Klinikum der
Johann Wolfgang Goethe Universität
Frankfurt am Main

microRNAs: novel therapeutics and diagnostics in cardiovascular disease

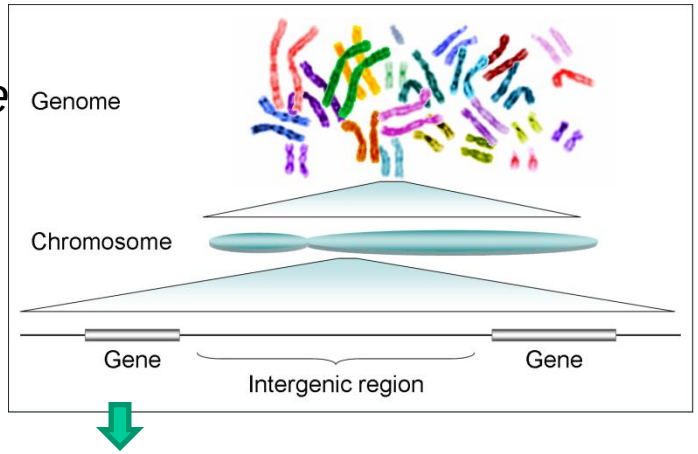
Stefanie Dimmeler

Conflict of interest: Miragen

Non-coding DNA & RNA and microRNAs

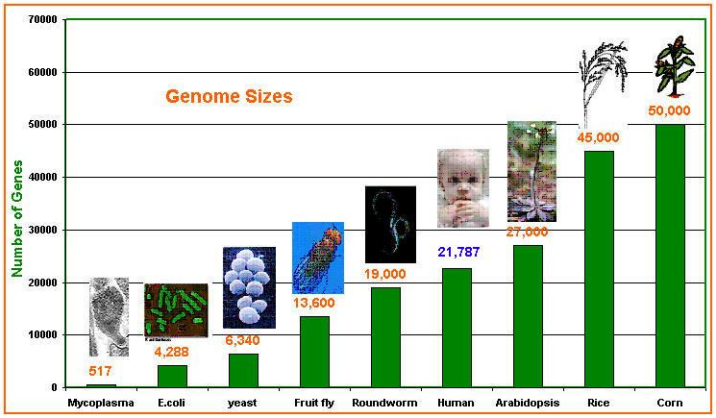
Human Genome Project: Craig Venter, 2003

3x10^9 bp

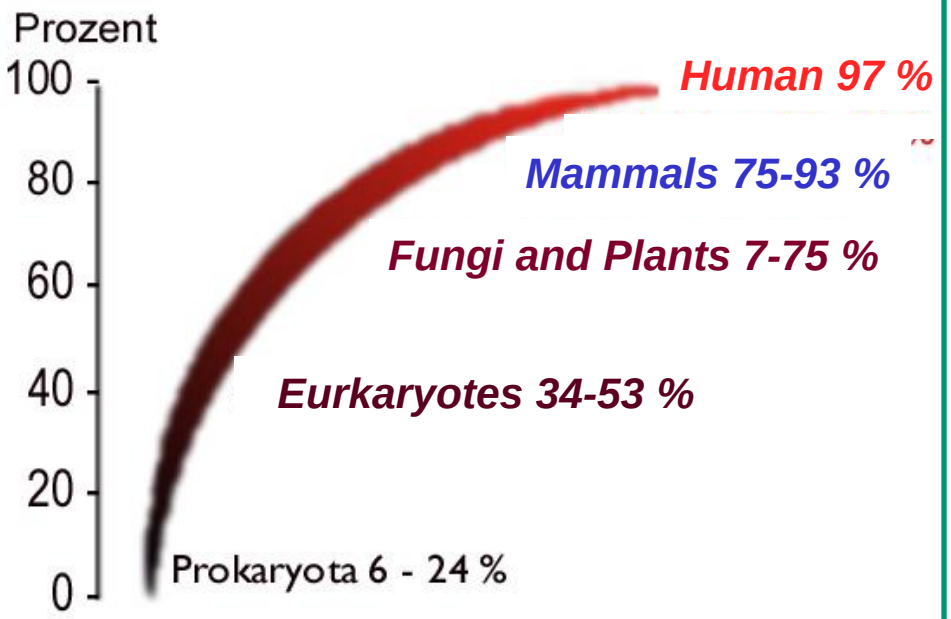


Proteins

Human Genome: ca. 25.000 Genes (only 2 x more than worms or flies)



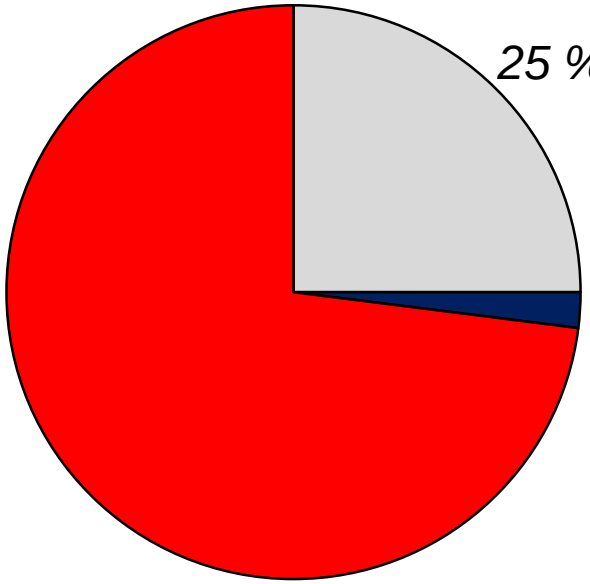
% non-coding DNA



„Junk“ DNA

Source: Wikipedia

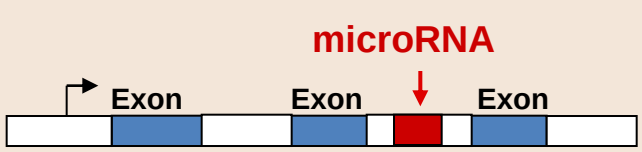
Non-coding RNAs

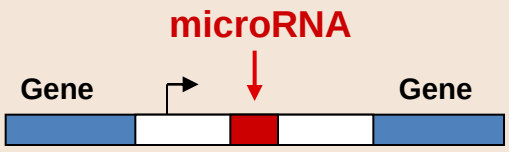


~ 73 %
Non-coding RNAs

- *tRNAs etc*
- *Long non-coding RNAs*
- *MicroRNAs (<200 nt)*

microRNAs: Processing and functions



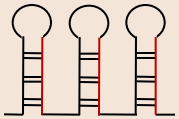


siRNA:

One target mRNA

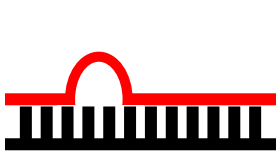
Primary-miRNA

Single miRNA

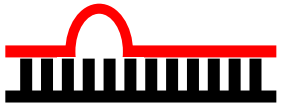


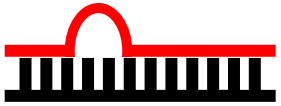
Cluster

microRNA:



up to hundreds of mRNAs





Nucleus

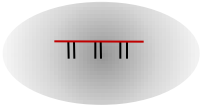
Drosha

Pre-miRNA

Dicer

miRNA Duplex

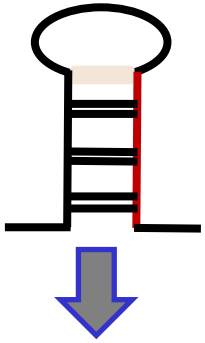
RISC Complex



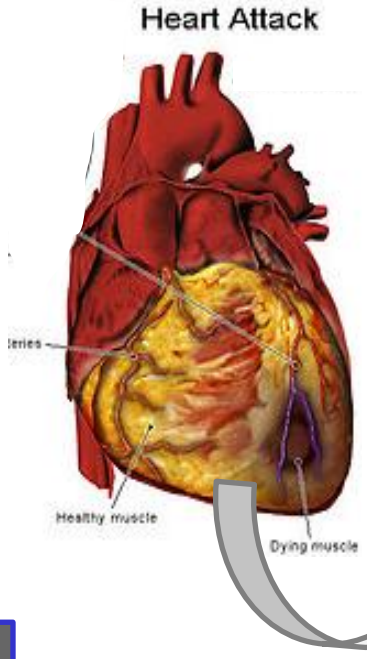
Translational Repression

mRNA Degradation

microRNA functions

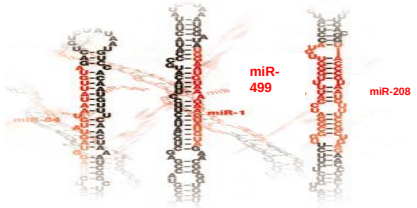


Biomarker



Cardiac-specific miRNA:
miR-208a

Muscle enriched miRNAs:
miR-1
miR-133a/b
miR-499



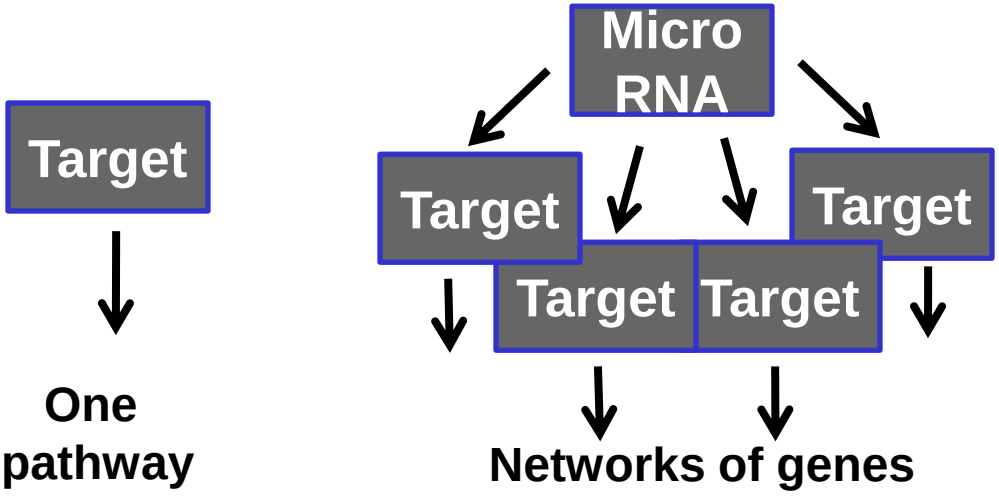
Release of miRNAs

(Dimmeler EHJ 2010)

Therapeutic option

Pharmacological/ Gene therapy

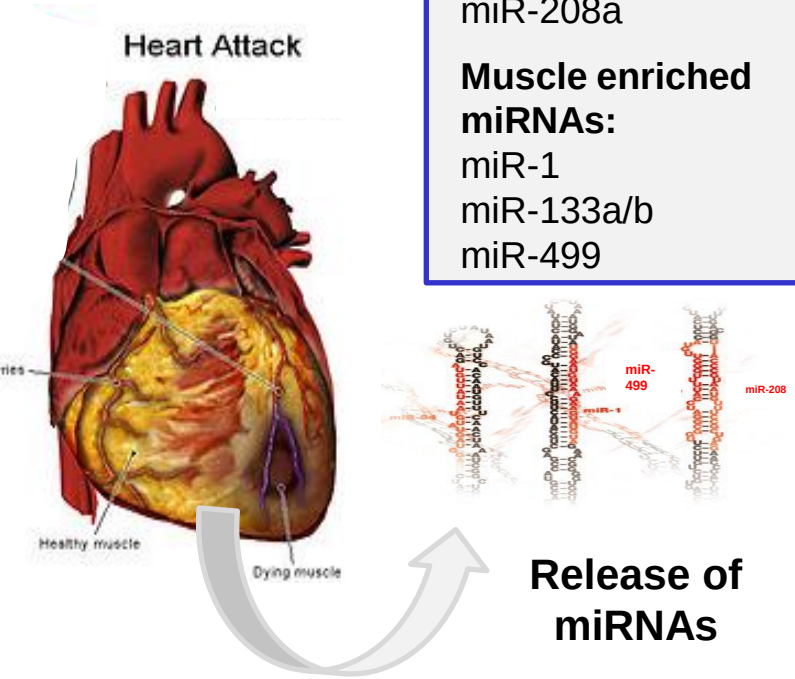
microRNA therapy



Release of microRNA during ACS

Cardiac-specific miRNA:
miR-208a

Muscle enriched miRNAs:
miR-1
miR-133a/b
miR-499



Release of miRNAs

(Dimmeler EHJ 2010)

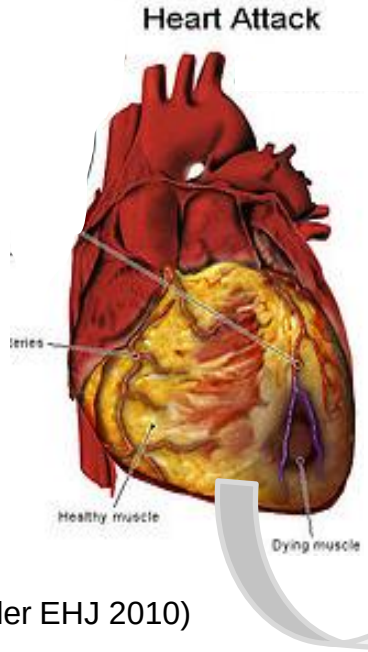
Study cohort	Groups and numbers of patients studied	Major Findings
AMI	33 pts with AMI 33 pts with stable CAD/others 30 healthy controls	miR-208a, miR-1, miR-133a, miR-499 ↑
AMI	33 pts with STEMI 17 healthy controls	miR-1, miR-133a, miR-133b, miR-499-5p ↑
AMI unstable angina	9 pts with STEMI 5 pts with unstable angina 10 healthy controls	miR-499↑ in STEMI (within 48h)
AMI	93 pts with AMI 66 healthy controls	miR-1 ↑
AMI	31 pts with AMI 20 healthy controls	miR-1 ↑
AMI	29 pts with ACS 42 pts without ACS	miR-1, miR-133↑
AMI	32 pts with AMI 36 pts with chest pain but normal angiogram	miR-208b, miR-133a, miR-1,miR-499 ↑ miR-223 ↓
AMI	20 pts. with AMI 20 pts. without AMI	Whole blood: miR-1291, miR-663b ↑
ACS	444 pts with ACS	In pts with ACS: miR-1, miR-133a/b, miR-208b↑
ACS	7 pts non-CAD 31 pts stable CAD 19 pts ACS	In pts with ACS: miR-133, miR-499, miR-208↑

Fichtlscherer et al, ATVB 2011

AMI/STEMI:
D´Alessandra et al EHJ 2010; Wang, EHJ 2010
Ai et al, BBRC 2010; Cheng et al, Clin Sci 2010;
Corsten et al, Circ Cardiovas Genet 2010 etc.

microRNA functions

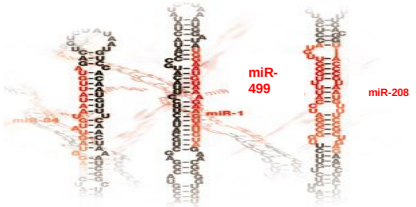
Biomarker



(Dimmeler EHJ 2010)

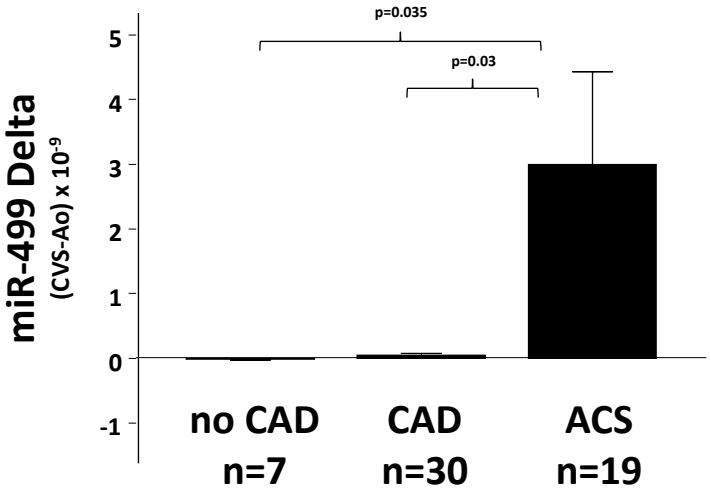
Cardiac-specific miRNA:
miR-208a

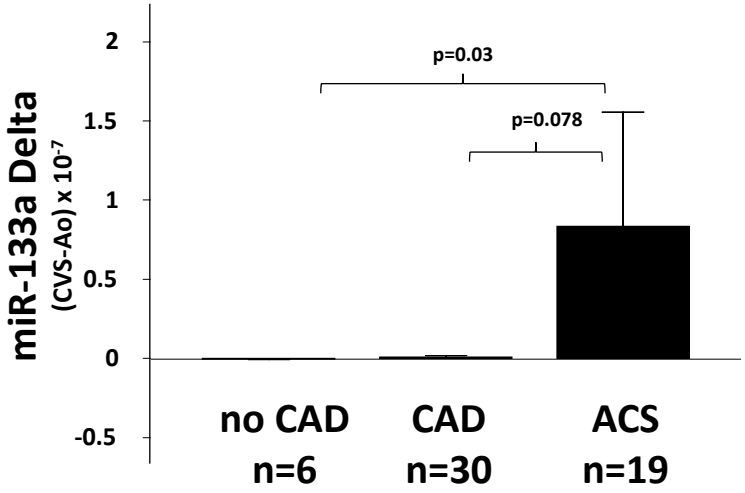
Muscle enriched miRNAs:
miR-1
miR-133a/b
miR-499



Release of miRNAs

Transcoronary gradient:
(Coronary venous sinus- Aorta)

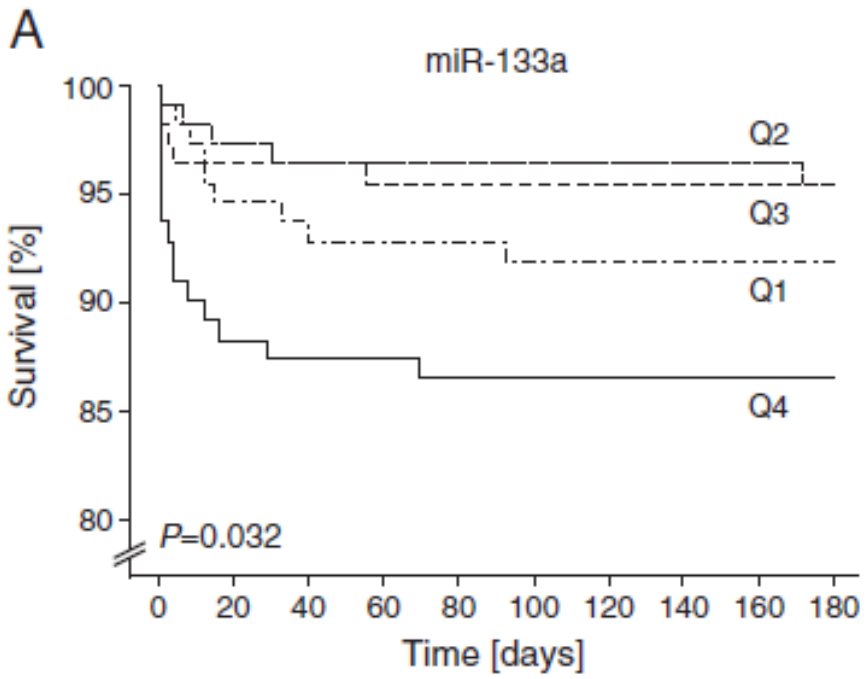


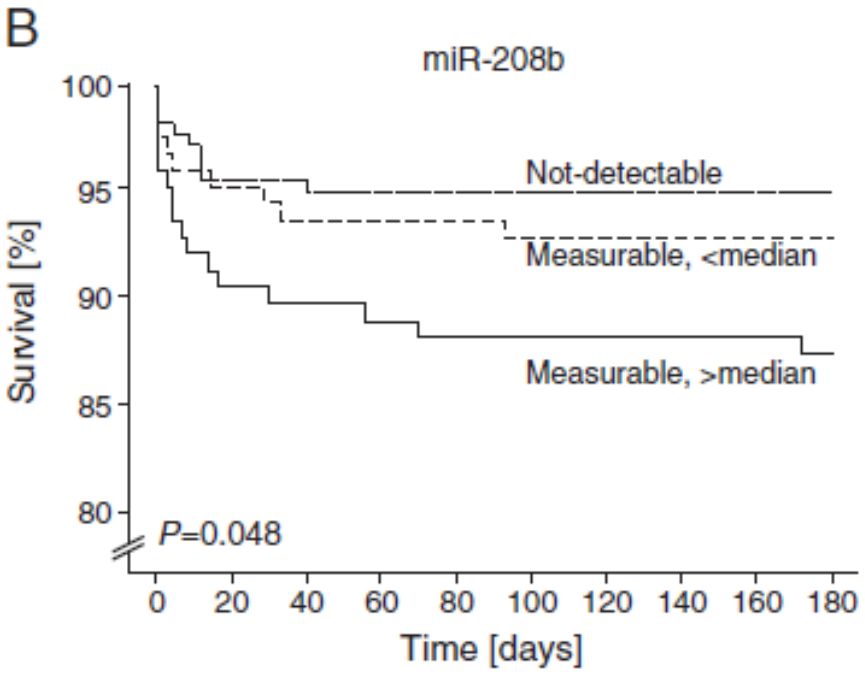


Circulation 2011

Prognostic relevance of circulating cardiac miRs?

444 patients with ACS –
association of cardiac/muscle miRs with death





Widera et al. JMCC (2011) 51:872

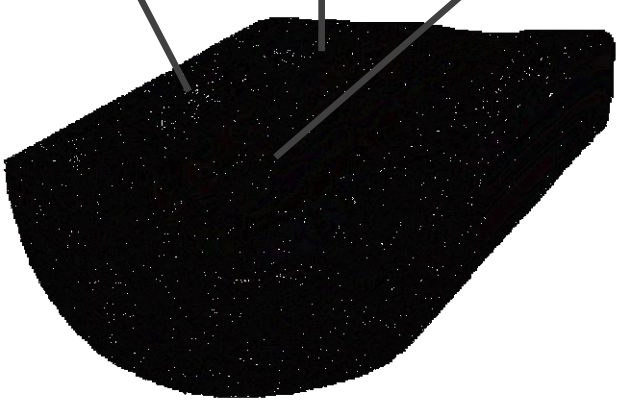
Regulation of vascular microRNAs

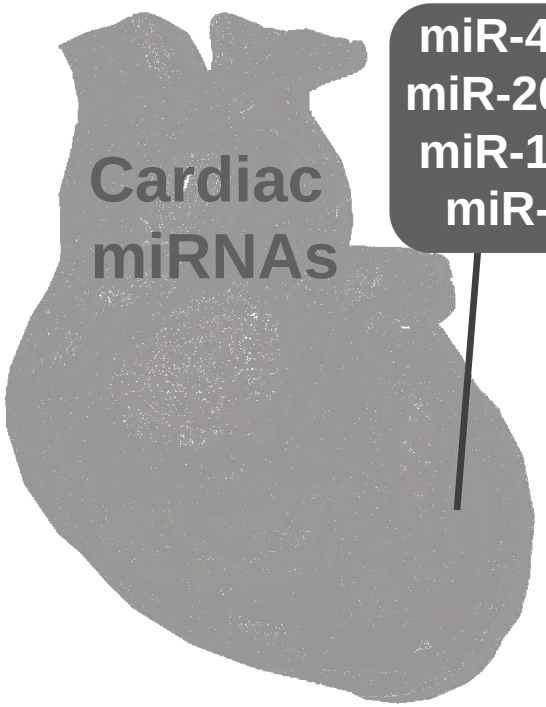
Smooth muscle | Endothelial | Inflammatory

miR-143
miR-145

miR-126
miR-92a

miR-155
miR-223





miR-499
miR-208a
miR-133
miR-1

Regulation of vascular miRNAs?

- As a marker for endothelial function ?
- As a biomarker for plaque instability ?

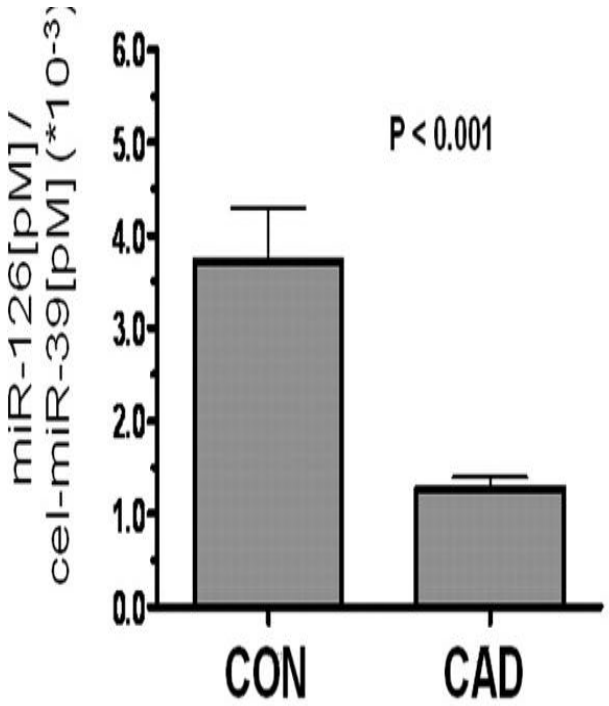
Vascular miRs are reduced in patients with CAD

miR-126:

- Expressed in ECs
- Essential for EC functions
- Anti-atherosclerotic
- Improves pro-angiogenic cell functions

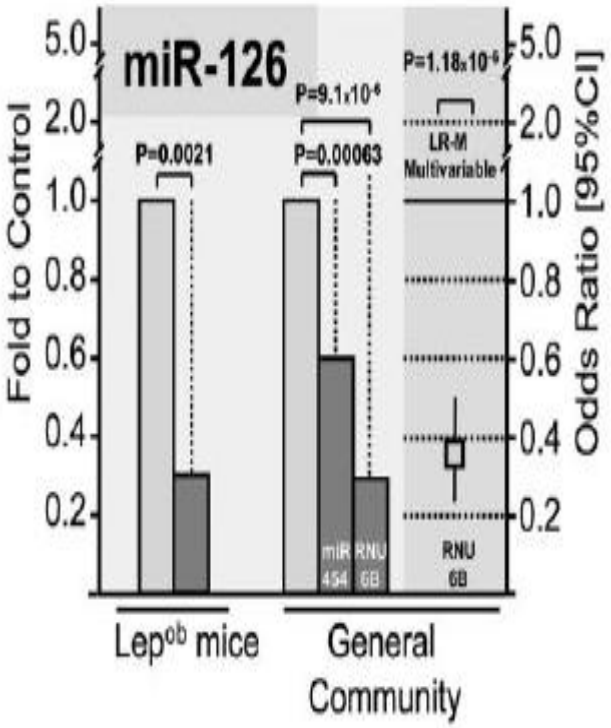
(Fish , 2008; Harris, 2008, Wang,2008,Zernecke, 2009 Landmesser Blood/Circulation 2012)

Patients with CAD



Fichtlscherer et al. (2010)
Circ Res 107:677

Patients with Diabetes



Zampetaki et al. (2010)
Circ Res 107:810

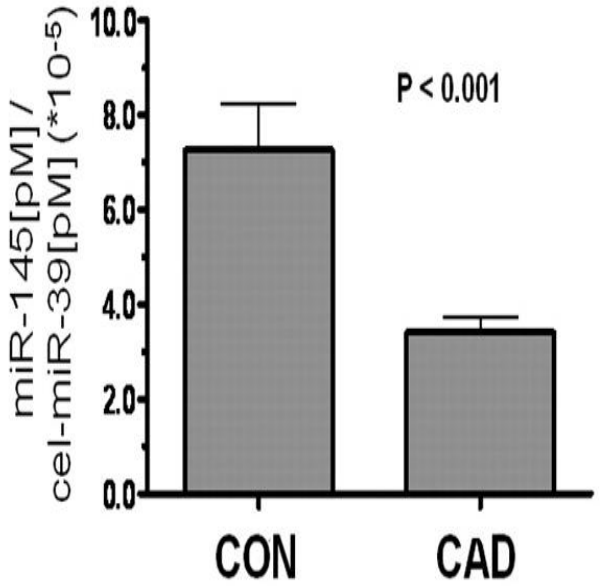
Vascular miRs are reduced in patients with CAD

miR-145:

- Expressed in SMC
- Prevents de-differentiation of SMCs
- Anti-atherosclerotic

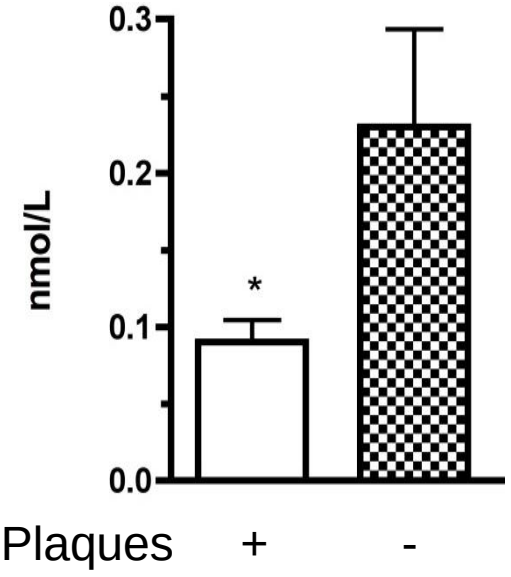
(Chen et al, 2009, Cordes et al, 2009, Böttger et al, 2009, Elia 2009)

miR-145 in CAD



Fichtlscherer et al. (2010)
Circ Res 107:677

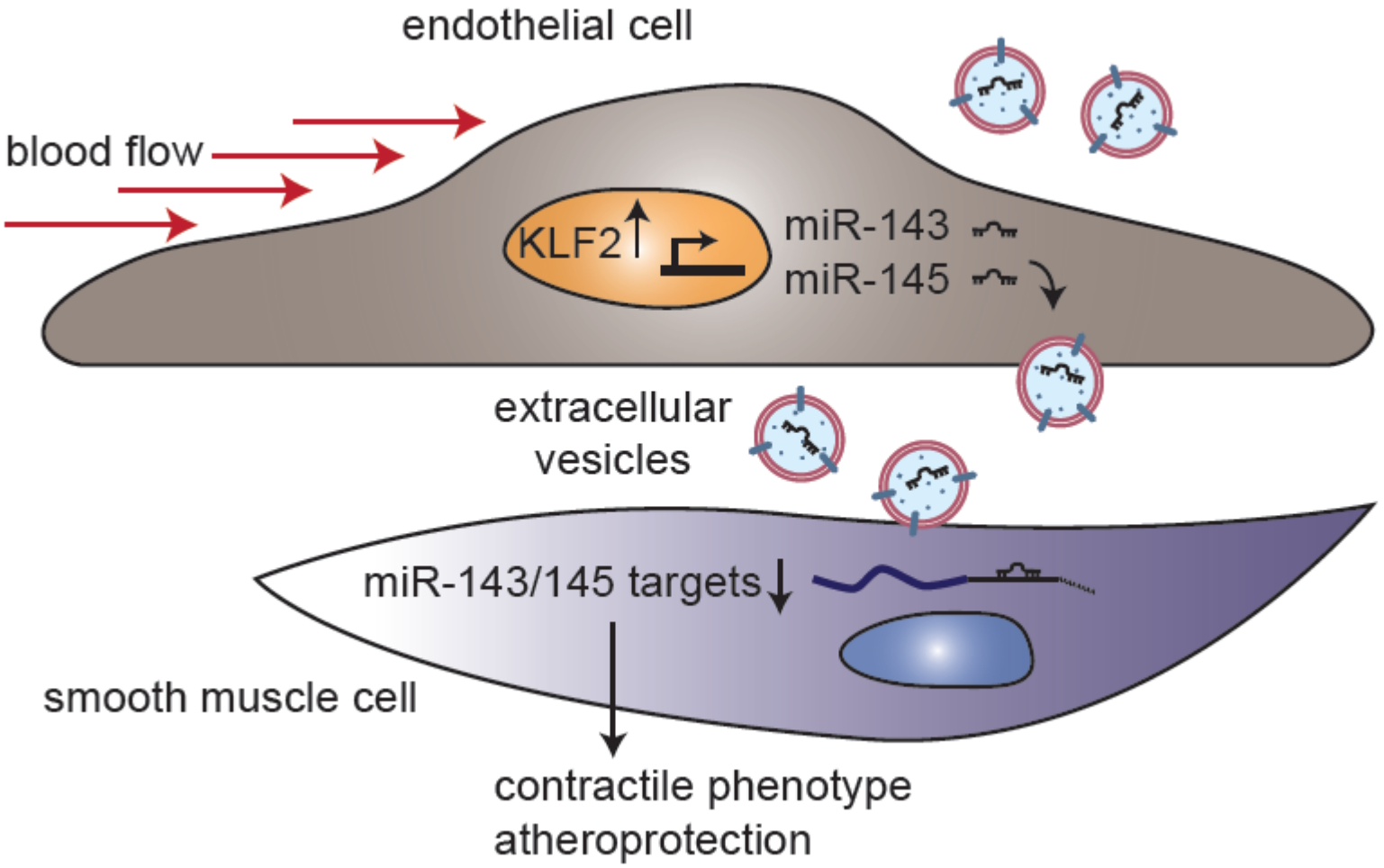
Individuals under-going cardiac CT



Rixe et al, AHA 2011

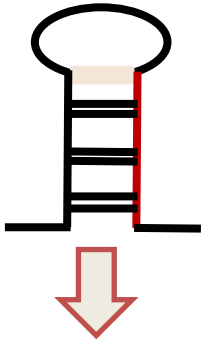
Circulating miRNAs: biomarkers or functional relevant mediators?

Klf2 and shear stress protect against atherosclerosis

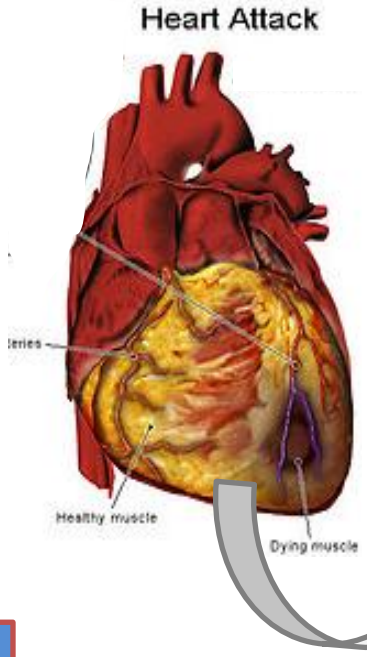


Hergenreider et al Nat Cell Biol 2012

microRNA functions

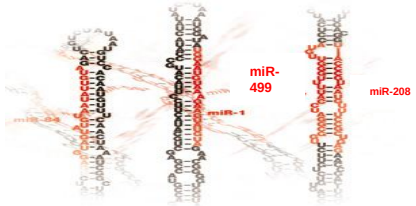


Biomarker



Cardiac-specific miRNA:
miR-208a

Muscle enriched miRNAs:
miR-1
miR-133a/b
miR-499



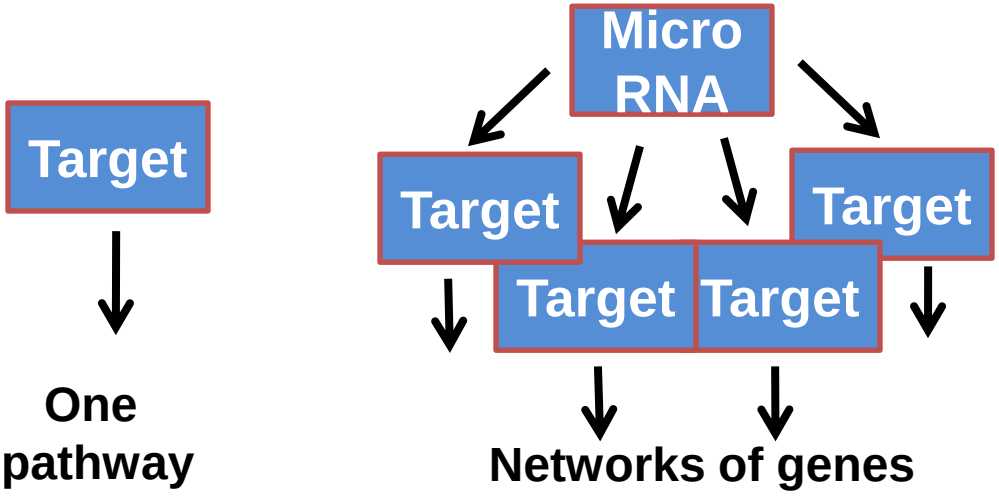
Release of miRNAs

(Dimmeler EHJ 2010)

Therapeutic option

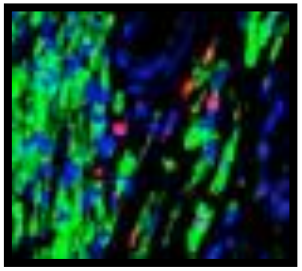
Pharmacological/ Gene therapy

microRNA therapy

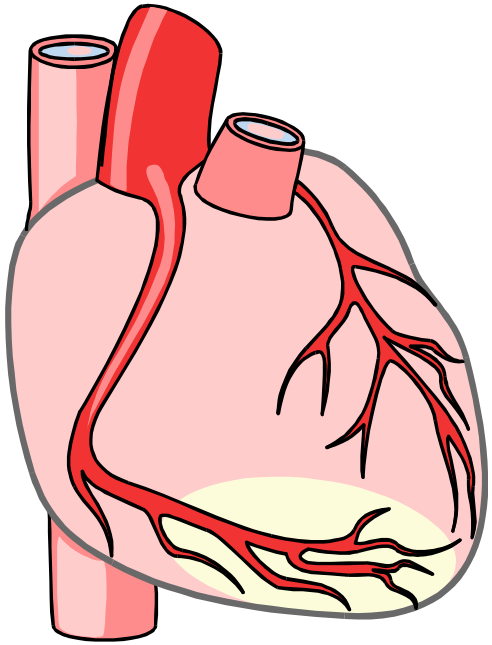


MicroRNAs postinfarction repair

Cell death

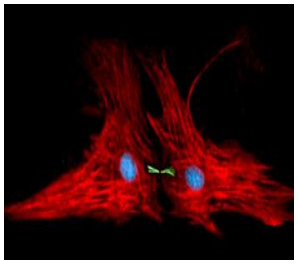


miR-15
miR-24
miR-34
miR-214
(calcium overload)

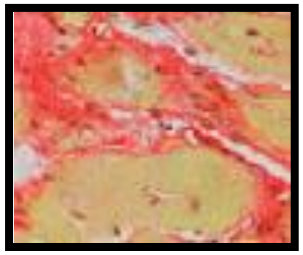


Cardiomyocyte proliferation

miR-15/
miR-195
miR-199
miR-590



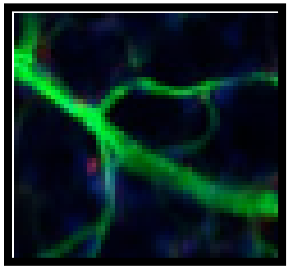
Fibrosis



miR-21
miR-101

Angiogenesis

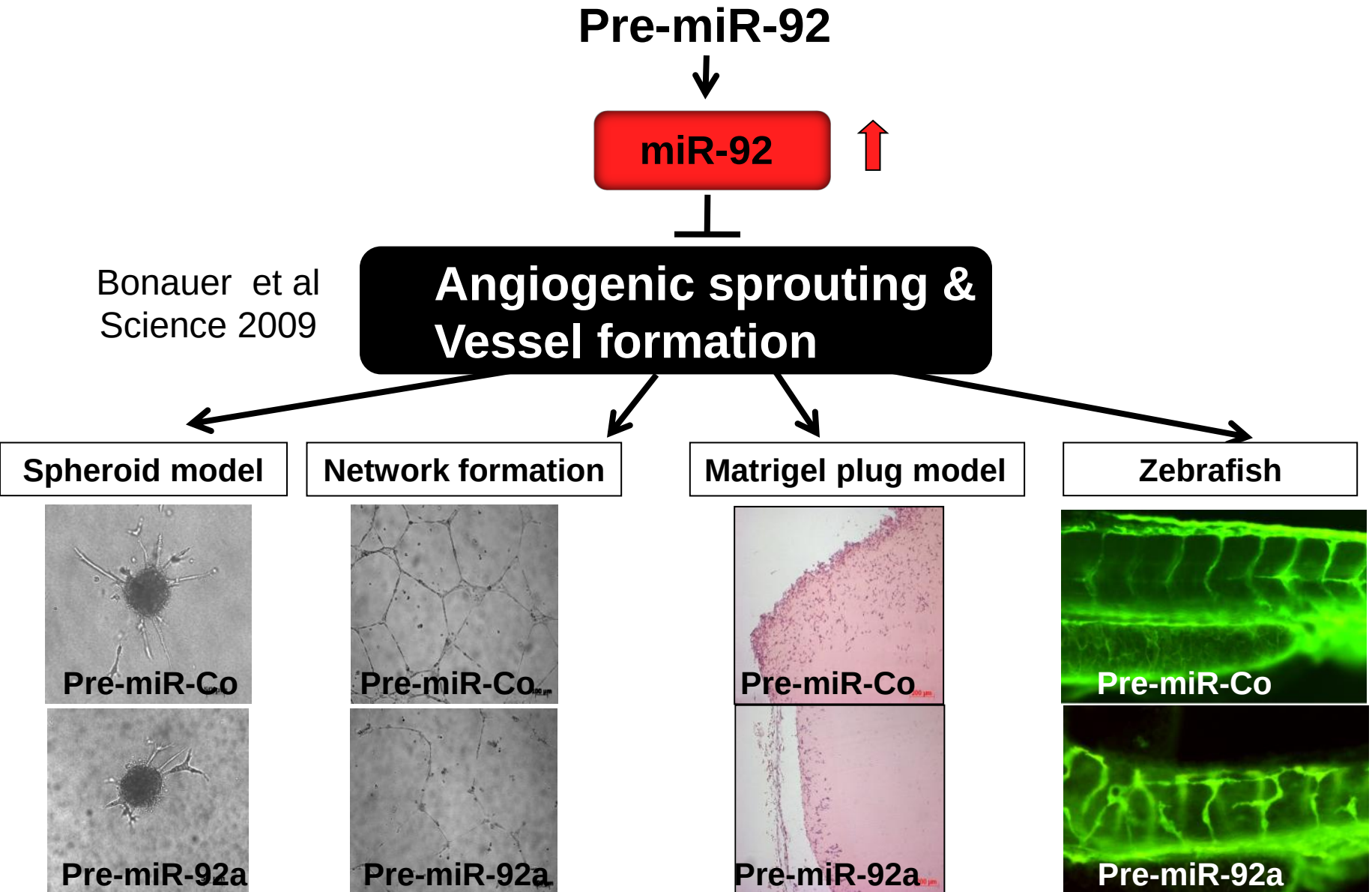
miR-15
miR-24
miR-92a



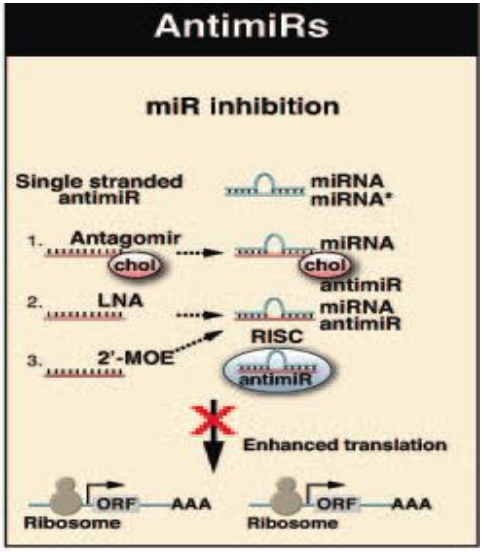
Cardiac reprogramming

miR-1
miR-208
miR-499

miR-92a regulates angiogenesis and vessel patterning

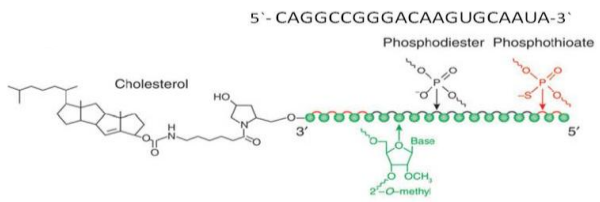


miR-92a inhibition by antimiRs

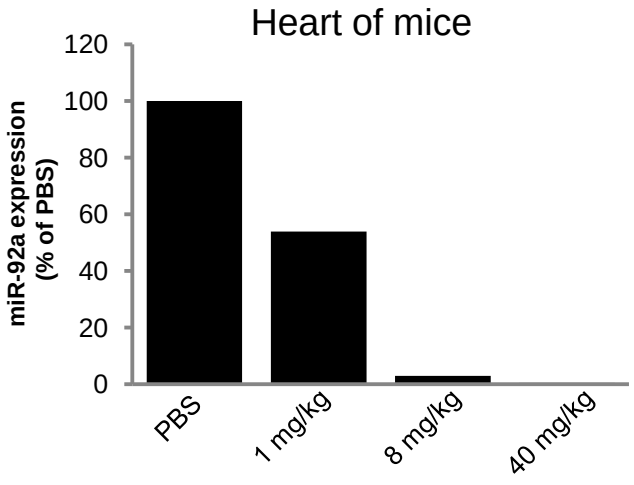


(van Rooij et al, *Circ Res*, 2008)

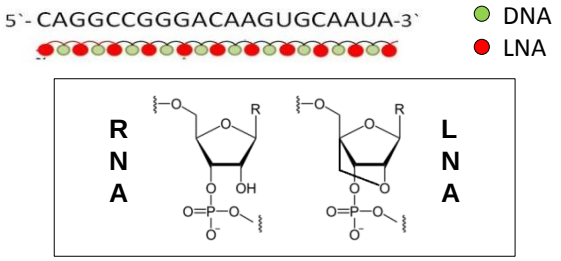
Antagomir-92a

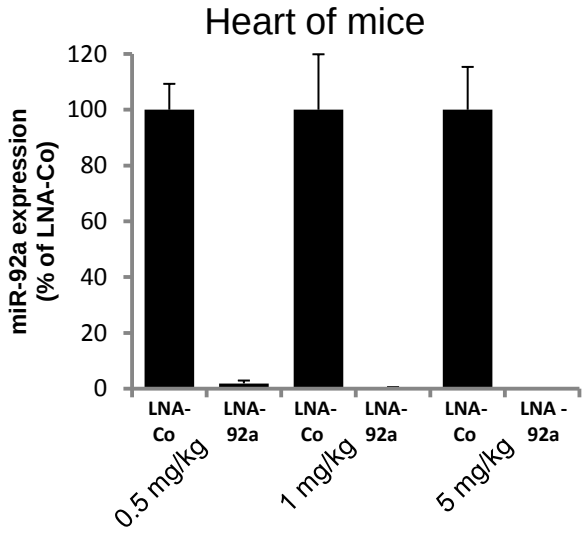


According to Krutzfeldt,et al. *Nature* 2005



LNA-92a





➔ **LNA –modified antimiRs have been tested in non-human primates and have been successfully used in a phase II study (AntimiR-122; Hepatitis)**

Antagomir-92 improves the recovery after ischemia

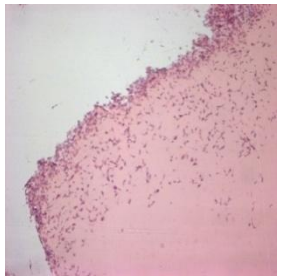
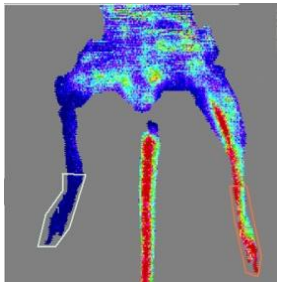
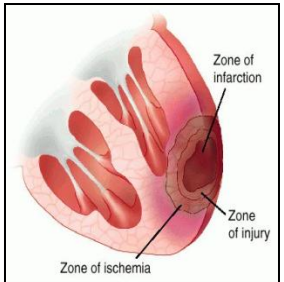
Antagomir 92a

miR-92a

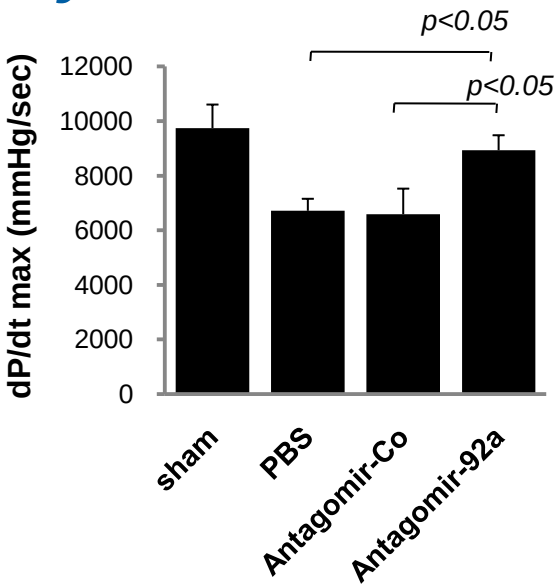
Bonauer et al, Science 2009

eNOS ← **Integrin α5** | **SIRT1** | **Others**

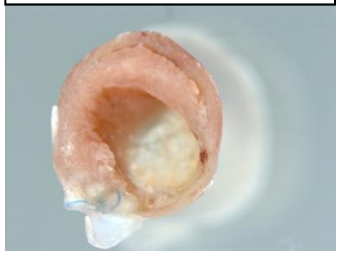
Improvement after ischemia

Matrigel plug Model	Hinterbein-ischämie	AMI
		

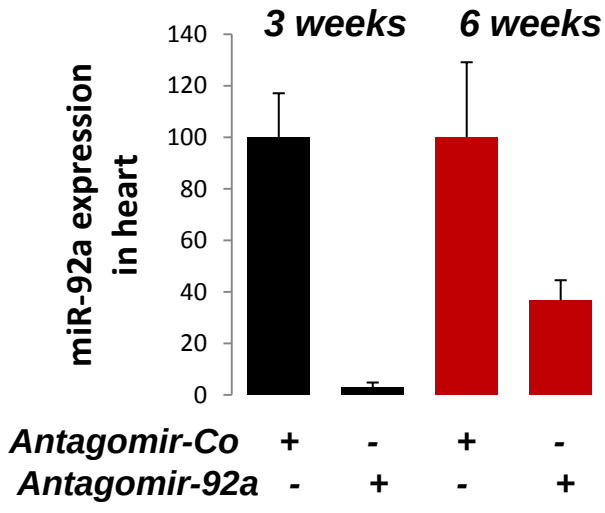
Myocardial infarction



Antagomir-Co



Antagomir-92a



Ischemia/reperfusion in pigs

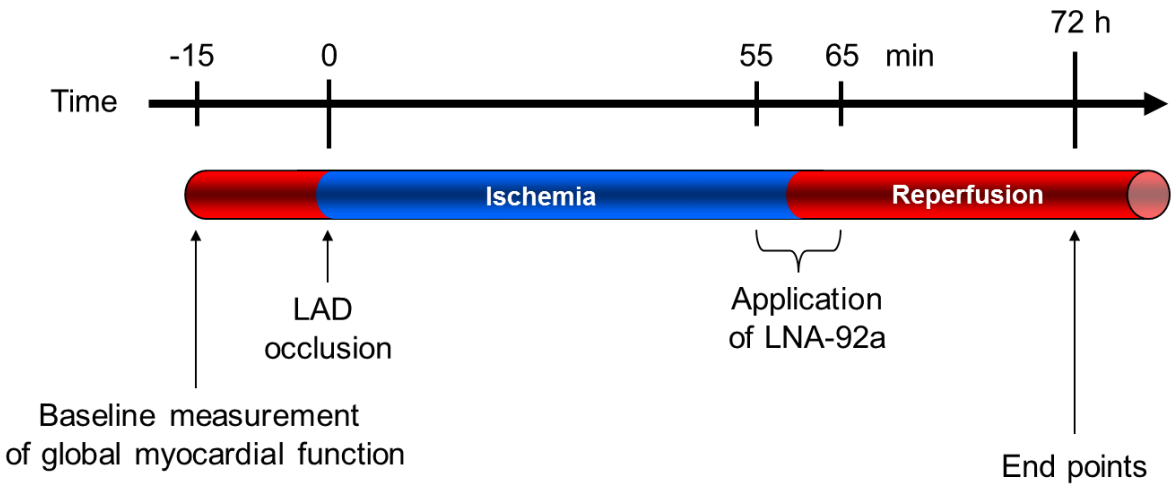
Animals: male pigs, German landrace, body weight: 20-25kg

1) Induction of acute myocardial infarction (AMI) by occlusion of the left anterior descending (LAD) using a PTCA balloon

2) After 55 minutes of ischemia: Infusion of LNA-92a (5 mg/kg heart weight; 0.03mg/kg body weight; 0.75mg/pig)

3) Reperfusion after 60 minutes of ischemia

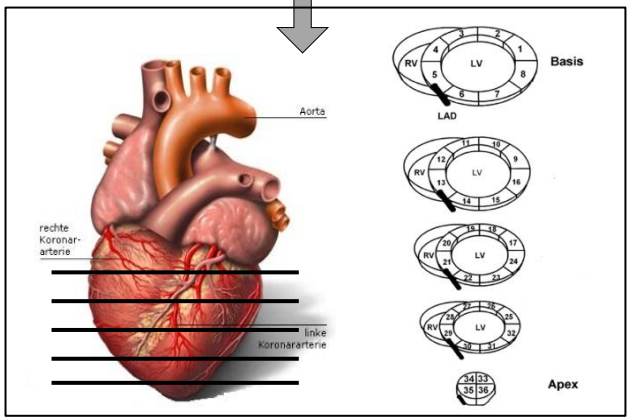
4) Harvest 72 hours after AMI



Endpoints:

- Infarct size
- Global myocardial function
- Regional myocardial function
- Inflammation
- Cell death

- Collection of tissue samples for qPCR, Western blot and Histology

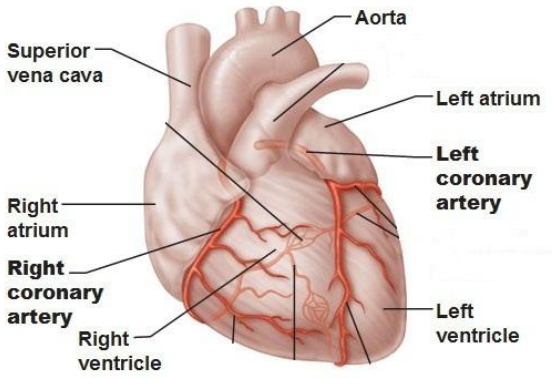


Rabea Hinkel & Christian Kupatt, Munich

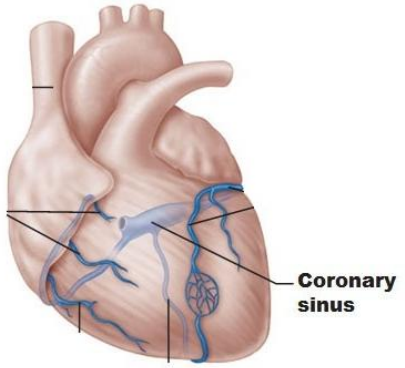
Experimental groups

Experimental groups:

anterograde



retrograde



Control groups:

1) **PBS (n=5)**

2) **LNA-Control (n=5)**

LNA-92a treated groups (n=5 each):

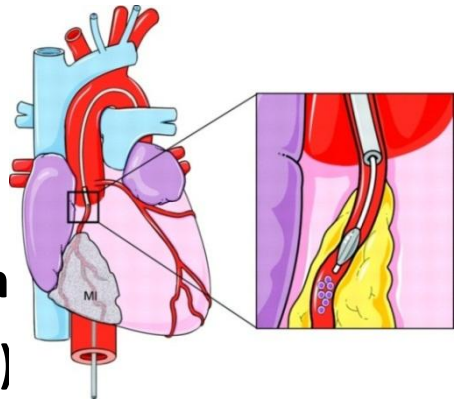
3) **i.v.** (Vena jugularis externa) **(n=5)**

4) **retrograde** via coronary sinus (catheterization) **(n=5)**

5) **anterograde** via left coronary artery (over-the-wire balloon) **(n**

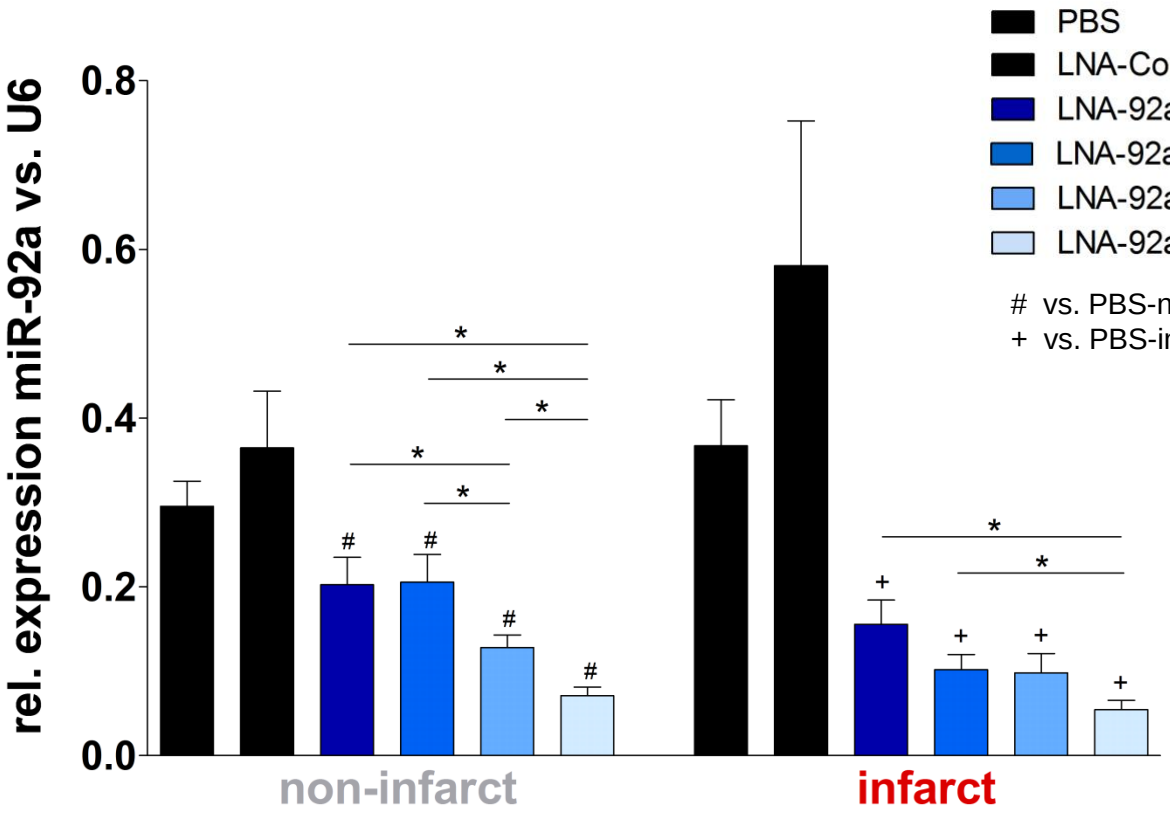
6) **anterograde** with **5 fold increased LNA-92a concentration (5x)**

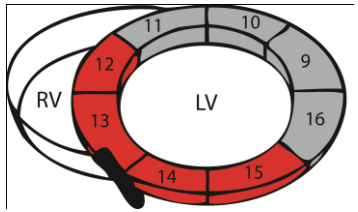
(0.15 mg/kg BW)

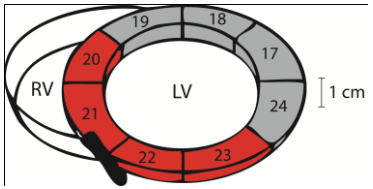


Rabea Hinkel & Christian Kupatt, Munich

LNA-92a inhibits miR-92a expression





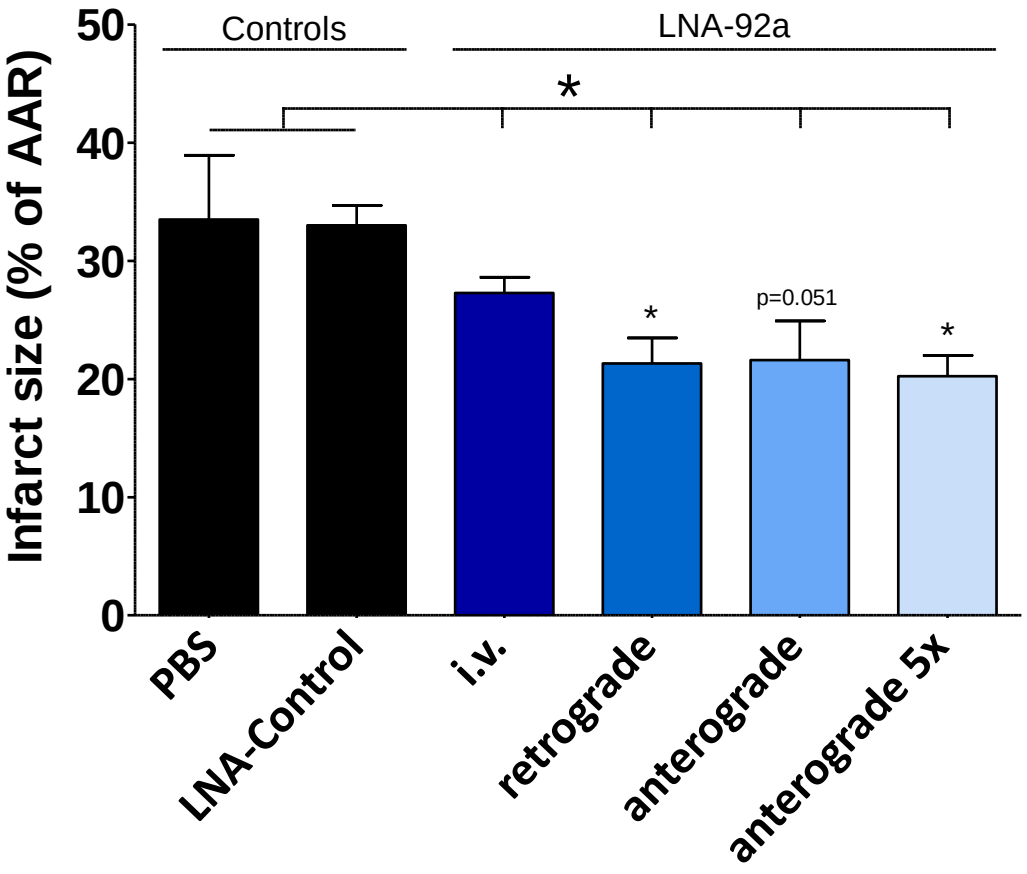


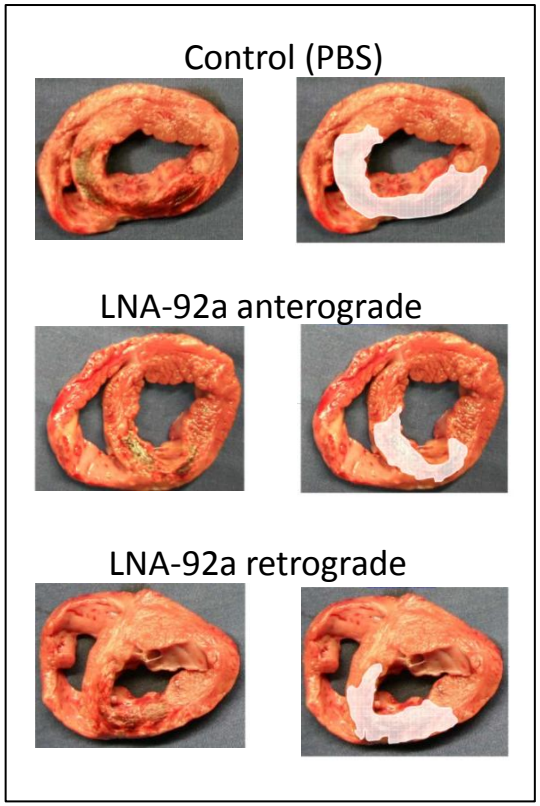
non-infarct regions: 9, 10, 11, 16, 17, 18, 19, 24
infarct regions: 12, 13, 14, 15, 20, 21, 22, 23

➔ **LNA-92a administration reduced the miR-92a expression in non-infarct as well as in infarcted regions of the heart**

LNA-92a reduces infarct size

Infarct size was measured using planimetry.



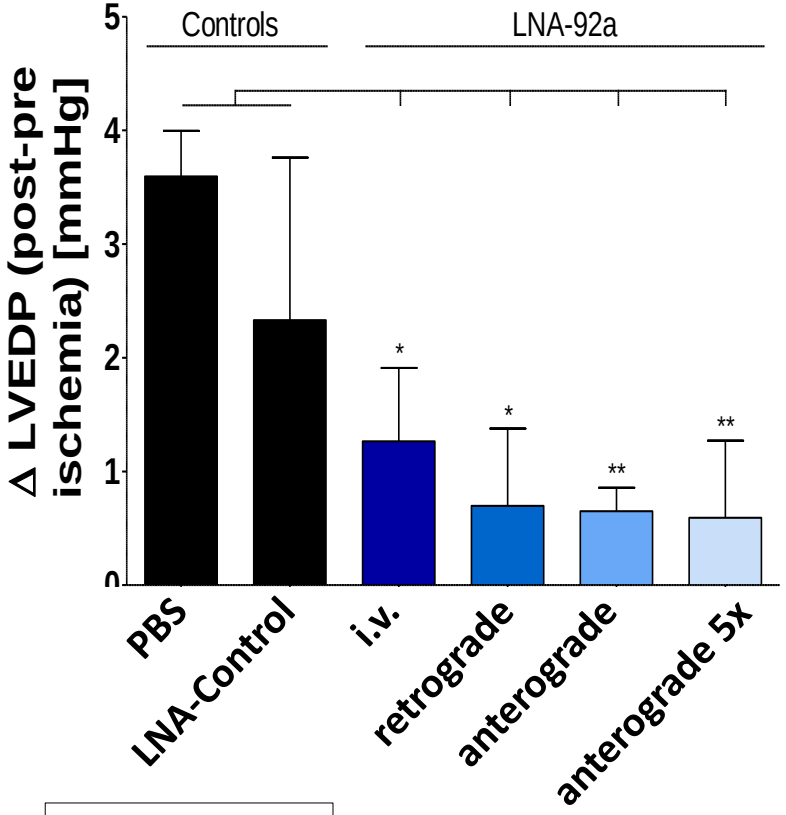


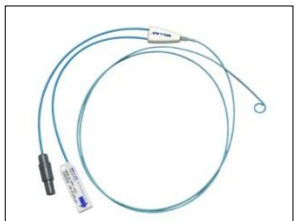
➔ **LNA-92a treatment after AMI reduces the infarct size**

➔ **Local administration of LNA-92a was more effective than i.v. application**

LNA-92a improves cardiac function

- **Global function**

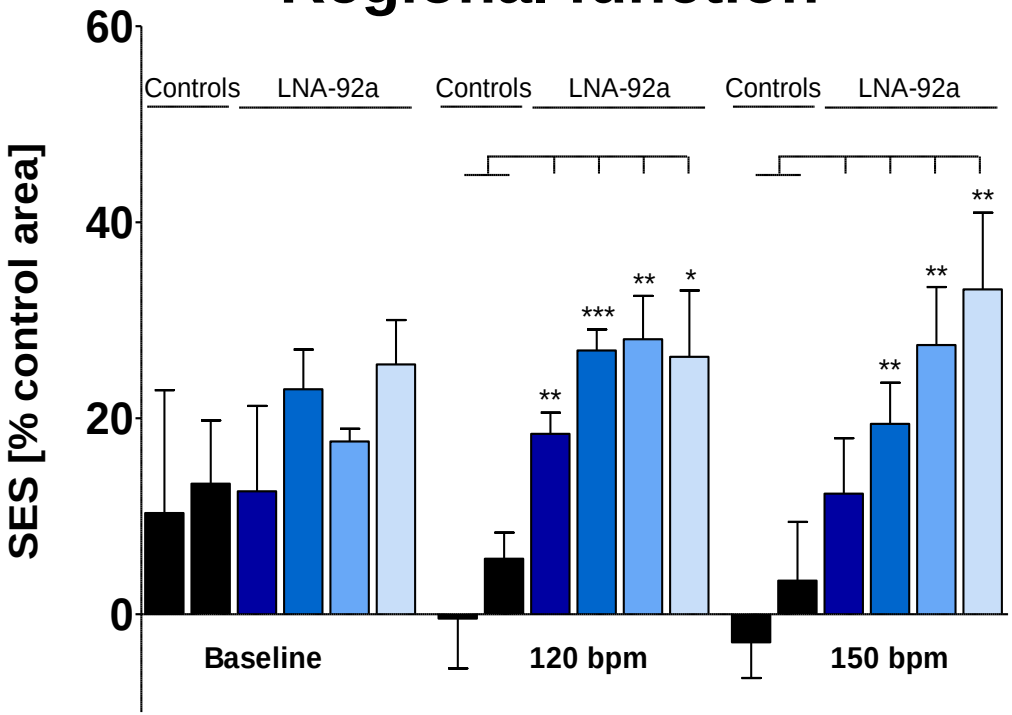




Millar pressure tip catheter

LVEDP = Left ventricular end diastolic pressure

- **Regional function**

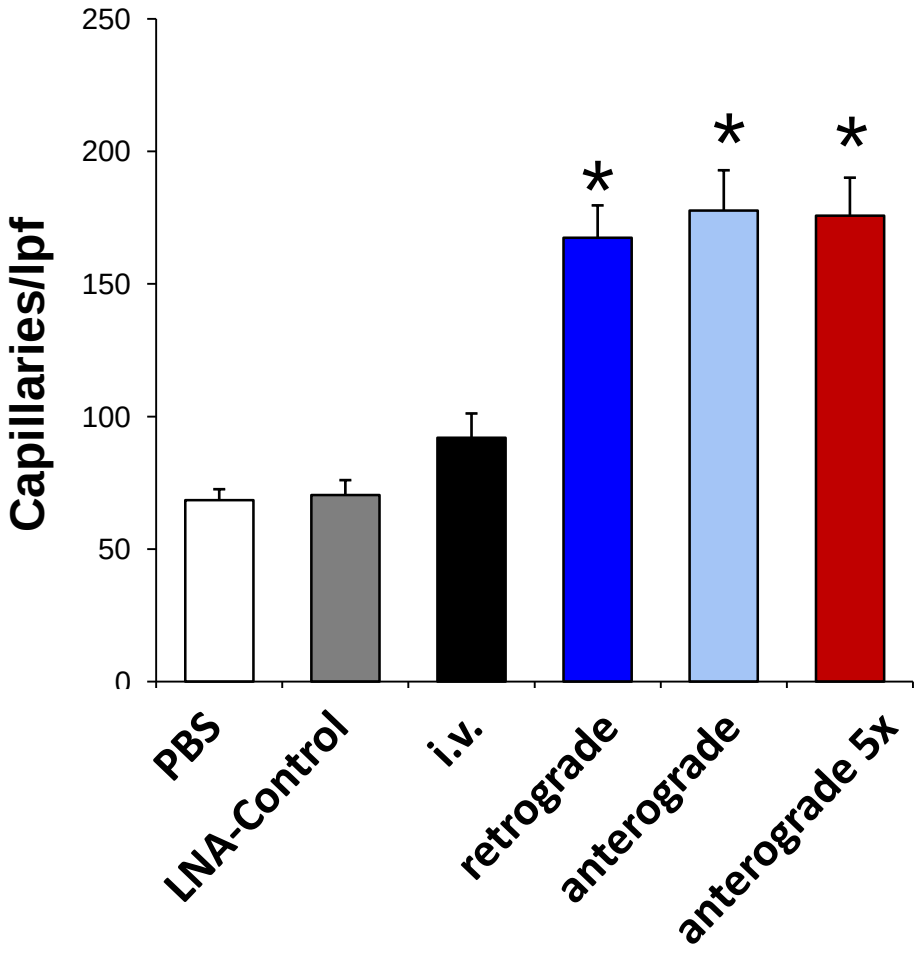


Sonometric crystals:Red: apical LAD-perfused region; Blue: non-ischemic RCx region

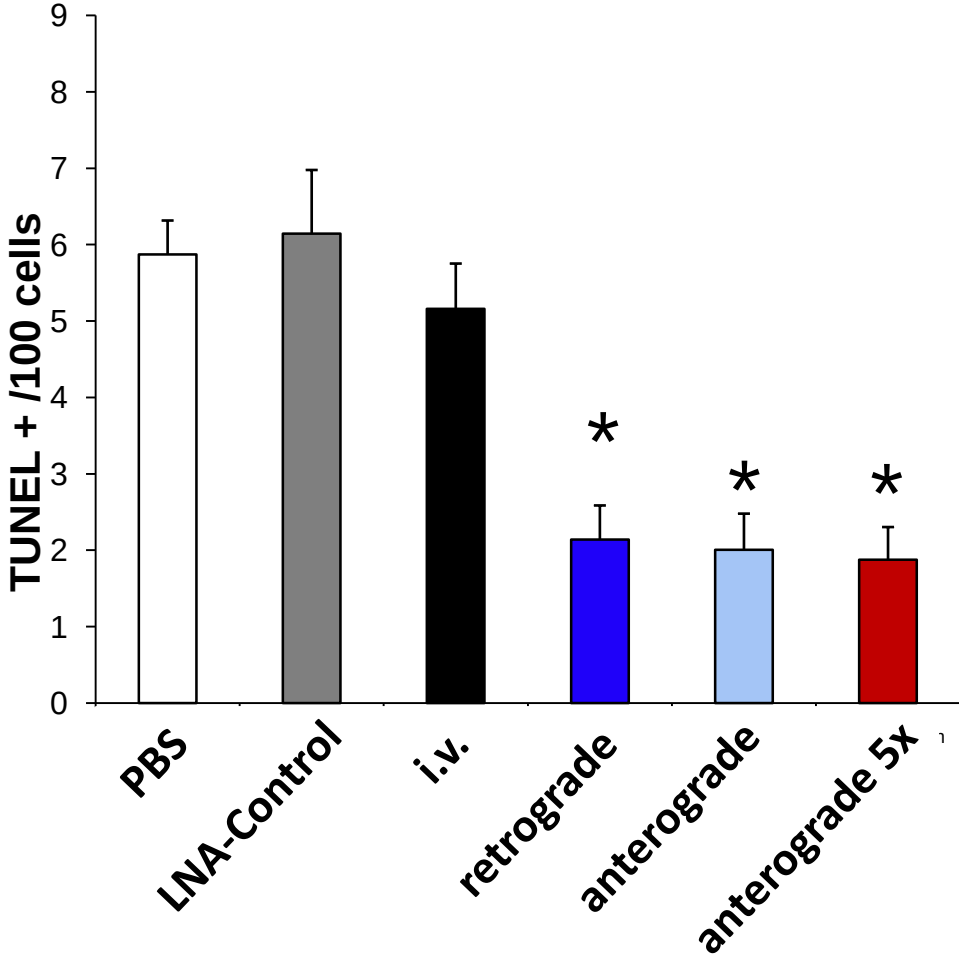
PBS
LNA-Control
LNA-92a i.v.
LNA-92a retrograde
LNA-92a anterograde
LNA-92a anterograde 5x

LNA-92a increases capillary density and improves cell survival

- **Capillary density**



- **Cell death**

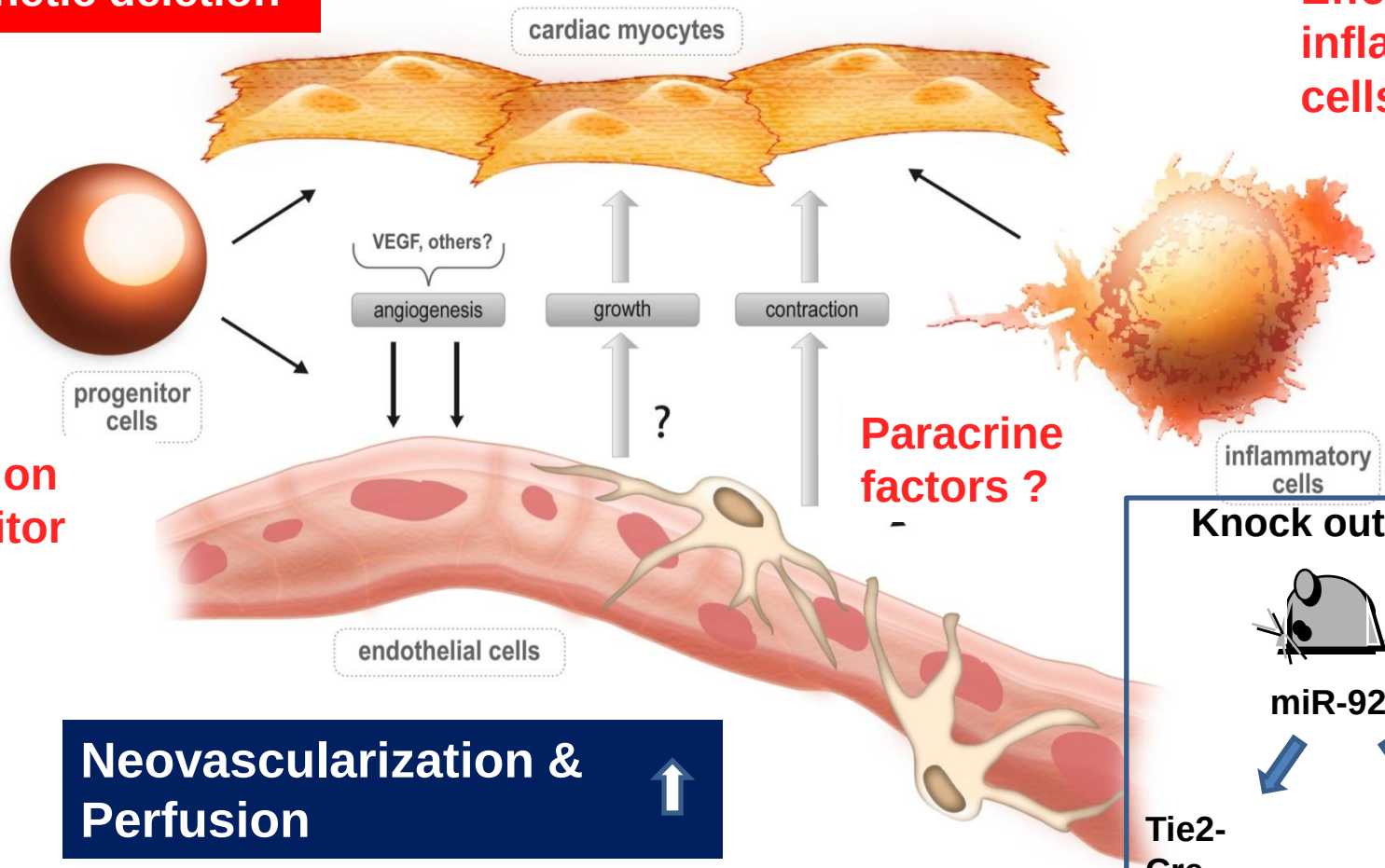


Inhibition of miR-92a enhances neovascularization and survival

- **miR-92a inhibition** *(LNA, antagomirs)*
- **Genetic deletion**

CM survival ⬆ (day 4 after AMI)

Effects on inflammatory cells?



Paracrine factors ?

Effects on progenitor cells?

Neovascularization & Perfusion ⬆

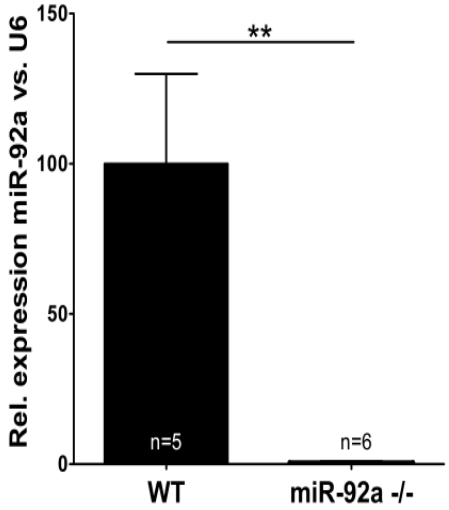
Knock out Mice

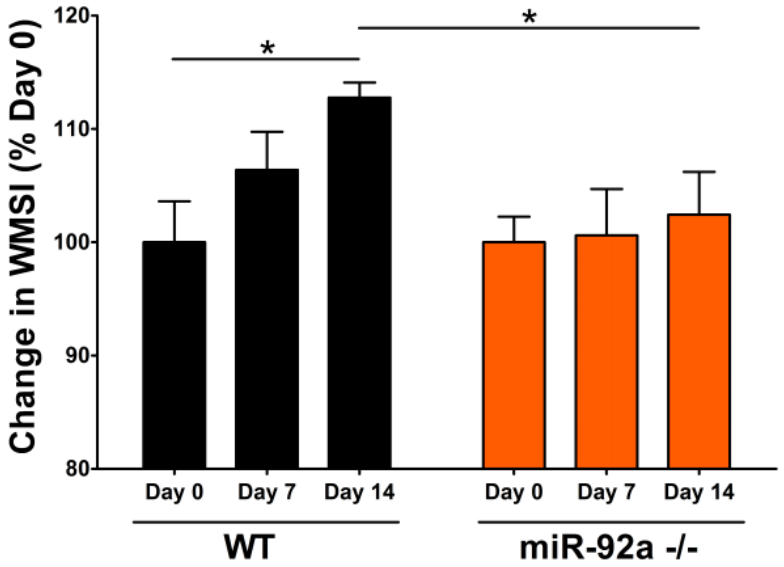
miR-92a$^{fl/fl}$

Tie2-Cre (endothelial)	aMHC-Cre (cardial)

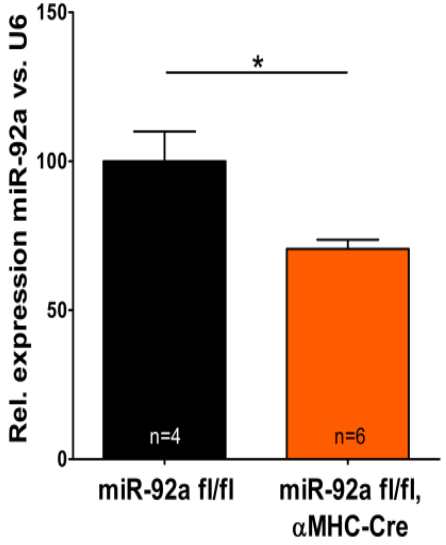
Improved functional recovery in miR-92-/- mice after AMI

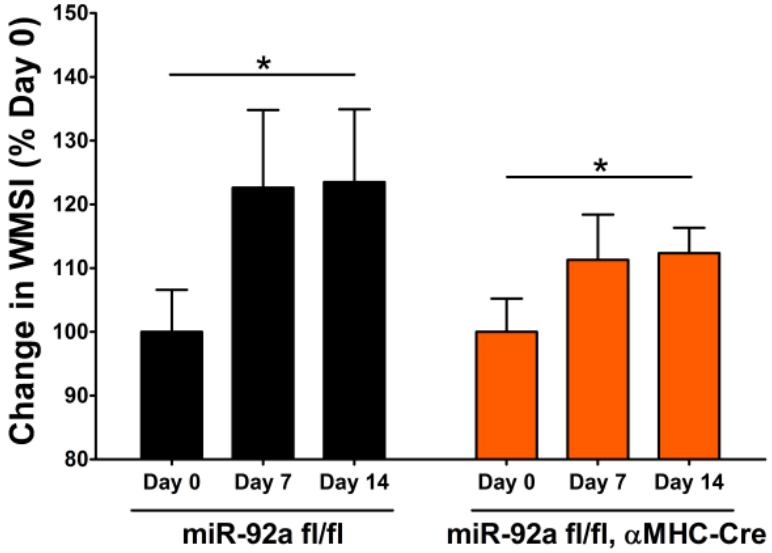
miR-92a-/- mice





Cardiac-specific deletion of miR-92a





Summary

•Improves recovery after ischemia and re-endothelialisation

AntimiR-92a

miR-92a

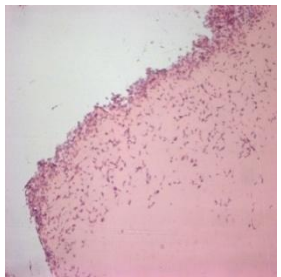
•No effect on tumor angiogenesis

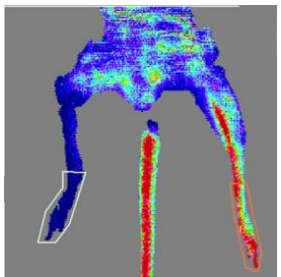
Tumor angiogenesis

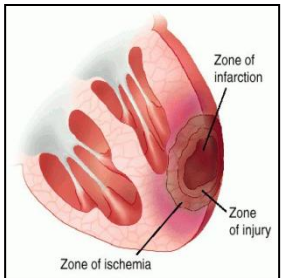
eNOS ← Integrin α5 | SIRT1 | Others

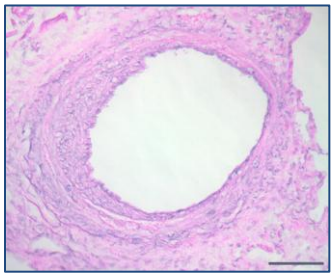
Functional recovery after ischemia

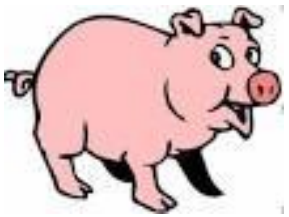
Matrigel plug model	Hindlimb ischemia	Myocardial infarction	Endothelialisation/Neointimaformation	Ischemia/ Reperfusion











Support:

Deutsche Forschungsgemeinschaft (SFB553, TR-SFB23)
Leducq Foundation: Transatlantik Network of Excellence
Excellence Cluster ECCPS
European Union: ERC Advanced Grant, Endostem

Klinikum der
Johann Wolfgang Goethe Universität
Frankfurt am Main

Collaborators:

Andreas Zeiher

Circulating miRs:

St. Fichtlscherer
David Leistern
Salvatore de Rosa
Laleh Gaheni

microRNA projects:

miR-17-92 cluster

Angelika Bonauer ***Carmen Doebele***

Eva-Marie Heinrich ***Daniela Penzkofer***

&
Rabea Hinkel, Christian Kupatt (Munich)
Eva van Rooij (Miragen)

Klf2 Team

Reinier Boon
Group leader

Ed Hergenreider

Anuradha Doddaballapur

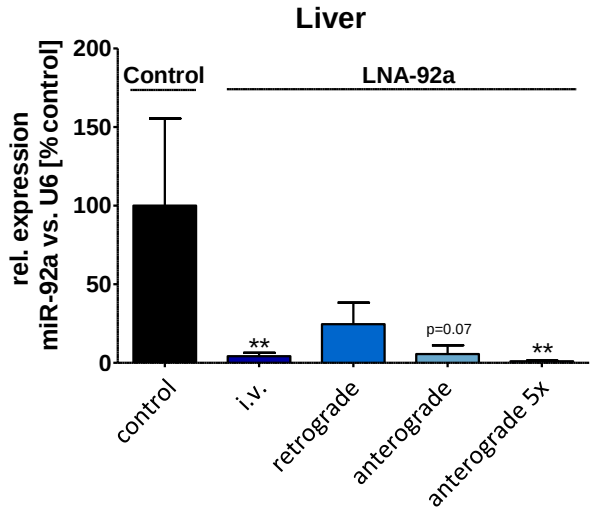
&
Thomas Böttger, Thomas Braun (Bad Nauheim)
Achilleas Frangakis (Frankfurt)
Manuel Mayr (London)

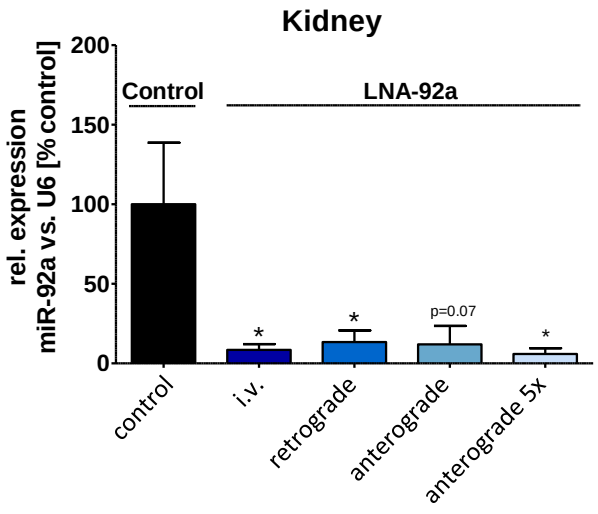
miR-27

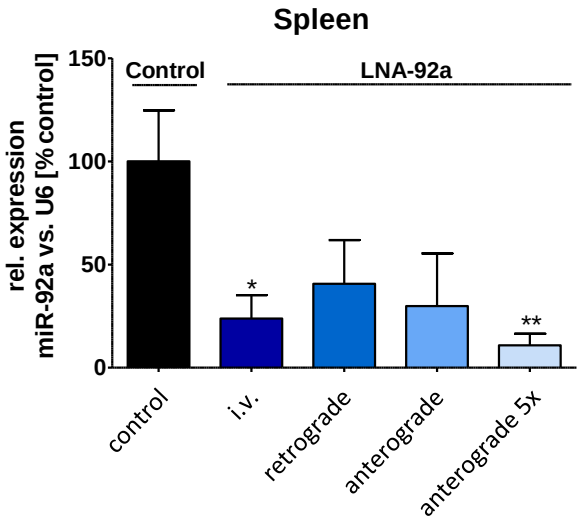
David Kaluza ***Carmen Urbich***

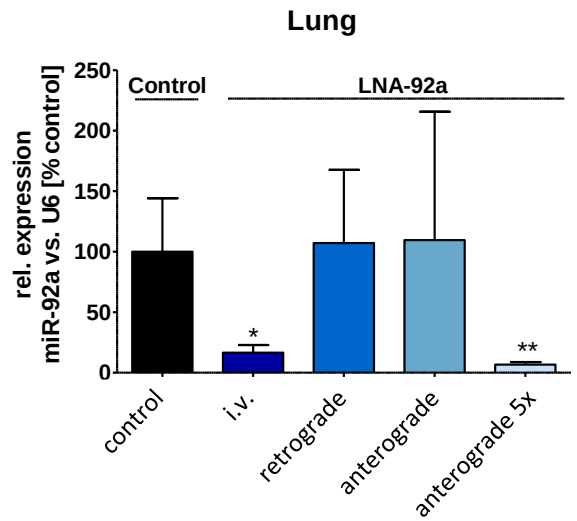
&
Jens Kroll, Helmut Augustin (Mannheim)
Timo Frömel
Ingrid Flemin (Frankfurt)

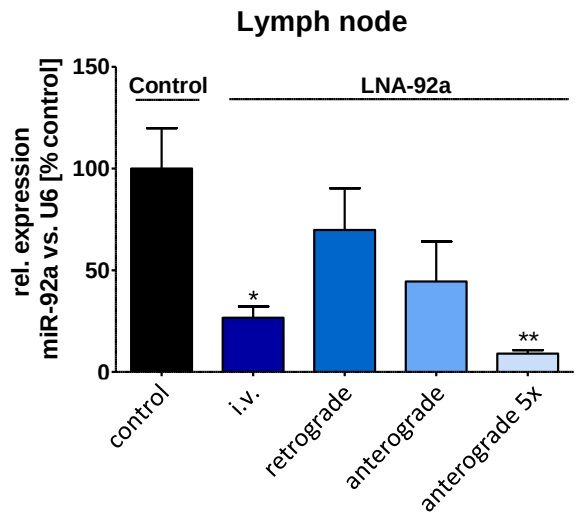
Experimental groups











- ➔ Reduction of miR-92a expression was strongest in liver and kidney
- ➔ Local LNA-92a administration has less systemic effects than i.v. infusion
- ➔ 5x LNA-92a has the strongest effects

➔ Therapeutic benefits of LNA-92a ?