Mesenchymal stem cell-derived extracellular vesicles, a potential new tool in regenerative medicine
Regenerative Medicine and Mesenchymal Stem Cells
MSCs und regenerative Medizin

Gebler et al. (2012). TiMM 18, 128-134

MSCs get captured in the Lung

Myocardial infarction ischemic stroke ...

Graft-versus-Host Disease Morbus Crohn ...

MSCs act in a paracrine manner
MSCs and regenerative Medicine

Gebler et al. (2012). TiMM 18, 128-134

MSCs and regenerative Medicine

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MSCs and regenerative Medicine

MSC-EVs

Graft-versus-Host Disease

Morbus Crohn

Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury

MSC-EVs

Graft-versus-Host Disease

Morbus Crohn

Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury

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Graft-versus-Host Disease

Morbus Crohn

Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury

MSC-EVs

Graft-versus-Host Disease

Morbus Crohn
MSCs and regenerative Medicine: MSCs might act via extracellular vesicles

http://www.ncmr.co.jp
Ludwig and Giebel (2012). IJBCB 44, 11-15

Myocardial infarction
ischemic stroke
...

Graft-versus-Host Disease
Morbus Crohn
...
The EV field is very young
Acute Graft-versus-host disease

Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells

Katarina La Blanc, Ida Rasmusson, Berit Sundberg, Cecilia Götherström, Moustapha Hassan, Mehmet Uzunel, Olof Ringdén

Adult bone-marrow-derived mesenchymal stem cells are immunosuppressive and prolong the rejection of mismatched skin grafts in animals. We transplanted haploidentical mesenchymal stem cells in a patient with severe treatment-resistant grade IV acute graft-versus-host disease of the gut and liver. Clinical response was striking. The patient is now well after 1 year. We postulate that mesenchymal stem cells have a potent immunosuppressive effect in vivo.

Lancet 2004; 363: 1439–41
See Commentary page 1411

A 9-year-old boy with acute lymphoblastic leukaemia in third remission received a transplant of blood stem cells from an HLA-A, HLA-B, HLA-DRβ1 identical, unrelated, female donor after conditioning with cyclophosphamide (120 mg/kg) and fractionated total body irradiation (3 Gy for 4 days). Immunosuppression included thymoglobulin (6 mg/kg) during the conditioning, followed by ciclosporin combined with four doses of methotrexate. On day 11 after allogeneic stem-cell transplantation, the patient developed a maculopapular rash of the thorax and back that progressed despite treatment with prednisolone (2 mg/kg daily). By
Acute Graft-versus-host disease

Allogeneic stem cell therapy exert Graft-versus Leukaemia effects, but also can lead to GvHD

Volunteer

Collection

Bone marrow (allogeneic)

MSC culture

http://www.ncmr.co.jp

Bone marrow (allogeneic)

EV Preparation

Administration

Ludwig and Giebel (2012). IJBCB 44, 11-15
In vitro and in vivo effects of MSC-EVs

Kordelas et al., Leukemia (2014).

Clinical MSC application:
0.5-2 x 10^6 cells/kg

80 kg patient: 0.4-1.6 x 10^8 cells

1 unit: exosomes 4.0 x 10^7 MSCs secrete within 48 hours

Application | time course | administered units
--- | --- | ---
1 | day 0 | 0.1 units
2 | day 2 | 0.2 units
3 | day 4 | 0.3 units
4 | day 7 | 0.4 units
5 | day 9 | 1 unit
6 | day 11 | 2 units
7 | day 14 | 4 units

Patienten PB-MNCs

Giebel/Paris, FSEV-2017
Are MSC-EVs therapeutically as effective as MSCs?
MSC-EVs in ischemic stroke treatment

Döppner et al., Stem Cell TM (2015)
Mesenchymal Stromal Cell-Derived Extracellular Vesicles Protect the Fetal Brain After Hypoxia-Ischemia

DAAN R.M.G. OPHELDERS, TIM G.A.M. WOLFS, REINT K. JELLEMA, ALEX ZWANENBURG, PETER ANDRIESSEN, TAMMO DELHAAS, ANNA-KRISTIN LUDWIG, STEFAN RADTKE, VERA PETERS, LEON JANSSEN, BERND GIEBEL, BORIS W. KRAMER

Key Words. Hypoxia-ischemia • Brain injury • Preterm • Mesenchymal stromal cells • Extracellular vesicles • Exosomes

STEM CELLS TRANSLATIONAL MEDICINE 2016;5:754–763

Full-length Article

Mesenchymal stem cell-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury

Karla Drommeltschmidt, Meray Serdar, Ivo Bendix, Josephine Herz, Frederik Bertling, Sebastian Prager, Matthias Keller, Anna-Krystin Ludwig, Vikas Duhan, Stefan Radtke, Kyra de Miroshedji, Peter A. Horn, Yohan van de Looij, Bernd Giebel, Ursula Fielderhoff-Müser
MSC-EV application in animal models

acute and chronic kidney failure ([Camussi; Bruno et al., 2009](Camussi; Bruno et al., 2009))
(Gatti et al., 2011; Bruno et al., 2012; He et al., 2012; Kilpinnen et al., 2013; Reis et al., 2012; Zhou et al., 2013; Zou et al., 2014)

myocardial infarction ([Lim and Kleijn; Lai et al., 2010](Lim and Kleijn; Lai et al., 2010))

hypoxia-induced pulmonary hypertension ([Lee et al., 2012](Lee et al., 2012))

hind limb ischemia ([Zhang et al., 2012](Zhang et al., 2012))

ischemic occlusion in a rat stroke model ([Xin et al., 2013](Xin et al., 2013))

survival of allogeneic skin grafts ([Zhang et al., 2013](Zhang et al., 2013))

liver damage ([Li et al., 2013; Tan et al., 2014](Li et al., 2013; Tan et al., 2014))

*E. coli* endotoxin-induced acute lung injury ([Zhu et al., 2014](Zhu et al., 2014))

re-epithelialization following skin burn ([Zhang et al., 2014](Zhang et al., 2014))

....
Articles on MSC-EVs (06.11.2017)
Search term: ("mesenchymal stem cells" and "exosomes") or ("mesenchymal stem cells" and "microvesicles") or ("mesenchymal stem cells" and "extracellular vesicles")
Conclusion

MSC-EVs provide therapeutic activities in many disease models, apparently in a comparable manner than MSCs.
Challenges

Translate MSC-EVs into the clinics
(initially to treat additional GvHD patients)
To-Dos

Optimization of MSC expansion protocols
(for the production of therapeutically active EVs)

Optimization of EV purification methods
(different methods, GMP)

Optimization of analyzing tools
(Analysis of extracellular vesicles using imaging flow cytometry)

Qualification of potency assays

Identification and qualification of surrogates
(proteins, lipids, microRNAs)

REGULATIONS, REGULATIONS, REGULATIONS

....
POSITION PAPER

Applying extracellular vesicles based therapeutics in clinical trials – an ISEV position paper

Thomas Lener¹,², Mario Gimona¹,², Ludwig Aigner¹, Verena Börger³, Edit Buzas⁴, Giovanni Camussi⁵, Nathalie Chaput⁶,⁷, Devasis Chatterjee⁸,⁹, Felipe A. Court¹⁰, Hernando A. del Portillo¹¹,¹², Lorraine O’Driscoll¹³,¹⁴, Stefano Fais¹⁵, Juan M. Falcon-Perez¹⁶,¹⁷, Ursula Felderhoff-Mueser¹⁸, Lorenzo Fraile¹⁹, Yong Song Gho²⁰, André Görgens³, Ramesh C. Gupta²¹,²², An Hendrix²³, Dirk M. Hermann²⁴, Andrew F. Hill²⁵, Fred Hochberg²⁶, Peter A. Horn³, Dominique de Kleijn²⁷, Lambros Kordelas²⁸, Boris W. Kramer²⁹, Eva-Maria Krämer-Albers³⁰, Sandra Laner-Plamberger¹,², Saara Laitinen³¹, Tommaso Leonardi³²,³³, Magdalena J. Lorenowicz³⁴, Sai Kiang Lim³⁵, Jan Lötvall³⁶, Casey A. Maguire³⁷, Antonio Marcilla³⁸,³⁹, Irina Nazarenko⁴⁰, Takahiro Ochiya⁴¹, Tushar Patel⁴², Shona Pedersen⁴³, Gabriella Pocsfalvi⁴⁴, Stefano Pluchino³², Peter Quesenberry⁸,⁹, Ilona G. Reischl⁴⁵, Francisco J. Rivera⁴⁶, Ralf Sanzenbacher⁴⁷, Katharina Schallmoser¹,², Ineke Slaper-Cortenbach⁴⁸, Dirk Strunk⁴⁹, Torsten Tonn⁵⁰, Pieter Vader⁵¹,⁵², Bas W. M. van Balkom⁵³, Marca Wauben⁵⁴, Samir El Andaloussi⁵²,⁵⁵, Clotilde Théry⁷,⁵⁶, Eva Rohde¹,²*, and Bernd Giebel³*

Citation: Journal of Extracellular Vesicles 2015, 4: 30087 - http://dx.doi.org/10.3402/jev.v4i3.30087
Concise Review: Developing Best-Practice Models for the Therapeutic Use of Extracellular Vesicles

Key Words. Stem cells • Cellular therapy • Clinical trials • Clinical translation • Extracellular vesicles • Therapeutics • Exosomes • Microvesicles
Challenges

Heterogeneity
Acute Graft-versus-host disease

Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells

Katarina Le Blanc, Ida Rasmusson, Berit Sundberg, Cecilia Götherström, Moustapha Hassan, Mehmet Uzunei, Olof Ringdén

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Acute Graft-versus-host disease

Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells


The mesenchymal stromal cells dilemma—does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road?

Galipeau (2013). Cytotherapy 15; 2-8
Mesenchymal Stem/Stromal cells (MSCs)

CD44  CD73  CD90
MFI:17.9  25.5  19.7

CD105  CD146
14.6  4.4

CD14  CD31

CD146  CD31

CD34  CD45

Morphology  Osteogenesis  Adipogenesis  Chondrogenesis

Radtke et al. (in preparation)
Mesenchymal Stem/Stromal cells (MSCs)
Heterogeneity of MSCs

Radtke et al., Cell Cycle 2016

Giebel/Paris, FSEV-2017
Heterogeneity of MSCs

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Radtke et al., Cell Cycle 2016
MSCs seem to provide a heterogeneous cell entity

Not all MSC sub-types seem to mediate clinical impacts

Do MSC-EV fractions differ in their therapeutic activity?
MSCs exert immune suppressive functions

Inhibitory effect of BM-MSCs on T-lymphocyte proliferation induced by allogeneic PBLs, DCs, or PHA

Di Nicola et al., Blood 2002
Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

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Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

0.05 Unit

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Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

Giebel/Paris, FSEV-2017
Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

MSC 1-20 ALT EV B1 based on cell equivalents (Units)

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Heterogeneity of MSCs
Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

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MSCs are expanded in human Platelet Lysate supplemented media

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Hemeda et al., Cytotherapy 2014, Radtke et al., ISBT Science Series 2014
Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

Effect related to source media volume MSC 1-20 ALT B1

Proliferation in %

source media volume in ml

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Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

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Unsupervised Clustering
Heterogeneity of MSC-EV functions
Modulation of PHA induced T cell proliferation

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Conclusion

Conventional T cell proliferation assays may not help to identify MSC-EV fractions with the highest therapeutic potentials.
Challenges

MSC-EV heterogeneity

Method

Analysis (Quality assurance)
Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy

Viktoriya Sokolova¹, Anna-Kristin Ludwig¹, Sandra Hornung³, Olga Rotan², Peter A. Horn¹, Matthias Epple²,³, Bernd Giebel¹,²

Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis

Rebecca A. Dragovic, MSc¹, Christopher Gardiner, PhD¹,², Alexandra S. Brooks, MD¹, Dione S. Tannetta, PhD², David J.P. Ferguson, PhD³, Patrick Hole, PhD³, Bob Carr, PhD³, Christopher W.G. Redman, MD, FRCGP, Adrian L. Harris, MD, PhD, FRCPEd, Peter J. Dobson, PhD, FInstP, CPhys², Paul Harrison, PhD³, Ian L. Sargent, PhD³,²
Human Hematopoiesis

Multi-Parameter Flow Cytometry / Sorting

Beckmann et al., Blood 2007; Görgens et al., Cell Reports 2013; Görgens et al, Stem Cell Reports 2014; Radtke et al., British J. Haematology 2015; Radtke et al., Cell Cycle 2016
Imaging Flow Cytometry
Amnis ImageStream X MkII

Görgens, A.
Science/AAAS
Technology Webinar Series
June 22, 2016
Analysis of extracellular vesicles including exosomes by imaging flow cytometry
Re-Evaluation von EV Isolation Protokollen

Suspension Cell Culture

2,000 x g Supernatant

10,000 x g Supernatant

0.22 µm Filtrate

100,000 x g Pellet

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Heterogeneity of MSC-EV fractions
Analyses at the single EV-level

Non-conditioned hPL containing media
MSC-conditioned hPL containing media
MSC-conditioned serum-free media

Giebel/Paris, FSEV-2017
To-Dos

Optimization of MSC expansion protocols
(for the production of therapeutically active EVs)

Optimization of EV purification methods
(different methods, GMP)

Optimization of analyzing tools
(Analysis of extracellular vesicles using imaging flow cytometry)

Qualification of potency assays

Identification and qualification of surrogates
(proteins, lipids, microRNAs)

REGULATIONS, REGULATIONS, REGULATIONS

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German Society for Extracellular Vesicles

It is the goal of GSEV to promote extracellular vesicles research and to support young academics within the field.

- Founded: March 2\textsuperscript{nd} 2017 in Düsseldorf at the IGLD meeting
- Founding session was visited by approx. 150 persons
- Number of Members: 8 founding members, member recruitment will start in June 2017
- Membership: no geographical restriction
- Sector of Members: academic, clinical and industry

www.extracellular-vesicles.de  Giebel/Paris,FSEV-2017


