Inflammation in heart failure:

Focus on fibroblasts

Carsten Tschöpe

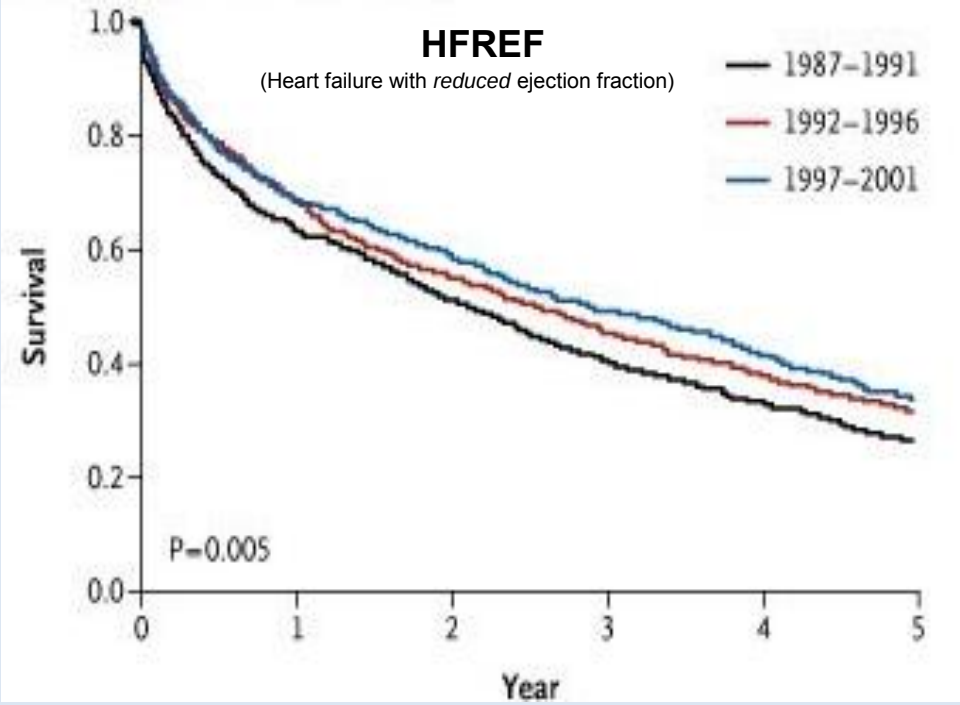
Kardiologie, Campus Benjamin Franklin

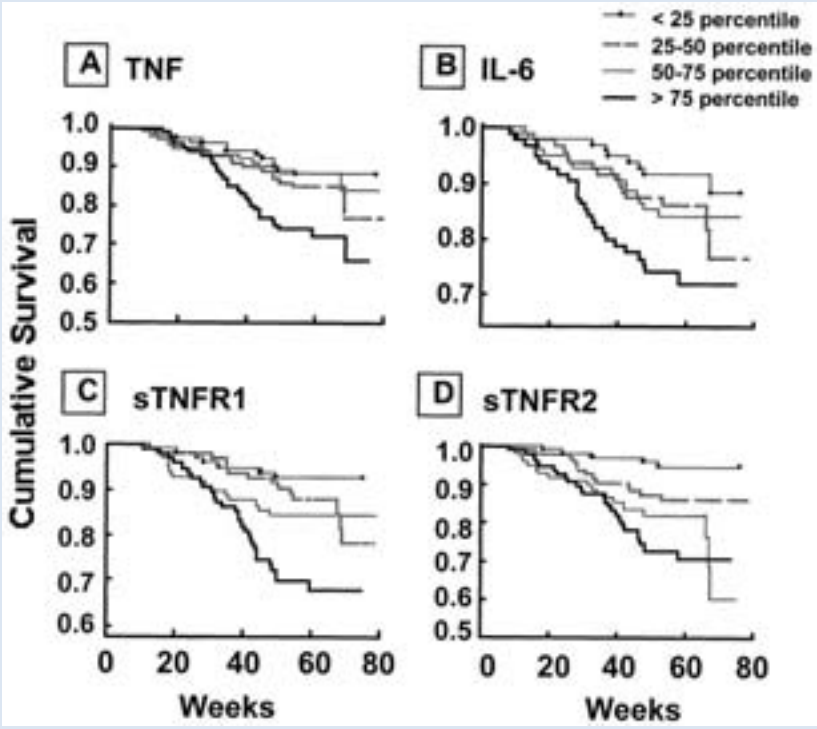
CHARITÉ

Berlin

Prognosis and heart failure

HFREF

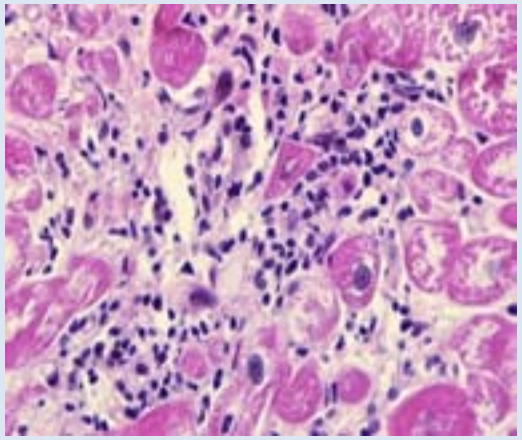




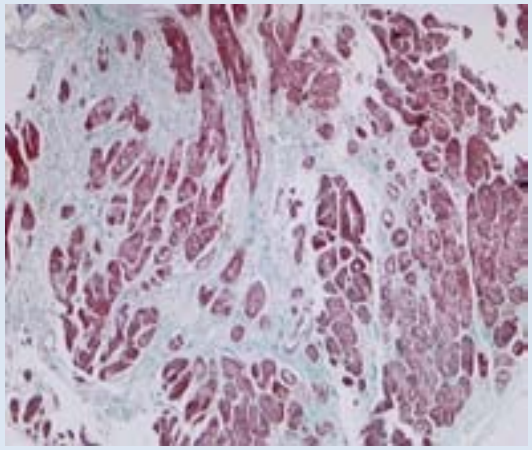
nach Owan T et al. N Engl J Med 2006;

Deswal et al. Circulation 2001

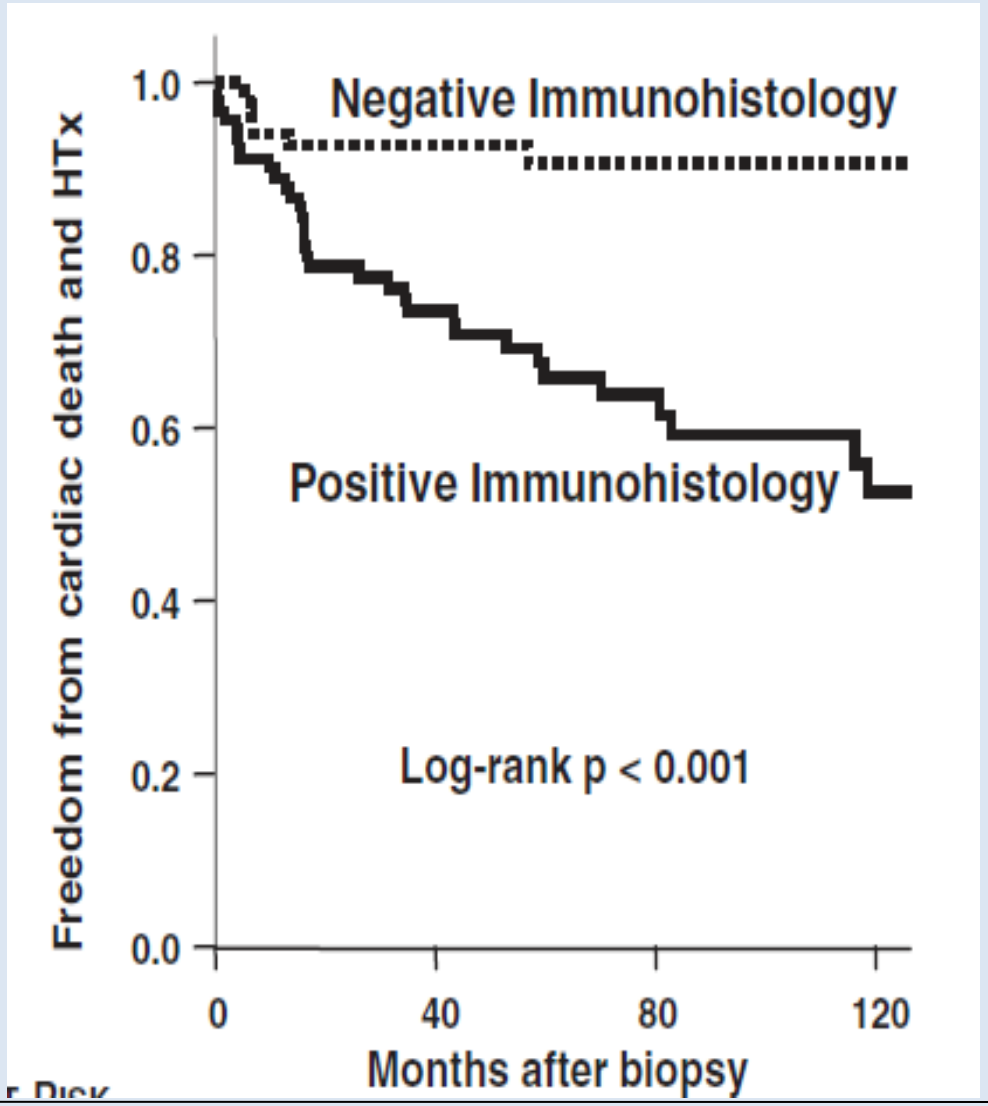
Inflammation is a negative predictor in inflammatory cardiomyopathy



Basal



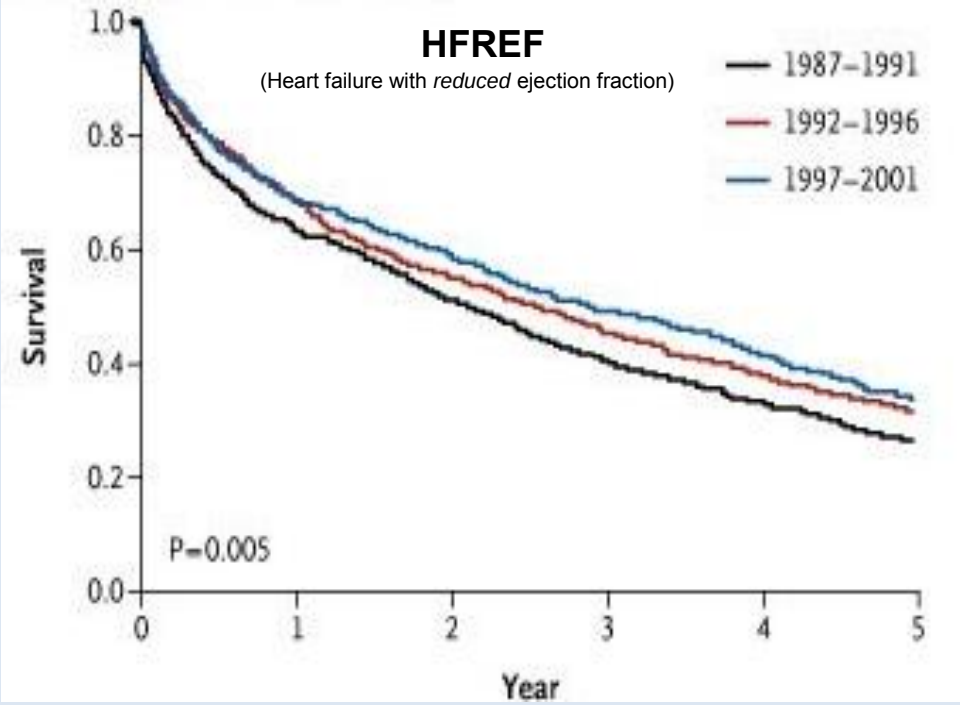
6 Monatsverlauf



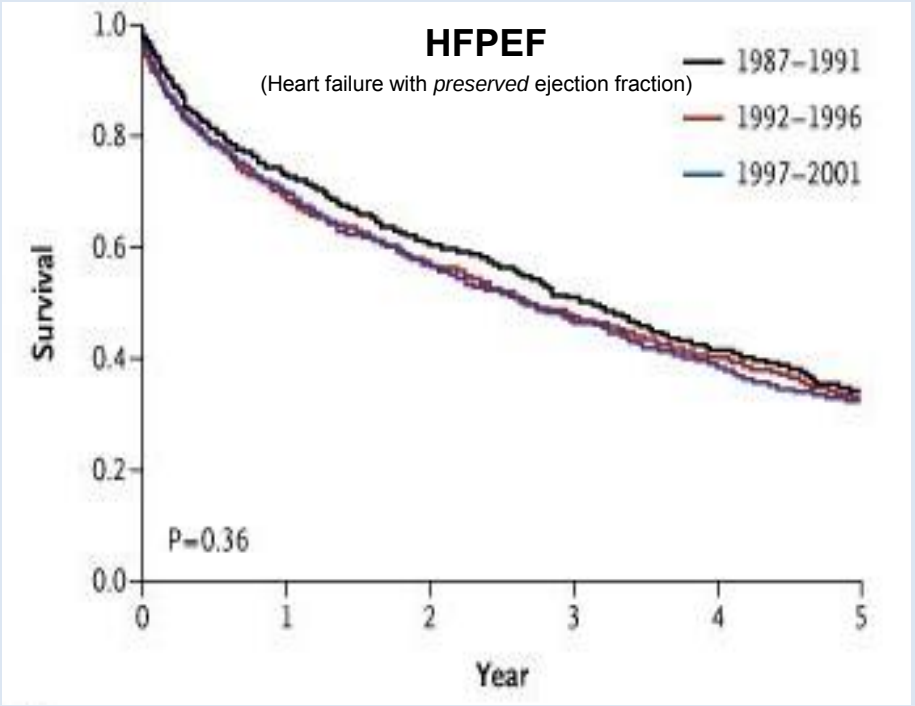
Kindermann et al. Circulation 2008

Prognosis and heart failure

HFREF

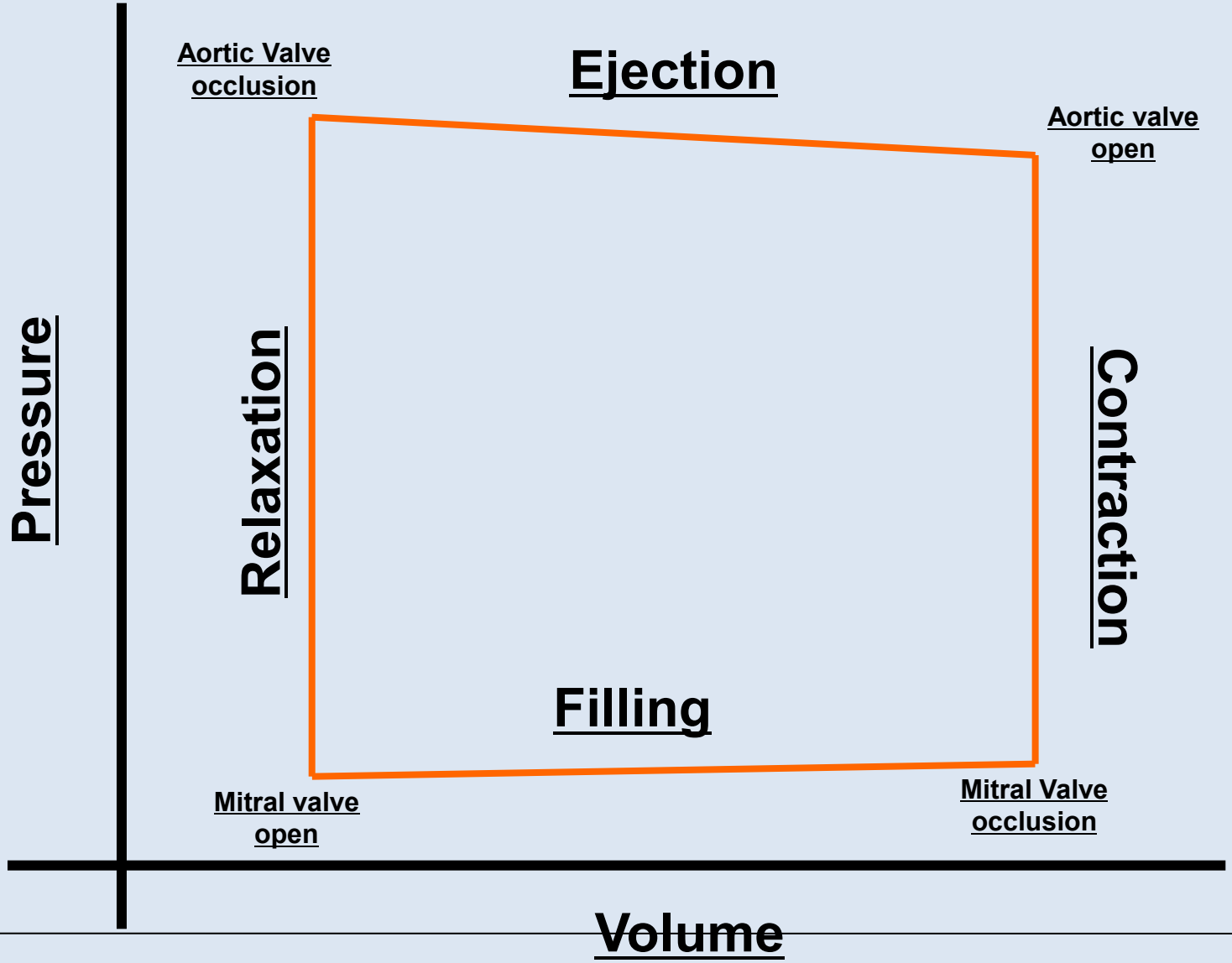


HFPEF

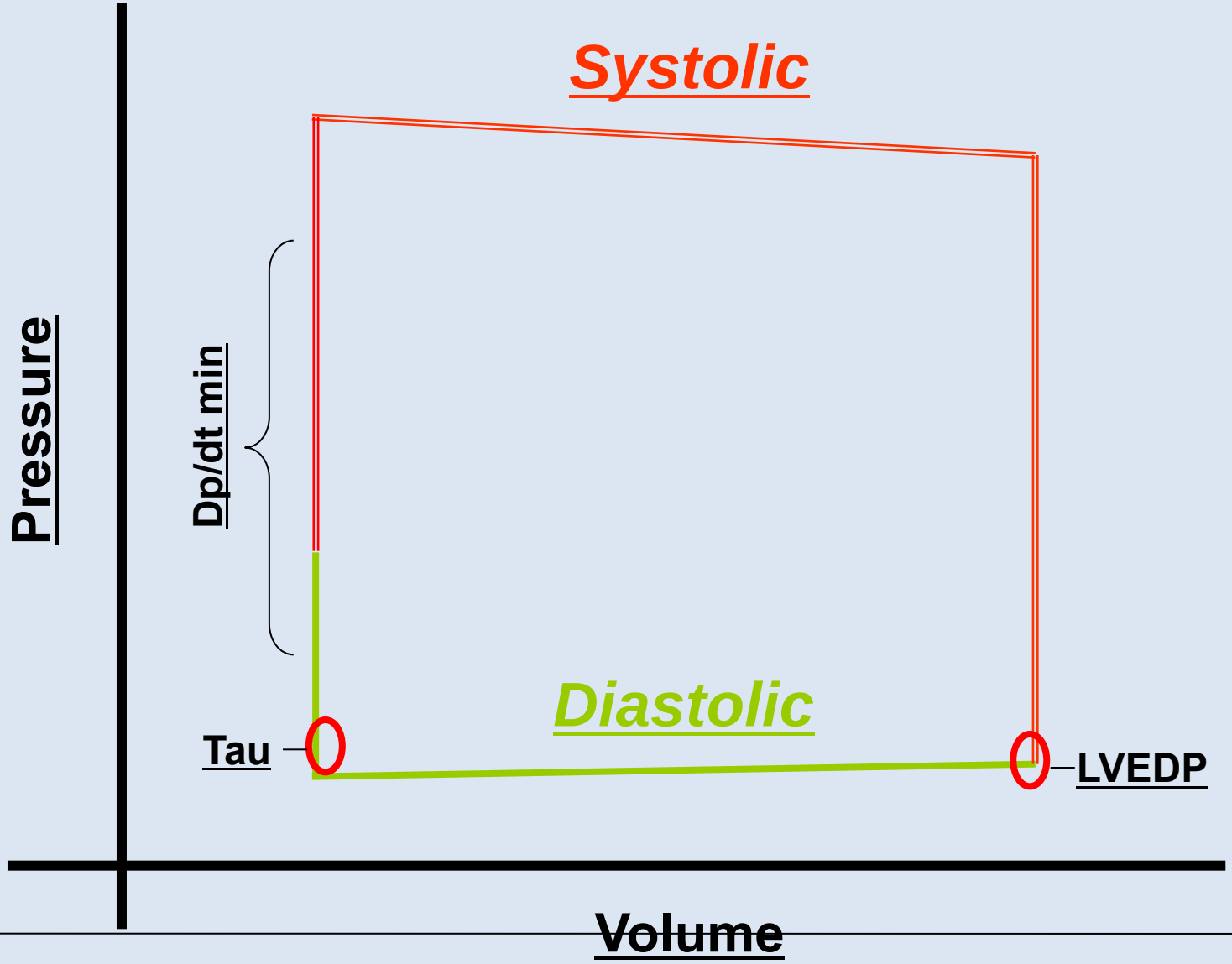


nach Owan T et al. N Engl J Med 2006;

Cardiac Working Diagram
(Pressure-Volume Relationship)

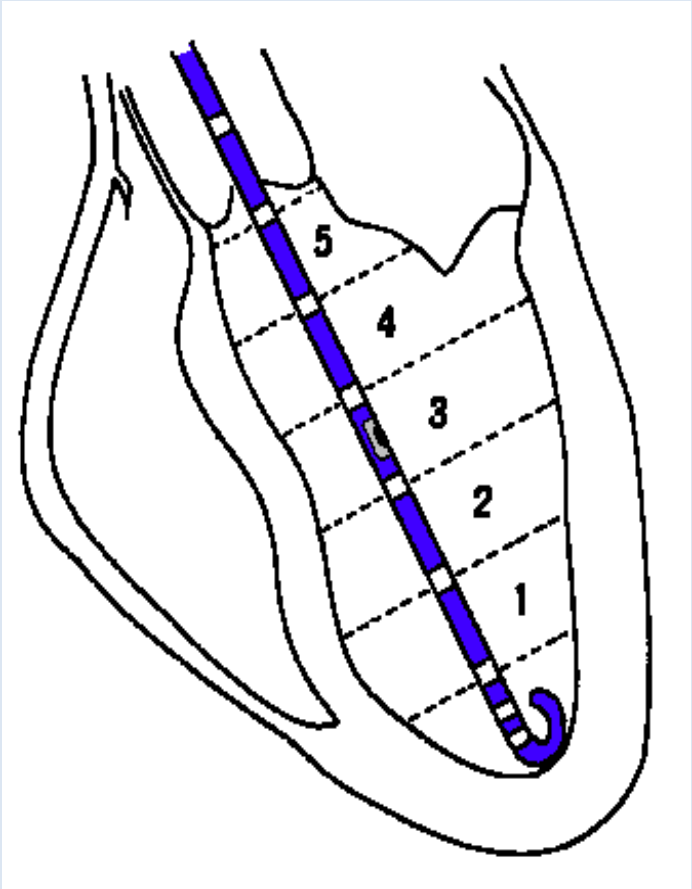


Cardiac Working Diagram
(Pressure-Volume Relationship)



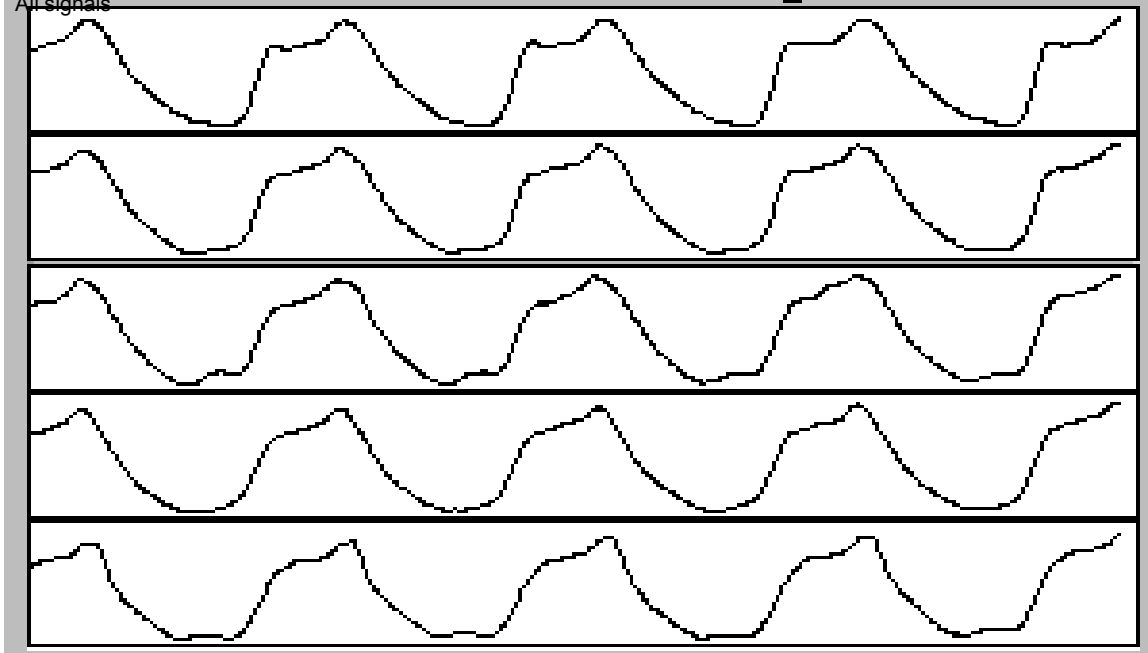
Conductance Catheter

LV pressure and volume measurement

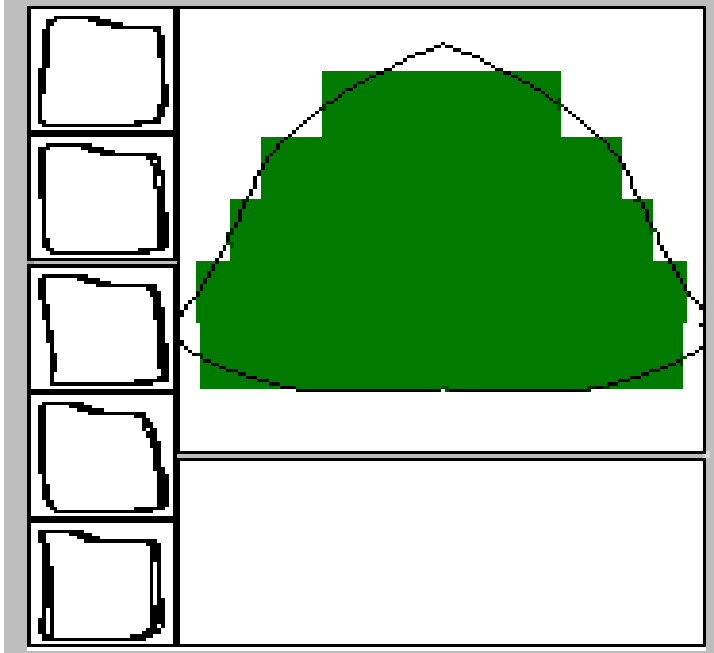


All signals

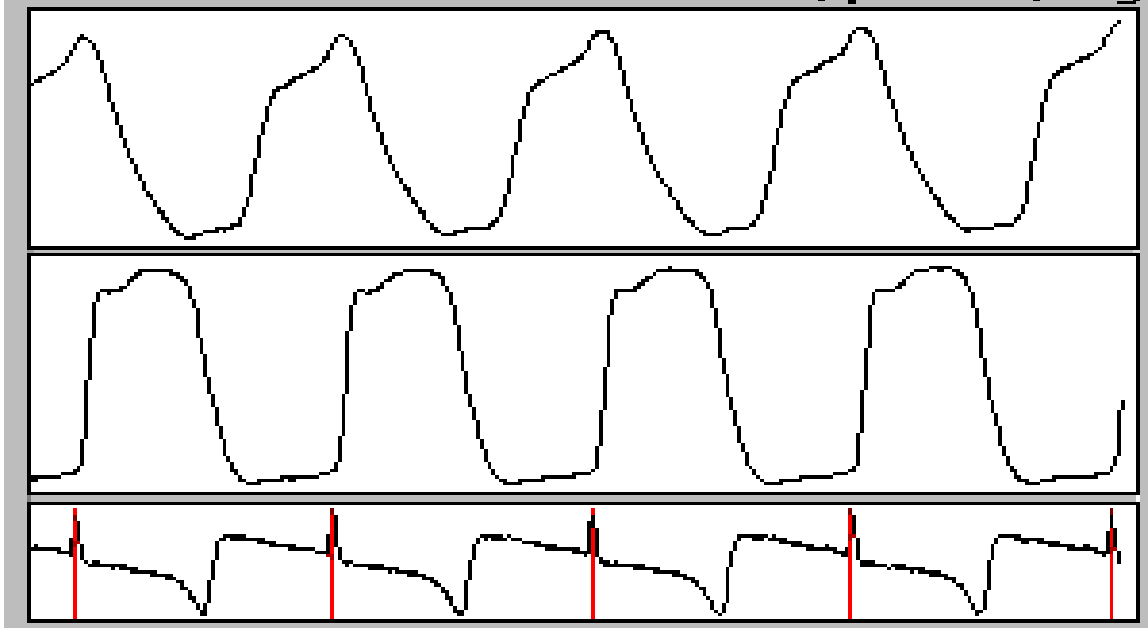
segmental volumes



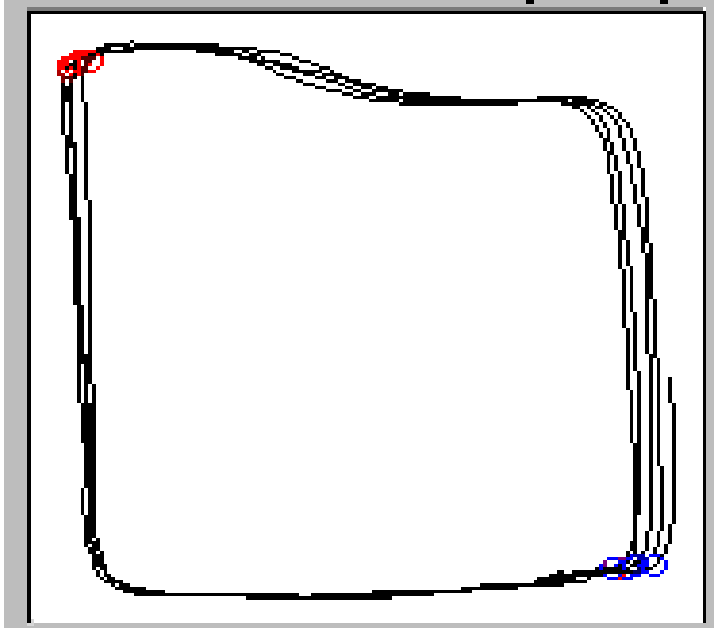
contour



volume, pressure, ecg

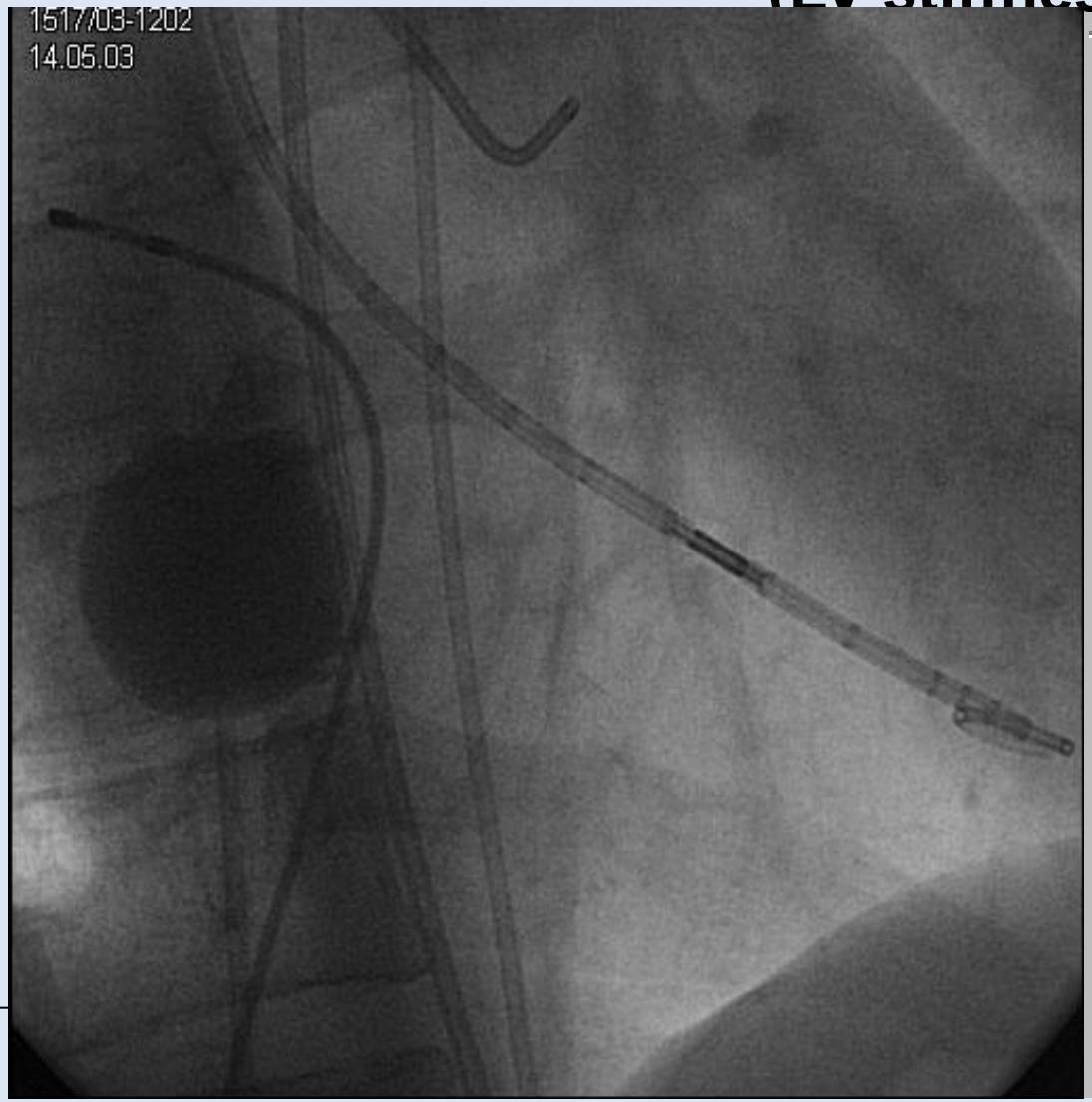


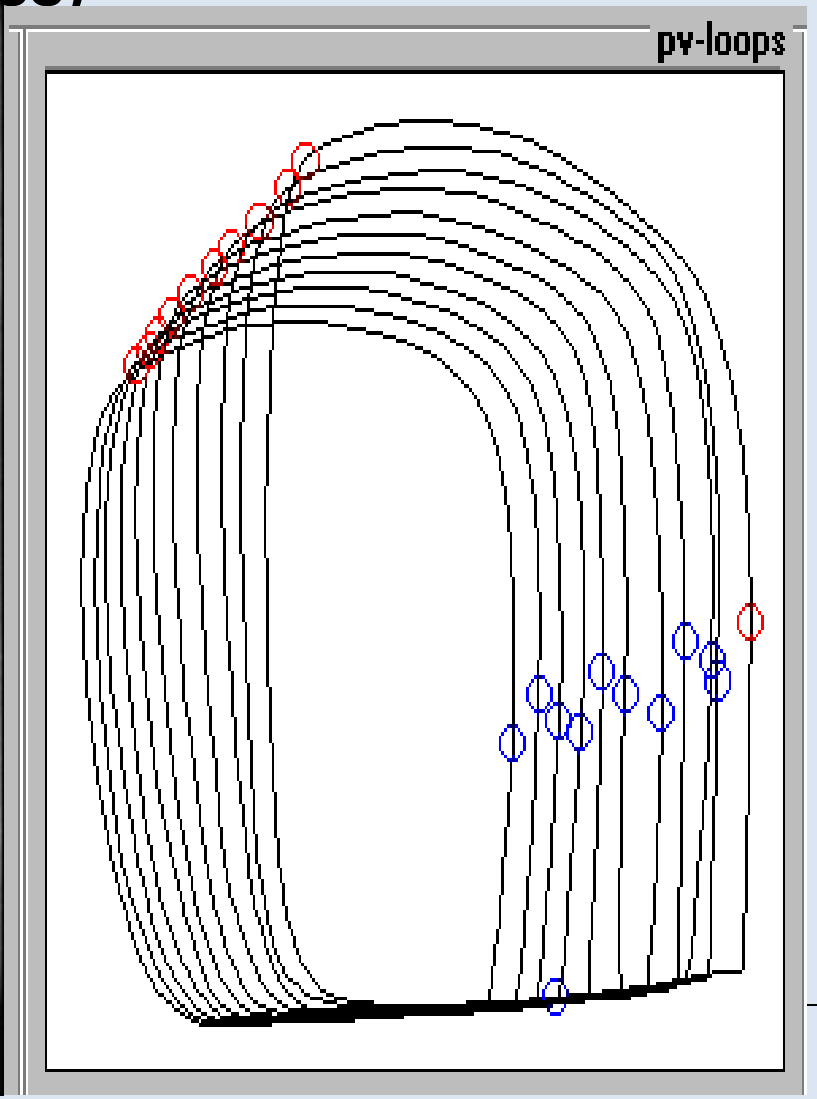
pv-loops



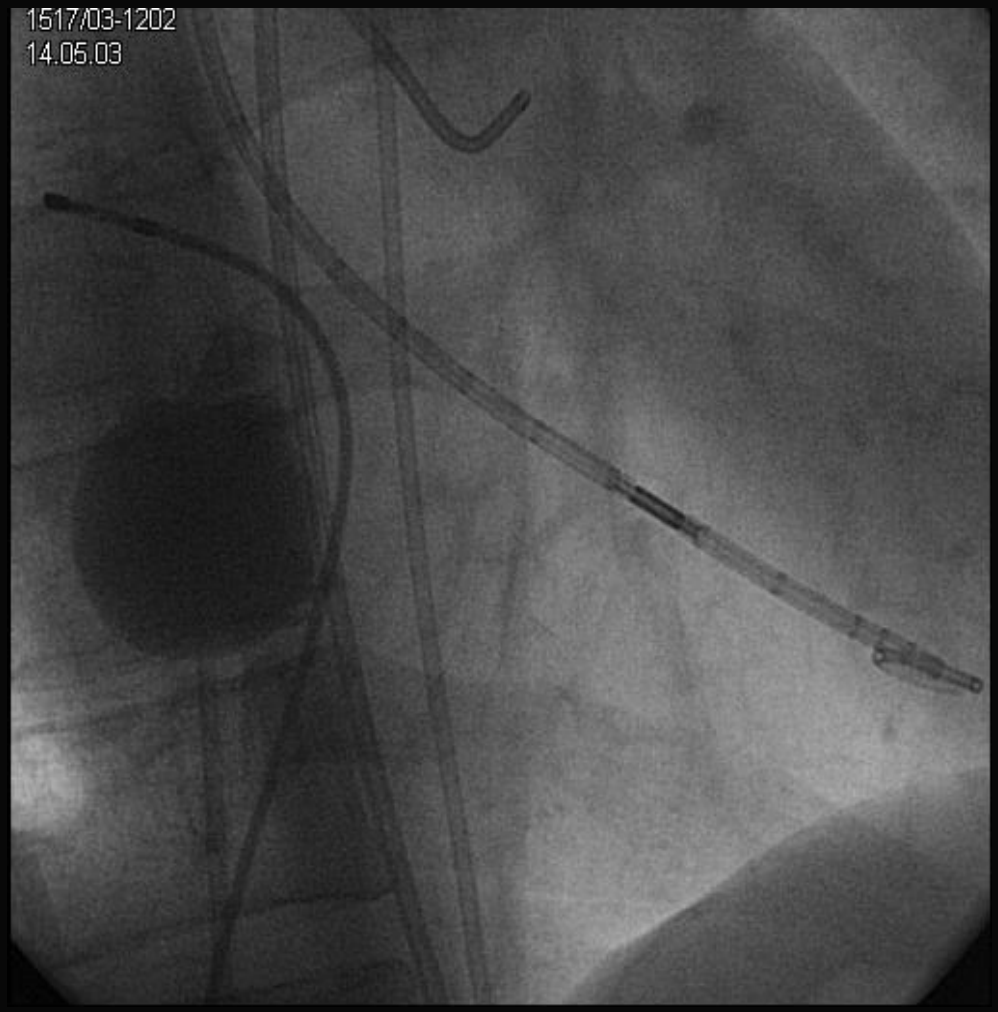
V. Cava Occlusion for transient preload reduction to evaluate end-diastolic pressure-volume relationship

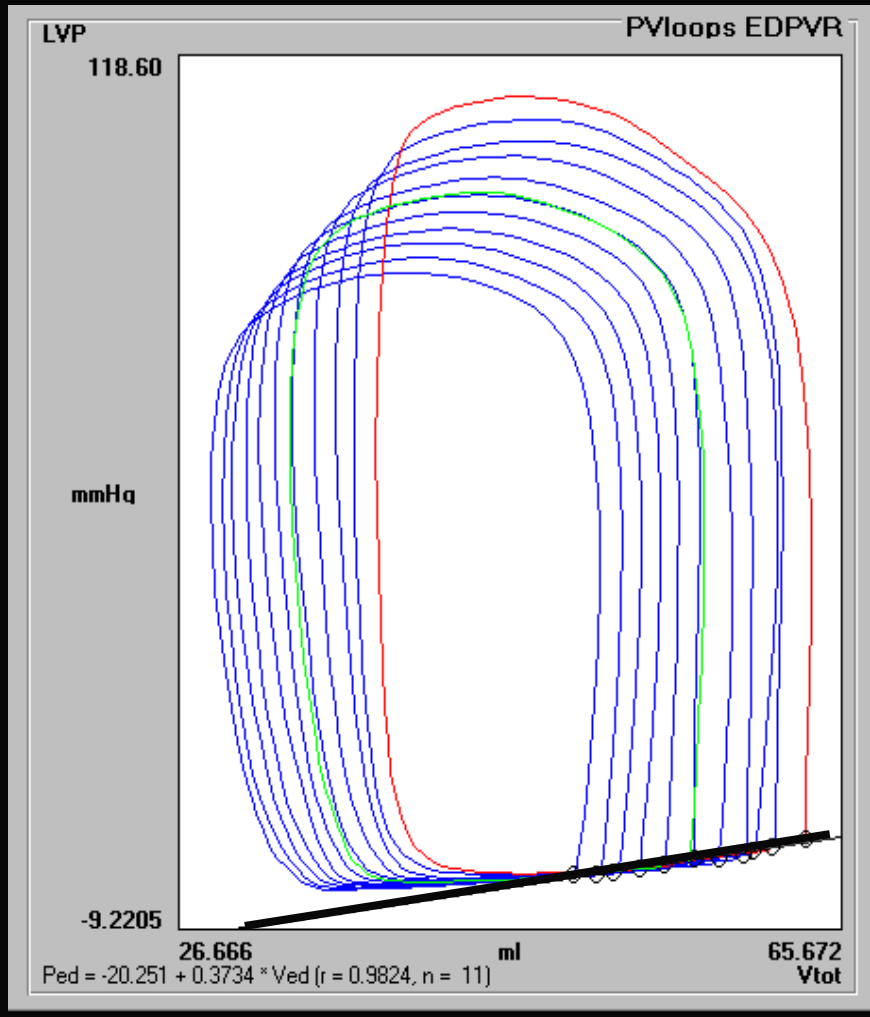
(LV stiffness)



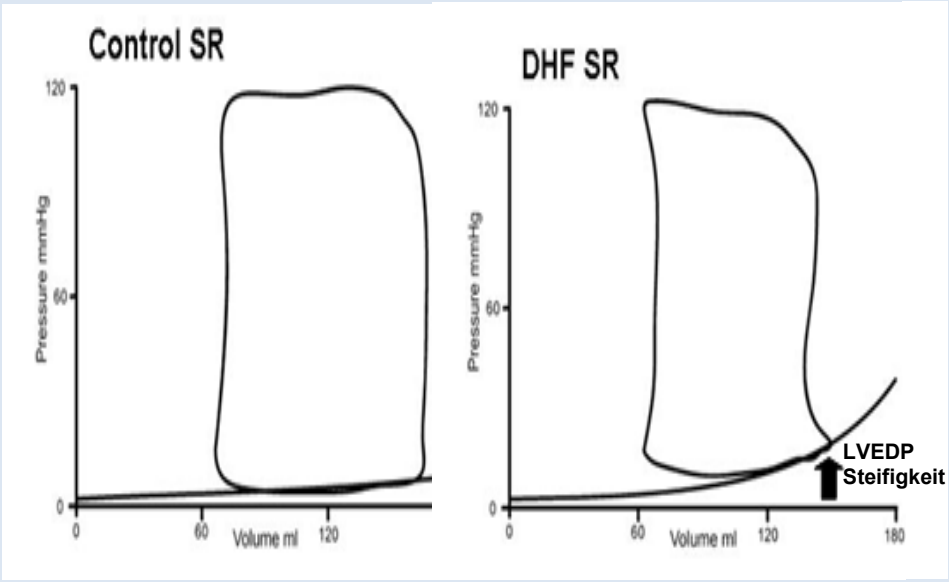


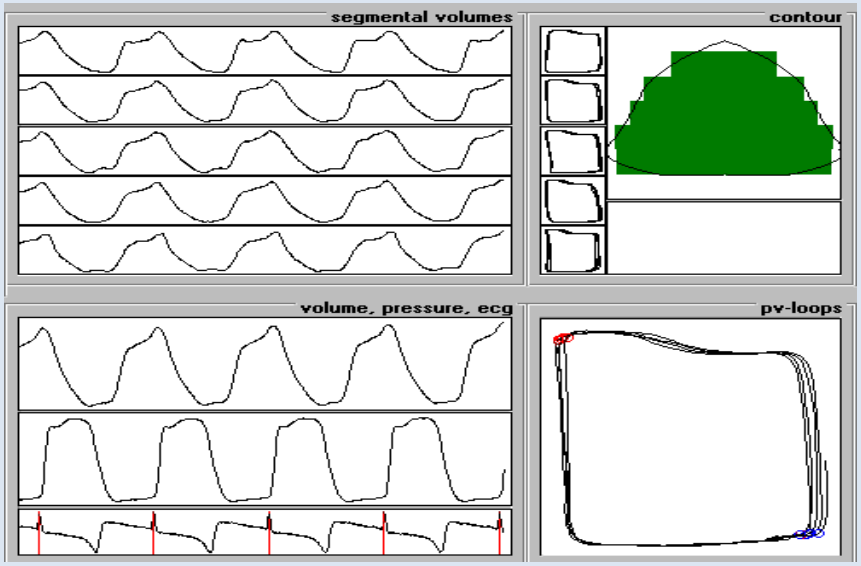
Direct measurment of LV stiffness
End-diastolic pressure-volume relationship

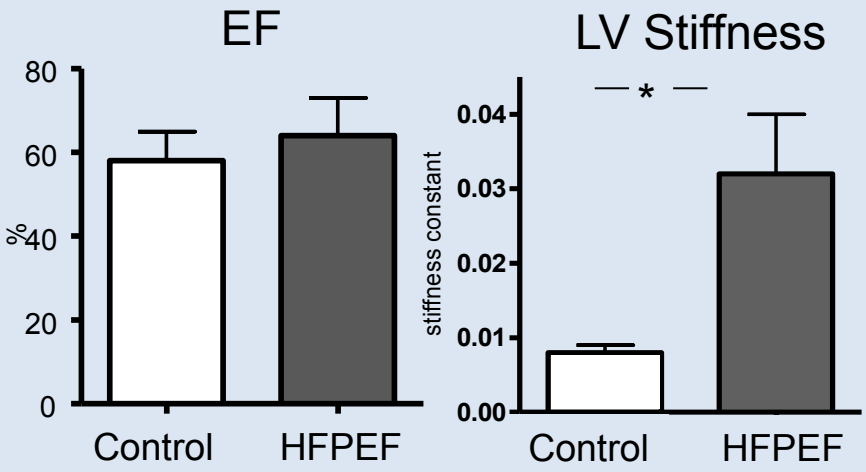




Hemodynamic characterisation of HFPEF





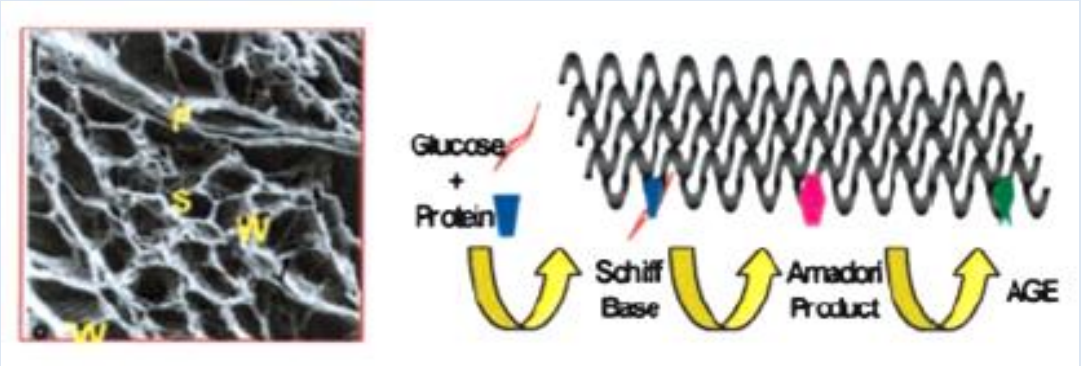


*n =70/ Gruppe, *P<0.05

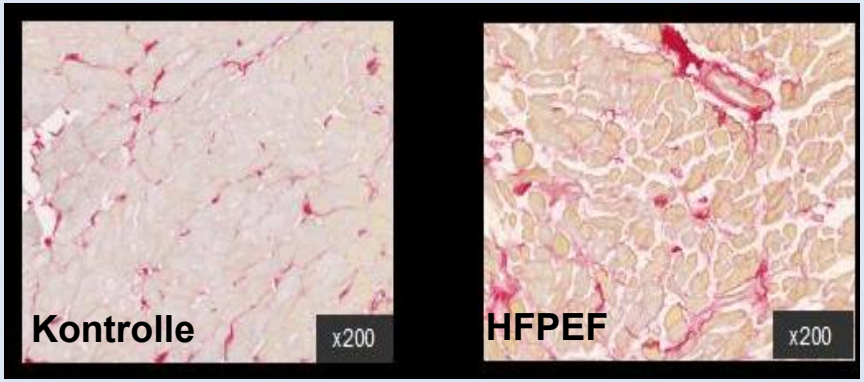
Kasner et al, Circulation 2007
Westermann et al, Circulation 2008

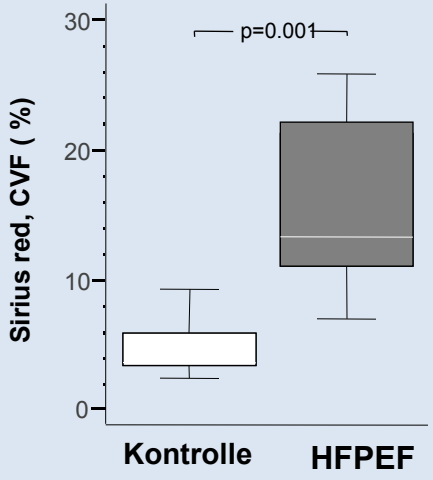
LV Stiffness: Mechanismens

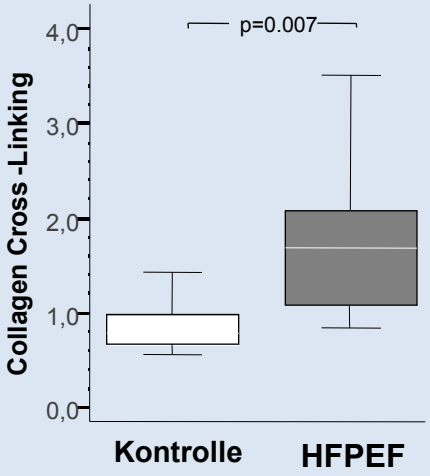
Matrix



LV Stiffness and collagen index in HFPEF



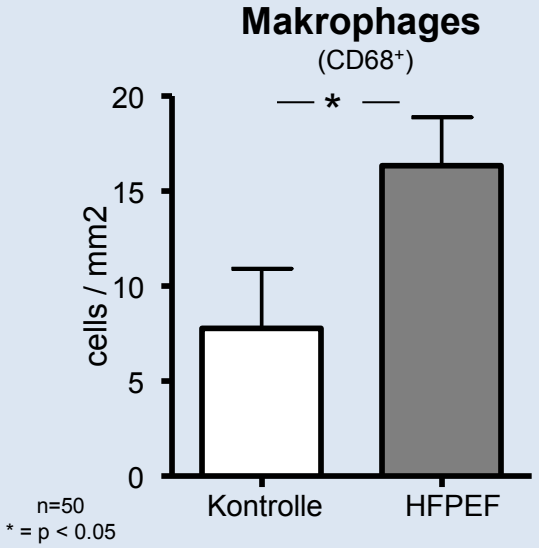


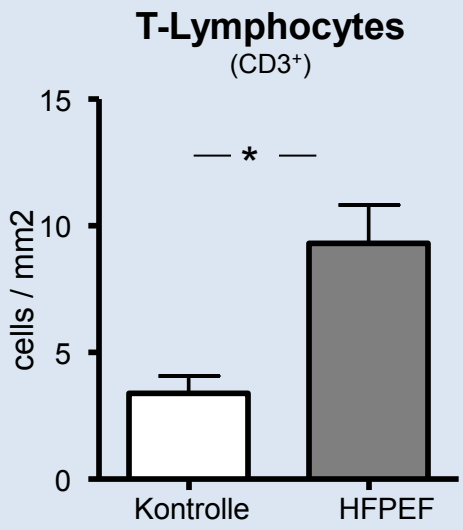


Kasner et al, JACC 2011

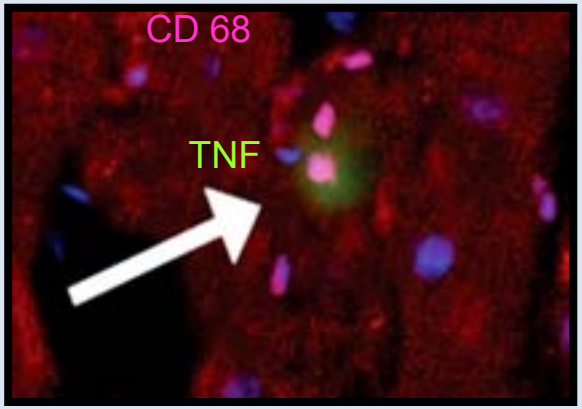
Westermann et al, Circulation 2008

Inflammation in endomyocardial biopsies of patients with HFPEF

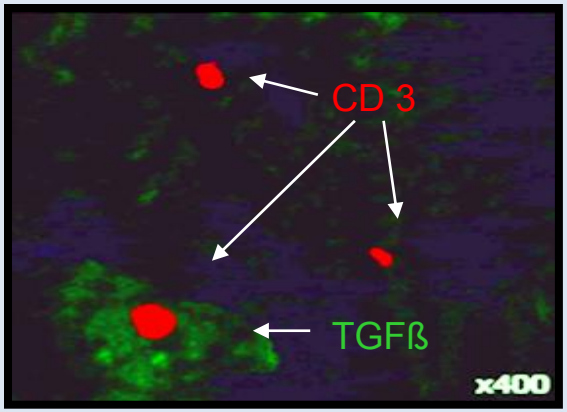




Colocalisation of TNF- α / CD 68 cells

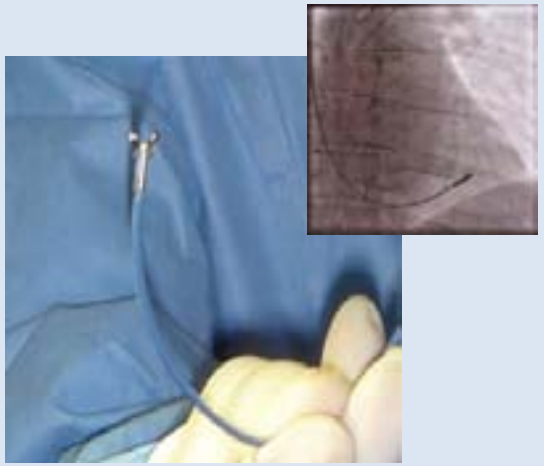


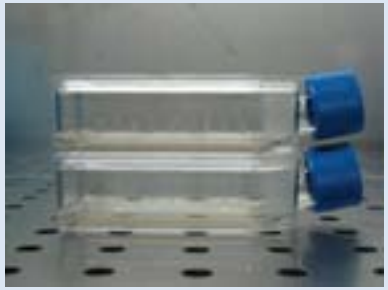
Colocalication of TGF-β / CD 3 cells



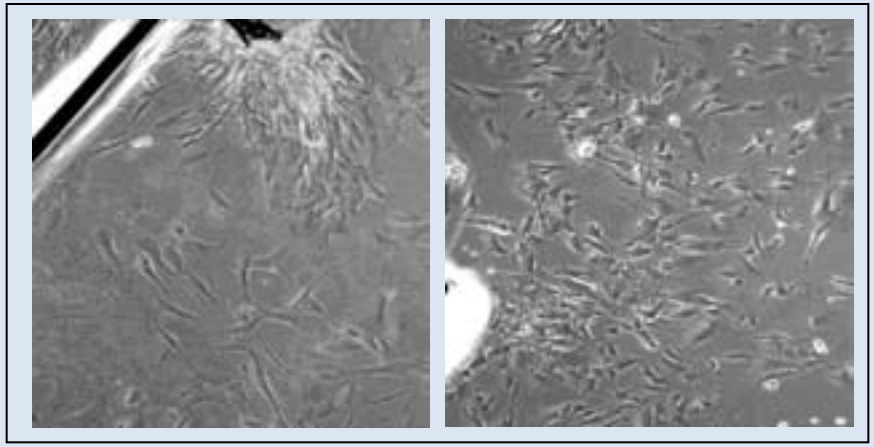
Westermann et al, Circulation HF 2011

Correlation: Matrix - inflammation?

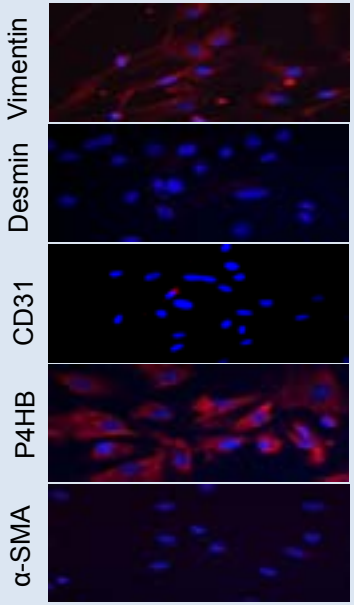




Primary human fibroblast cell culture

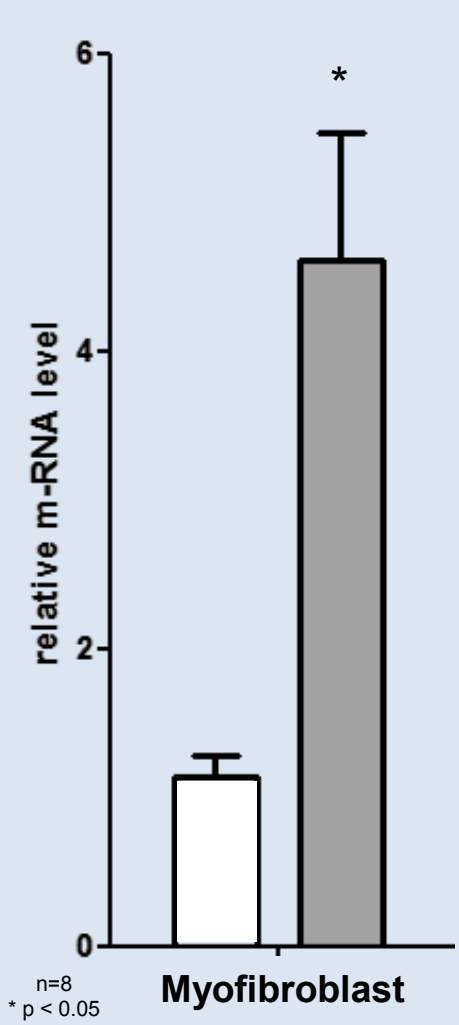


P4HB+
Vimentin +
Desmin –
CD 31-
α SMA-

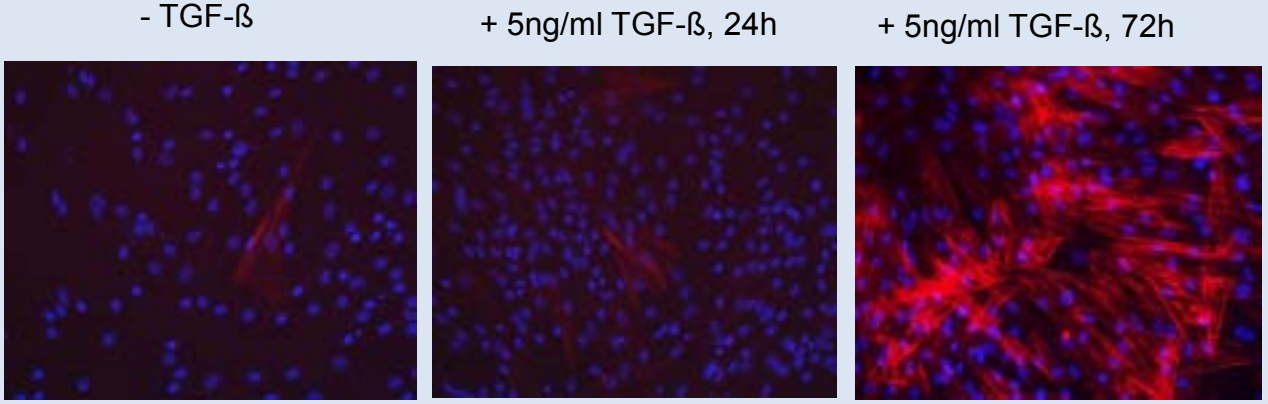


Linder et al, Biochem Res Int 2012

Role of growth Factor–β (TGF- β)

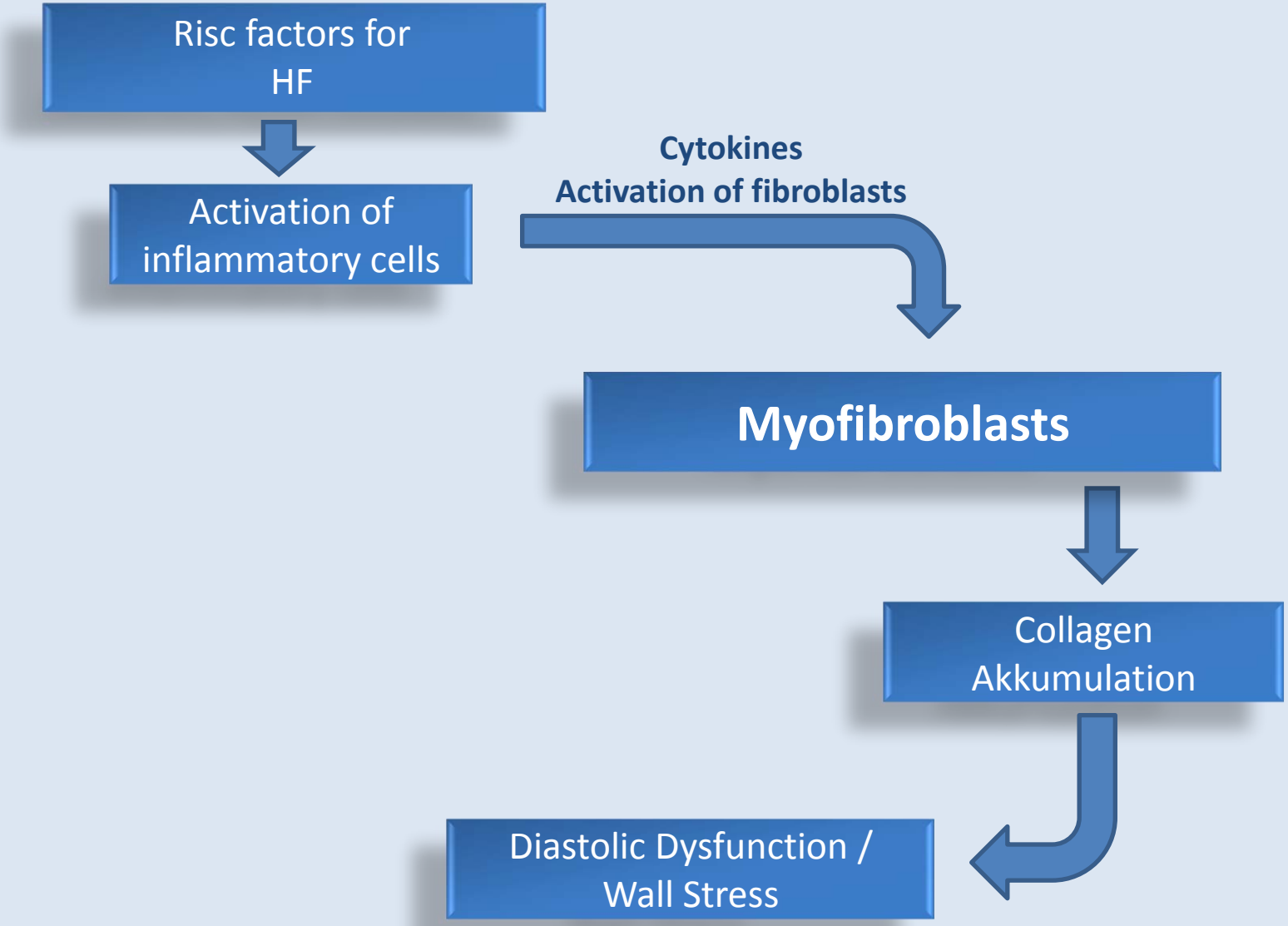


In vitro Fibroblast after TGF-β Stimulation (alpha-SMA)

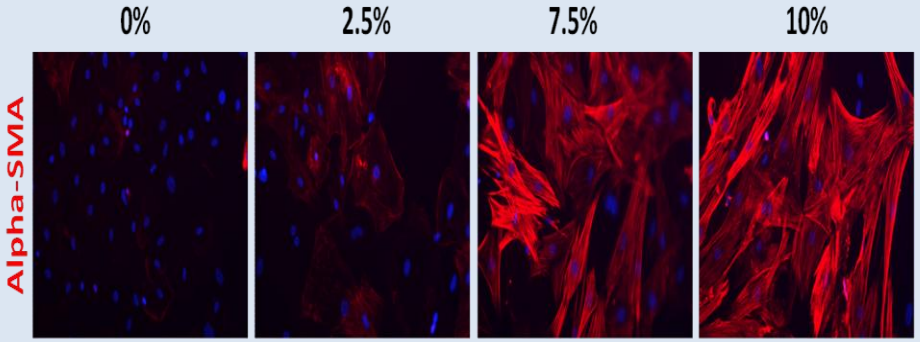


Westermann et al, Circulation HF 2011

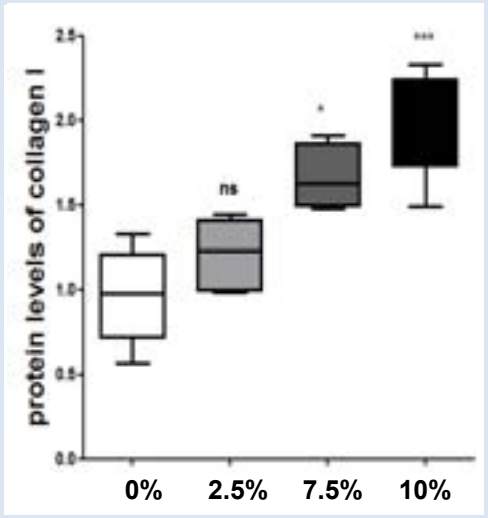
Mechanism in heart failure:
Fibrosis and Inflammation



Mechanic stress activates myofibroblasts



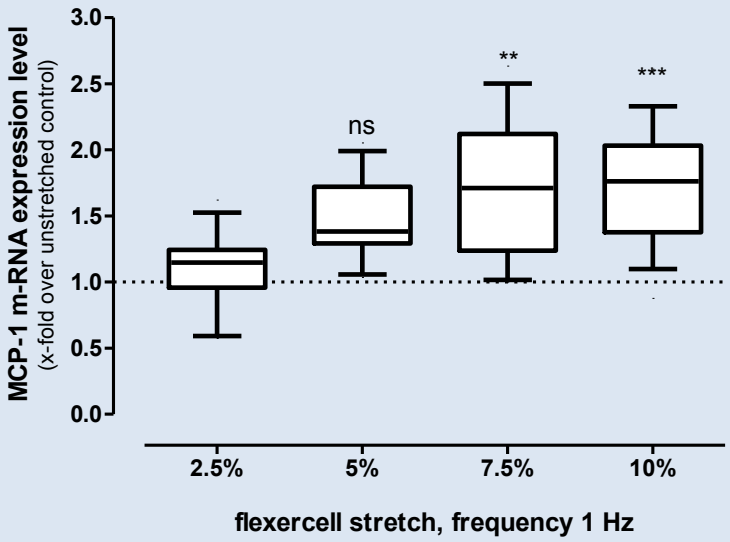
Production of collagen

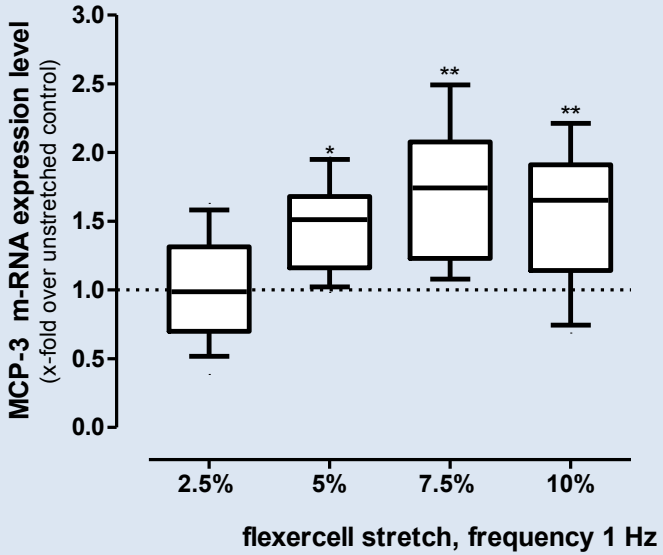


Strech intensity

Westermann et al, Circulation HF 2011

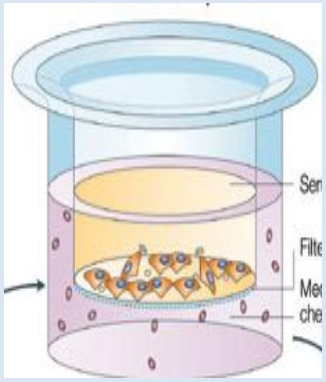
Mechanic stress induces chemokine production

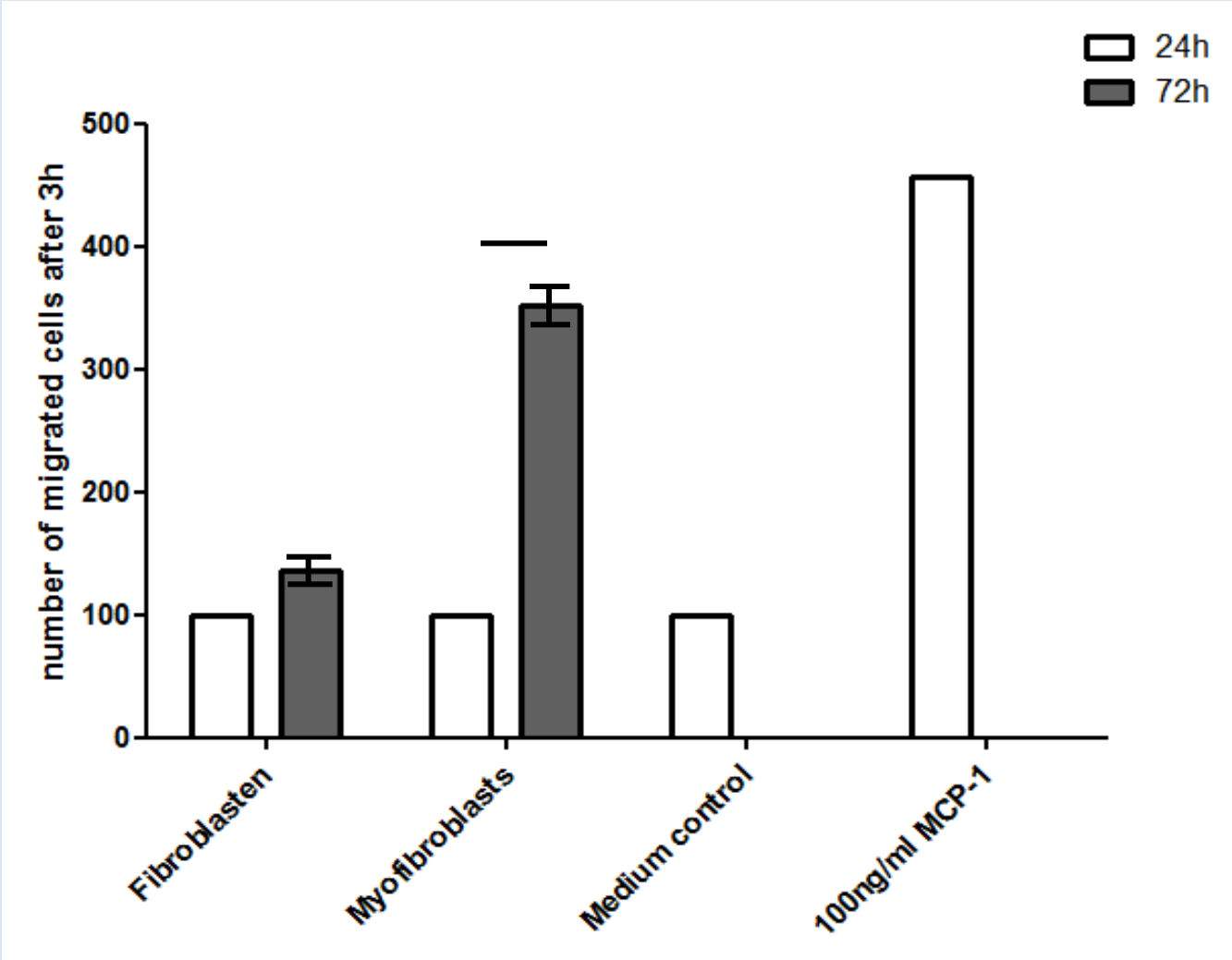




Westermann et al 2012

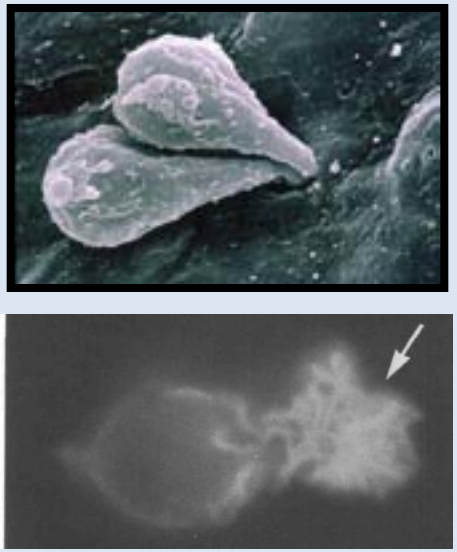
Migration of lymphocytes following the supernatant of myofibroblasts





Westermann et al 2012

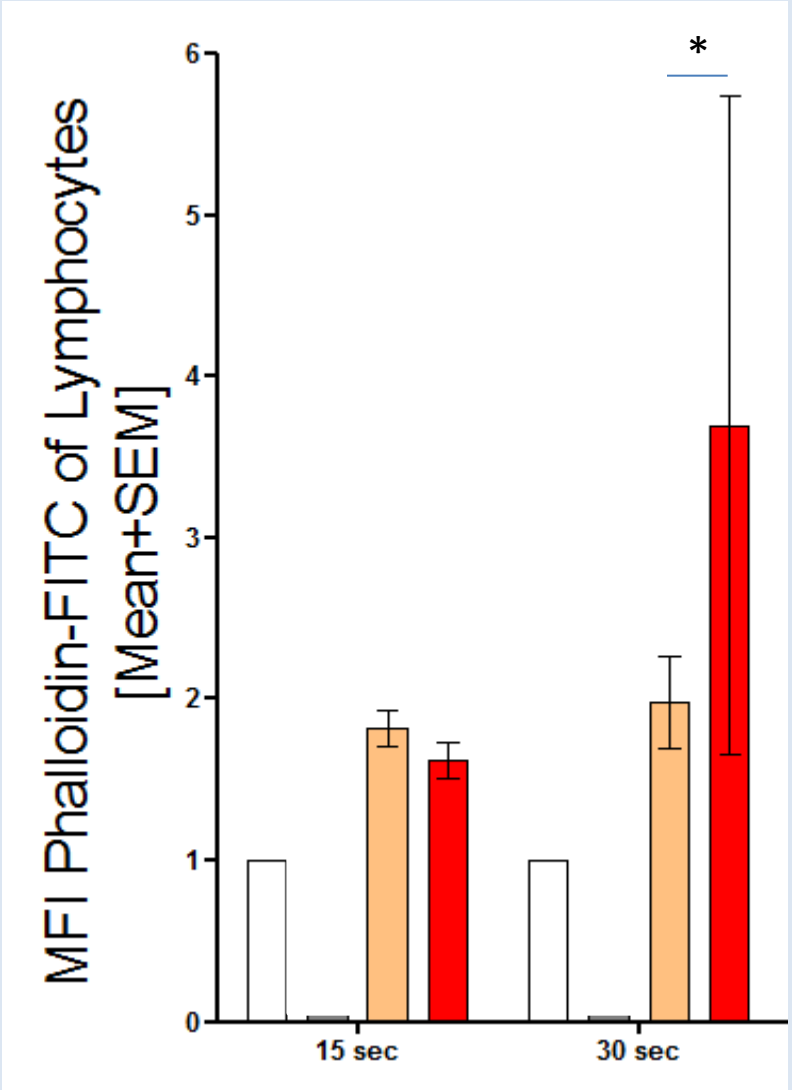
Increased actin polymerization of PBMCs as a sign of increased cell mobility after incubation with the supernatant of myofibroblasts



Incubated with medium

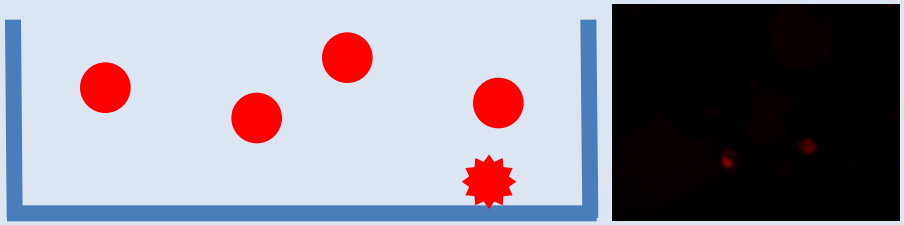
Supernatant of fibroblasts

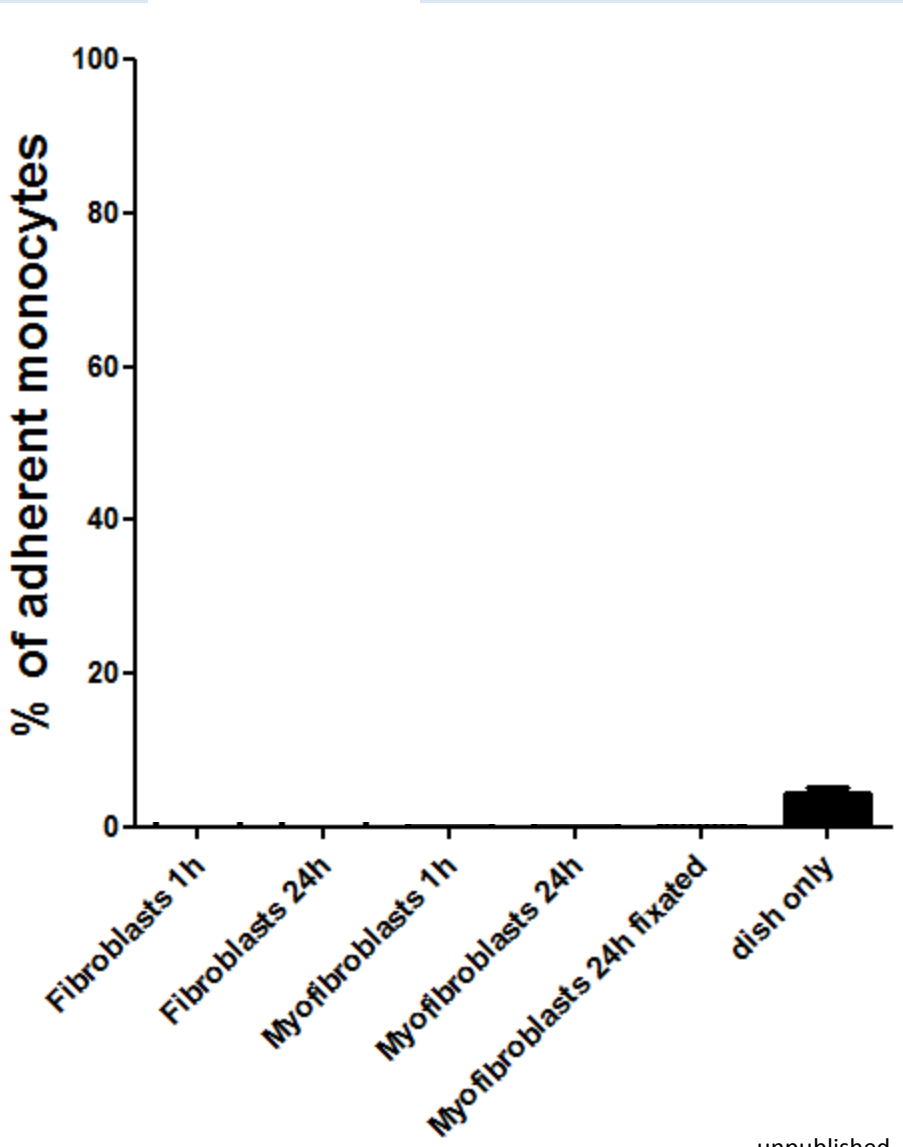
Supernatant of myofibroblasts



unpublished

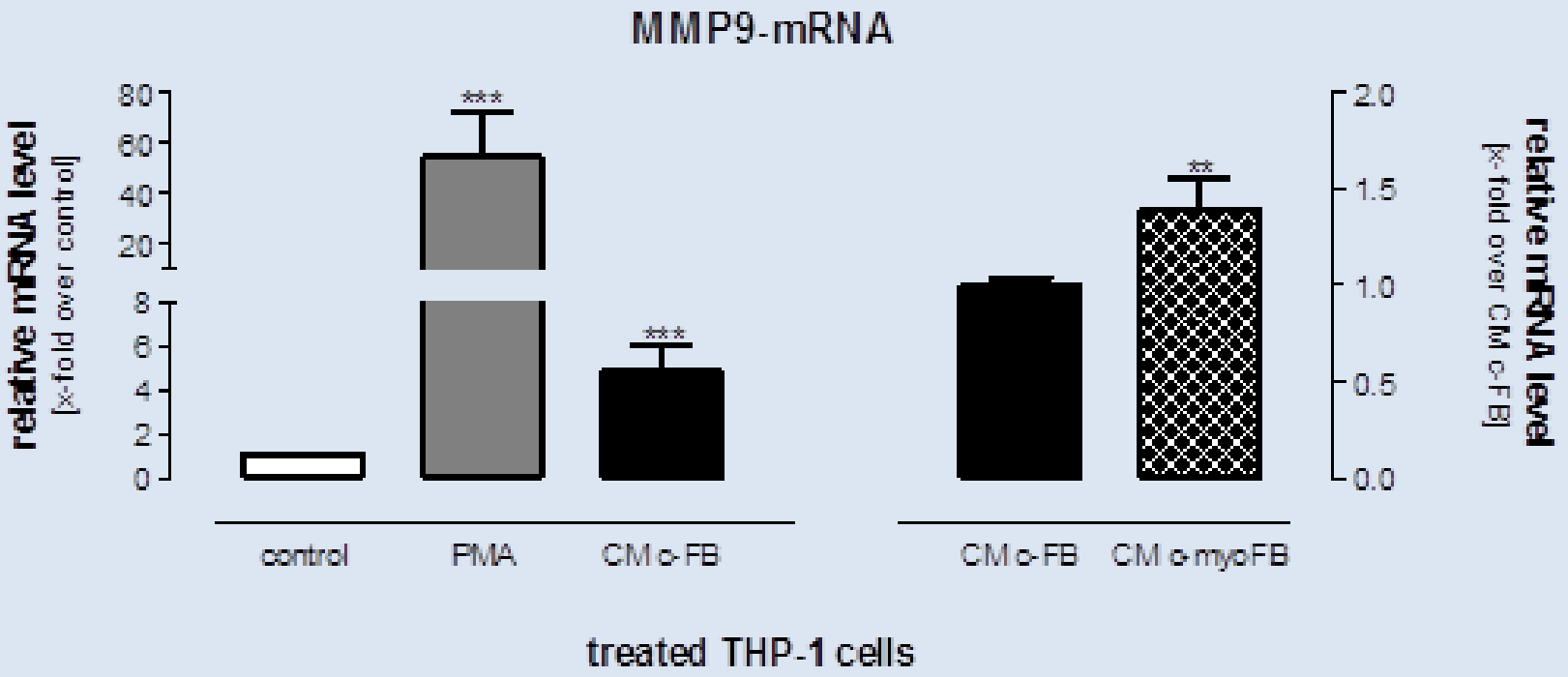
Direct adhesion of monocytes (THP-1) on myofibroblasts





unpublished

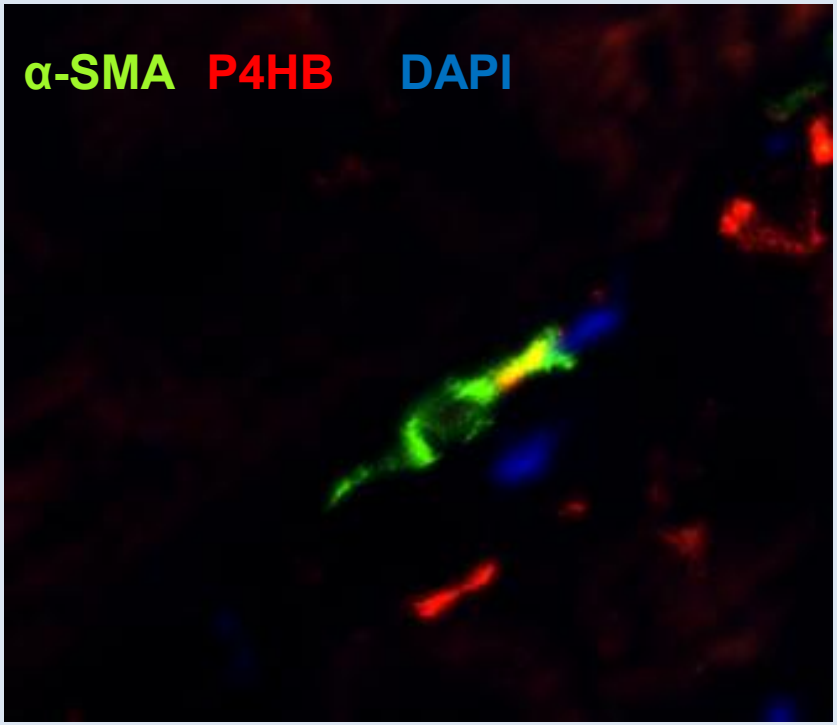
Monocytes (Th1-Zellen) produce MMP-9 after treatement with the supernatant of myofibroblasts



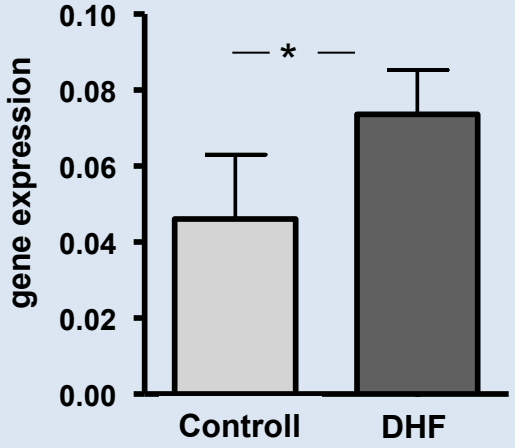
Identification of myofibroblasts in humanen cardiac biopsies

Migration and Chemotaxis

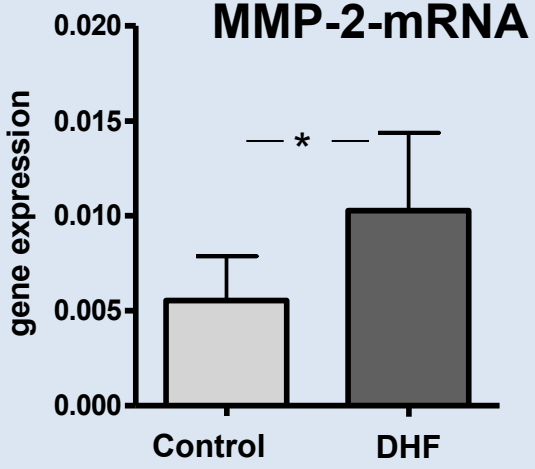
Myofibroblasts



MCP2-mRNA

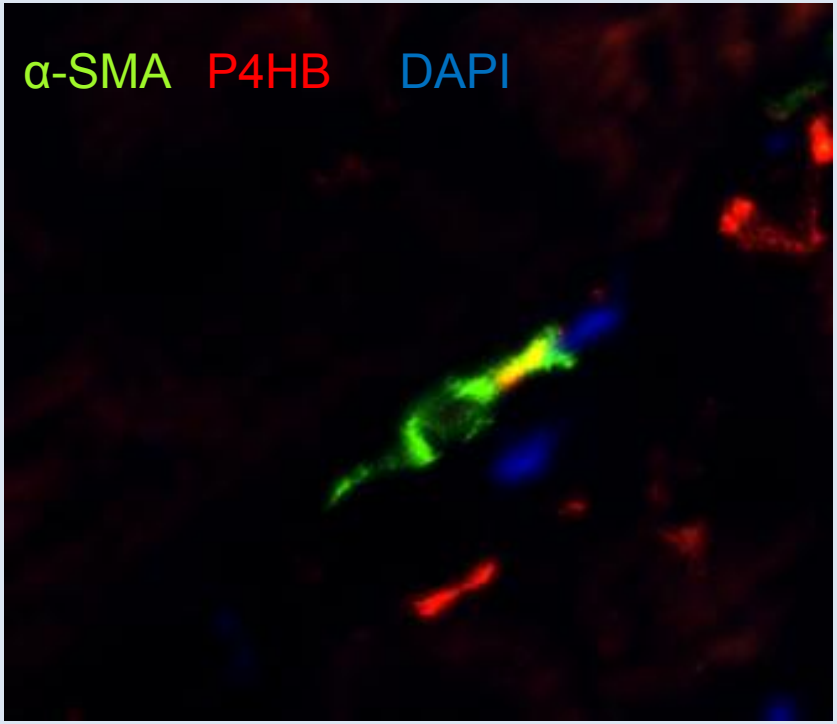


MMP-2-mRNA

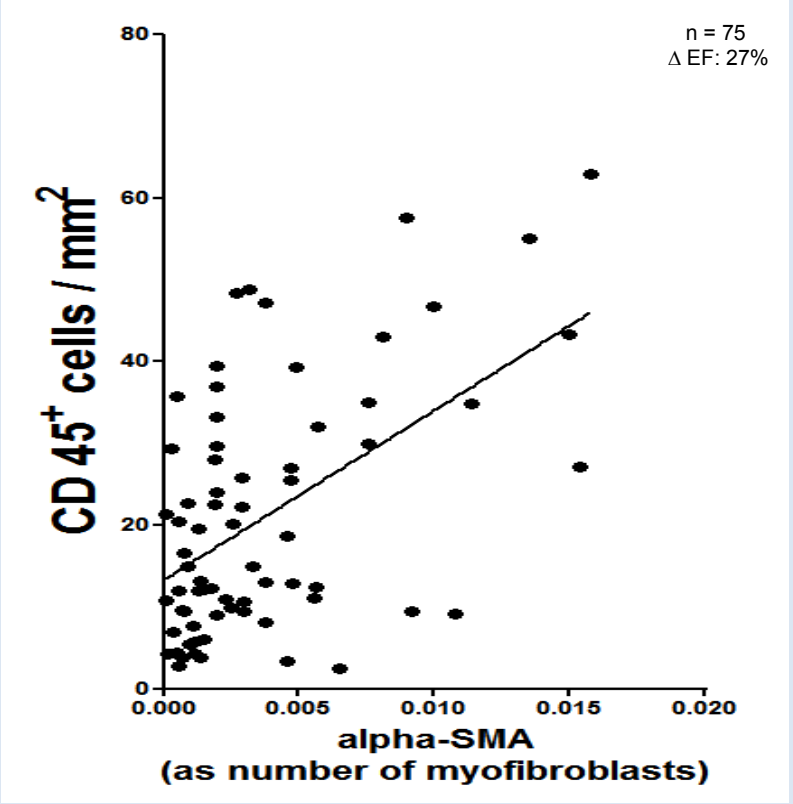


Identification of myofibroblasts in humanen cardiac biopsies

Myofibroblast

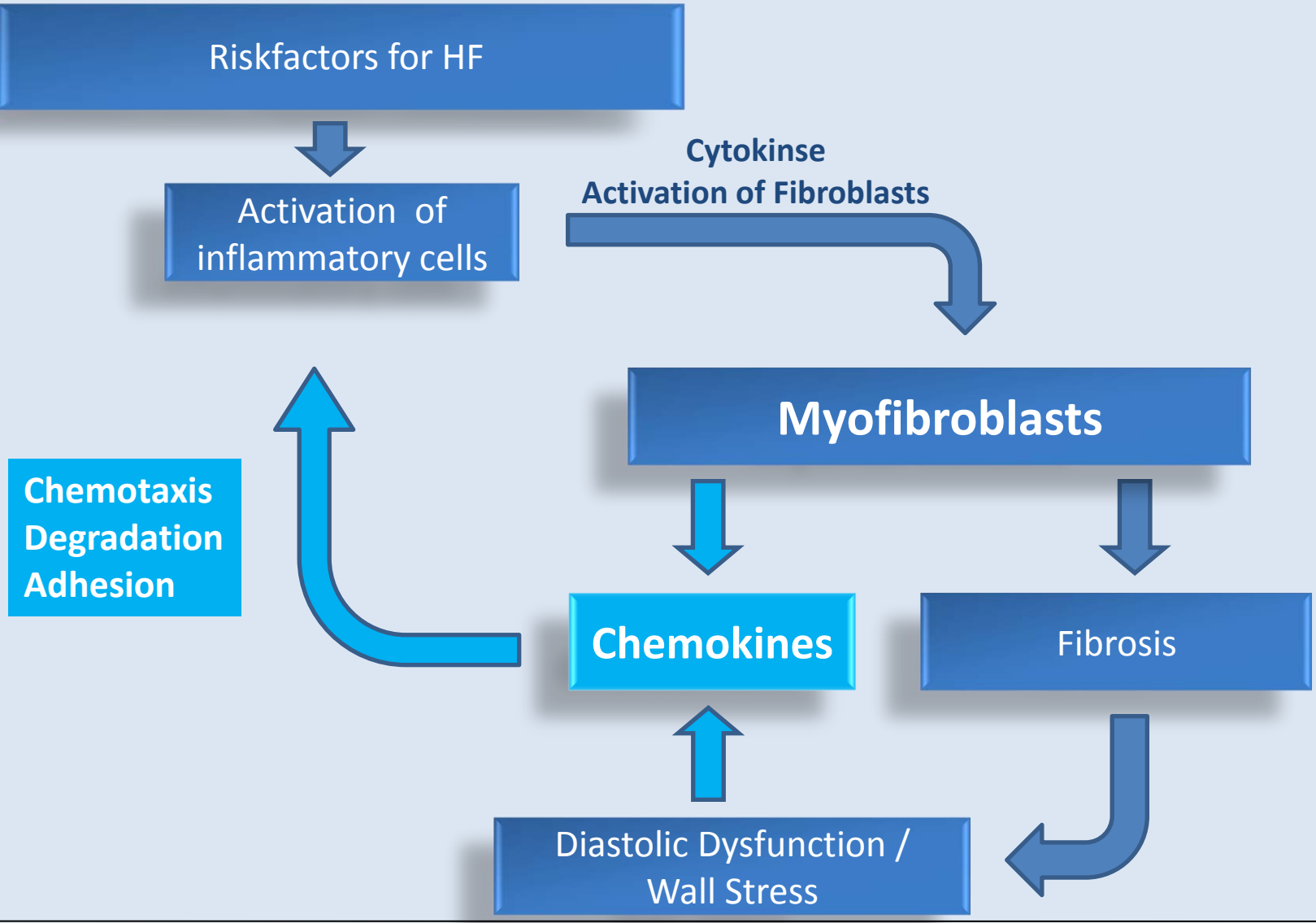


Number of myofibroblasts correlates with the number of invaded inflammatory cells

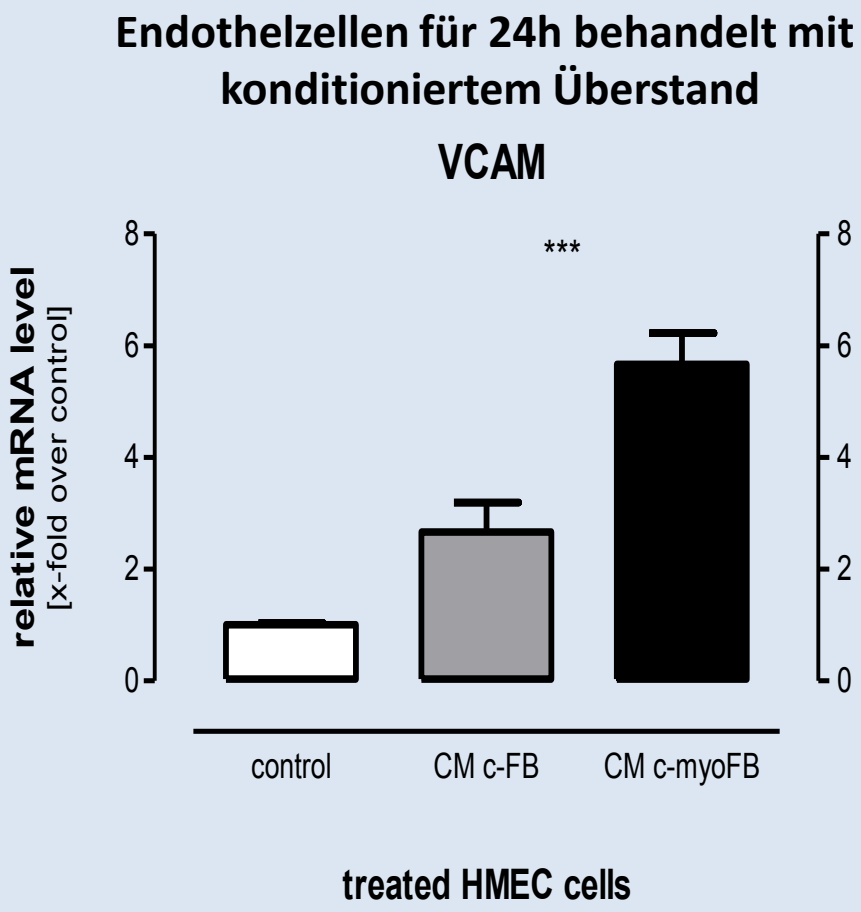


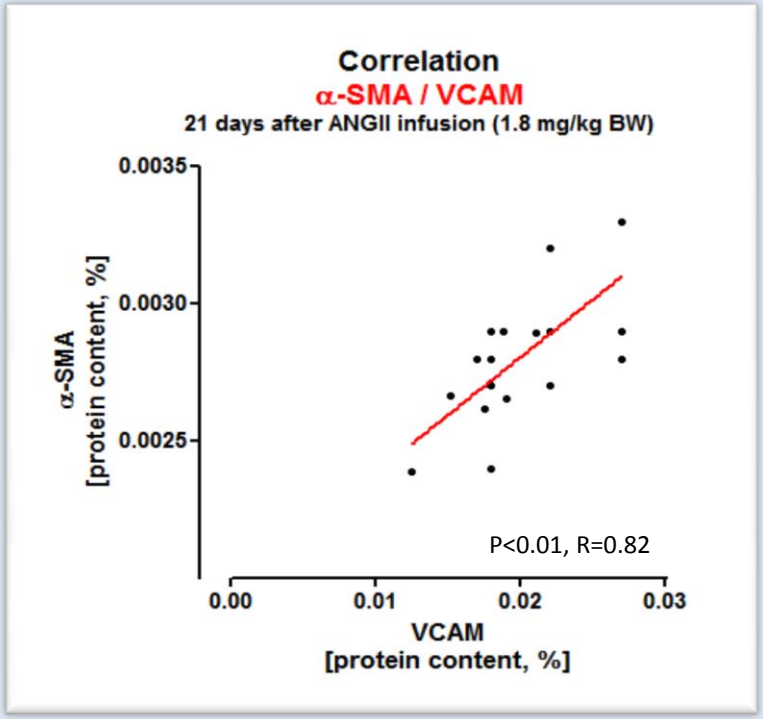
Westermann et al, Circulation HF 2011

Fibrosis and Inflammation as a Circulus vitiosus

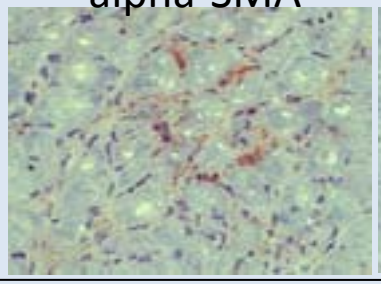


Increased expression of adhesionsmolecules after incubation of endothelial cells with the supernatant of myofibroblasts

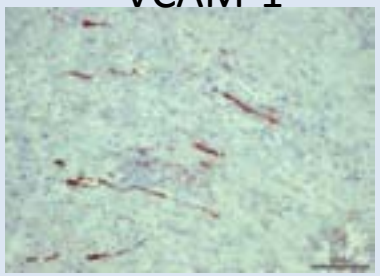




alpha-SMA

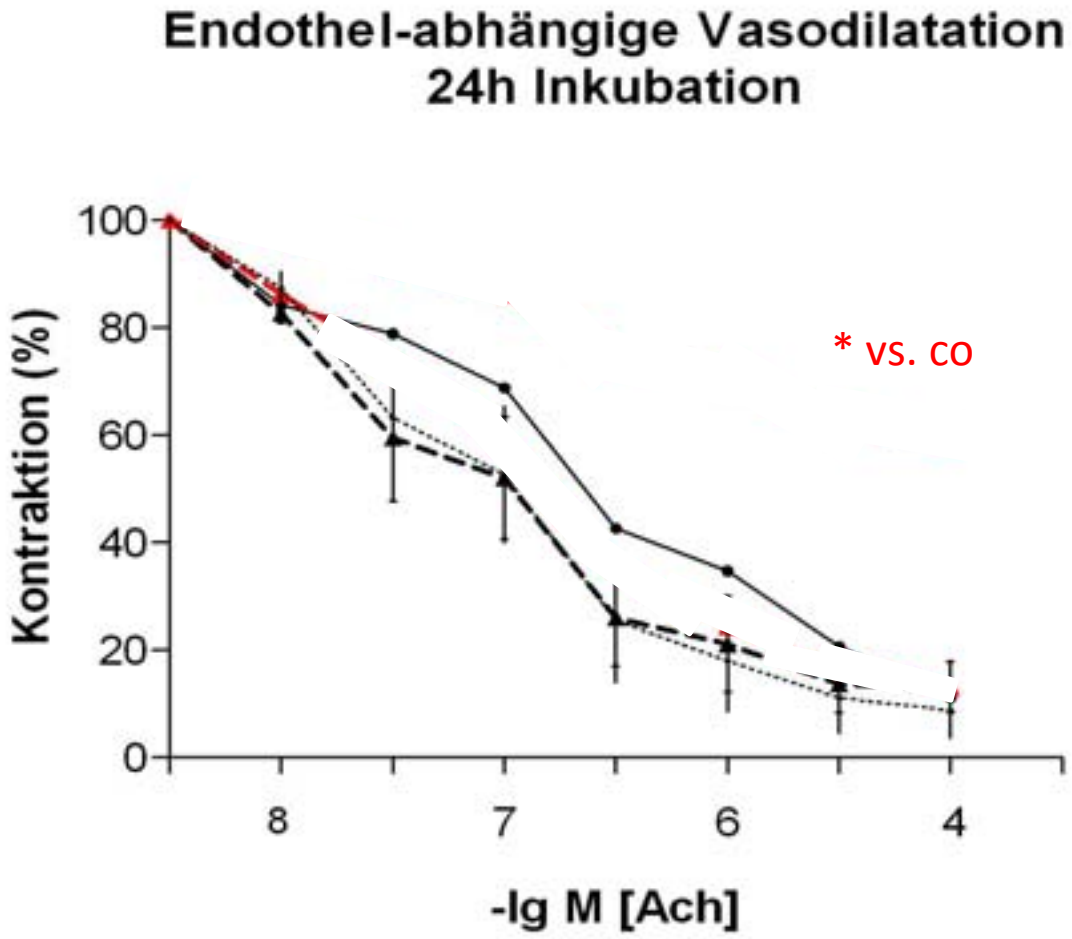


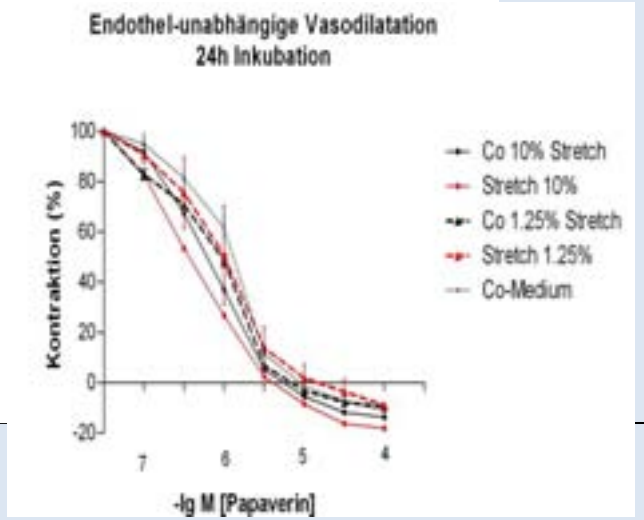
VCAM-1



Westermann et al 2012

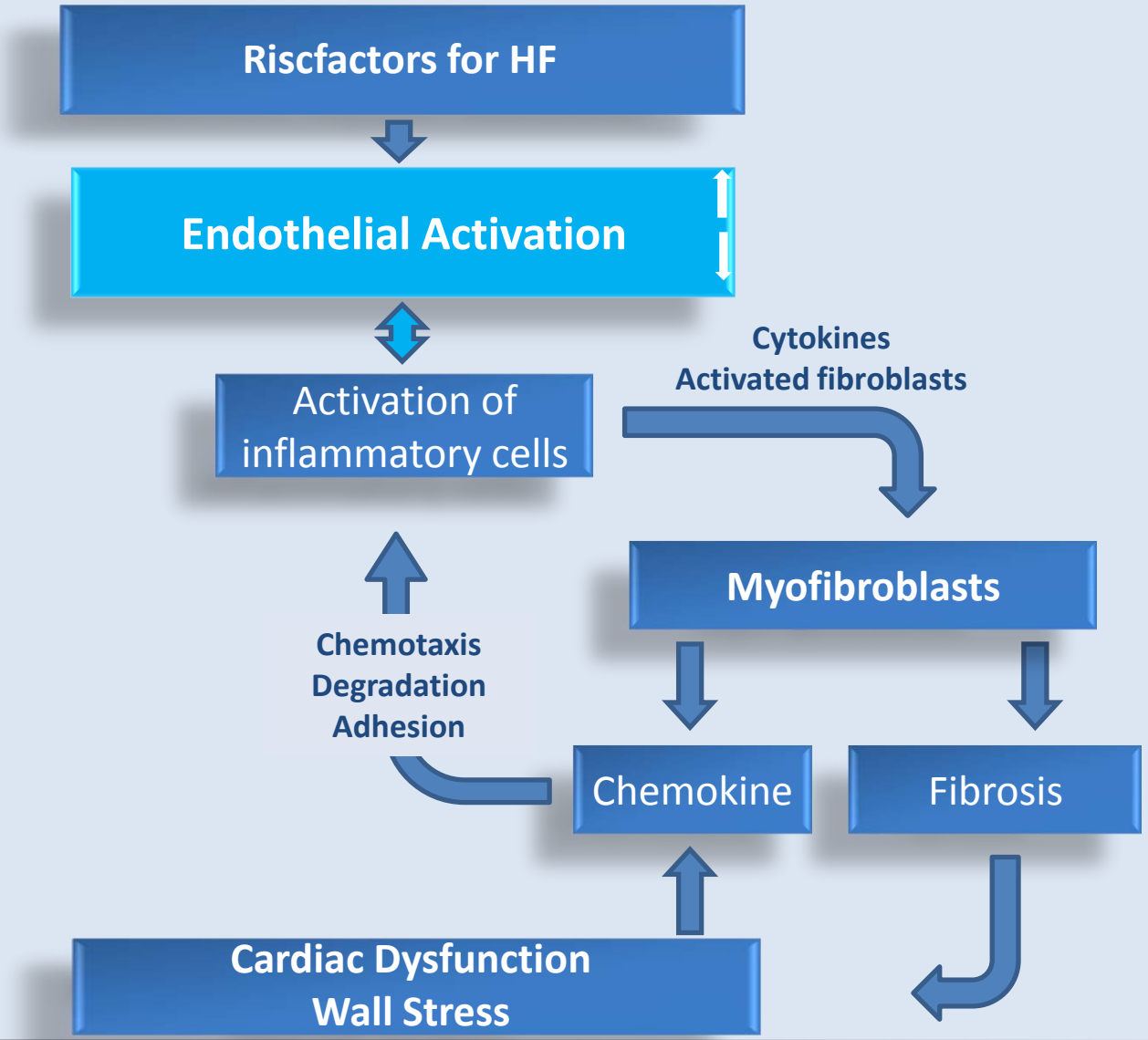
Endothelial dysfunction *in vitro* after stimulation with the supernatant of myofibroblasts





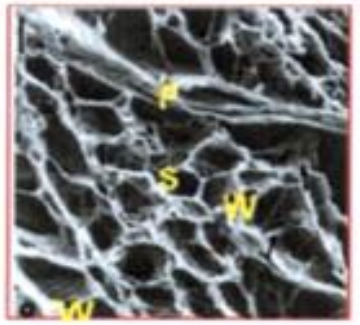
Westermann et al 2013

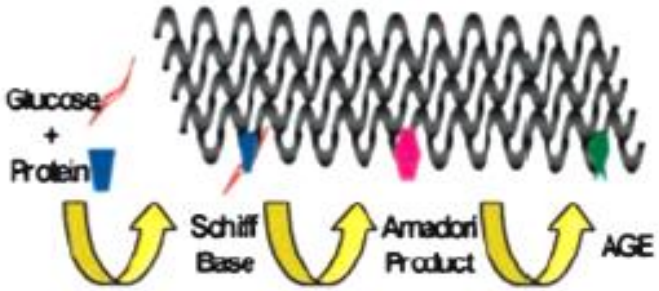
Myofibroblast and vascular in heart failure



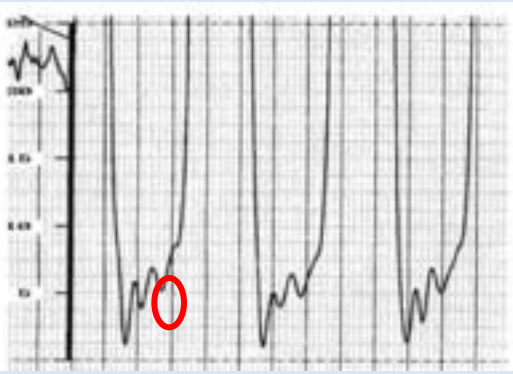
LV Stiffness: Mechanisms

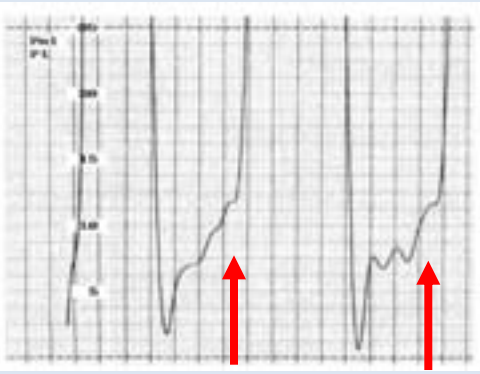
Matrix



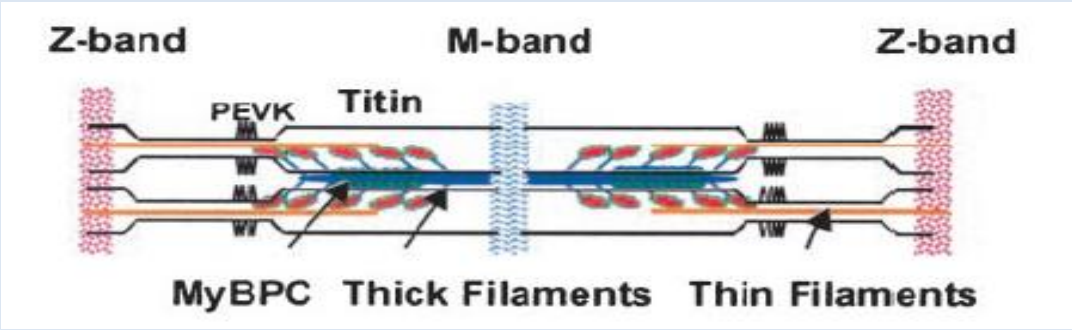


Endothelium

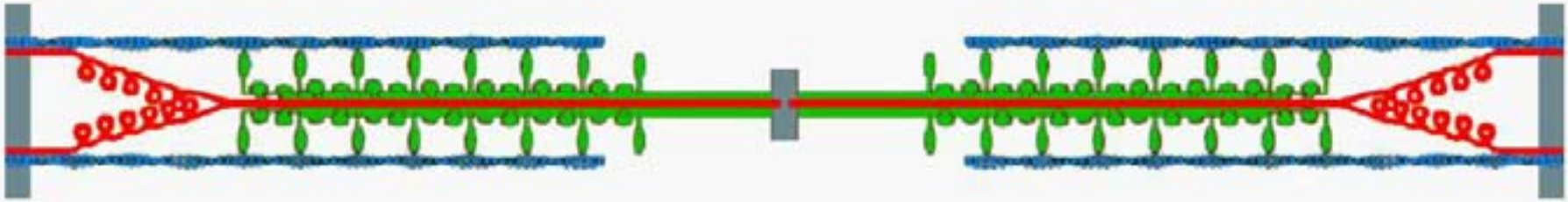


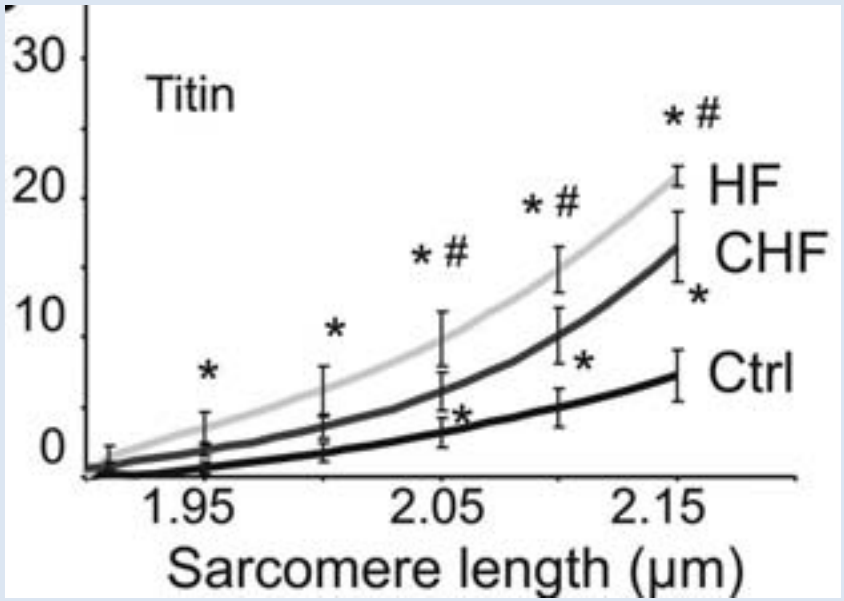


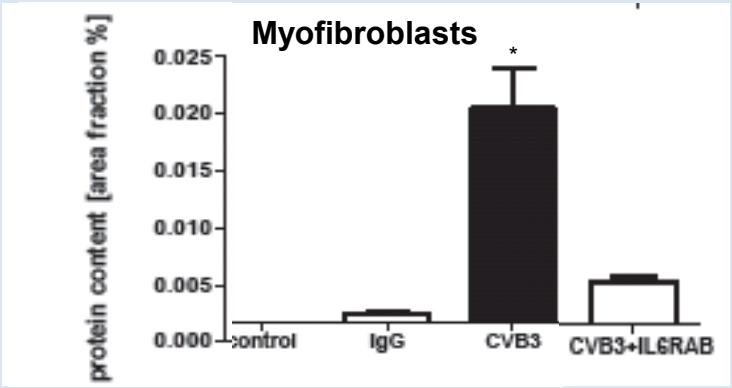
Myocyt

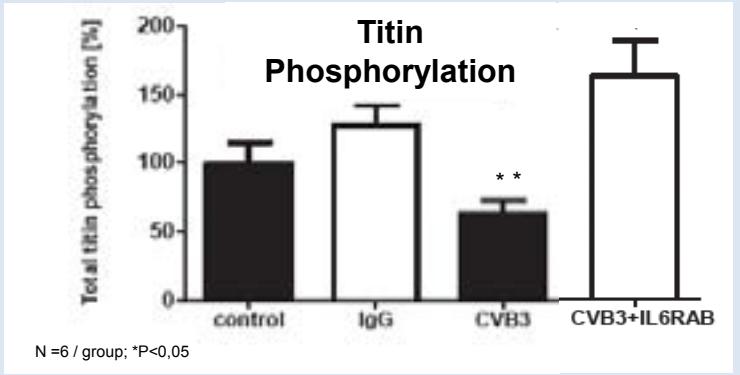


Titin function and inflammatory Stress





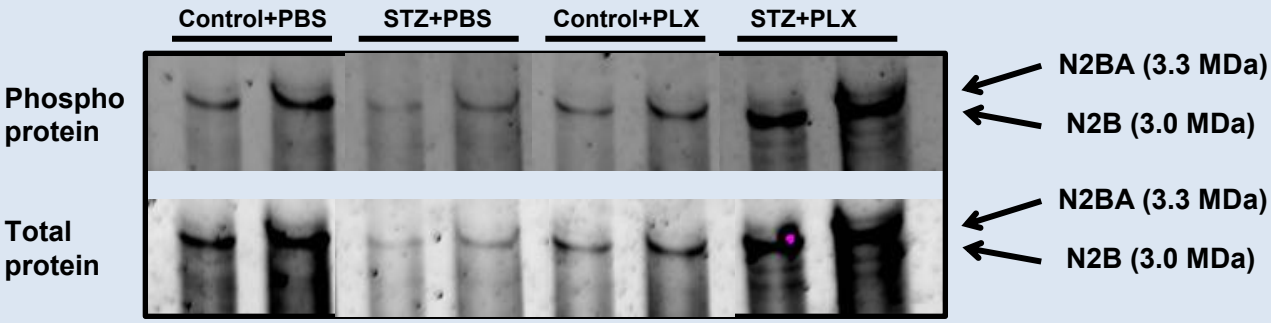


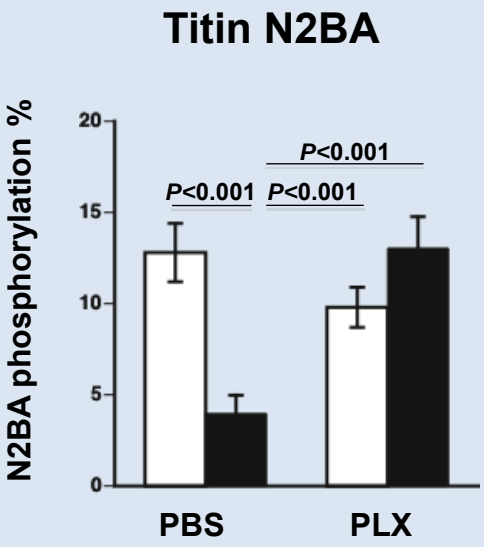


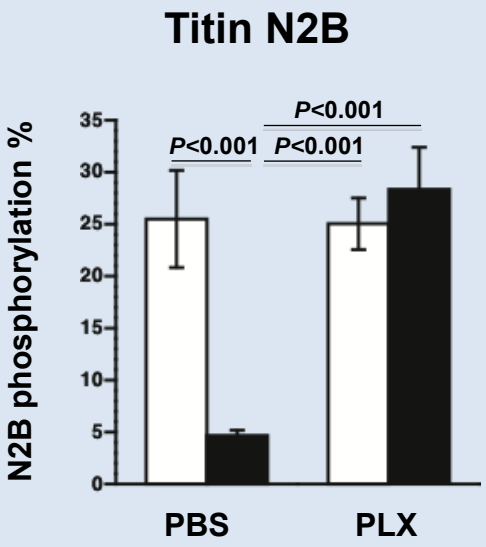
Paulus & Tschöpe JACC 2013

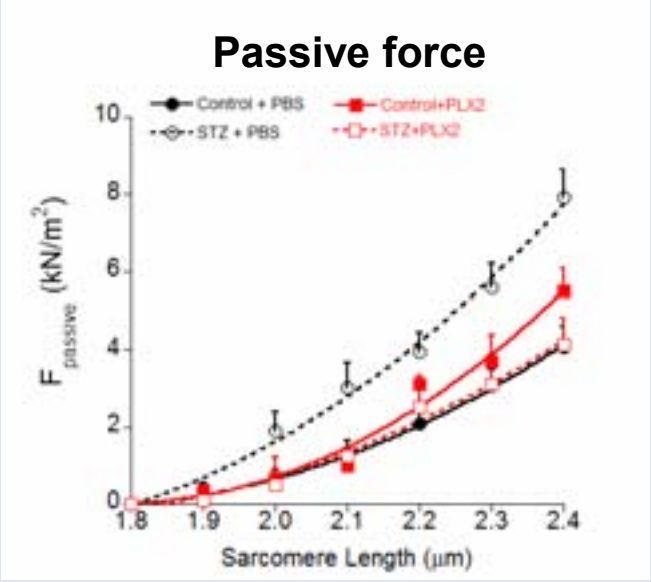
Becher et al, BRC in press

PLX restore hypophosphorylation of titin N2BA and N2B in STZ-induced diabetic mice



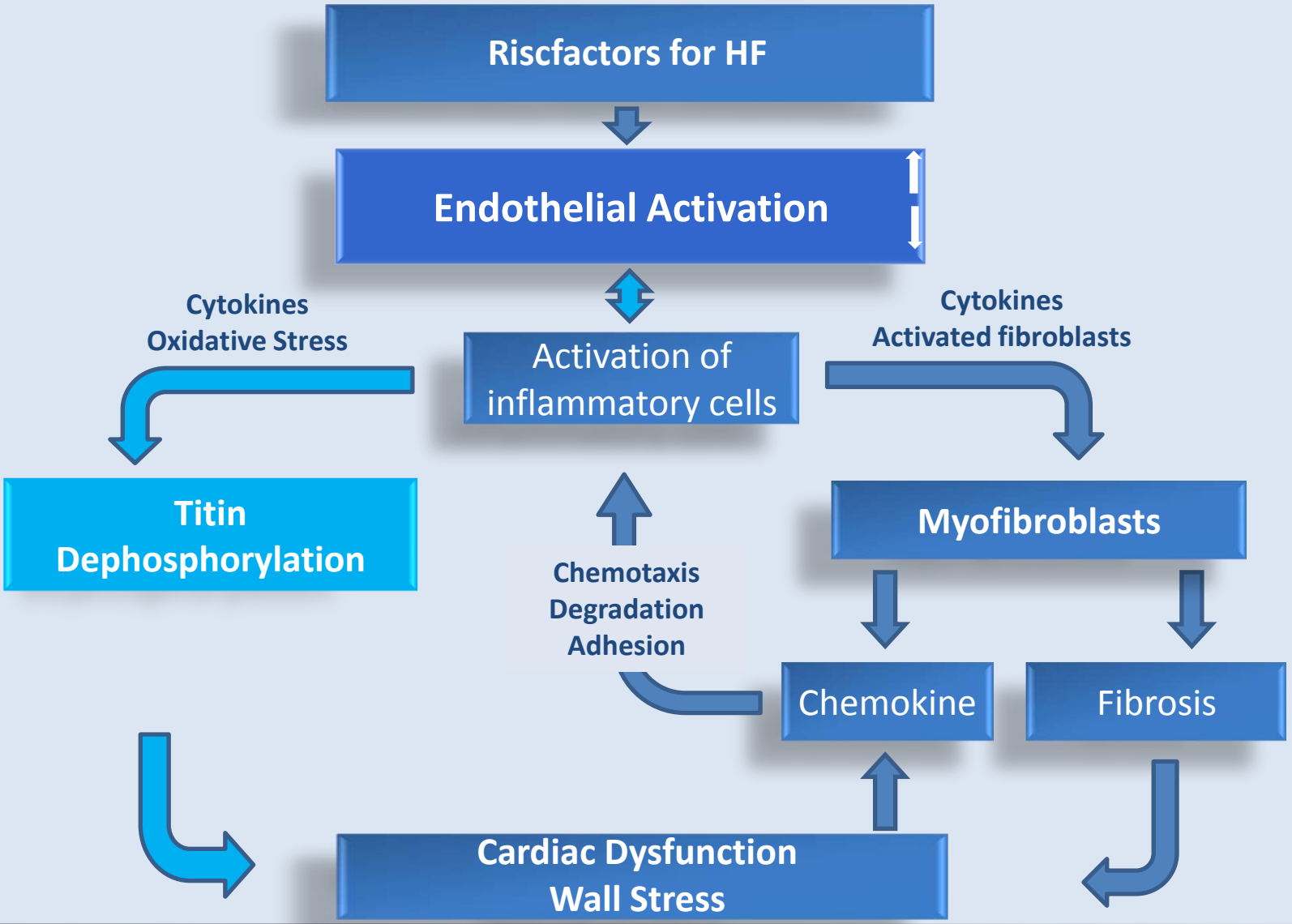






C. Tschöpe

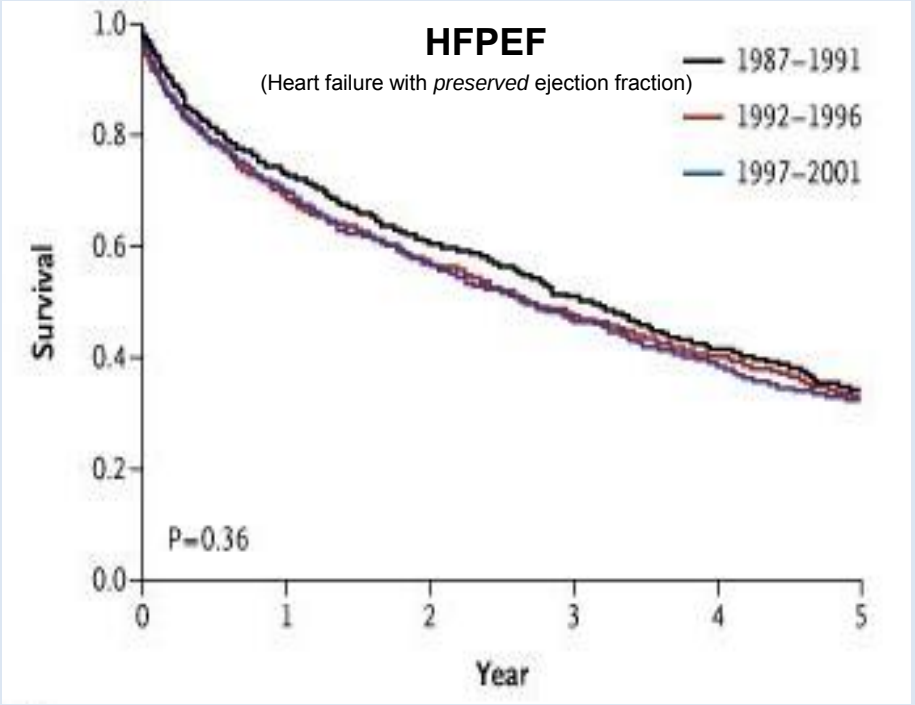
Myofibroblast and Inflammation in heart failure



C. Tschöpe

Prognosis and heart failure

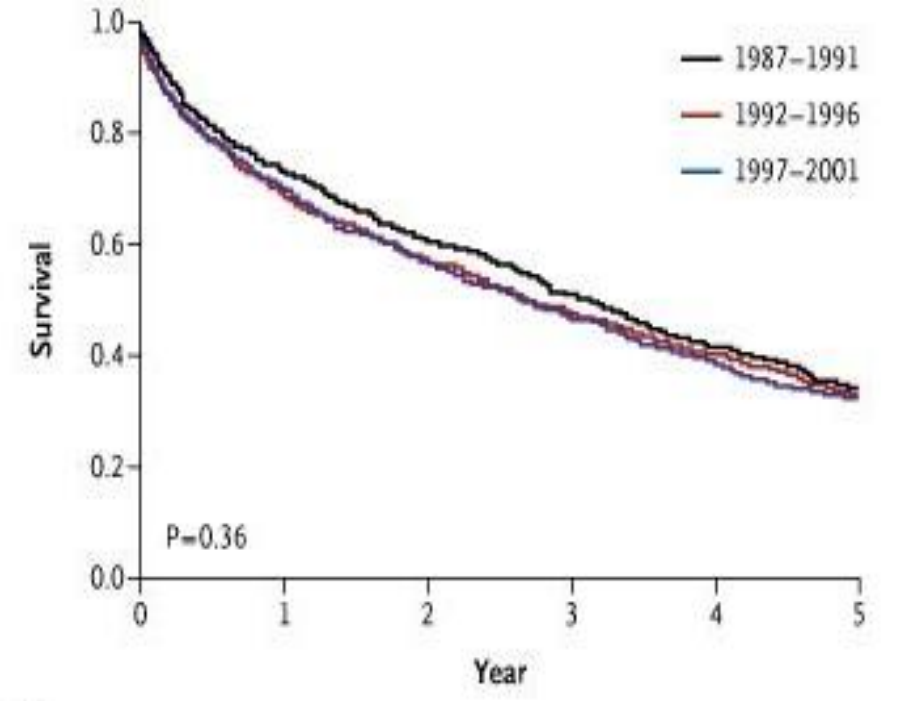
HFPEF

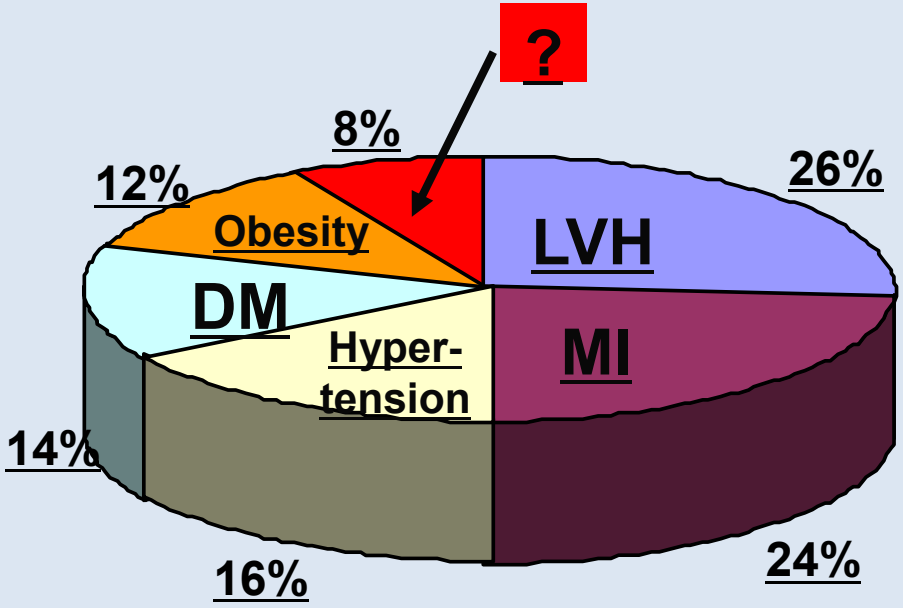


nach Owan T et al. N Engl J Med 2006;

Etiology of isolated diastolic dysfunction

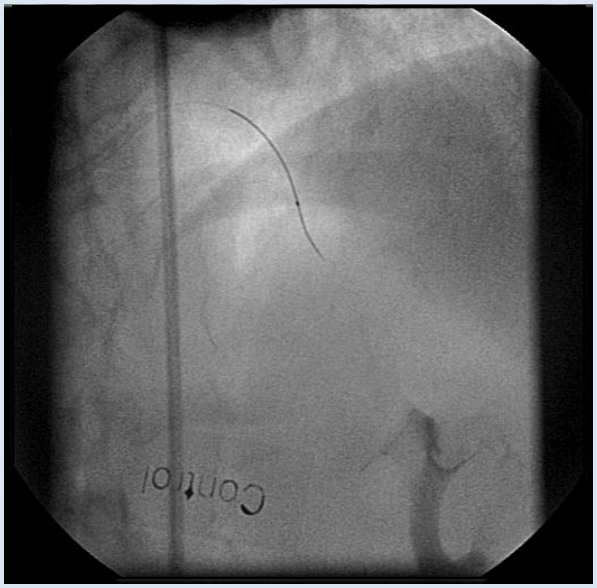
HFPEF

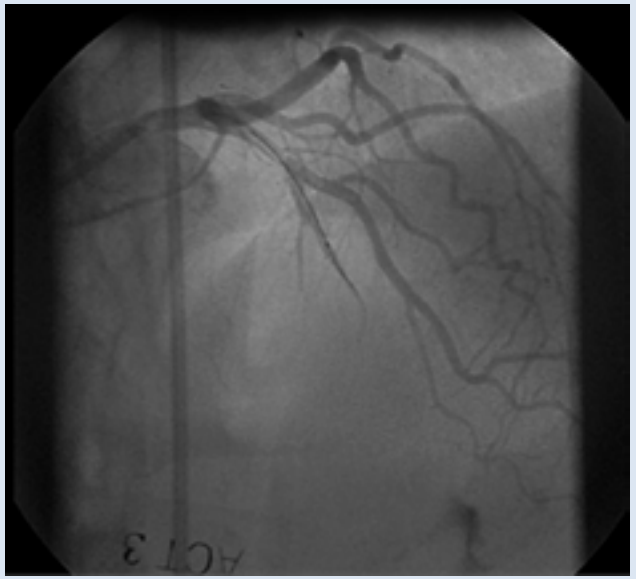


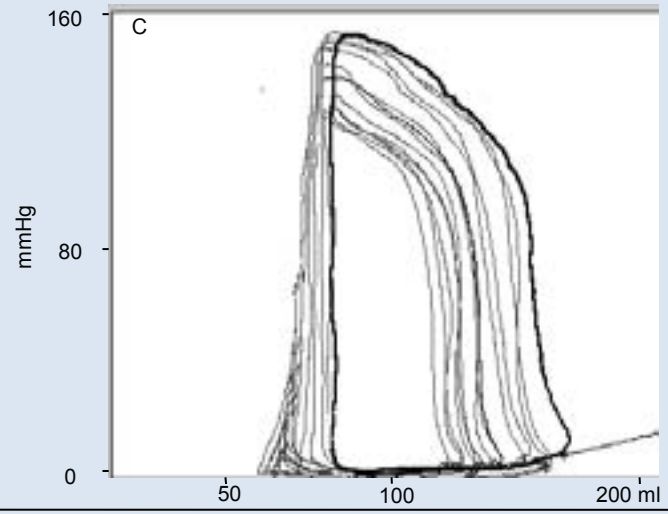


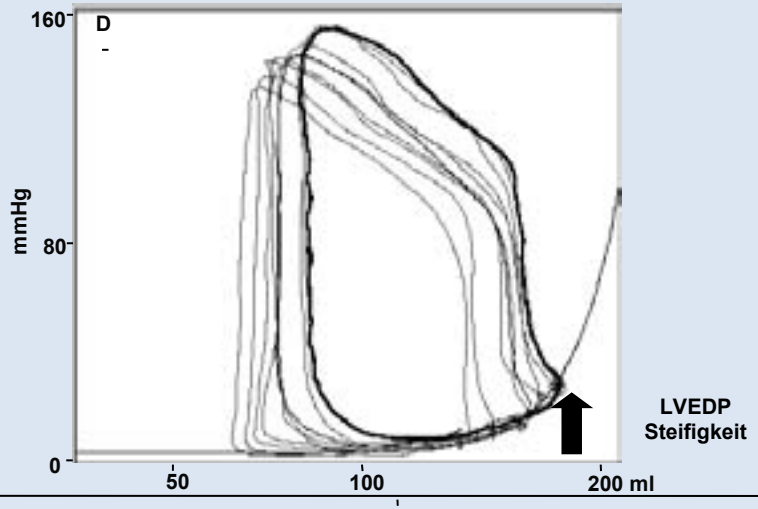
Adapted Fischer M Eur J Heat Fail 2002

Endothelial Dysfunction







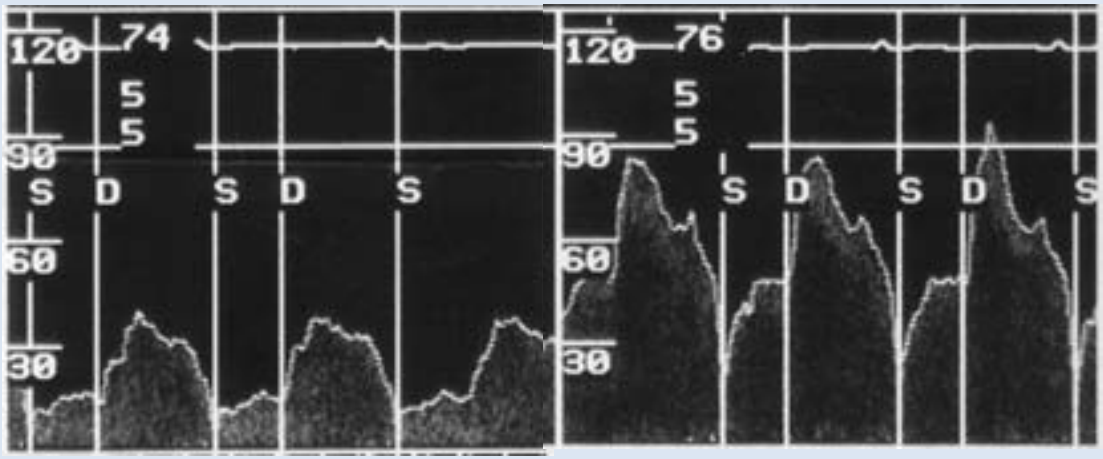


Tschöpe et al, Circulation 2005

Basal **Acethylcholine**

(7.2 µg/min)

ABV:28

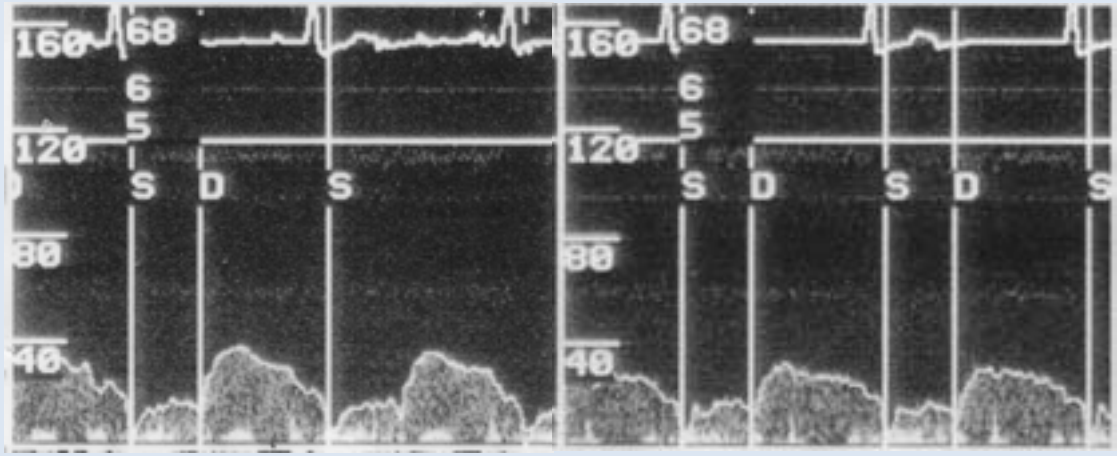


ABV:62

ABV: Average peak velocities

Regular endothelial function

ABV:20



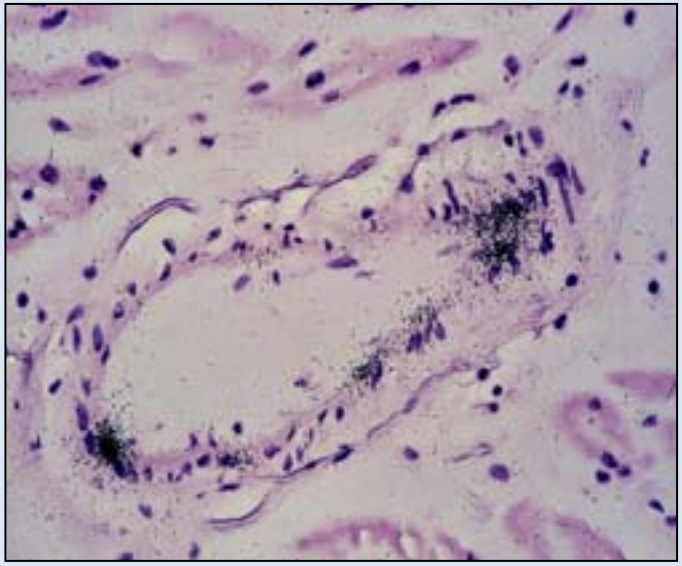
ABV:21

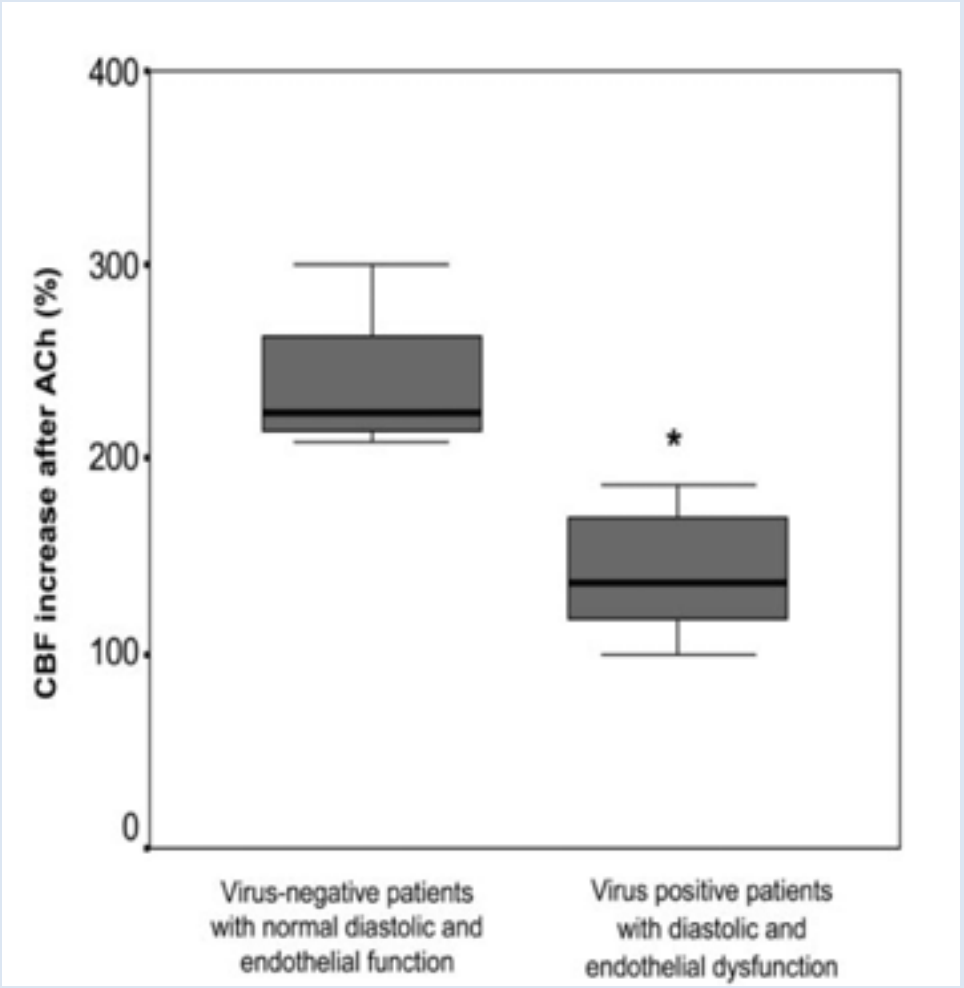
Endothelial Dysfunction

Reduced flow reserve and endothelial dysfunction in cardiac Parvovirus B19 Infection

Vaskulotropism of PVB 19

(In situ Hybridization)
Kandolf et al.



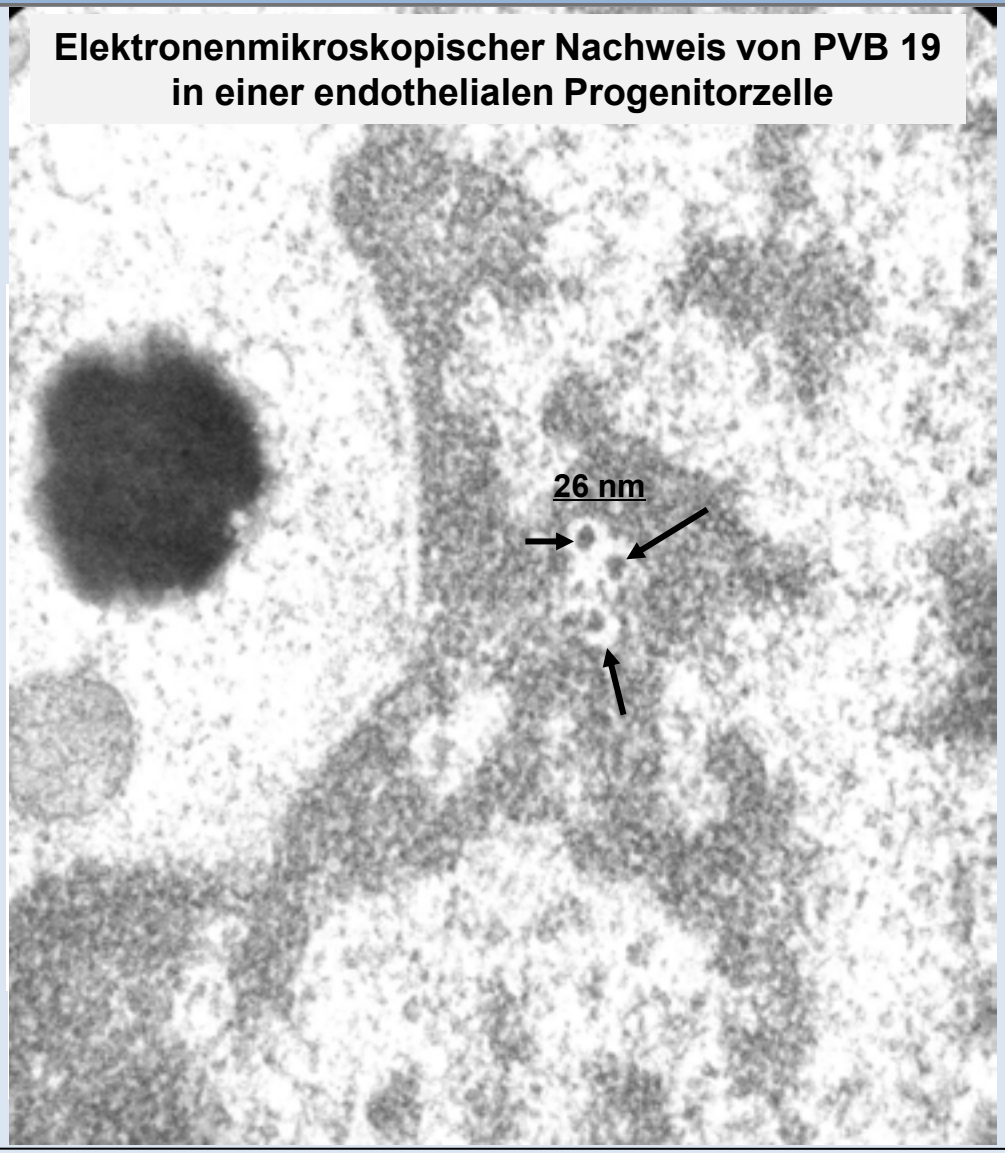


N = 60 /Gruppe; * p< 0.05

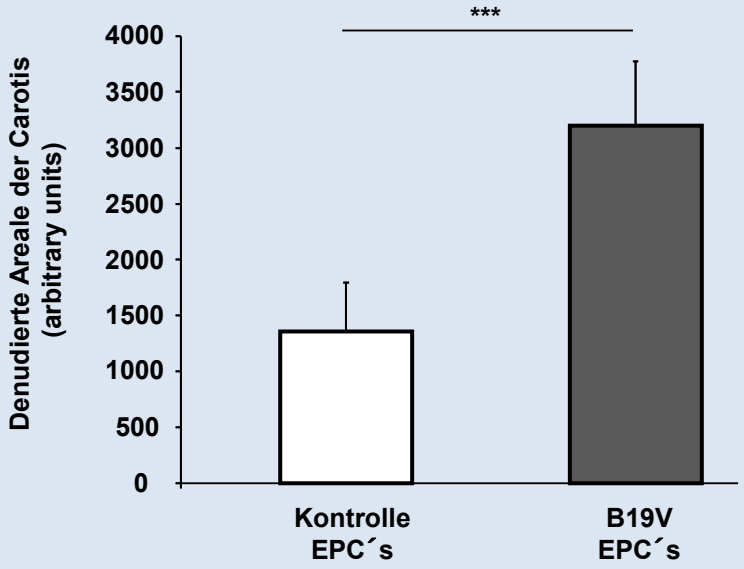
Tschöpe et al, Circulation 2005

PVB 19 – infected endothelial progenitor cells
Impact on impaired endothelial regeneration

Elektronenmikroskopischer Nachweis von PVB 19 in einer endothelialen Progenitorzelle



Nicht re-endotheliasierte Areale der Carotis Läsion



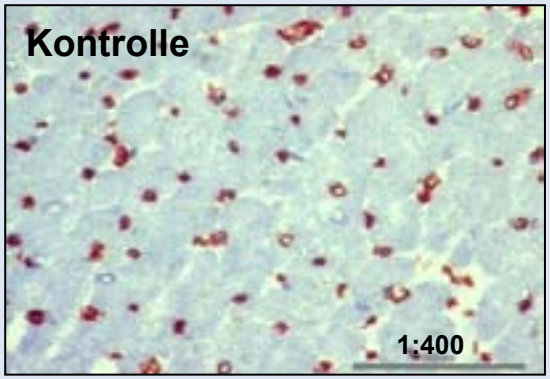
EPC: endotheliale Progenitorzelle; n = 10 Gruppe; *** P< 0.001

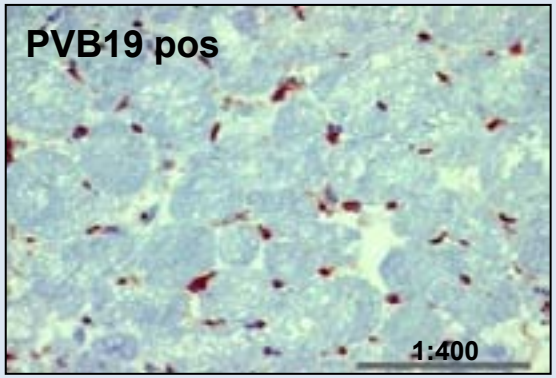
Zobel et al, 2012

Schmidt-Lucke et al, 2012

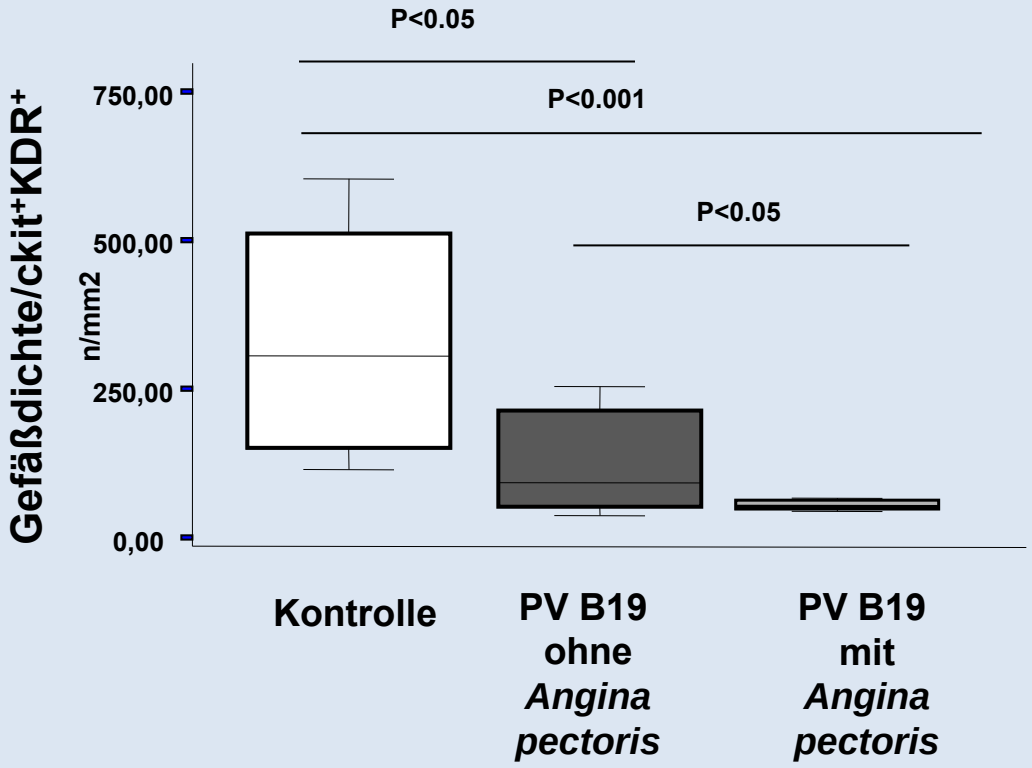
Vascular density and clinical symptoms in PV B 19 patients

Vascular density





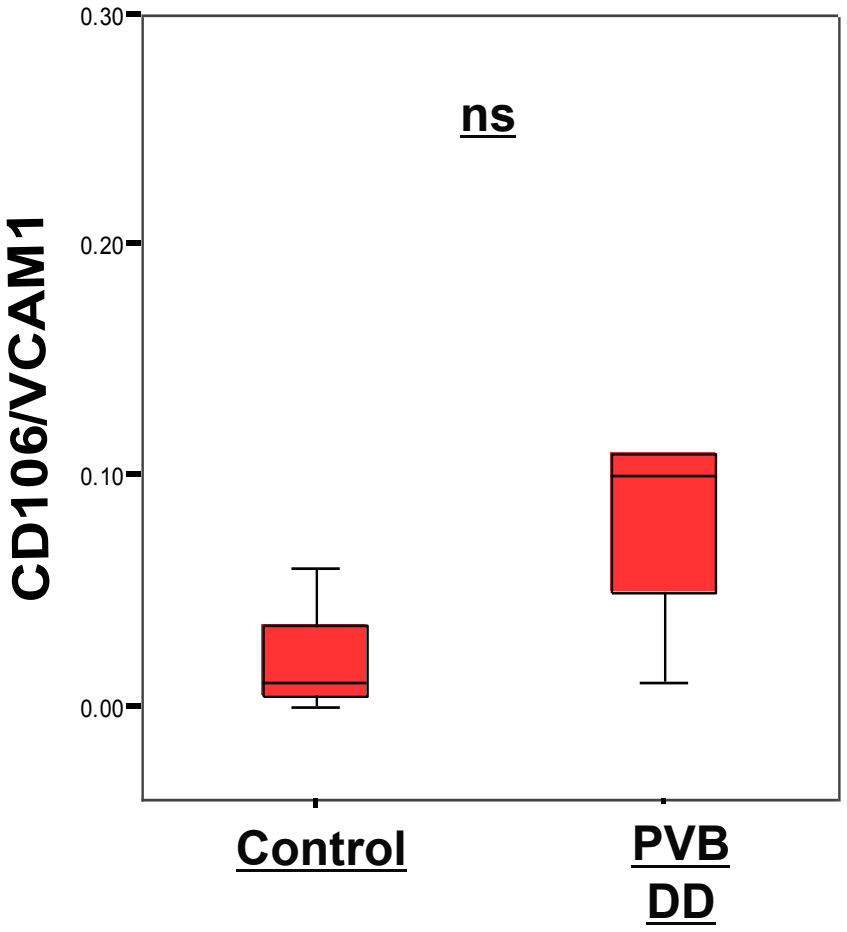
Vascular densiry and angina pectors symptoms



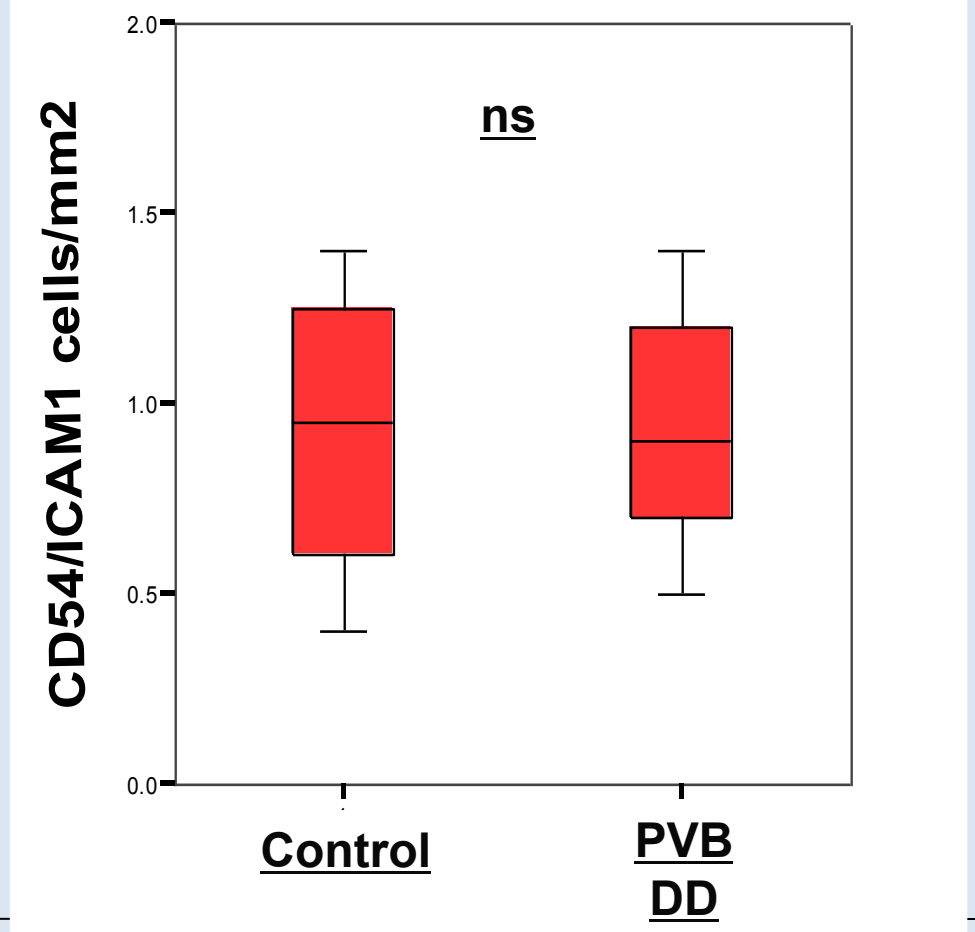
Schmidt-Lucke et al , 2012 in press

No myocardial inflammation in patients with and without diastolic dysfunction and viral persistance

VCAM



ICAM



Tschöpe, Noutsias et al. 2005

Mechansims ?

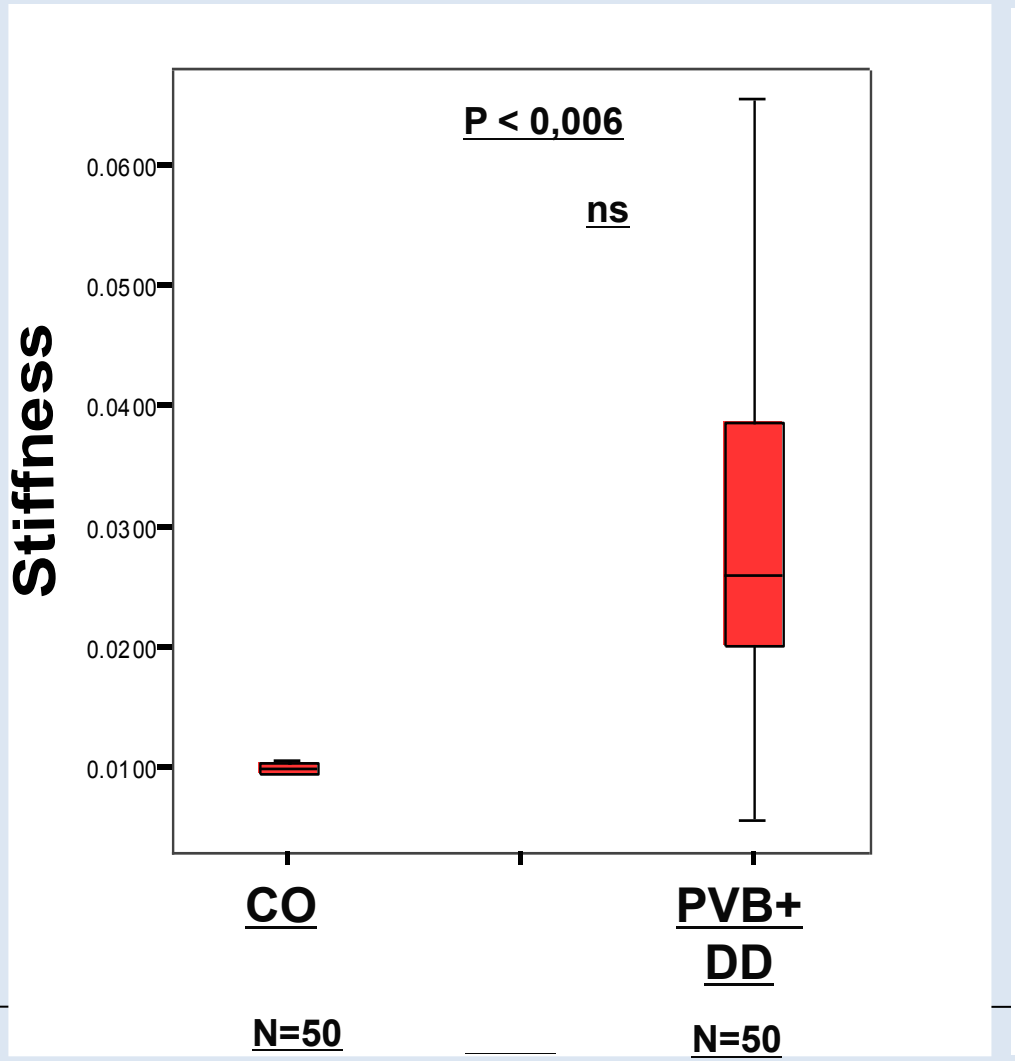
Fibrosis ?

Inflammation ?

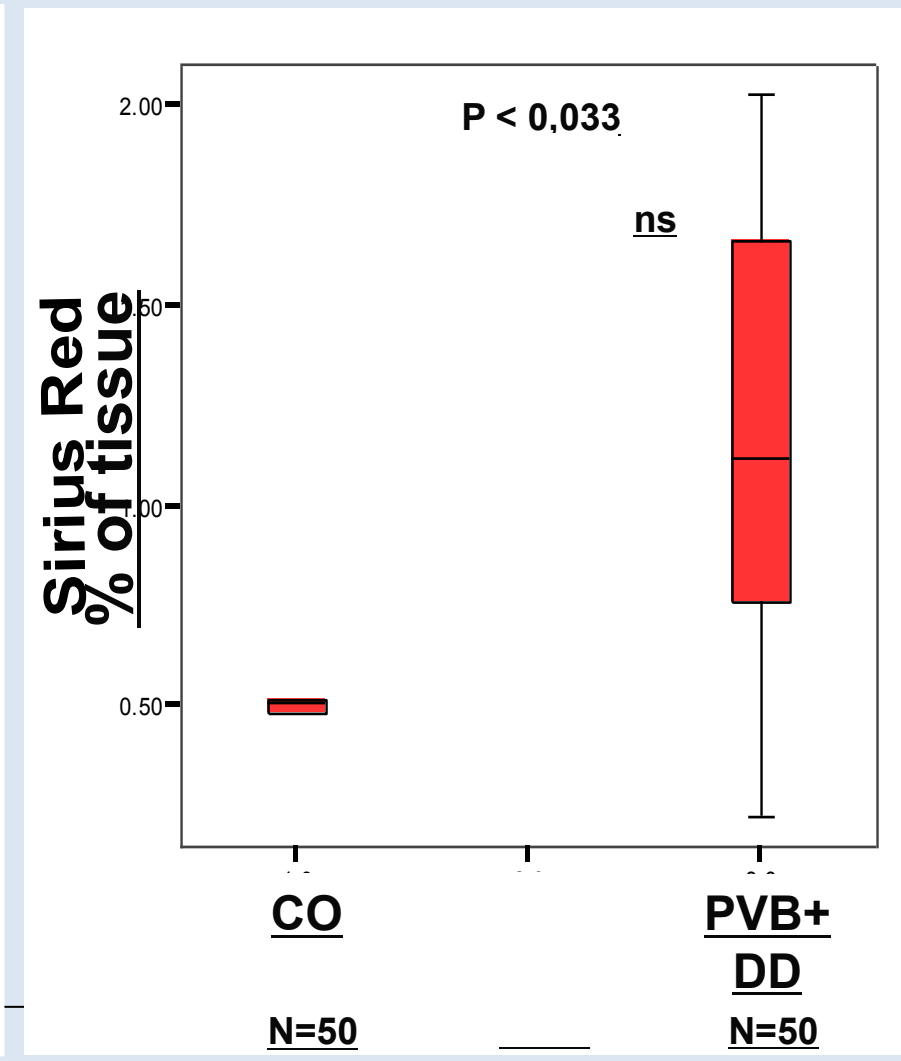
Endothelial Dysfunction ?

LV stiffness and cardiac fibrosis in patients with and without diastolic dysfunction and viral persistance

Cardiac passive stiffness

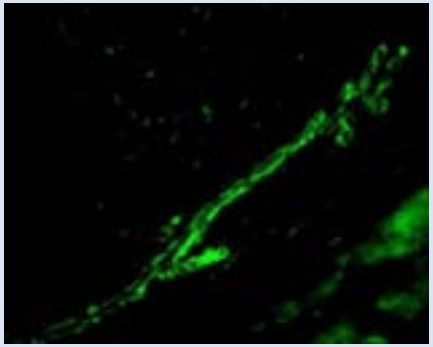


Total collagen content

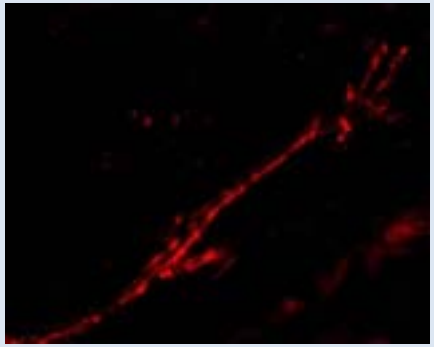


Tschöpe, Westermann, Pauschinger et al. 2005

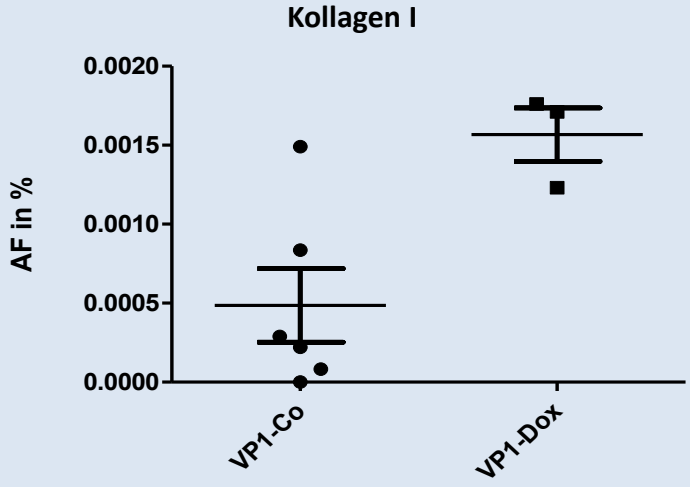
Transgene Mausmodelle:
Parvovirus B19 - induzierte Kardiomyopathie

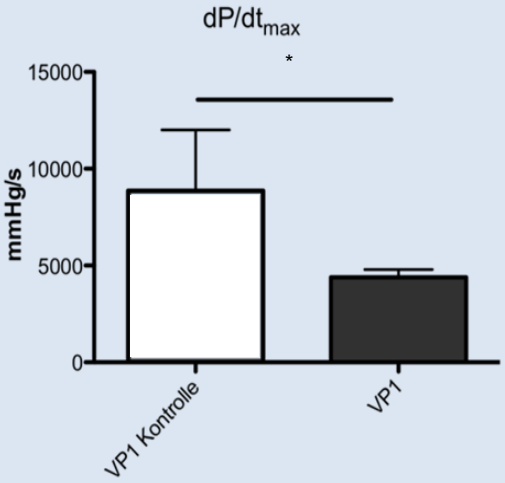


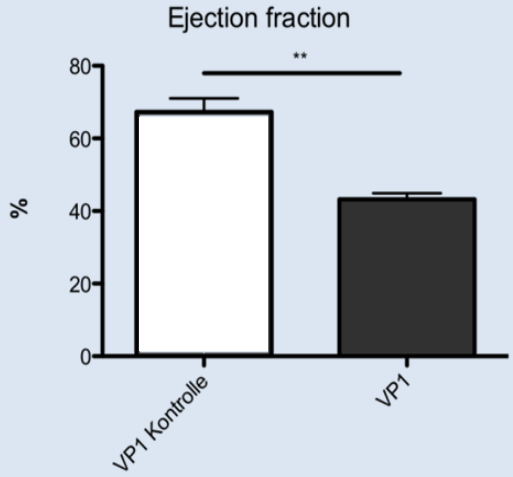
VP1 Expression



CD106/VCAM-1



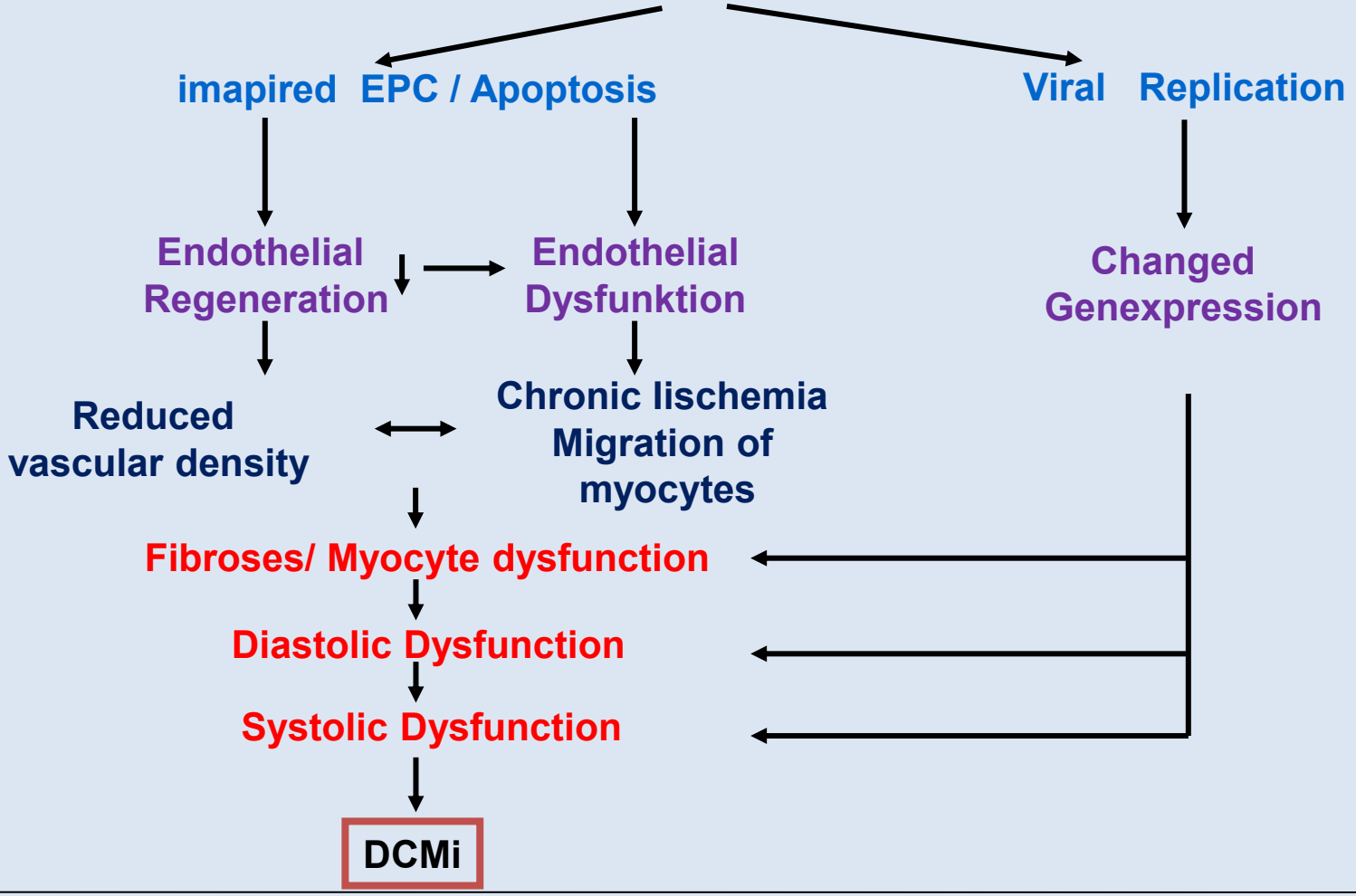




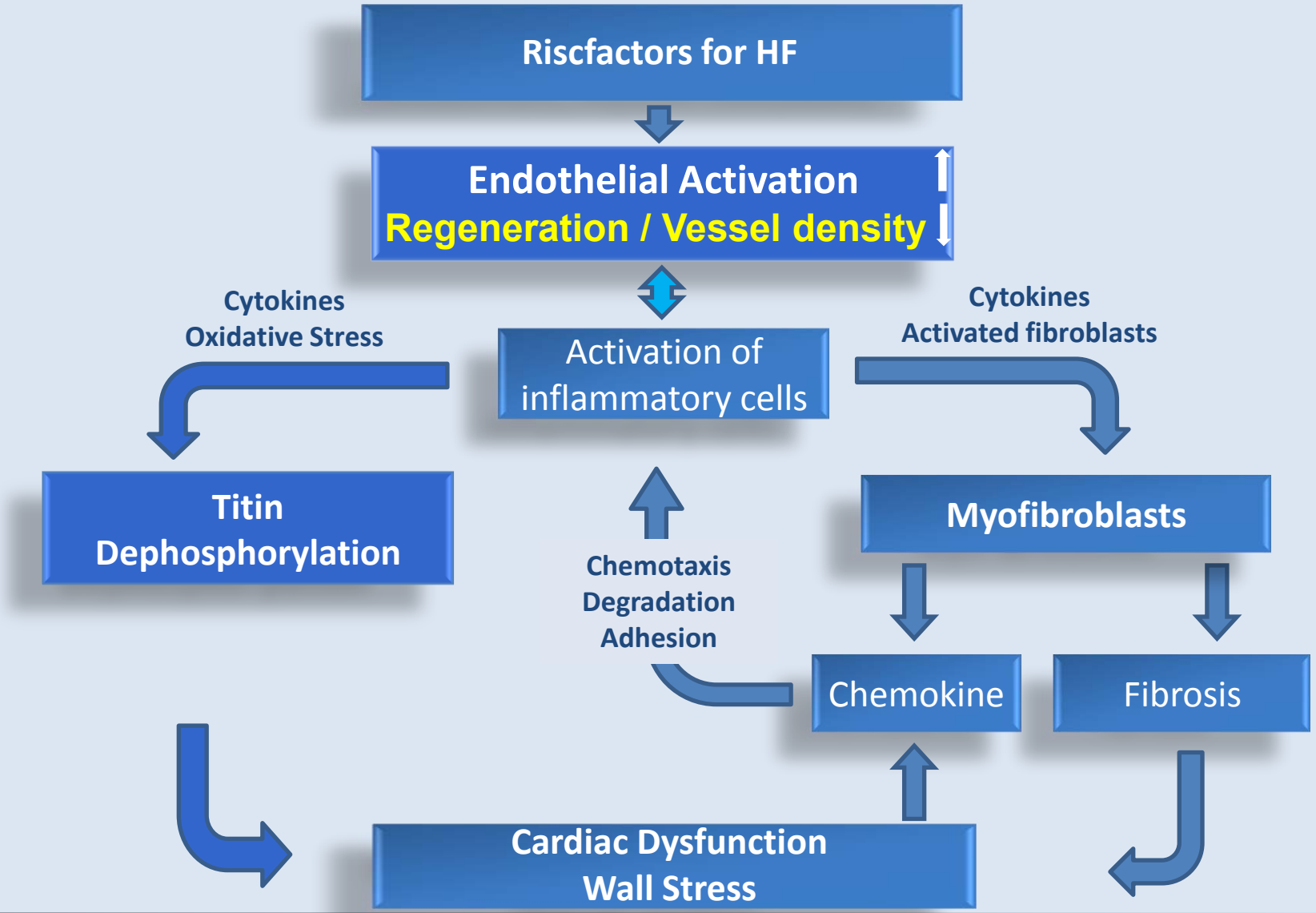
Bock et al. 2012

Model of inflammatory cardiomyopathy

Infektion and impaired EPC



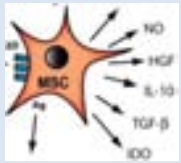
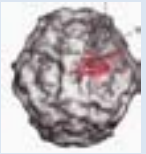
Myofibroblast and Inflammation in heart failure



C. Tschöpe

Development of new anti-inflammatory and antifibrotic therapy options for the future

Cardiac inflammation

Cytokine-System	Innate Immunsystem	Matrix Proteine (MMP´s)	Regeneration	Virustoxizity
				 PV B19 Coxsacki B
IL1β, IL2, IL4, IL6, IL10, IL23, TNFα	TLR-4, -9 TRIF, MYD-88 NOD2 S100 A8/A9	MMP-2, MMP-9 Biglycan	Mesenchymale Stromazelle T reg´s CAP´s	Interferon ß Telvibudine

IL: Interleukin, TNF; Tumor Nekrose faktor, TLR: Toll-like Rezeptor, NOD: Nucleotide-binding oligomerization domain-containing protein, MMP: Metalloproteinase, MSC: Mesenchymale Stromazelle, Treg´s: regulatve T-Zelle; CAP´s: cardiac derived adherent proliferating cell

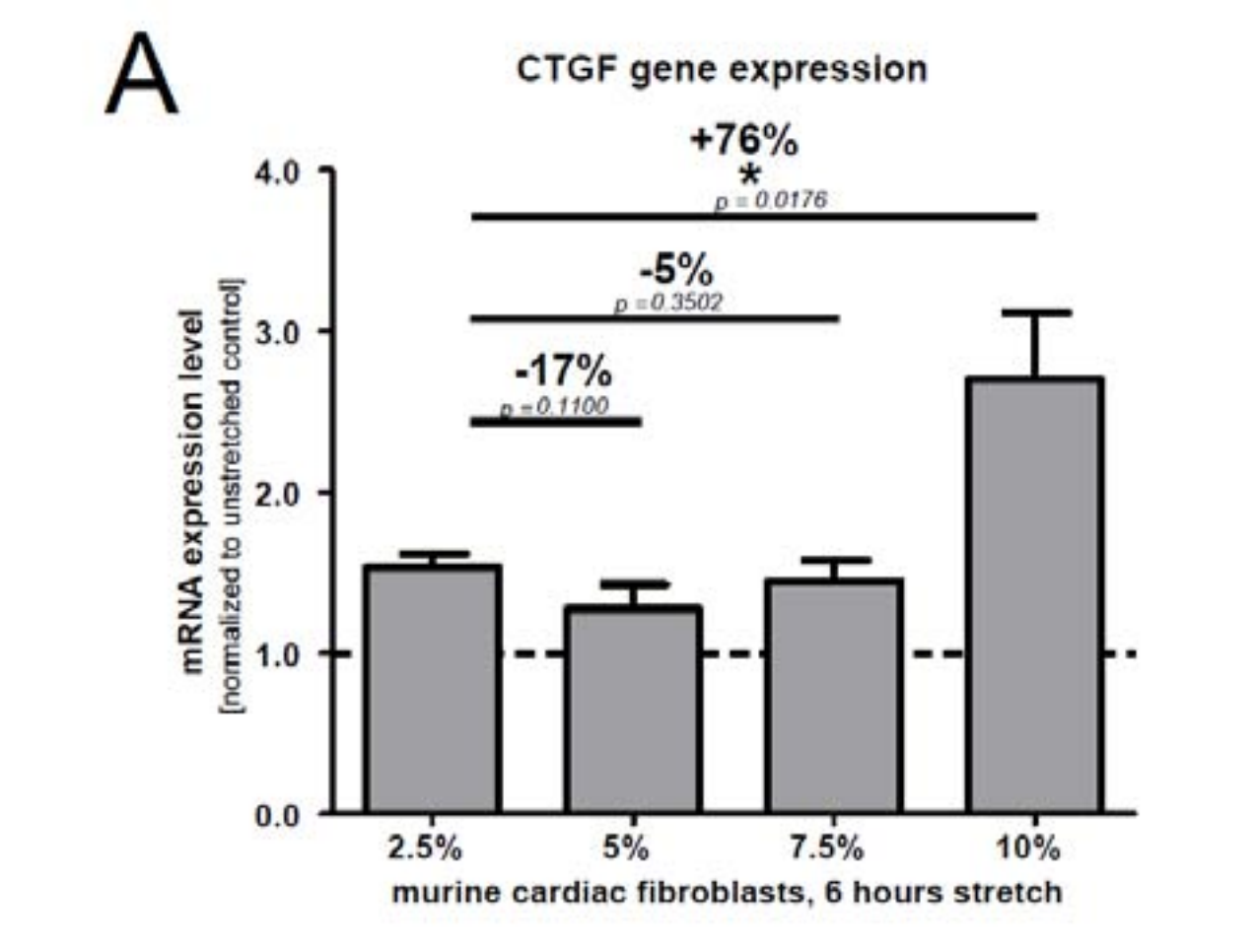
Li et al. Eur J Pharmacol 2007
Koch et al. Int Immunopharm 2010
Riad et al. J Immunol 2011

Westermann et al. Circulation 2011
Van Linthout et al. Eur Heart J 2011
Savvatis et al. PlosOne 2012

Myofibroblast as a therapeutic target ?

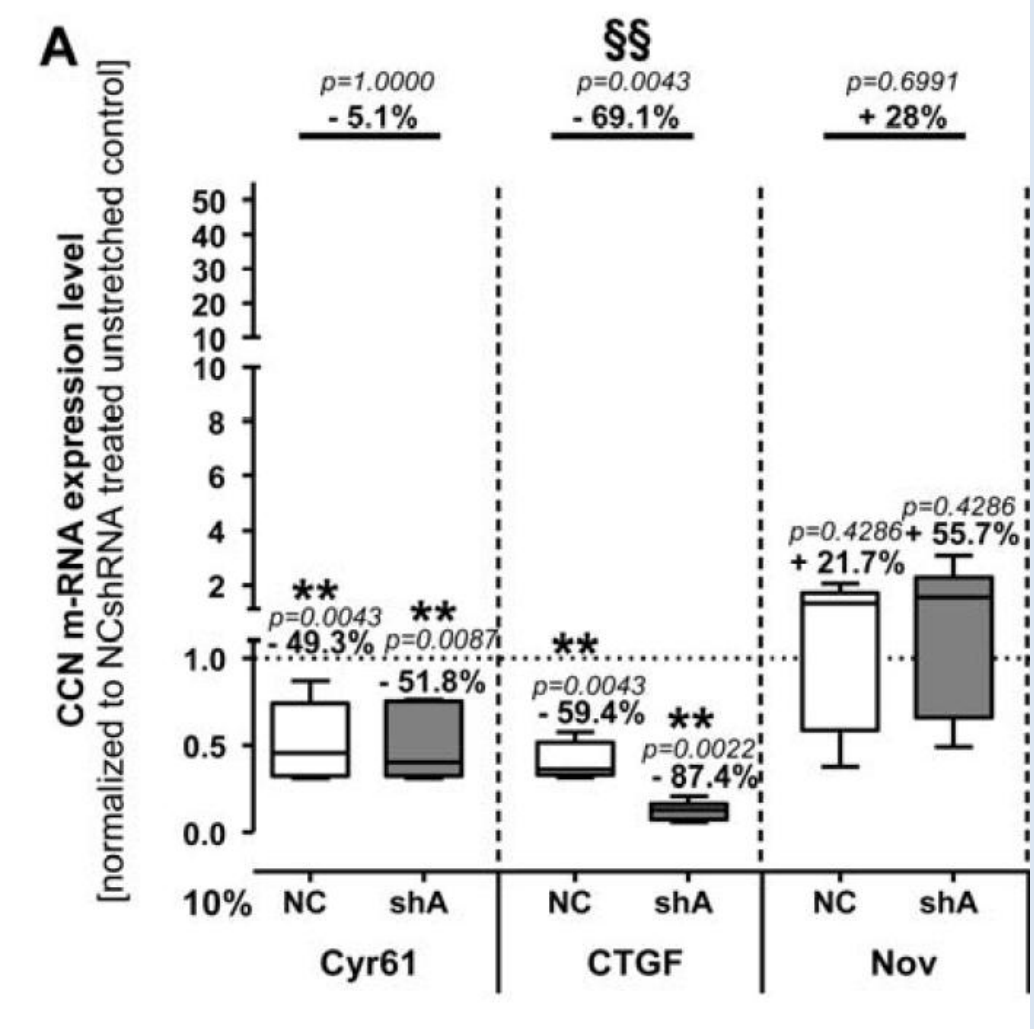
Deregulated genes WITHOUT significant correlation to LV-EF						
Gene Symbol	Regulation	Factor (AFFX)	q-value (AFFX)	Gene Name	Correlation with EF (r-value)	Network
CCN1	**up**	**3.6**	**1.943**	**CYR61 = Member 1 of CCN Gene Family with 6 known members**	0.5690	CCN1
THBS1	up	3.4	3.868	Thrombospondin 1 = a protein interacting with CCN1 domain III	0.3609	CCN1
ITGB1	up	2.5	1.994	Integrin - β1 = a protein interacting with CCN1 domains II, III, IV	0.1868	CCN1
BDNF	up	2.4	3.641	Brain-derived neurotrophic factor	0.2603	CCN1
CD47	up	2.1	4.108	Integrin-associated signal transducer	0.4442	CCN1
FGF2	up	2.1	2.868	Fibroblast growth factor (basic)	0.5027	CCN1
PDGFC	up	2.0	3.469	Platelet-derived growth factor C	0.3051	CCN1
CCN2	**up**	**2.0**	**0.3592**	**Connective Tissue Growth Factor = Member 2 of CCN Gene Family**	0.3592	
CXCL14	down	5.9	1.943	Chemokine (C-X-C motif) ligand 14	0.1787	CCN1

CTGF (CCN2) upregulation in fibroblast after strech in vitro



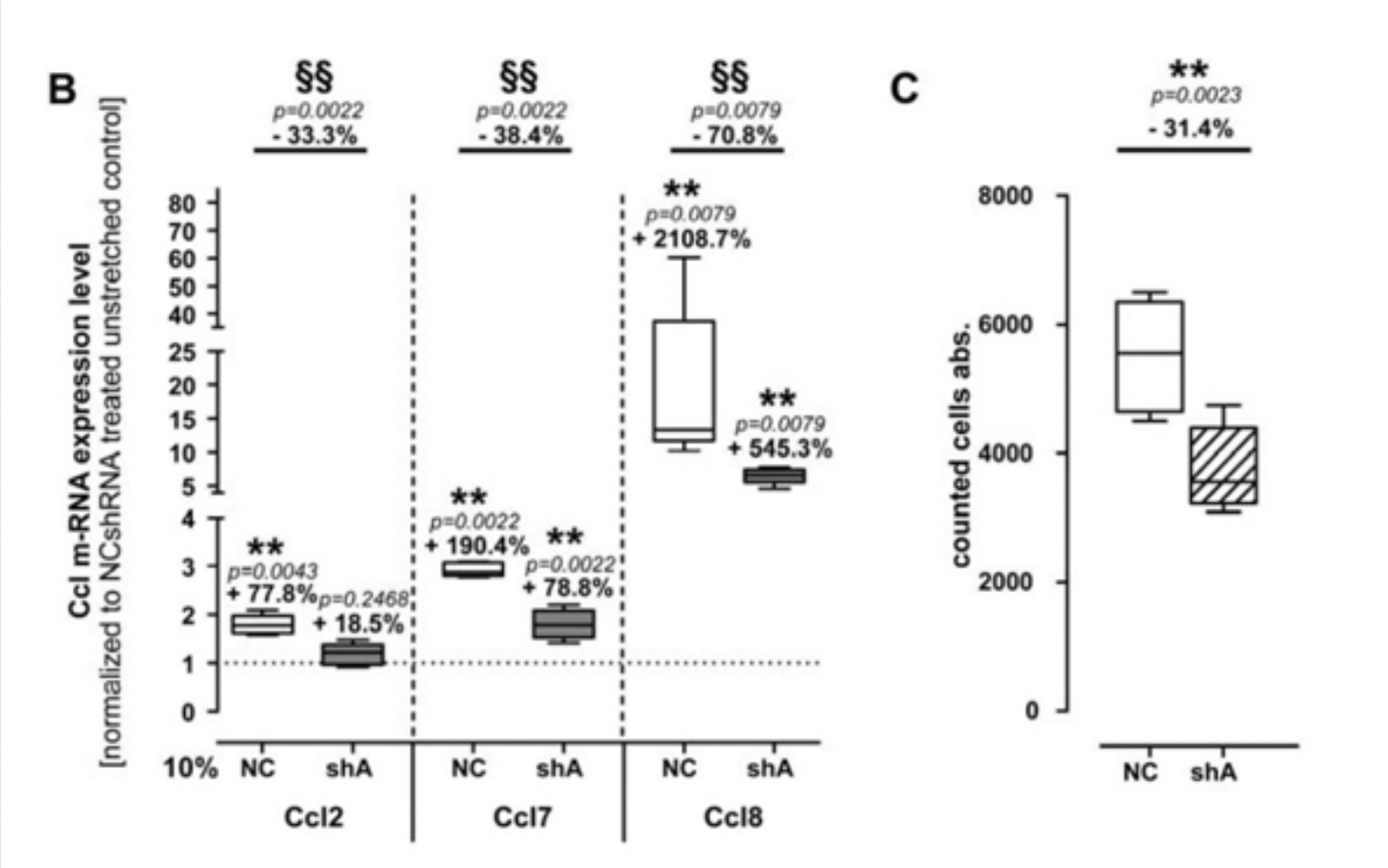
Cooperation AG Poller, Charite, unpublished

New anti-CTGF intervention option in the future via cardiac RNA interefrence



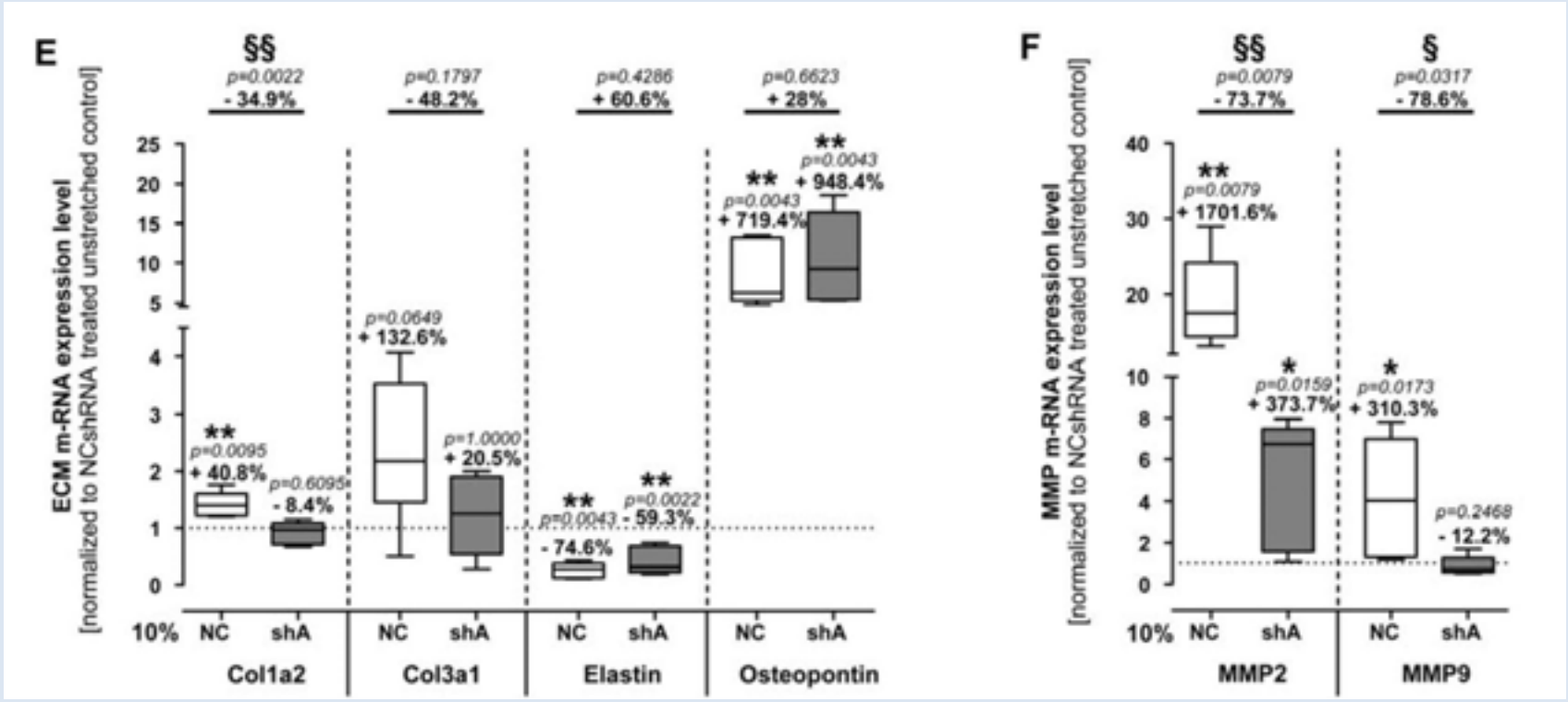
Cooperation AG Poller, Charite, unpublished

Anti-CTGF RNAi knockdown normalizes chemokine production



Cooperation AG Poller, Charite, unpublished

Anti-CTGF RNAi knockdown normalizes collagen and MMP expression



Cooperation AG Poller, Charite, unpublished

Conclusion

1. **Myofibroblasts are chemo-active, activates lymphocytes and can incuse endothelial dysfunction.**

2. **The number of myofibroblasts correlate with the degree of collagen expression as well as with the number of invading inflammatory cells in the heart.**

3. **Therefore, myofibroblasts are inflammatory supporter cells**

4. **They are a therapeutic target to modulate cardiac fibrosis and inflammation.**

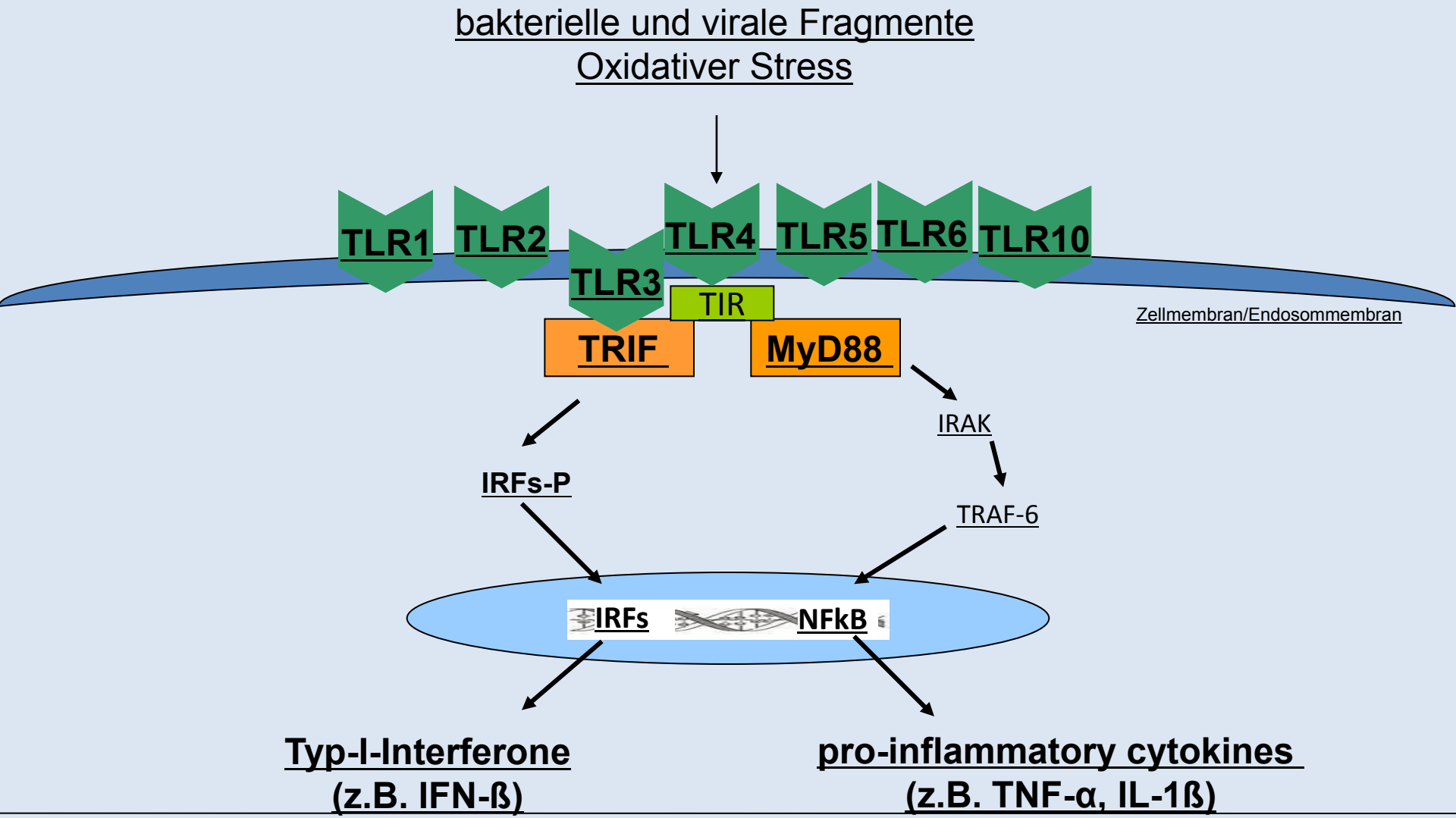
Charite, Campus Benjamin Franklin, Kardiologie

Danke
Dirk Westermann
Diana Lindner
Christine Zietsch
Nadine Orin
Kerstin Puhl

Funded by:
SFB TR 19 „Inflammatory Cardiomyopathy“
EU FP7-call „Diastolic Heart Failure“

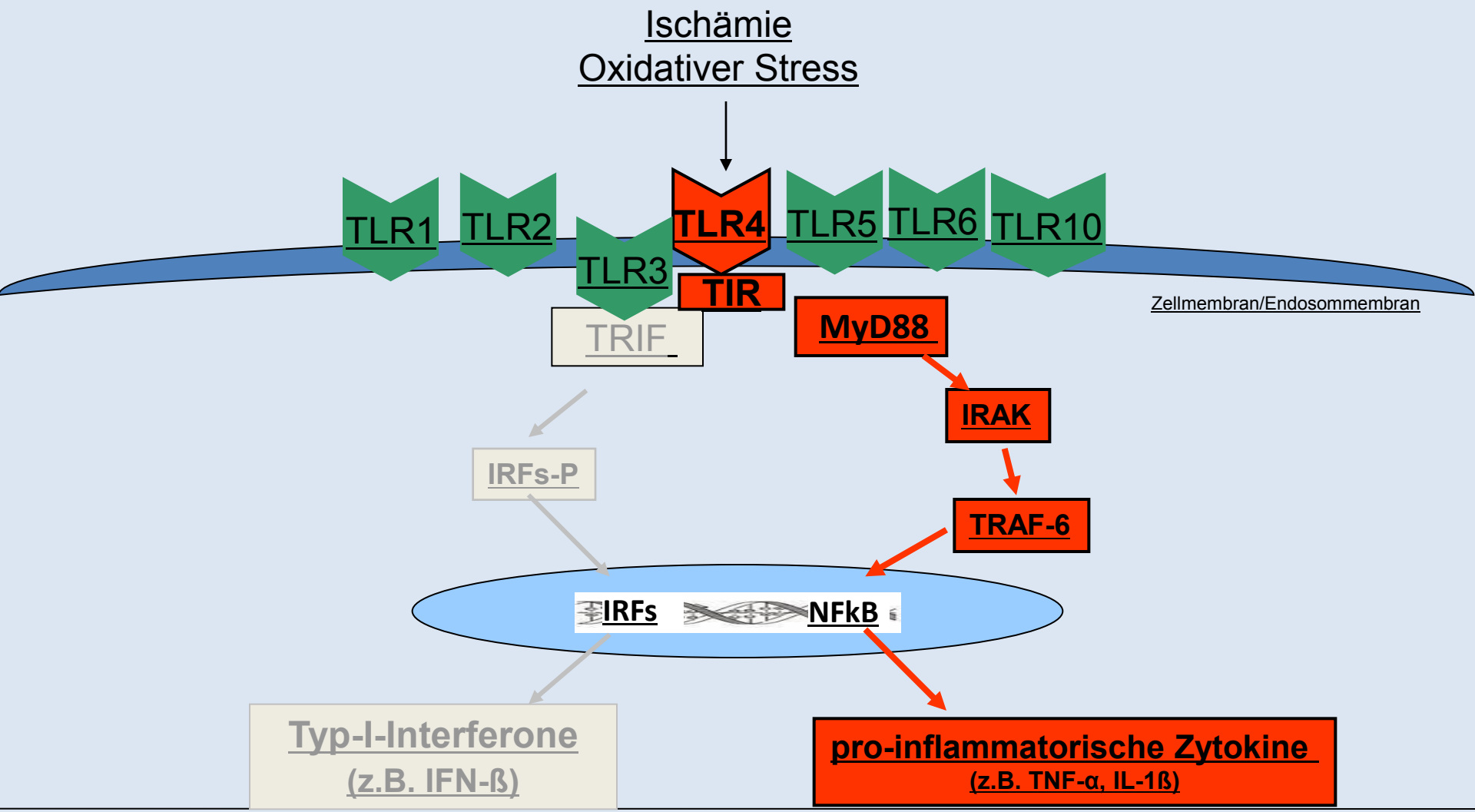
Thanks

Innate Immunsystem: Toll-Like Receptors



modifiziert nach: O`Neill et al., Nat Rev Immunol 2007,

Toll like Rezeptor 4
bei der ischämischen Kardiopathie

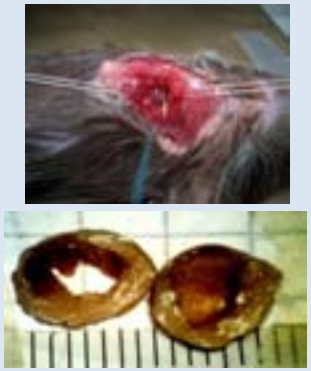


modifiziert nach: O`Neill et al., Nat Rev Immunol 2007,

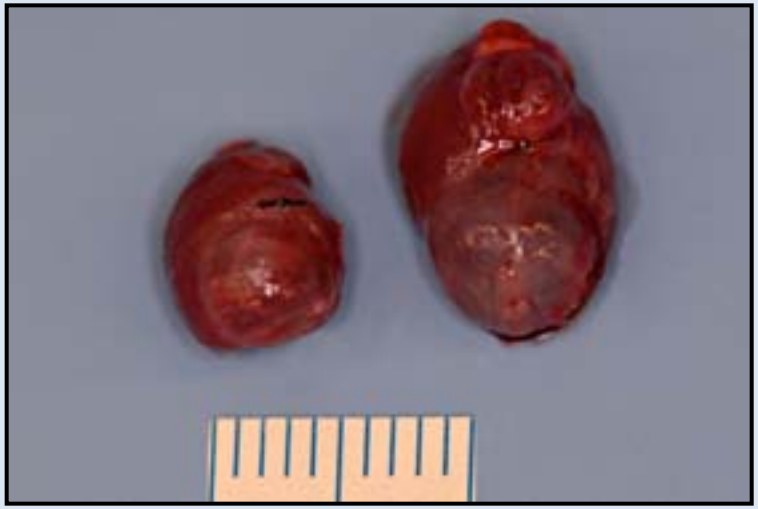
Das TLR System als kritischer Mortalitätsfaktor beim Myokardinfarkt: Toll-like Rezeptor 4

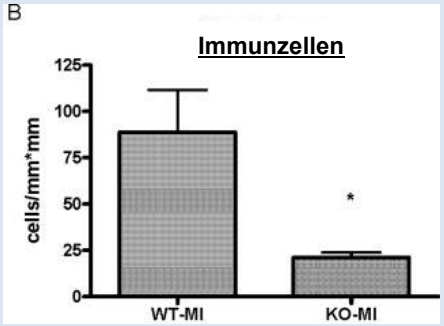
TLR4 -/-

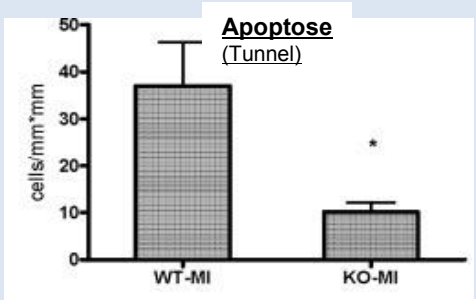
C57BL/10ScN

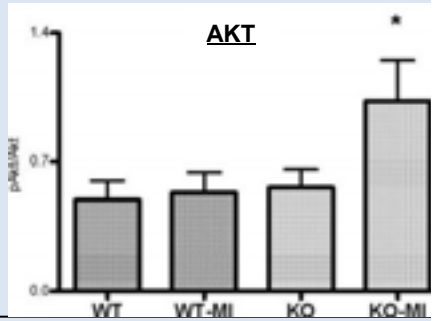


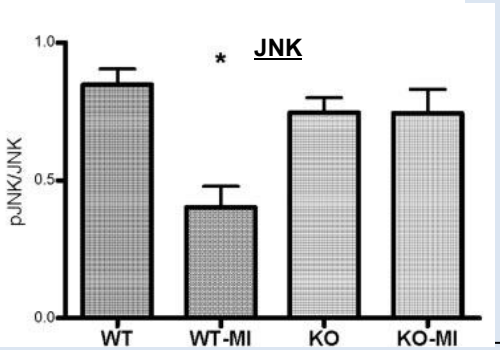
TLR4 -/- TLR4

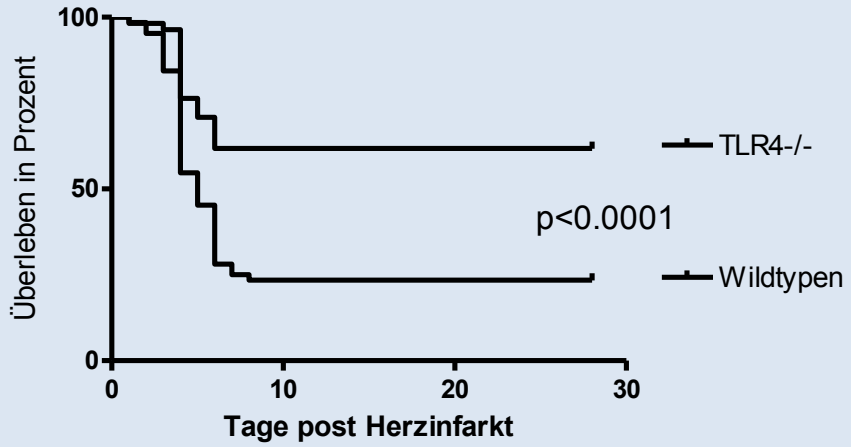










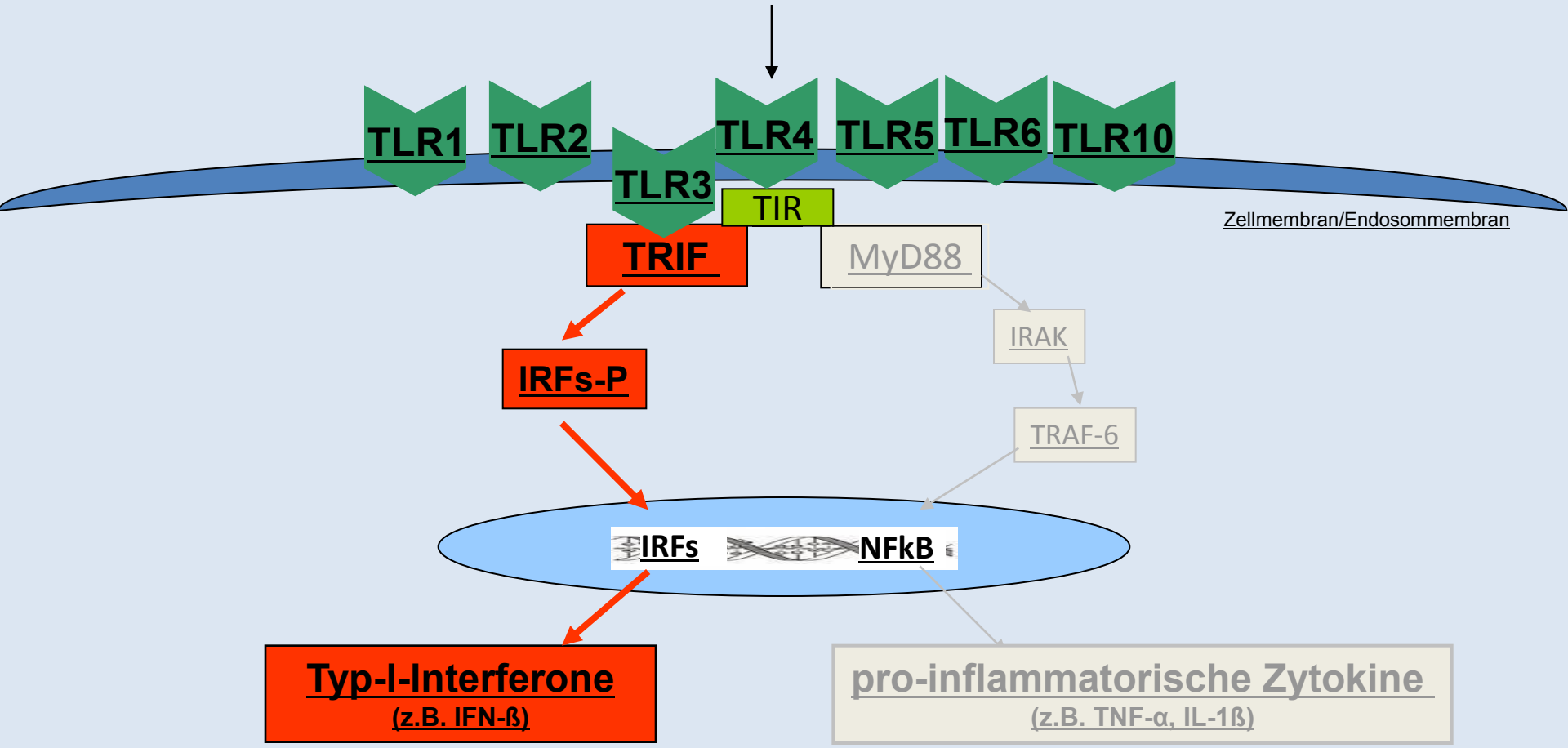


n = 25 / Gruppe

Riad & Tschöpe et al. Journal of Immunology 2008

Projektförderung: TR-SFB 19: B5/Z3

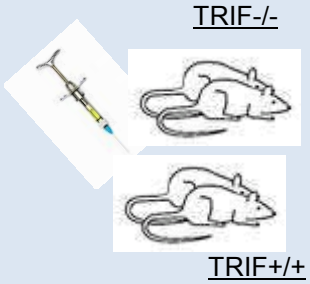
TIR domain-containing adaptor inducing IFN-ß (TRIF) bei der viralen Myokarditis

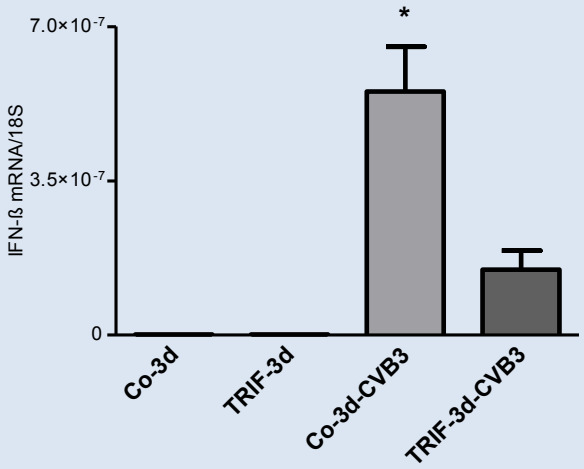


modifiziert nach: O`Neill et al., Nat Rev Immunol 2007,

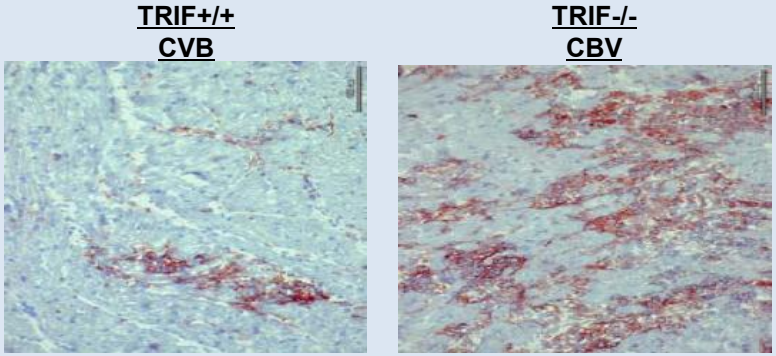
TIR domain-containing adaptor inducing IFN-ß (TRIF) bei der viralen Myokarditis

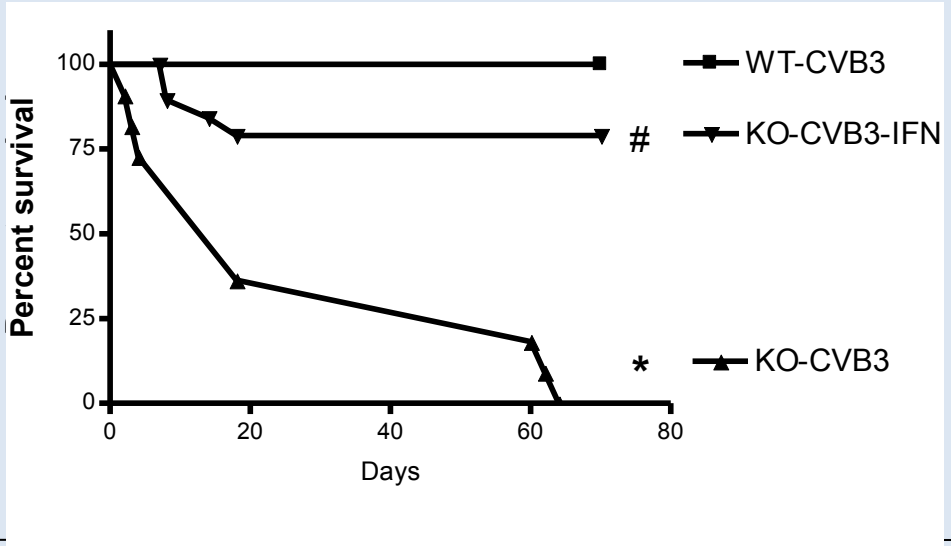
Coxsackievirus
(5*10^5 PFU i.p.)





Kardiale Entzündungsreaktion

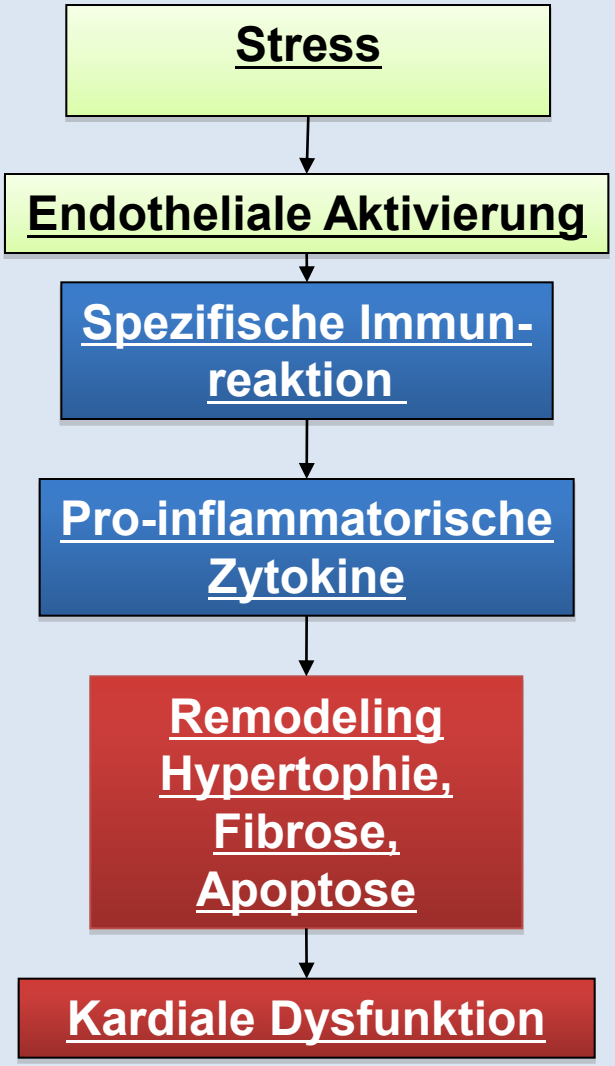




Riad & Tschöpe et al. Journal of Immunology 2011

Projektförderung: TR-SFB 19: B5/Z3

Mögliche zukünftige therapeutische Ansätze

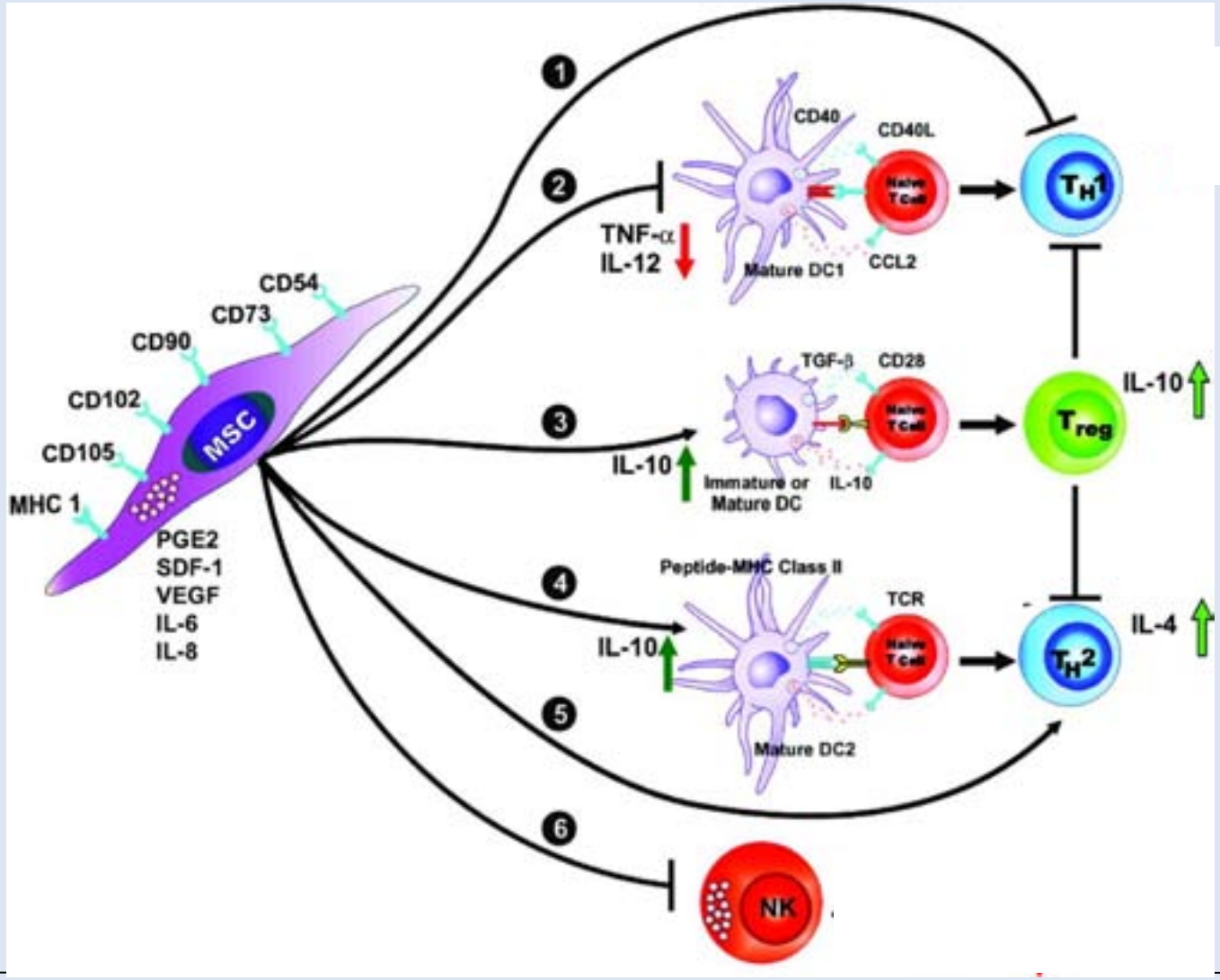


B1 Antagonisten
TLR Rezeptor Antagonisten
Interferone

Zytokininhibitoren
NO Enhancer
p38 Inhibitoren
selekt. MMP-Inhibitoren

Zelltherapeutischen Ansatz ?

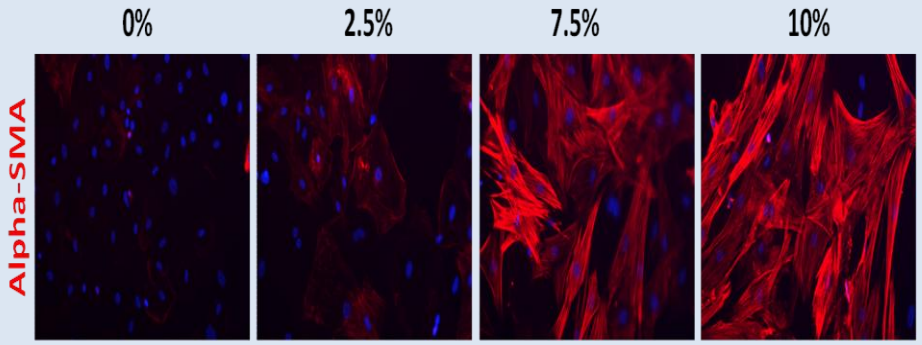
Immunmodulatorische Effekte von mesenchymalen Stammzellen



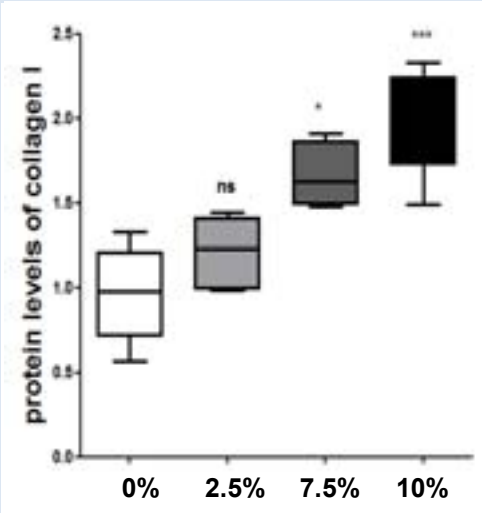
Diabetes mellitus
Pneumonie
Arthritis
Multiple Sklerose
Transplantation
Niereninsuffizienz
Infarkt

Review: Abdi et al. Diabetes 2008

Role of mechanic stress

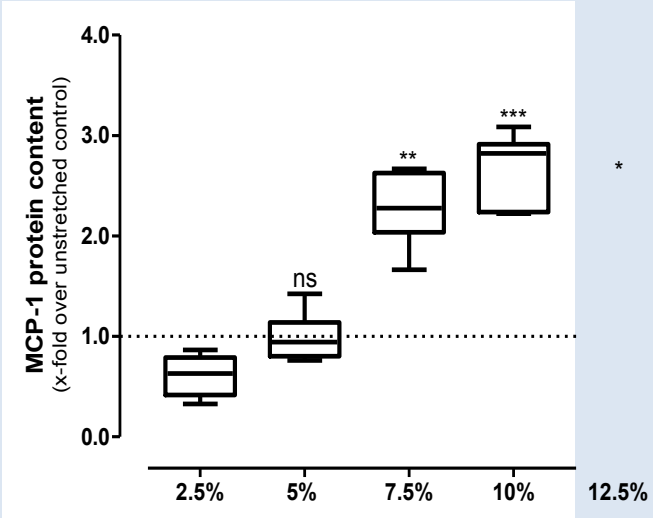


Stimulation of collagene production



Stretch

Stimulation of chemokines production

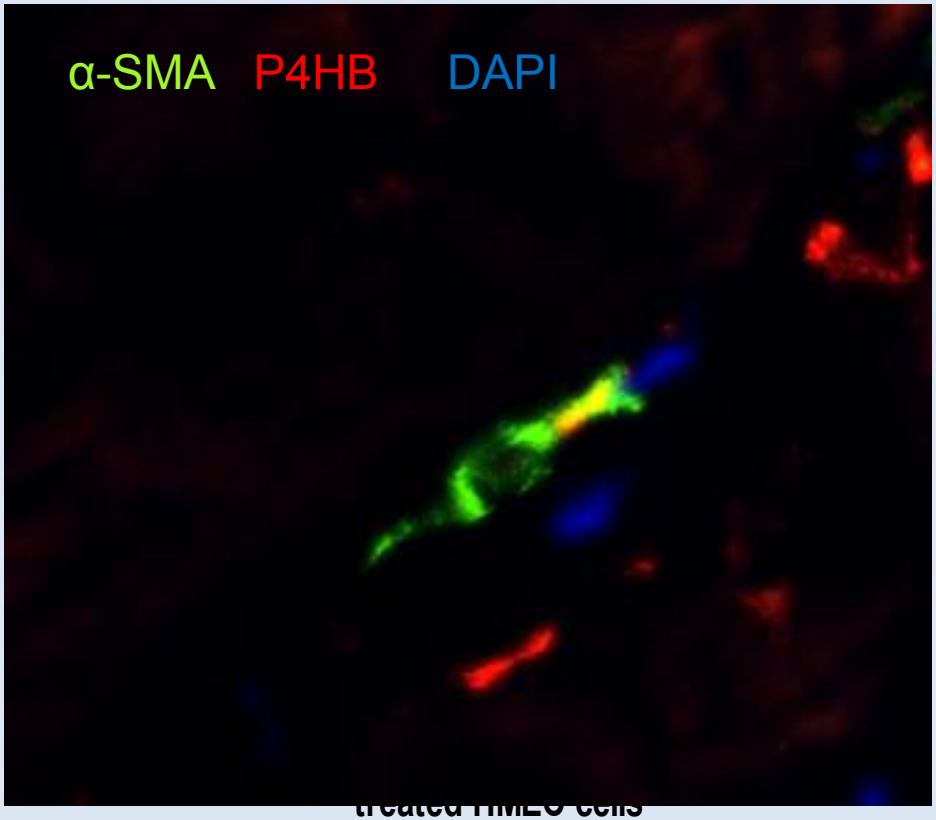


Stretch

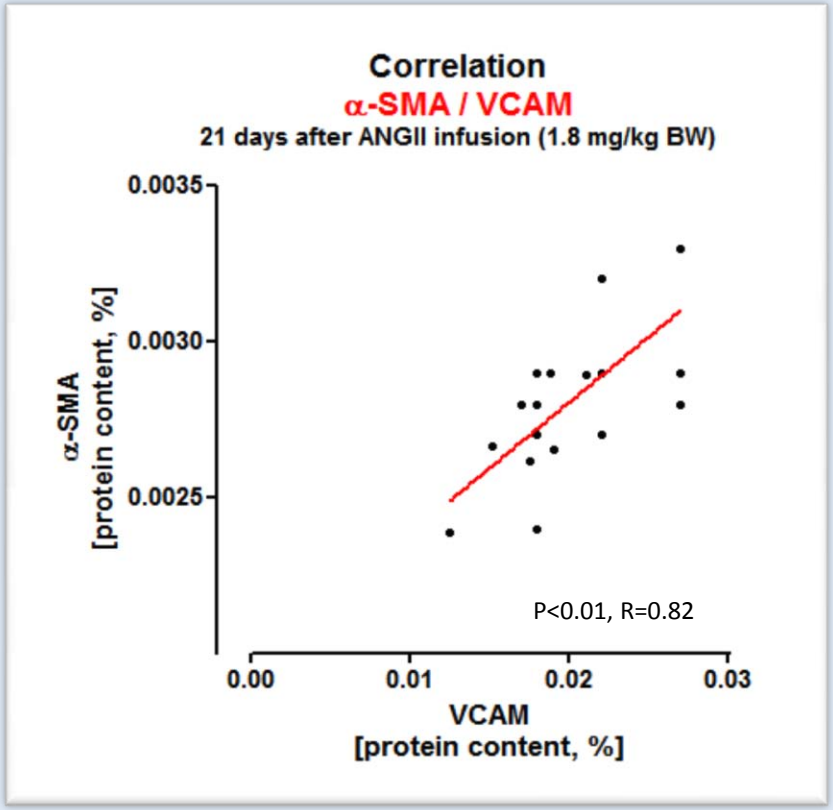
flexercell stretch, frequence 1Hz

Westermann et al, Circulation HF 2011

Myofibroblast and endothelial activation

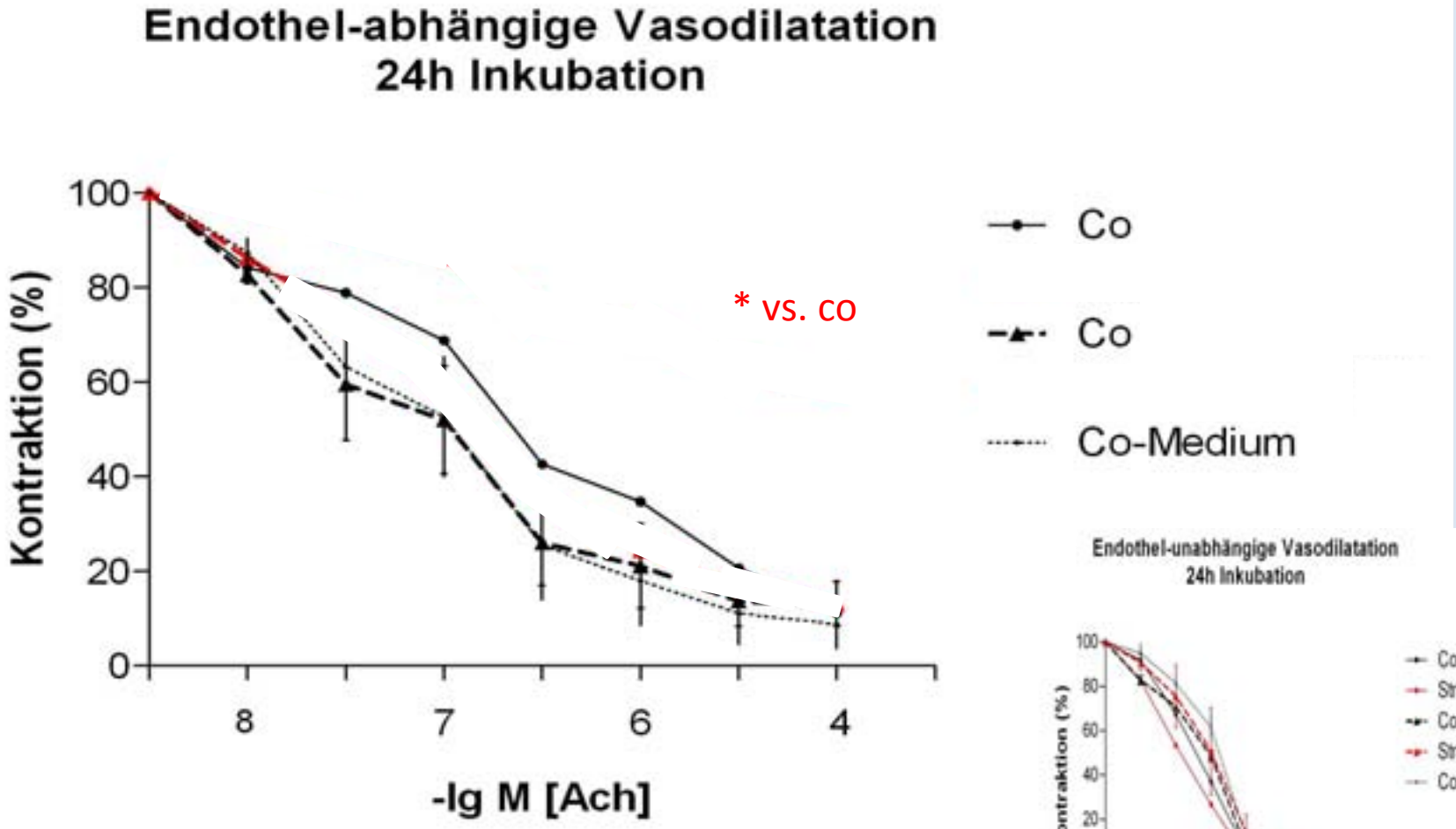


treated HMEC cells



Westermann et al 2012

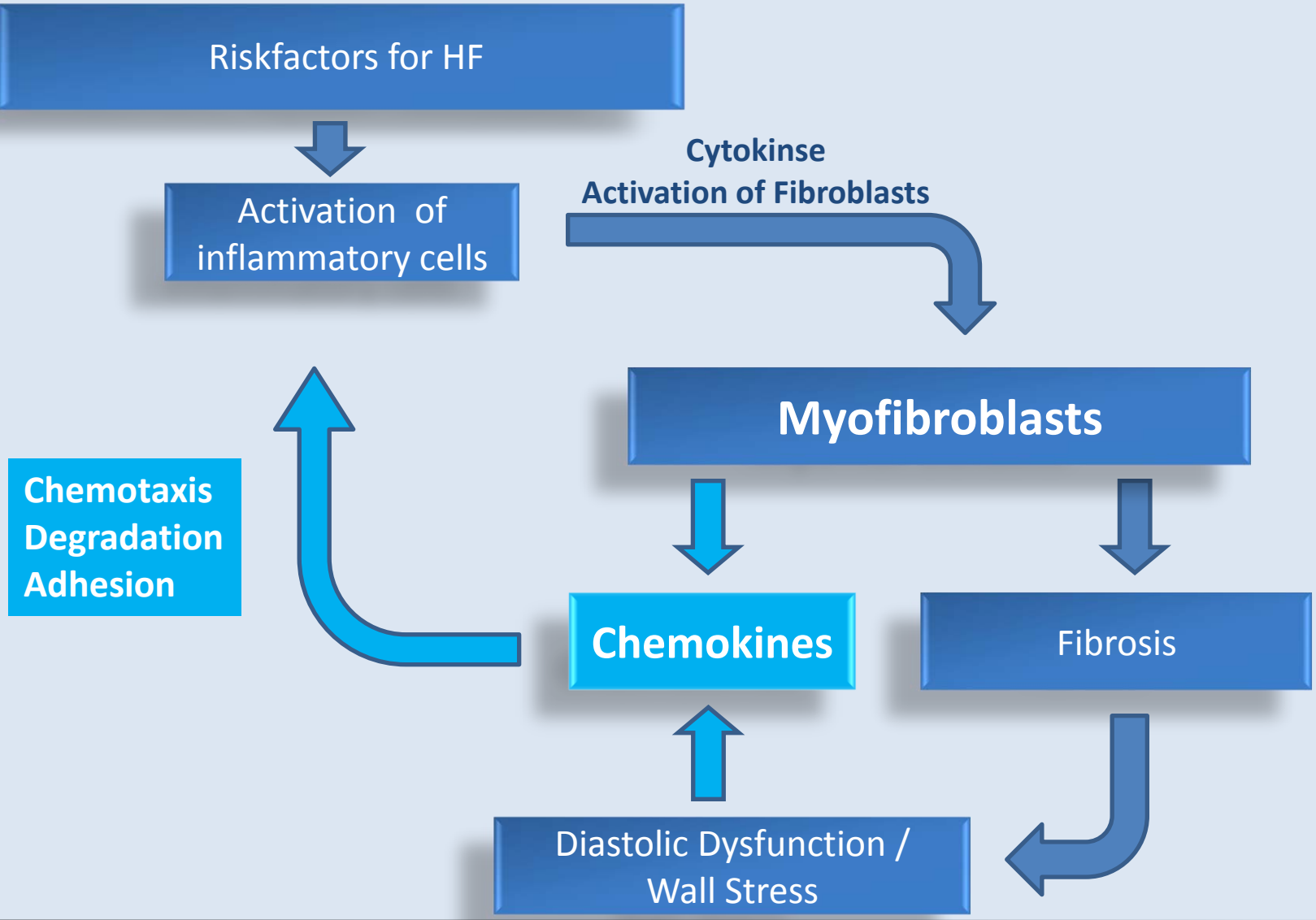
Endothelial Dysfunction *in vitro*



Westermann et al 2012

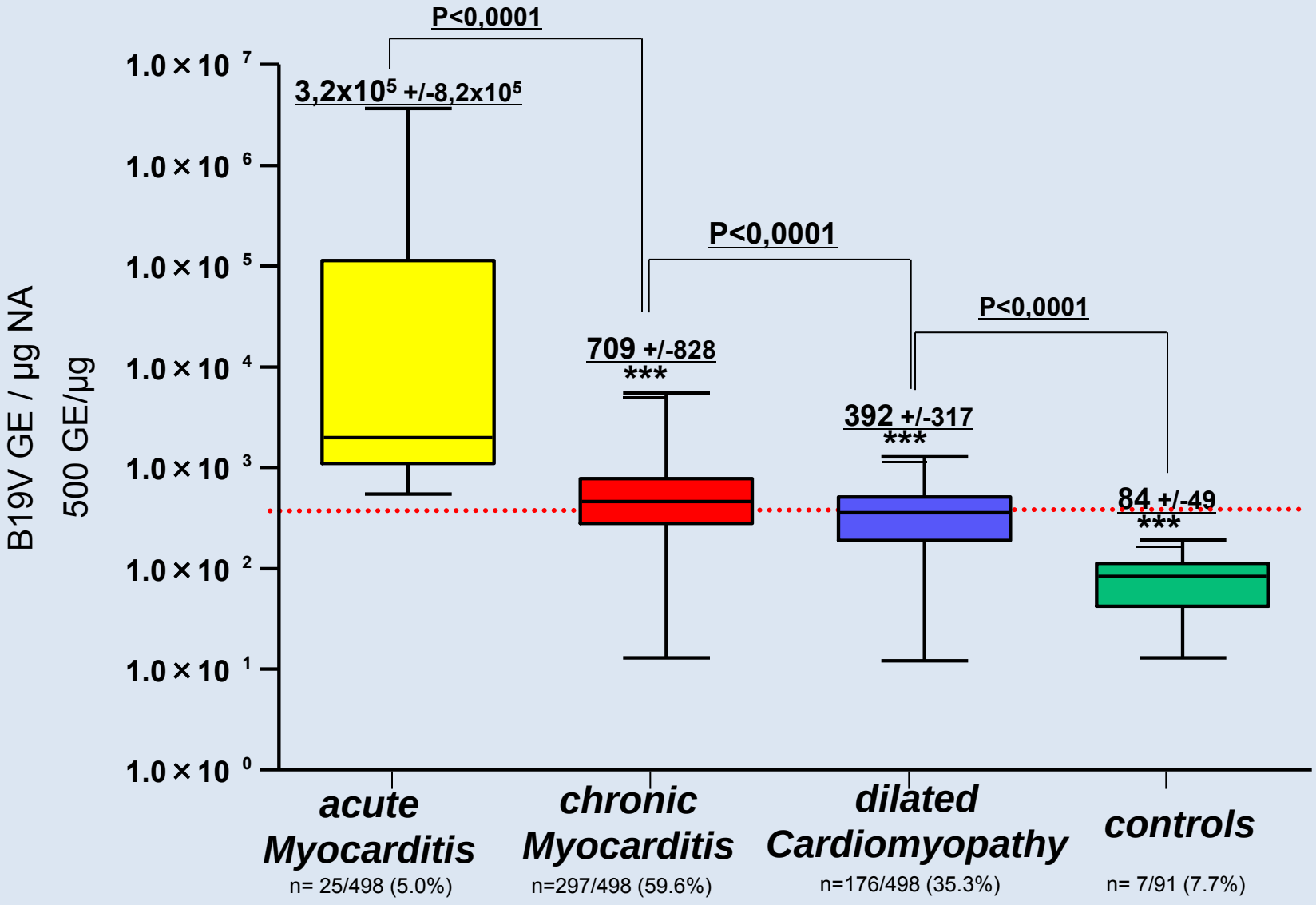
Identification of myofibroblasts in humanen cardiac biopsies

Fibrosis and Inflammation as a Circulus Vitiosus



When is the Parvo B19 infection of the heart of clinical significance?

Kardiale Parvovirus B19 – DNA Last von Patienten mit Myokarditis oder inflammatorischer dilatativer Kardiomyopathie



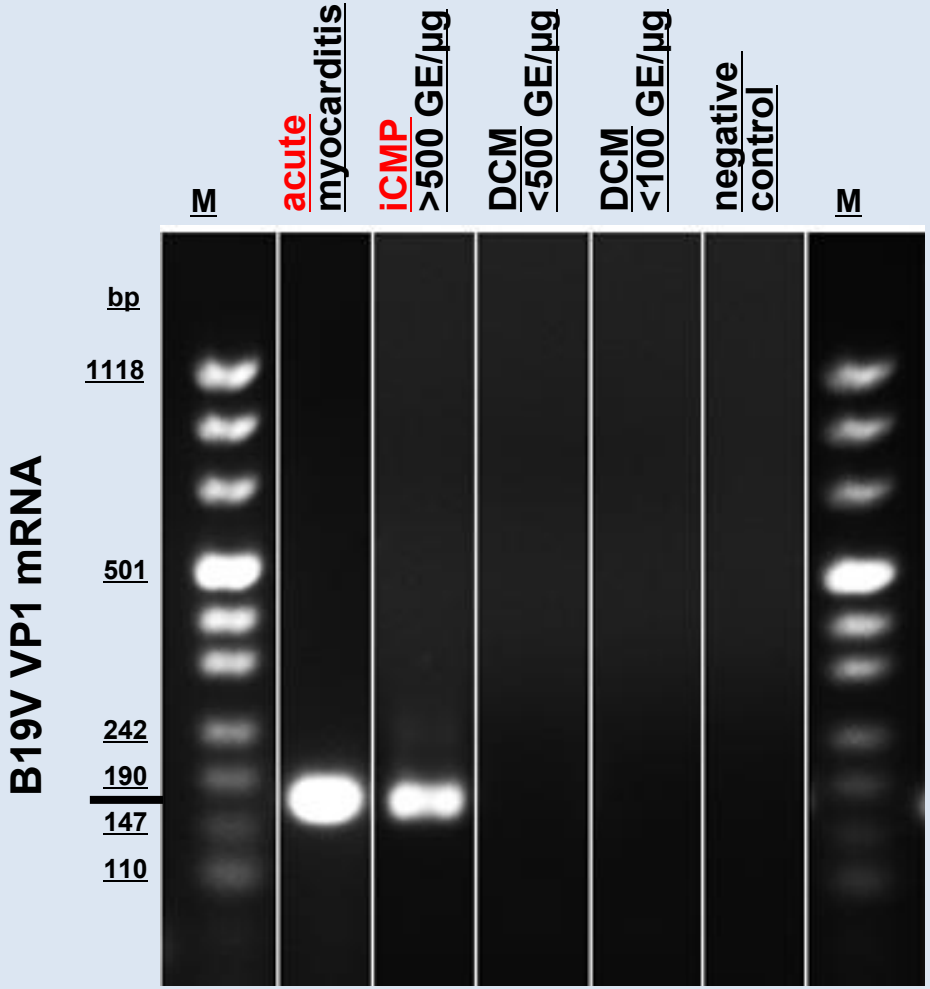
Bock et al NEJM 2010

Virus Typen spezfische Reaktion auf eine Interferon-Therapie

	n	"Clearance"	Virus Last Reduktion
Gesamt	88	40 (46%)	57 (65%)
Enterovirus	24	24 (100%)	
Adenovirus	9	9 (100%)	

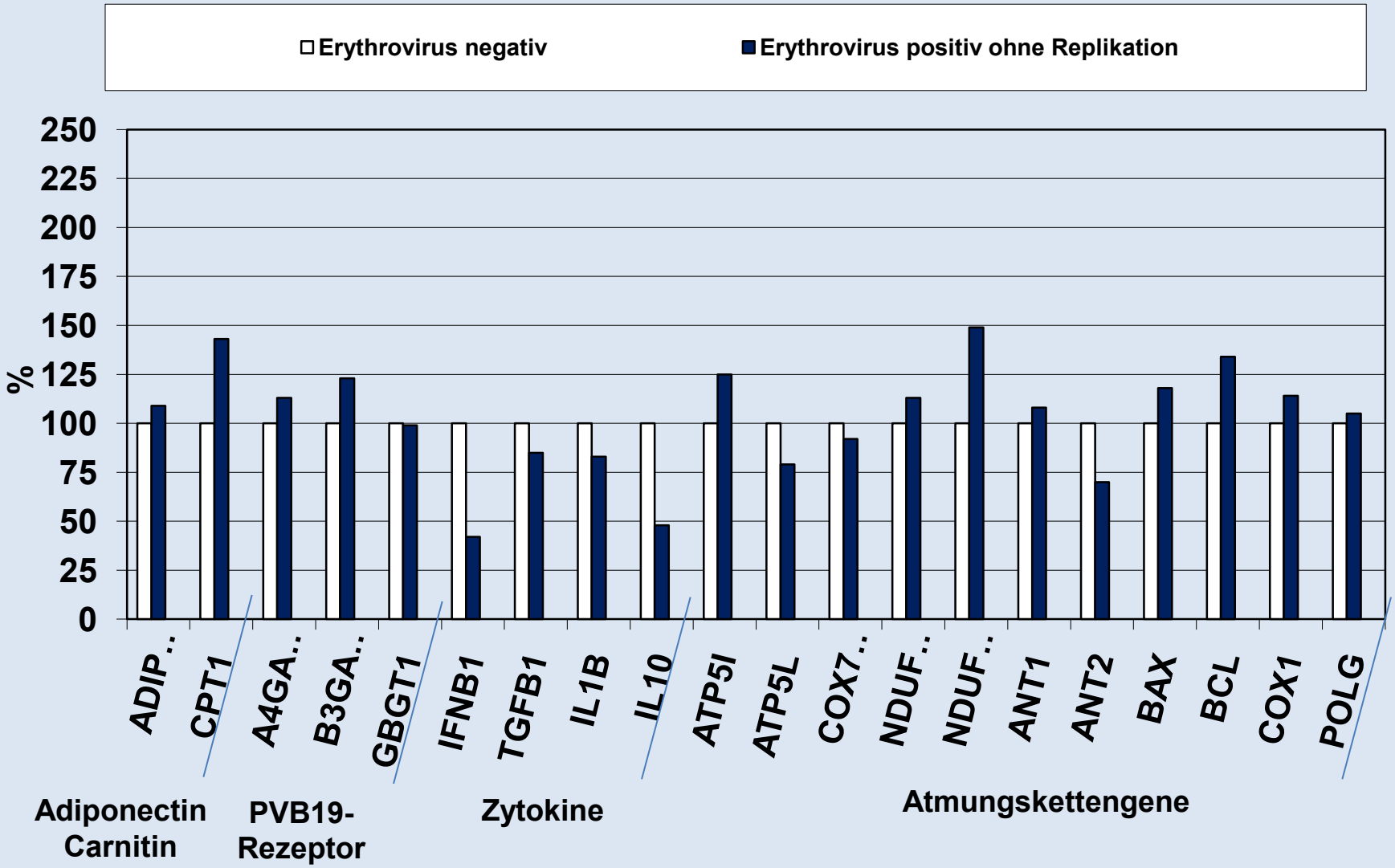
Kühl et al 2012

Nachweis von Parvovirus B 19 mRNA in endomyokardialen Biopsien
Hinweis auf aktive Replikation



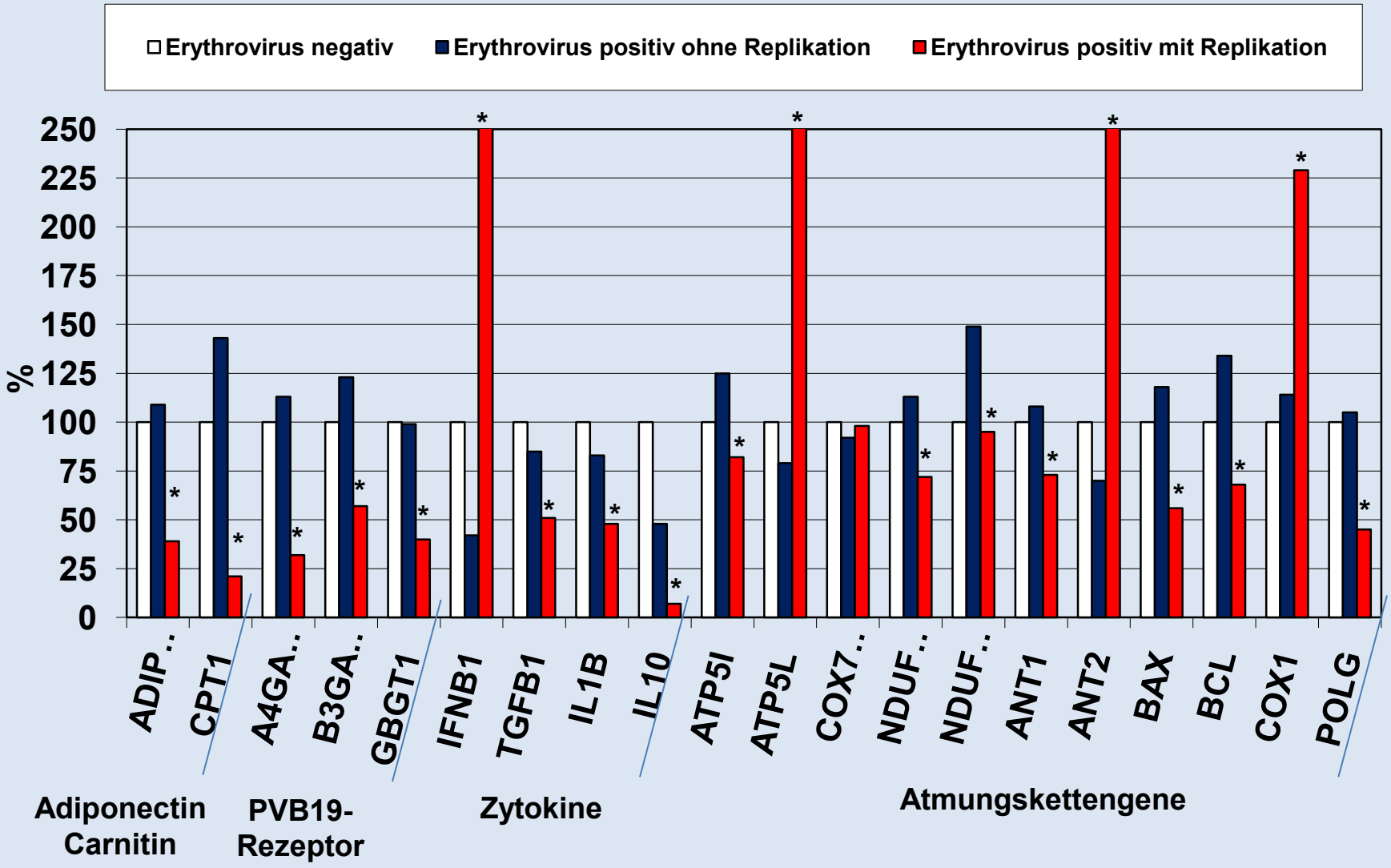
Bock et al 2010
Kühl et al 2012

Regulation der Genexpression bei transkribierter Parvovirus-RNA



C. Tschöpe

Regulation der Genexpression bei transkribierter Parvovirus-RNA



C. Tschöpe

Nucleosid Analogon:

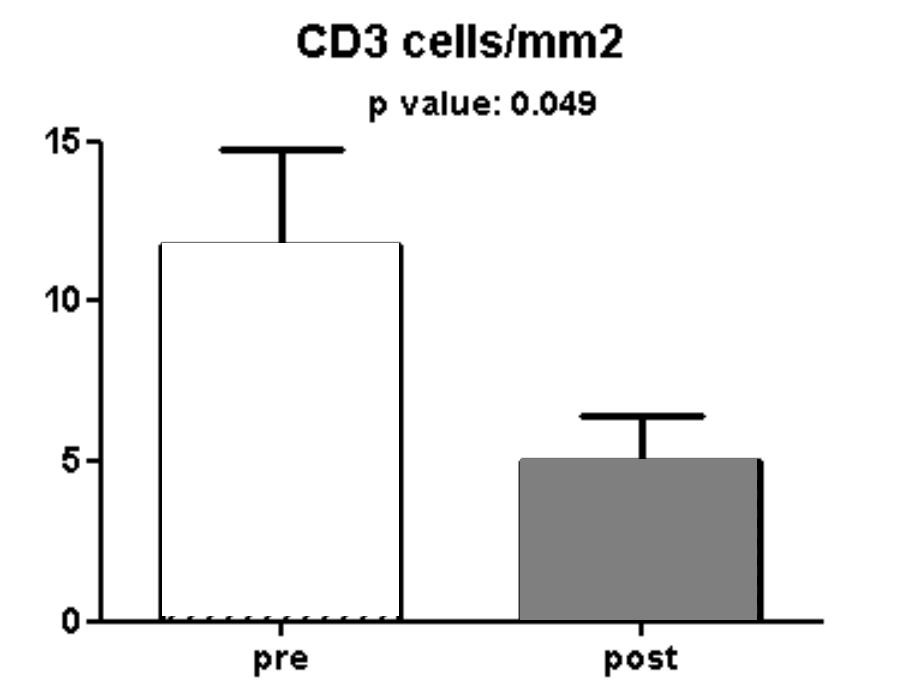
Telbivudine

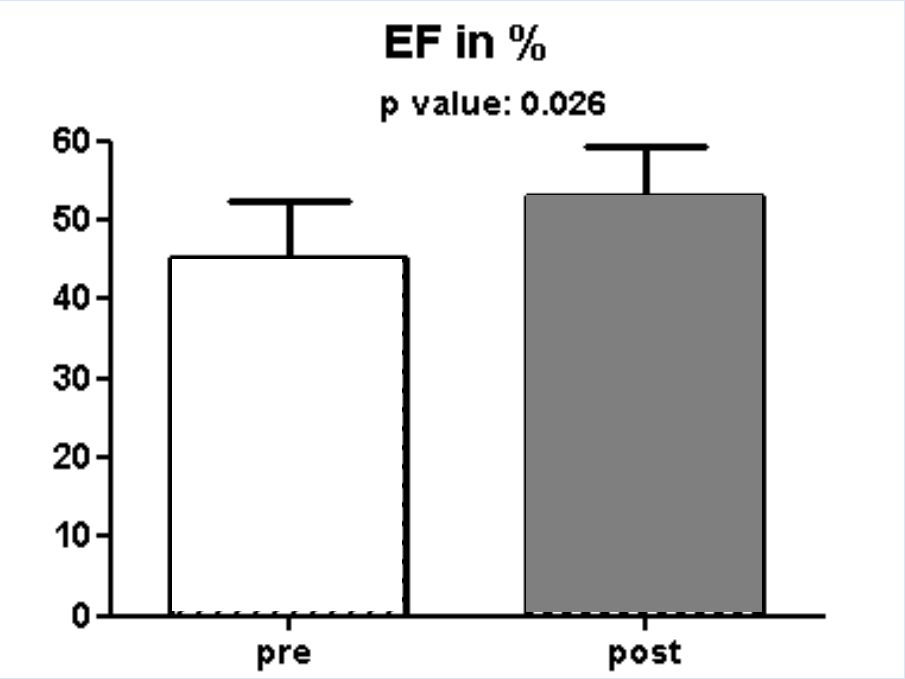
Telbivudine Treatment of Parvovirus B19-positive Cardiomyopathy

B19V load in patients with silenced transcriptional activity (B19V-mRNA) during Telbivudine treatment

	Baseline biopsy	Follow up biopsy	p
B19V load (copies/µg DNA)	2543	1188	<0.05
B19V mRNA (copies/µg RNA)	249	0	<0.01

Effekt von Telvibudine in behandelten Patienten mit chronischer kardialer Parvovirus B19 Infektion





n = 30

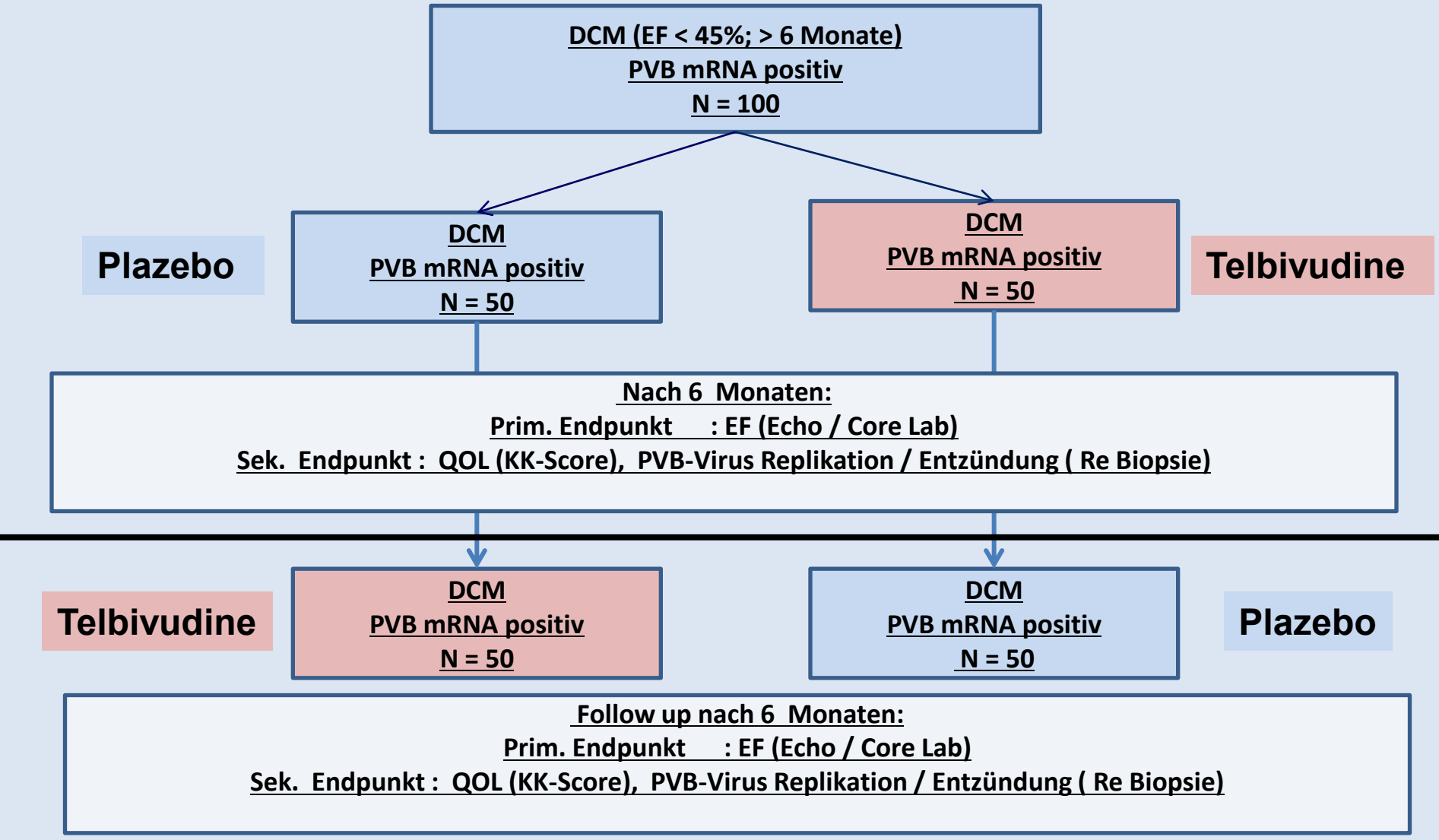
*T*elbivudine Treatment *o*f *P*arvovirus B19-positive *C*ardiomyopathy

DZHK
DEUTSCHES ZENTRUM FÜR HERZ-KREISLAUF-FORSCHUNG E.V.

ToPIC - Study

Phase II Studie

*T*elbivudine Treatment *o*f *P*arvovirus B19-positive *C*ardiomyopathy

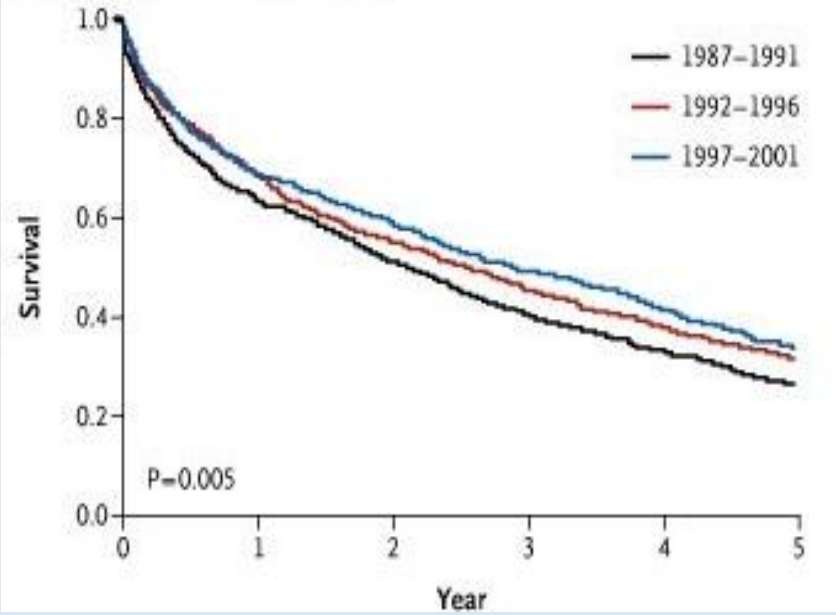


C. Tschöpe

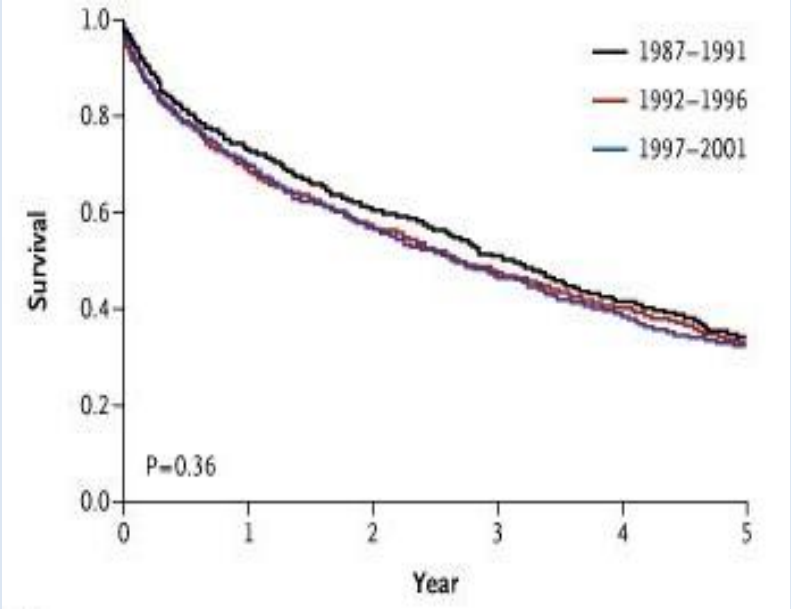
DZHK
DEUTSCHES ZENTRUM FÜR HERZ-KREISLAUF-FORSCHUNG E.V.

Heart failure treatment today?

HREF



HFPEF



Owan T et al. N Engl J Med 2006;

Task

Identification of new mechanisms
Personalized medicine / Biopsy-guided
Genetic/Epigentic
Systeme medicine

Merci

Kooperationspartner/Funding:

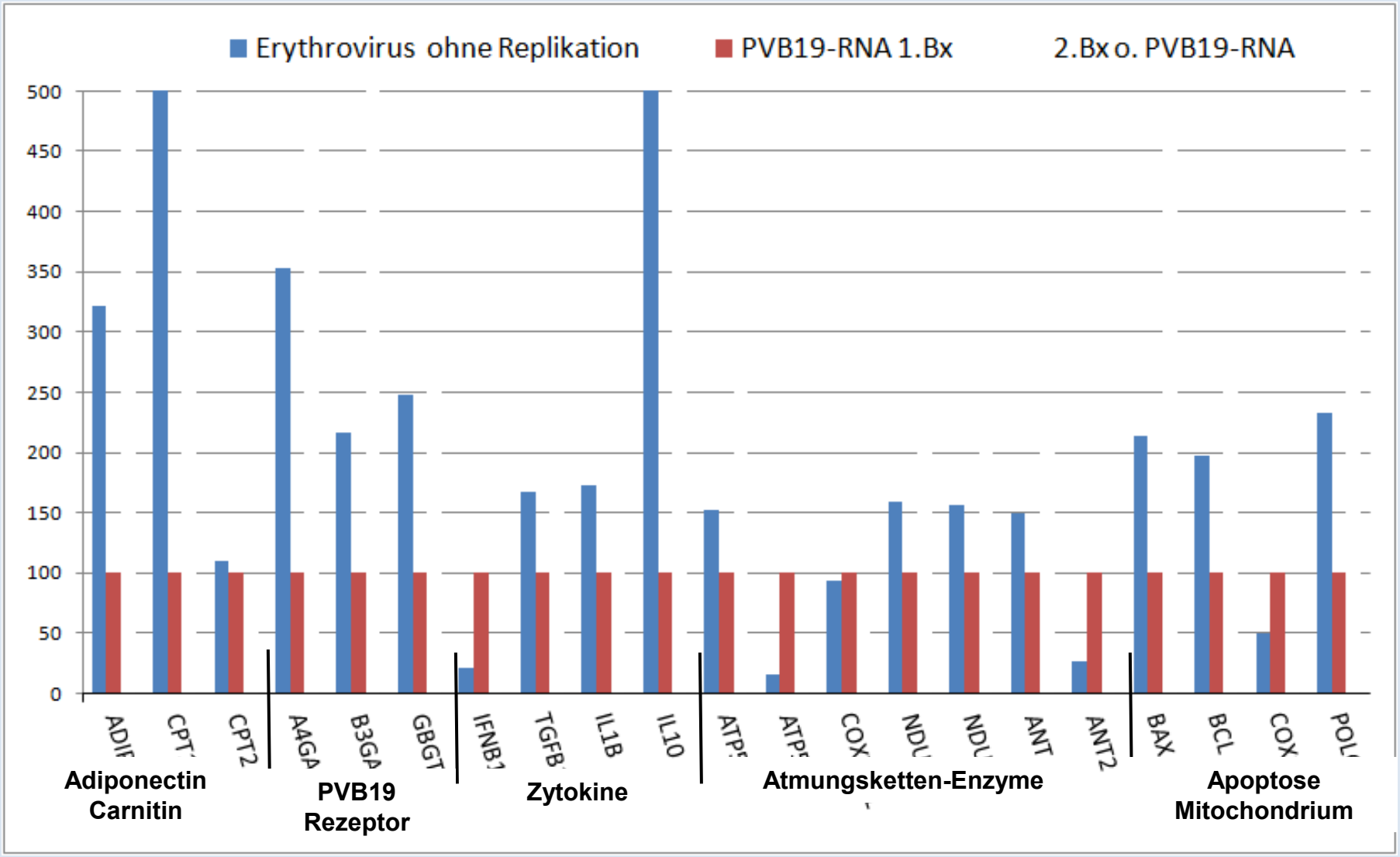
TR-SFB 19: inflammatorische Kardiomyopathie
BCRT/BMBF: Immunsystem u. Herz
EU FP7: Diabetes mellitus u. diastolische Herzinsuffizienz
EU FP7: Stammzelle u. Diabetes mellitus
DZHK: Inflammatorische Kardiomyopathie

Summary and Conclusion

VB19 genomes were predominant in patients with unexplained
olated diastolic dysfunction in our study group.

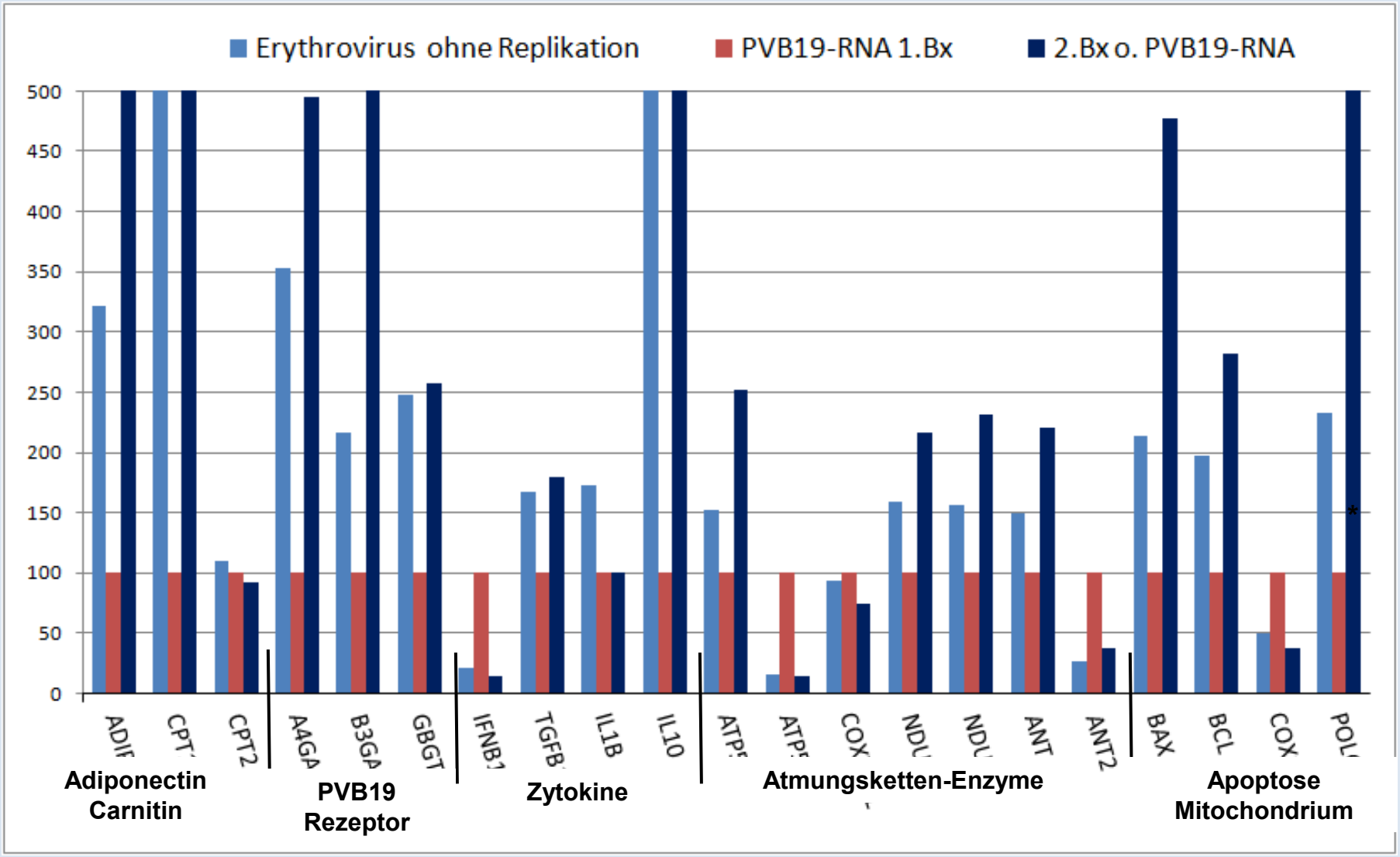
strong association with the incidence of endothelial dysfunctio
as obvious, consistent with the hypothesis that PVB19-induced
ndothelial dysfunction may be a possible pathomechanism
nderlying diastolic dysfunction.

Gegenregulation der Gene nach Abklingen der PVB19-mRNA (Follow-up Biopsien)



C. Tschöpe

Gegenregulation der Gene nach Abklingen der PVB19-mRNA (Follow-up Biopsien)



Inflammatory Cardiomyopathy Conclusion I

-The detection of viral genome and or inflammation in the myocardium has been shown by multivariate regression analysis to be an independent predictor of clinical outcome.

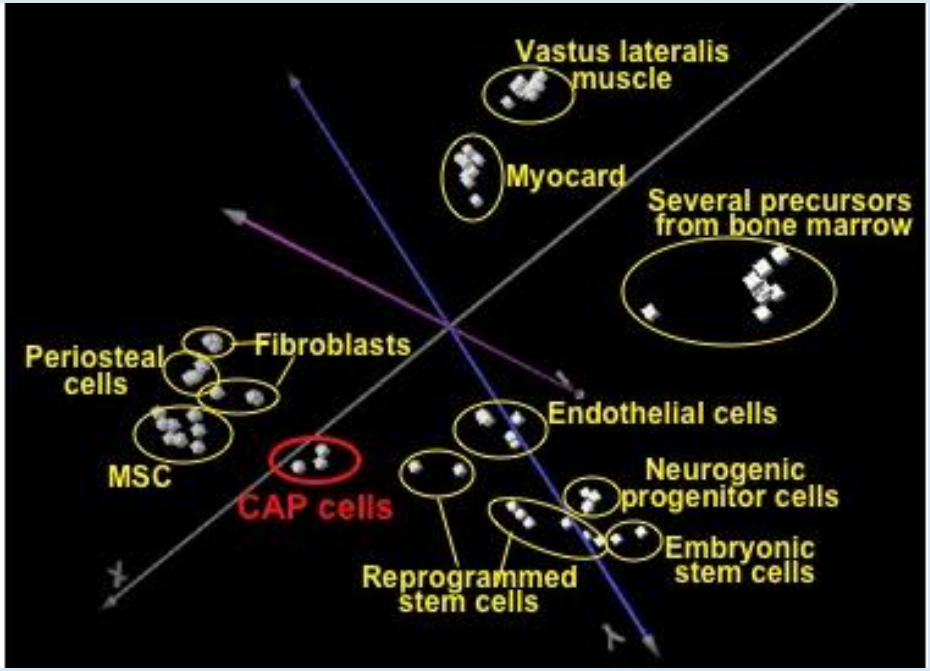
- This cannot be detected by echo/MRI

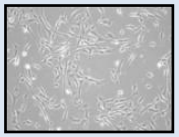
Inflammatory Cardiomyopathy Conclusion III

- However, any rational immunomodulatory therapeutic regimen for inflammatory cardiomyopathy must consider the underlying pathogenesis based on
  - histological,
  - immunohistological and
  - viral

evaluation of endomyocardial biopsies.

Cardiac Adherent Proliferating Cells (CAP´s): Anti-fibrotische und anti-entzündliche Effekte

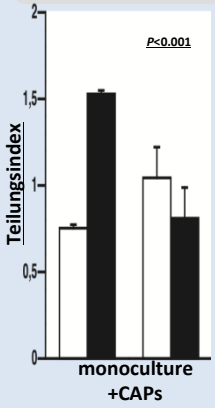




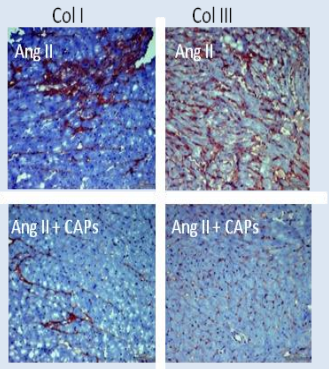
CAP cells

FIBROSE

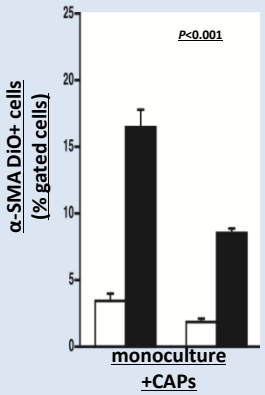
Fibroblasten Proliferation



Kollagensynthese

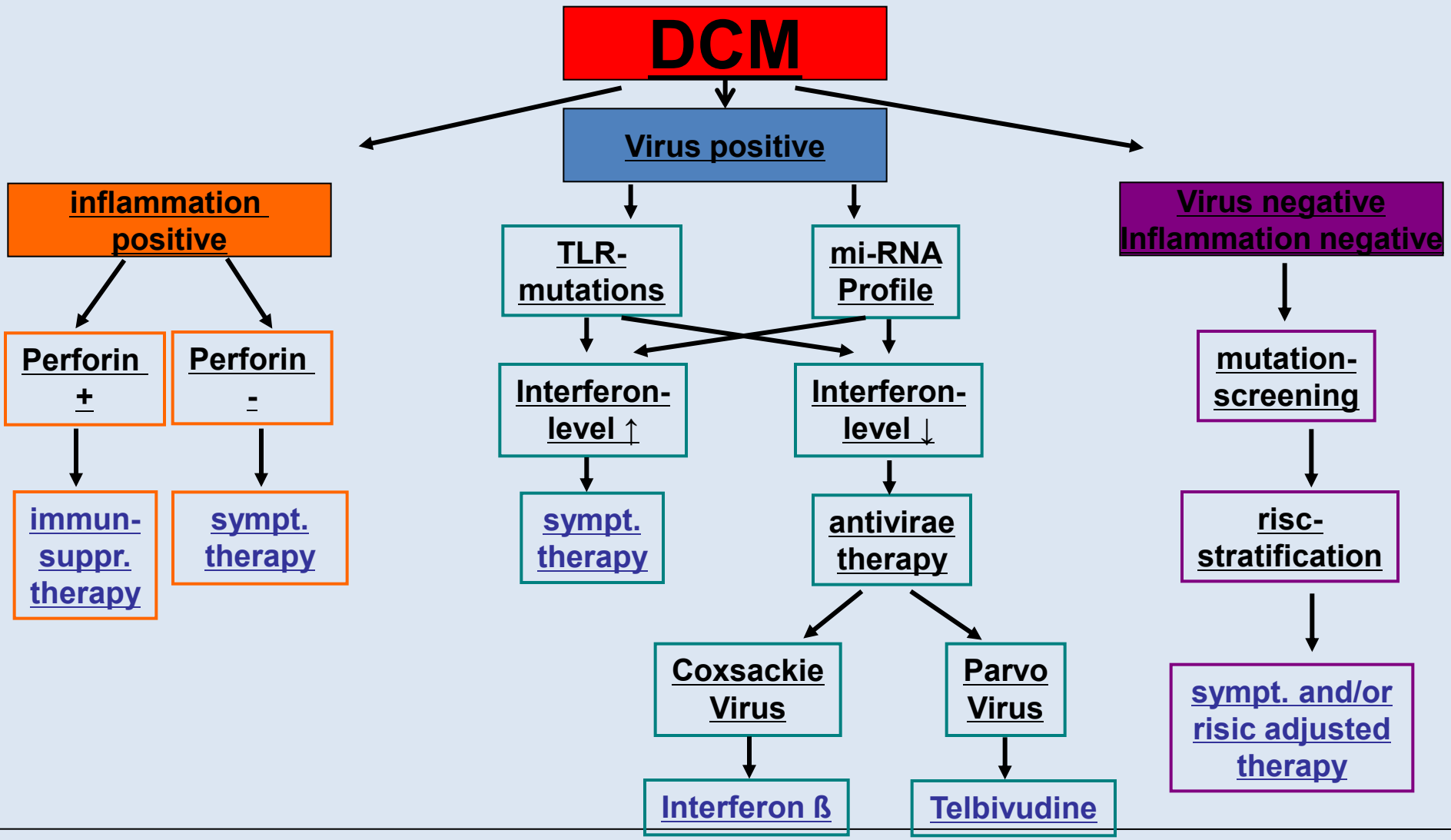


Myofibroblast Transdifferentiation



Haag et al. J Cell Biochemistry 2010
Milteva et al. PLOS One 2011

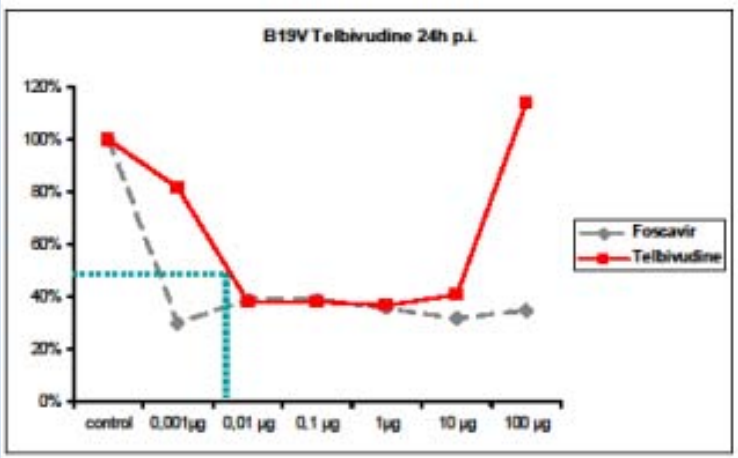
Personalised medicine in Dilated Cardiomyopathy

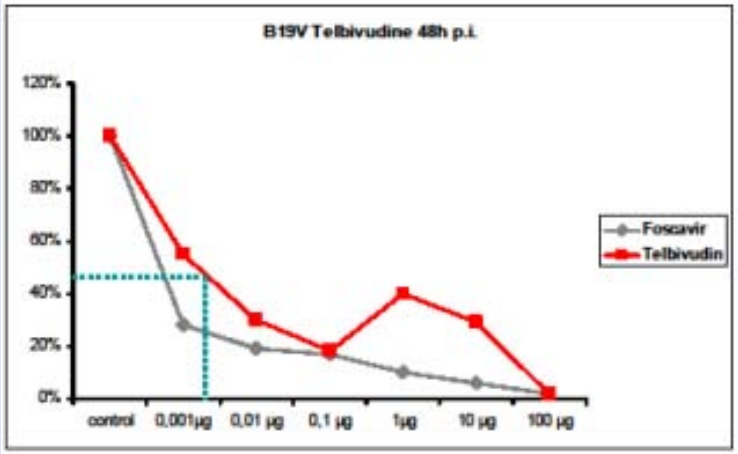


Schultheiss et al EHJ, 2011

Susceptibility and inhibitory concentration (IC) of Telbivudine on parvovirus B19 replication in endothelial cell culture experiments

Determination of IC50 of telbivudine in cell cell culture





IC_{50} < 0.001 µg (2 µM) comparable to HBV

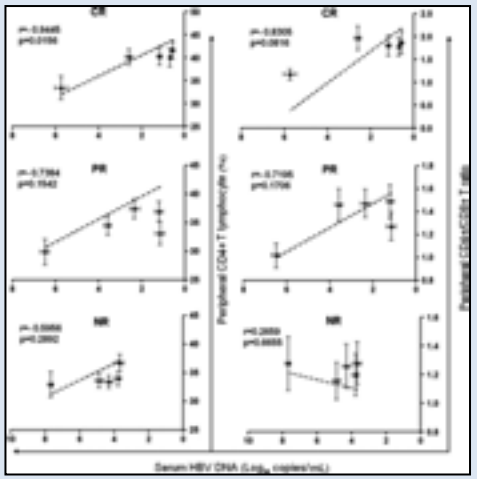
Proposed mechanisms of telbivudine

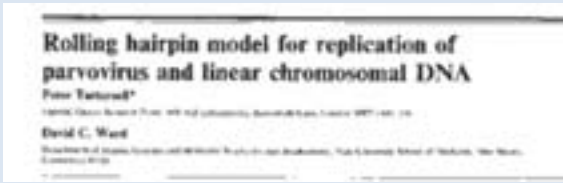

Antiviral Research

Effect of telbivudine therapy on the cellular immune response in chronic hepatitis B

Yu Chen, Xuefen Li, Bo Ye, Xianzhi Yang, Wei Wu, Baode Chen, Xiaoping Pan, Hongzui Cao, Lanjuan Li

Telbivudine can probably interact with host immune system to control B19V replication

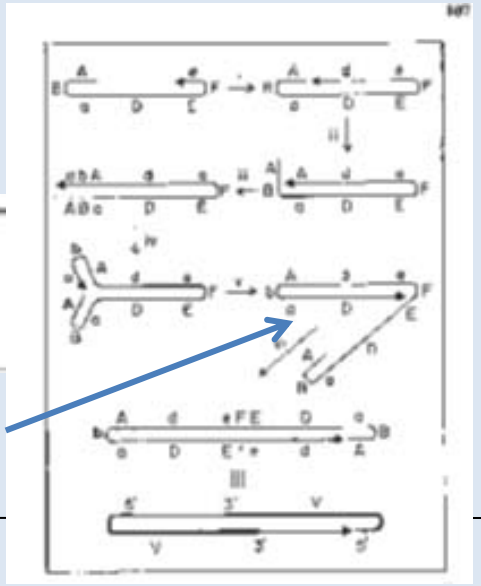



Rolling hairpin model for replication of parvovirus and linear chromosomal DNA

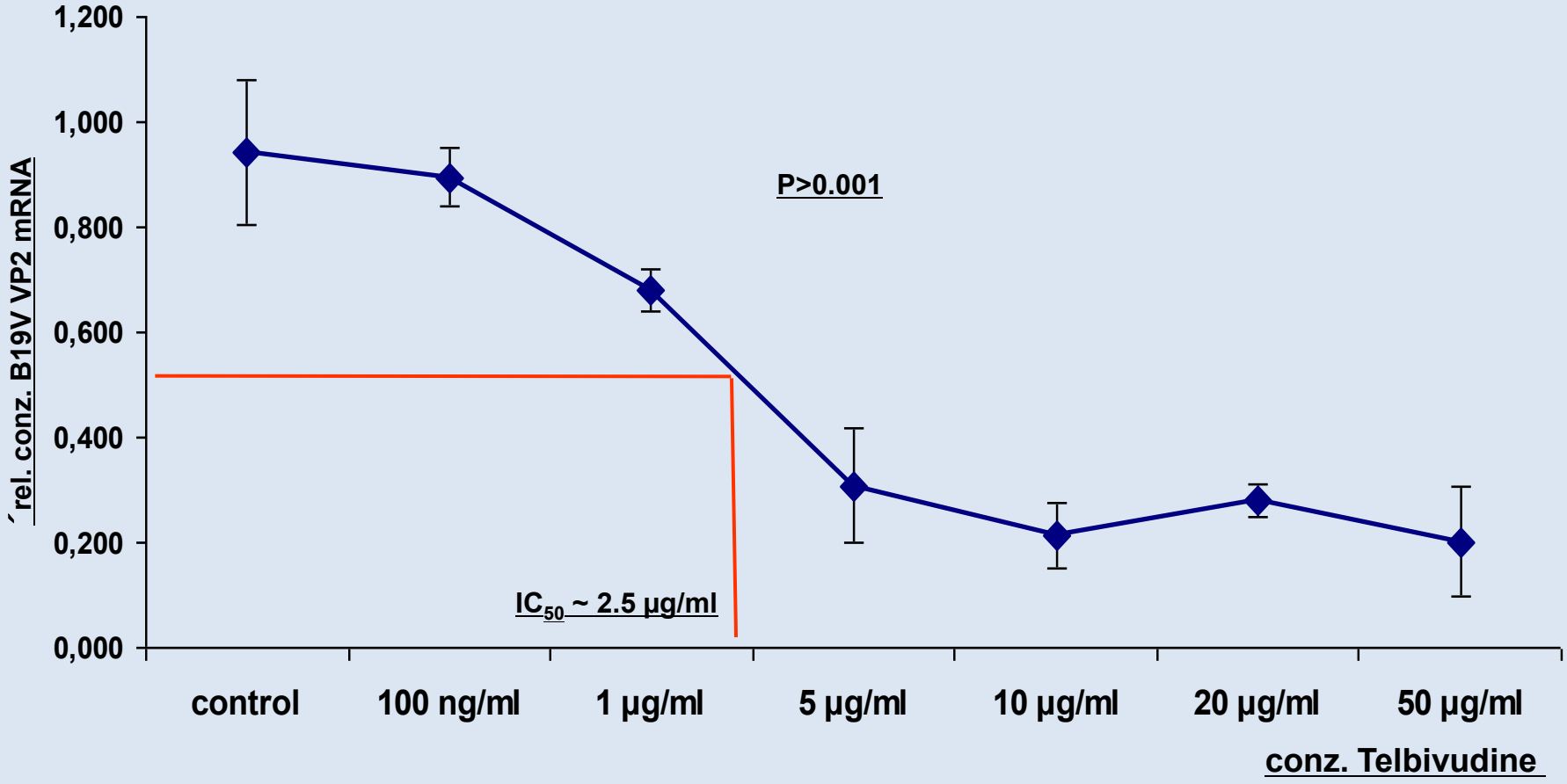
Peter Tattersall*

David C. Ward

Telbivudine can probably interact with DNA-synthesis during rolling hairpin replication

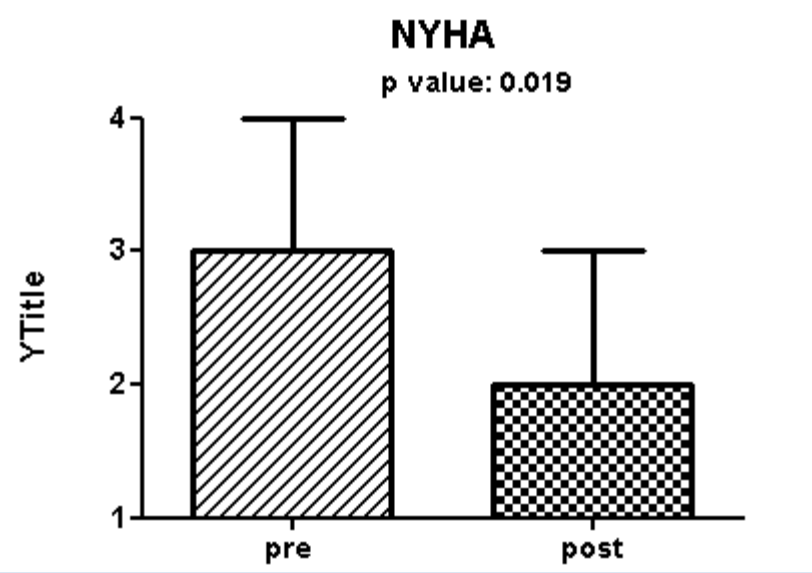


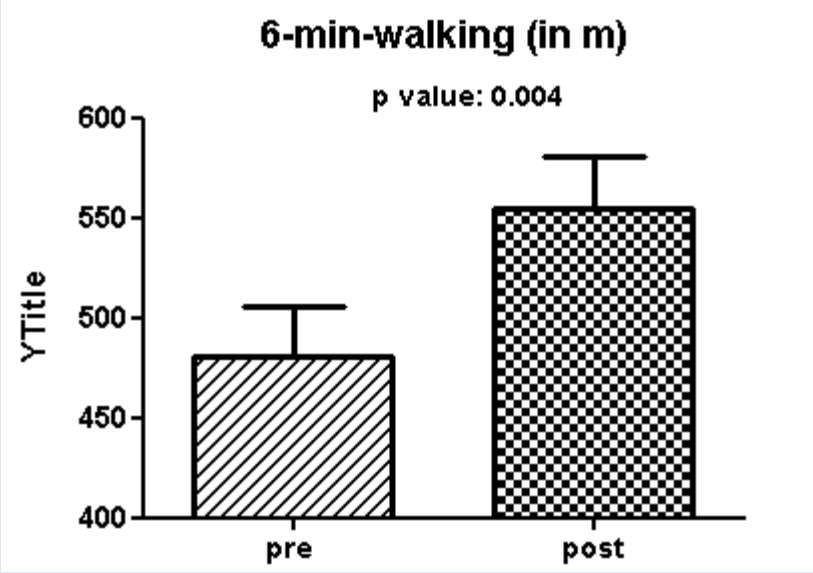
Inhibition of Parvovirus B19 Replication by Telbivudine Treatment in B19V-infected endothelial Cell Culture (hMEC-1)



Clinical improvement of Telbivudine-treated patients with subacute/chronic disease, n=7*

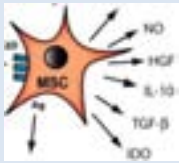
(Telbivudine treatment: 24 weeks, n=7)





Entwicklung neuer Interventionsziele zur Therapieoptimierung der Zukunft bei der Herzinsuffizienz

Kardiale Inflammation

Zytokin-System	Angeborene Immunsystem	Matrix Proteine (MMP´s)	Virustoxizität	Regeneration
			 PV B19 Coxsacki B	
IL1β, IL2, IL4, IL6, IL10, IL23, TNFα	TLR-4, -9 TRIF, MYD-88 NOD2 S100 A8/A9	MMP-2, MMP-9 Biglycan	Interferon ß Telvibudine	Mesenchymale Stromazelle T reg´s CAP´s

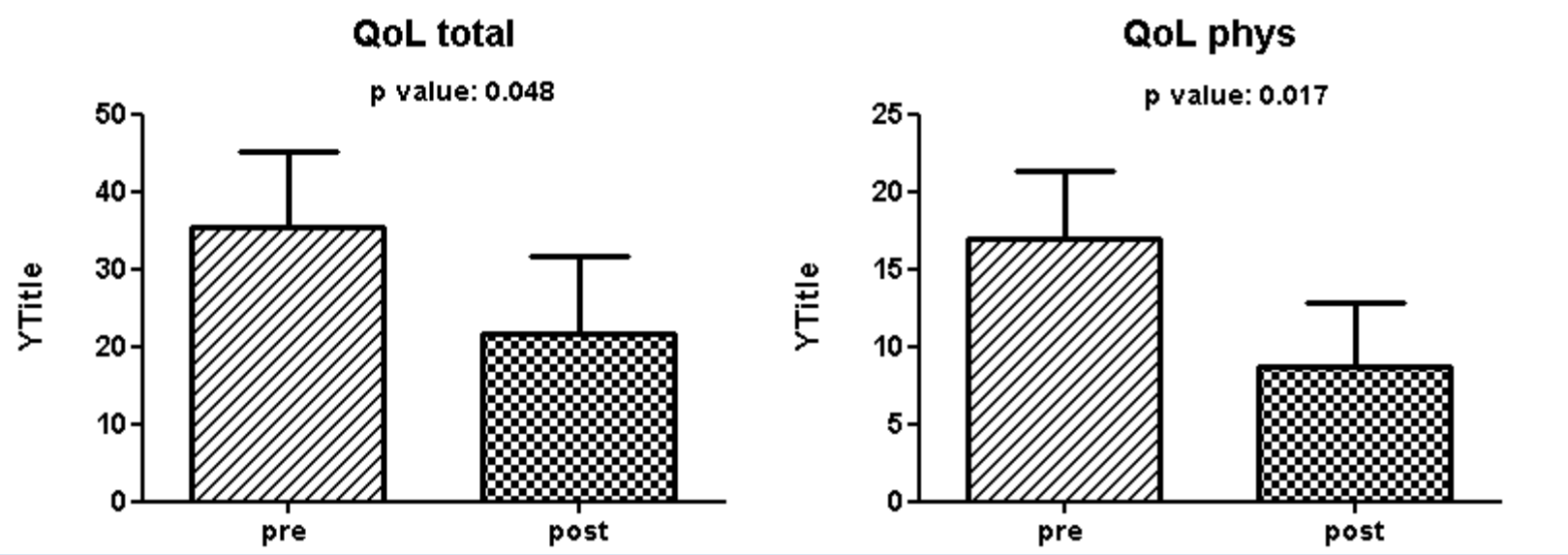
IL: Interleukin, TNF; Tumor Nekrose faktor, TLR: Toll-like Rezeptor, NOD: Nucleotide-binding oligomerization domain-containing protein, MMP: Metalloproteinase, MSC: Mesenchymale Stromazelle, Treg´s: regulatve T-Zelle; CAP´s: cardiac derived adherent proliferating cell

Li et al. Eur J Pharmacol 2007
Koch et al. Int Immunopharm 2010
Riad et al. J Immunol 2011

Westermann et al. Circulation 2011
Van Linthout et al. Eur Heart J 2011
Savvatis et al. PlosOne 2012

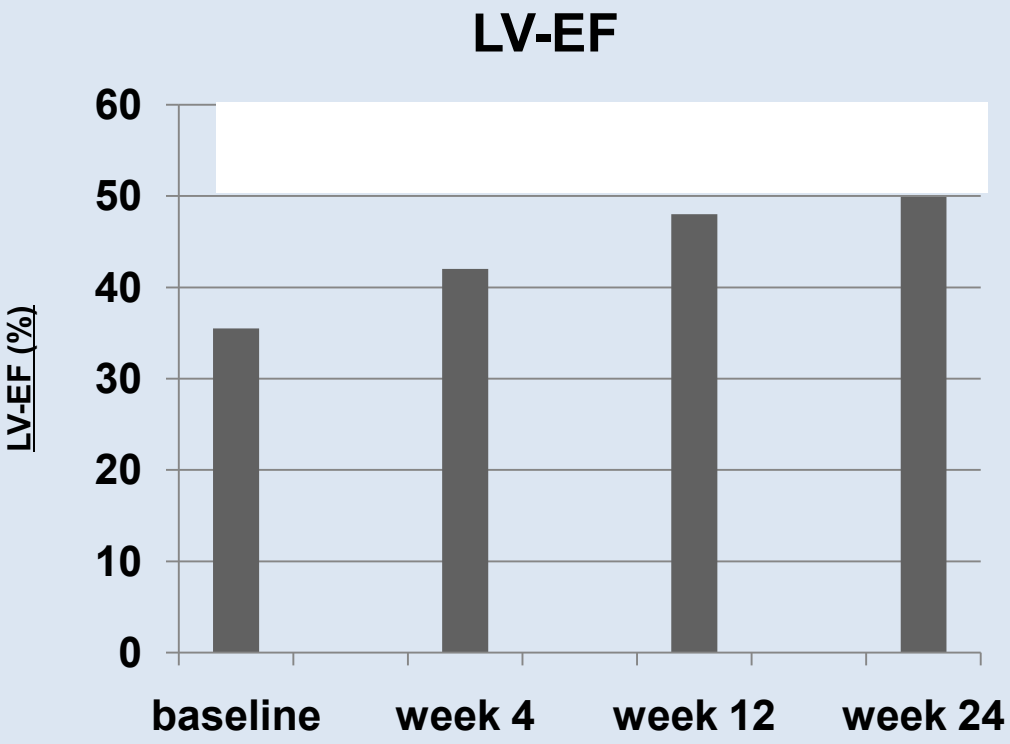
Clinical improvement of Telbivudine-treated patients with subacute/chronic disease, n=7*

(Telbivudine treatment: 24 weeks, n=7)

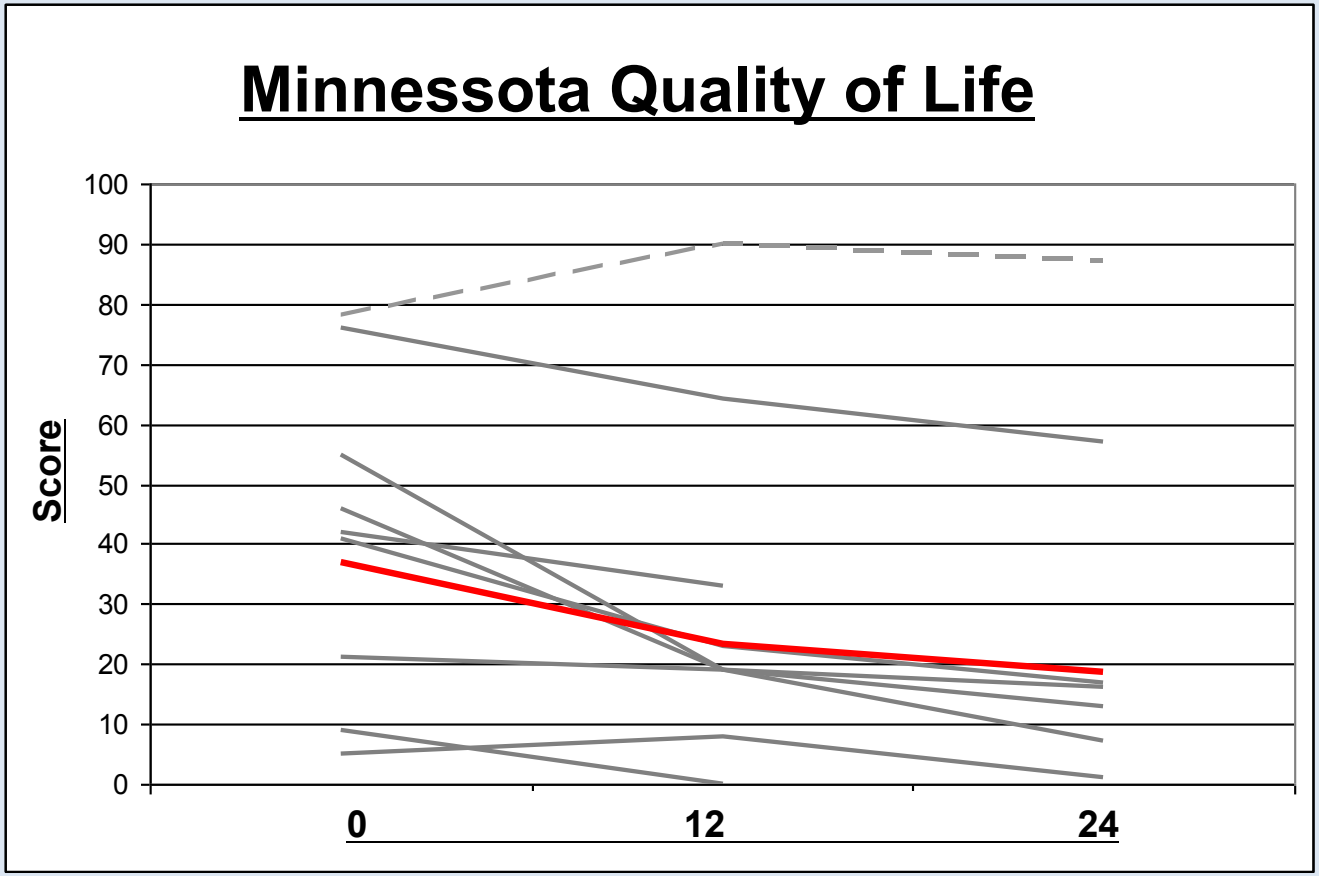


Hemodynamic changes in Telbivudine-treated Patients with subacute/chronic disease

*** Acute B19V infections excluded**

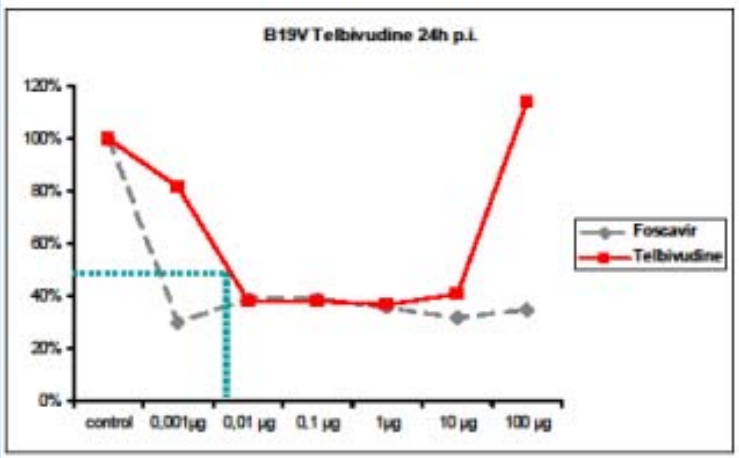


Symptomatic improvement (QoL)



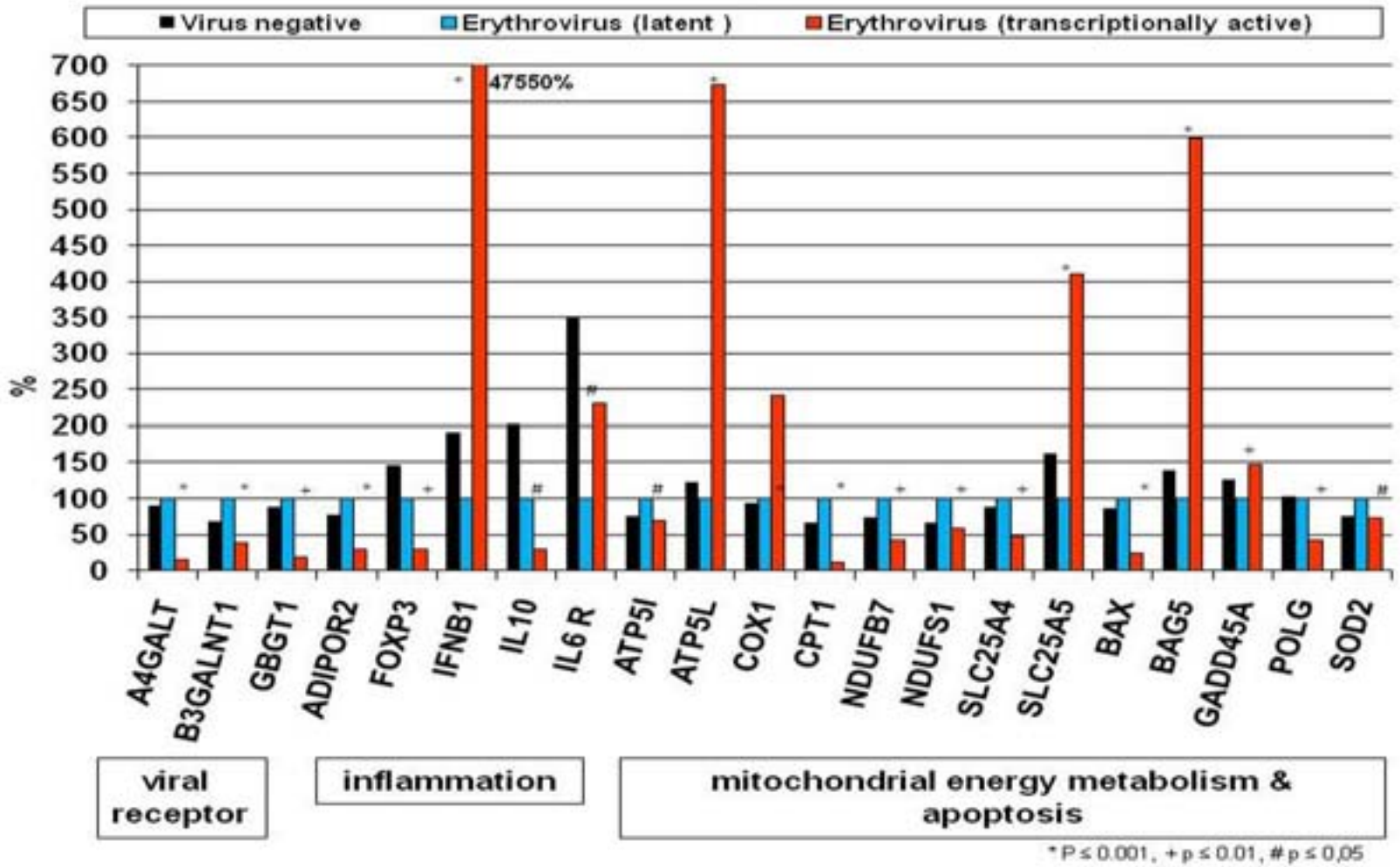
Susceptibility and inhibitory concentration (IC) of Telbivudine on parvovirus B19 replication in endothelial cell culture experiments

Determination of IC50 of telbivudine in cell cell culture



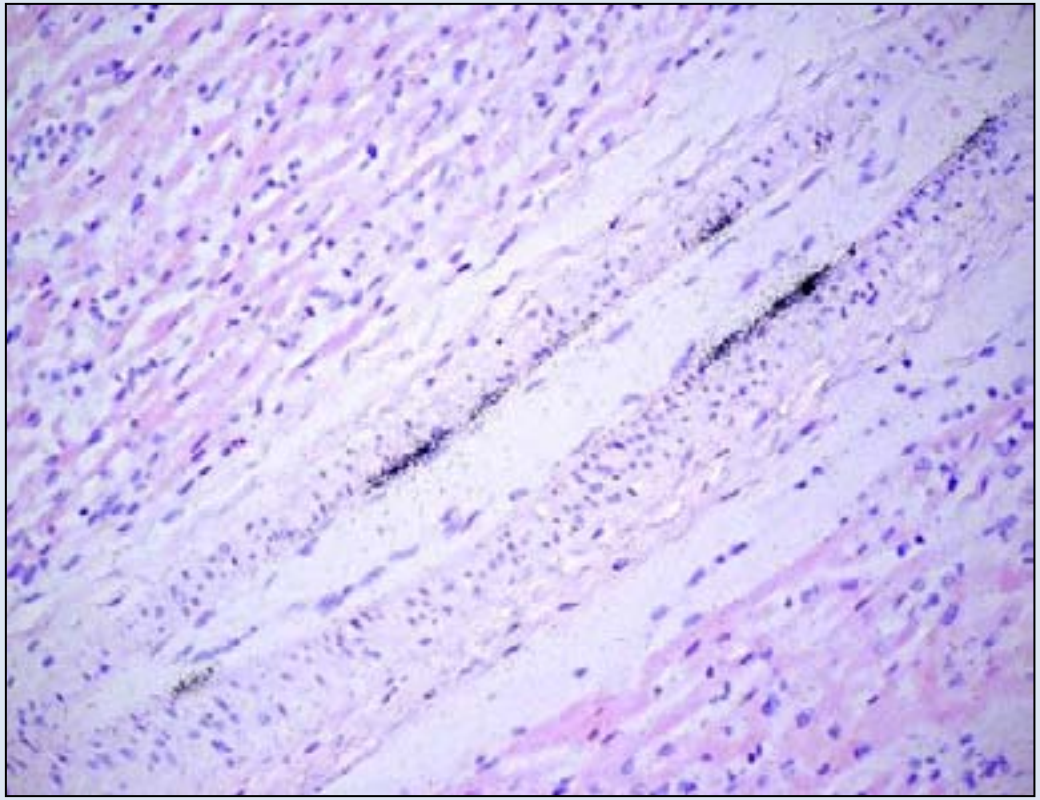
IC_{50} < 0.001 µg (2 µM) comparable to HBV

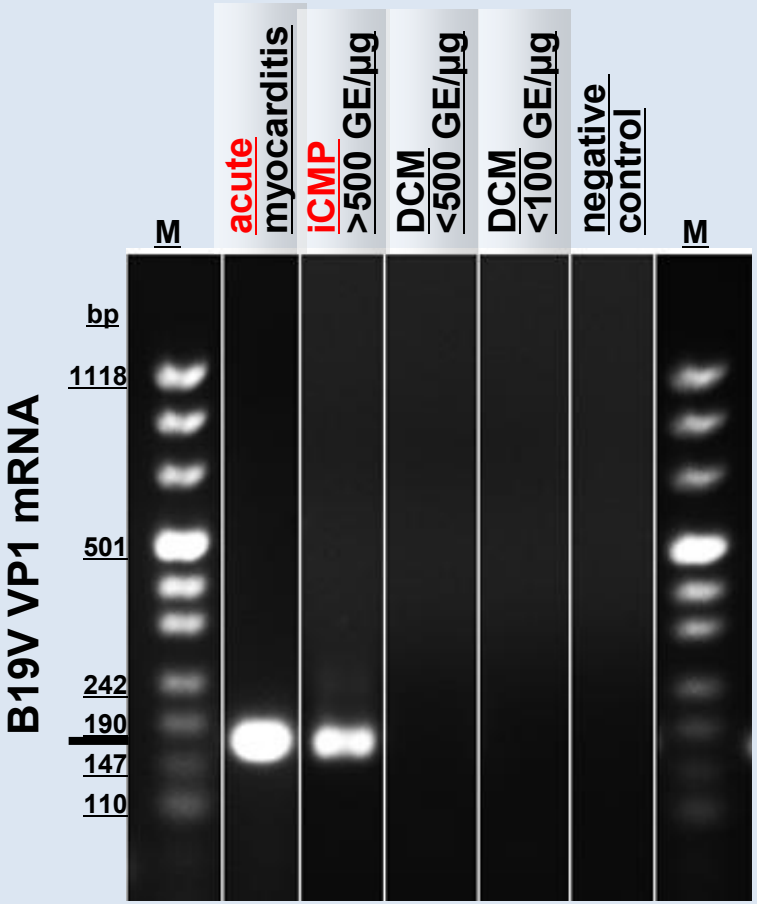
Veränderung der kardialen Genexpression ist assoziiert mit transkribierter Parvovirus-RNA



Nachweis von Parvovirus B 19 mRNA in endomyokardialen Biopsien: Hinweis auf aktive Replikation

In situ Hybridisierung (PVB DNA)

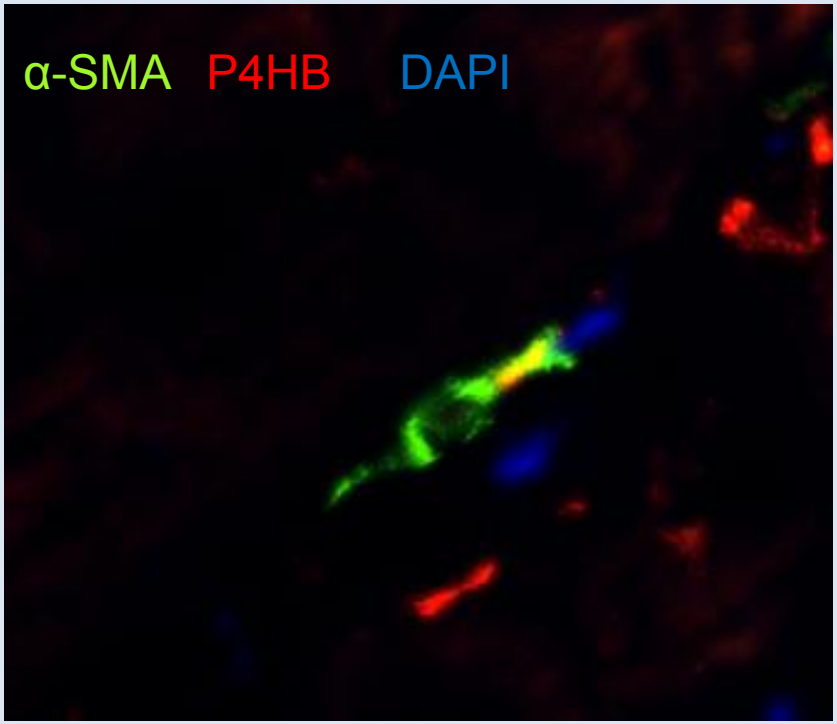




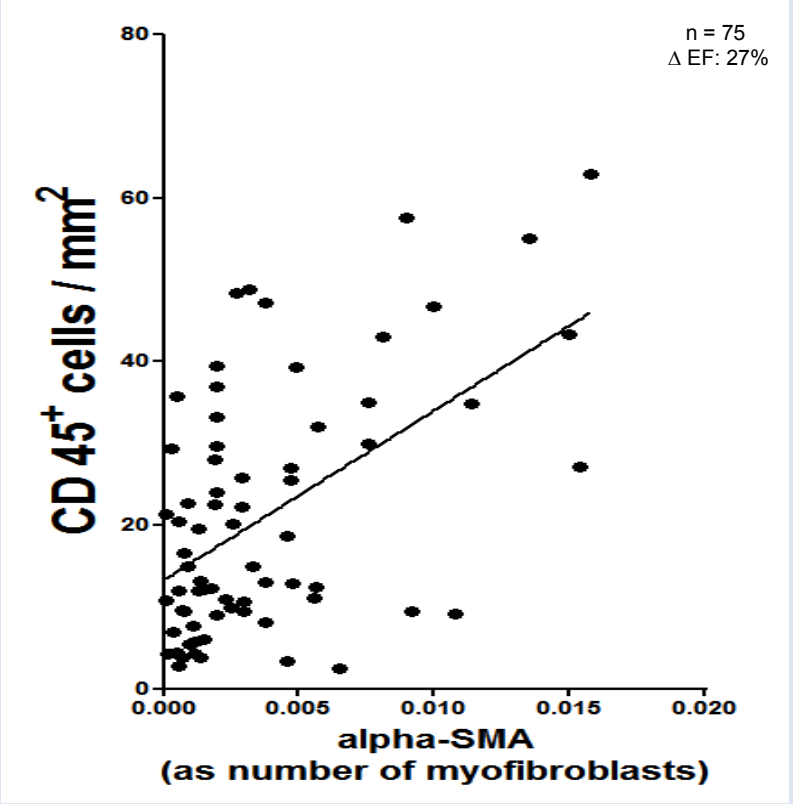
Bock et al 2010
Kühl et al 2012

Nachweis von aktivierten Myofibroblasten in humanen endomyokardialen Biopsien

Myofibroblast



Myofibroblasten Anzahl korreliert mit endomyokardialer Inflammationsreaktion



Westermann et al, Circulation HF 2011