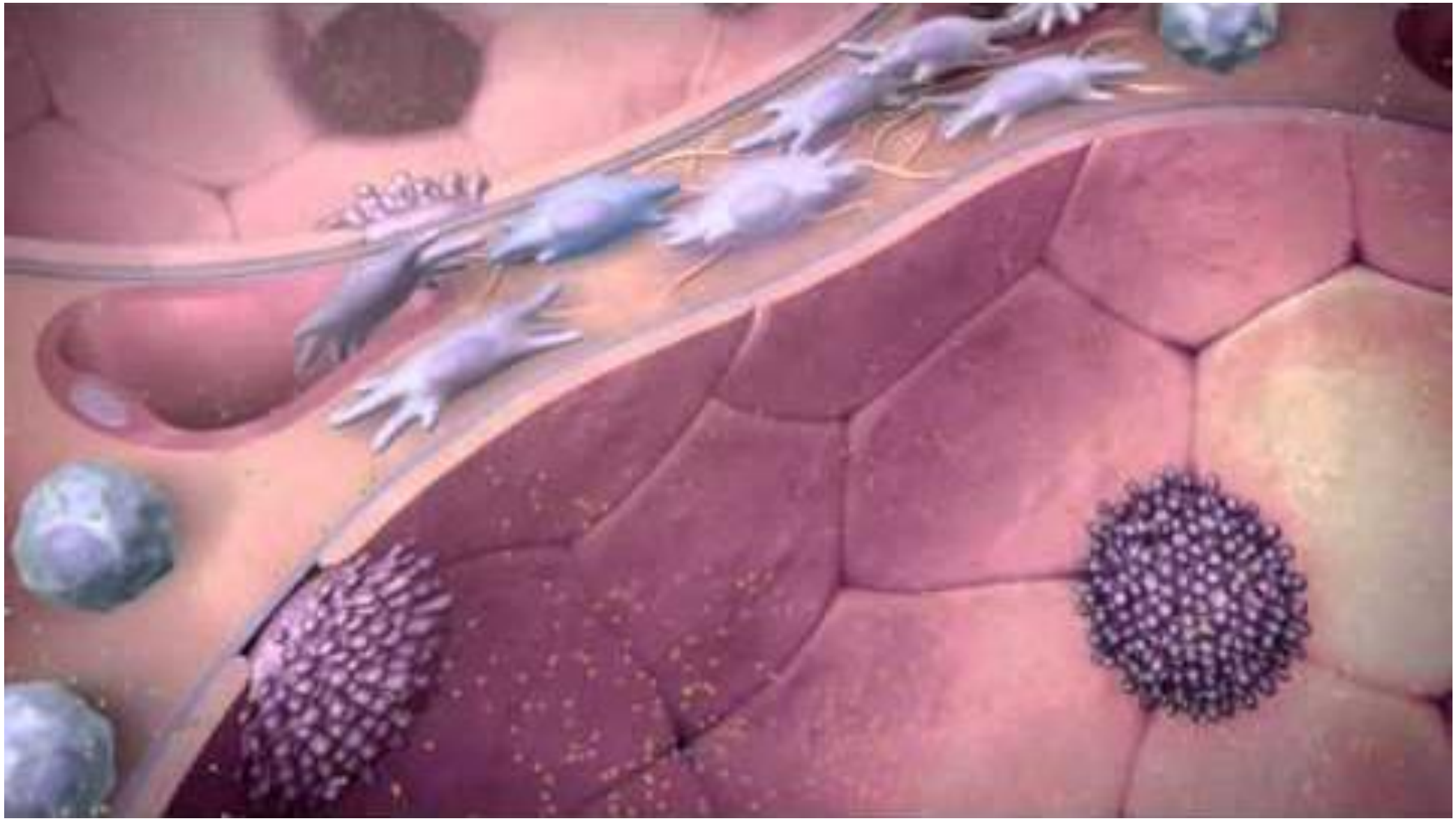
The secretome of induced pluripotent stem cells reduces lung fibrosis in part by hepatocyte growth factor

A. Gazdhar, I. Grad, L. Tamo, M. Grugger, A. Feki, T. Geiser



Introduction

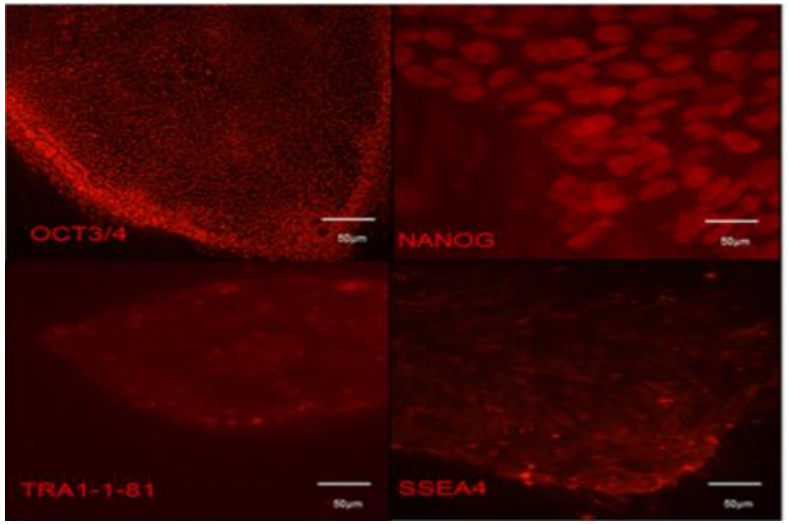
- **Lung fibrosis**: Chronich progressive scaring
  - Epithelial injury -> Failure of the aveolar epithelium to re-epithelialize -> improper wound healing -> scaring of lung parenchyma
  - Currently no treatment available to reverse lung destruction
- **Cell-based therapy**:
  - Hepatocyte growth factor (HGF) has antifibrotic propreties; low HGF Expression in fibrotic lung; [1]
  - iPSCs – potential to differentiate into any cell type[2]
  - iPSCs have transcriptional memory -> using cell-conditioned media obtained from stem cells can have similar therapeutic effect, but better for clinical translation[3]

- 1 **HGF Expressing Stem Cells in Usual Interstitial Pneumonia Originate from the Bone Marrow and Are Antifibrotic,** Amiq Gazdhar,Njomeza Susuri, Katrin Hostettler, Mathias Gugger,Lars Knudsen,, Michael Roth,Matthias Ochs,, Thomas Geiser
- 2: **Induction of pluripotent stem cells from adult human fibroblasts by defined factors., 2007,** Takahashi K[1], Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S.
- 3: Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells, 2011,

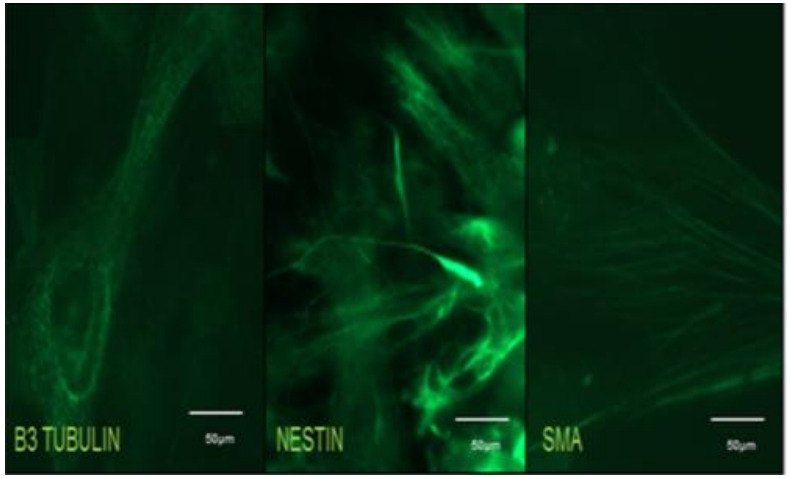
Methods

- Commercially available cell lines of human foreskin fibroblasts
  - Cells were expanded and viraly transfected to induce pluripotency
  - Cells were differentiated in-vitro, to examine if iPSC are able to differentiate into all cell types
  - Immunostained with OCT ¾, NANOG, SSEA4, TRA-1-81
  - 10-12 iPSC colonies were grown – iPSC cell-conditioned media (iPSCcm) was collected
  - Stained with Annexin-Propidium iodide- to measure cell death and apoptosis
  - Mice have been treated intratrachelly with bleomycin to induce pulmonary fibrosis
    - 1 group was treated with iPSC-cm, other two with control media
  - iPSC-cm was incubated with HGF antibodies

Results

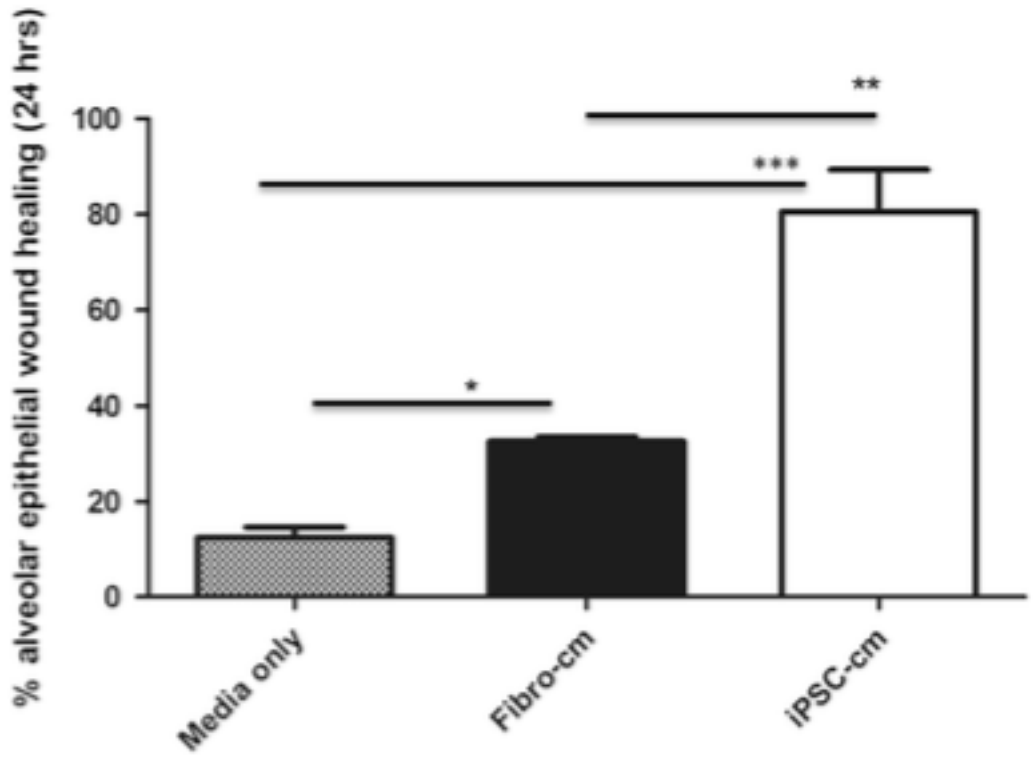


- Positive for pluripotent markers OCT4, Nanog, SSEA4, TRA-1-81



- iPSC are able to differentiate into all 3 germ layers (B3 tubulin-ektoderm marker; Nestin- endoderm marker; SMA-mesoderm marker)

Results- in vitro



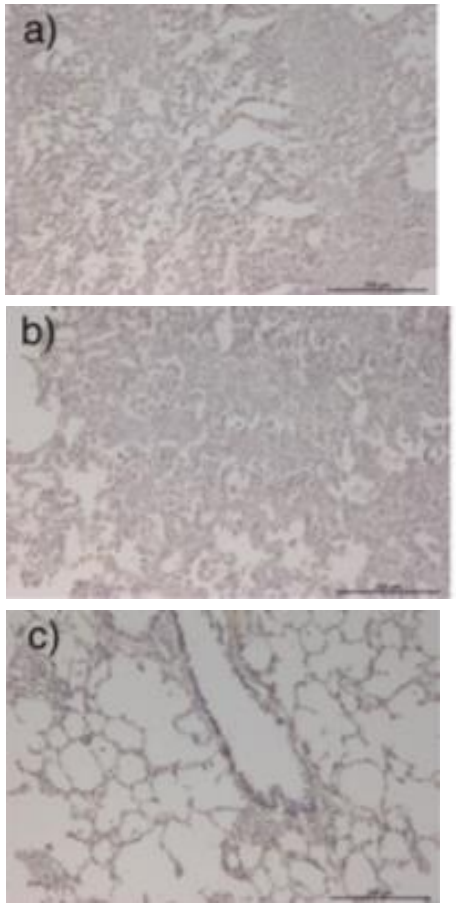
- iPSC-cm improves alveolar epithelial wound repair in vitro

*p<0,001

**p<0,001

***p<0,0001

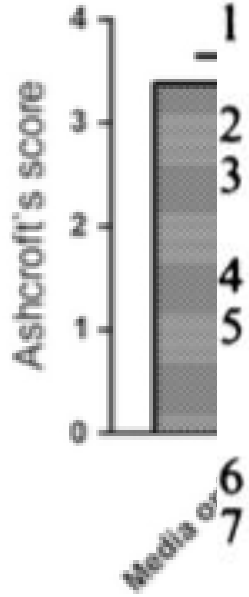
Results- in vivo



Histology:
a- media only
b- Fibroblast-cm
c- iPSC-cm

-> significant decrease of fibrosis in iPSC-cm treatment

Results- in vivo



Grade of fibrosis	*Histological features*
0	Normal lung
1	Minimal fibrous thickening of alveolar or bronchiolar walls
2	
3	Moderate thickening of walls without obvious damage to lung architecture
4	
5	Increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses
6	
7	Severe distortion of structure and large fibrous areas; "honeycomb lung" is placed in this category
8	Total fibrous obliteration of the field

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: score

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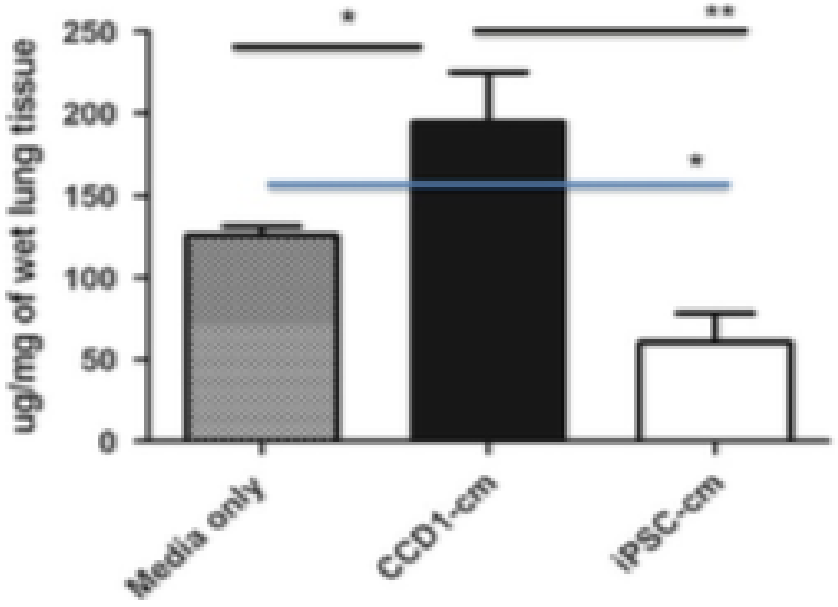
Results- in vivo



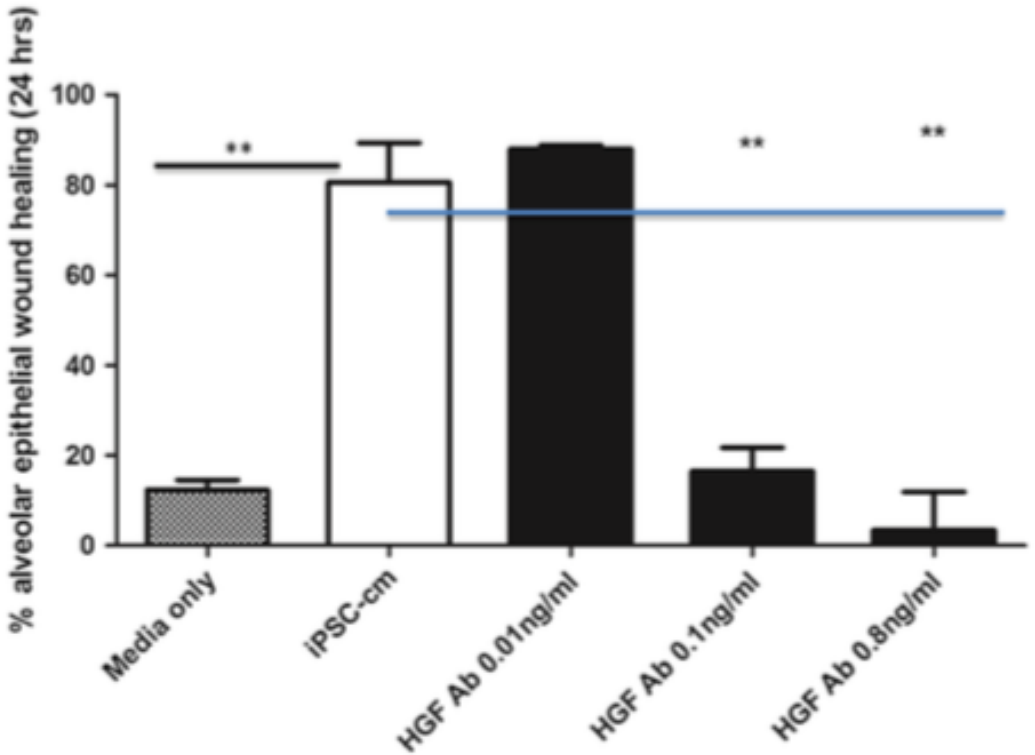
- Collagen content measurement

-> reduced after treatment with iPSC-cm compared with media control

*p<0,01

**p<0,001

Results- Hepatocyte growth factor (HGF)



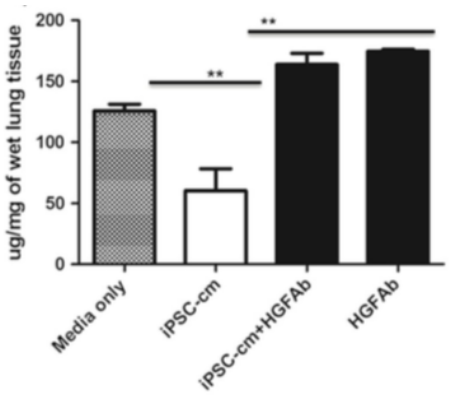
- In vitro:
  - Specific inhibition of HGF using HGF neutralizing antibodies resulted in significant *dose-dependent* decrease of iPSC-cm-induced of epithelial repair

-> central role for HGF in epithelial repair-inducing acitivities

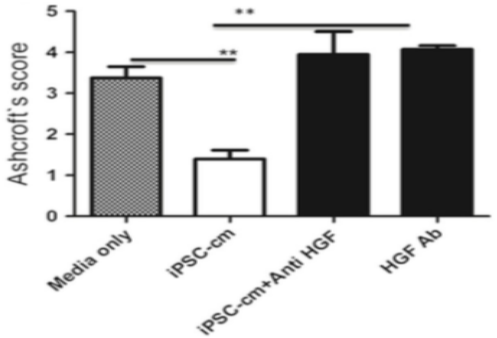
**p<0,001

Results- Hepatocyte growth factor (HGF)

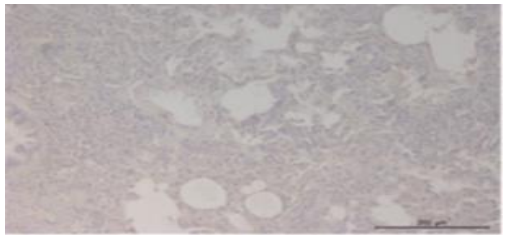
a



b



c



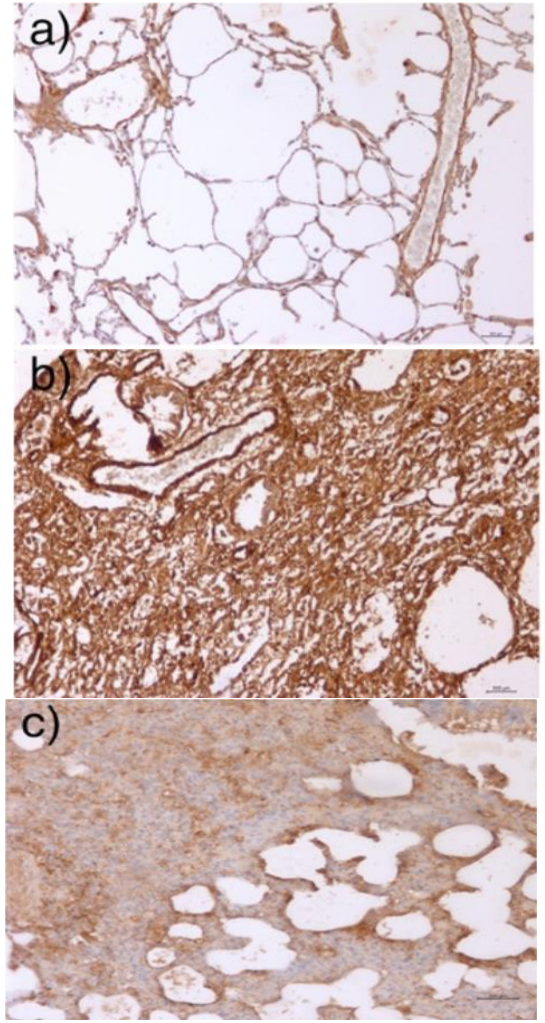
- In vivo:

The presence of HGF neutralizing antibodies- collagen content increased, as well es Ashcroft score (a, b)

- HGF antibodies alone have no antifibrotic effect (c)

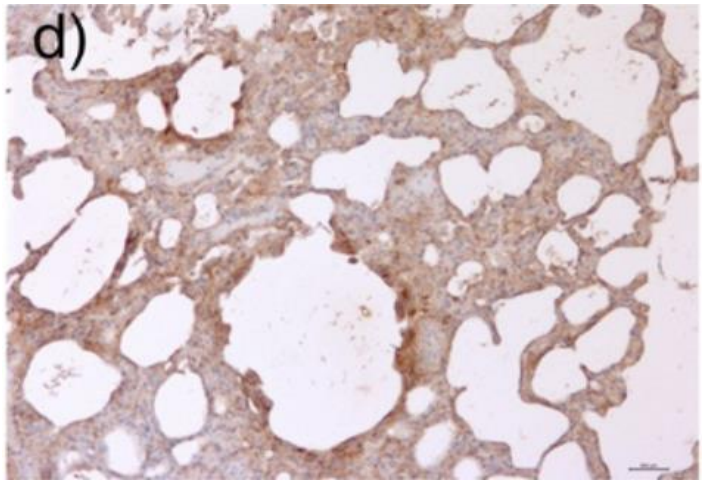
**p<0,001

Results- Myofibroblasts and TGFβ1



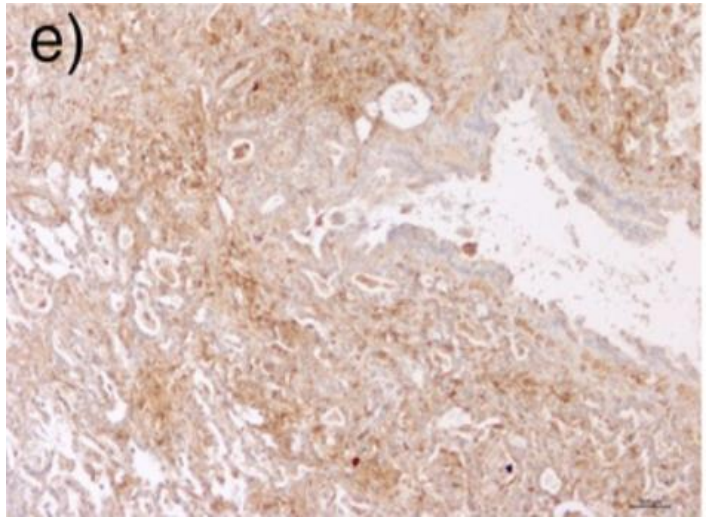
After treatment with iPSC-cm (a) reduction of myofibroblasts in the injured areas of the lung has been showed compared with the CCD1-cm-treated group (b) and media control (c).

Results- Myofibroblasts and TGFβ1



- d- iPSC-cm pretreated with HGF neutralizing antibodies

-> increase of TGFβ1 and myofibroblast expression compared to iPSC-cm alone



- e- HGF neutralizing antibodies treatment alone did not reduce myofiboblast expression in fibrotic tissue

Discussion/Summary

- This study shows that iPSC-cm treatment induces alveolar epithelial repair in vitro and reduces lung fibrosis in vivo, in part by a HGF-dependent mechanism.
  - In vitro: improved epithelial wound repair after iPSC-cm treatment
  - In vivo: collagen level and Ashcroft‘s score decreased after iPSC-cm treatment
    - Hypothesis that antifibrotic effect was due to HGF-dependent mechanism was supported after results were abolished after adding the HGF-antibodies

Discussion

-> treatment with iPSC-cm is very effective in the bleomycin-injured rat lungs, indicating that the secreted mediatores have strong therapeutic effect -> good subsitute for cell therapy

- Detailed analysis of iPSC-cm and long-term effects is needed
- Patient-tailored therapy possible: patient‘s own somatic cells can be reprogrammed to iPSCs, thus circumventing the immune response that is expected with therapies using cell donores.

    -> using patient‘s own iPSC-cm offers promising, safe and effective therapeutic option

Mesenchymal Stem Cells in Neurodegenerative Diseases

Rotem Volkmann, Daniel Offen

Introduction

- Neurodegenerative diseases: progressive decline in neuronal function, brain atrophy, abnormal deposition of proteins
  - Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and multiple system atrophy (MSA) - common cellular and molecular mechanisms
- Problems of finding adequate therapy:
  - Cause of neuronal death is obscure
  - Early diagnosis is impeded due to lack of efficiant biomarkers
  - Secondary effects (chronic inflammation)
  - Crossing blood-brain-barrier (BBB) and targeting spesific cell types in CNS (efficient vectors required)

Introduction

- Mesenchymal Stem Cells (MSCs)
  - Multipotent, capable of self -renewal
  - Able to greatly expand, but no toxicity or tumorrigenicity
  - Capability to migrate towards neural lesions due to attraction by chemokines
  - Paracrine secretion- clinical potential by regulation immunomodulation, apoptosis etc

Introduction

- Multiple reports showed improvement in various models of neurodegenerative diseases or acute brain insults upon transplanting MSCs in rodent models
  - Improved survival rates, declined pathology, rescued cognitive function
  - Mechanisms remain debatable
    - Majority of this diseases display complex etiology-> multiple beneficial roles of MSCs
    - Induction of neurogenesis, modulation of iflammation, prevention of misfolded protein aggregation

Induction of Neuronal Regeneration

- PD: loss of dopaminergic neurons in Substantia nigra
- ALS: degeneration of motor neurons in the brainstam and spinal cord
- AD: global neuronal loss in the cerebral cortex and hippocampus
- HD: degeneration of projection neurons in the dorsal striatum

-> neural regeneration is a major therapy strategy

- MSCs manipulated to differentiate into functional neurons- replacement of damaged neural tissue

Induction of Neuronal Regeneration

- Ex vivo differentiation of human MSCs into dopamine secreting and acetylcholine secreting neuronal-like cells[1]
  - Alternatively: genetic modification: transdifferation of bone-marrow-derived MSCs using ectopic expression of neuronal subtype-specific transcription factors[2]
    - e.g. LMX1 for dopaminergic phenotype, neurogenin1 for ischemic brain

1: Trzaska KA, Kuzhikandathil EV, Rameshwar P. Specification of a dopaminer- gic phenotype from adult human mesenchy- mal stem cells. STEM CELLS 2007;25:2797– 2808. doi:10.1634/stemcells.2007-0212

2: Barzilay R, Ben-Zur T, Bulvik S et al. Len- tiviral delivery of LMX1a enhances dopami- nergic phenotype in differentiated human bone marrow mesenchymal stem cells. Stem Cells Dev 2009;18:591–602. doi:10.1089/ scd.2008.0138.

Induction of Neuronal Regeneration

- Induction of endogenous Neurogenesis: encourage neural stem cells to generate the appropriate neurons
  - Identify the factors that enhace endogenous neurogenesis:
    - Brain derived Neurotrophic factors (BDNFs) together with vascular endothelial growth factor (VEGF) has shown to increase the generation of new neurons

    -> injecting bone-marrow-derived MSCs (hBM-MSCs) into gyrus dentatus of healthy mice: promotion and differation of stem cells due to elevated secretion of NTFs such as VEGF, FGF2, etc[1]

1. Munoz JR, Stoutenger BR, Robinson AP et al. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippo- campus of mice. Proc Natl Acad Sci USA 2005;102:18171–18176. doi:10.1073/ pnas.0508945102.

Induction of Neuronal Regeneration

- Autism spectrum disorder model: after hBM-MSCs transplantation, mice have shown improved neurogenesis in hippocampus and improved cognitive function
  - Dramatic improvement in social behaviour and cognitive function 3 weeks following transplantation[1]
- Rat model of cerebral ischemia- enhaced neurogenesis associated with induced levels of NT1, VEGF following transplantation[2]

->neurogenesis was mediated by NTF secretion

1: Segal-Gavish H, Karvat G, Barak N et al. Mesenchymal stem cell transplantation pro- motes neurogenesis and ameliorates autism related behaviors in BTBR mice. Autism Res 2016;9:17–32. doi:10.1002/aur.1530.

2: Cova L, Armentero M-T, Zennaro E et al. Multiple neurogenic and neurorescue effects of human mesenchymal stem cell after trans- plantation in an experimental model of Par- kinson's disease. Brain Res 2010;1311:12–27. doi:10.1016/j.brainres.2009.11.041

Enhanced NTFs Secretion

- Improve neuronal survival, neuroprotection, reduction in oxidative stress, modulation of the inflammatory response
- Ex vivo protocol for programming astrocytic-like cells termed NTFs-secreting MSCs (MSC-NTFs)- elevate secretion of BDNF, GDNF, insulin-like growth factor ect.[1]
  - Conditioned media from MSC-NTFs promotes neural protection against oxidative stress and inhibits proliferation of immune cells in response to multiple sclerosis related antigens; promote survival after optic nerve injuries as well as sciatic nerve injuries; [2,3]

1: Sadan O, Bahat-Stromza M, Barhum Y et al. Protective effects of neurotrophic factor-secreting cells in a 6-OHDA rat model of Parkinson disease. Stem Cells Dev 2009; 18:1179–1190. doi:10.1089/scd.2008.0411.

2: Barhum Y, Gai-Castro S, Bahat-Stromza M et al. Intracerebroventricular transplantation of human mesenchymal stem cells induced to secrete neurotrophic factors attenuates clinical symptoms in a mouse model of multiple sclero- sis. J Mol Neurosci 2010;41:129–137. doi: 10.1007/s12031-009-9302-8.

3: Levkovitch-Verbin H, Sadan O, Vander S et al. Intravitreal injections of neurotrophic factors secreting mesenchymal stem cells are neuroprotective in rat eyes following optic nerve transaction. Investig Ophthalmol Vis Sci 2010;51:6394–6400. doi:10.1167/iovs.09- 4310.

Immunomodulation and Neuroinflammation

- Microglia activated-migrate along chemotactic gradient- recruit circulating immune cells- secrete proinflammatory cytokines, chemokines and reactive oxygen species- able to perform phagocytosis- chronic inflammation and neuronal death
- Cumulative evidence suggests that inflammation plays a major role in the progression of several neurodegenerative diseases
  - Enhanced microglia and astrocyte activation, proinflammatory cytokines, lymphocyte infiltration, etc

-> modulation of immune response toward an anti-inflammatory state emerges as a potential disease-modifying therapeutic strategy for neurodegeneration

Immunomodulation and Neuroinflammation

- Umbilical cord blood-derived MSCs (hUCB-MSCs) in an acute model of AD showed reduced levels of microglial and astrocytic activation as well as apoptosis[1]
- Familial AD model mice show much elevated levels of proinflammatory cytkines TNFalpha, IL-1-beta when compared to wild type mice- significantly reduced after transplantation with hUCB-MSCs[2]
  - + expression of anti-inflammatory markers IL-4, Arg-1. YM-1, etc was induced
- hMSCs also maintain BBB integrity- morphogenic changes in astrocytes and endothelial cells

-> another therapeutic advance for hMSCs following acute/chronic inflammation

1: Lee HJ, Lee JK, Lee H et al. The therapeutic potential of human umbilical cord blood- derived mesenchymal stem cells in Alzheimer's disease. Neurosci Lett. 2010;481:30–35. doi: 10.1016/j.neulet.2010.06.045. Epub 2010 Jun 19

2: Lee HJ, Lee JK, Lee H et al. Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impair- ment in an Alzheimer's disease mouse model through modulation of neuroinflammation. Neu- robiol Aging 2012;33:588–602. doi:10.1016/ j.neurobiolaging.2010.03.024

Immunomodulation and Multiple Sclerosis (MS)

- MS: chronic, autoimmune and neurodegenerative disease of the CNS, in which immune cells,(auto- reactive CD4+ T-helper cells), infiltrate into the CNS and pro- mote an inflammatory response, resulting in myelin injury and axonal loss
  - Modulating the immune response, through a shift from a proinflammatory response, mediated by cytokines secreted by T-helper 1 (Th1) cells, to an anti-inflammatory response, mediated by cytokines secreted by T-helper 2 (Th2) cells
- hBM-MSCs transplantation: migrate from the blood stream into CNS and localize to white matter demyelination sites[1]
  - Decrease in leukocyte infiltration, inhanced Th2 cytokine profile, oligodendrogenesis and IL4 and IL10 secretion; NFTs secretion

- 1.: Bai L, Lennon DP, Eaton V et al. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. Glia 2009;57: 1192–1203. doi:10.1002/glia.20841.

Protein Aggregate Clearance

- abnormal aggregation of proteins is a major hallmark of neurodegenerative diseases
- PD : formation of Lewy bodies, mostly composed of the a-synuclein protein
- HD : expansion of CAG repeats in the N-terminus of the huntingtin gene, resulting in protein polyglutamination
- AD: is associated with both extracellular amyloid plaques
- ALS: involves the intraneuronal formation of inclusions, mostly in spinal MNs, containing TAR DNA-binding protein 43 (TDP-43), FUS and SOD1 proteins

-> reducing abnormal protein aggregates by preventing aggregation as well as by imposed clearance of aggregating proteins is a main strategy for neurodegenerative disease therapy

Protein Aggregate Clearance

- Transplantation of hMSCs into AD model:
  - Secretion of Neprilysin (Ab-degrading enzyme) alongside with reduced Ab plaques in the hippocampus, emonstrating a role for hMSCs in enhancing the cells endogenous proteolytic machinery [1]
- hBM-MSCs in Ab-inoculated mice increased the survival of hippocampal neurons and reduced the levels of Ab due to mechanisms of autophagy (cellular pathway involved in protein and organelle degradation)[2]

1: Kim J-Y, Kim DH, Kim JH et al. Soluble intracellular adhesion molecule-1 secreted by human umbilical cord blood-derived mesen- chymal stem cell reduces amyloid-b plaques. Cell Death Differ 2012;19:680–691. doi: 10.1038/cdd.2011.140

2: Shin JY, Park HJ, Kim HN et al. Mesen- chymal stem cells enhance autophagy and increase b-amyloid clearance in Alzheimer disease models. Autophagy 2014;10:32–44. doi:10.4161/auto.26508.

Efficacy and Limitations of hMSCs-Based Therapy

- Efficient cell source for therapy, safely transplanted, no major ethical concerns, possess a wide therapeutic potential
  - meeting several key aspects of neurodegeneration, such as neuroprotection, immunomodulation, and protein aggregate clearance
- Recent comparative analyses have indicated variable protein secretion patterns and identified additional markers that differentially express in hMSCs derived from various origins (Bone marrow, adipose tissue)
  - may result in heterogeneous clinical outcomes->future studies should be performed to determine the relevant hMSCs sources

Efficacy and Limitations of hMSCs-Based Therapy

- Factors associated with hMSCs survival in the CNS were not identified yet
  - hMSCs survival in the CNS following transplantation was repeatedly reported to be limited up to several months in rodent models (due to immune response, hypoxic stress)

- hMSCs are repeatedly reported to home toward lesion sites and secrete a broad range of molecules that modulate various aspects of diseases
  - the use of hMSCs as vehicles for delivery of therapeutics into lesion area will provide an efficient platform for targeted therapy in various neurodegenerative diseases.

Table 1. Naïve human mesenchymal stem cells in murine models of neurodegeneration

	Authors	Cell source	Model animal	Suggested mechanism	Clinical improvement
Alzheimer's	[61]	Umbilical cord blood	Aβ-inoculated mice	Reduced glia activation, oxidative stress, and apoptosis	Improved learning/memory performance
Alzheimer's	[36]	Umbilical cord blood	APP/PS1 mice	Reduced Aβ and p-tau deposition Modulation of microglia activation	Improved spatial learning and memory decline
Alzheimer's	[89]	Umbilical cord blood	APP/PS1 mice	sICAM-1 mediated upregulation of neprilysin Induced Aβ clearance	Increased neuronal survival in vitro
Alzheimer's	[93]	Not mentioned	Aβ-inoculated mice	Induction of autophagy Induced Aβ clearance	Enhanced hippocampal neurons survival
Alzheimer's	[37]	Not mentioned	Aβ-inoculated mice	Enhanced WNT signaling Enhanced endogenous neurogenesis	Improved working memory
Parkinson's	[34]	Bone marrow	6-OHDA mice	NTF secretion Enhanced endogenous neurogenesis	Enhanced dopaminergic neurons survival
Parkinson's	[36]	Bone marrow	MPTP-induced mice	Enhance EGFR expression Enhanced endogenous neurogenesis	Enhanced dopaminergic neurons survival
Parkinson's	[94]	Bone marrow	MPTP-induced mice	Induction of autophagy Induced α-synuclein clearance	Enhanced dopaminergic neurons survival
Parkinson's	[63]	Bone marrow	α-Synuclein-inoculated mice	Modulation of microglia activation MSCs IL-4 secretion Induced α-synuclein clearance	Increased neuronal survival
Stroke	[35]	Bone marrow	Acute ischemia rats	NTF secretion Enhanced endogenous neurogenesis Reduced apoptosis	Reduced infarct volume Functional recovery in NSS
Stroke	[65]	stable bone marrow derived B10 line	Acute ischemia rats	Reduced microglia activation Reduced NFκB signaling	Reduced infarct volume
Stroke	[71]	Adipose	Acute ischemia rats	BBB integrity maintenance Reduced neutrophil infiltration Reduced endothelial vasculature damage	Enhanced neuronal survival Improved functional behavior
Stroke	[70]	Bone marrow	LPS induced rats	BBB integrity maintenance Reduced neutrophil infiltration modulation of microglia activation	Enhanced neuronal survival
MS	[41]	Bone marrow	EAE mice	NGF mediated axonal protection	Reduced mortality Reduced disease severity Reduced neuronal loss
MS	[73]	Bone marrow	EAE mice	Reduced immune cell infiltration Induced Th2 immune response Enhanced oligodendrogenesis	Deceleration in disease progression
MS	[74]	Adipose	EAE mice	Decreased spinal cord inflammation Decreased demyelination Induced Th2 immune response Neurotrophic factor secretion Enhanced oligodendrogenesis	Reduced disease severity Reduced axonal loss
MS	[75]	Bone marrow	EAE mice	Reduced demyelination	Decreased white matter lesions Reduced disease severity
MS	[78]	Placenta	EAE mice	Neuroprotection Immunomodulation mediated by reduced TSG6 expression	Reduced disease severity
MS	[77]	Decidua	EAE mice	Decreased inflammatory infiltration Immunomodulation	Reduced disease severity

Table 2. Ex vivo manipulated human mesenchymal stem cells in murine models of neurodegeneration

	Authors	Source	Ex vivo manipulation	Model animal	Suggested mechanism	Clinical improvement
Parkinson's	[18]	Bone marrow	NICD transduction and NTF induction	6-OHDA rats	GDNF secretion Dopamine secretion	Decreased apomorphine induced rotations
Parkinson's	[5]	Bone marrow	MSC-NTF	6-OHDA rats	NTF secretion	Decreased amphetamine induced rotations Reduced dopamine depletion Enhanced striatal regeneration
Parkinson's	[52]	Bone marrow	NICD and GDNF transduction	6-OHDA rats	GDNF secretion	Decreased amphetamine induced rotations Dopamine-fibers rejuvenation
Stroke	[17]	Bone marrow	Neuronal differentiation by NGN1 overexpression	Acute Ischemia rats	Enahnced endogenous neurogenesis MSC neuronal differentiation Anti inflammation	Improved motor recovery Reduced ischemic core
MS	[51]	Bone marrow	MSC-NTF	EAE mice	NTF secretion Immunomodulation	Increased survival Delayed symptom onset
MS	[79]	Adipose	IL-4 transduction	EAE mice	Induced Th2 immune response	Reduced disease severity
MS	[80]	Adipose	IL-10 transduction	EAE mice	Induced Th2 immune response	Reduced disease severity
ALS	[53]	Bone marrow	GDNF and VEGF transduction	SOD1 rats	NTF secretion	Increased survival Slower motor deterioration Improved NMJ innervation Enhanced motor neuron survival
Huntington	[48]	Bone marrow	MSC-NTF	QA-induced rats	NTF secretion	Reduced striatal atrophy Decreased apomorphine induced rotations
Huntington	[54]	Bone marrow	BDNF transduction	YAC128 and R6/2 mice	BDNF secretion	Increased survival Reduced anxiety behavior Decreased striatal atrophy Enhanced neurogenesis
Schiatic nerve injury	[51]	Bone marrow	MSC-NTF	Schiatic nerve crushed rats	Neurotrophic factor secretion	Improved motor functions Lateral reflex recovery Axonal regeneration Reduced NMJ degeneration
Optic nerve injury	[50]	Bone marrow	MSC-NTF	Optic nerve transectioned rats	NTF secretion	Increased survival of retinal ganglion cells

Table 3. Human mesenchymal stem cells in human clinical trials

	Authors	Cell source	Administration	Number of patients enrolled	Clinical outcome
MS	[95]	Allogenic, Umbilical cord blood	Intravenous injection	13 in treatment group, 10 in control group	Improved functional performance Reduced relapse occurrence Enhanced Th2 response
MS and ALS	[98]	Autologous, Bone marrow	Intathecal and Intravenous injections.	15 in MS treatment group, 19 in ALS treatment group	Improved functional performance in MS patients Slower disease progression rate in ALS patients Enhanced anti-inflammatory response
MS	[97]	Autologous, Bone marrow	Intrathecal injection	7 patients in the treatment group	Clinical stability Enhanced peripheral blood FoxP3 expression
MS	[99]	Autologous, Bone marrow	Intravenous injection	10 patients in the treatment group	Improved visual acuity Increased optic nerve area
MSA	[36]	Autologous, Bone marrow	Intra-arterial and Intravenous injections	11 in treatment group, 16 in placebo group	Smaller increase in clinical severity. Reduced size of glucose hypo-metabolism areas.
Parkinson's	[106]	Autologous, Bone marrow	Stereotactic injection into the lateral ventricles	7 patients in the treatment group	Steady improvement in clinical score in 3/7 patients
Stroke	[3]	Autologous, Bone marrow	Intravenous injection	5 in treatment group, 25 in control group	Decreased brain atrophy Improved functional performance
Stroke	[61]	Autologous, Bone marrow	Intravenous injection	16 in treatment group, 36 in control group	Reduced mortality Improved functional performance
Stroke	[105]	Autologous, Bone marrow	Intravenous injection	12 patients in the treatment group	Improved neurological scores Reduced infarct size
ALS	[101]	Autologous, Bone marrow	Intrathecal injection	8 patients in the treatment group	Slower disease progression rate Elevated anti-inflammatory markers
ALS	[100]	Autologous, Bone marrow	Intrathecal injection	7 patients in the treatment group	Slower disease progression rate
ALS	[103]	Autologous, Bone marrow, ex vivo differentiated	Intramuscular and intrathecal injections.	26 patients in the treatment group	Slower disease progression rate
ALS	Brainstorm Cell therapeutics	Autologous, Bone marrow, ex vivo differentiated	Intramuscular and intrathecal injections.	36 in treatment group, 12 in control group	Decreased inflammatory markers Slower disease progression rate

Thank you for your attention!