

# Therapeutic Capacity of Apoptotic Mononuclear Cell Secretome in Experimental Spinal Cord Injury

Doctoral viva

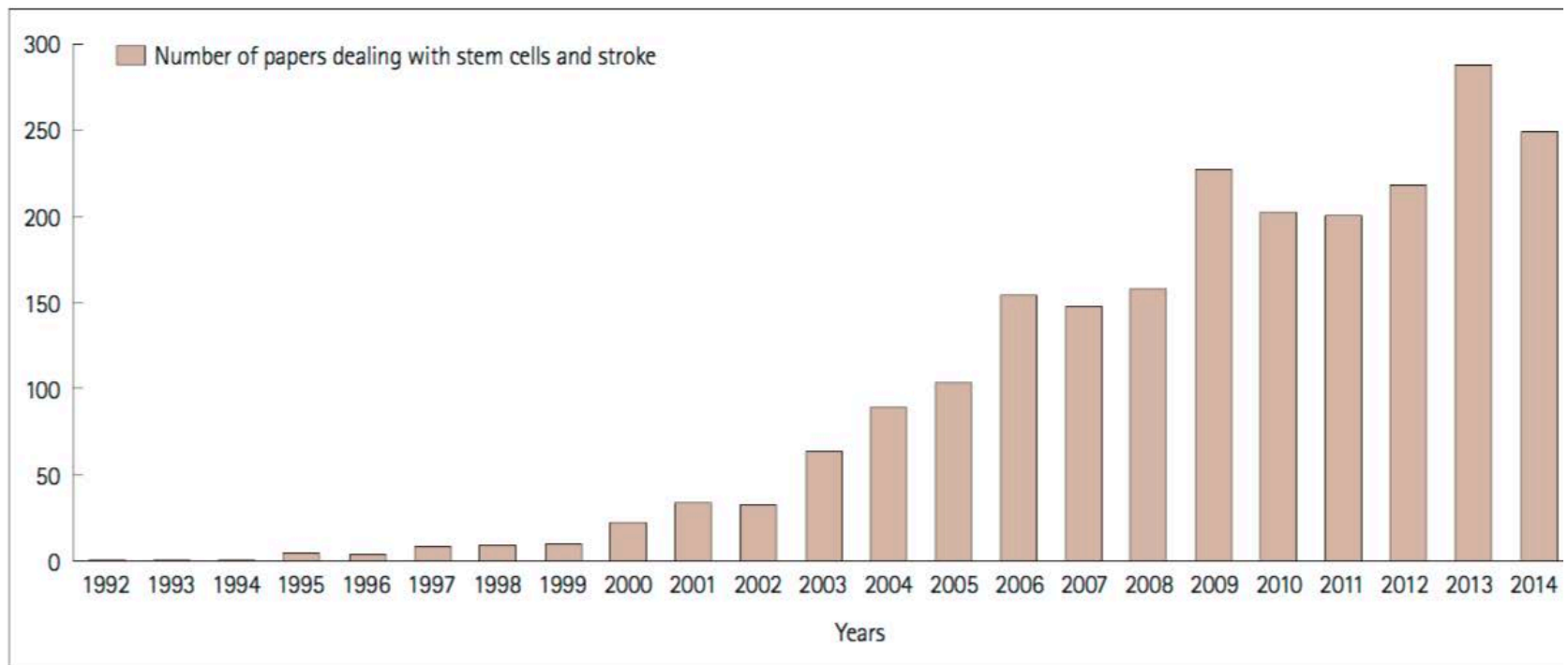
Thomas Haider, M.D.

CHRISTIAN DOPPLER LABORATORY  
for the Diagnosis & Regeneration of Cardiac and Thoracic Diseases  
Medical University of Vienna  
[www.meduniwien.ac.at/applied-immunology](http://www.meduniwien.ac.at/applied-immunology)

Vienna, March 29, 2016

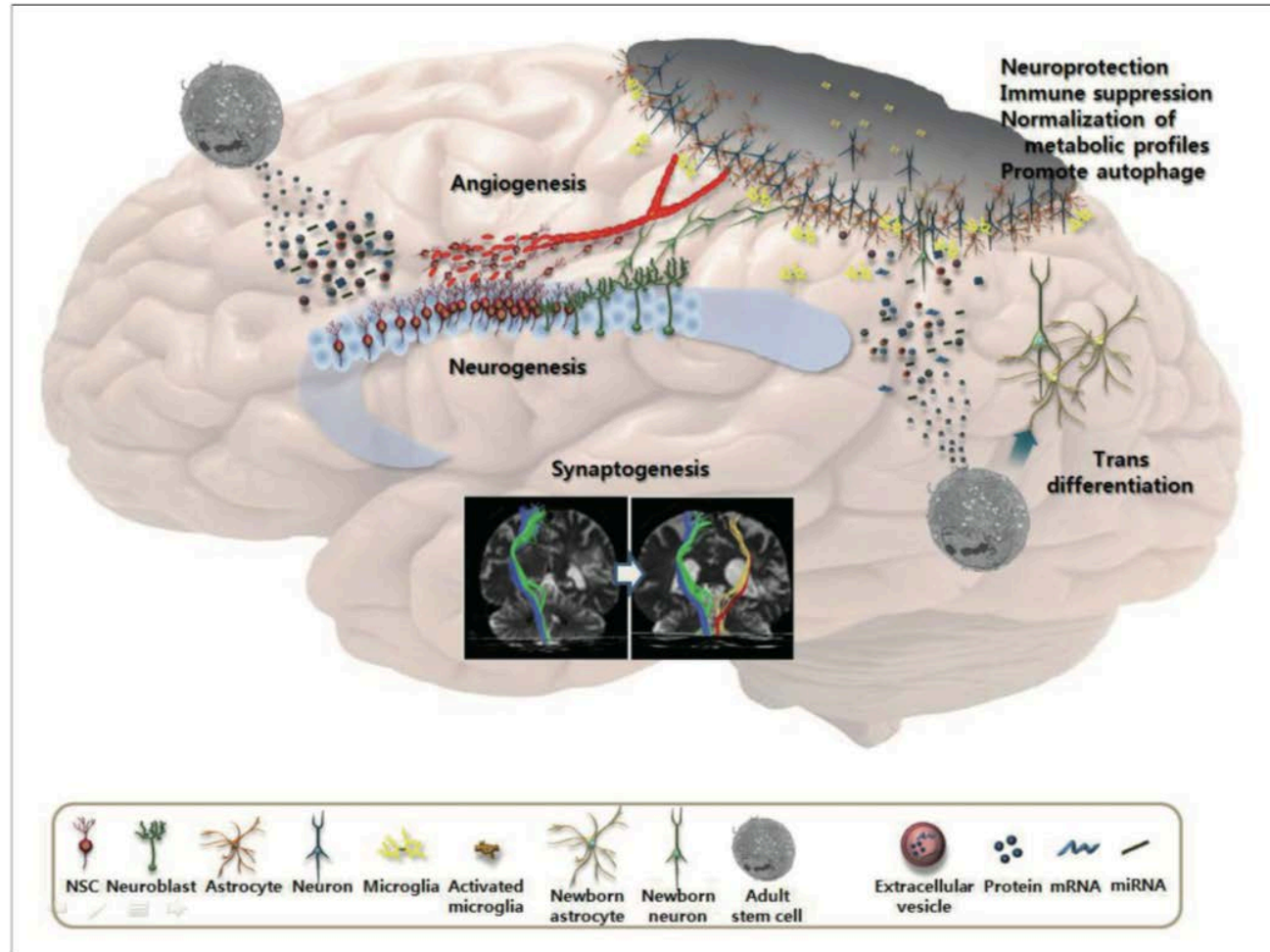
# Introduction

## Stem Cell Therapy



J Clin Neurol 2016;12(1):14-20

# Introduction



J Clin Neurol 2016;12(1):14-20

# Introduction

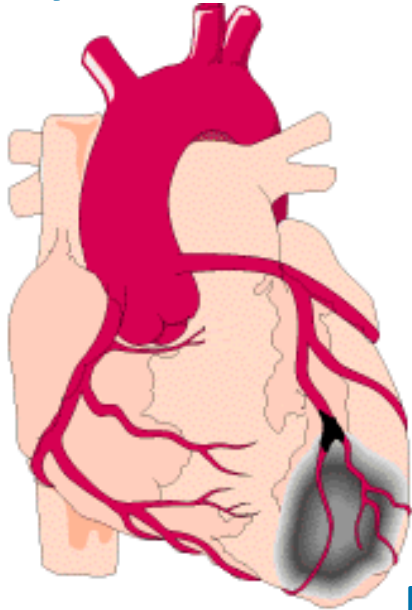
**Table 1** | Randomized trials in patients with acute myocardial infarction or ischemic heart failure

Trial name	Number of patients	Cell type	Dose	Route of delivery	Timing of delivery	Primary end point	Comments
<i>Acute myocardial infarction</i>							
BOOST	60	nBMC	128 ml	i.c.	Day 6 ± 1	LVEF ↑	Effect diminished after 18 and 61 months
REPAIR-AMI	187	mnBMC	50 ml	i.c.	Day 3–6	LVEF ↑	NA
Leuven-AMI	66	mnBMC	130 ml	i.c.	Day 1	LVEF ↔	Regional contractility ↑ Infarct size ↓
ASTAMI	97	mnBMC	50 ml	i.c.	Day 6 ± 1	LVEF ↔	NA
FINCELL	77	mnBMC	80 ml	i.c.	Day 3	LVEF ↑	NA
REGENT	117	mnBMC (unselected vs CD34 <sup>+</sup> /CXCR4 <sup>+</sup> )	50–70 ml (unselected) 100–120 ml (selected)	i.c.	Day 3–12	LVEF ↑ with both cell types	NA
HEBE	189	mnBMC vs mnPBC	60 ml (mnBMC) 150 ml (mnPBC)	i.c.	Day 3–8	Regional contractility ↔	NA
<i>Ischemic heart failure</i>							
MAGIC	97	SkM	400 or 800 × 10 <sup>6</sup>	i.m.	>Week 4	LVEF ↔	LVEDV ↓ LVESV ↓
TOPCARE-CHD	58	mnBMC vs CPC	50 ml	i.c.	Month 81 ± 72	LVEF ↑ (mnBMC) LVEF ↔ (CPC)	NA

Nat. Rev. Cardiol. 2010; 7: 204-215.

# Introduction

## Myocardial Infarction



**Necrosis**

Attraction of immune cells

Secretion of pro-inflammatory cytokines

IL-1 IL-6 TNF- $\alpha$

Amplification of inflammation

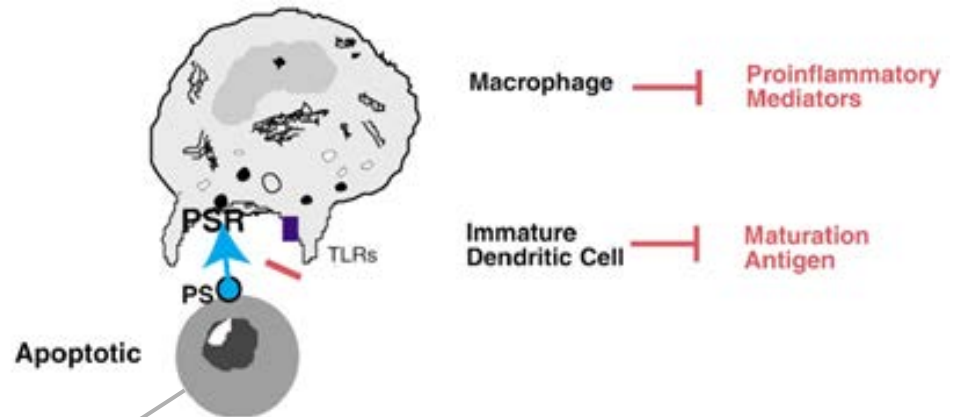
*Inhibition of pro-inflammatory Signals*

## The Dying Stem Cell Hypothesis

by Anker *et al.*

up to 25% of all transplanted cells are in the state of apoptosis

apoptotic cells induce transient immunosuppression



J Am Coll Cardiol. 2005 Nov 15;46(10):1799-802.

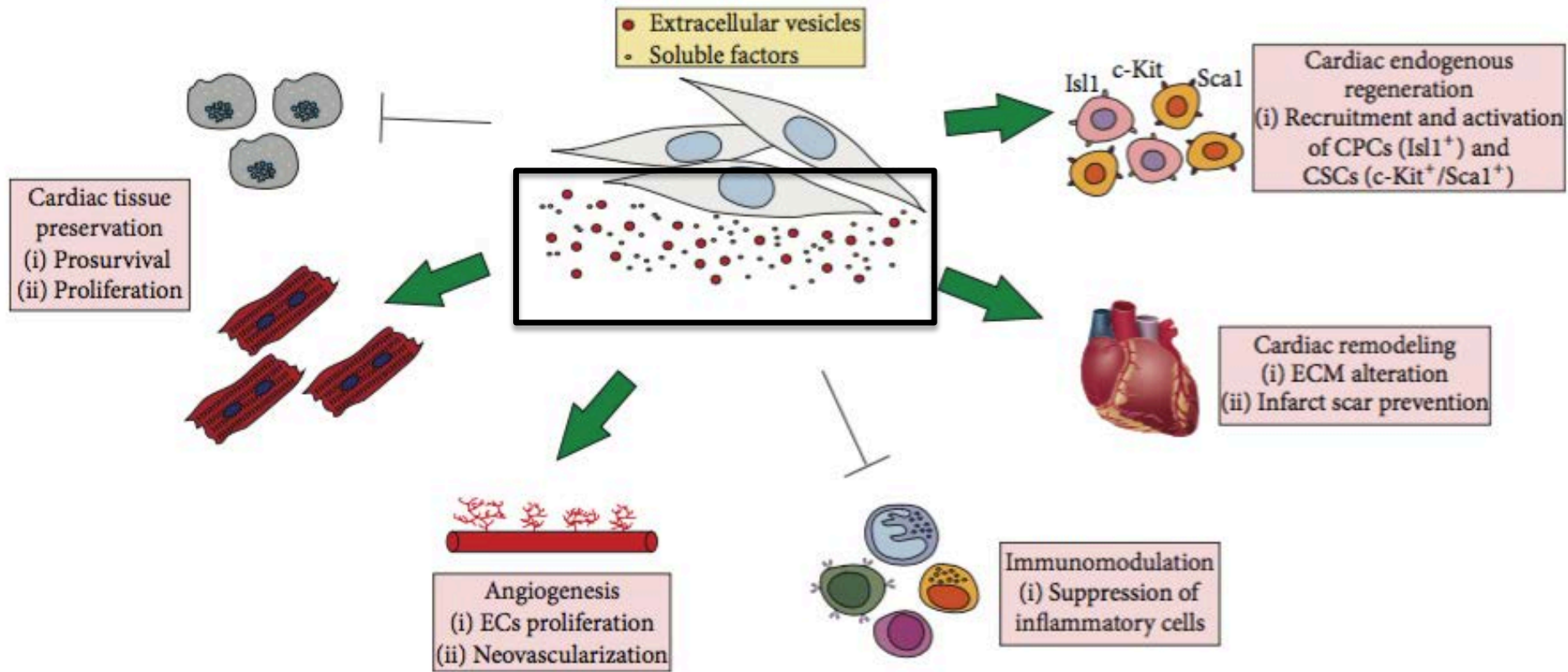
J Clin Invest. 2001 Oct;108(7):957-62.

# Introduction

## Stem Cell Engraftment and Transdifferentiation?

- Low number of transplanted cells
- Permanent engraftment not observable
- Therapeutic effect in less than 72h
- Secretome (CM) alone delivers comparable results

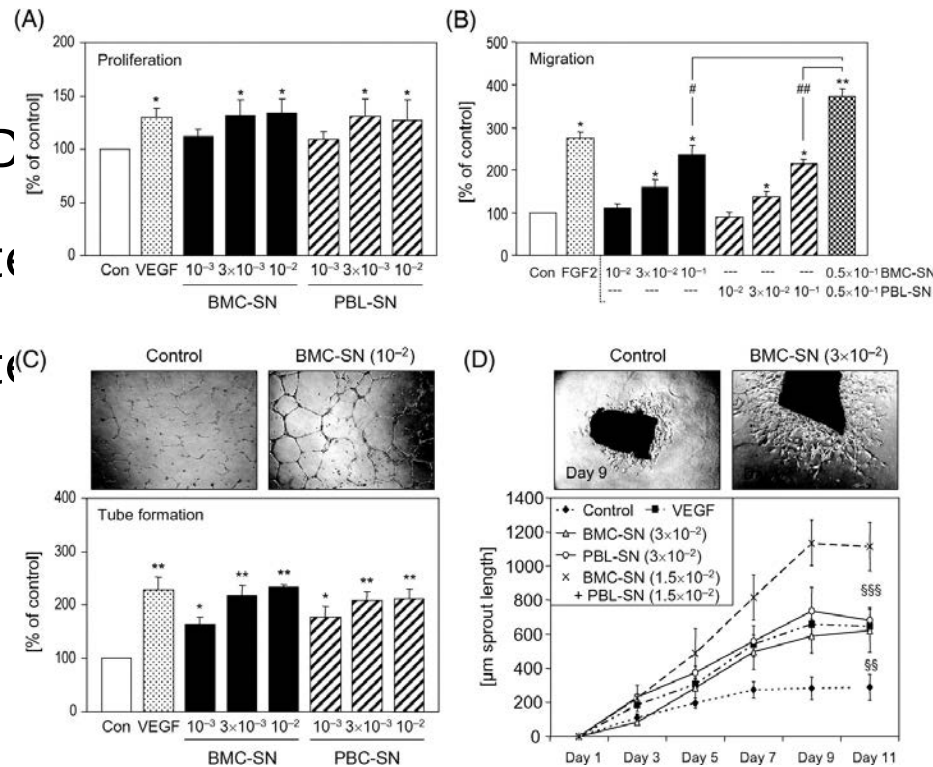
# Introduction



# Introduction

## Comparability of BMCs and PBMCs Secretome

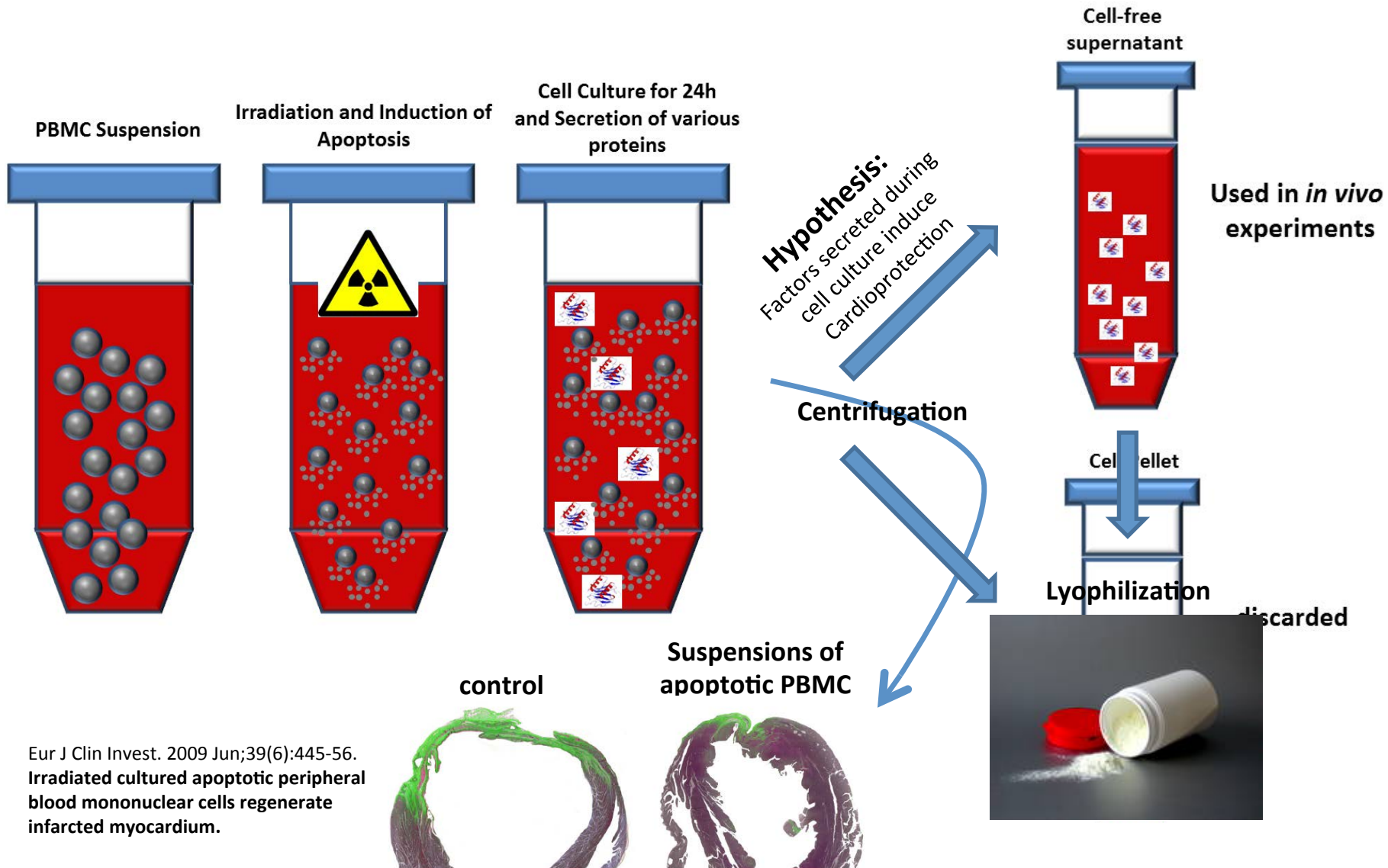
- Secretome: D
- 25 secretome factors
- 10 secretome factors



factors  
tome  
etome



# Introduction



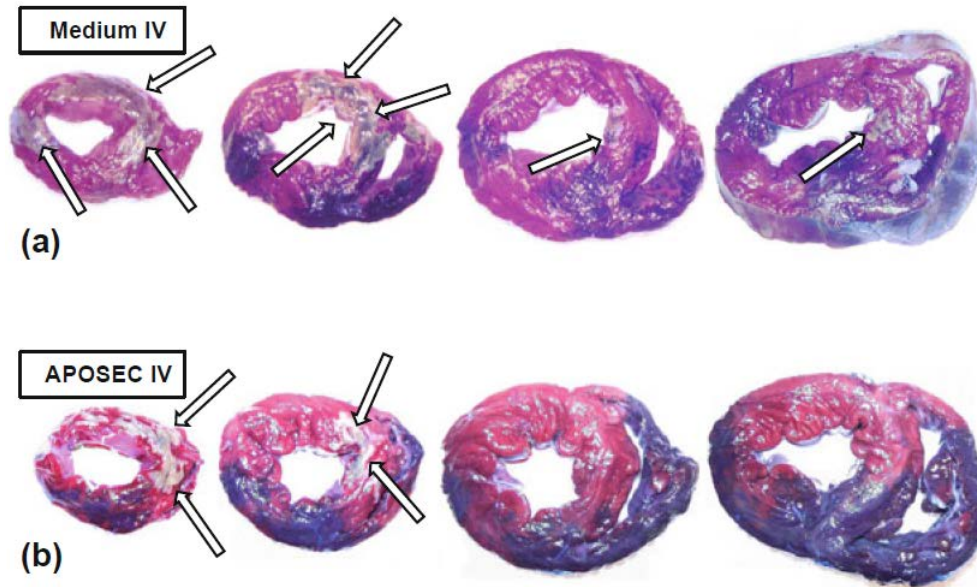
Eur J Clin Invest. 2009 Jun;39(6):445-56.  
Irradiated cultured apoptotic peripheral blood mononuclear cells regenerate infarcted myocardium.

# Introduction

- Attenuation of acute myocardial infarction

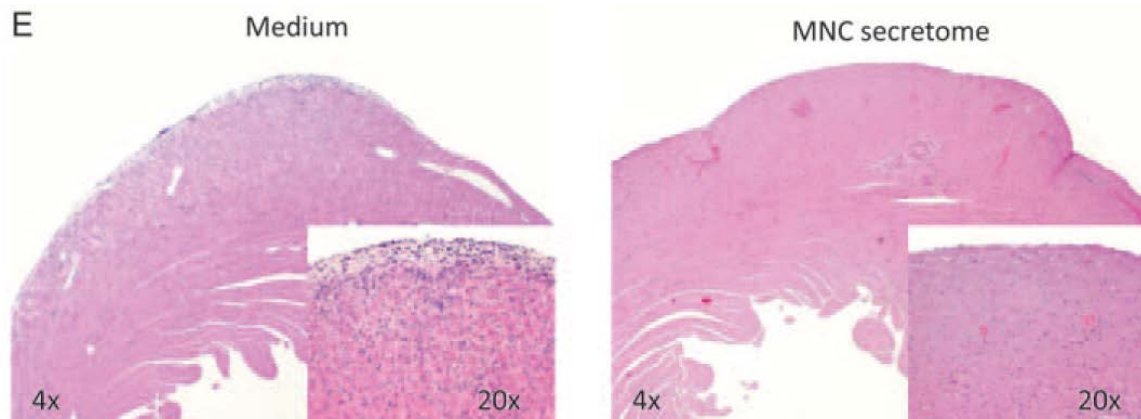
(Lichtenauer et al; Basic Res Cardiol. 2011)

Macroscopic analysis after 24 hours



# Introduction

- Attenuation of acute myocardial infarction  
(Lichtenauer et al; Basic Res Cardiol. 2011)
- Immunosuppression in an experimental myocarditis model  
(Hoetzencker et al; Eur Heart J. 2013)

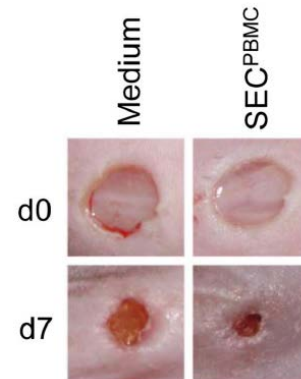
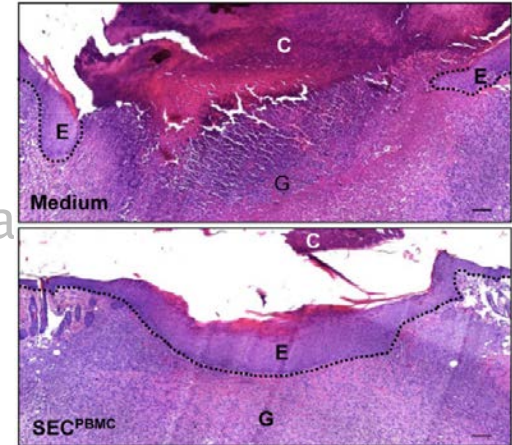


# Introduction

- Attenuation of acute myocardial infarction  
(Lichtenauer et al; Basic Res Cardiol. 2011)
- Immunosuppression in an experimental myocarditis model  
(Hoetzencker et al; Eur Heart J. 2013)
- **Inhibition of MVO and thrombocyte activation**  
(Hoetzencker et al; Bas Res Cardiol 2012)

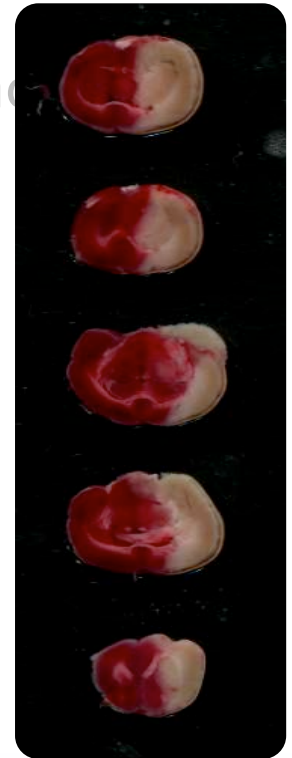
# Introduction

- Attenuation of acute myocardial infarction  
(Lichtenauer et al; Basic Res Cardiol. 2011)
- Immunosuppression in an experimental myocardial infarction  
(Hoetzencker et al; Eur Heart J. 2013)
- Inhibition of MVO and thrombocyte activation  
(Hoetzencker et al; Bas Res Cardiol 2012)
- **Wound healing in a murine and porcine wound model**  
(Mildner et al; PLoS One. 2013)

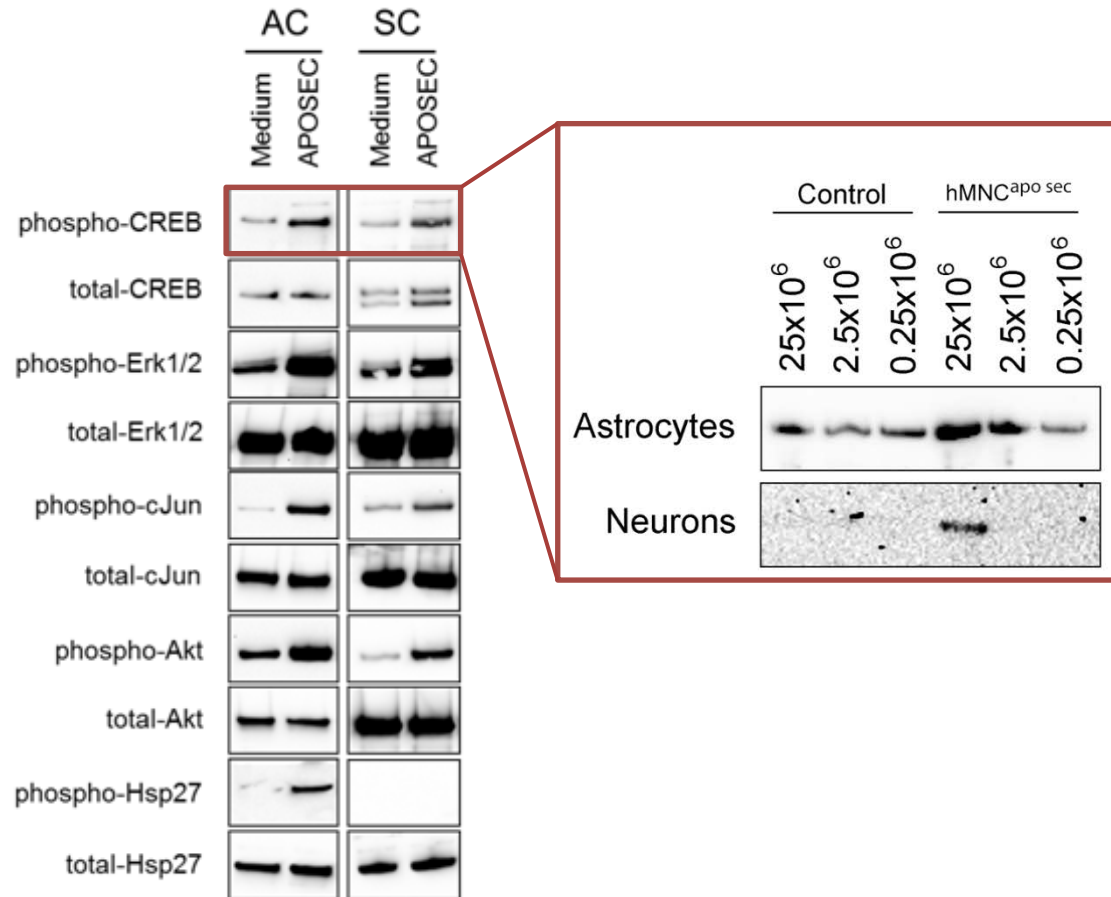


# Introduction

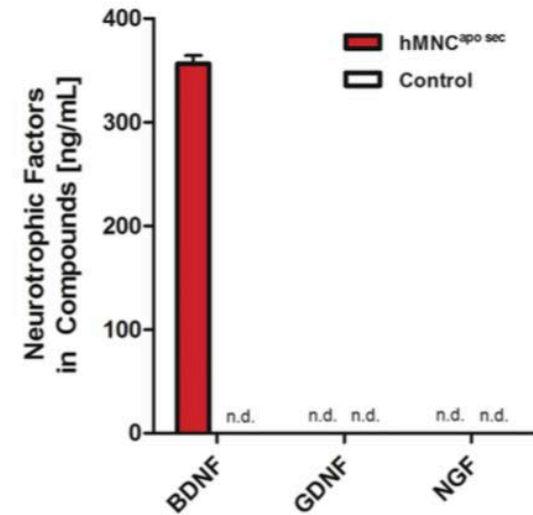
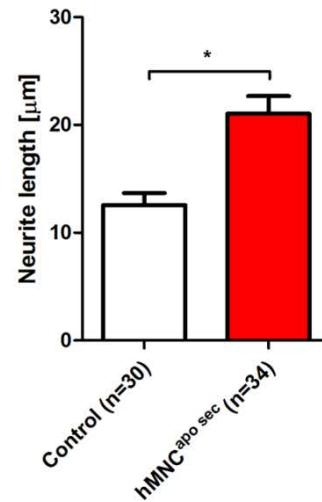
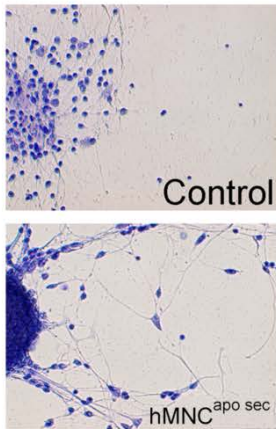
- Attenuation of acute myocardial infarction  
(Lichtenauer et al; Basic Res Cardiol. 2011)
- Immunosuppression in an experimental myocarditis model  
(Hoetzencker et al; Eur Heart J. 2013)
- Inhibition of MVO and thrombocyte activation  
(Hoetzencker et al; Bas Res Cardiol 2012)
- Wound healing in a murine and porcine wound model  
(Mildner et al; PLoS One. 2013)
- **Experimental stroke model**  
(Altmann et al; F1000Res. 2014 Jun 19 [revised 2014 Oct 28])



# Introduction



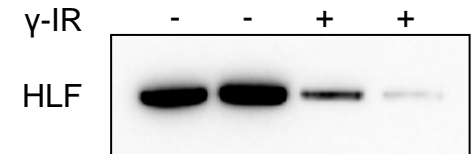
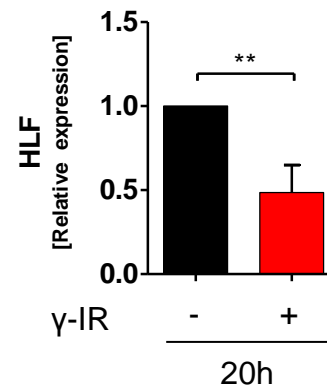
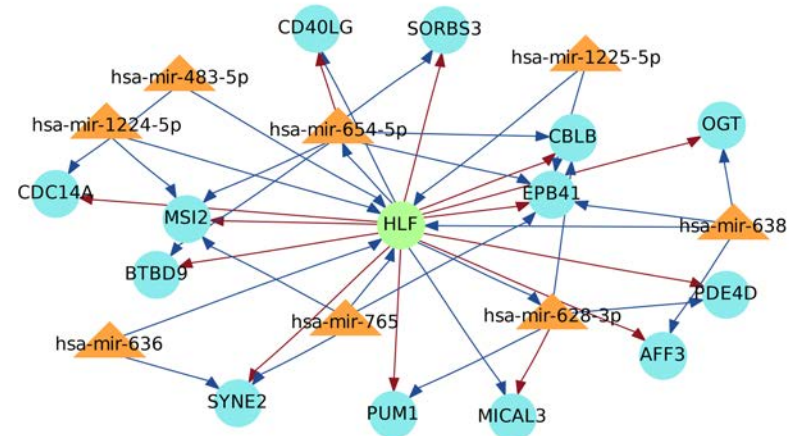
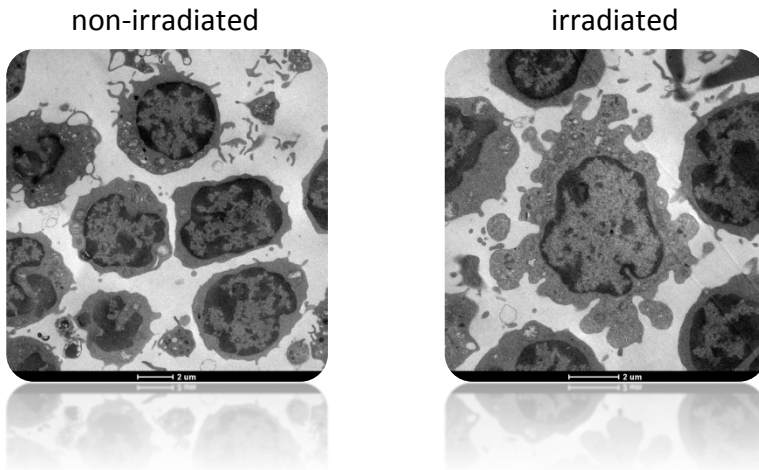
# Introduction





# Introduction

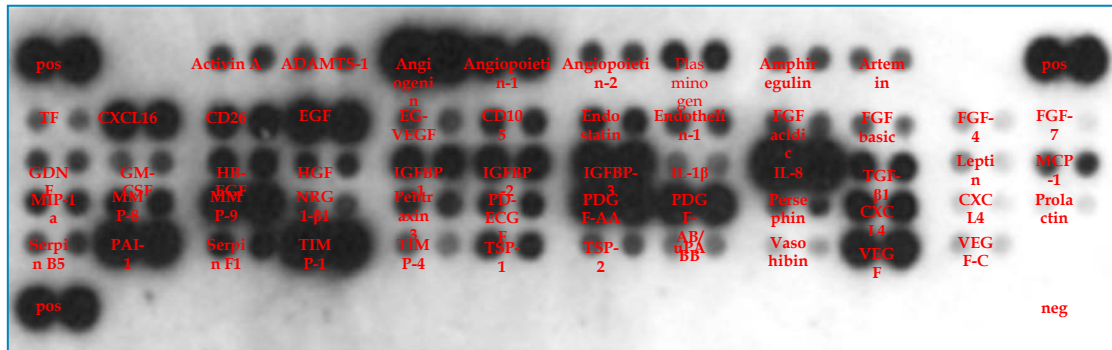
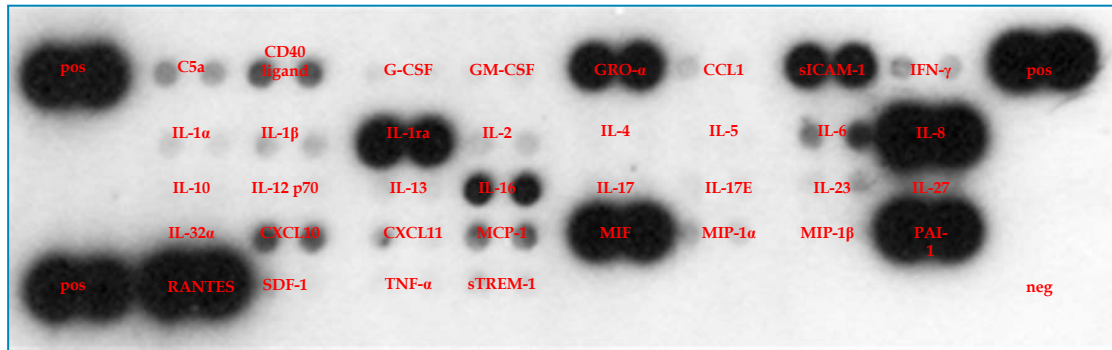
## Induction of Apoptosis



Beer et al. BMC Genomics 2014

# Introduction

## Compounds of the Secretome



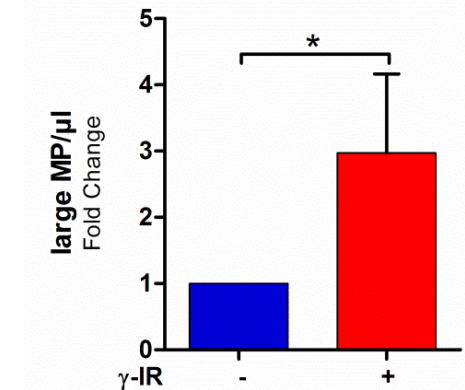
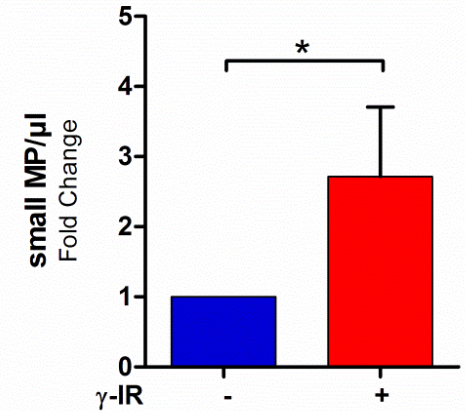
	2,5*10 <sup>6</sup>
IL-8 (pg/ml)	2305,8 ±136,4
GRO-alpha (pg/ml)	487,7 ±89,5
ENA-78 (pg/ml)	37857,5 ±12734,2
MCP-1 (pg/ml)	739,9 ±175,5
NAP-2 (µg/ml)	9,9 ±0,5
RANTES (pg/ml)	22251,2 ±3641,9
sICAM-1 (pg/ml)	2068,2 ±415,2
VEGF <sub>165</sub> (pg/ml)	640,1 ±35,2
IL-16 (pg/ml)	1254,2 ±77,6
IL-1ra (pg/ml)	410,7 ±167,0
IL-10 (pg/ml)	7,1 ±0,5
IGF-I (pg/ml)	5,8 ±3,2
HGF (pg/ml)	72,9 ±19,1
FGF-2 (pg/ml)	534,2 ±11,6
TGF-beta (pg/ml)	87,3 ±20,4
MMP9 (pg/ml)	3612,3 ±597,7
MIF (pg/ml)	20147,5 ±1140,2
PAI-1 (pg/ml)	5060,6 ±3247,5
SDF-1 (pg/ml)	148,5 ±7,1

Lichtenauer et. Al Basic Res Cardiol. 2011 Nov;106(6):1283-97.

# Introduction

## Compounds of the Secretome

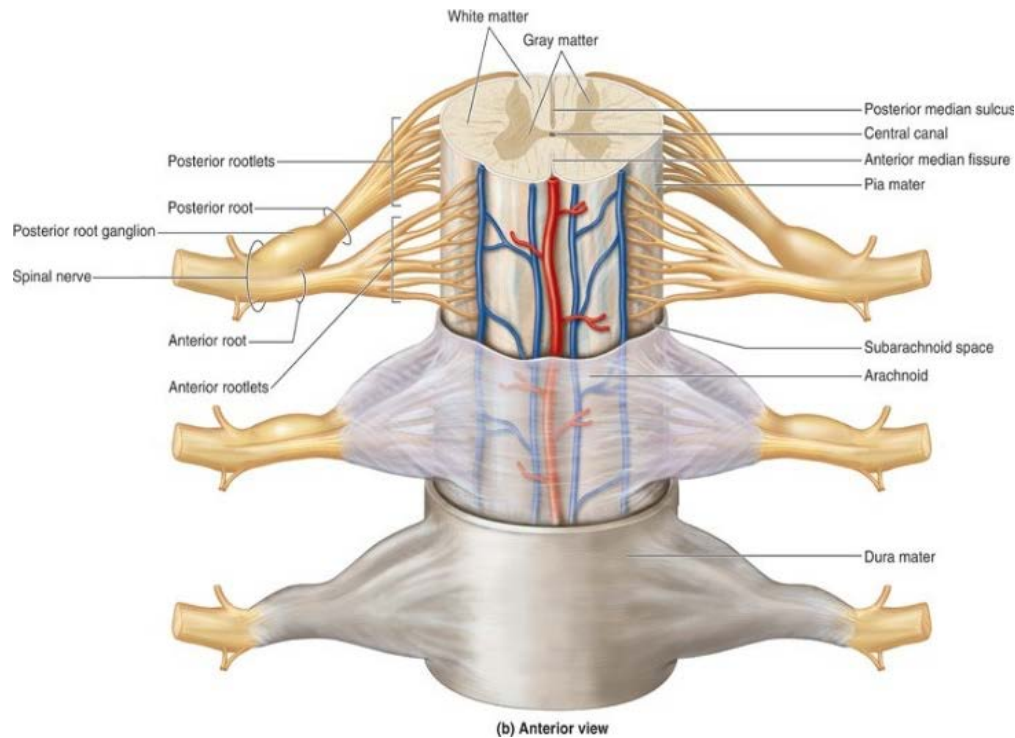
- Secreted Proteins
- Oxidized Lipids
- Extracellular vesicles
  - Microparticles
  - Exosomes



Beer et al. BMC Genomics 2014

# Introduction

## Spinal Cord Injury



# Introduction

## Spinal Cord Injury



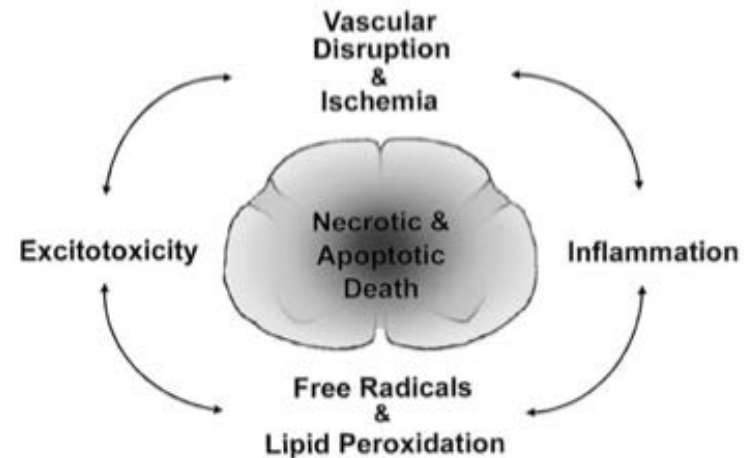
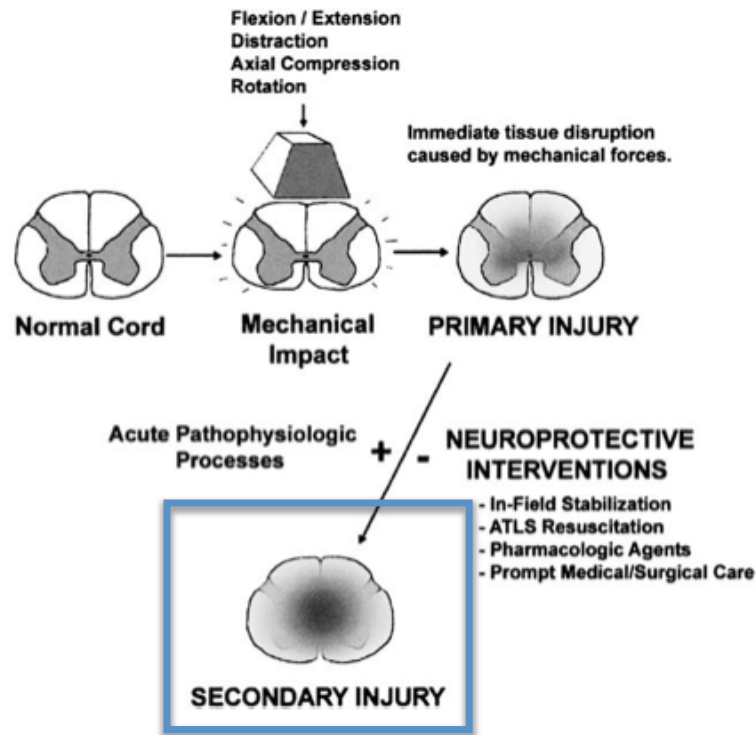
- 50 per 1 million annually worldwide (200/year in AUT)
- Average age of patients : 31 years
- 50% experience total loss of motor function
- 2 out of 3 – cervical spinal cord affected



J Neurosurg. 1991; 75(1): 15-26.  
J Neurotrauma. 2004; 21(10): 1355-70.

# Introduction

## Spinal Cord Injury



The Spine Journal 2004; 4(4): 451-64.

# Introduction

## Spinal Cord Injury – Treatment

- Early surgical decompression
- Corticosteroids (?) – Methylprednisolone (MP) regime
- Rehabilitation

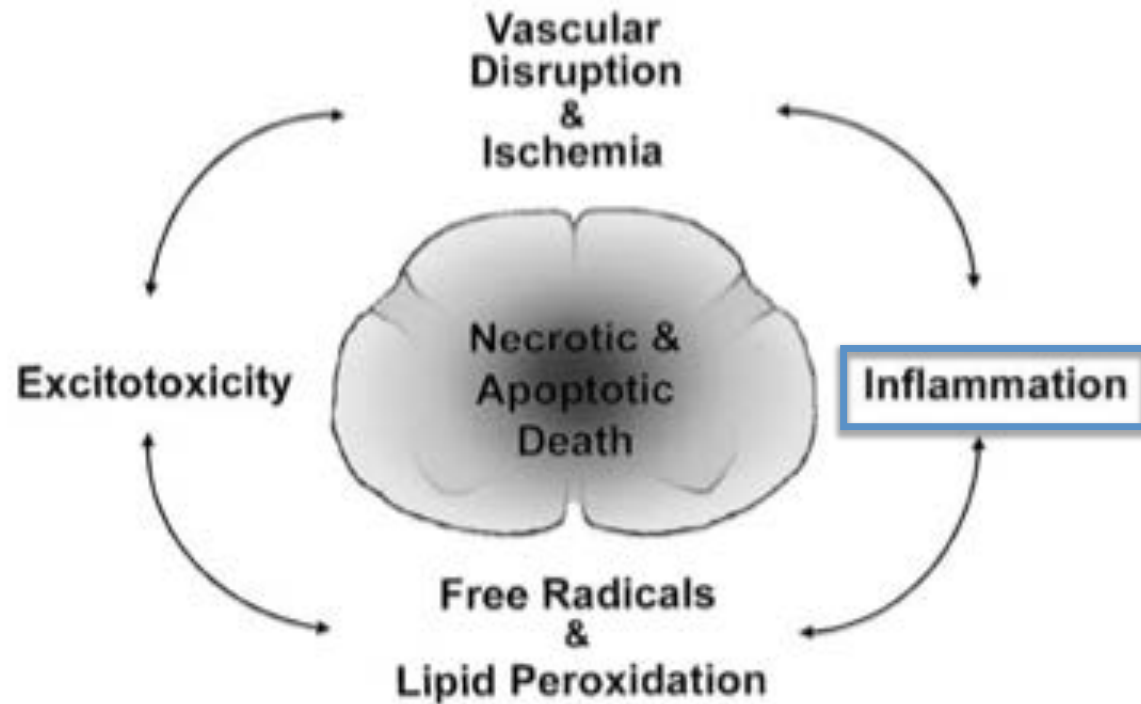
# Introduction

## Spinal Cord Injury – Treatment

- 30 years of SCI research -> 1 approved treatment option (MP) aside from surgical therapy
- Recommendation for the use of MP in SCI was revoked in 2013 by the AANS

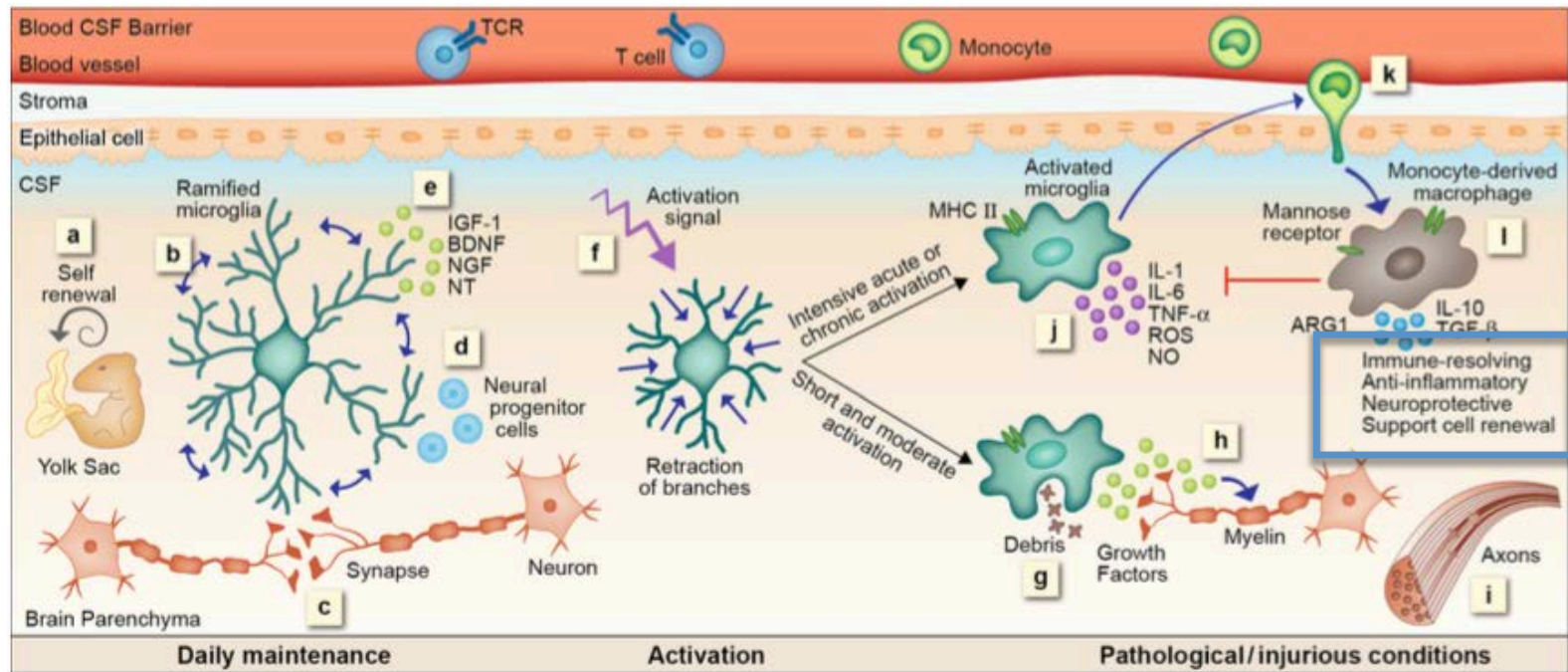


# Introduction



The Spine Journal 2004; 4(4): 451-64.

# Introduction



Front. Cell. Neurosci. 2013; 4.

# Hypothesis

**Compounds of secretome of apoptotic peripheral blood mononuclear cells exert therapeutic capacity**

# Methods

Contusion Injury Model

BBB-Score



J Neurotrauma. 2003; 20(2): 179-93.

J Neurotrauma. 1995; 12(1): 1-21.

# Methods

Venous Blood Withdrawal



Cell Separation



Irradiation



Incubation for 24h



Centrifugation



Lyophilization



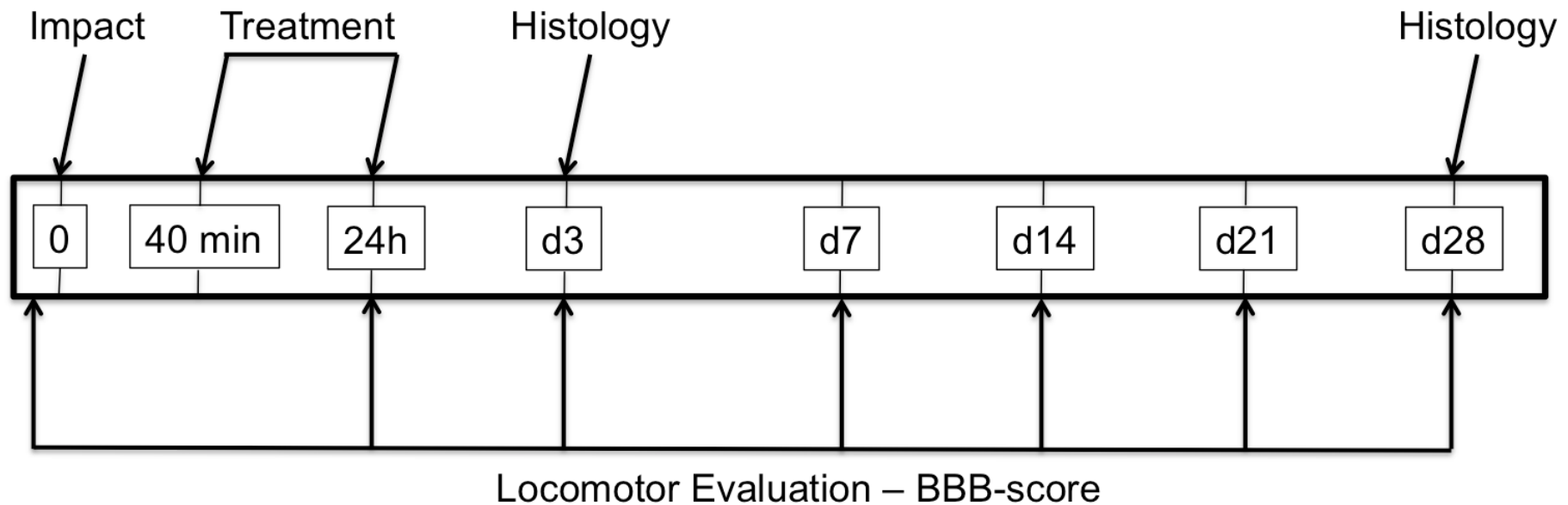
Lyophilized MNC-secretomes



Virus Elimination (GMP)

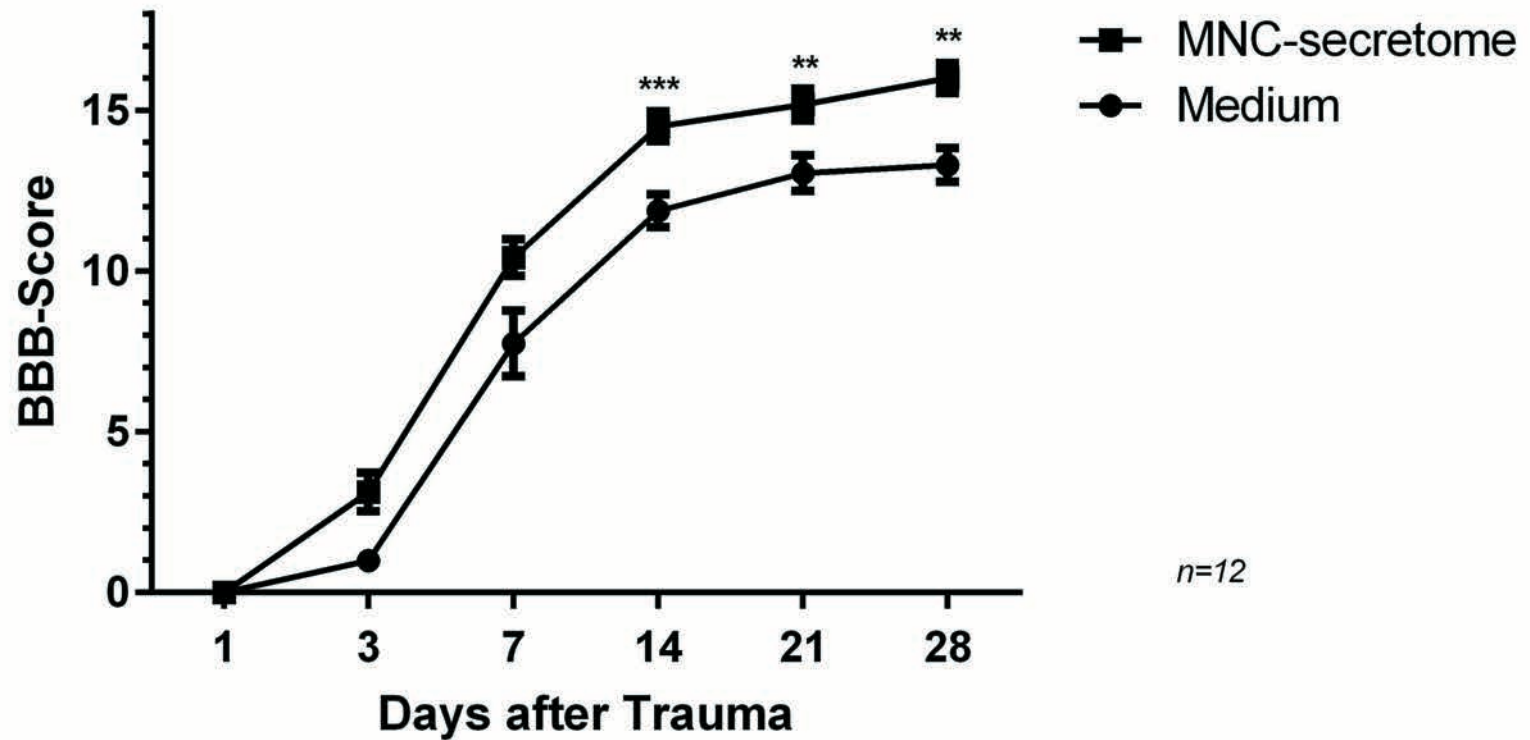


# Methods



# -RESULTS-

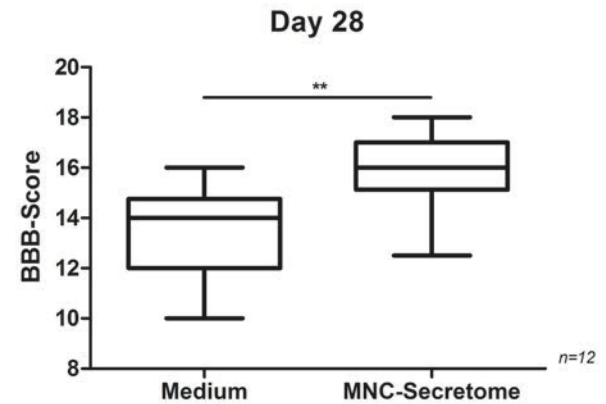
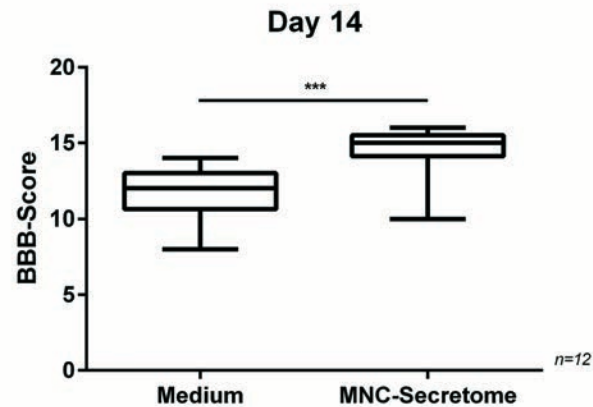
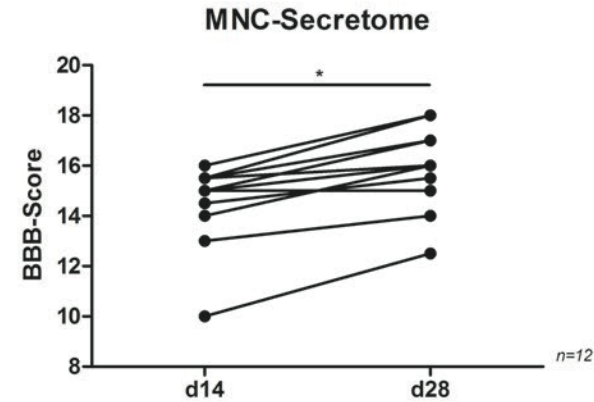
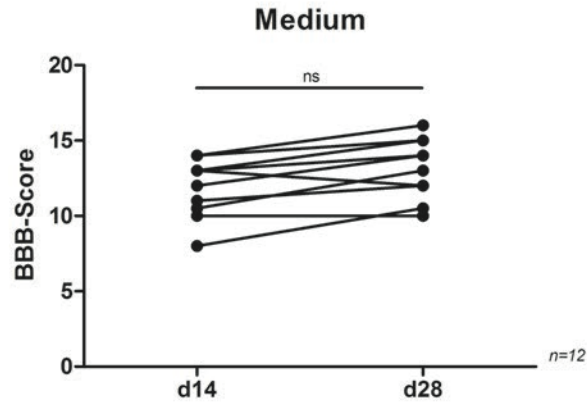
# Results



Exp Neurol. 2015; 267: 230-242.

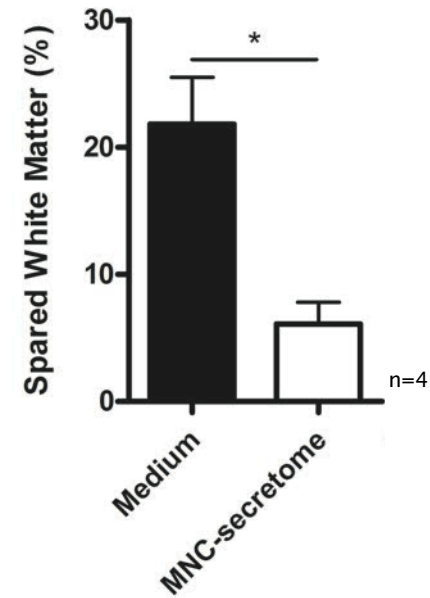
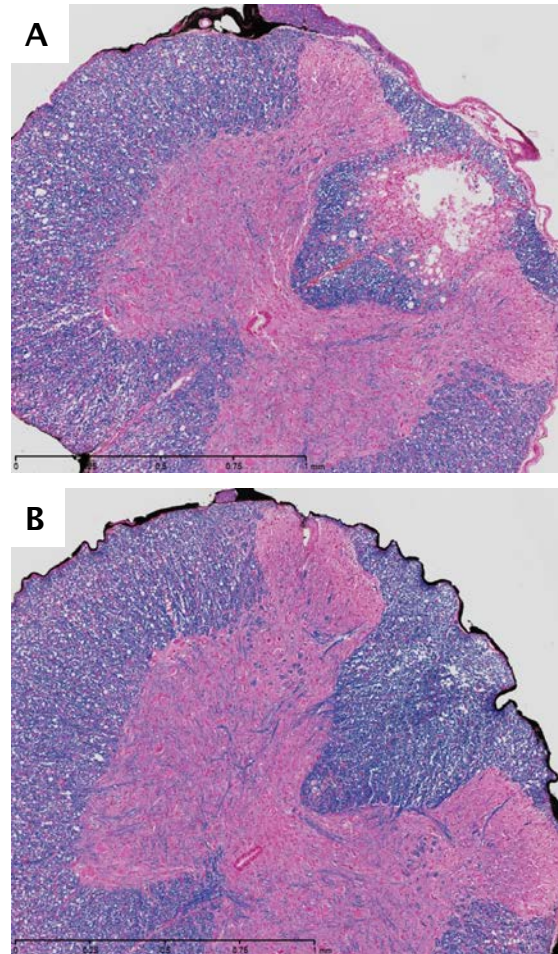


# Results



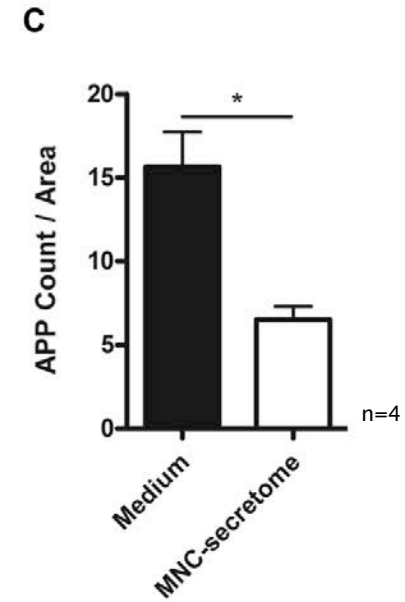
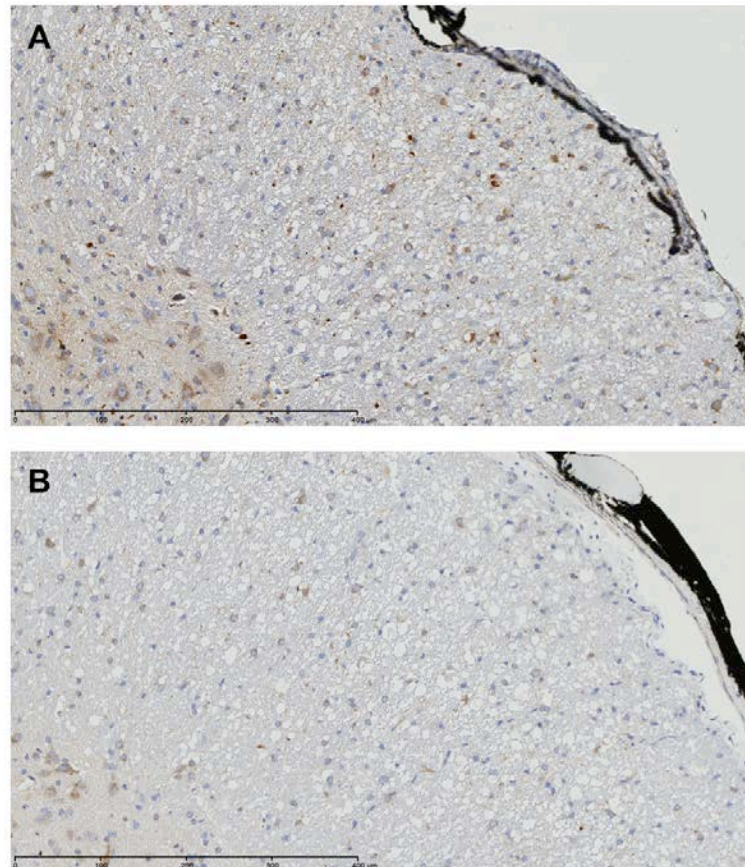
Exp Neurol. 2015; 267: 230-242.

# Results



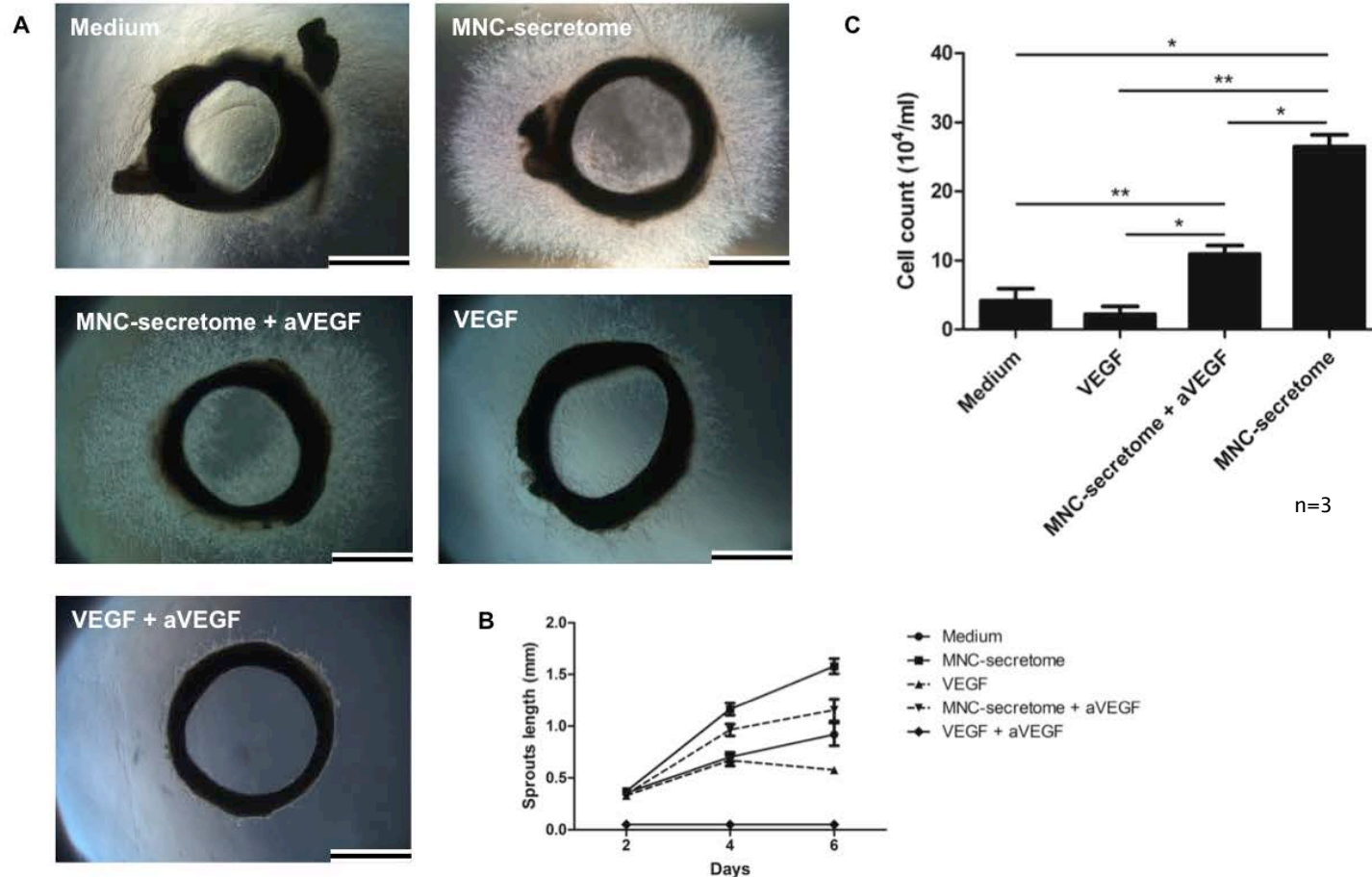
Exp Neurol. 2015; 267: 230-242.

# Results



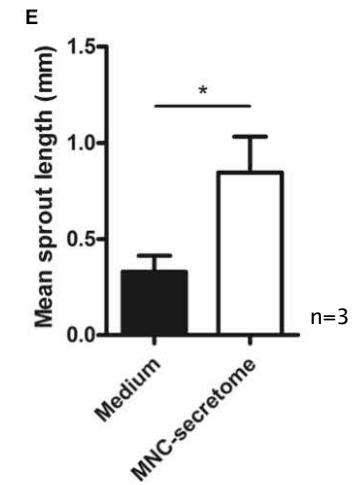
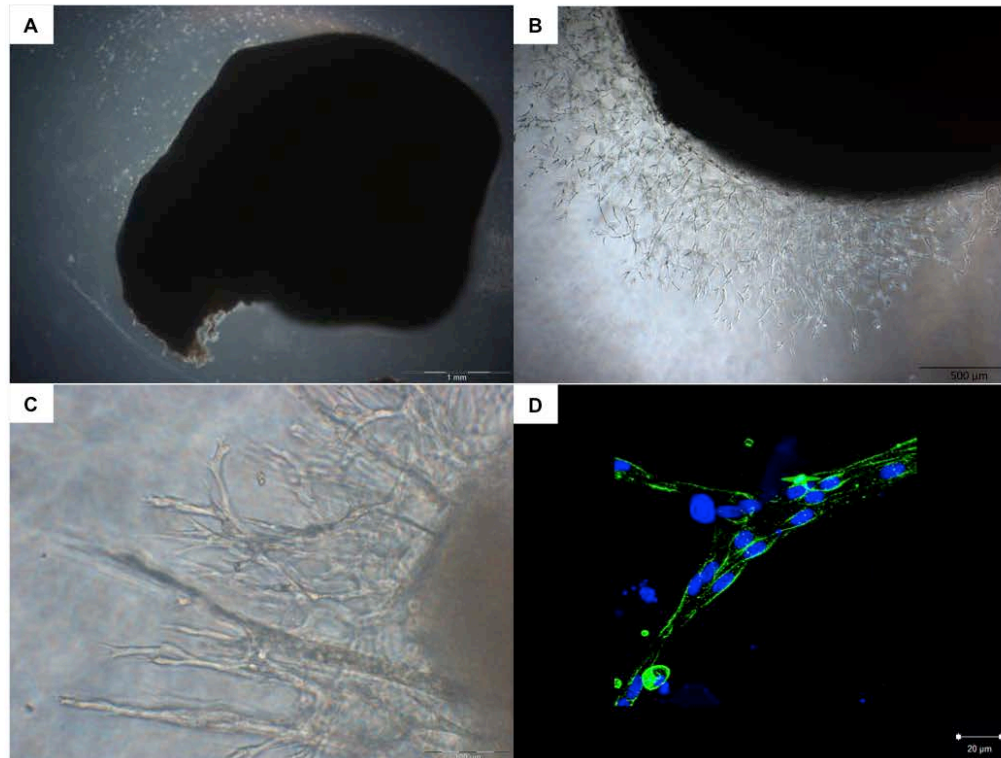
Exp Neurol. 2015; 267: 230-242.

# Results



Exp Neurol. 2015; 267: 230-242.

# Results

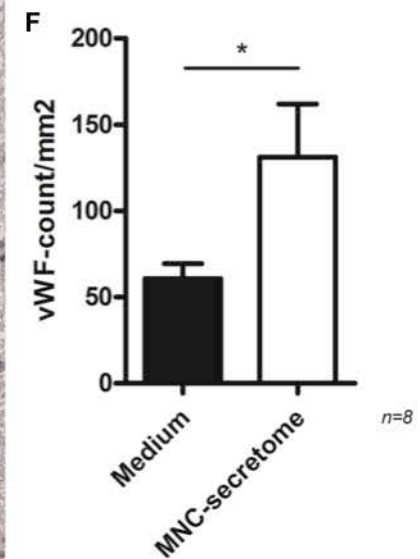
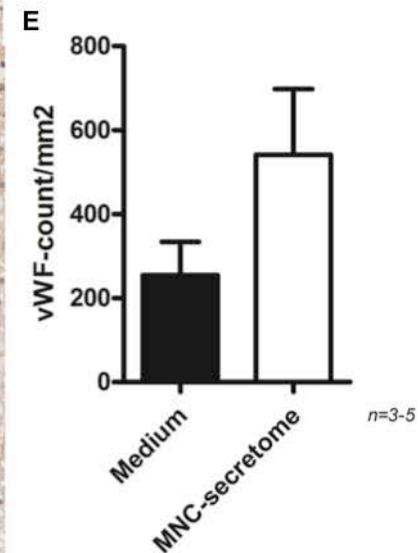
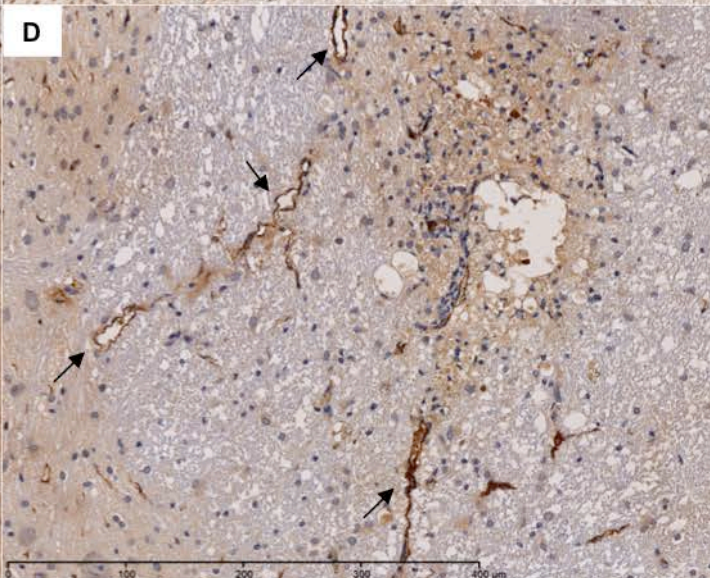
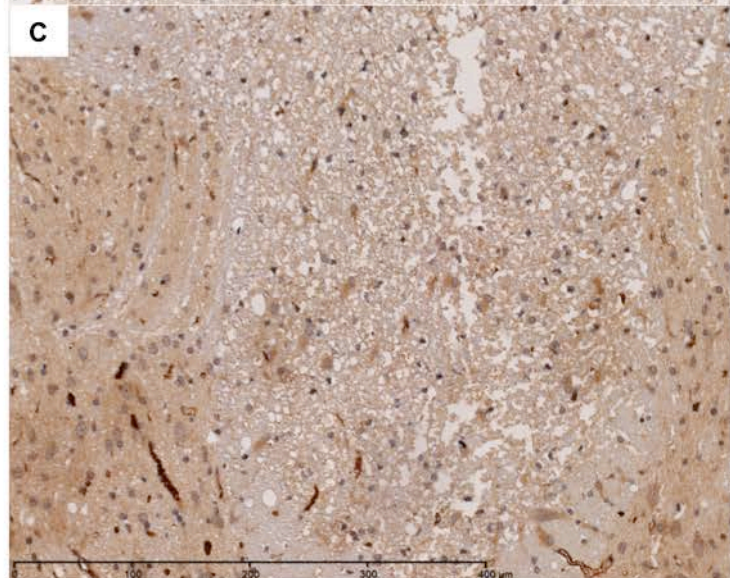
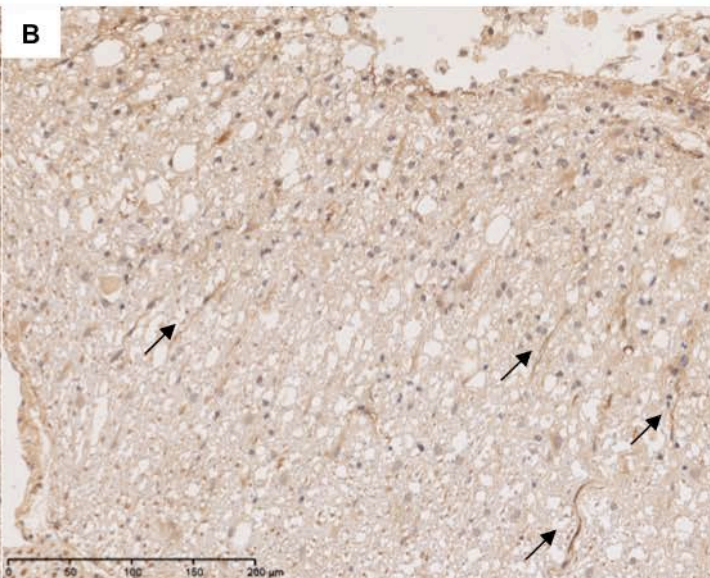
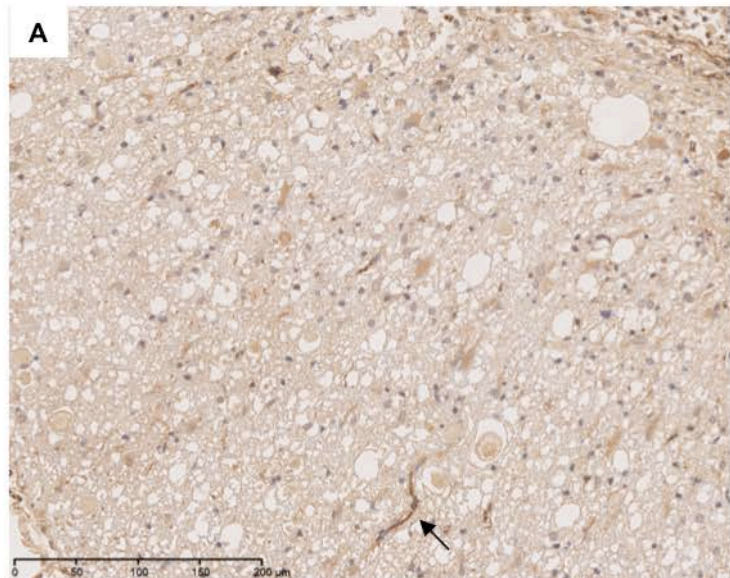


Exp Neurol. 2015; 267: 230-242.

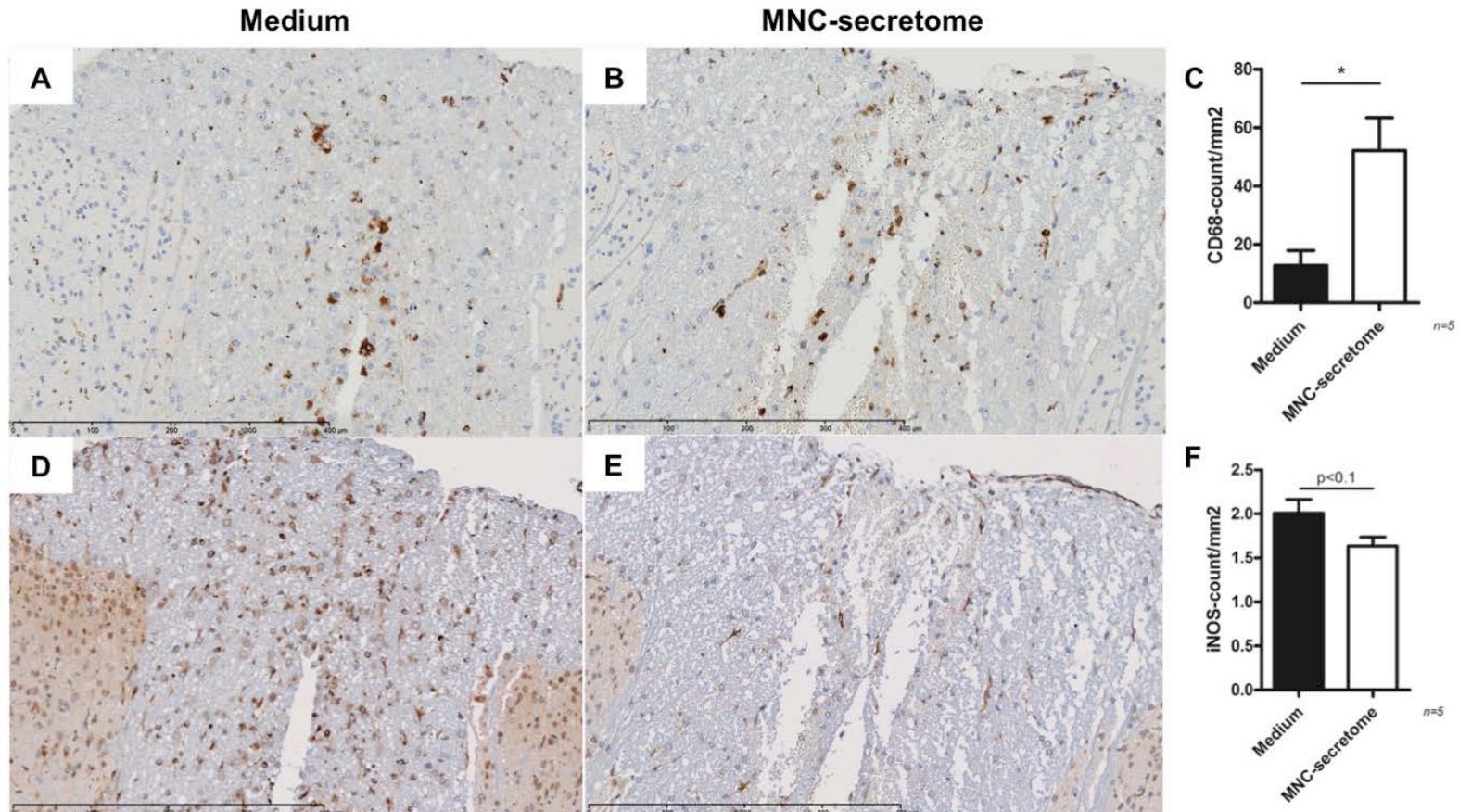
# Results

Medium

MNC-secretome

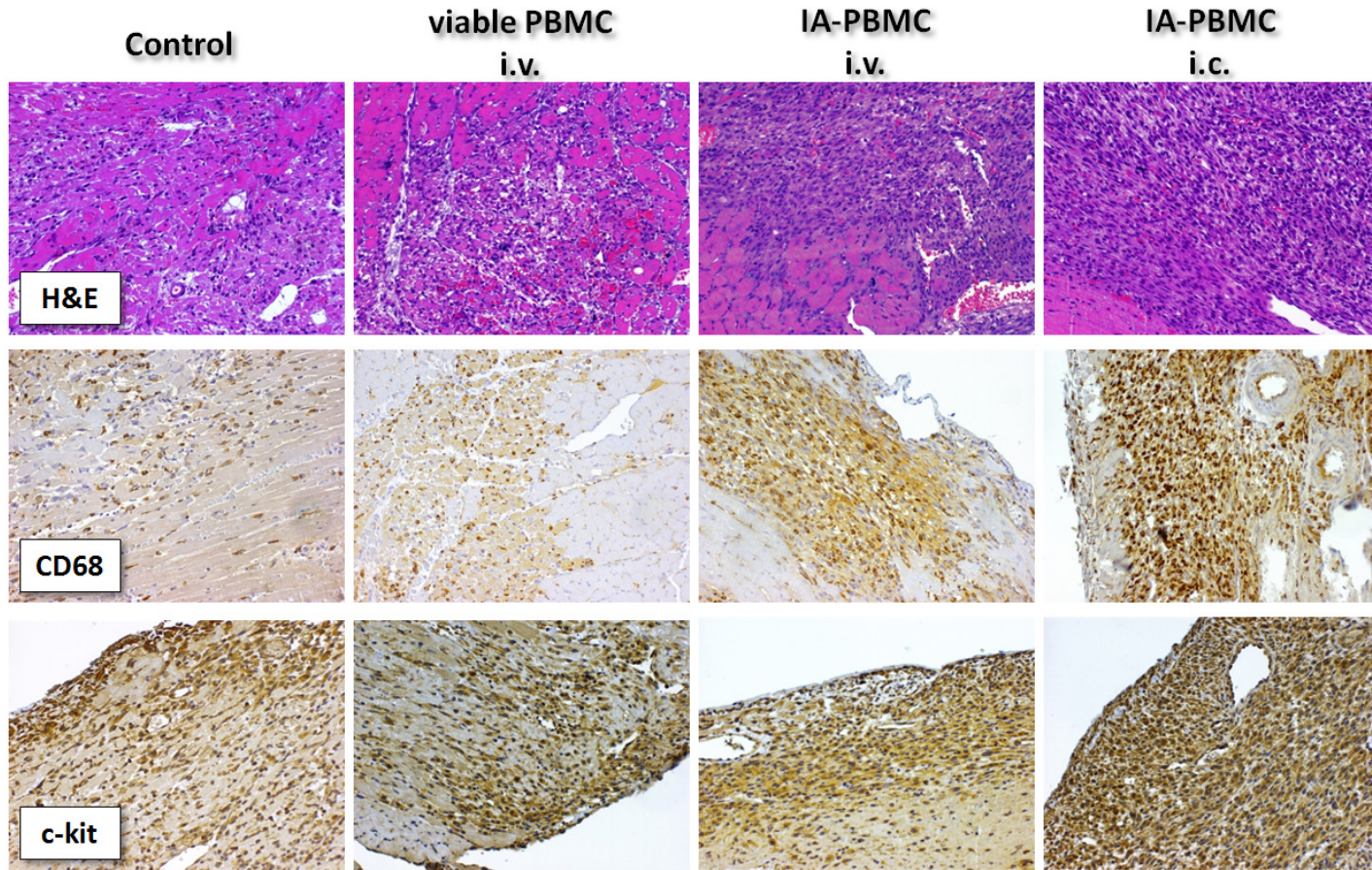


# Results



Exp Neurol. 2015; 267: 230-242.

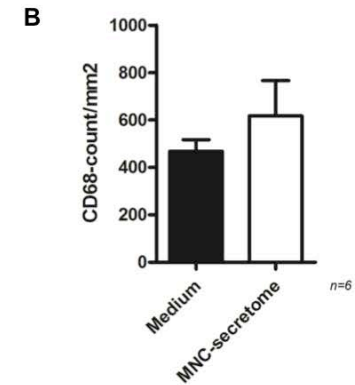
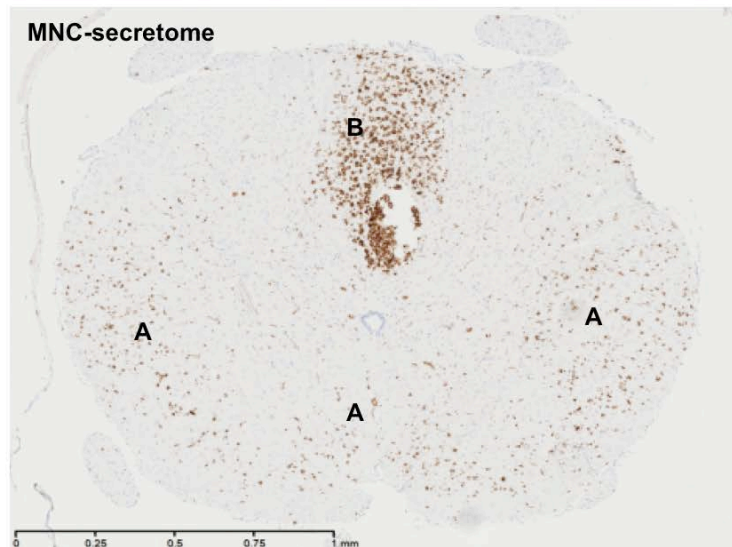
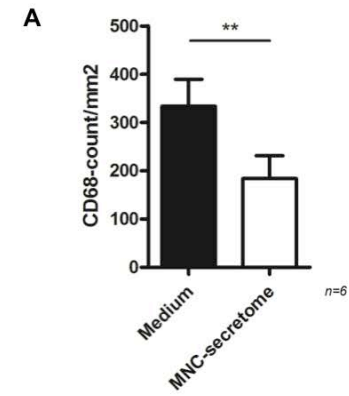
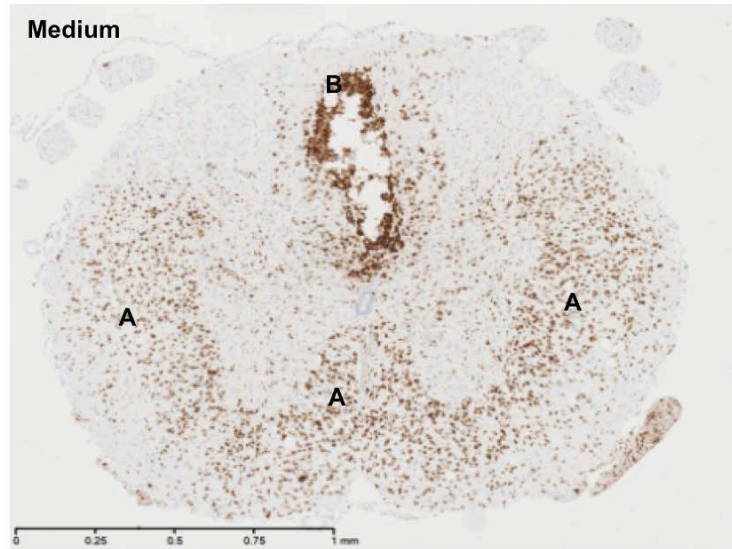
# Previous Results



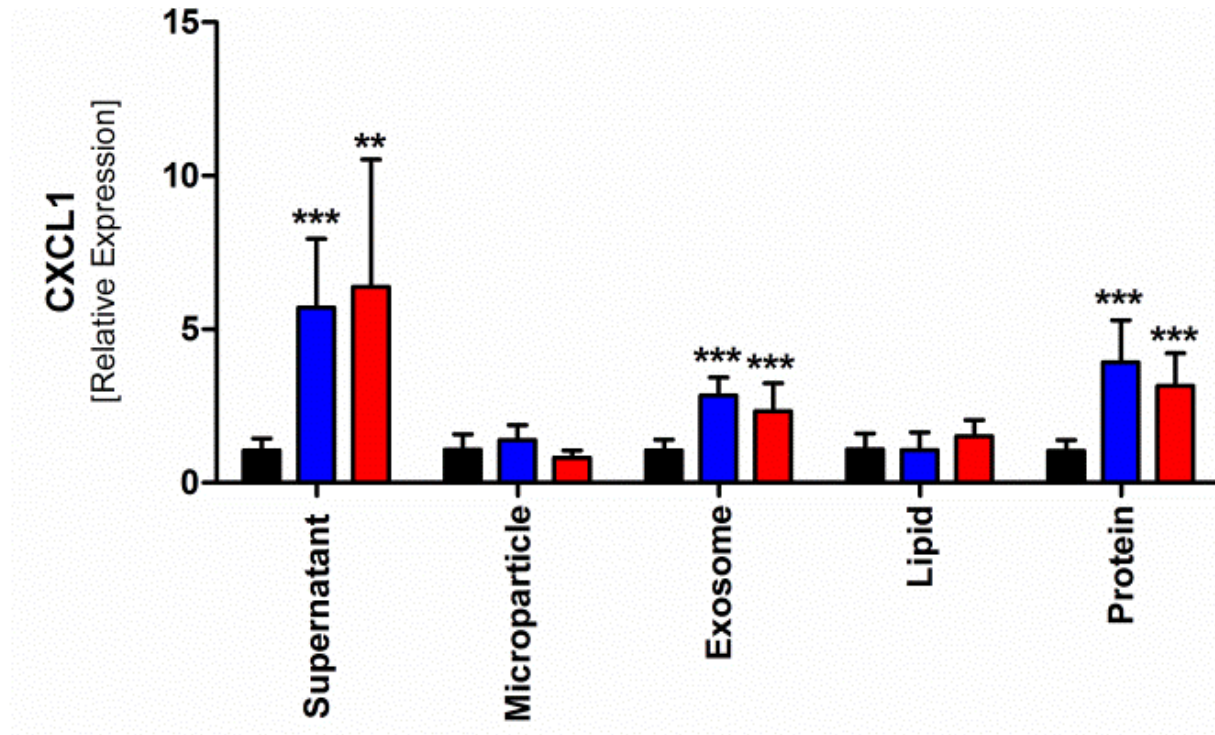
Basic Res Cardiol. 2011 Jun;106(4):645-55.



# Results

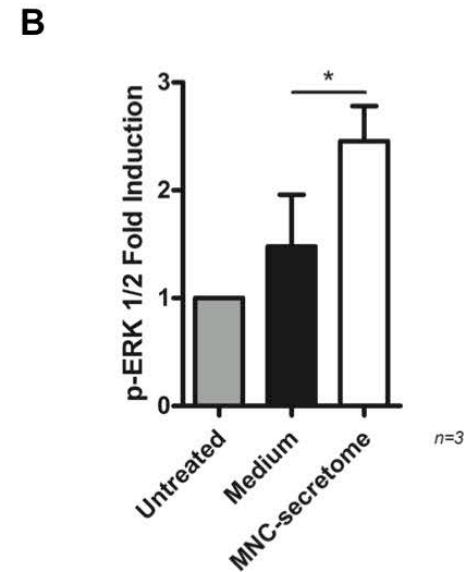
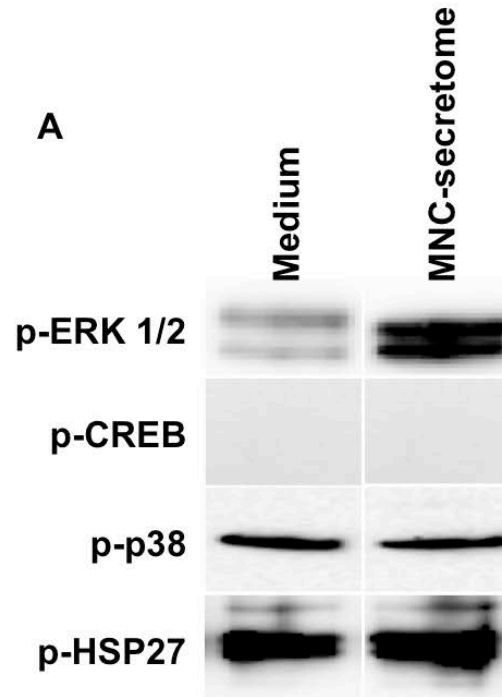


# Results



Exp. Nat. Biol. Med. 2014; 267: 2302-2312

# Results

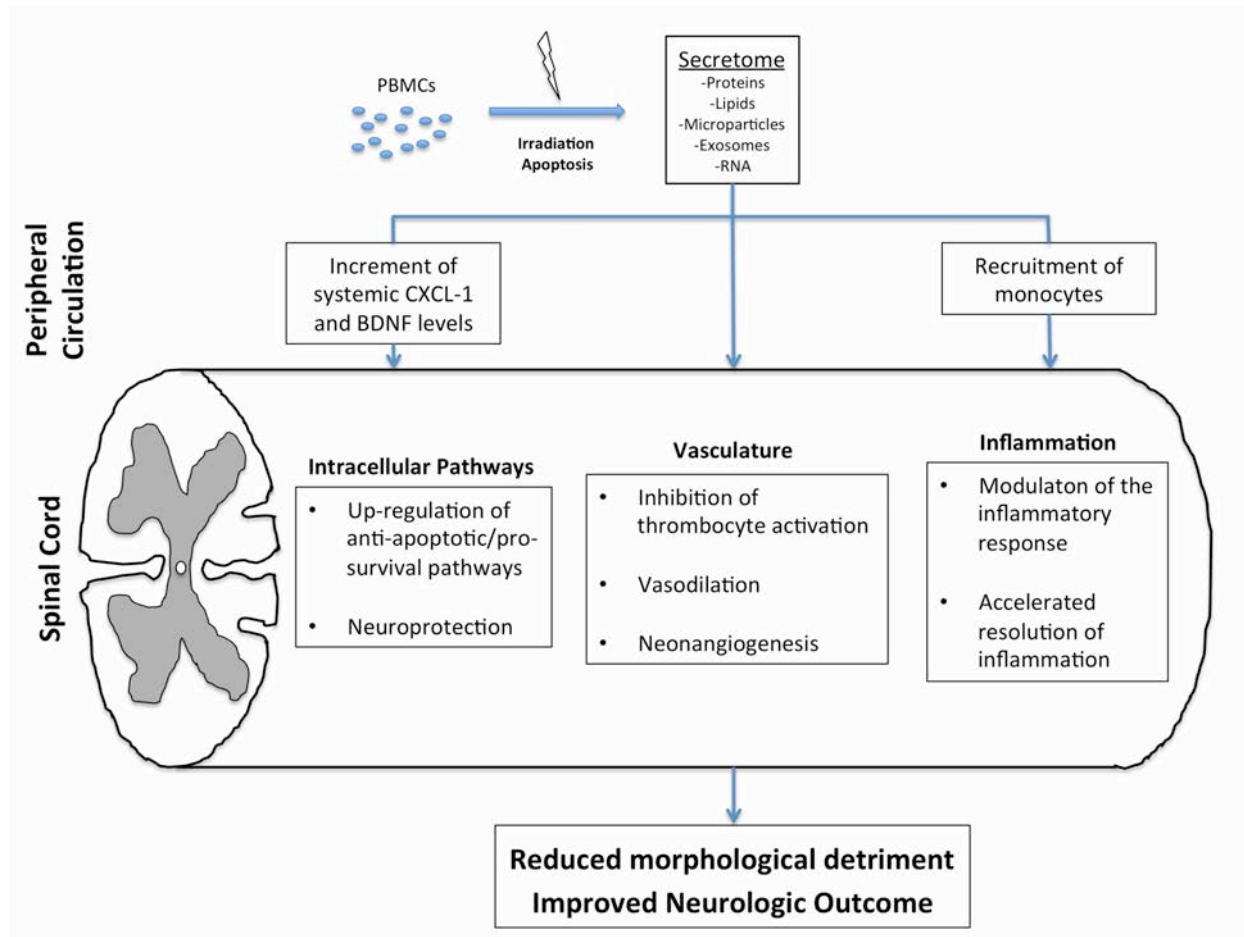


Exp Neurol. 2015; 267: 230-242.

# Summary

- Secretome of apoptotic PBMCs previously attributed with
  - Cytoprotection/Inhibition of apoptosis
  - Immunomodulation
  - Inhibition of microvascular obstruction/  
thrombocyte activation
- Translatable to Spinal Cord Injury

# Summary



TTND. 2016; 3:e1198.

# Summary

- Secretome of apoptotic PBMCs lead to
  - Improvement of neurologic outcome
  - Attenuation of morphological damage
  - Improvement of vascularity
  - Recruitment of peripheral monocytes

-> Multilayered therapy

# Outlook

- Crossing of the blood-brain barrier?
- Alternative administration route/Combination with novel approaches
- Mechanisms involved in monocyte recruitment?
- Translation to bed-side

# Acknowledgments



## **Christian Doppler Laboratory** for Cardiac and Thoracic Diagnosis and Regeneration

Hendrik Jan Ankersmit  
Patrick Altmann  
Andreas Mitterbauer  
Matthias Zimmermann  
Lucian Beer  
Mahdi Mohammad Kasiri

## **Institute of Neurology/Neuropathology**

Romana Höftberger  
Irene Leißer  
Eva Dassler  
Gerda Ricken  
Mirjam Lutz

## **Department of Transfusion Medicine**

Beate Rüger  
Tanja Buchacher

## **Department of Pediatrics**

Gert Lubec

## **Department of Dermatology**

Michael Mildner  
Bahar Golabi

## **Center for Brain Research**

Simon Hametner

## **LBI Experimental and Clinical Traumatology**

Heinz Redl  
Jens Hartmann  
Markus Rossmann

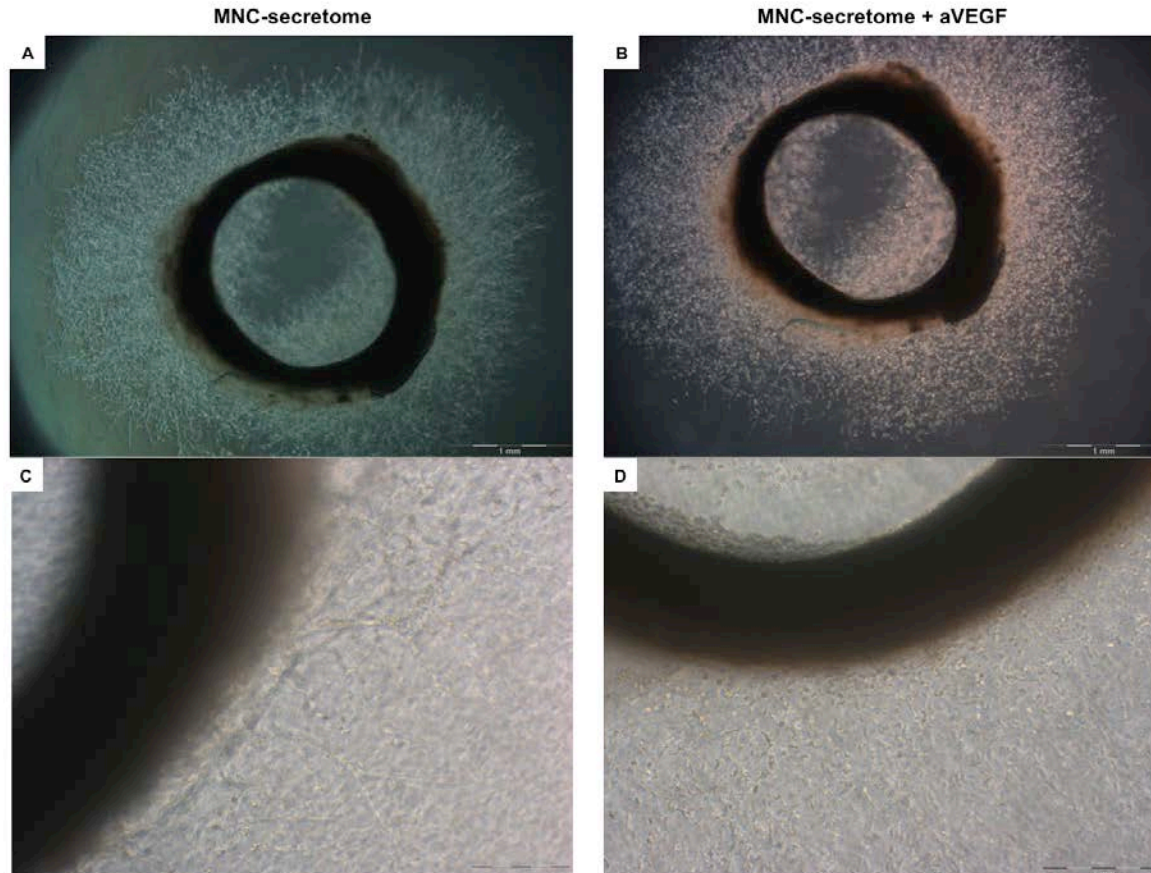
## **Red Cross Blood Transfusion Service Linz**

Christian Gabriel



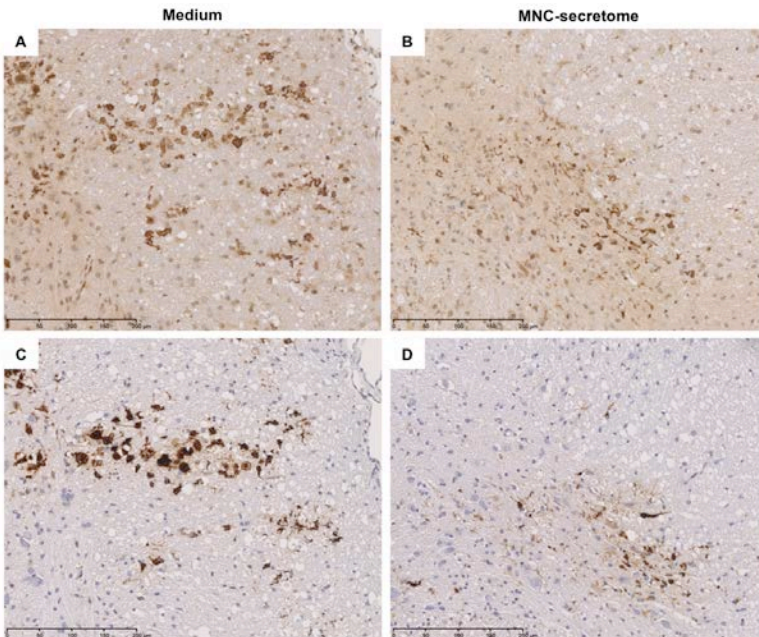


# Addendum



Exp Neurol. 2015; 267: 230-242.

# Addendum

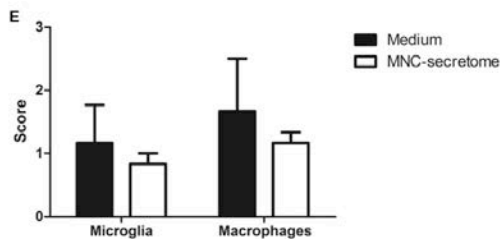


## TNF and Increased Intracellular Iron Alter Macrophage Polarization to a Detrimental M1 Phenotype in the Injured Spinal Cord

Antje Kroner,<sup>1</sup> Andrew D. Greenhalgh,<sup>1</sup> Juan G. Zarruk,<sup>1</sup> Rosmarini Passos dos Santos,<sup>1</sup> Matthias Gaestel,<sup>2</sup> and Samuel David<sup>1,\*</sup>

<sup>1</sup>Centre for Research in Neuroscience, The Research Institute of the McGill University Health Center, 1650 Cedar Avenue, Montreal, Quebec, H3G 1A4, Canada

<sup>2</sup>Institute of Biochemistry, Hannover Medical School, 30625 Hannover, Germany



Exp Neurol. 2015; 267: 230-242.