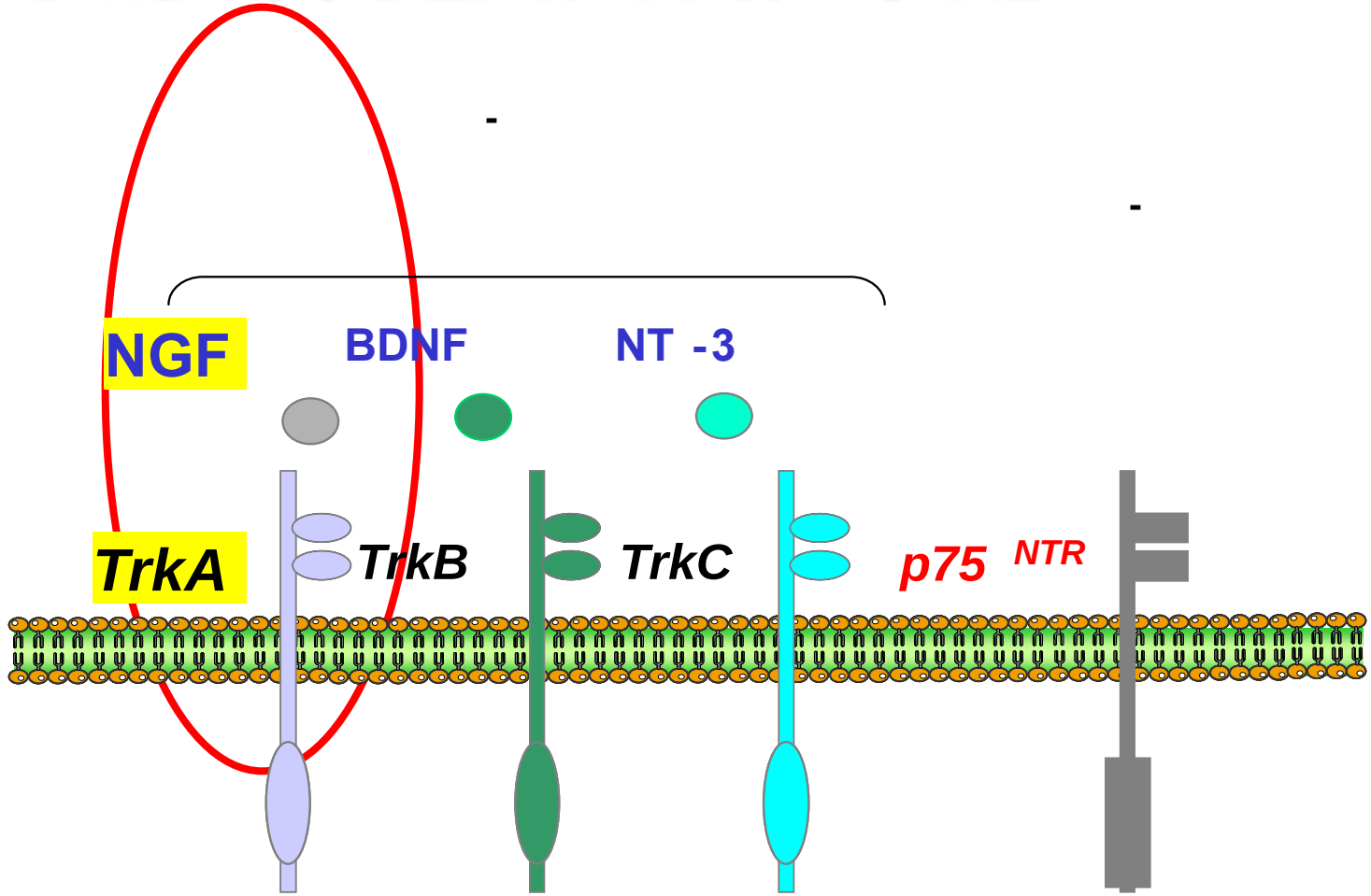
Integrative Physiology

Circulation Research
Journal of the American Heart Association

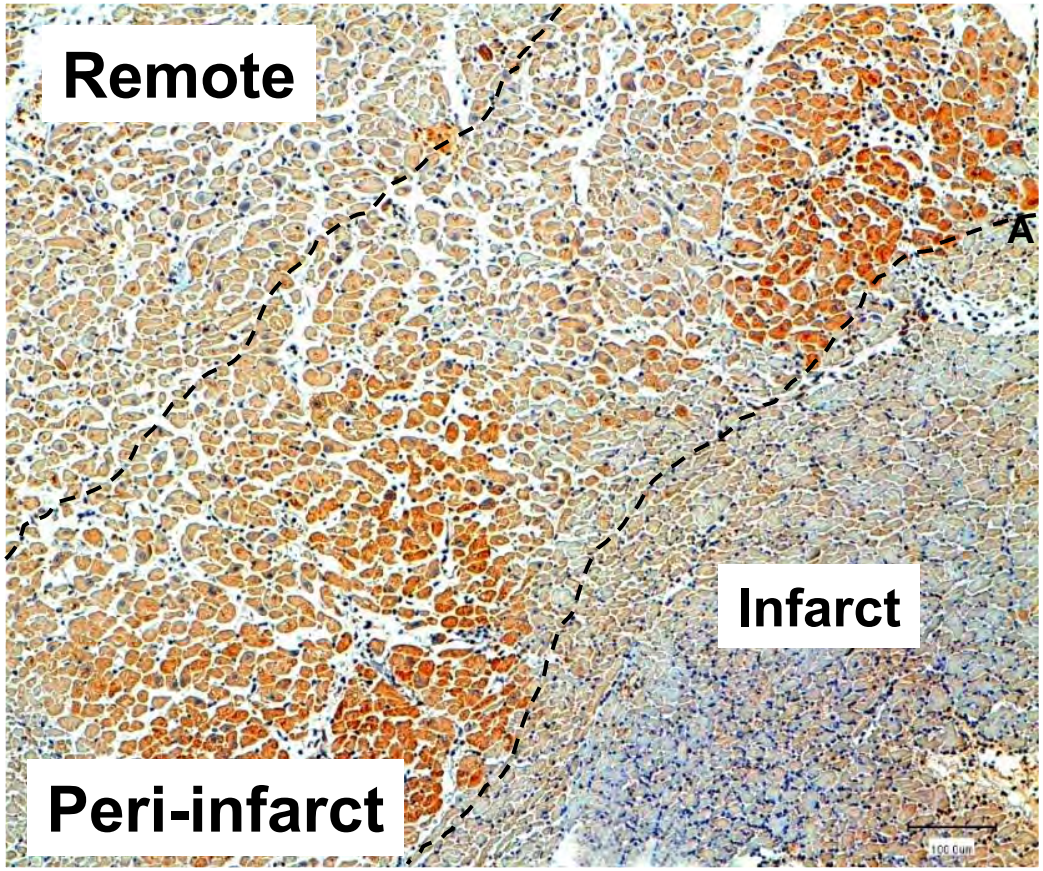
2010

Nerve Growth Factor Promotes Cardiac Repair following Myocardial Infarction

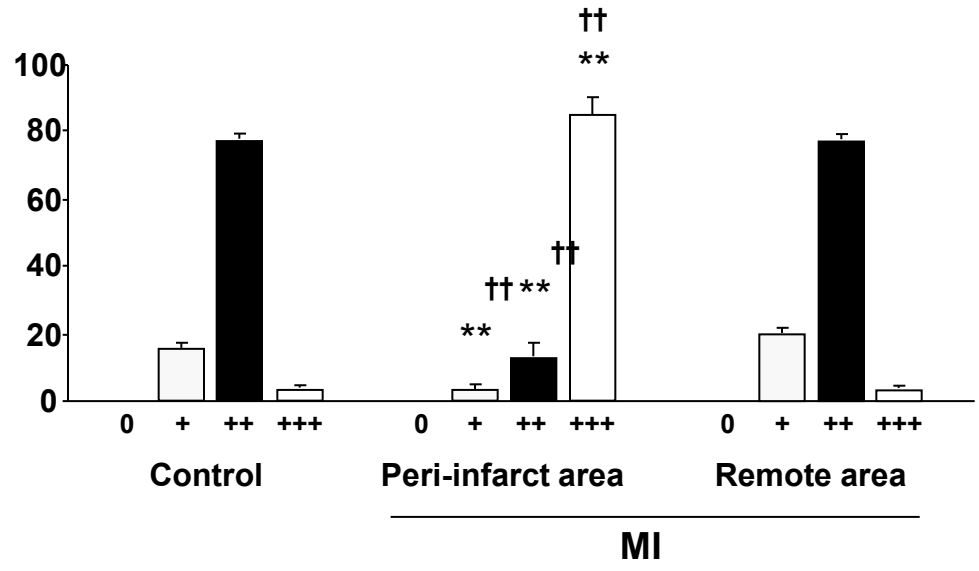
Marco Meloni, Andrea Caporali, Gallia Graiani, Costanza Lagrasta, Rajesh Katare, Sophie Van Linthout, Frank Spillmann, Ilaria Campesi, Paolo Madeddu, Federico Quaini, Costanza Emanueli



NGF is present in the human heart and upregulated in the peri-infarct area

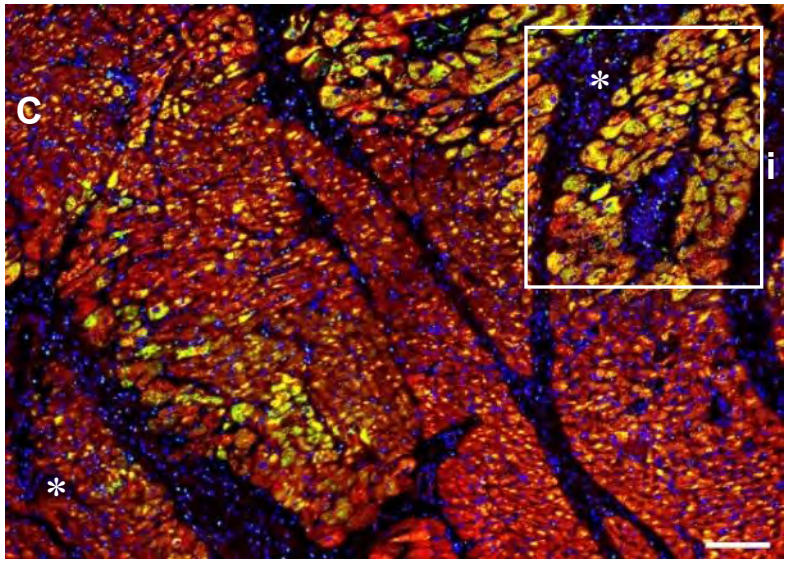


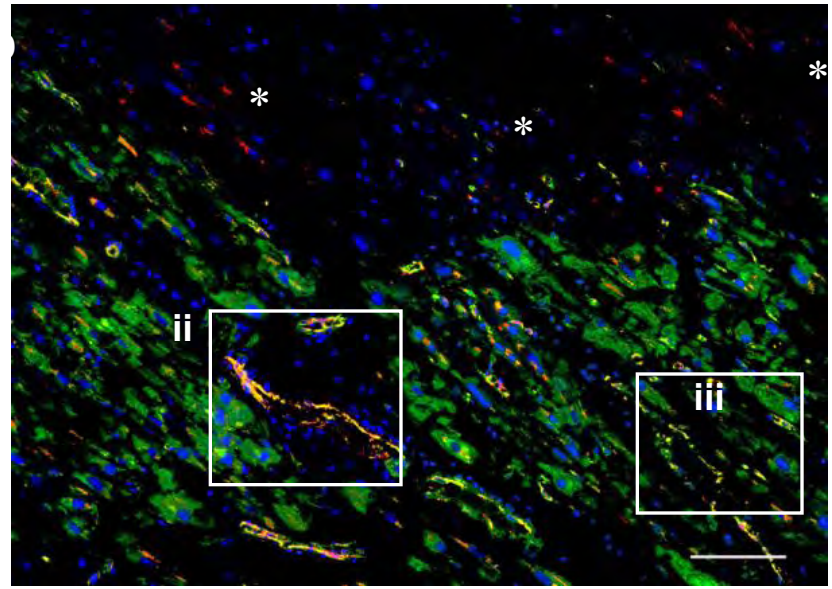
Distribution of cardiomyocytes according to their NGF expression level



†† P<0.01 vs Control ** P<0.01 vs Remote area

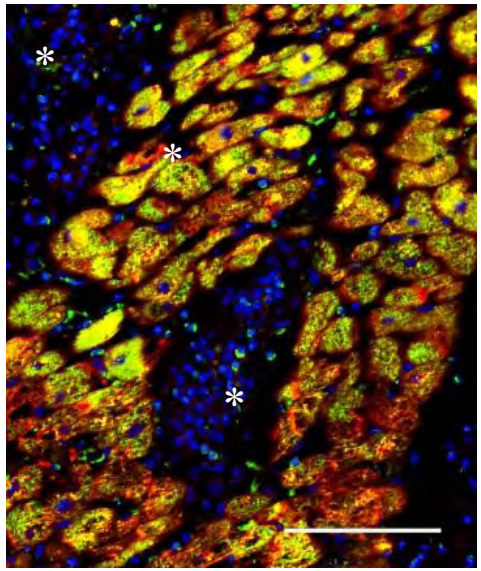
TrkA expression by cardiomyocytes and ECs in human MI

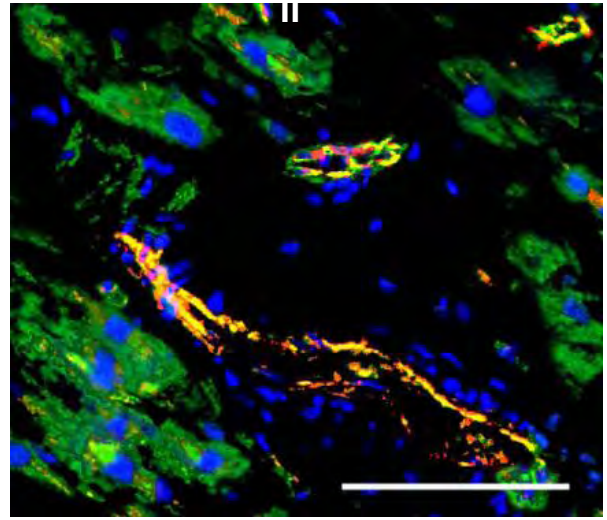


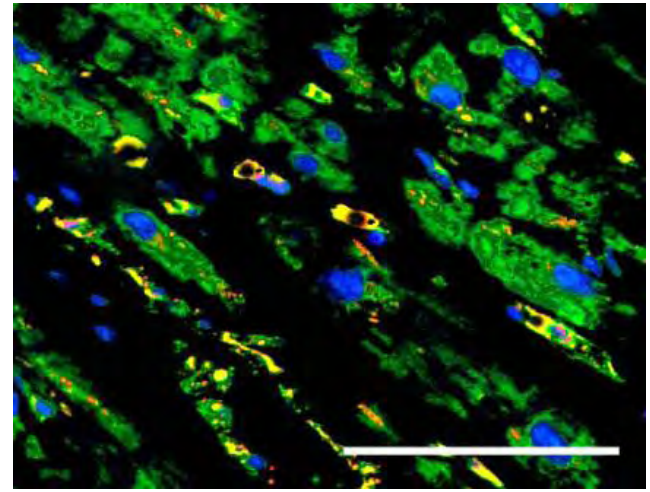


TrkA
CD34

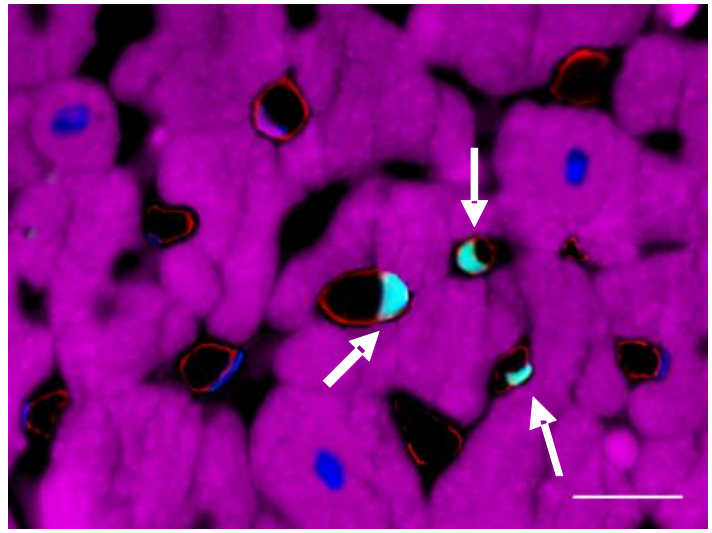
TrkA
α-sarc



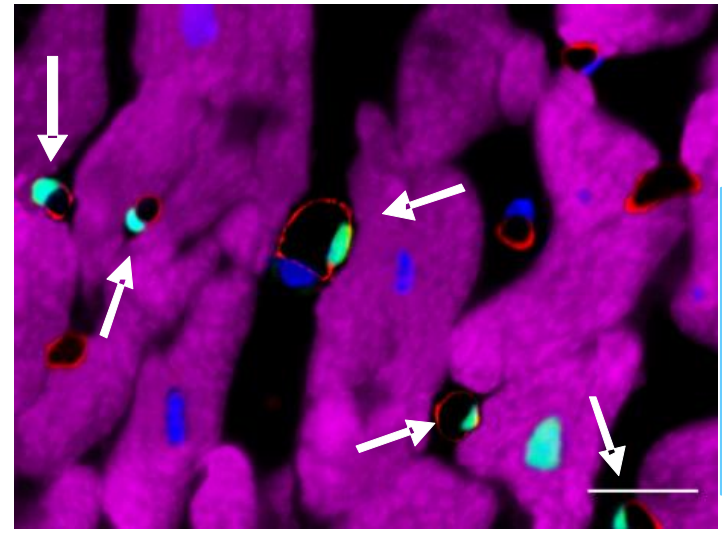




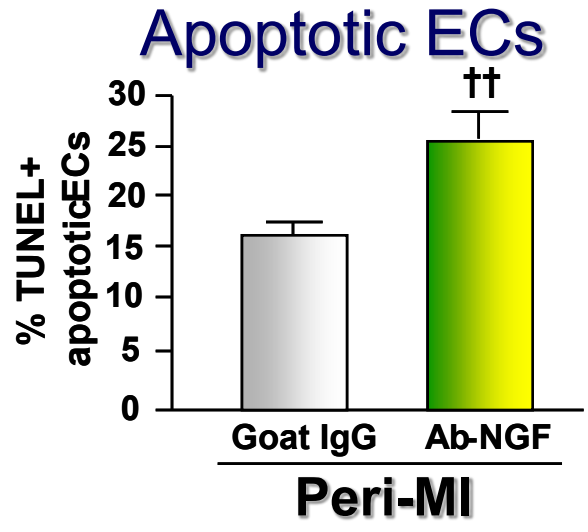
Endogenous NGF promotes endothelial cell survival in the post-MI myocardium (experiment with NGF neutralizing Ab)



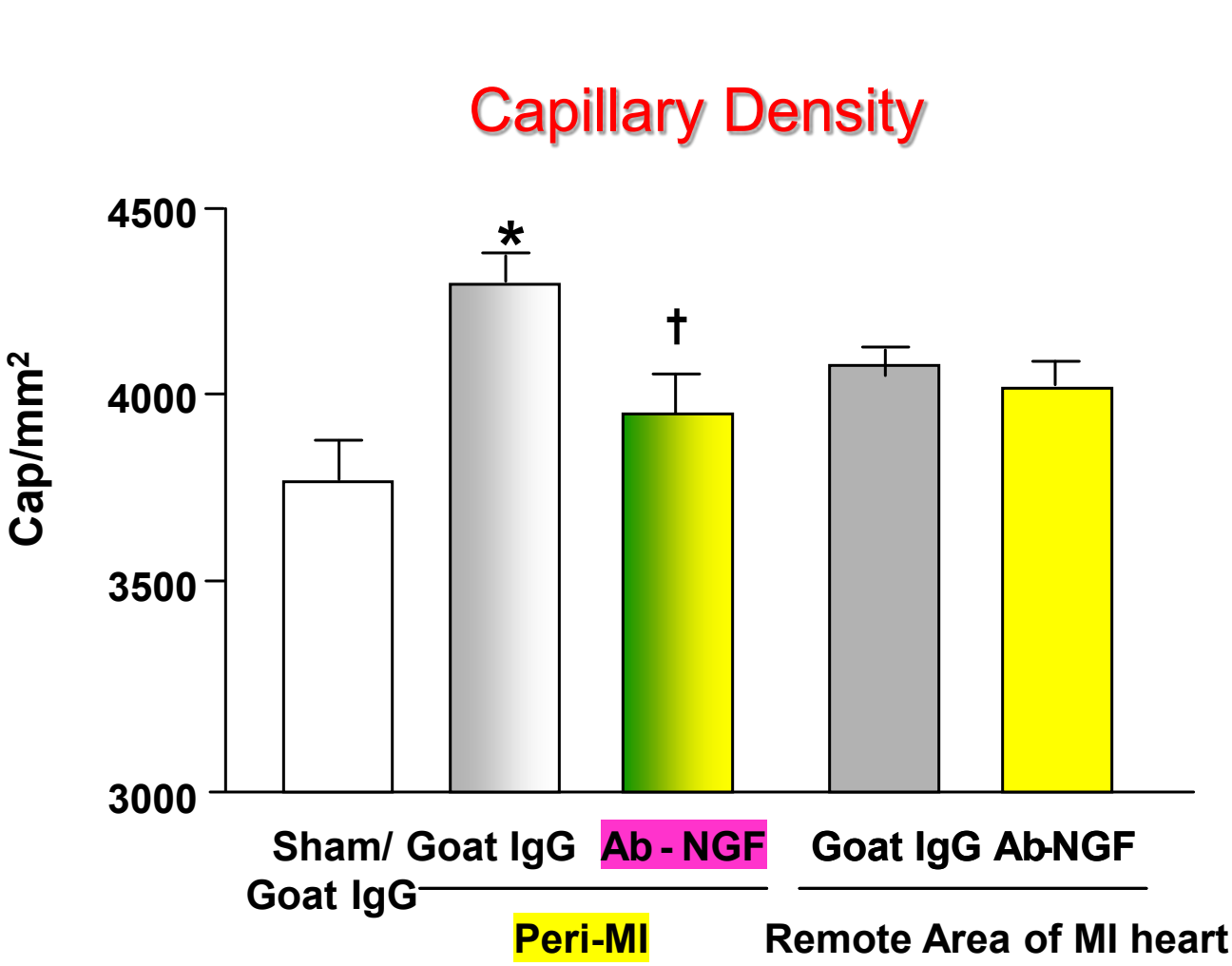
MI/Goat IgG

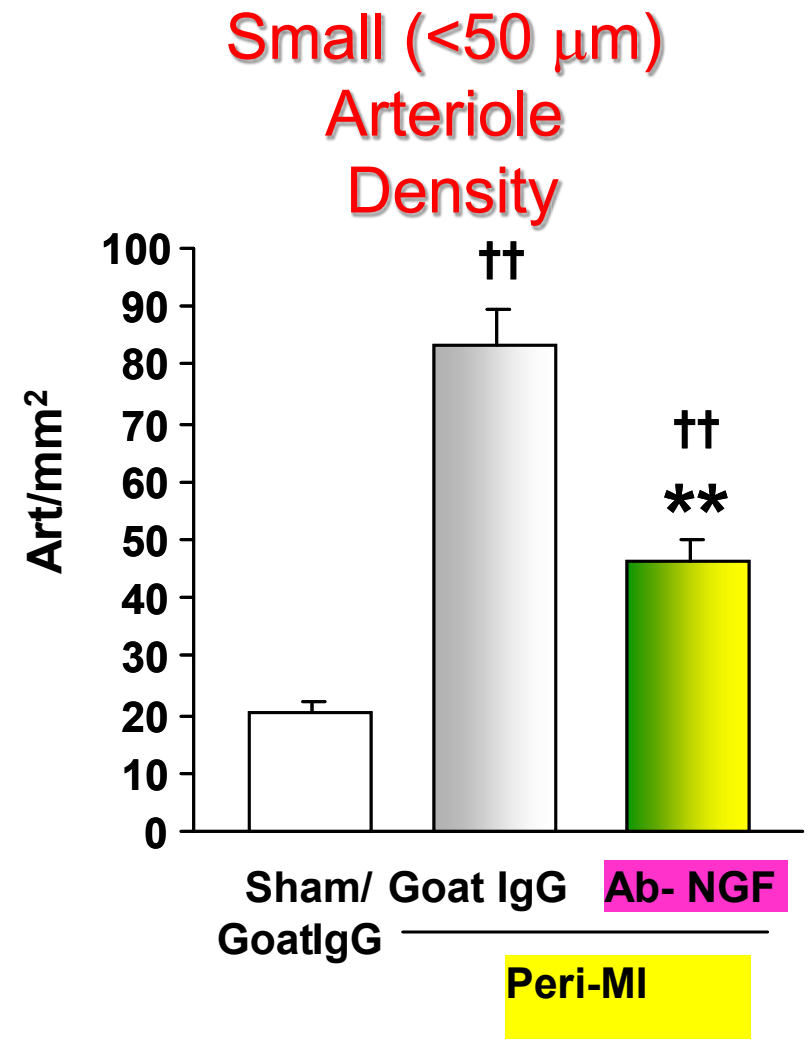


MI/Ab-NGF

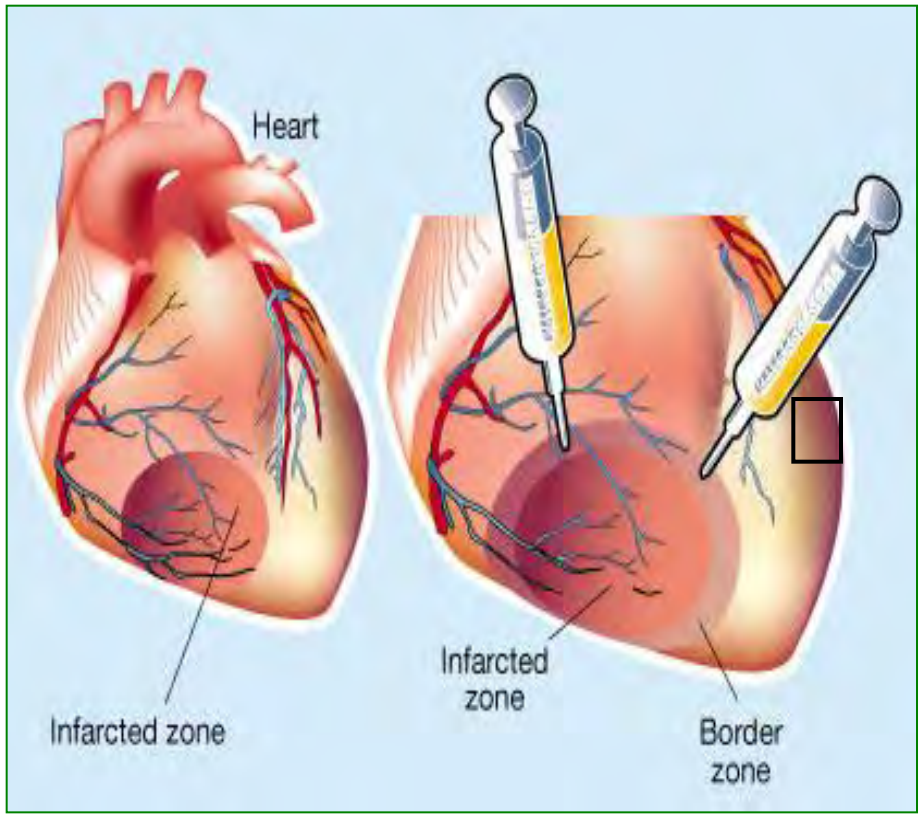


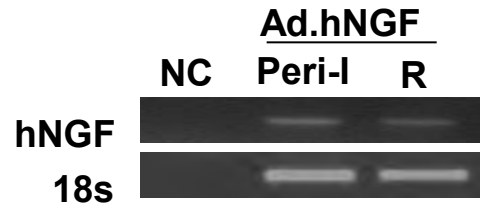
Endogenous NGF promotes reparative angiogenesis in the post-MI myocardium





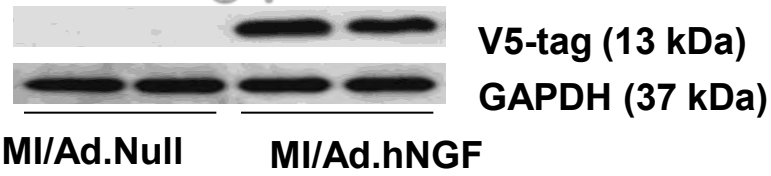
Ad-mediated intra-myocardial NGF gene transfer in mice



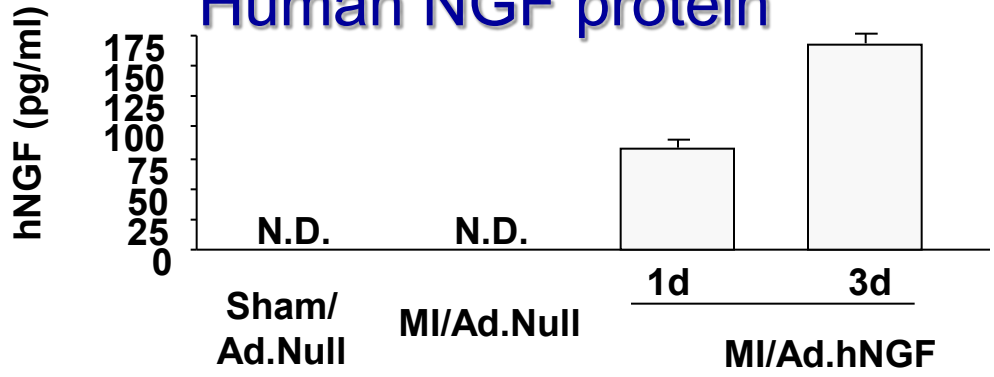


Human NGF mRNA

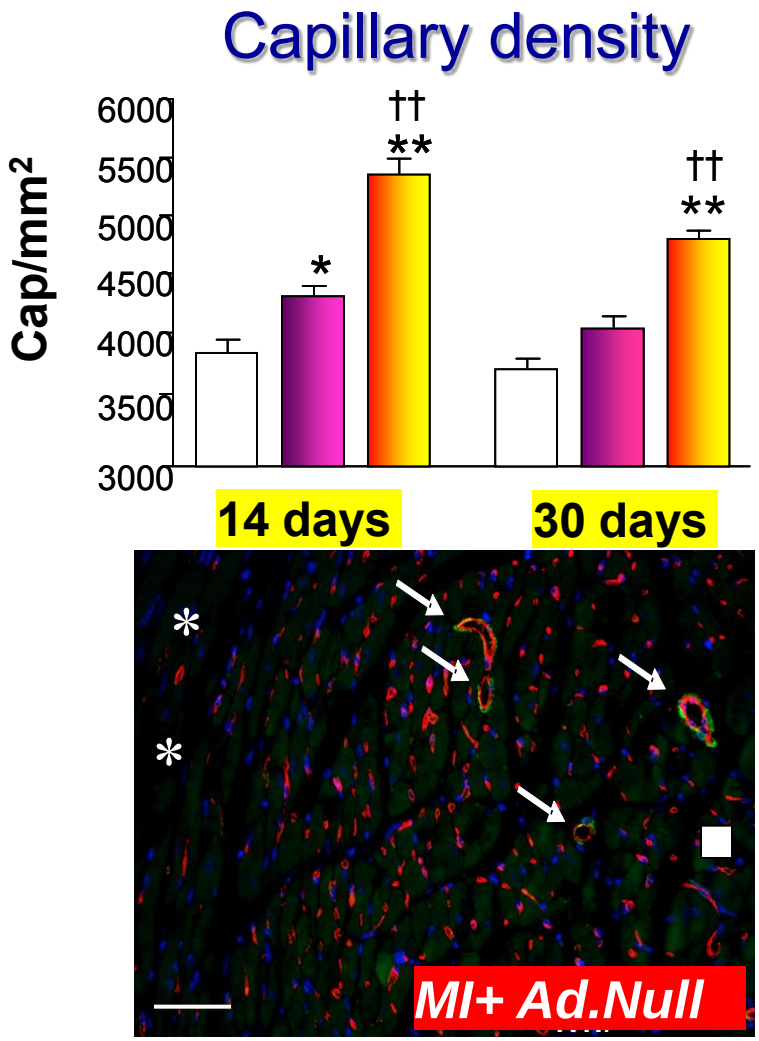
V5-tag protein

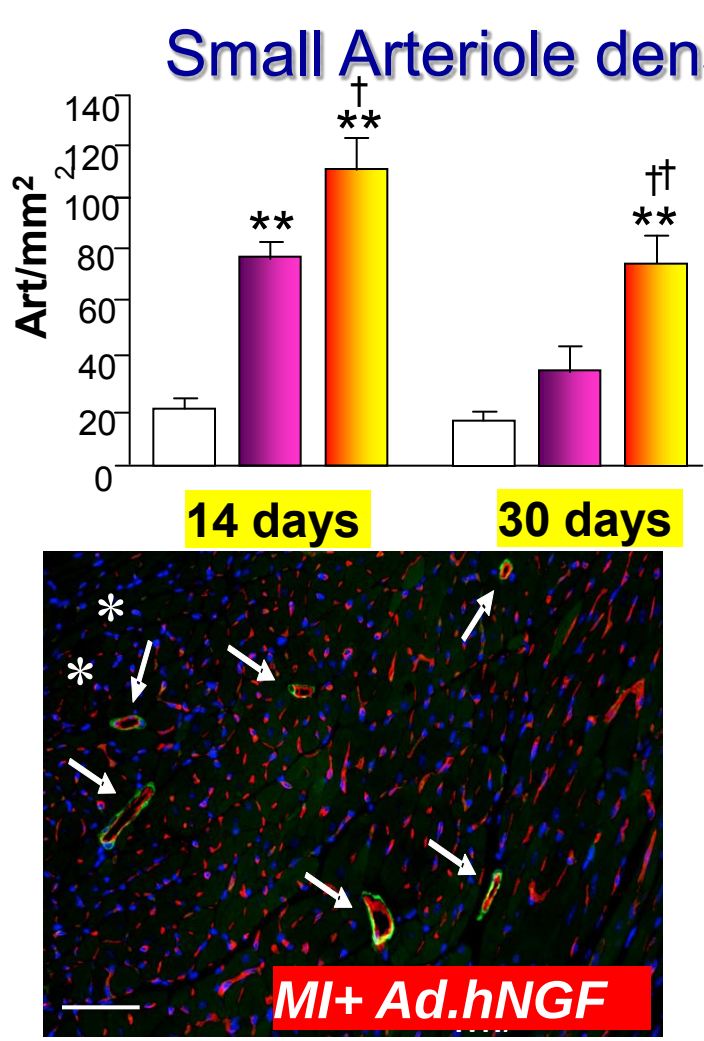


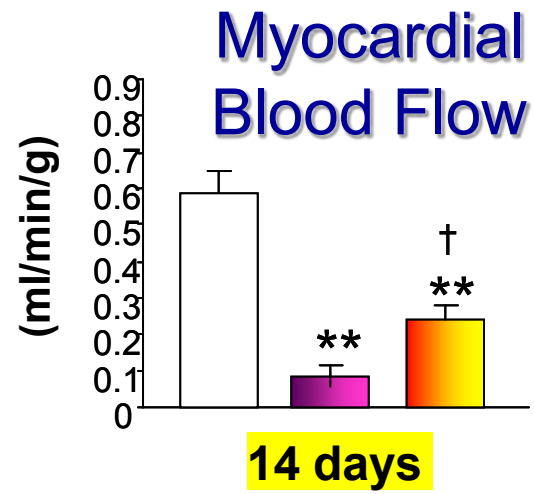
Human NGF protein



NGF gene therapy improves post-MI angiogenesis and blood flow





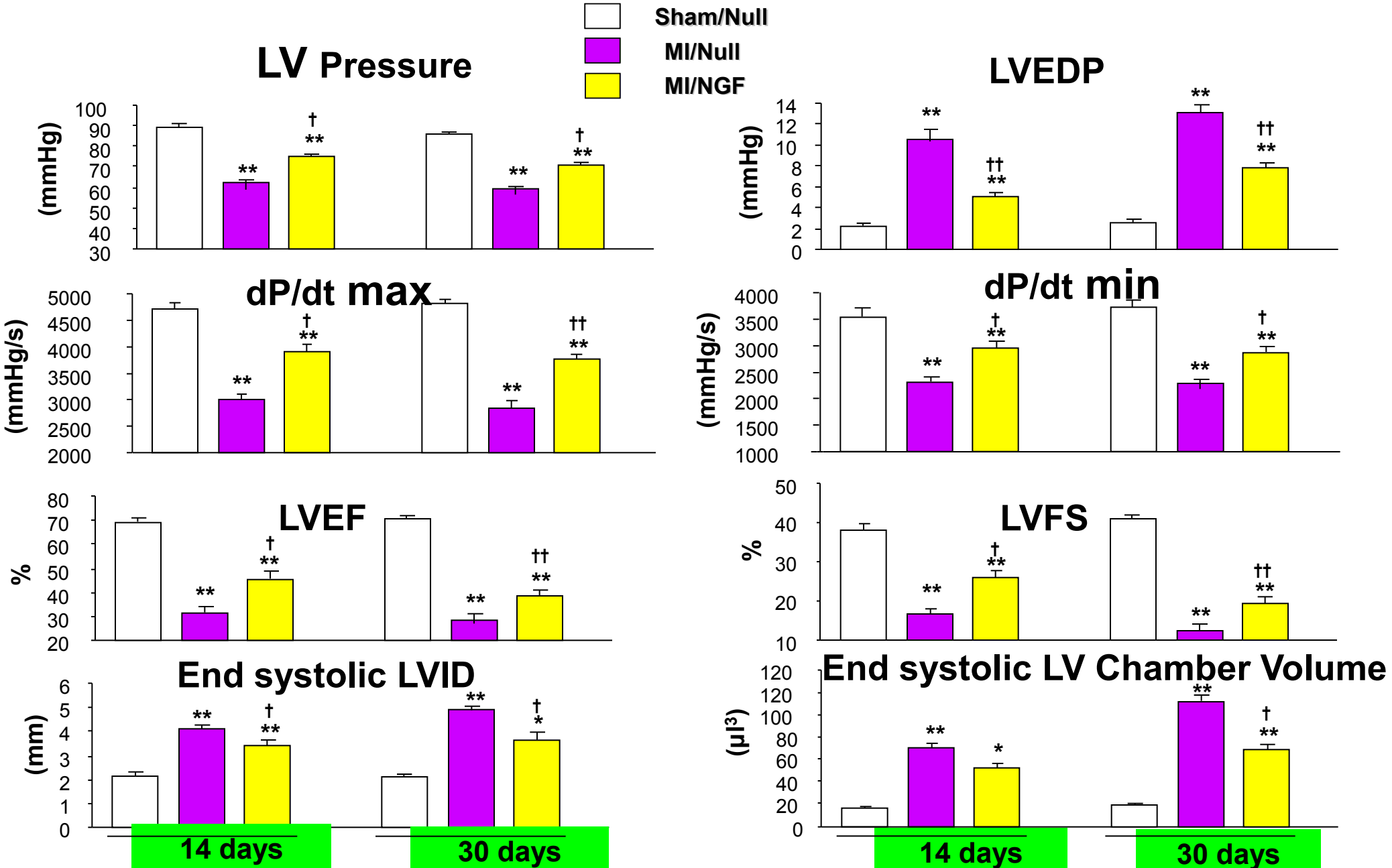


Sham/Null

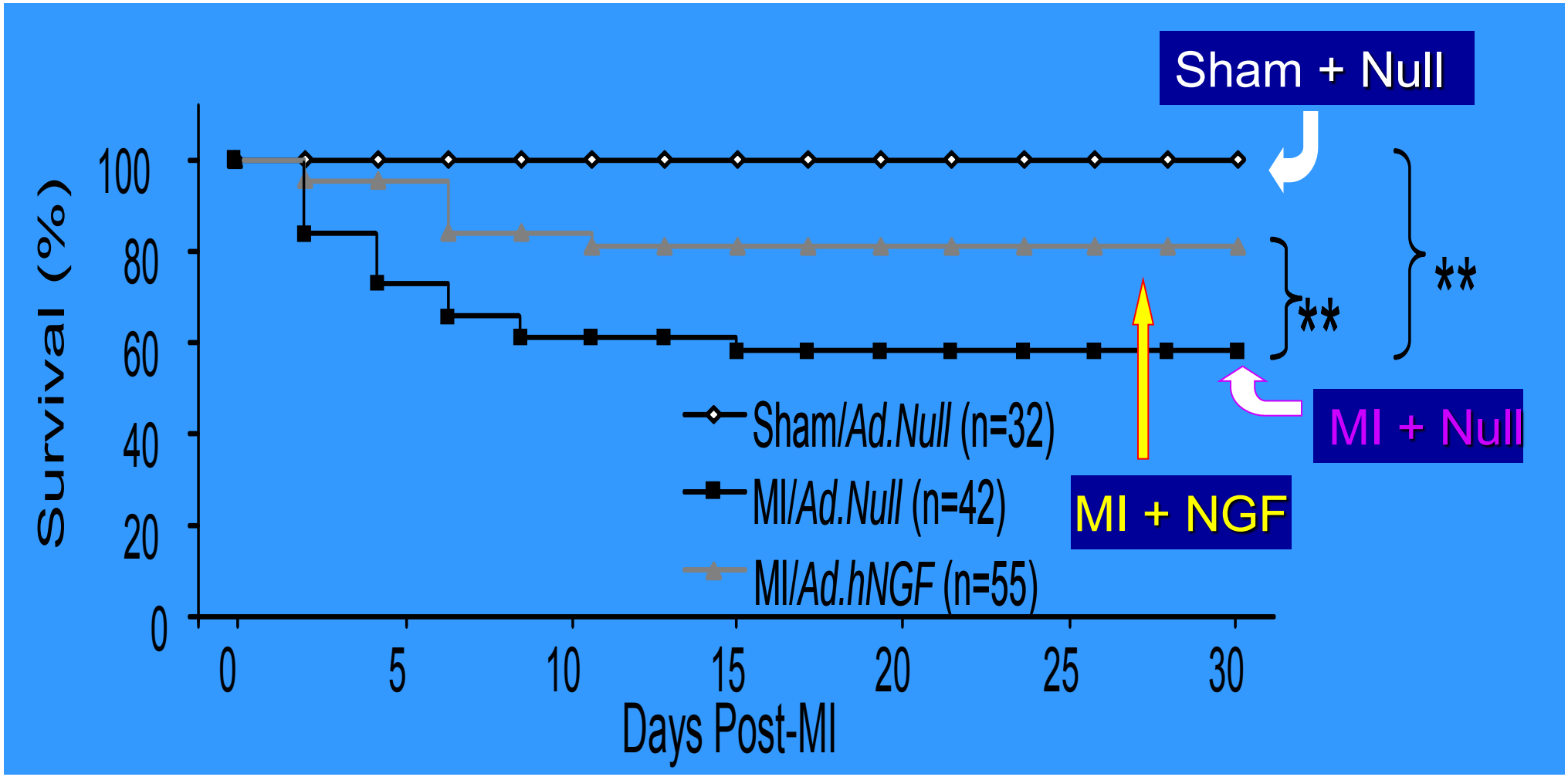
MI/Null

MI/NGF

NGF therapy improves post-MI LV function and remodeling



NGF gene therapy improves post-MI survival

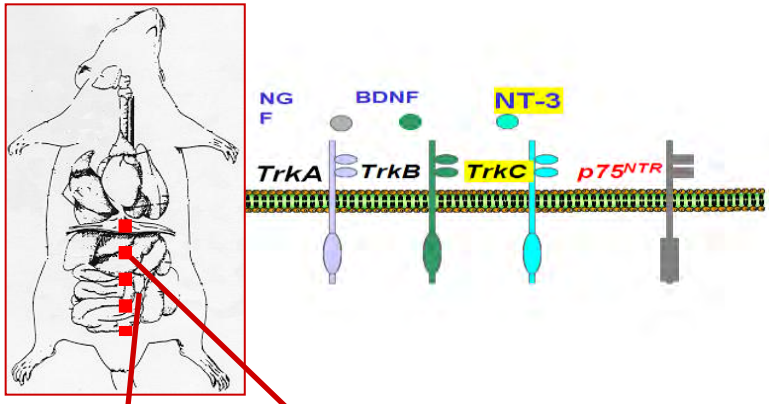


NGF therapy:

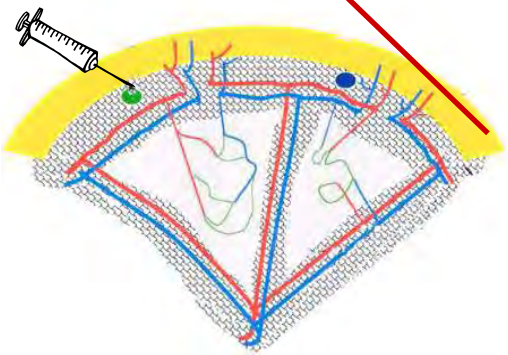
Next steps in view of clinical translation are:

- work in a large (pig) animal model
- selection of best vector for gene delivery
- design of good phase I clinical trial
- get approval from regulatory authorities and
- find money to do the work

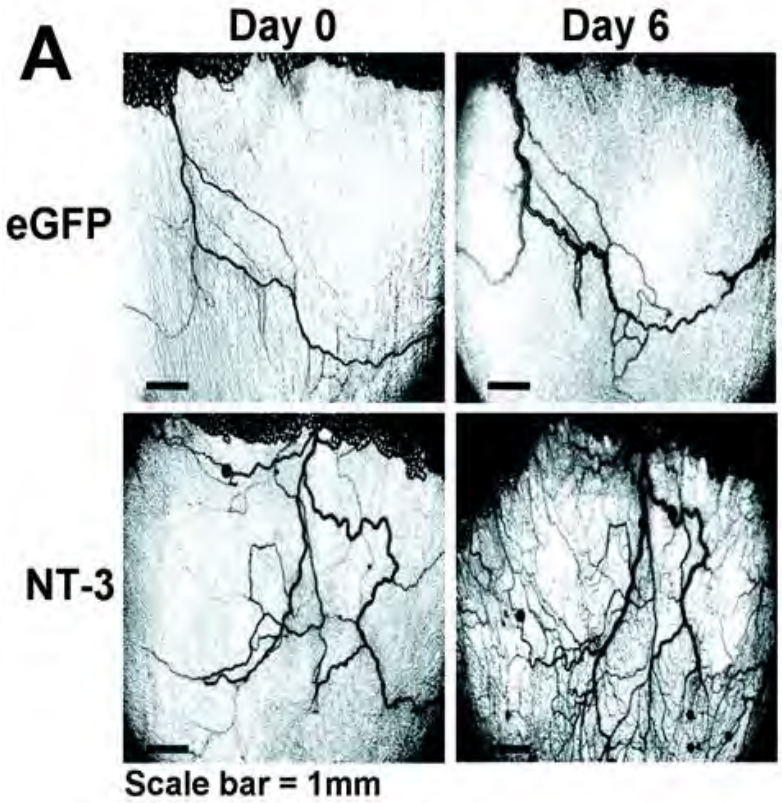
NT-3 promotes the growth of “mature neovessels” in a rat mesentery model

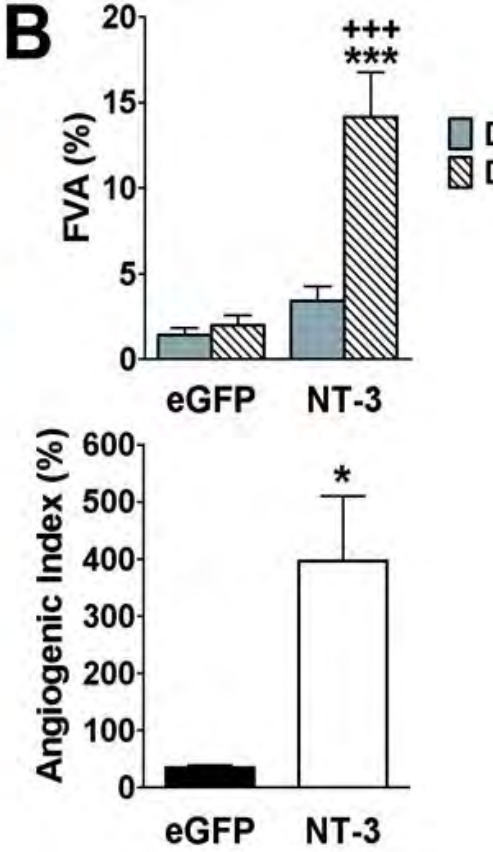


Laparotomy



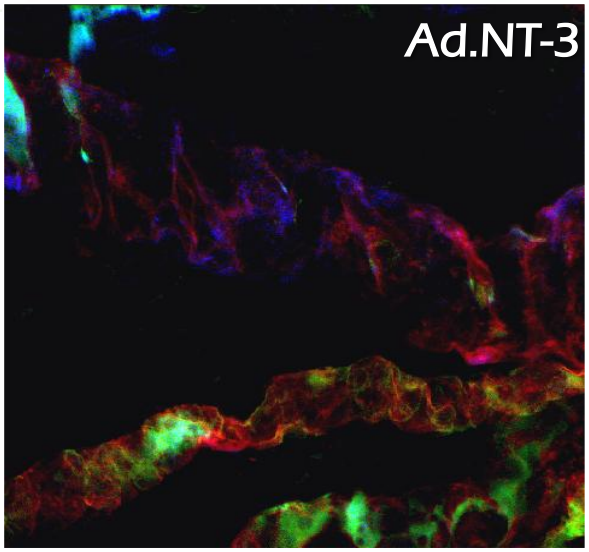
Cristofaro & Emanueli. ATVB, 2010

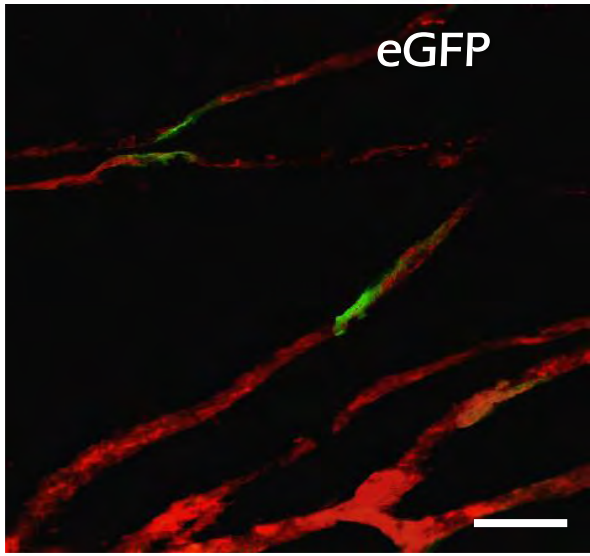




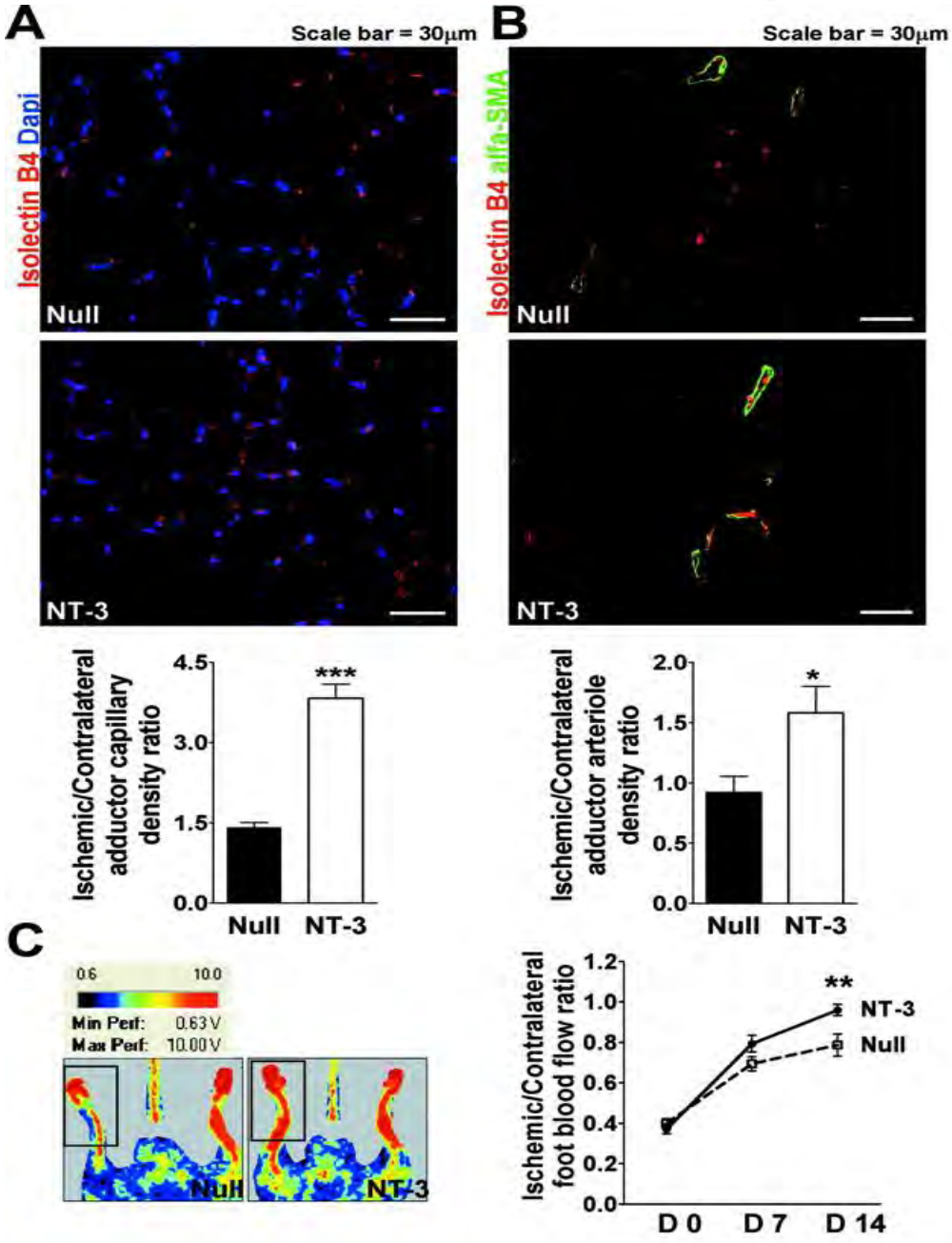
Scale bar = 40μm

NG2
alfa-SMA
Isolectin B4

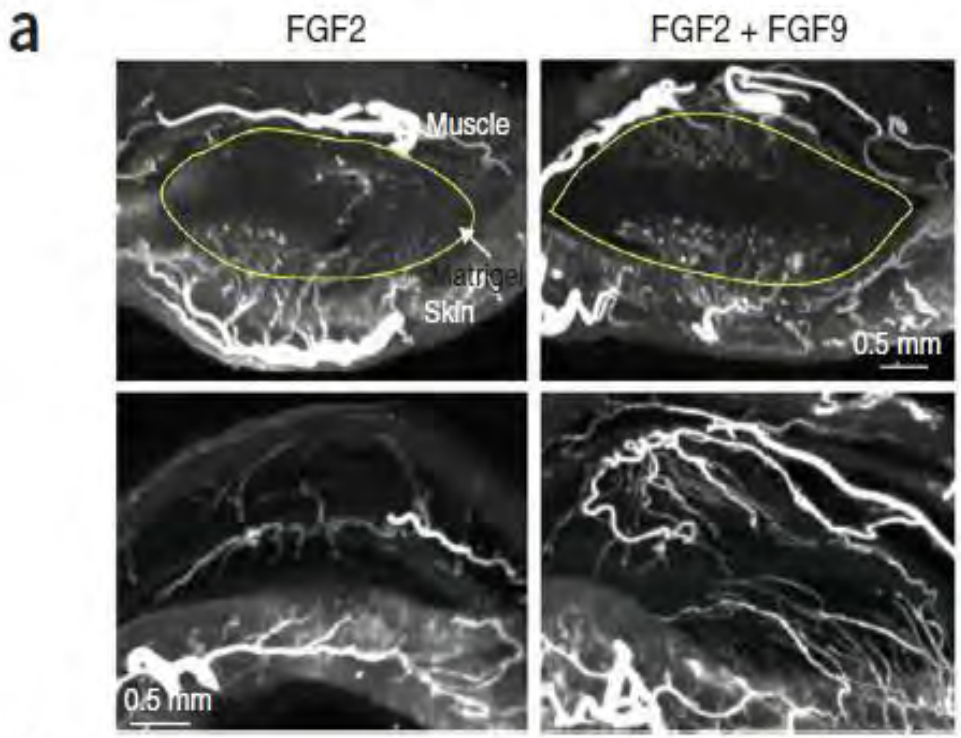


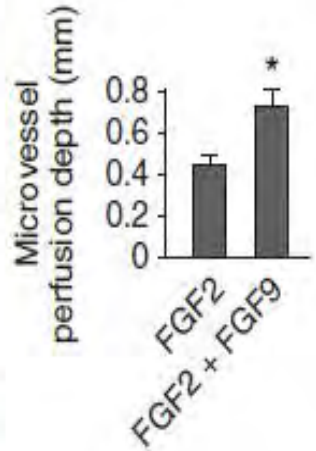


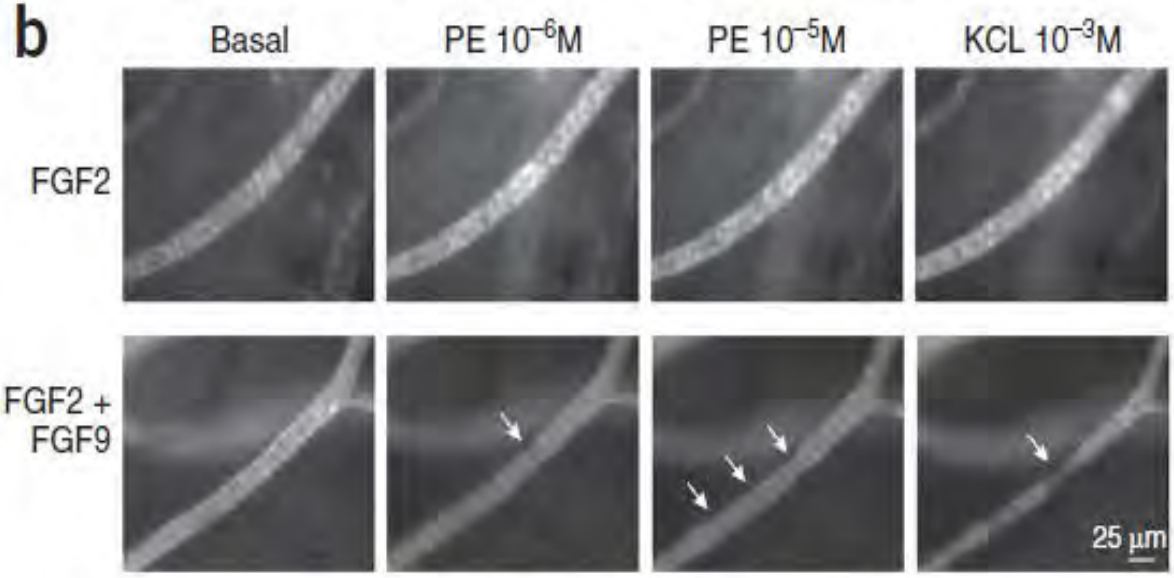
Ad.NT-3 enhances postischemic neovascularisation



Cristofaro et al
ATVB, 2010



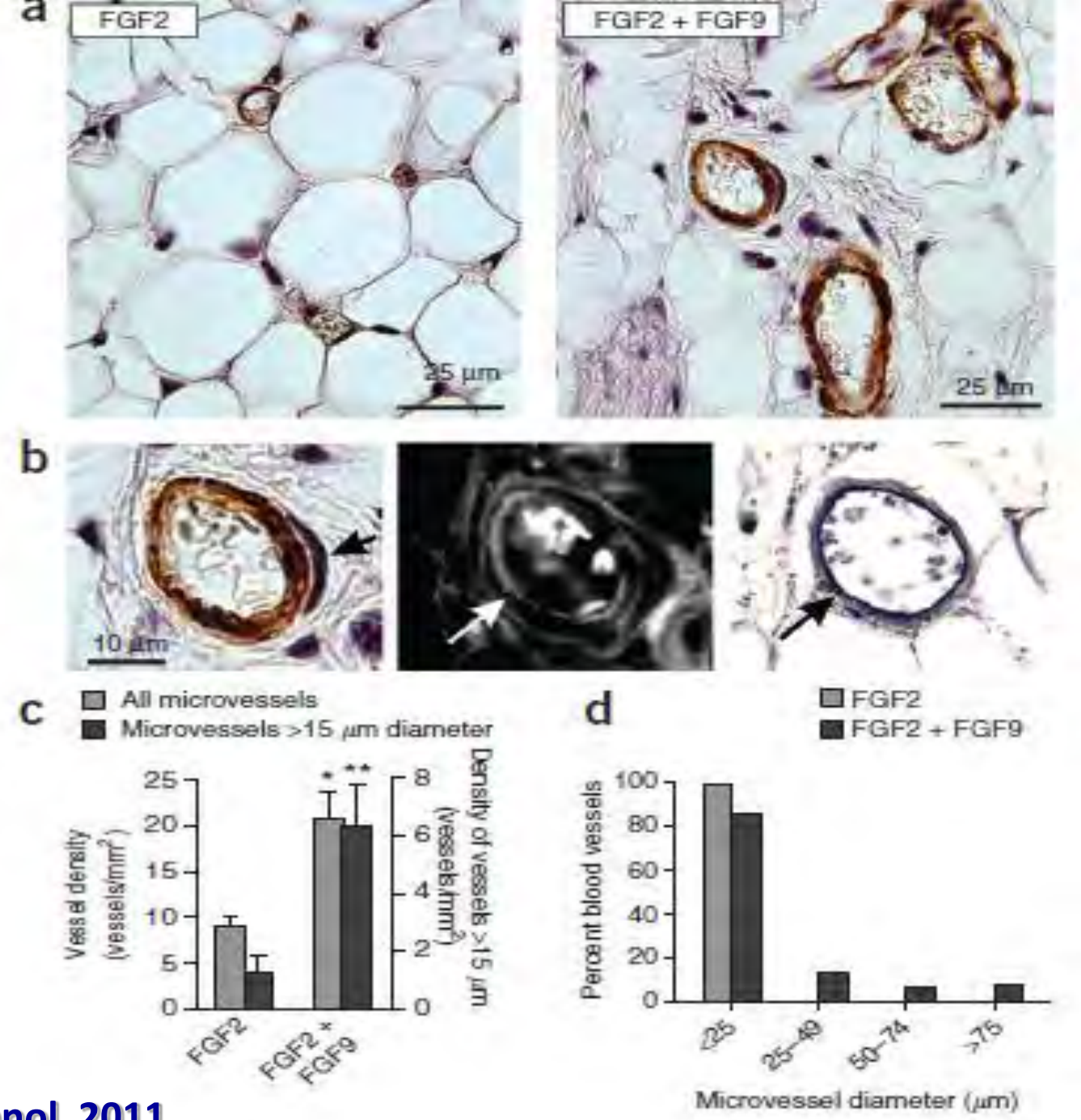




FGF-9, differently from other FGFs, does not initiate angiogeneis, but it acts on mural cells to produce new muscularised vessels able to bring blood flow and react to vasodilators and vasoconstrictors

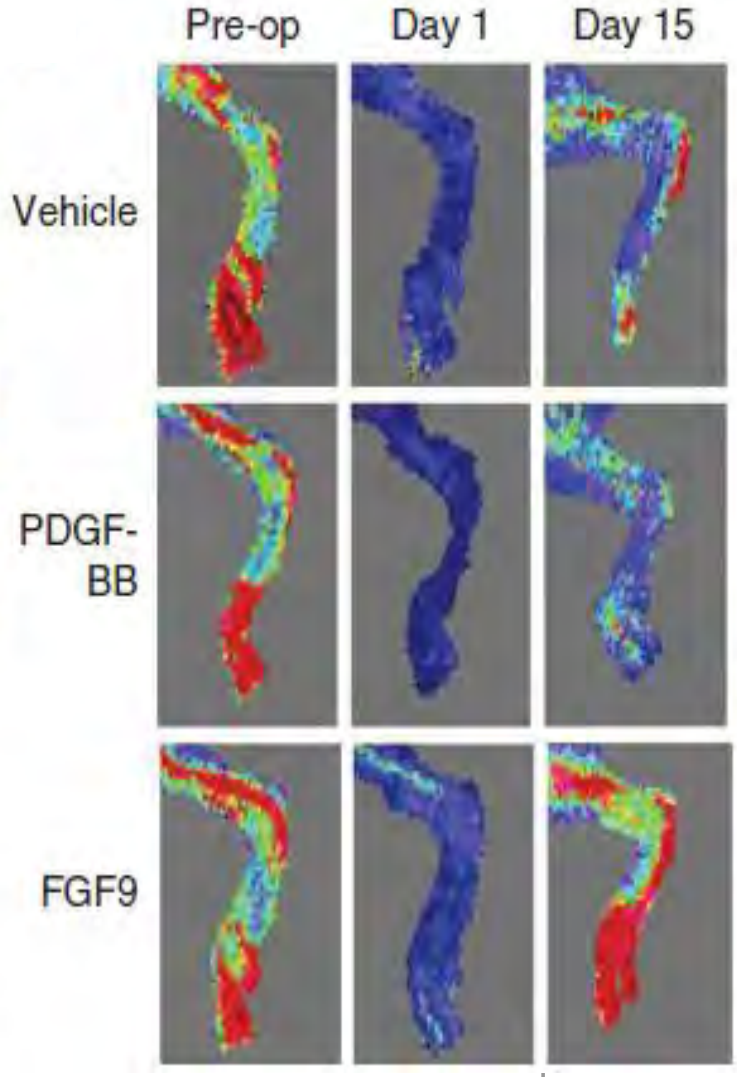
Frontini & Pickering, Nat Biotechnol, 2011

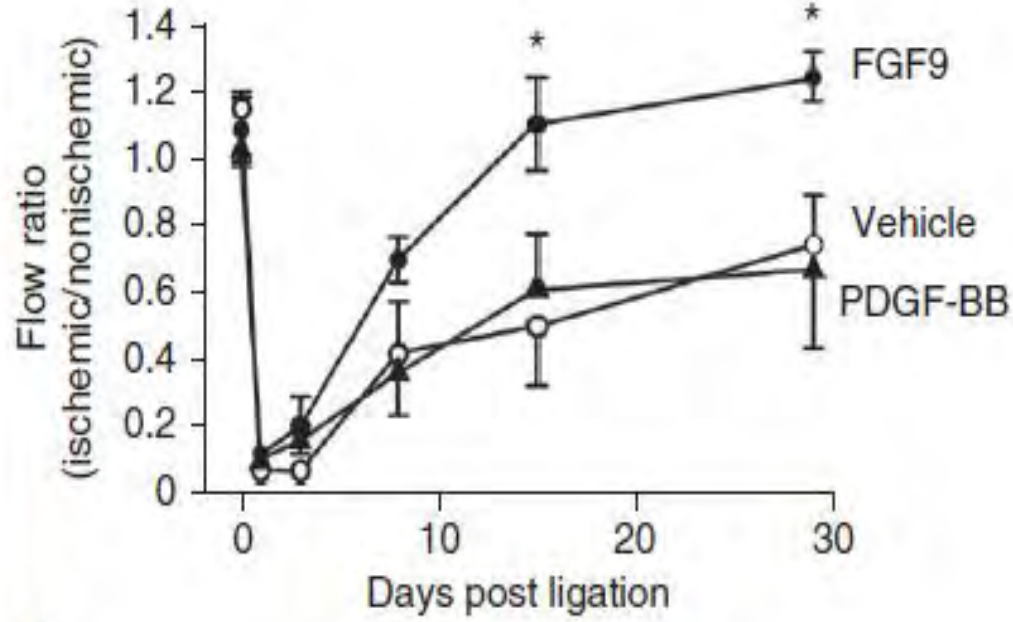
FGF-9-induced new vessels in matrigel pugs live up to one year in vivo



Frontini & Pickering, Nat Biotechnol, 2011

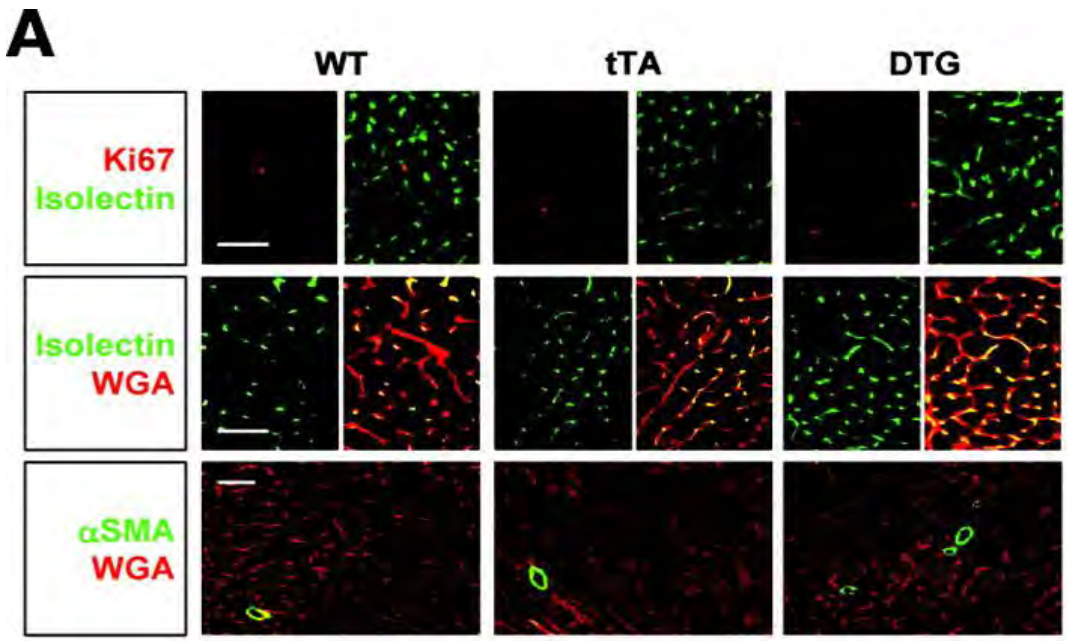
FGF-9 improves post-ischaemic blood flow recovery of mouse hindlimbs

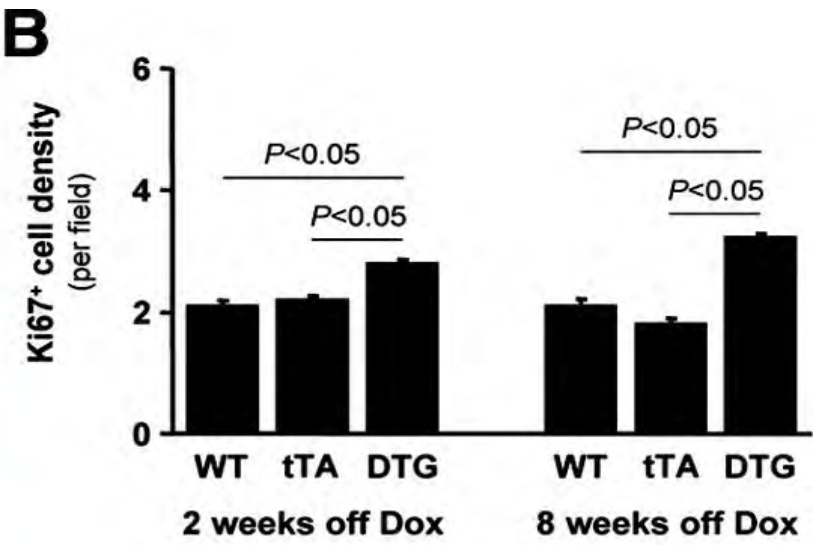


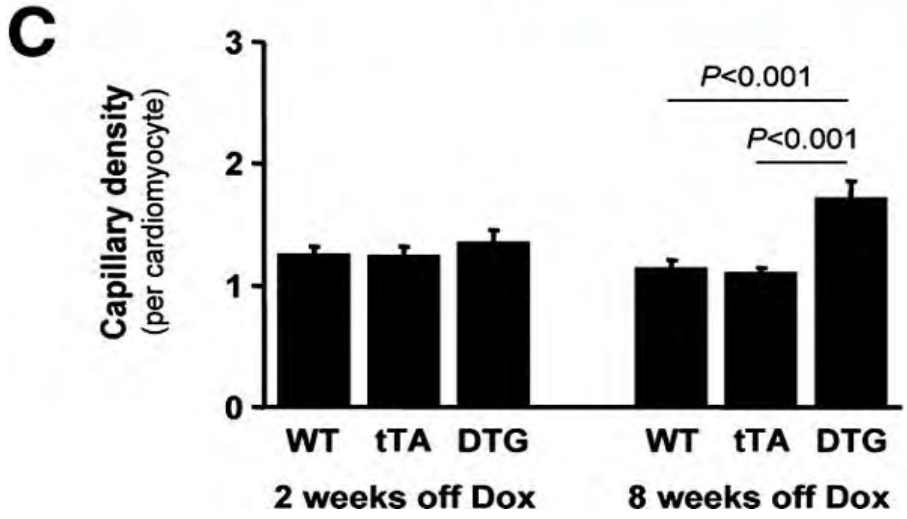


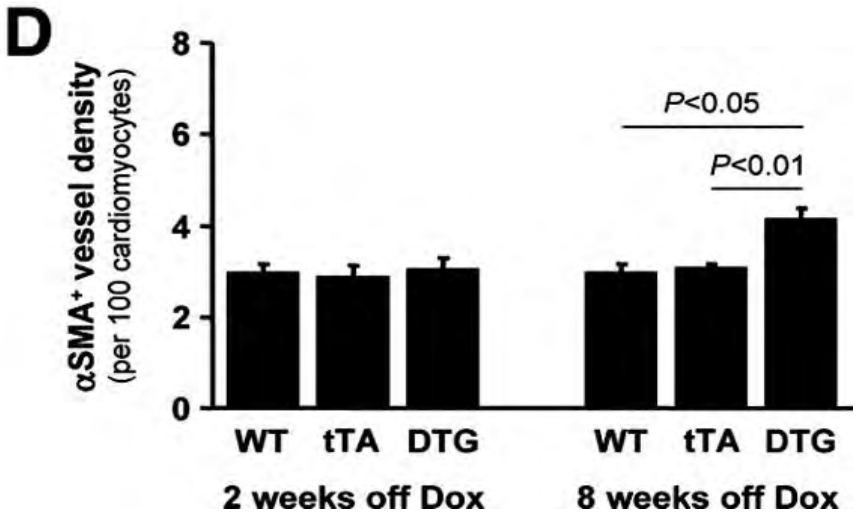
Frontini & Pickering, Nat Biotechnol, 2011

FGF9 enhances microvessel density in the myocardium



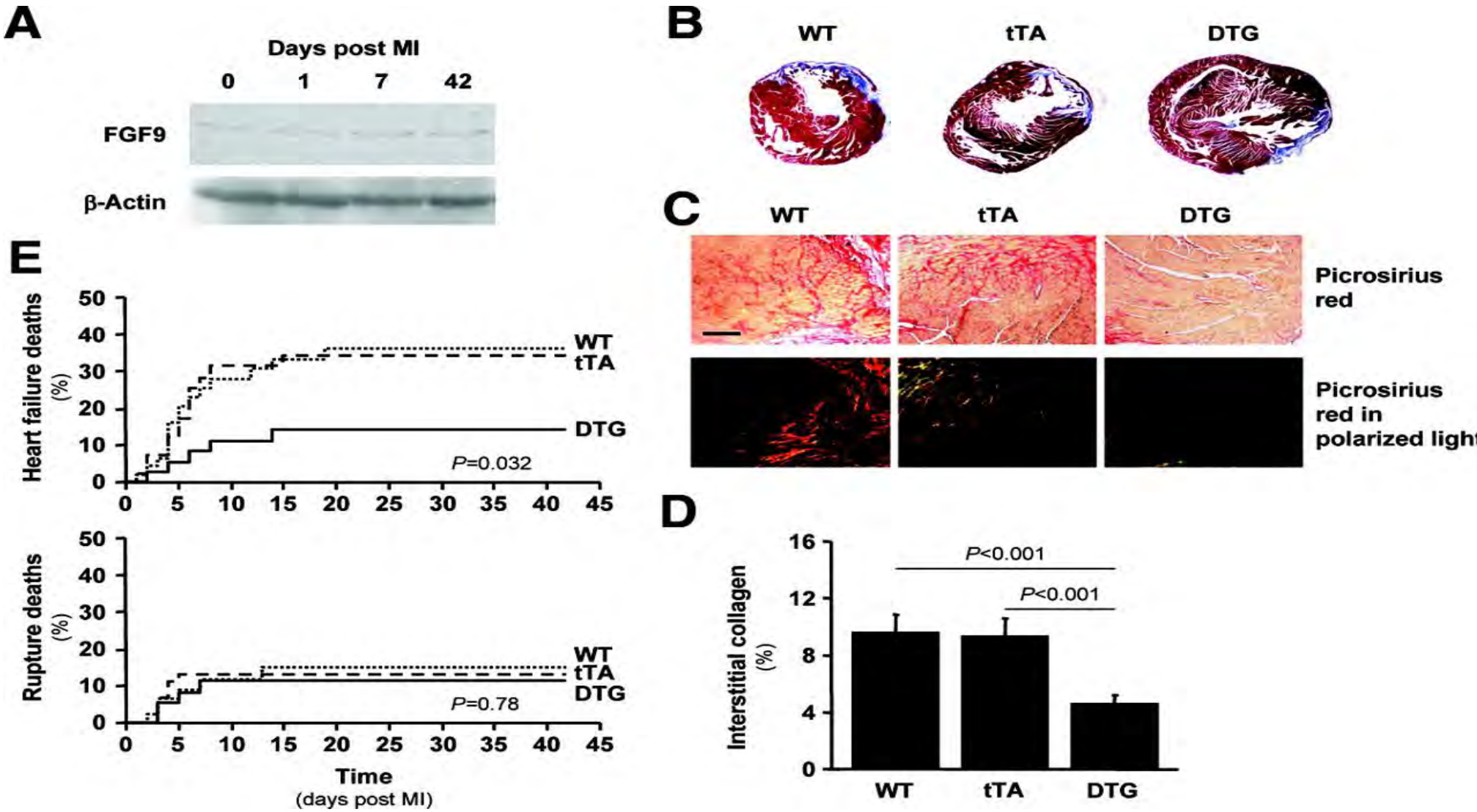






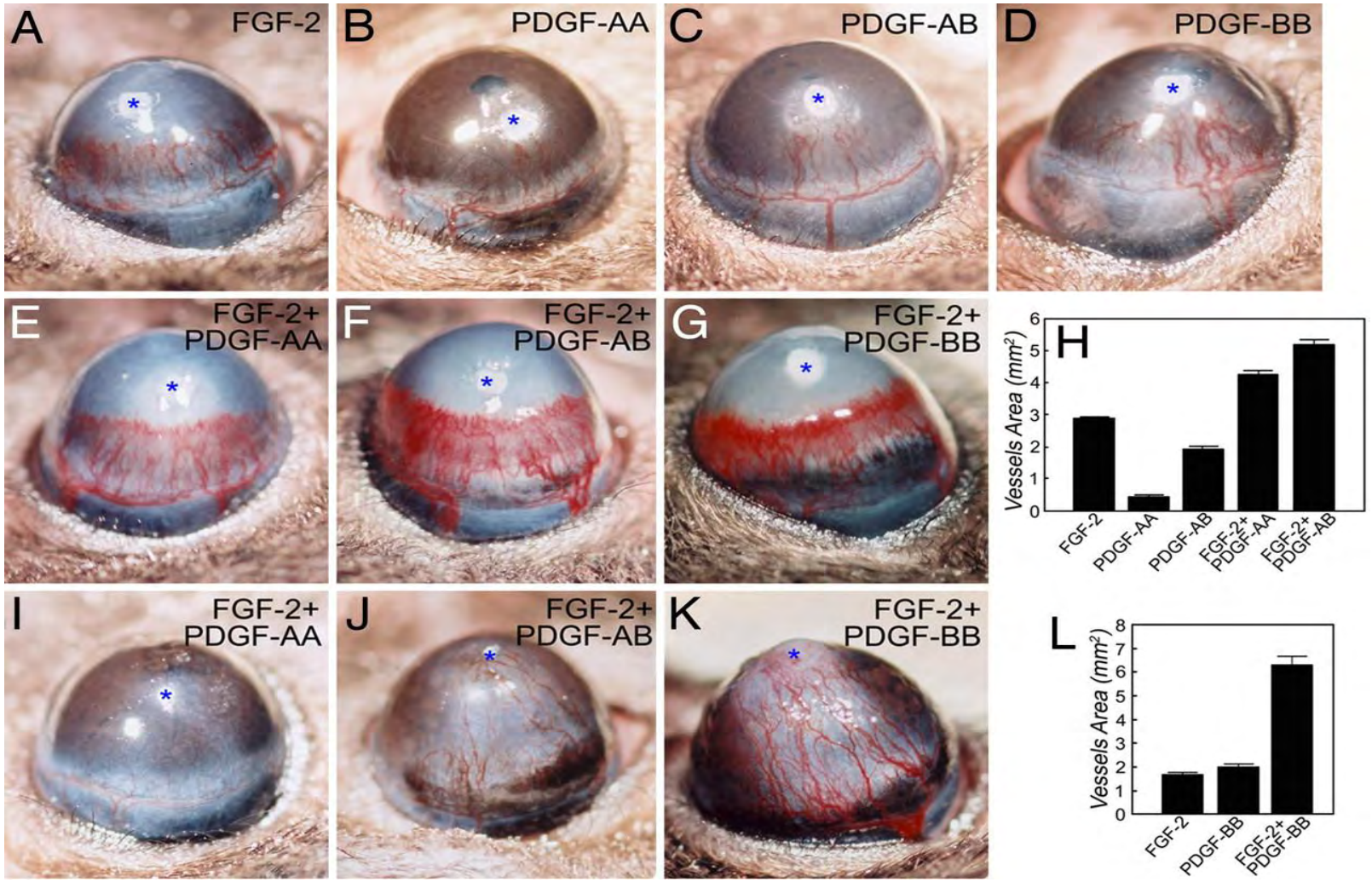
Korf-Klingebiel, M. et al. Circulation 2011;123:504-514

FGF9 reduces interstitial fibrosis and reduces heart failure mortality after MI



Korf-Klingebiel, M. et al. Circulation 2011;123:504-514

Combinatory approaches for angiogenesis



Lu H et al. PNAS 2007;104:12140-12145

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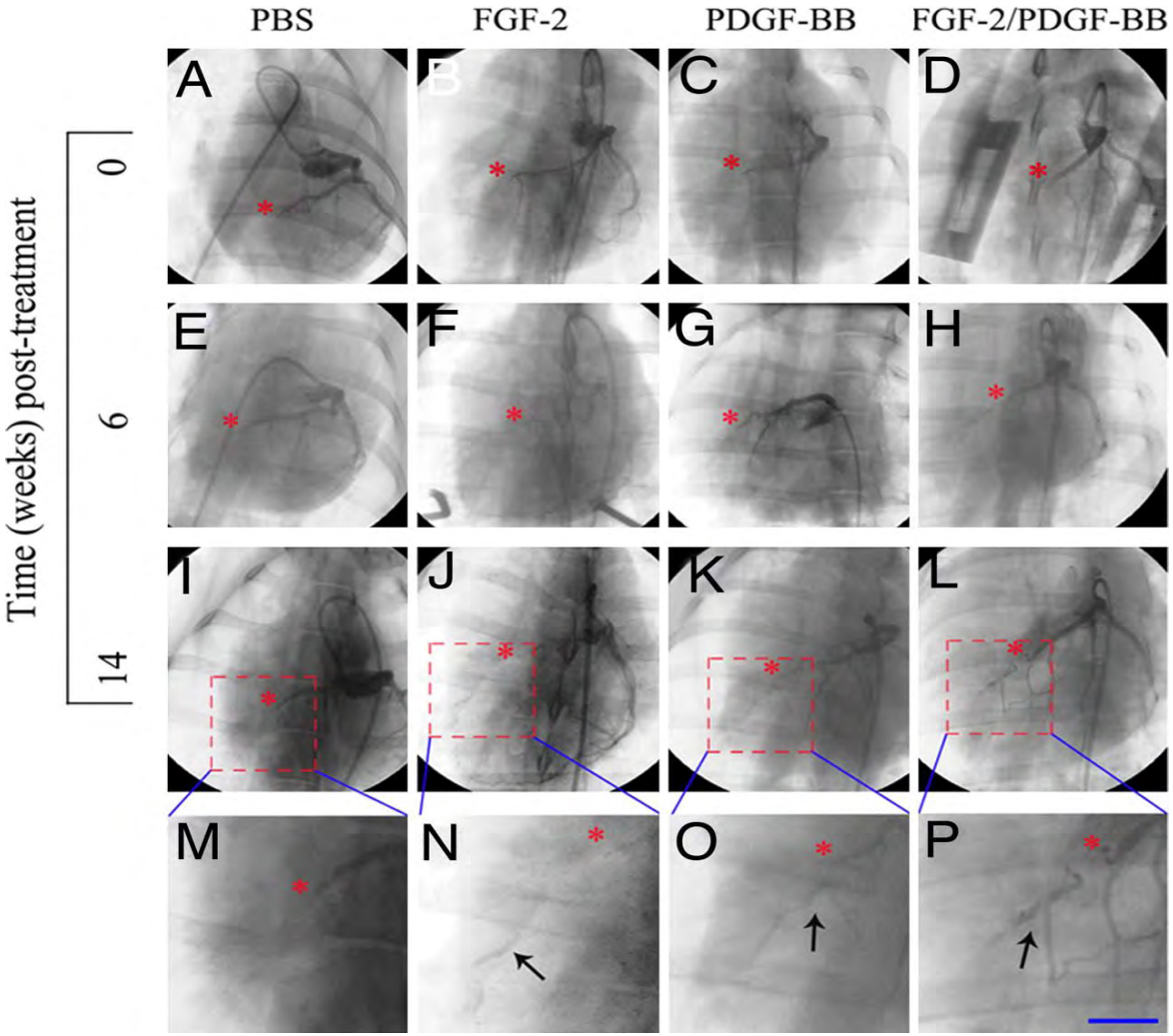
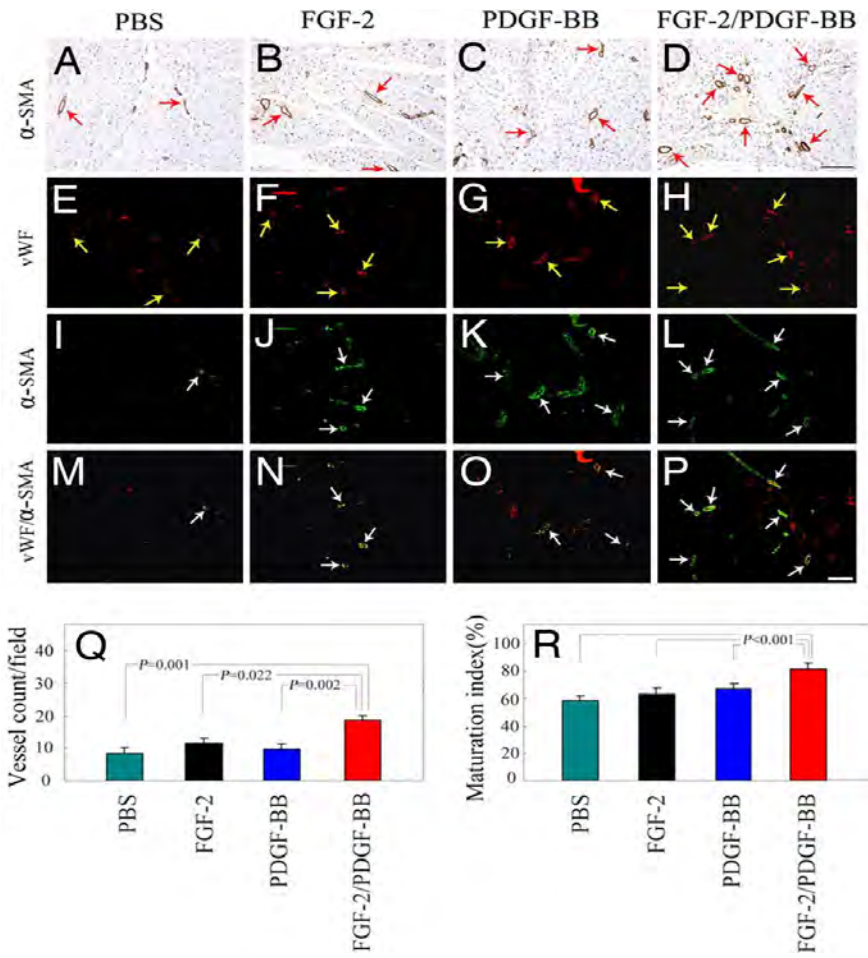
This looks promising as it works in a large animal model





Lu H et al. PNAS 2007;104:12140-12145

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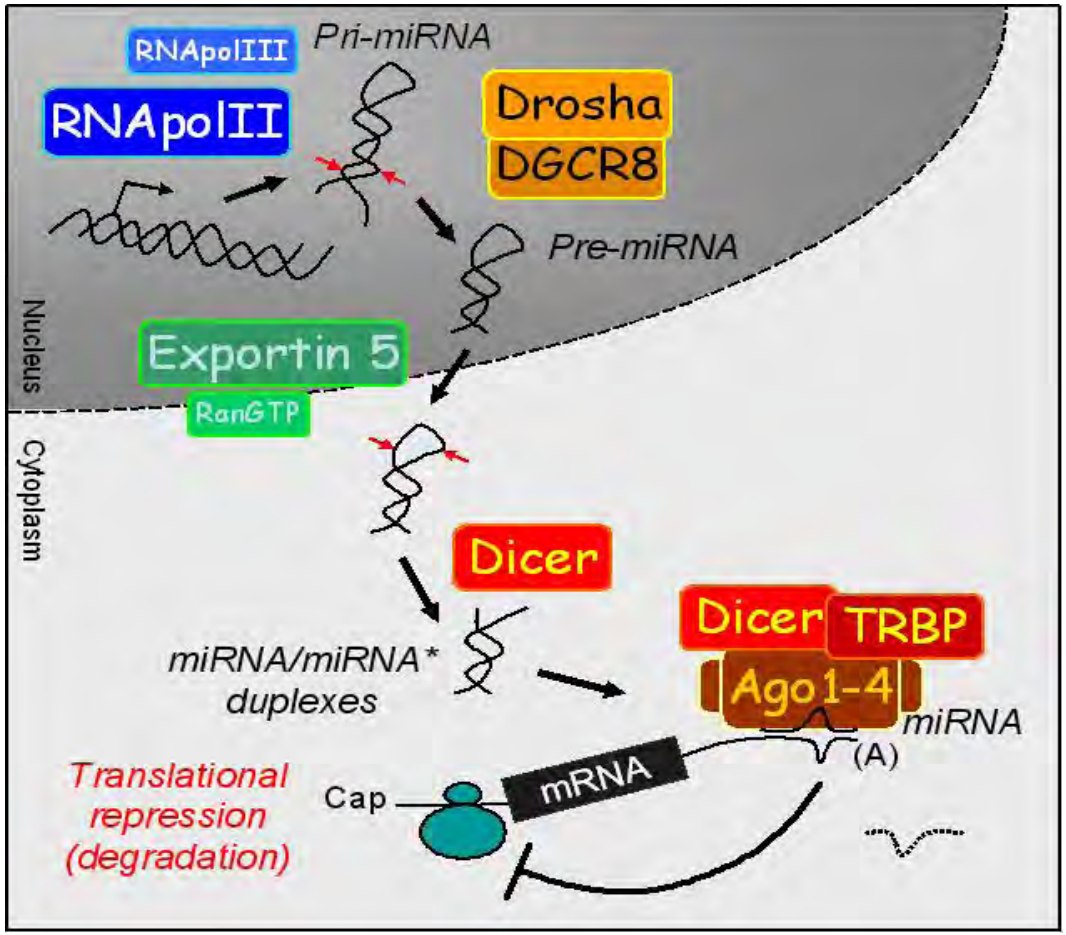
microRNAs in post-ischaemic vascular repair

microRNAs (miRNAs): basic things to know



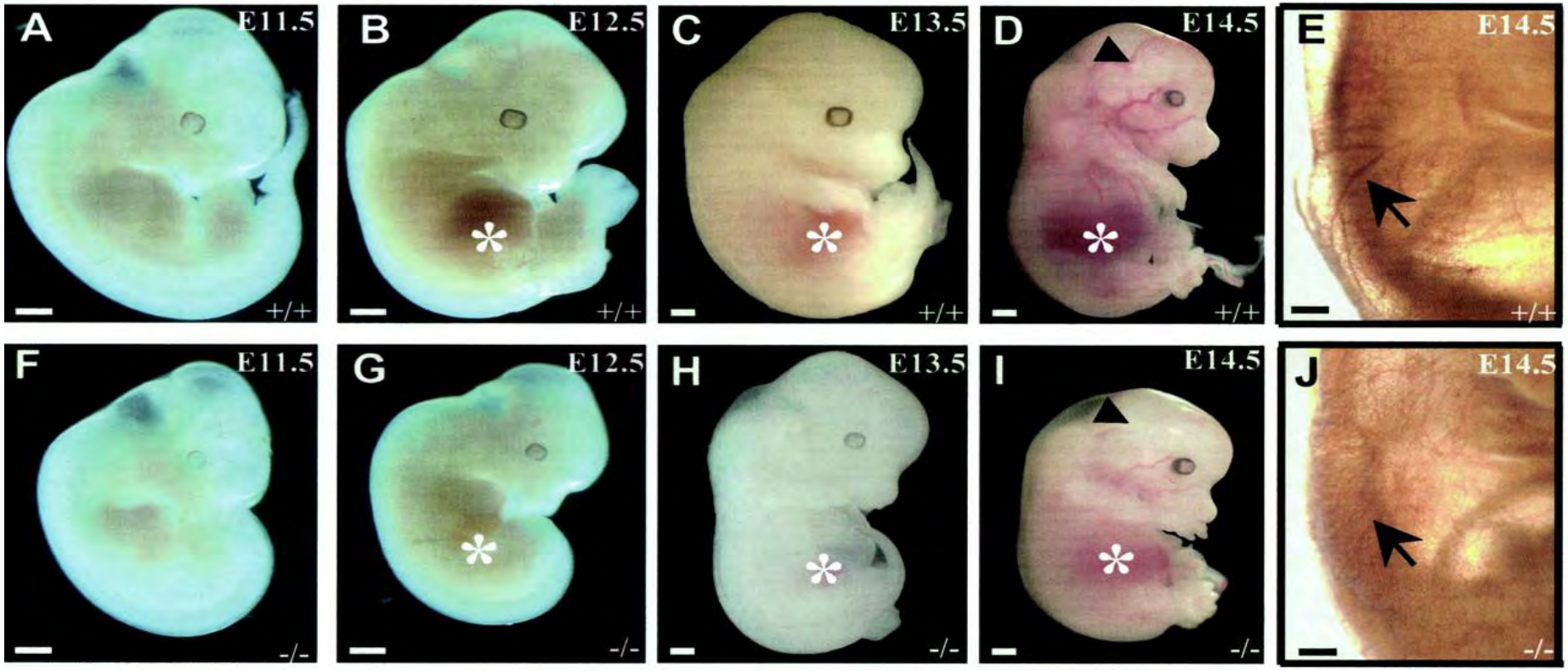
miRNAs are endogenous, small, non-coding RNAs, which negatively control gene expression of target genes.

miRNAs are either produced as pri-miRNAs by miRNA genes or by intragenic sequences of protein genes.
Often miRNAs are clustered in polycistronic units that generate a large primary transcript containing more than one miRNAs.

miRNAs are processed as described in left figure
Mature miRNAs act of target mRNAs for degradation and/or translational repression. miRNAs binding sites usually lie in 3'UTR of target mRNA and exhibit imperfect or perfect complementarity

Evidences for a regulatory role of miRNAs in angiogenesis

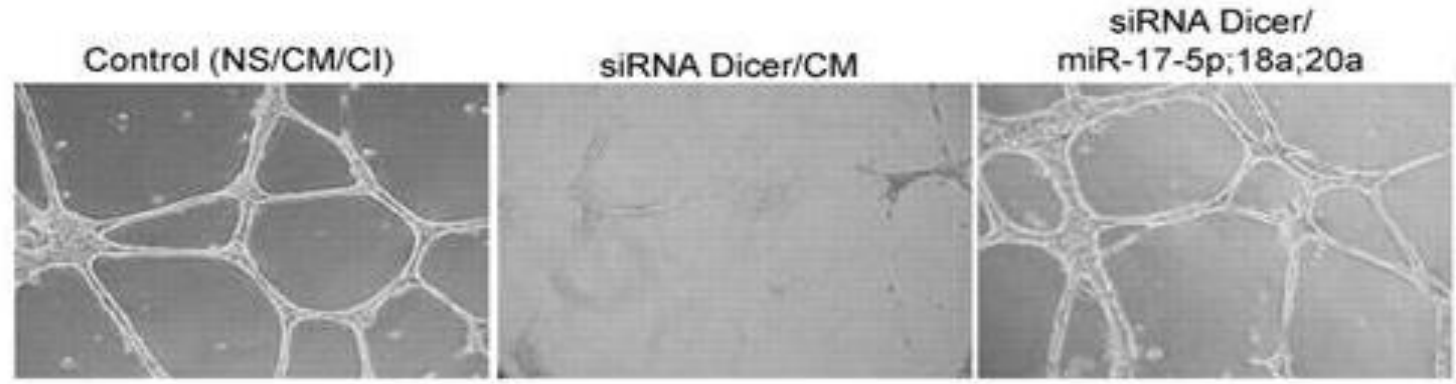
Retarded development and defective angiogenesis in dicerex1/2 embryos



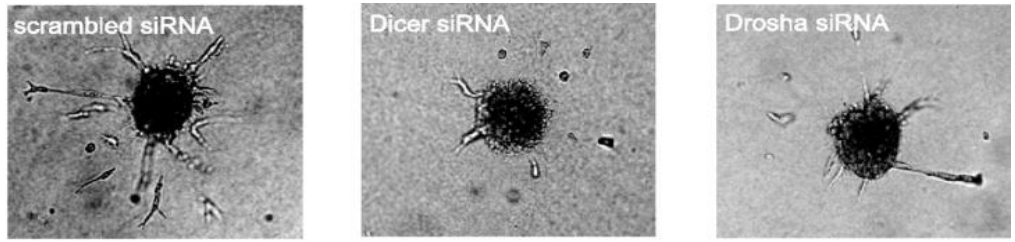
Dicer is responsible for the generation of short interfering RNAs (siRNAs) from long double-stranded RNAs. It is also involved in miRNA maturation

The first evidence implicating miRNAs in the regulation of angiogenesis came from analysis of mice homozygous for a hypomorphic allele of *Dicer*. Angiogenesis was dramatically impaired and mice died between days 12.5 and 14.5 of gestation

Yang W J et al. J. Biol. Chem. 2005;280:9330-9335

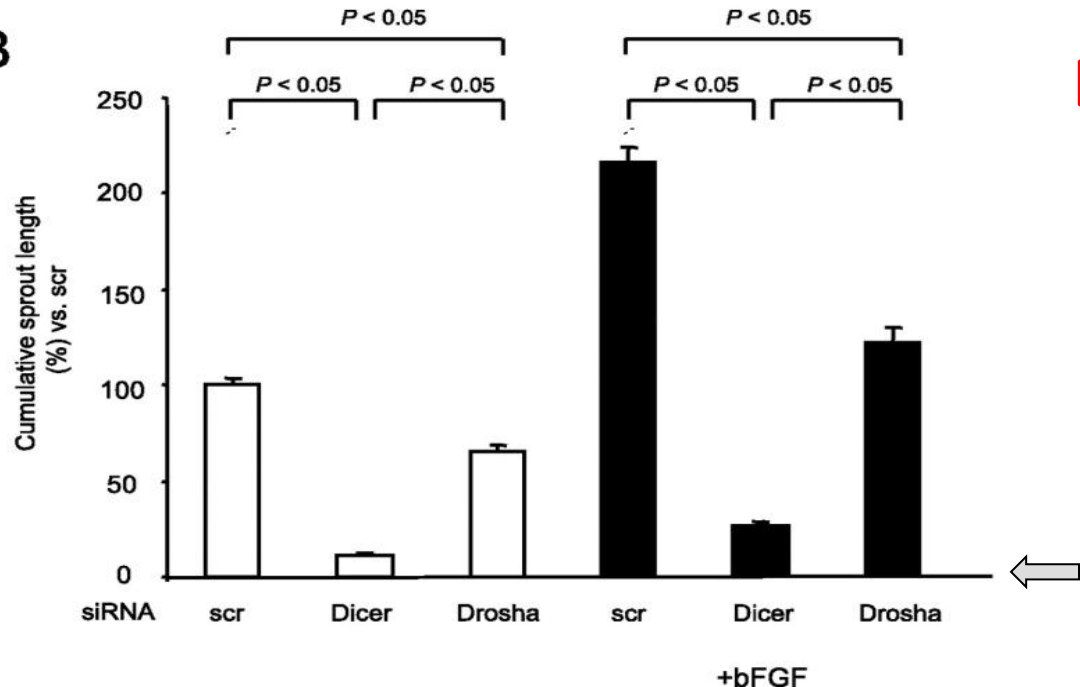


A



Suárez Y et al. PNAS 2008;105:14082-14087

B

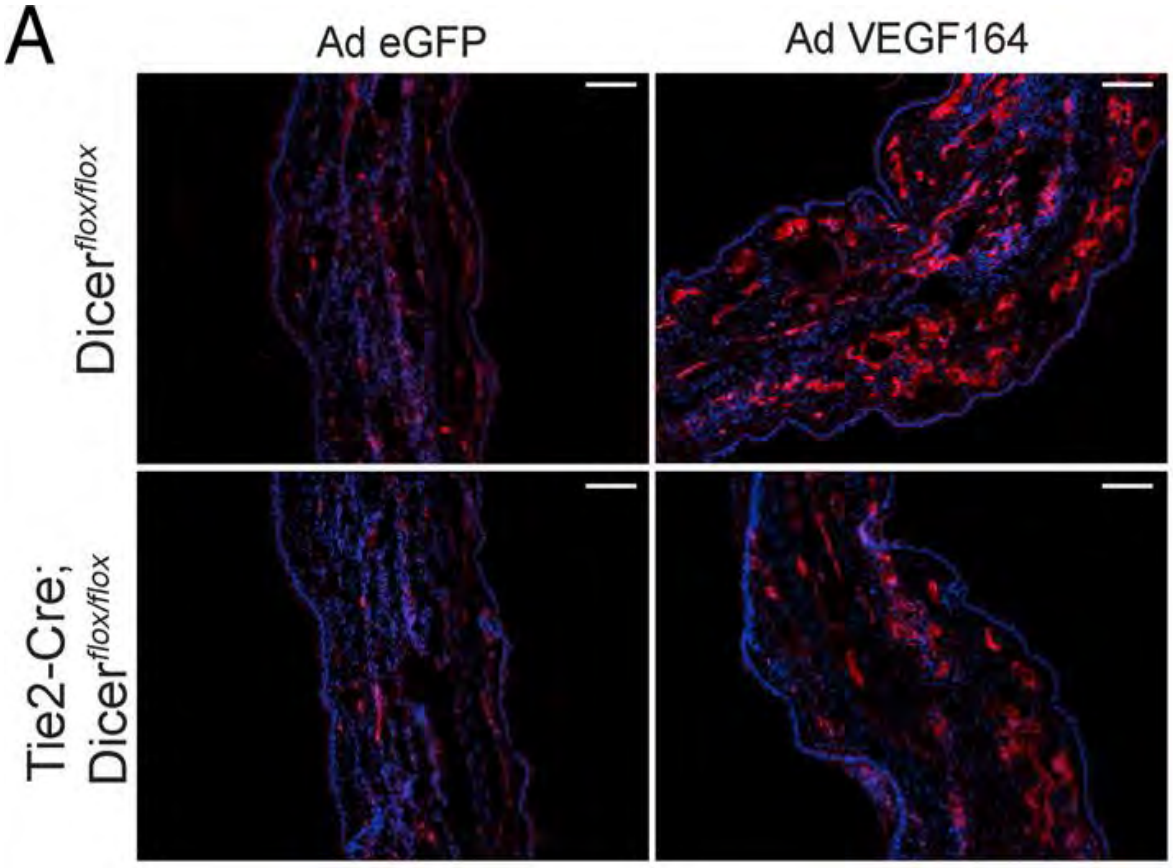


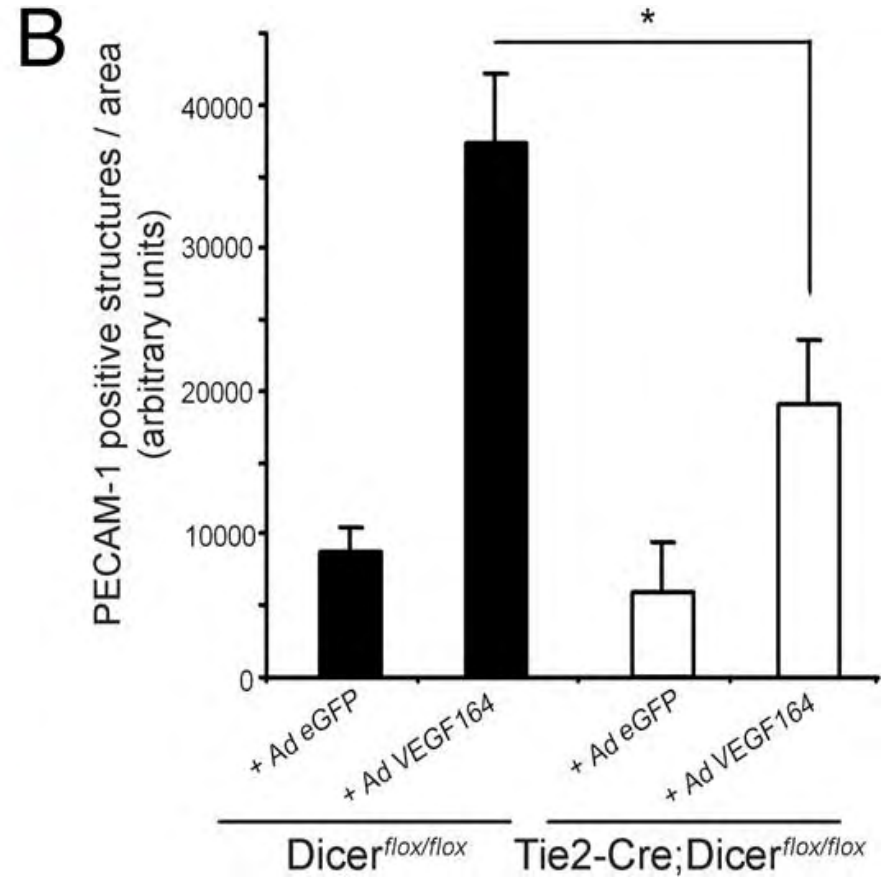
Drosha or Dicer inactivation impairs angiogenesis from HUVECs

Kuehbacher A et al. Cir Res 2007

Endothelial-selective Dicer inactivation reduces VEGF-induced angiogenesis in vivo

Tie2-Cre;Dicer*flox/flox* mice (control: Dicer*flox/flox* mice)

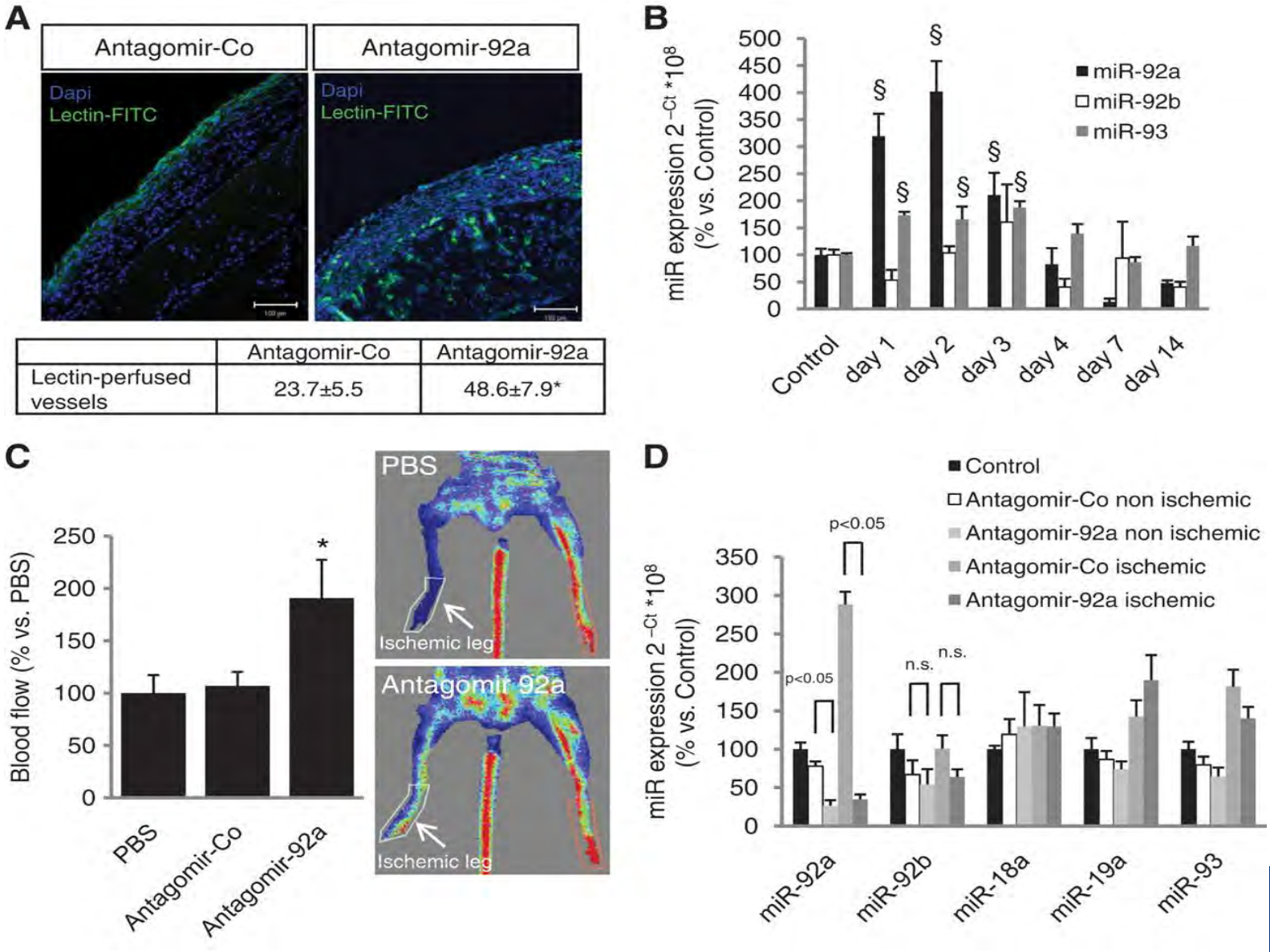




Ad.eGFP and Ad.VEGF-A injected in mouse ears.
PECAM staining (red) of endothelial cells

Suárez Y et al. PNAS 2008;105:14082-14087

Inhibition of miR-92a enhances angiogenesis in mice.

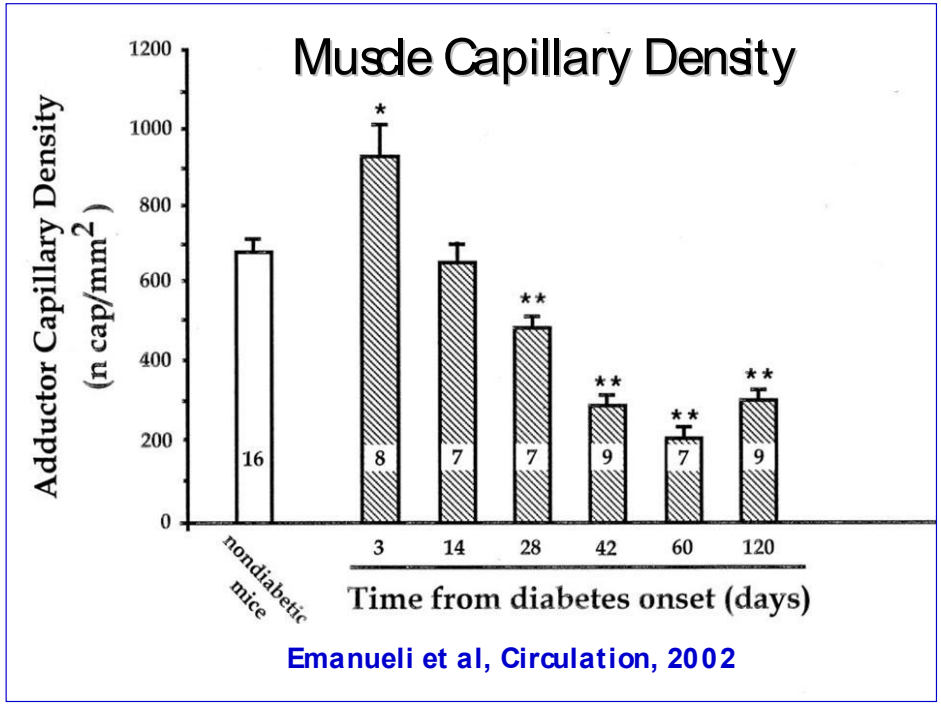


	Antagomir-Co	Antagomir-92a
Lectin-perfused vessels	23.7±5.5	48.6±7.9*

A Bonauer et al. Science 2009;324:1710-1713

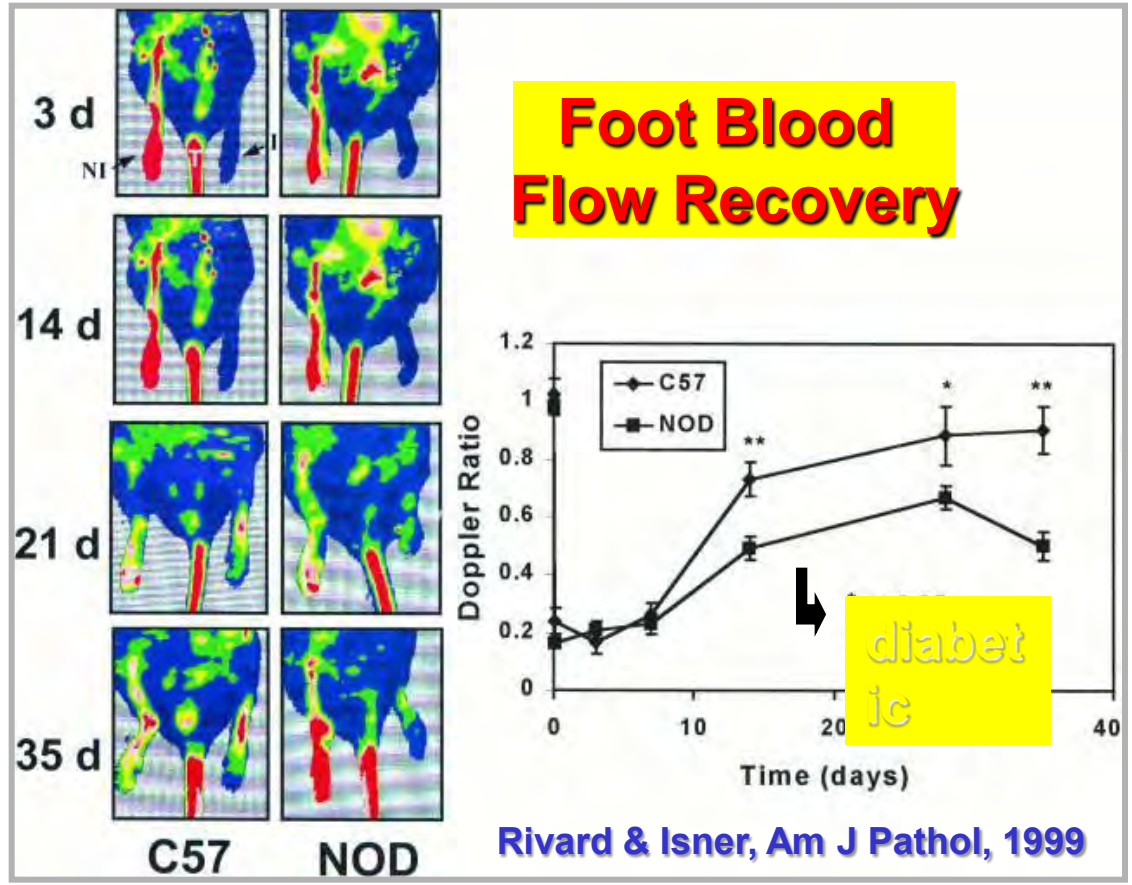
Science
AAAS

Are microRNAs involved with diabetes endothelial dysfunction and limb ischaemia?



Emanueli et al, Circulation, 2002

Microangiopathy



Rivard & Isner, Am J Pathol, 1999

Impaired post-ischaemic angiogenesis and blood flow recovery

Circulation 2011
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Molecular Cardiology

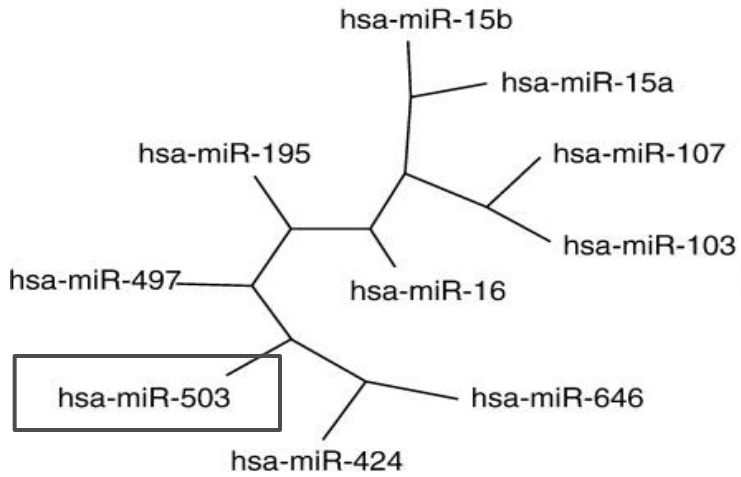
Deregulation of microRNA-503 Contributes to Diabetes Mellitus–Induced Impairment of Endothelial Function and Reparative Angiogenesis After Limb Ischemia

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 Madeddu, MD; Fabio Martelli, PhD; Costanza Emanueli, PhD

miR-503

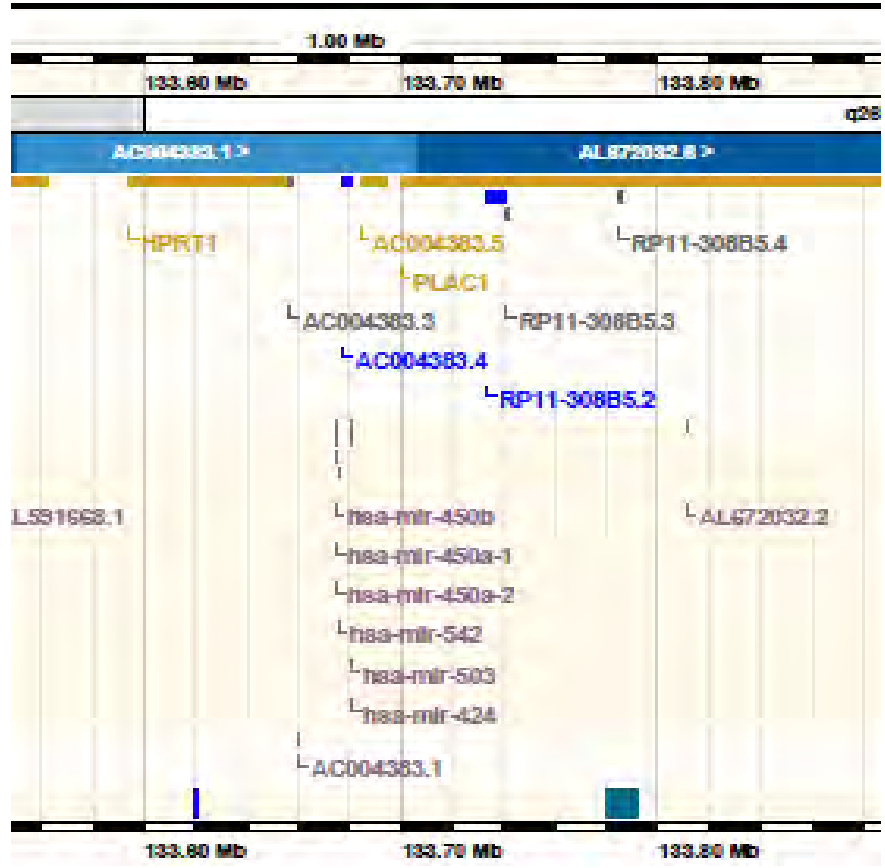
Member of miR-15/107 group:
AGCAGC seed sequence

hsa-miR-107	AGCAGC	AUUGUACAGGGCUAUCA
hsa-miR-103	AGCAGC	AUUGUACAGGGCUAUGA
hsa-miR-15a U	AGCAGC	ACAUAAUGGUUUGUG
hsa-miR-15b U	AGCAGC	ACAUCAUGGUUUACA
hsa-miR-16 U	AGCAGC	ACGUAAAUAUUGGCG
hsa-miR-195 U	AGCAGC	ACAGAAAUAUUGGCC
hsa-miR-497 C	AGCAGC	ACACUGUGGUUUGU
hsa-miR-503 U	AGCAGC	GGGAACAGUUCUGCAG
hsa-miR-424 C	AGCAGC	AAUUCAUGUUUUGAA
hsa-miR-646 A	AGCAGC	UGCCUCUGAGGC



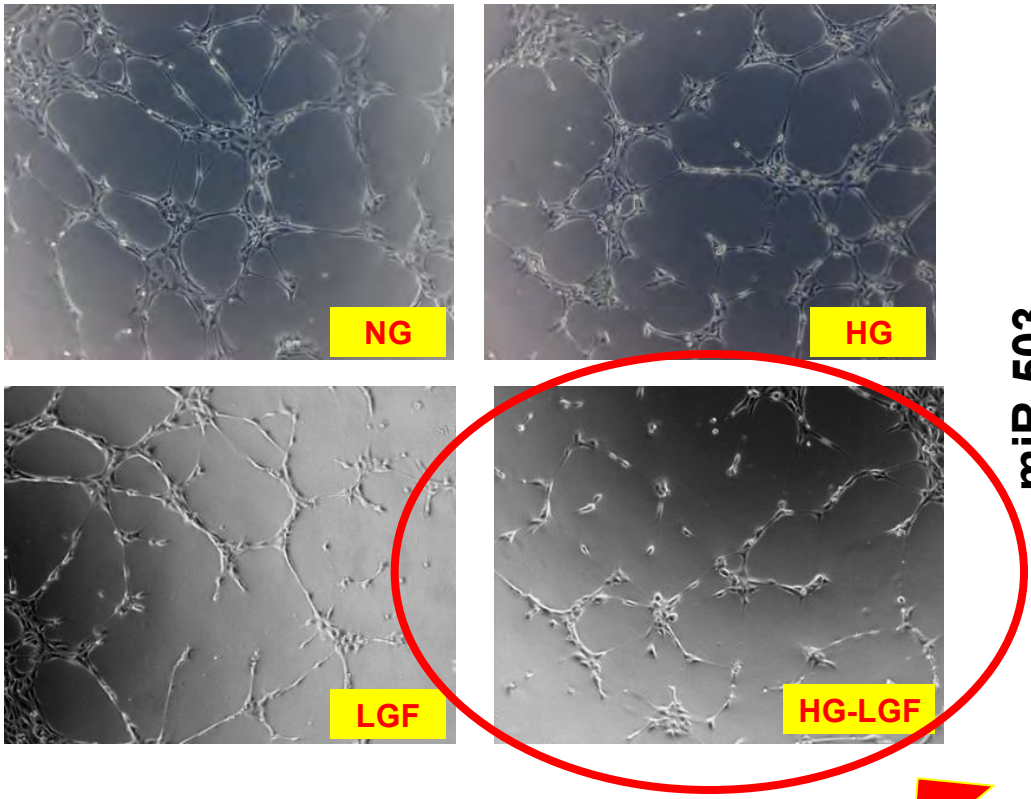
Finnerty JR et al. JMB 2010

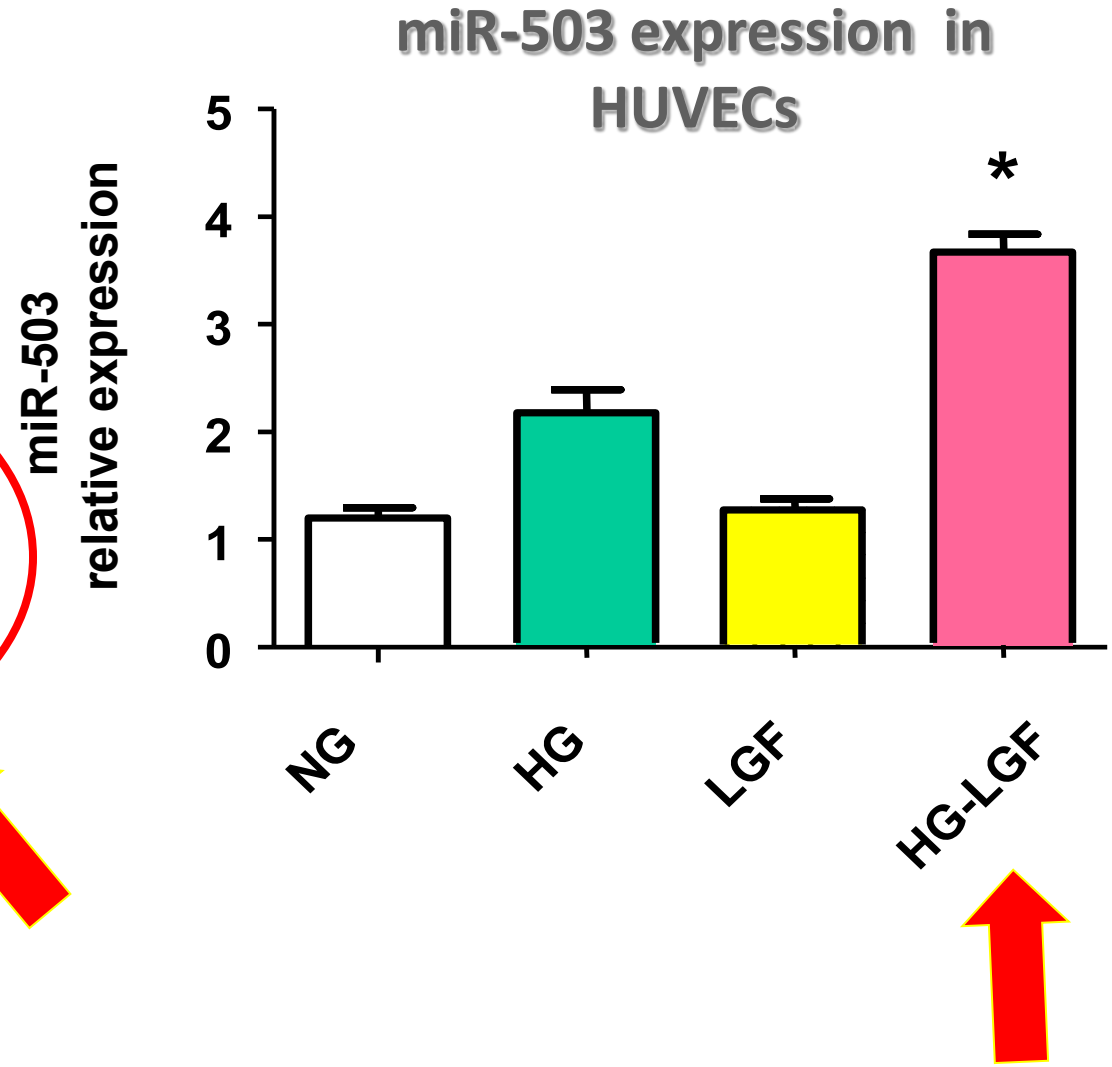
Chr X; intergenic
Cluster: miR-424-503



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

miR-503 expression in ECs is increased by culture conditions mimicking hyperglycemia and ischaemia





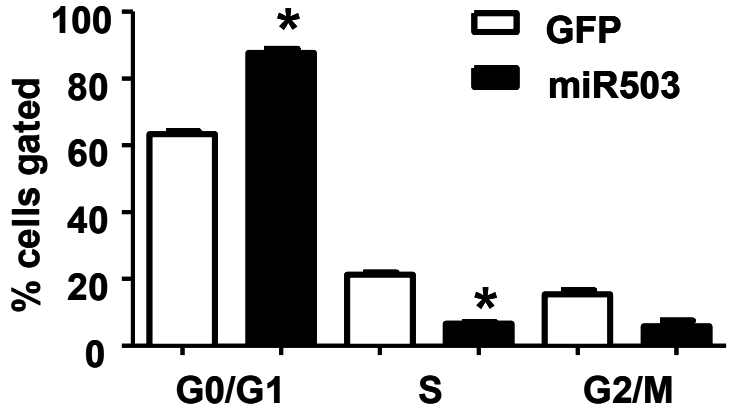
NG= normal glucose, 5 mM

HG= high glucose, 25 mM

LGF = reduced growth factors

Consequences of miR-503 overexpression in endothelial cells

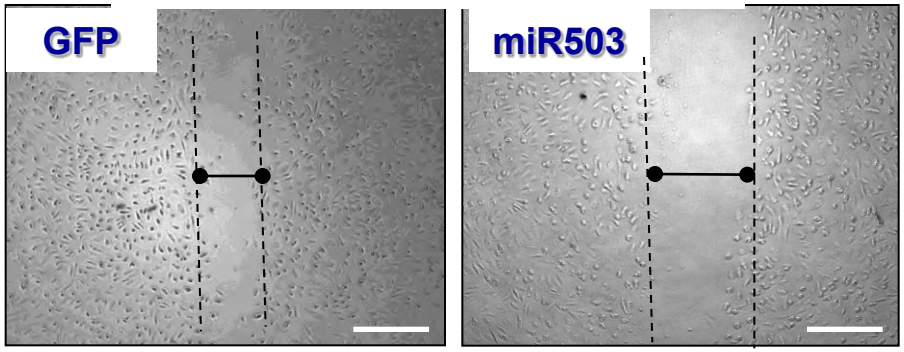
Impaired EC cycle



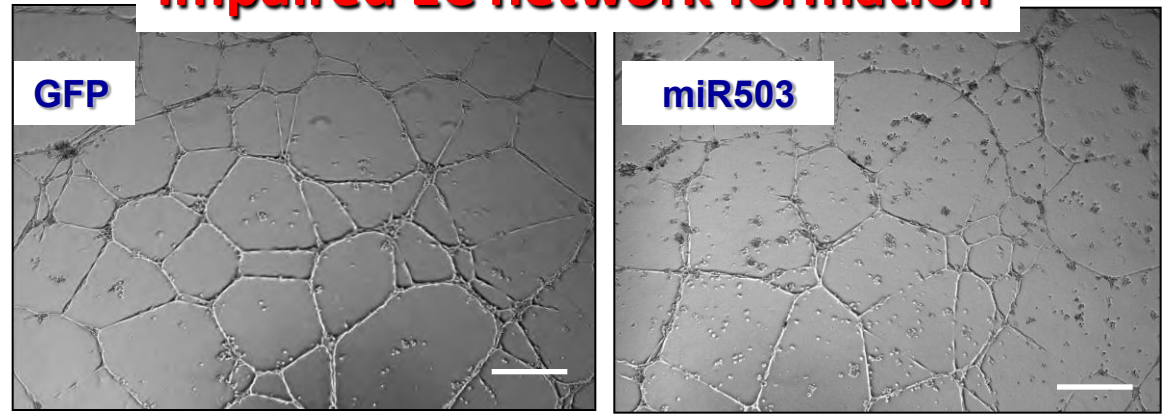
miR-503 target genes

675-682 cdc25A 3'UTR 5' ...CGCUGUGGUACUGGGGCUGCUGCUAU...
has-miR-503 3' GACGUCUUGACAAGGGCGACGAU
248-254 CCNE1 3'UTR 5' ...GUGCGUGCUCCCGAUGCUGCUAU...
has-miR-503 3' GACGUCUUGACAAGGGCGACGAU
486-492 CCNE1 3'UTR 5' ...AACUGUUUUGUAAGUGCUGCUAU...
has-miR-503 3' GACGUCUUGACAAGGG-----CGACGAU

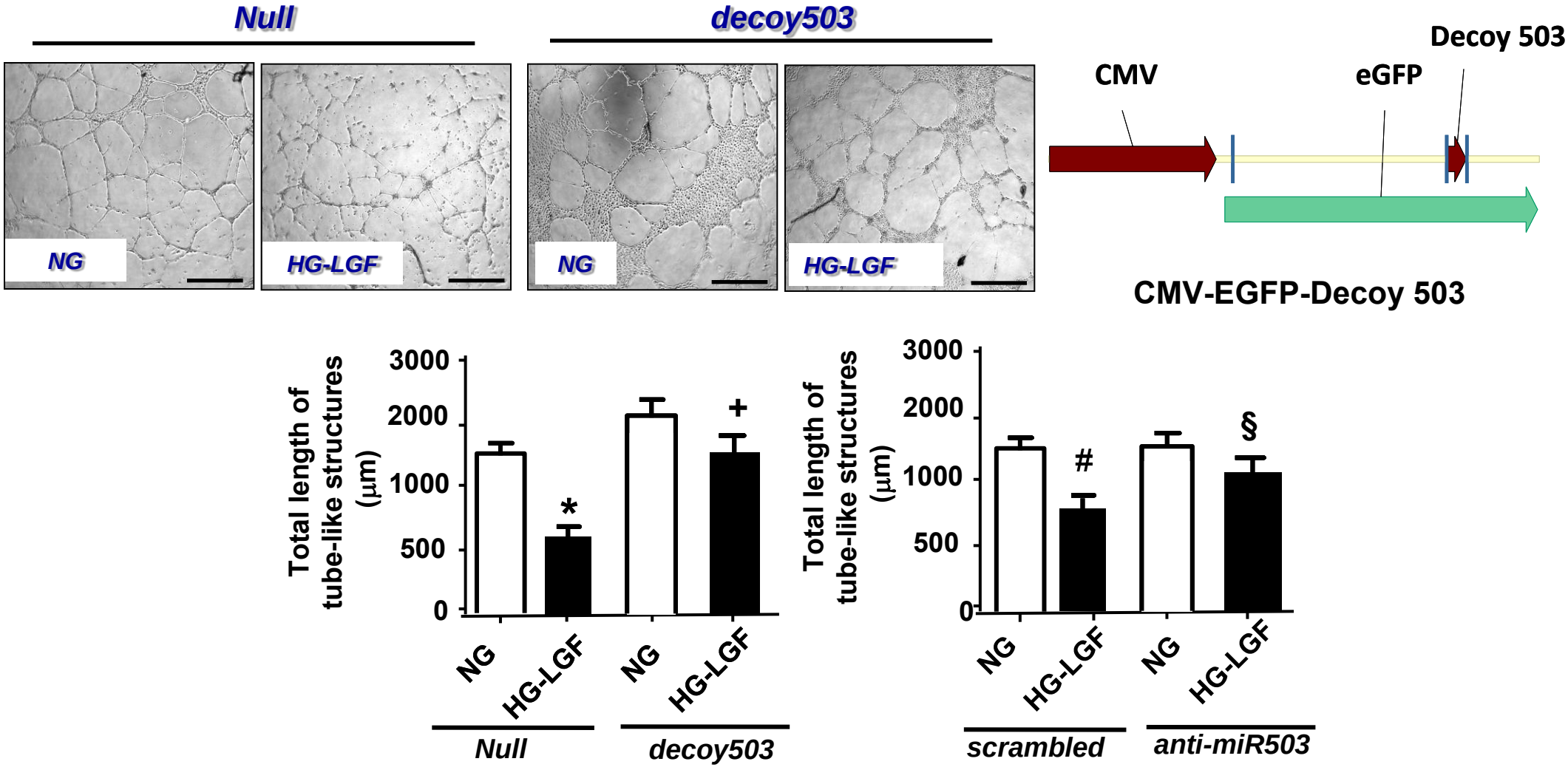
Impaired EC migration



Impaired EC network formation

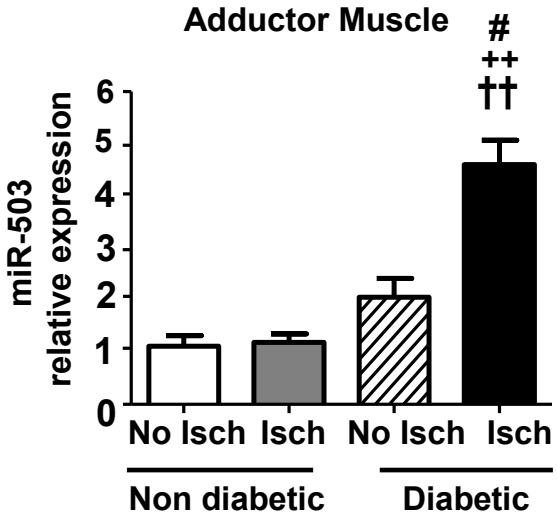


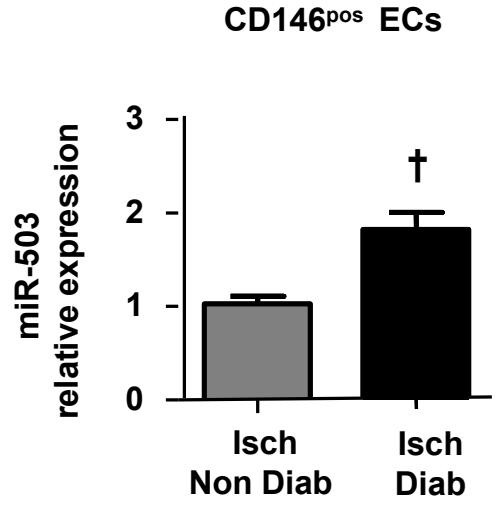
miR-503 inhibition improves angiogenesis in the presence of "diabetes" and "ischaemia" (*in vitro*)



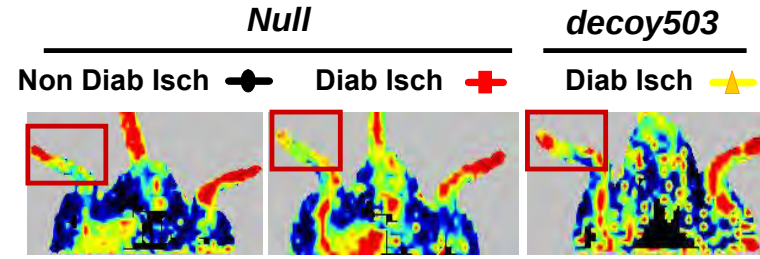
Local gene therapy with a decoy for miR-503 improves post-ischaemic angiogenesis in diabetic mice

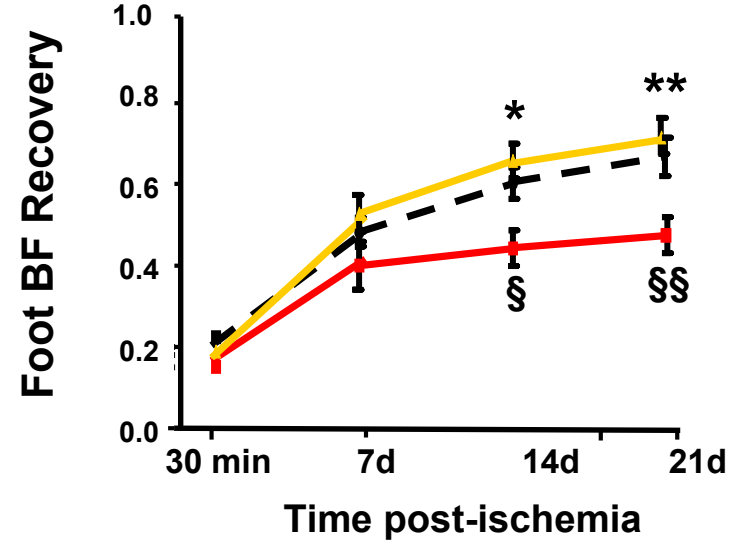
miR-503 expression



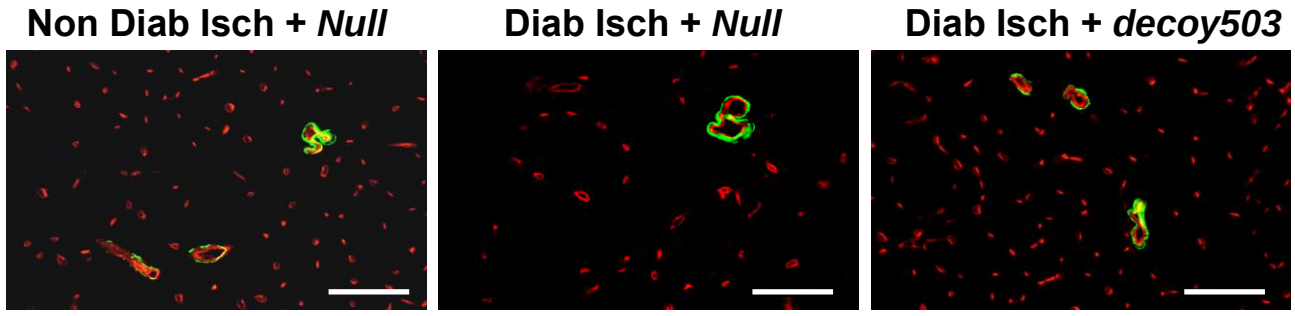


Blood flow recovery

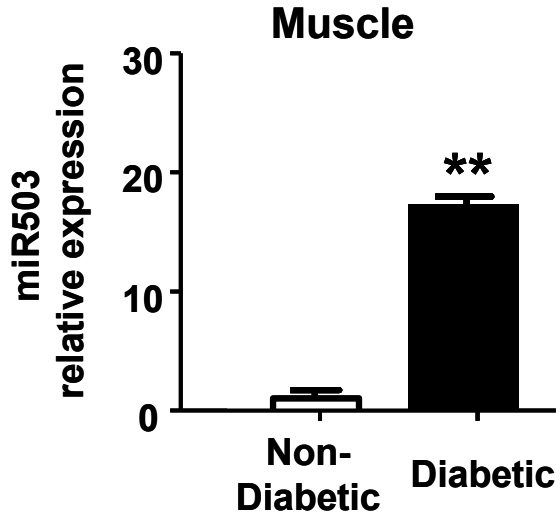


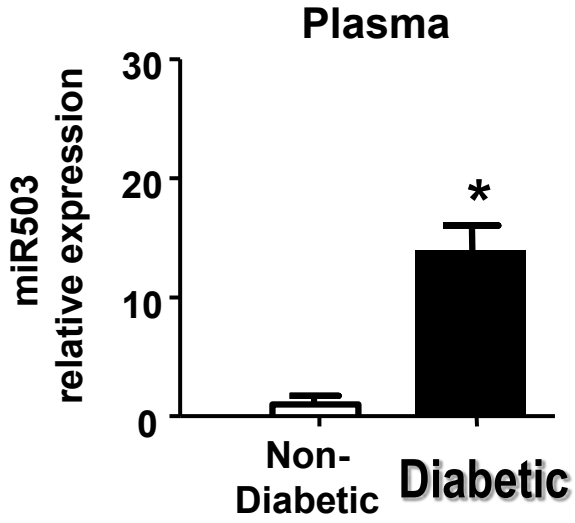


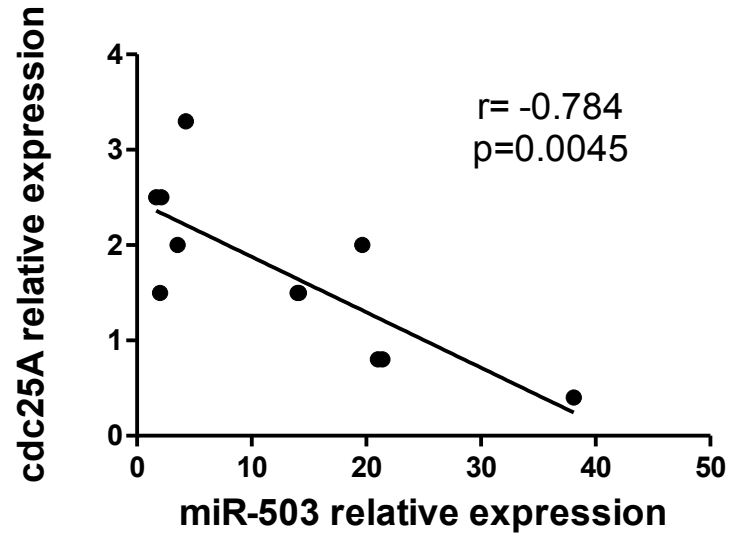
Limb microvessels

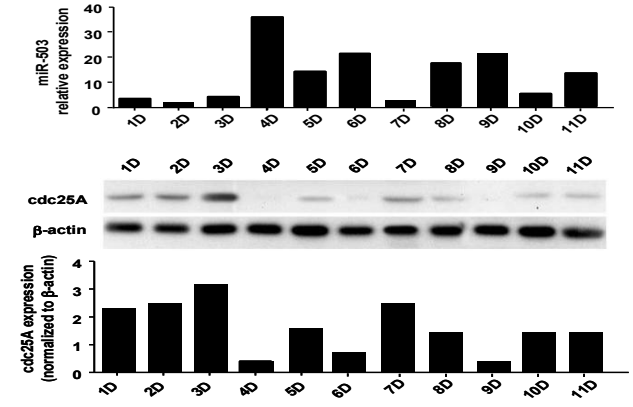


miR-503 expressional changes in diabetes and critical limb ischaemia



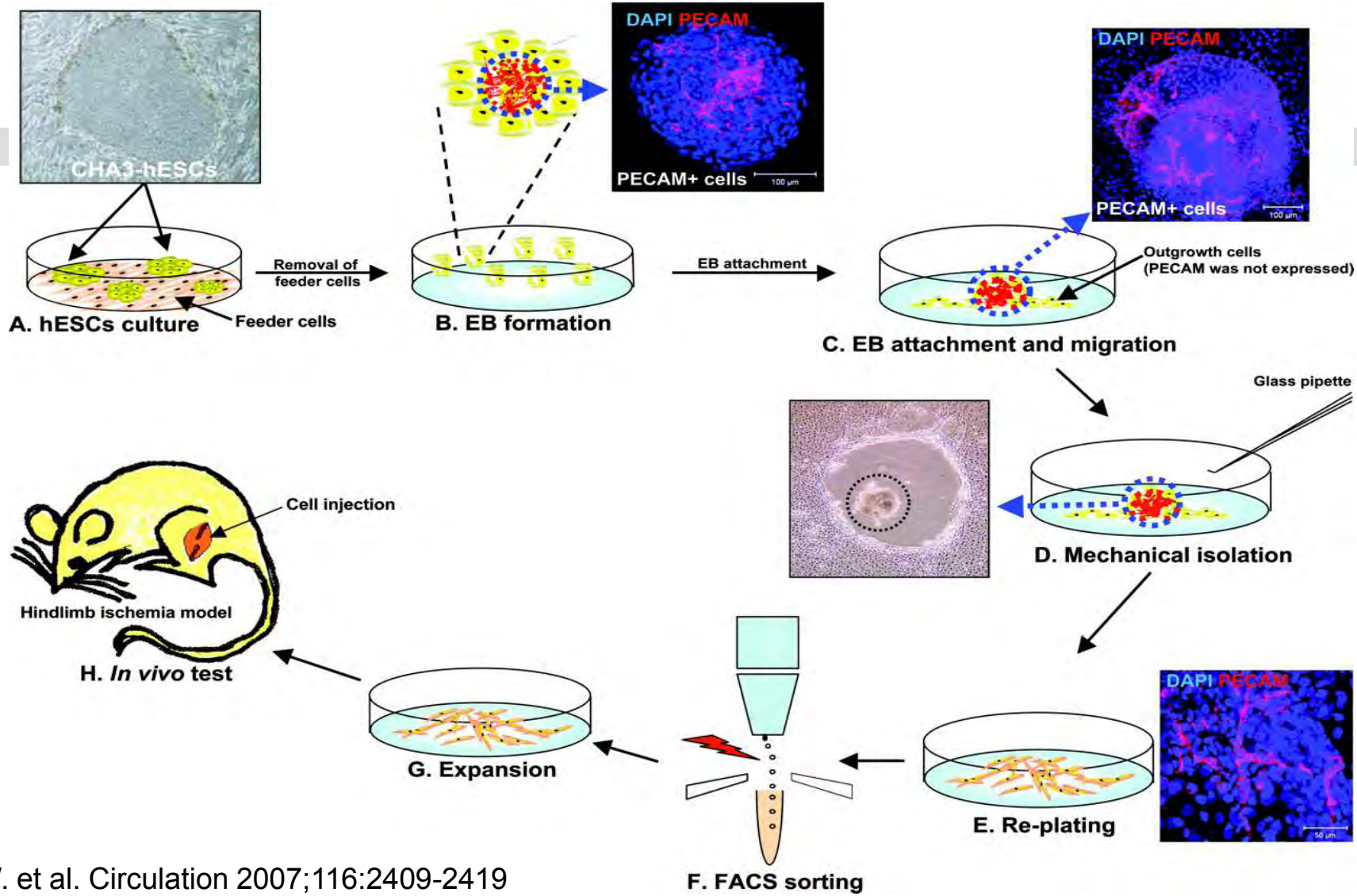






New cell therapy products

Initial methods for differentiating hES cells toward ECs



Cho, S.-W. et al. Circulation 2007;116:2409-2419

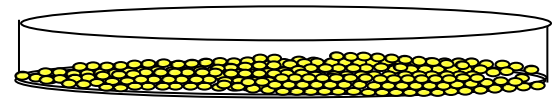
New Protocol for hESC Differentiation

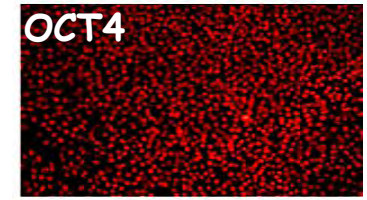
IN VITRO

Baker lab
Glasgow

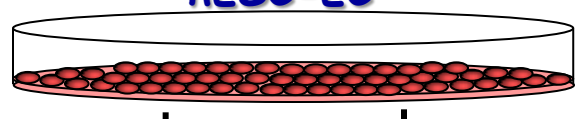
collaboration

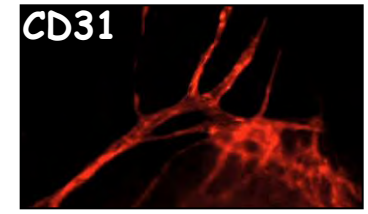
PLURIPOTENT hESC

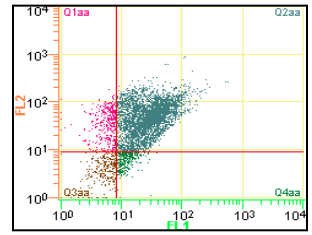


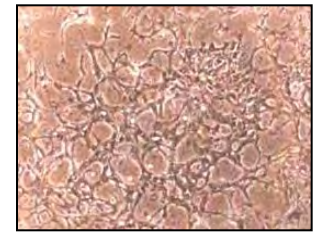


hESC-EC



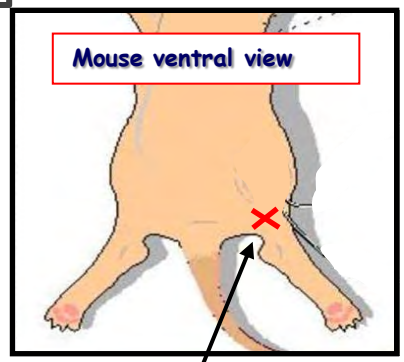




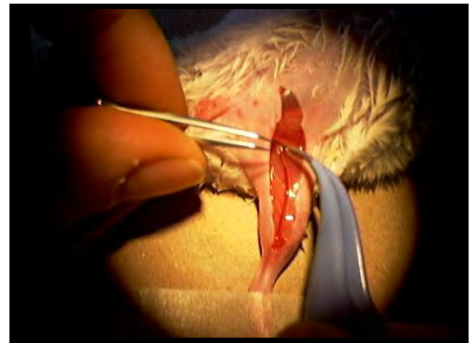


Emanueli lab Bristol

IN VIVO



Ligation of the left femoral artery

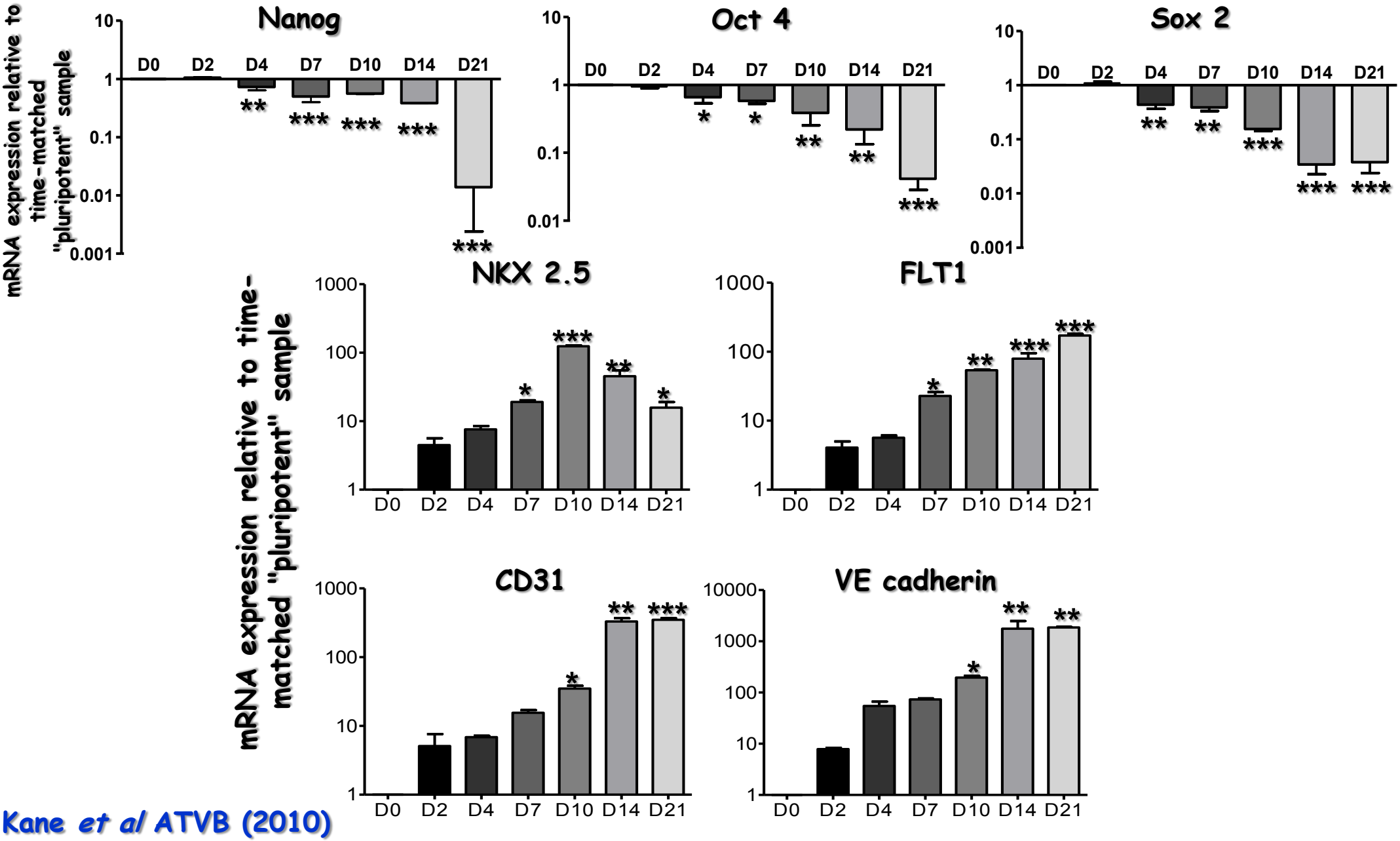


Measurement of foot perfusion (Laser Doppler) and clinical outcome

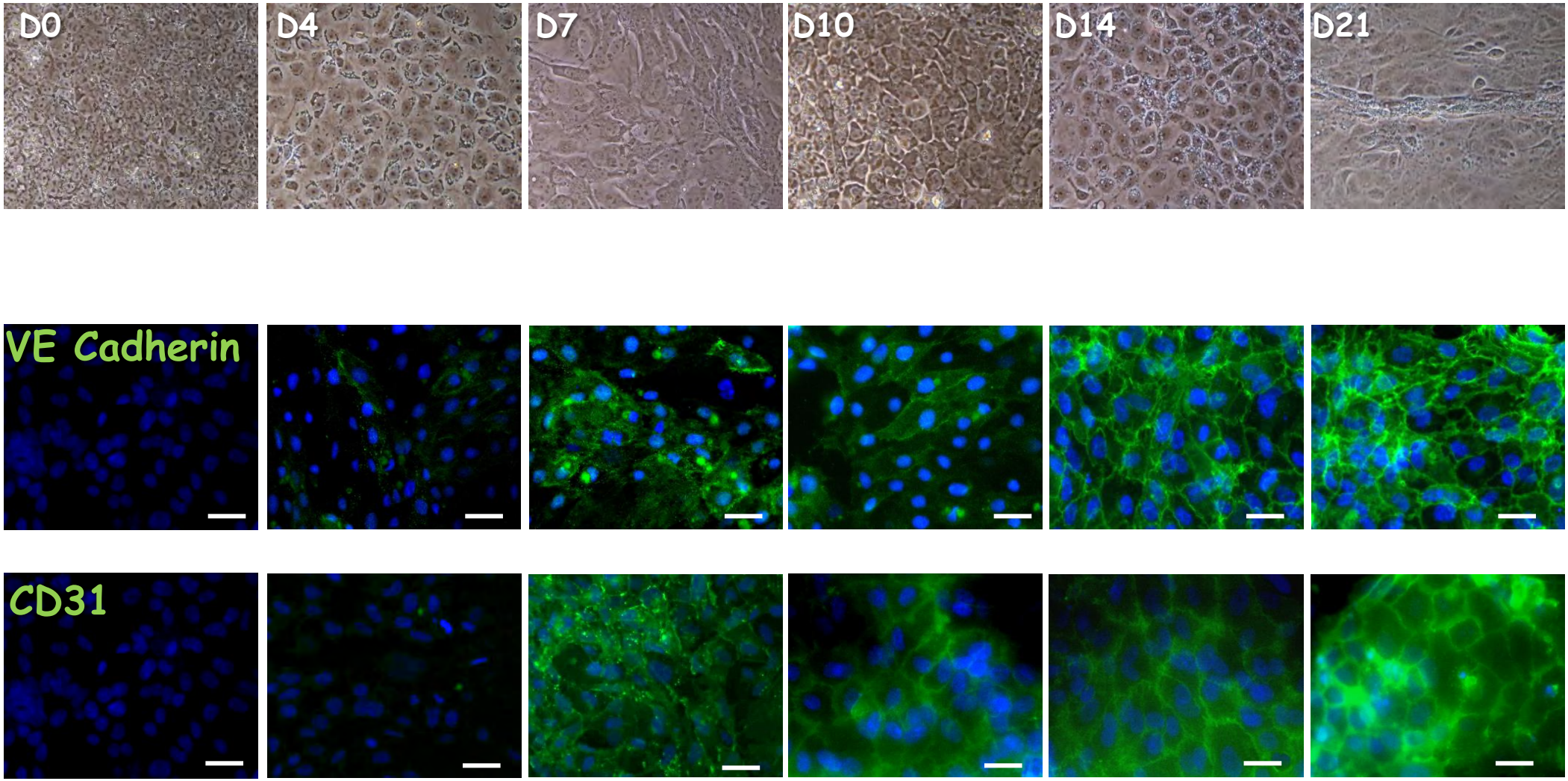
0 7 14

Sacrificing, perfusion/fixation and tissue collection

Differentiation toward EC evokes loss of pluripotency markers



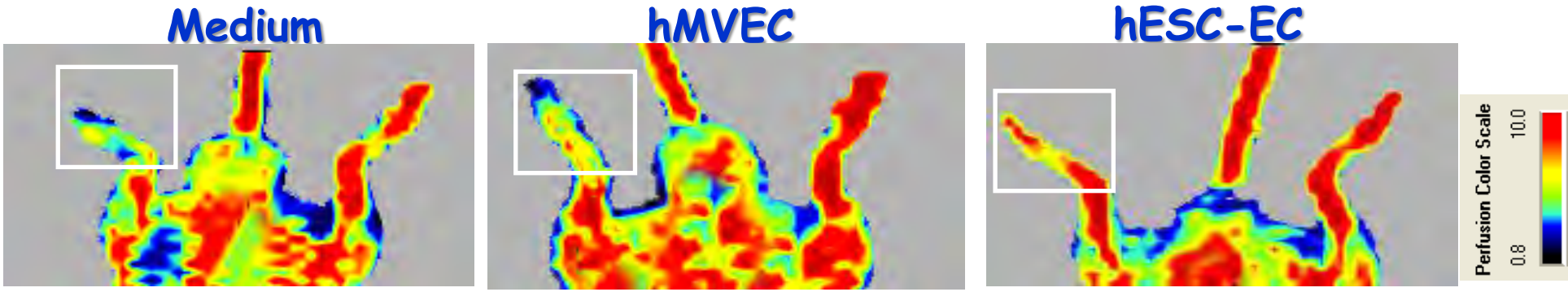
Kane *et al* ATVB (2010)

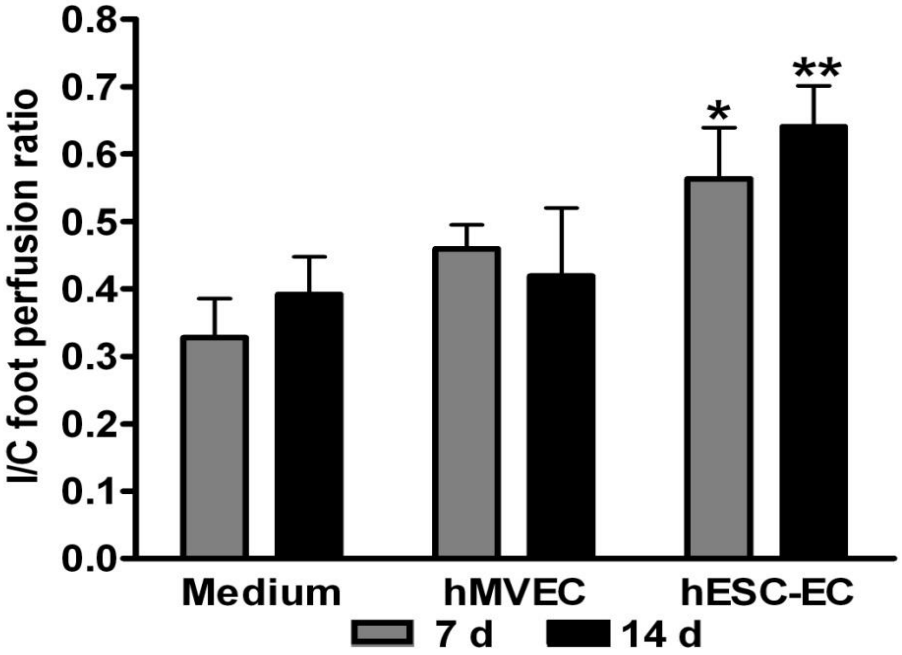


Kane *et al* ATVB (2010)

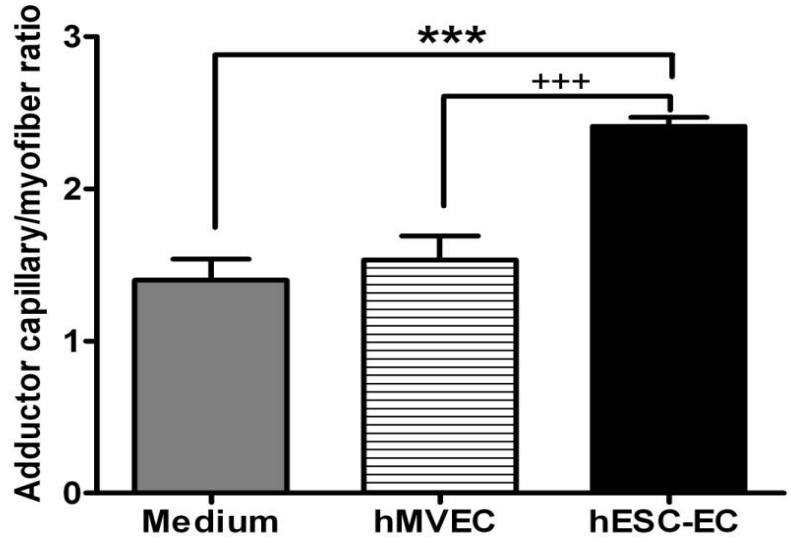
Scale bar = 20 μm

Injection of 10d hESC-EC cells improves blood flow to hindlimb ischemia model



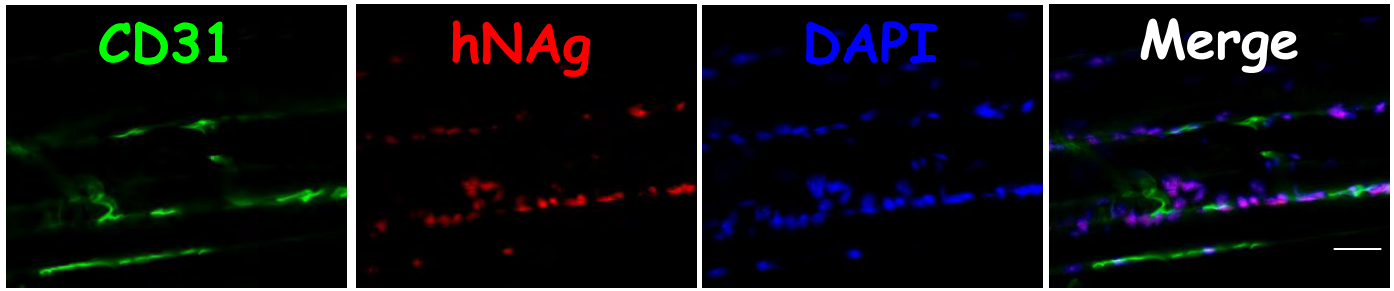


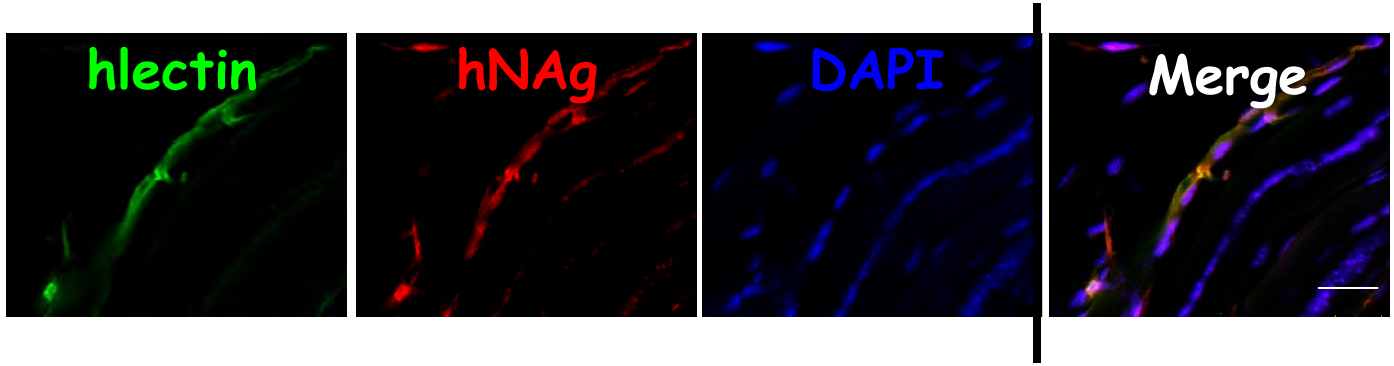
Increased foot perfusion ratio in hESC-EC, but not medium or MVEC

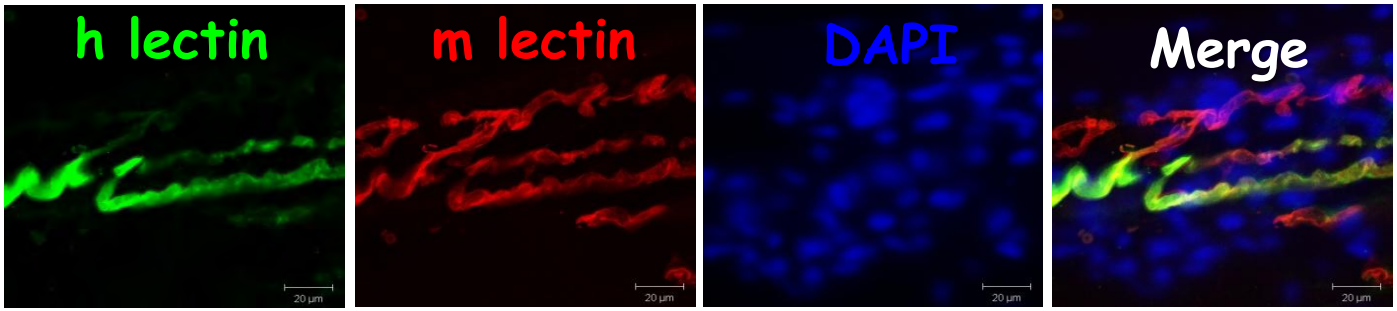


Increased neovascularisation with hESC-EC injection

Injection of 10d hESC-EC cells improves blood flow to hindlimb ischemia model





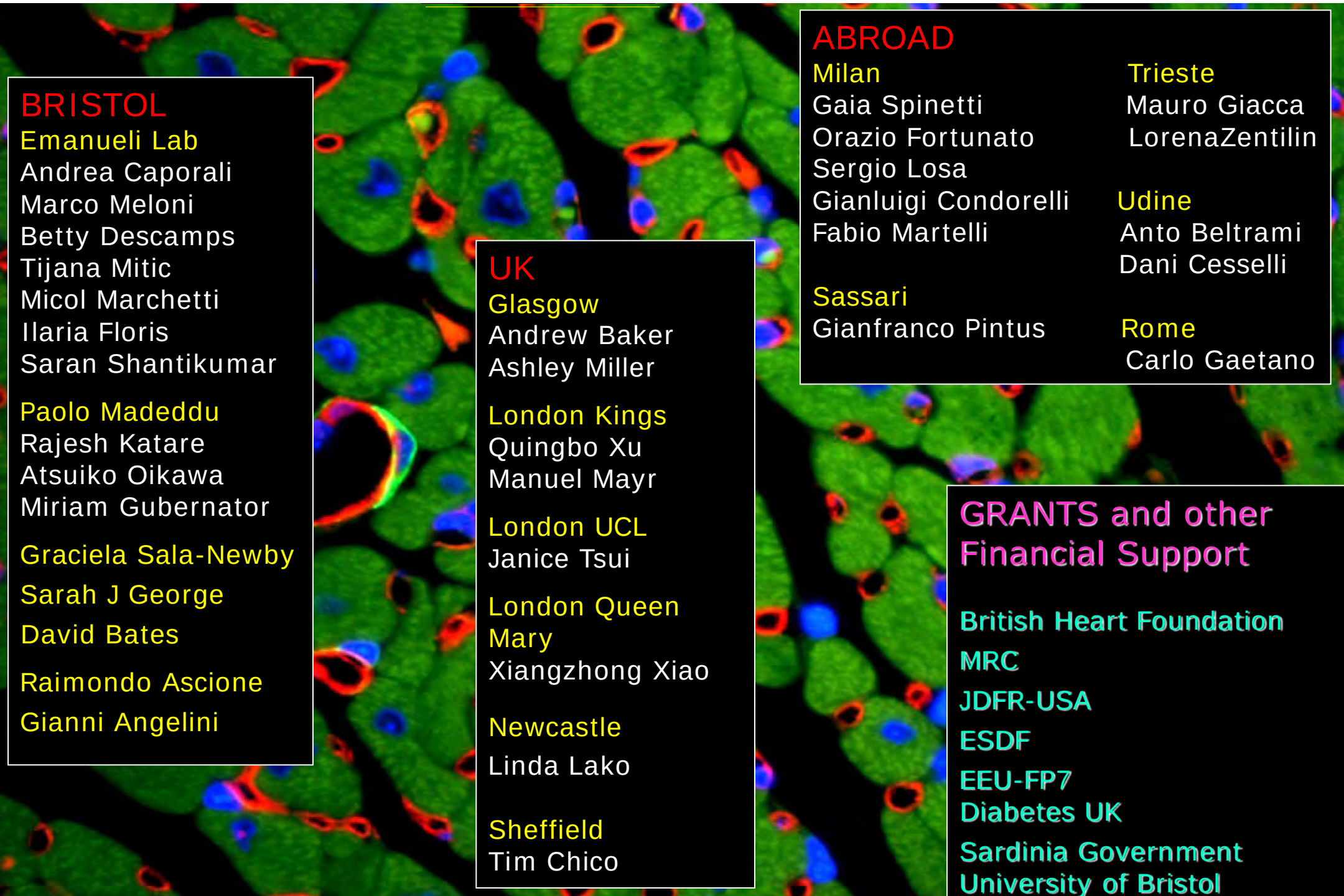


Incorporation of hESC-EC cells into CD31 +ve or hlectin +ve vessels in adductor muscle proximal to ischemia

Ischemia improvement is most likely due to cell engraftment within neovasculature

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.



BRISTOL

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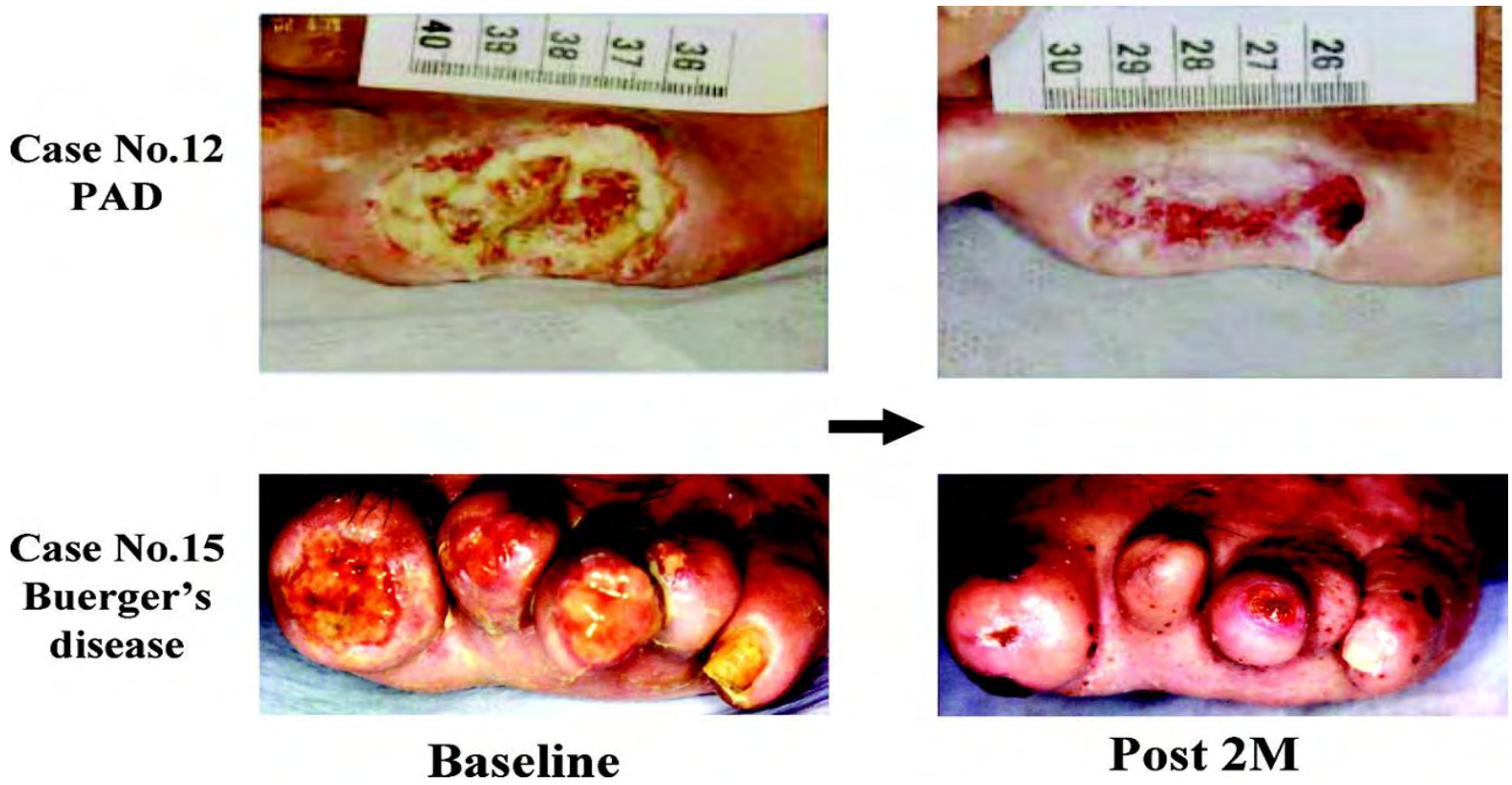
Diabetes UK
Sardinia Government
University of Bristol

Typical examples of changes in ischemic ulcers in patients: HGF gene therapy (phase I/II clinical trial)



Morishita, R. et al. Arterioscler Thromb Vasc Biol 2011;31:713-720

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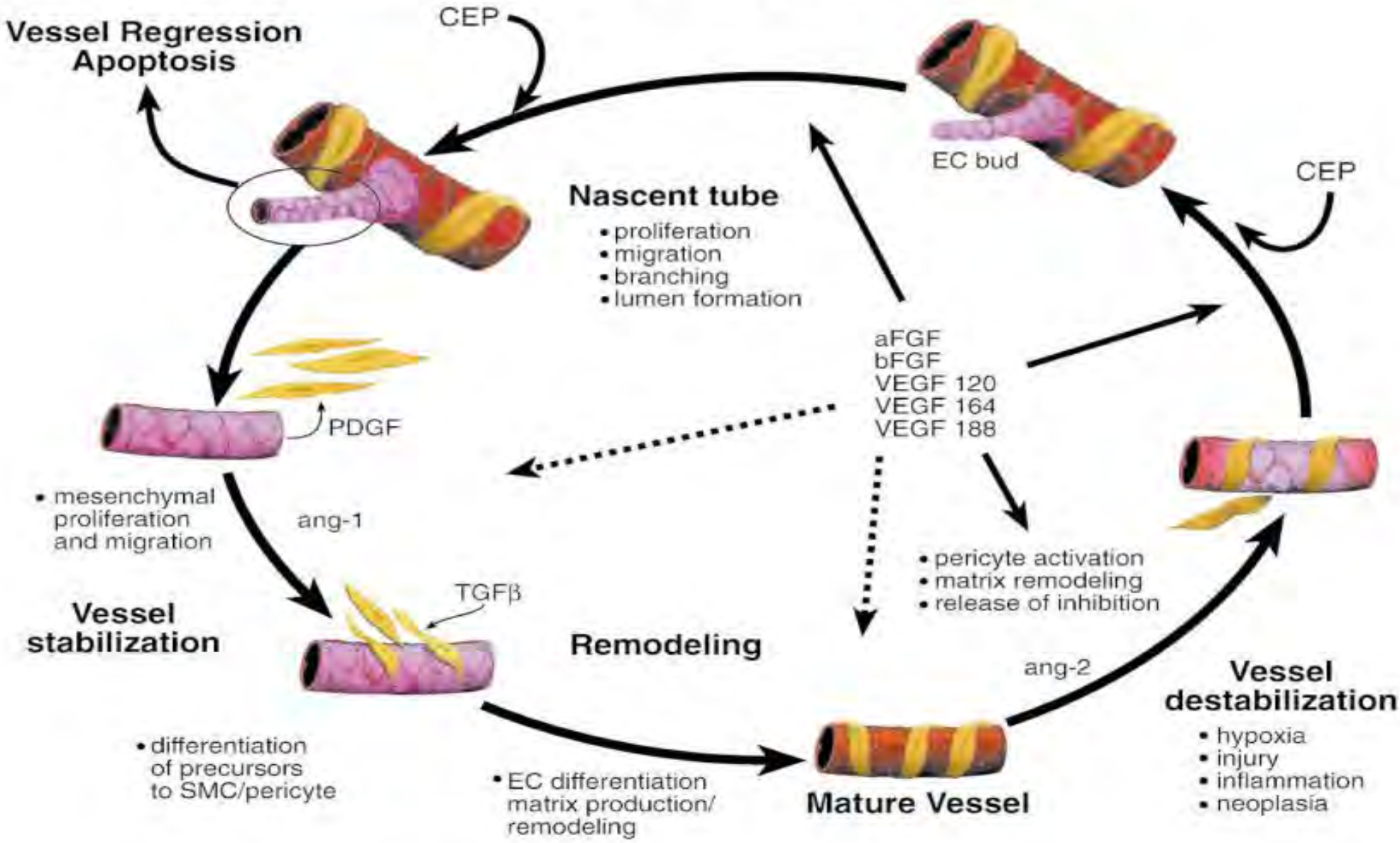
Learn and Live

Formation of a Stable Vessel



Current Controlled Trials in Cardiovascular Medicine