ADSCs as cell therapy in Traumatic Brain Injury (TBI)

The potential of ADSCs in modulation of the immune answer following TBI

Traumatic Brain Injury (TBI)

primary insult

Fracturs
Contusion
Axonal Damage

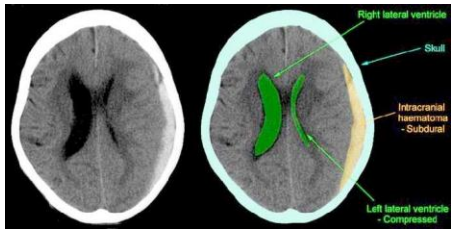
secondary damage

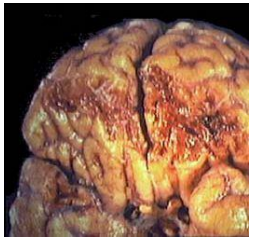
Inflammation
Necrosis
Ischemia
Neuronal Cell Death

repair regeneration

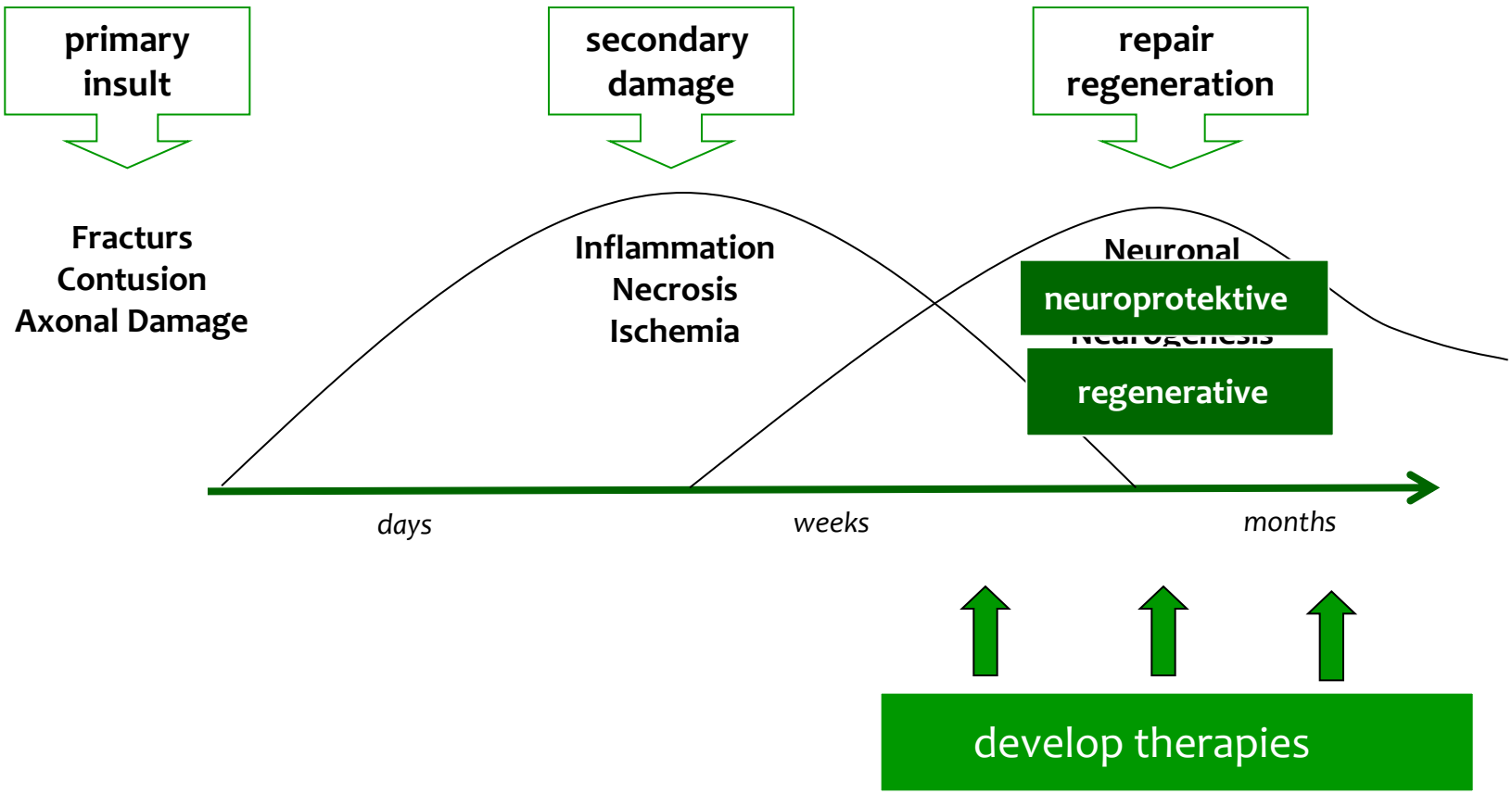
Neuronal
Growth
Neurogenesis

days *weeks* *months*





Traumatic Brain Injury (TBI)



Translational Approach

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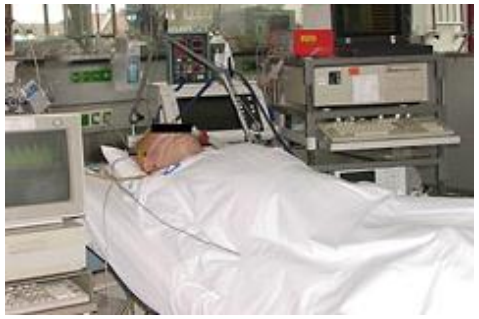
Medical Studies

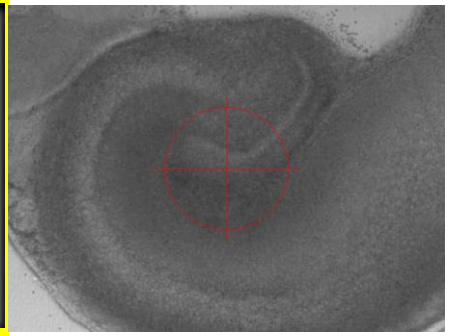
Brain Injured patients



Experimental Modells

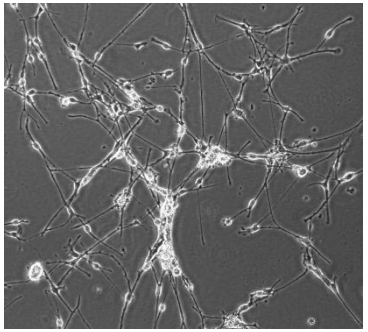
Brain Injury Model

Organotypical Cultures



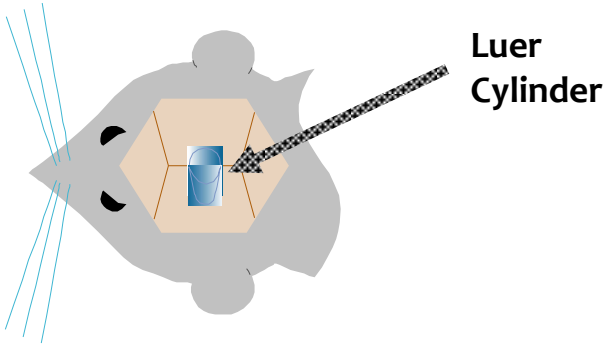
Cell- und Molecular Approaches

Stem- and Primary-Cell Cultures

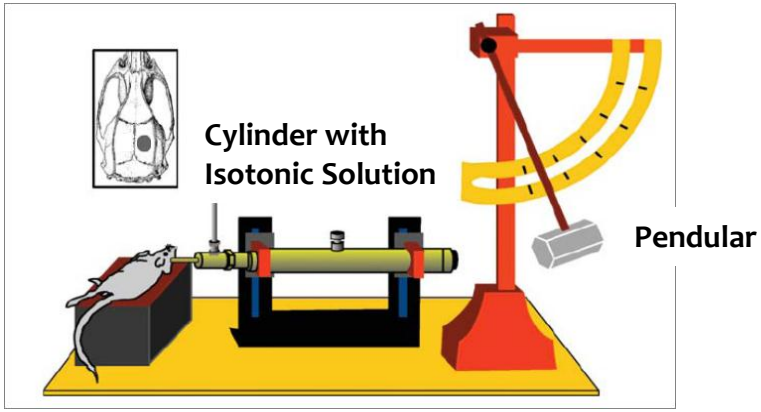


Questions /Results

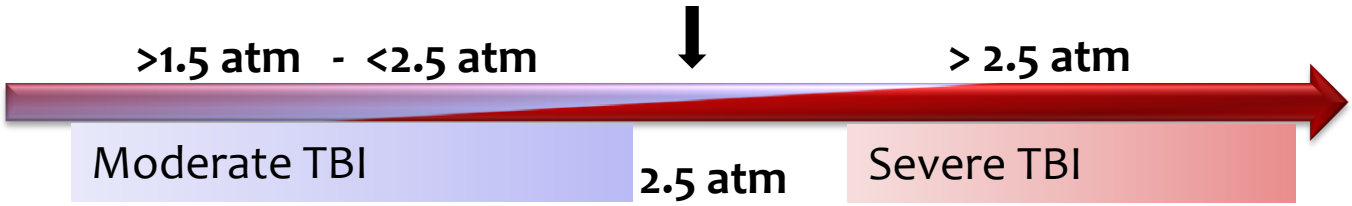
Experimental Trauma Model



Fluid Percussion Injury



Adopted from Thompson et al, Journal of Neurotrauma, Vol. 22(1), 2005



TBI Model (rat)
Lateral Fluid Percussion (LFP)

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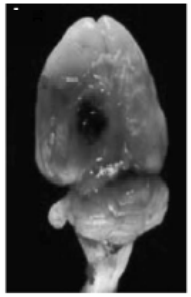
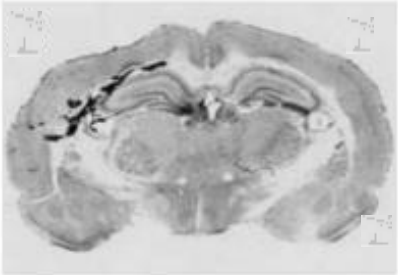
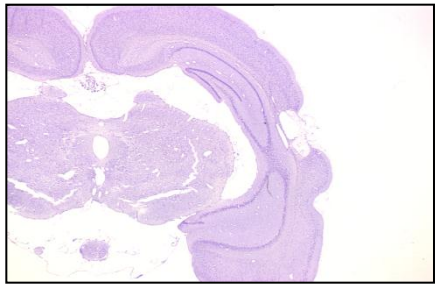


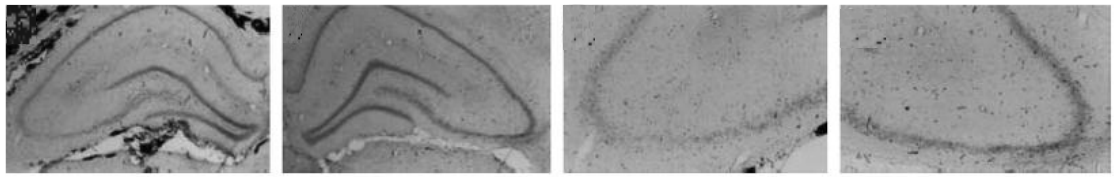
subdurale hematoma





cortical contusion;
hematoma;
shift of middle line;
diffuse axonal damage

defined lesion
due to necrosis
(7 weeks)



Loss of neurones in the hippocampus

Embryonic Stem Cell Transplantation

HYPOTHESIS

Undifferentiated murine ES cells, implanted after an **experimental TBI,** will **integrate into the injured brain, differentiate** and **substitute lost brain cells.**

This will lead to a neurologic improvement of motor and cognitive function.

Method *(Transplantation)*

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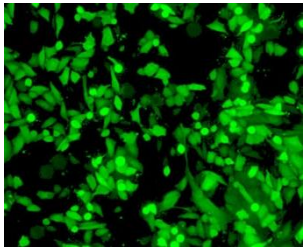
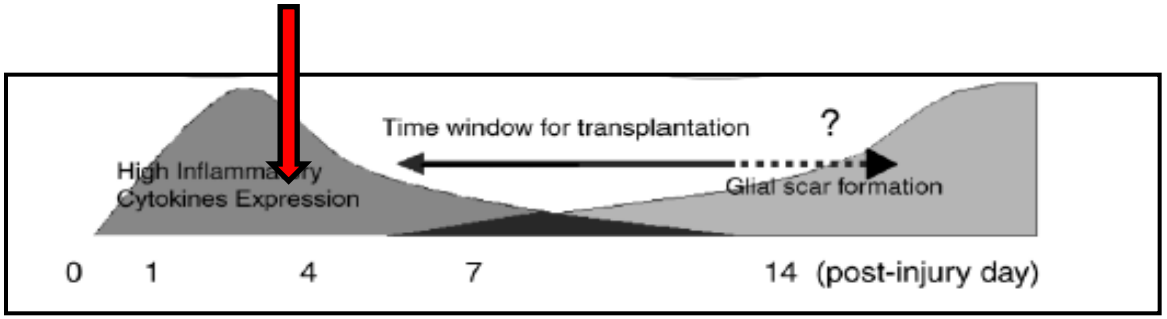
- **Undifferentiated murine ES cells**

- **D3 cell line - stably transfected with enhanced-GFP under controll of actin-promotor (green-fluorescing cells)**

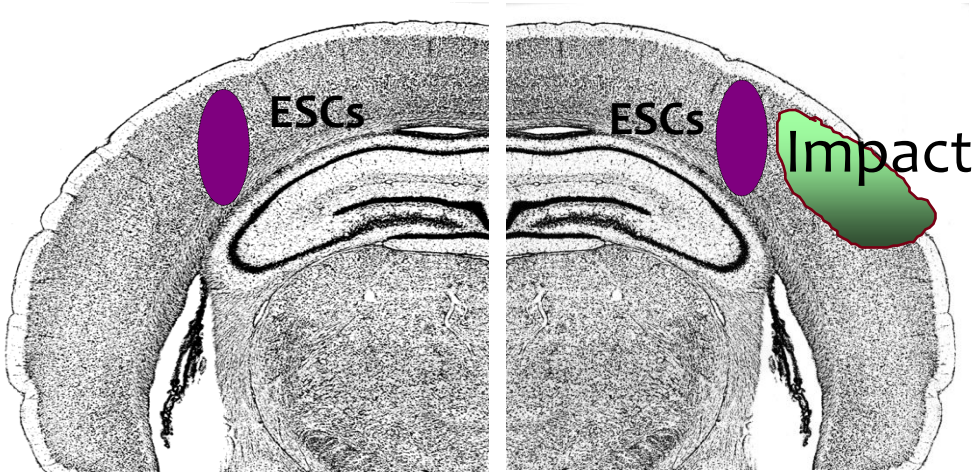




(Okano, J Neurosc Res, 2002)

- **Stereotactic implantation on 3rd day after TBI**

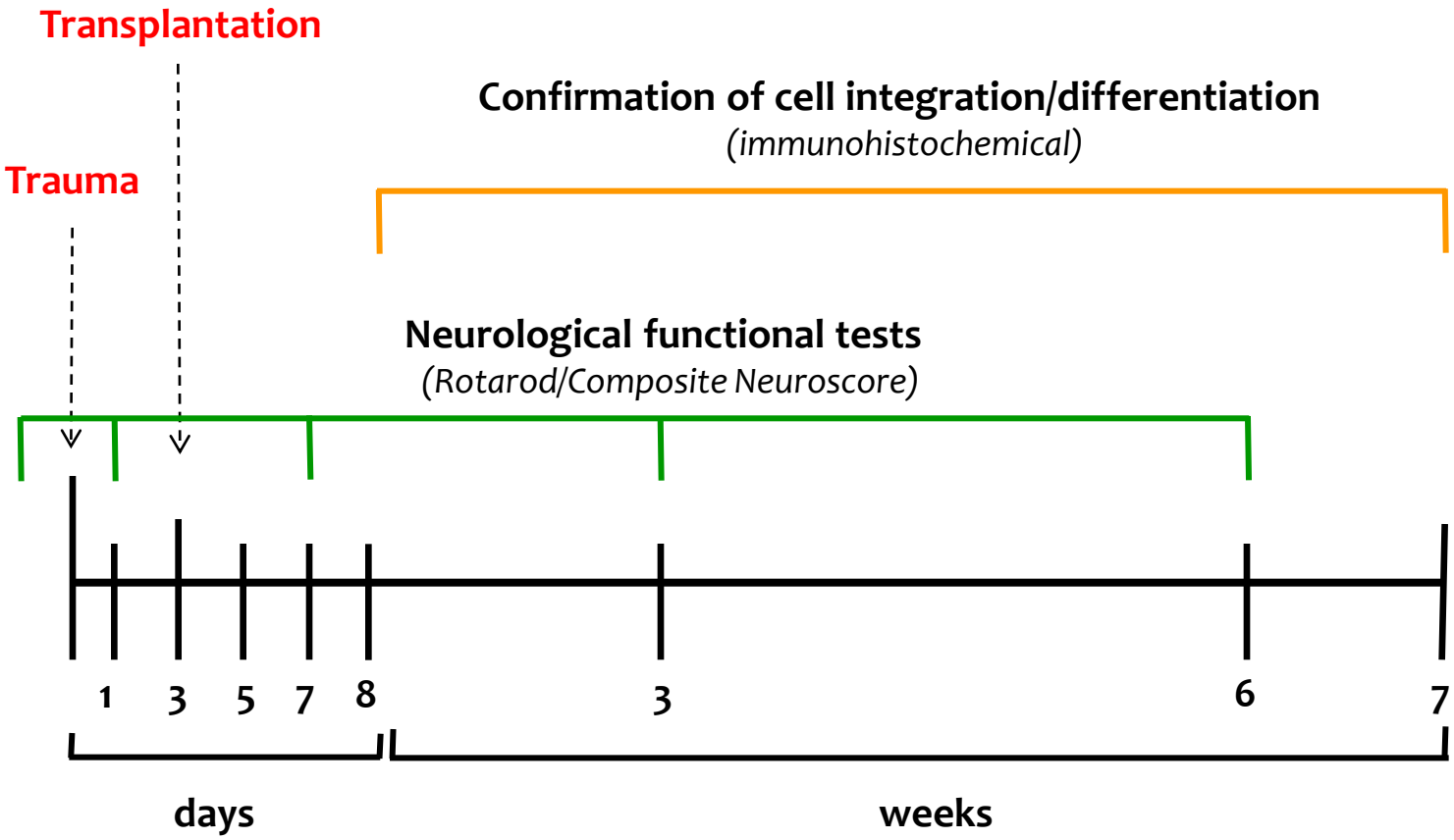
Method *(Transplantation)*



- **100 000 cells in 1 uL PBS injected into a ipsi/contralateral cortex**

Immunosuppresion with CyclosporinA - daily 10mg/kg body weight - for 2 weeks - post-TX

Experimental Set-up



Neurological functional tests

Sensorimotor function

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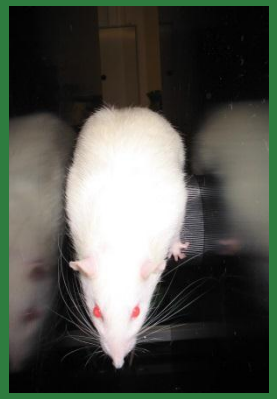
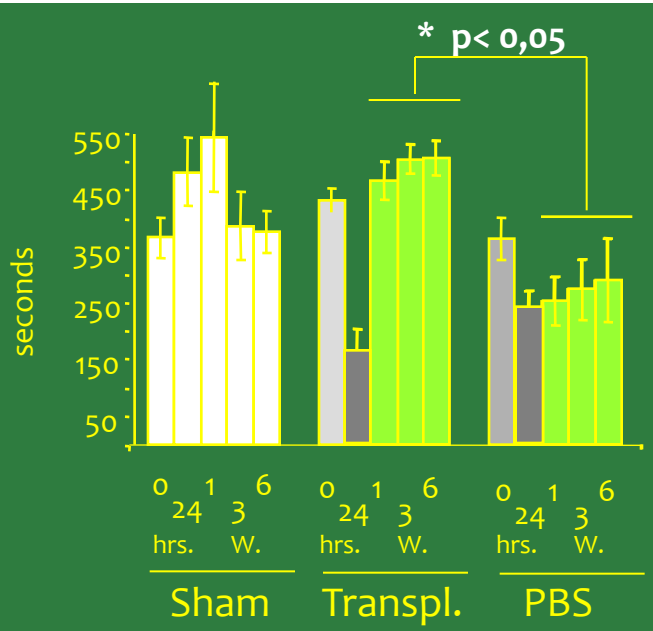
Rotarod





Motorfunction

Neurological functional tests

Sensorimotor function

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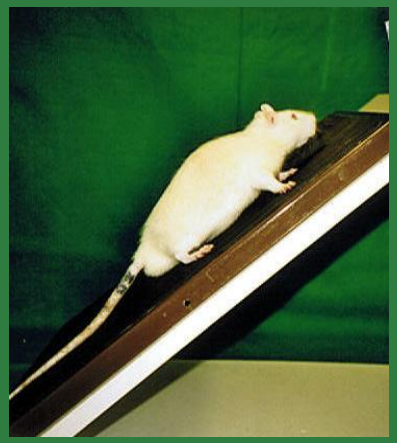
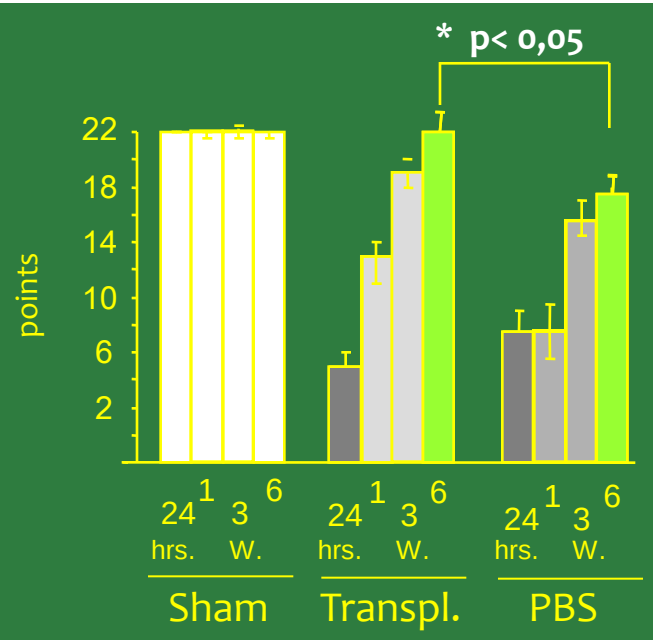
Composite Neuroscore

maximum score = 24



Motorfunction



1) left and 2) right forelimb contraflexion,
3) left and 4) right hindlimb flexion,
5) left and 6) right resistance to lateral pulsion,
7) the ability to stand on an inclined plane

Immunohistochemical Analyses

Confirmation of Cell Integration

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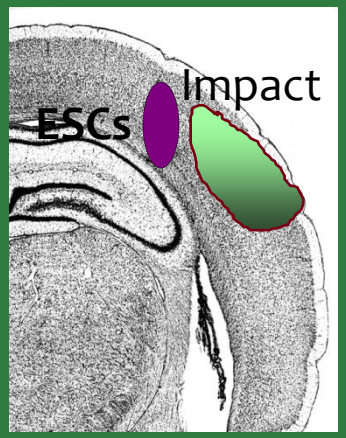
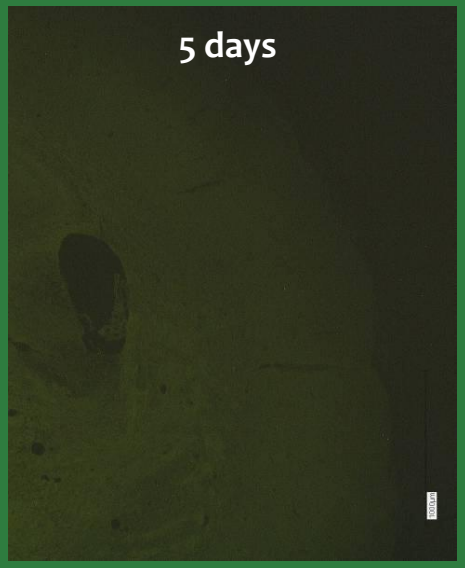
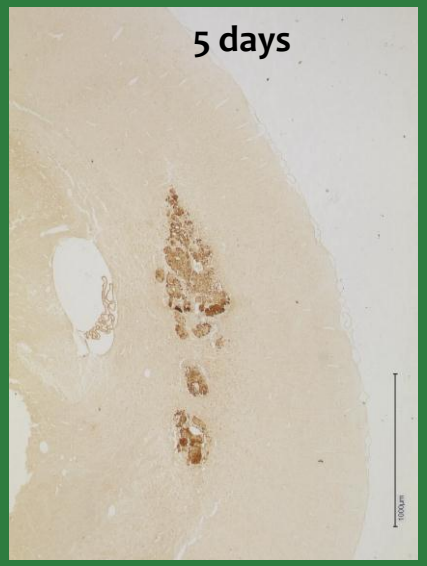
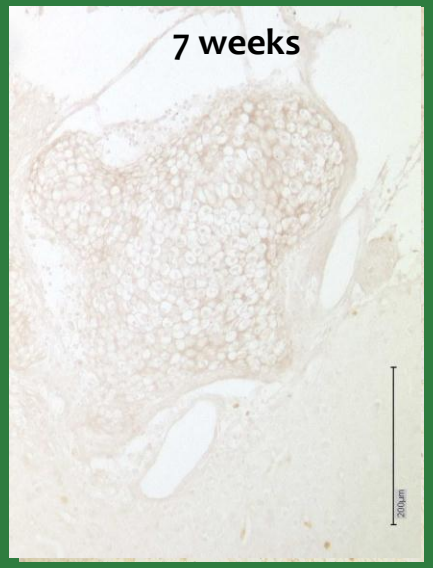




native fluorescence





non-invasive tumor
GFP-negative

What happened to the cells?

Immunohistochemical Analyses

Inflammatory Reaction

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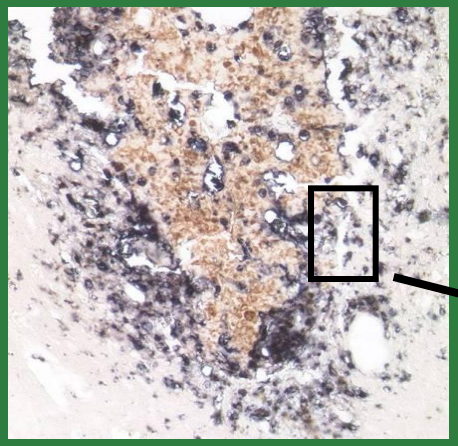
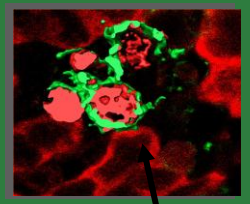
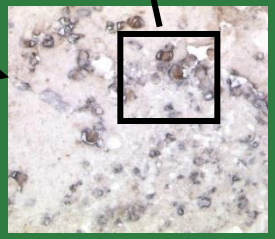
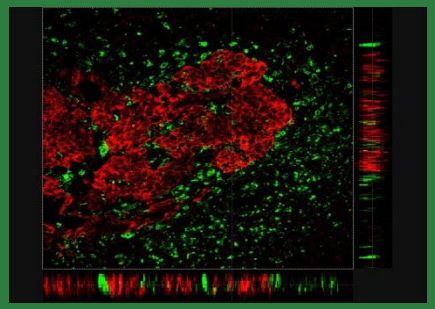
5 days









anti-GFP-DAB/ED1-NBT-BCIP activated macrophages

Confocal Fluorescence Microscopy

Short Summary

Significant improvement of the motoric function until 6 weeks after transplation of embryonic stem cells

No differentiation of stem cells
No integration of stem cells
No migration

After 7 weeks, majority of stem cells were phagocytosed

Remaining cells developed into tumor tissue

Cell Analysis in vitro

Neurotrophic Factors

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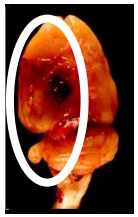
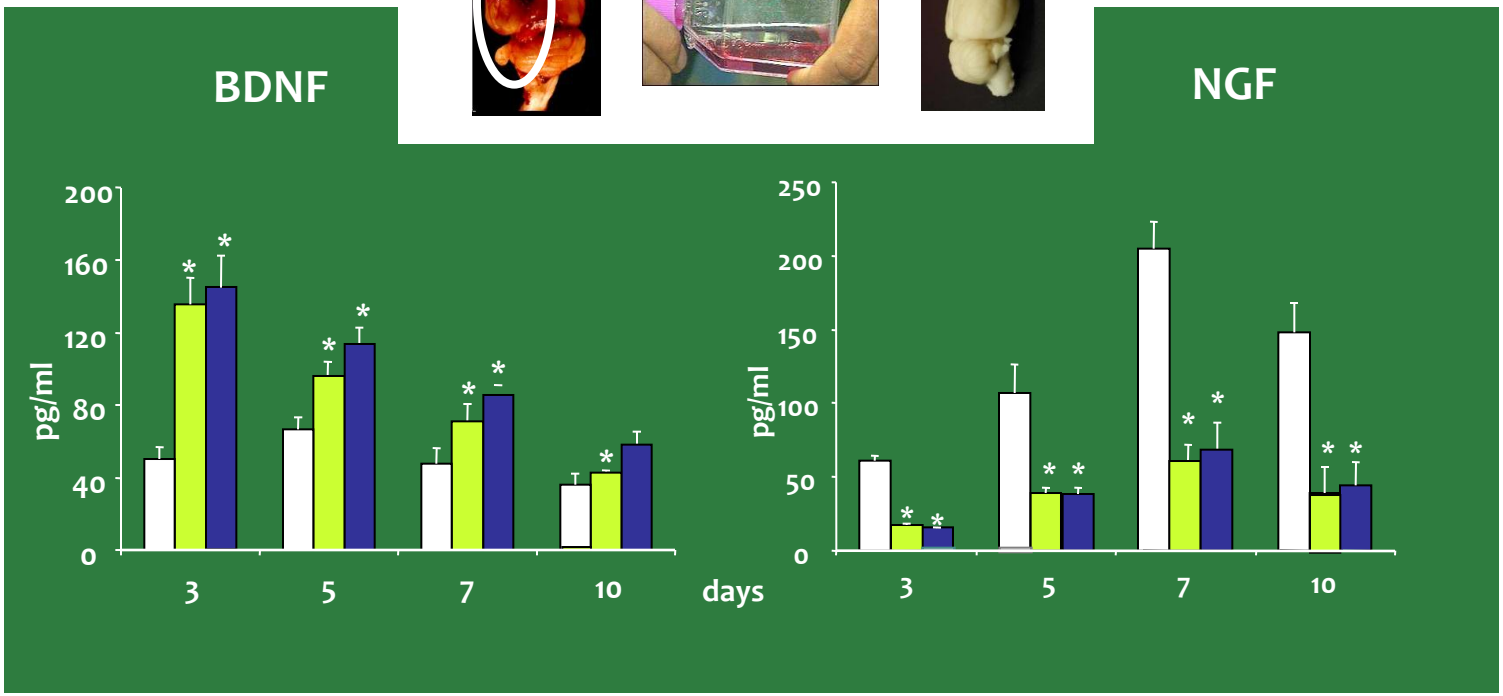
Extract ESCs Extract





Short Summary

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Embryonic stem cells are phagocytosed in the inflamm
microenvironment of the traumatized brain

Protective effects are probably m by the release of
neuroprotective factors

Stem cell re not phagocytosed might develop into
t

Transplantation of ESCs following traumatic brain injury are clinically irrelevant

Why are ADSCs clinically more relevant as cell therapy post-TBI

ADSCs for cell therapy following TBI

- autologous and allogenic sources
- immunosuppressive properties that reduce inflammation in injured tissue
- capable of secreting factors that facilitate the regrowth of neurons in the brain
- secretion of protective factors can be enhanced by stimulation of ADSCs with pro-inflammatory factors
- improvement of motor (possibly) cognitive function

ADSCs Example
(intravenous application following TBI)

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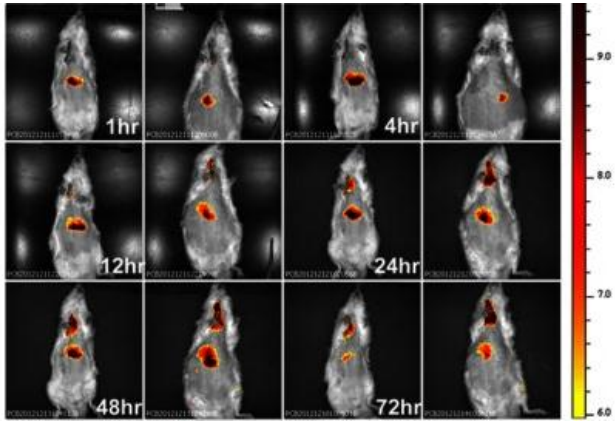
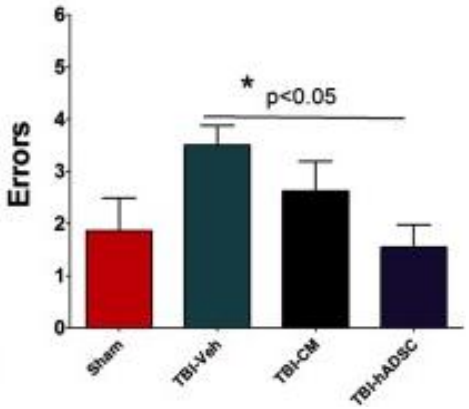
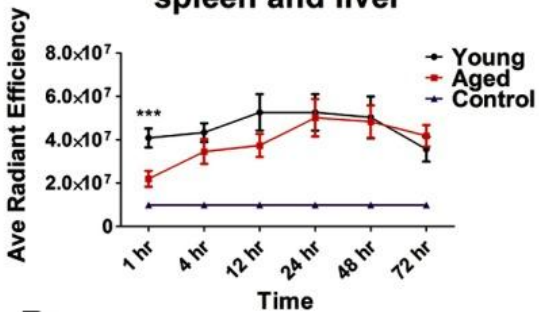


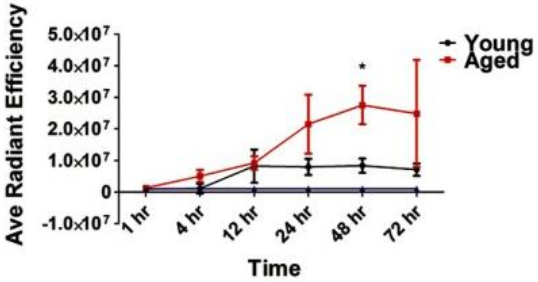
Cognitive function



***In vivo* DiR-ADSC cells in spleen and liver**



***In vivo* DiR-ADSC cells in Head**



Naoki Tajiri et al. J Neurosci. 2014 Jan 1; 34(1): 313–326.

Hypothesis

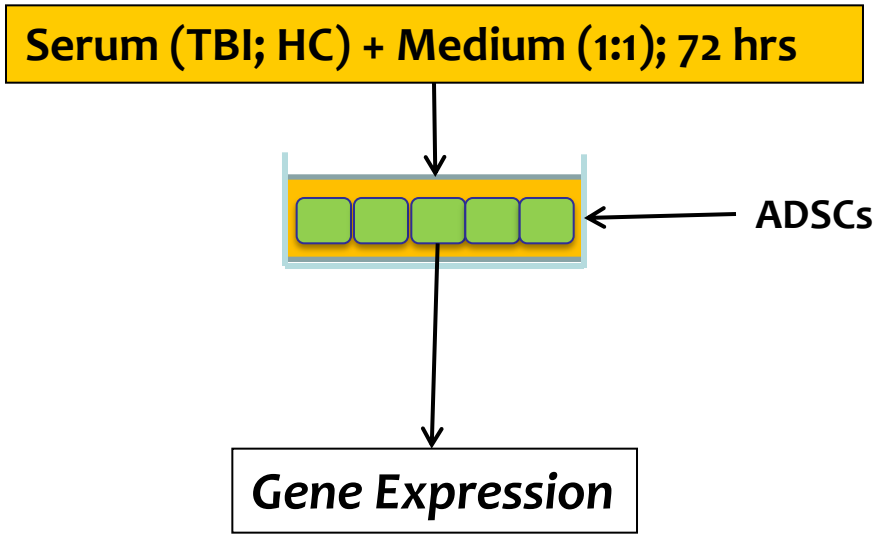
Protective Mechanism of ADSCs is mediated by an immune modulatory function

Immune modulatory function can be increased by in vitro preconditioning of ADSCs with patient serum

Activated monocytes/macrophages of TBI patients will be modulated into protective phenotype by injection (intravenous, intraventricle) of preconditioned ADSCs or the respective secretom

Verify/falsify hypothesis (1)

Pre-conditioning of ADSCs (in vitro)



Expression of distinct factors in preconditioned adipose derived stem cells

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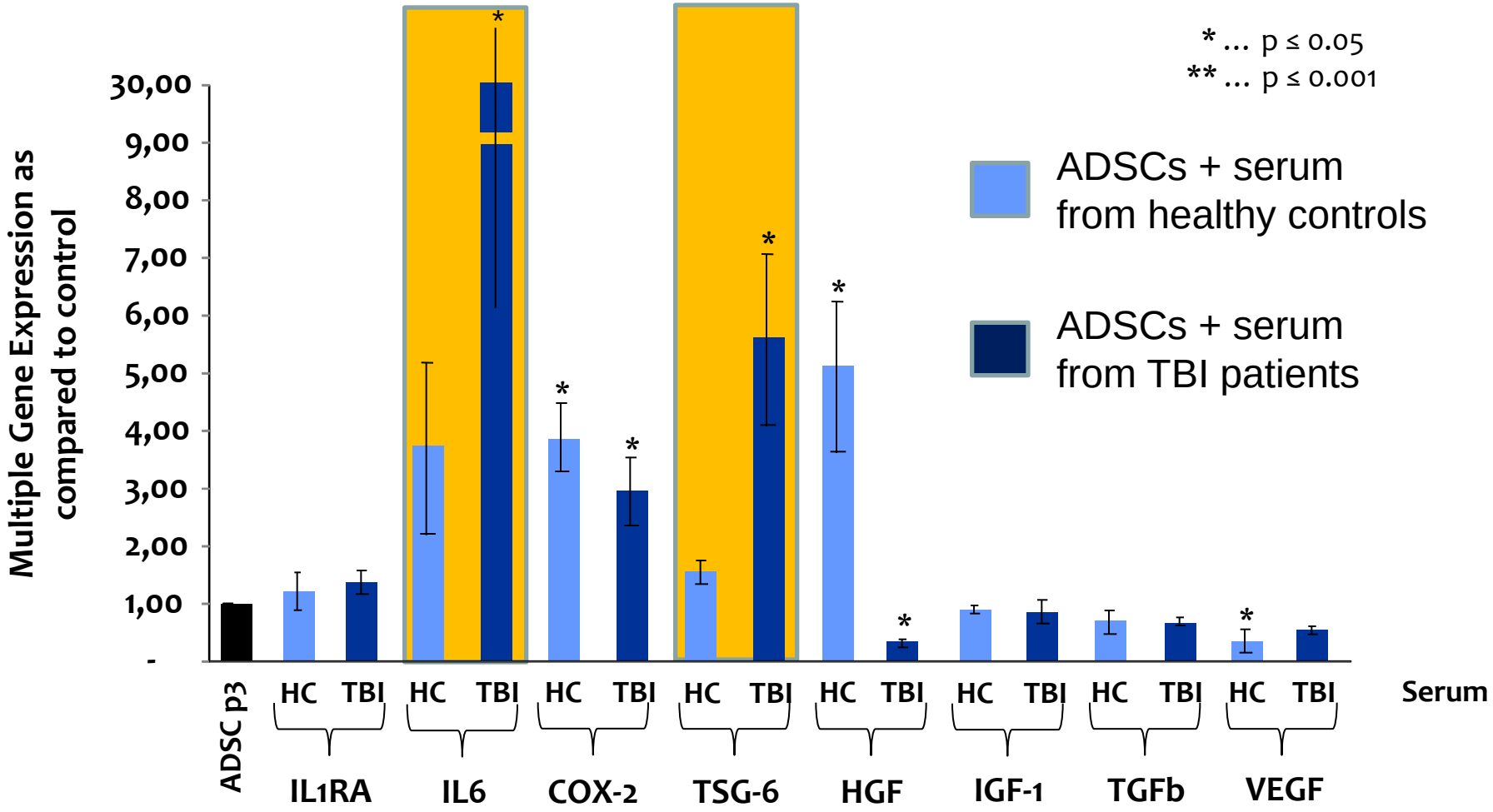

Immune Modulation of Macrophages by the ADSCs secretome

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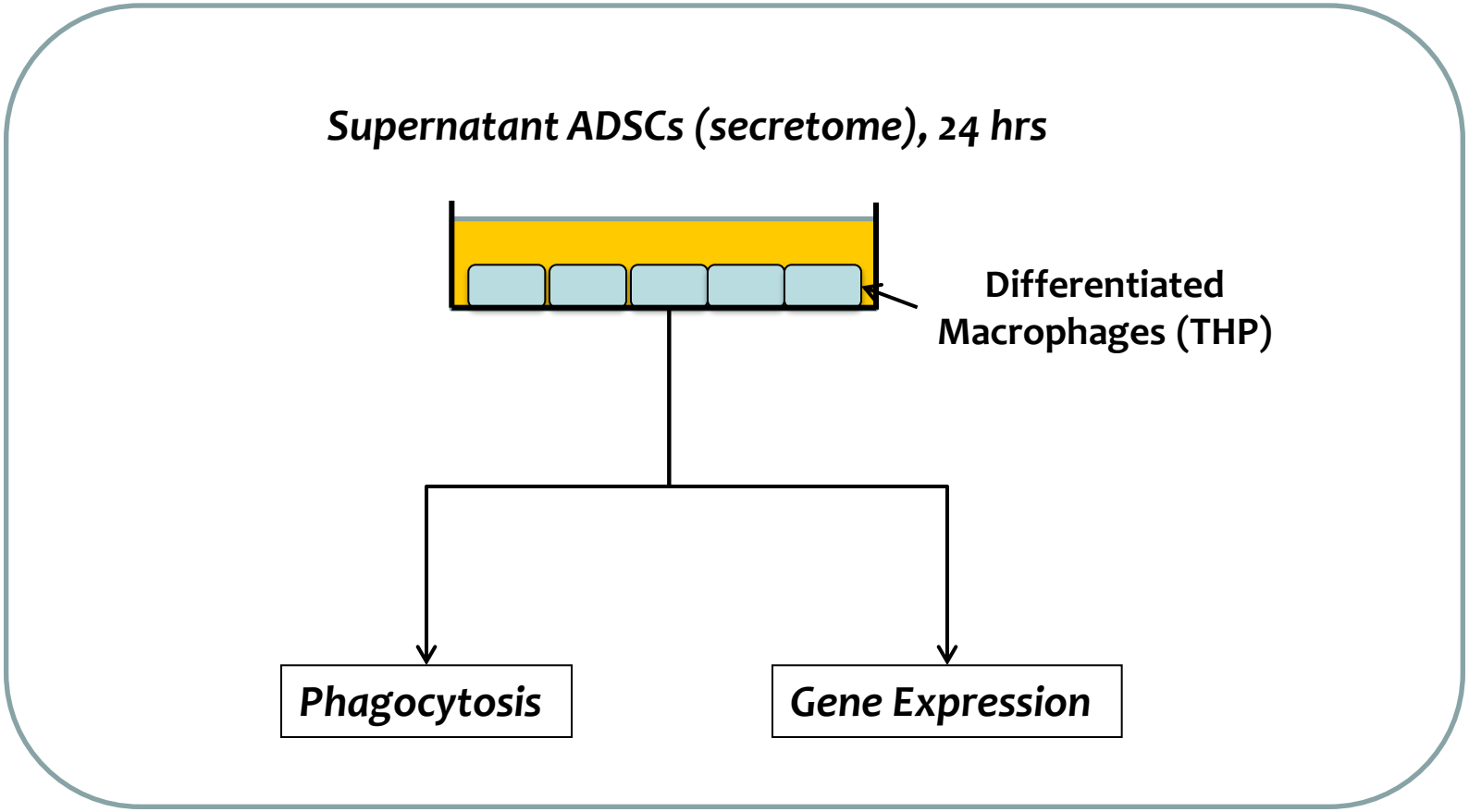

Phagocytotic potential of macrophages following incubation with ADSC scretome

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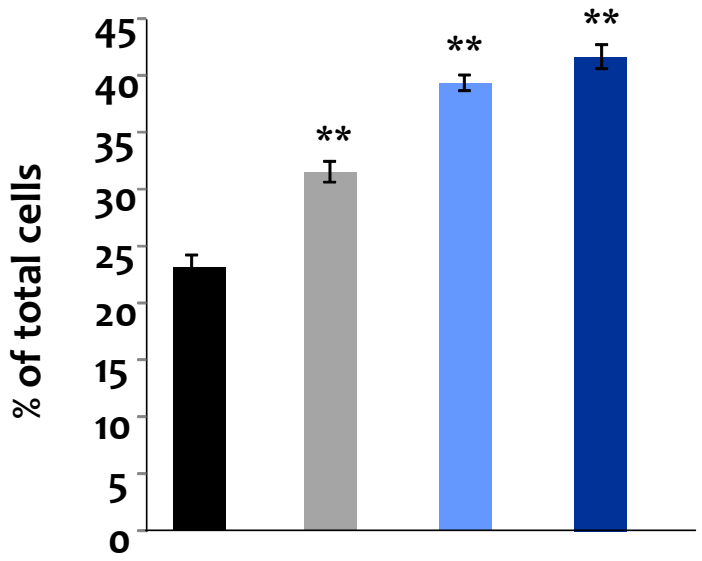
CD11b

* … p ≤ 0.05
** … p ≤ 0.001



Macrophages (MP) untreated	+	-	-	-
ADSCs secretome (untreated)	-	+	-	-
ADSCs secretome + HC-Serum	-	-	+	-
ADSCs secretome +TBI-Serum	-	-	-	+

Gene expression in Macrophages stimulated with ADSC Secretome

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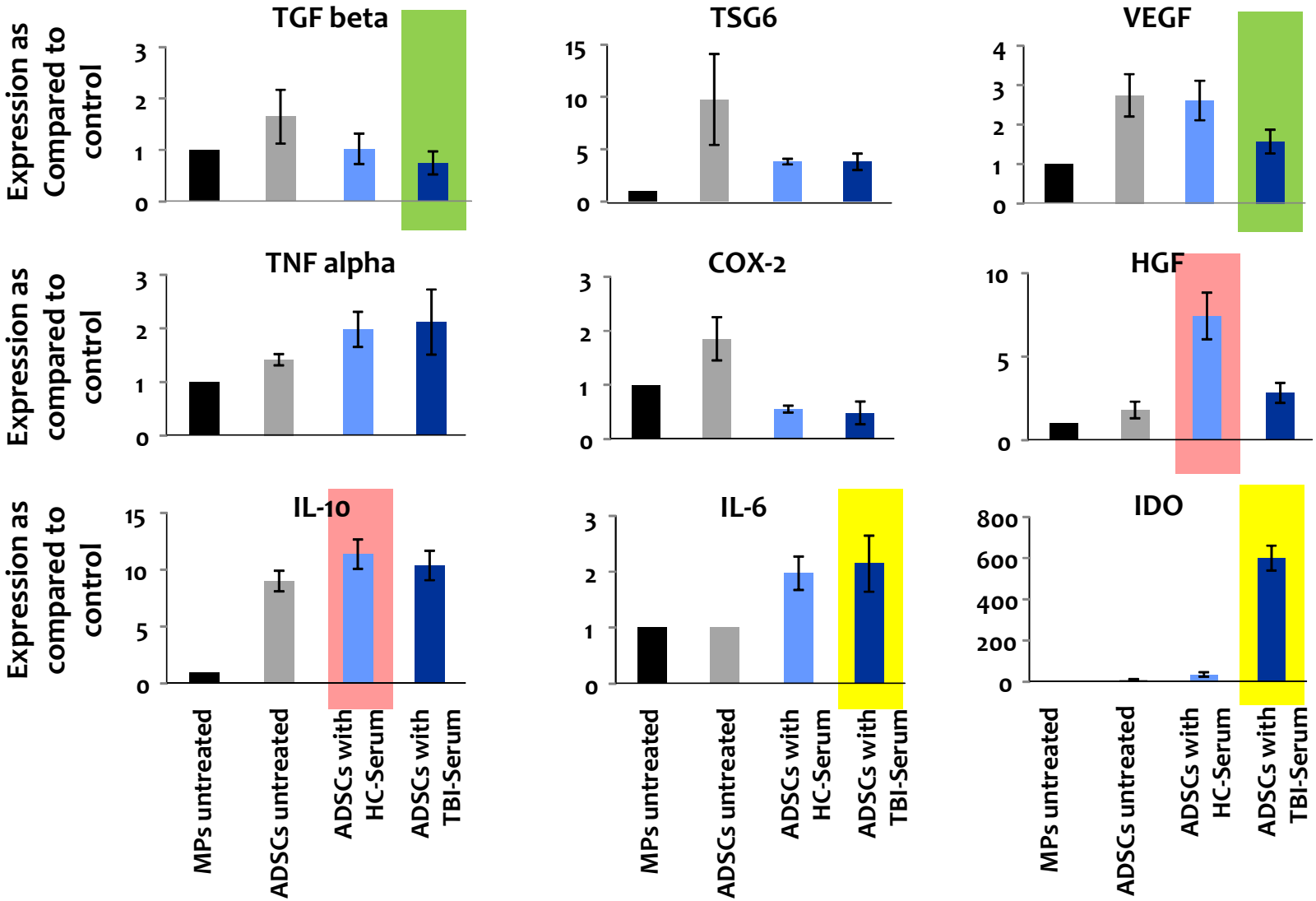


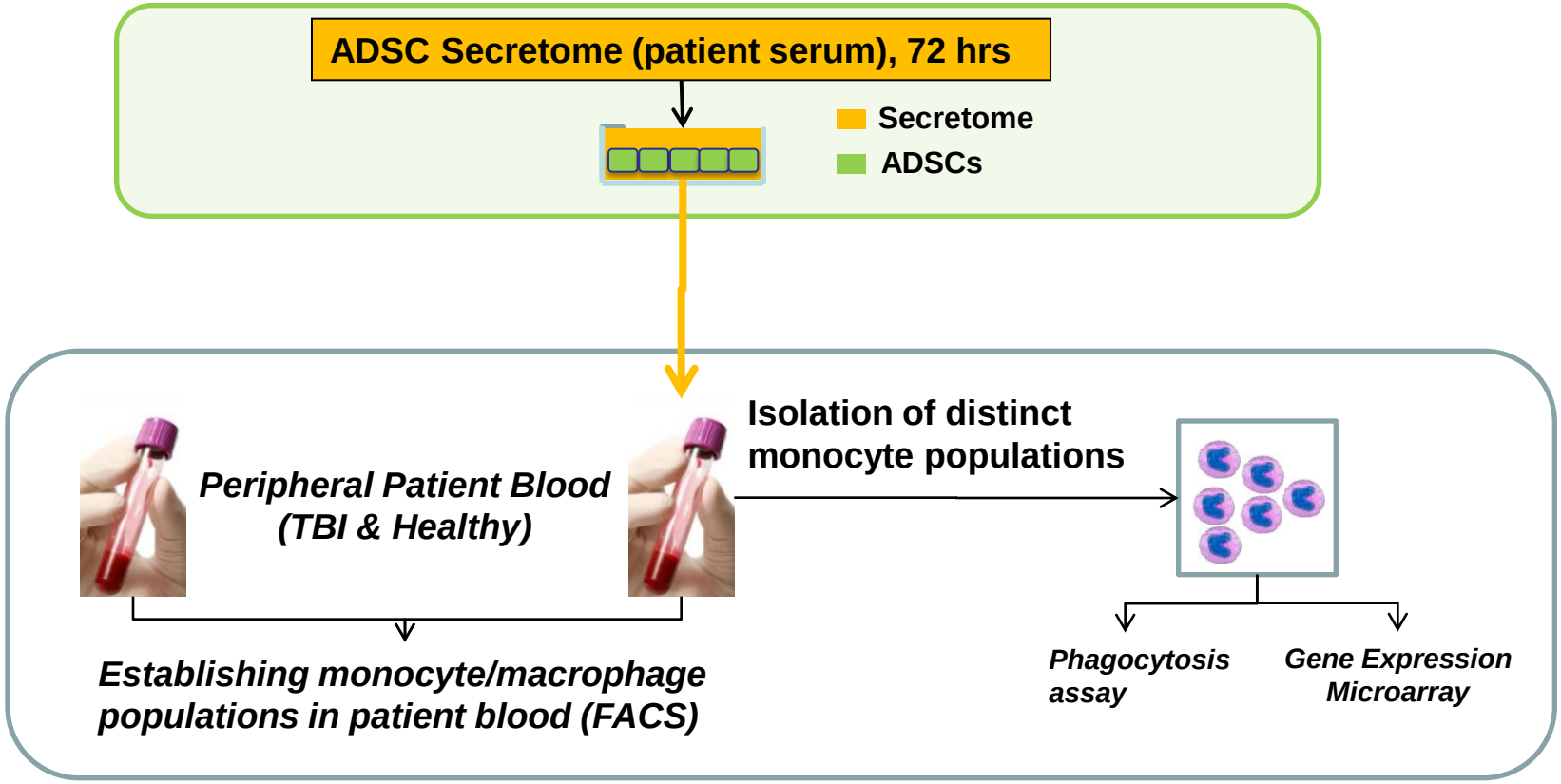
Short Summary

ADSCs can be pre-conditioned by patient serum to increase the release of potentially protective factors

The factors in the preconditioned ADSC secretome modulate gene expression in macrophage cell cultures

Does that also work with native blood derived macrophages?

Pre-conditioning of ADSCs



Ex vivo

Time dependent changes in macrophage populations following TBI

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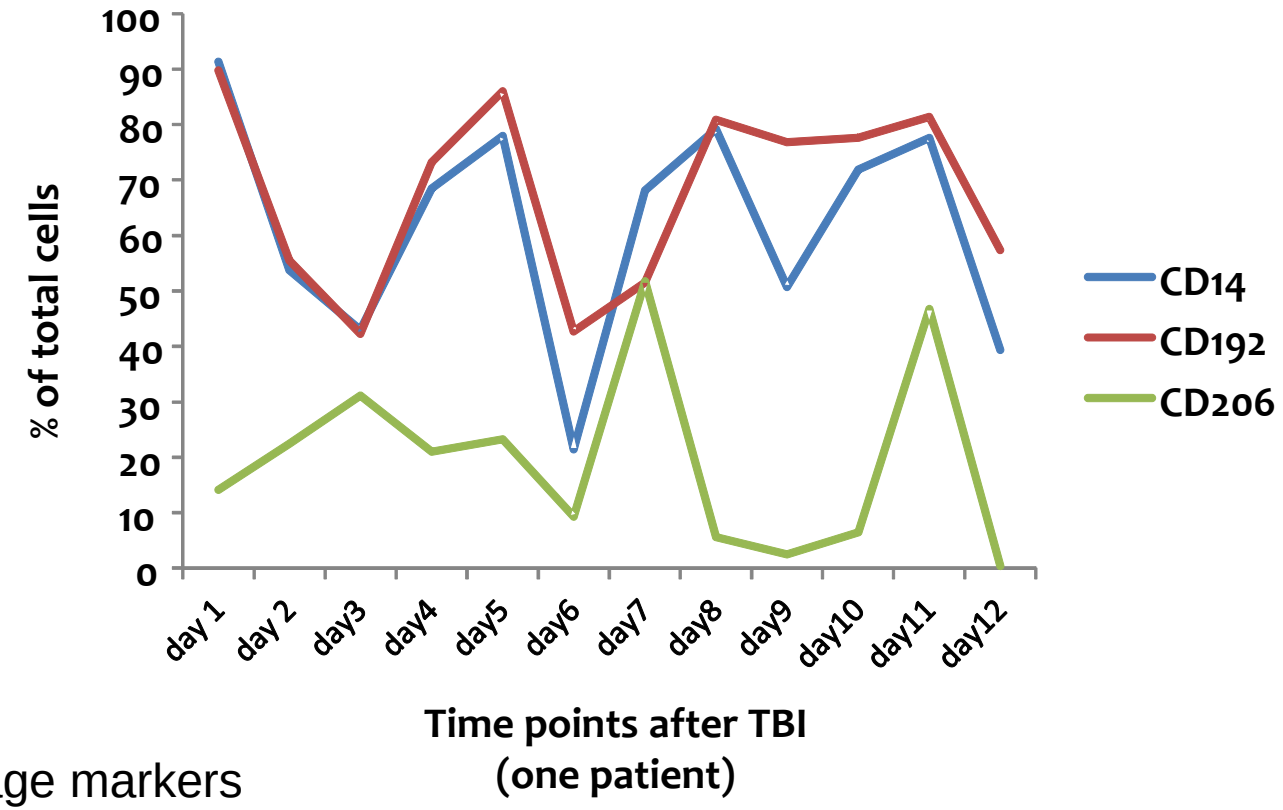

Macrophage markers

CD14: Opsonin receptor function
CD192: Chemokine receptor type 2
CD206: C-type lectin

Further results next year

Thank you very much for your attention ☺

ute.schaefer@medunigraz.at