Regenerative Medizin: Therapie mit humanen Zellen/Faktoren
(Human Cell-Based Medicinal Products)

Eva Rohde, Paracelsus Medizinische Privatuniversität / Salzburger Landeskliniken
Universitätsklinik für Blutgruppenserologie und Transfusionsmedizin

Spinal Cord Injury - Tissue Regeneration Center Salzburg, SCI-TReCS
LETTER OF INTENT

FOUNDATION OF THE
“SPINAL CORD INJURY AND TISSUE REGENERATION
CENTER SALZBURG (SCI-TRECS)” AT THE
PARACELSUS MEDICAL UNIVERSITY

GRÜNDUNGSBESCHLUSS FÜR DAS
QUERSCHNITT- UND
GEWEBEREGENERATIONSZENTRUM SALZBURG
DER PARACELSUS MEDIZINISCHEN PRIVATUNIVERSITÄT

Salzburg, November 22, 2011
Spinal Cord Injury - Tissue Regeneration Center Salzburg
SCI-TReCS Mandate

- SCI-related clinical research projects
- Providing best state of the art treatment and care of patients with spinal cord injury (SCI)
- Developing novel integrative therapeutic strategies for tissue regeneration and functional repair after organ injury
PMU / SCI-TReCS
Basic Science & Development
Under Constructions (IX/2013)
SCI-TReCS Road Map

• Internationally Recognized Scientific Location
  Within 5 - 10 Years Attracting Further Specialists
• Focus on SCI & Regenerative Medicine
• Persisting Multidisciplinary Efforts Should Result in Successful
  Basic Science Output & Long-term Clinical Projects

Years 1 – 3
Establishing SCI-TReCS
Design of 1st
Clinical Trials

Years 3 – 5
Consolidation
Starting with
Clinical Trials?

Beyond Year 5
Ongoing & Emerging Clinical Trials
IPR Royalties, 3rd Party-Financing
New Partners & Sponsors
SCI-TReCS Mission

Practising Multi-Disciplinarity

- **Basic Research (Discovery)**
  - An interdisciplinary effort bridging basic, preclinical, translational and clinical research on SCI and tissue regeneration

- **Translation**
  - Clinical trials to translate promising research into clinical therapies

- **Application**
  - Focus on quality of life of SCI patients
  - Development of innovative electronic/computational, pharmacological, cell-rehabilitation-based therapies

- **Patient Care**
  - Best practice care for SCI patients
  - Closed care circles for SCI patients
SCI-TReCS Approach

Practising Multi-Disciplinarity

Basic Research (Discovery)

Translation

Application

Patient Care

Clinical Wards: Traumatology, Neurology, Urology, ...

Neuro-Surgery, Rehabilitation Centers, Nursing Sciences, ...

Experimental & Clinical Cell Therapy

Molecular Regenerative Medicine

Experimental Neuro-Regeneration

Bone & Tendon Regeneration

GMP-Facility / Transfusion Medicine
Spinal Cord Injury and Tissue Regeneration Center
Salzburg (SCI-TReCS)
Organigram Draft – Version 1.0

International Scientific Advisory Board

Local Scientific Steering Committee

SCI-TReCS Operating Director
(TBA) Administrative & Coordinating Support
for Scientific Units and Core Facilities

Clinical Scientific Units
Hospital wards

Acute Surgery
Pelvic Floor Neurostimulation
Neuro-Rehabilitation
Neurophysiology and Paraplegiology
Neuro-Imaging

Preclinical Scientific Units
Strubergasse

Molecular Regenerative Medicine
Exp. SCI & Preclinical Neuro-Rehab.
Clinical & Experimental Cell Therapy
Bone & Tendon Regeneration

Core Facilities:

Small Animal Imaging
Flow cytometry
Animal Surgery
Microscopy
GMP-Facility
Histology
Molecular Biology
Bio-Hazard
Bio-Informatics -Statistics
$1 \times 10^9 = \text{human setting}\ 2.8\text{m}^2$

$1 \times 10^6 = \text{mouse setting, 10cm}^2$
Defined GMP-Requirements

- Personell
  - Responsibilities
  - Quality Training / Awareness
  - Continuous Improvement

- Facilities
  - Defined Lab Conditions
  - Defined Climate Condition

- Human Cell-based Medicinal Products (=pharmaceutical definition)
  - Process validation
  - Product licence (IMPD)
  - CT, Ethical Issues
  - Documentation / 30y traceability
GMP-Requirements (cell products)

Chapter 16:
Animal protein-free expansion of human mesenchymal stem / progenitor cells

Katharina Schallmoser,1,2 Nathalie Etchart,1,2,3 Dirk Strunk1,3 and Eva Rohde1,4

1Stem Cell Research Unit
2University Clinic of Blood Group Serology and Transfusion Medicine
3Department of Hematology and Stem Cell Transplantation, University of Internal Medicine
Medical University of Graz

1.4

GMP-COMPLIANT PROPAGATION OF HUMAN MULTIPOTENT MESENCHYMAL STROMAL CELLS

Eva Rohde, Katharina Schallmoser, Christina Bartmann, Andreas Reinisch, and Dirk Strunk
Medical University of Graz, Graz, Austria

Pharmaceutical Manufacturing Handbook: Regulations and Quality, edited by Shayne Cox and
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Regenerative Therapy Using Blood-Derived Stem Cells

Series: Stem Cell Biology and Regenerative Medicine
Alan, David S.; Strunk, Dirk (Eds.)
<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Source</th>
<th>Pages</th>
<th>DOI</th>
<th>Publication Date</th>
</tr>
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<tbody>
<tr>
<td>Human platelet lysate can replace fetal bovine serum for clinical-scale expansion of functional mesenchymal stromal cells</td>
<td>Schallmoser Katharina; Bartmann Christina; Rohde Eva; et al.</td>
<td>TRANSFUSION; Volume: 47 Issue: 8; Pages: 1436-1446; DOI: 10.1111/j.1537-2995.2007.01220.x</td>
<td>14</td>
<td>AUG 2007</td>
<td></td>
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<tr>
<td>Immune cells mimic the morphology of endothelial progenitor colonies in vitro</td>
<td>Rohde Eva; Bartmann Christina; Schallmoser Katharina; et al.</td>
<td>STEM CELLS; Volume: 25 Issue: 7; Pages: 1746-1752; DOI: 10.1634/stemcells.2006-0833</td>
<td>8</td>
<td>JUL 2007</td>
<td></td>
</tr>
<tr>
<td>Human Alternatives to Fetal Bovine Serum for the Expansion of Mesenchymal Stromal Cells from Bone Marrow</td>
<td>Bieback Karen; Hecker Andrea; Koekoemer Asli; et al.</td>
<td>STEM CELLS; Volume: 27 Issue: 9; Pages: 2331-2341; DOI: 10.1002/stem.139</td>
<td>0</td>
<td>2009</td>
<td></td>
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<tr>
<td>Humanized system to propagate cord blood-derived multipotent mesenchymal stromal cells for clinical application</td>
<td>Reinisch Andreas; Bartmann Christina; Rohde Eva; et al.</td>
<td>REGENERATIVE MEDICINE; Volume: 2 Issue: 4; Pages: 371-382; DOI: 10.2217/17460751.2.4.371</td>
<td>5</td>
<td>JUL 2007</td>
<td></td>
</tr>
<tr>
<td>Two steps to functional mesenchymal stromal cells for clinical application</td>
<td>Bartmann Christina; Rohde Eva; Schallmoser Katharina; et al.</td>
<td>TRANSFUSION; Volume: 47 Issue: 8; Pages: 1426-1435; DOI: 10.1111/j.1537-2995.2007.01219.x</td>
<td>7</td>
<td>AUG 2007</td>
<td></td>
</tr>
<tr>
<td>Rapid large-scale expansion of functional mesenchymal stem cells from unmanipulated bone marrow without animal serum</td>
<td>Schallmoser Katharina; Rohde Eva; Reinisch Andreas; et al.</td>
<td>TISSUE ENGINEERING PART C-METHODS; Volume: 14 Issue: 3; Pages: 185-196; DOI: 10.1089/tengtec.2008.0060</td>
<td>1</td>
<td>SEP 2008</td>
<td></td>
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<tr>
<td>Humanized large-scale expanded endothelial colony-forming cells function in vitro and in vivo</td>
<td>Reinisch Andreas; Hofmann Nicola A.; Oberauf Anna C.; et al.</td>
<td>BLOOD; Volume: 113 Issue: 26; Pages: 6716-6725; DOI: 10.1182/blood-2008-09-181362</td>
<td>0</td>
<td>JUN 25 2009</td>
<td></td>
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<td>Replicative senescence-associated gene expression changes in mesenchymal stromal cells are similar under different culture conditions</td>
<td>Schallmoser Katharina; Bartmann Christina; Rohde Eva; et al.</td>
<td>HAEMATOLOGICA:THE HEMATOLOGY JOURNAL; Volume: 95 Issue: 6; Pages: 867-874; DOI: 10.3324/haematol.2009.011692</td>
<td>0</td>
<td>JUN 2010</td>
<td></td>
</tr>
</tbody>
</table>
Process Optimization

STEP I

BM aspiration
day 0

4 x 2.5mL heparinized BM Aspiration diluted immediately (without density gradient) in

α-MEM / 10% HPL

α-MEM / 10% FBS

1 x 10^6 MNC / 60mL / 225cm² in
10 - 20 x T225 or 1 - 2 CF-4 (< 10^6 BM-MNC/cm²)

1° SEEDING
day 0

8 x 10^6 MSC / 225cm²

1 x 10^6 MSC / 225cm²

1° HARVEST
Δ 10 - 16 days

STORE: n x 3x10⁵ MSC_HPL aliquots

STORE: n x 1x10⁶ MSC_Isc aliquots

STEP II

2° SEEDING
day 0

α-MEM / 10% HPL

α-MEM / 10% FBS

3 x 10⁵ MSC / 1m²

3 x 10⁶ MSC / 1m²

2° HARVEST
Δ 11 - 15 days

3.0 - 5.4 x 10⁶ MSC_HPL

0.5 - 1.1 x 10⁶ MSC_FBS

Optimization II

Figure 12.3
Inverse Correlation of Seeding Density to MSC Proliferation.

<table>
<thead>
<tr>
<th>Seeding Density</th>
<th>day 1</th>
<th>day 3</th>
<th>day 5</th>
<th>day 10</th>
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<tbody>
<tr>
<td>1000/cm²</td>
<td>![Image]</td>
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<tr>
<td>100/cm²</td>
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<tr>
<td>10/cm²</td>
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<tr>
<td>1/cm²</td>
<td>![Image]</td>
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</tbody>
</table>

Choice of Supplements

Figure 12.4
MSC Proliferation Capacity Depends on Seeding Density in Xenogeneic FBS and Human Platelet Lysate-supplemented Cultures.

MSC cultured for 13 days

Identity/Purity
Phenotype Characterization

Figure 12.5
Immune Phenotype of Human MSC.

Morphology & Proliferation

Figure 12.6
Morphologic Evaluation of MSC.

Figure 12.8
Immunomodulatory Function of MSC

Serum Supplementation versus Serum-Free Culture

Figure 1.

A

Population doublings

Cloning efficiency %

0 2 4 6 8 10 12 14 16 18 20

Serum-free Medium α-MEM + FBS α-MEM + pHPL

B

Serum-free Medium α-MEM + FBS α-MEM + pHPL

MSC Detachment  
Efficiency & Viability

Proliferation & Migration

MSC ECFC 0 min. 24 hrs.
Proliferation & Migration

M. Gimona, Transfusion Medicine Salzburg
Proliferative and Migratory Capacity

xCELLigence System, Impedance Measurement
“Real-time Cell-based Assays”

M. Gimona & T. Lener, Transfusion Medicine Salzburg
Basic Research

STEM CELL RESEARCH UNIT
Medical University of Graz, Austria

Andreas Reinisch
Nicole Hofmann
Nathalie Etchart
Daniela Thaler
Margret Frühwirth
Rokhsareh Rohban
Christina Bartmann
Katharina Schallmoser
Anna Ortner
Monica Farrell
Claudia Url
Birth Feihauer

Dirk Strunk

HPL
Human Platelet Lysate

Exosomes
mRNA/miRNA/Protein Containing Microvesicles

IgG
bFGF
EGF
HGF
CTGF
IGF-1
TGF-β
VEGF-A, -C
PDGF
Factor V
Multimerin
Factor VIII
MMPs
α2- 
Macroglobulin
Plasminogen
PAI-1
CXCL-5
MIP-1α
RANTES
CCL17
IL-8
P-Selectin
Von Willebrand Factor
Thrombospondin
Fibrinogen
Integrin αIIbβ3
Integrin αvβ3
Fibronectin

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MSC-ECFC signaling in vivo: Modulating Neovasculogenesis?

Macroscopy

HE

CD31

accepted in: PLOS ONE 2013
R. Rohban, E. Rohde, D. Strunk, Graz-Salzburg
Revealing the Active Substances of Pooled Human Platelet Lysate, pHPL
Cell Culture / Differentiation / Regeneration

A Whole blood donation

Whole blood unit

Centrifugation
4,250 x g, 15 min, 22°C

Plasma
Buffy coat
Red blood cells

Separation

4 Buffy coat units + 1 Plasma unit or Alternative solution

Pooling

Centrifugation
340 x g, 6 min, 22°C

B Single donor platelet apheresis

PLATELET CONCENTRATE

Separation and Leukocyte depletion

= 1x10⁹ Plts/mL

≈ 300 mL

C pHPL

= 3 - 4 L

Aliquoting

Second Freeze / Thaw Step

Cen trifugation
4,000 g, 15 min, 4°C

= 20 x 150 mL

PLATELET FRAGMENTS

= 3 x 30 mL

- the microenvironment decisively modulates cell fate

- cells contribute to their own microenvironment
  (ECM, soluble factors, microvesicles)

Thus, the differentiation of stem and progenitor cells can be seen as a consequence of at least three regulatory processes:

1) Mechanotransduction  (response to ECM, stiffness etc.)

2) Growth factor receptors  (response to the receptor mediated induction of signal transduction cascades)

3) Exosomes  (response to intracellularly delivered RNA species and proteins)
**Characterization of Stem Cell Exosomes**

**Nanoparticle Tracking Analysis (NTA)**

Exosomes are mRNA/miRNA/Protein containing small vesicles delivering "Molecular Cargo" between cells.

**Exosome Western Blots**

- CD9

Particle size ~ 30-100nm

Mean: 130nm +/- 18nm
Purification of stem cell exosomes from conditioned media

Conditioned Culture Medium

- Supernatant #1
  - Supernatant #2
  - Supernatant #3 (discarded)

Pellet 1 (discarded)

- Pellet 2 (discarded)

- Pellet 3
  - 2 x wash and centrifugation @ 120,000 x g
  - Exosomes used for experiments
Studying Osteogenesis- / Adipogenesis- Modulation with Exosomes

Osteo-Induction (+HPL) + BM-MSC exosomes + ECFC exosomes

Adipo-Induction + BM-MSC exosomes + ECFC exosomes

Manuscript in preparation
M. Gimona, T. Lener & D. Peckl-Schmid, D. Streif, M. Öller, K. Schallmoser E. Rohde, Salzburg
## Uptake of heterotypic stem cell exosomes

<table>
<thead>
<tr>
<th>BM-MSPC with UC-ECFC exosomes</th>
<th>UC-ECFC with BM-MSPS exosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Phalloidin" /></td>
<td><img src="image2" alt="Phalloidin" /></td>
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<tr>
<td><img src="image3" alt="PKH67 control" /></td>
<td><img src="image4" alt="PKH67 control" /></td>
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<td><img src="image5" alt="Phalloidin" /></td>
<td><img src="image6" alt="Phalloidin" /></td>
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<tr>
<td><img src="image7" alt="PKH67" /></td>
<td><img src="image8" alt="PKH67" /></td>
</tr>
</tbody>
</table>

**Immunofluorescence microscopy images**

- **red:** Alexa 568 phalloidin
- **green:** PKH67
Heterotypic Exosomes Increase Proliferation (xCELLigence® Analyses)

BM-MSPC

UC-ECFC

Cell culture conditions: alpha MEM + 10% human platelet lysate (HPL)
Cell index values determined by impedance measurements using the xCELLigence System from ACEA after 3 days in culture
Cell counting performed after 14 days in culture
Exosomes & Osteogenic Lineage Induction

Exosomes do NOT induce differentiation

Uninduced

Uninduced + BM-MSPC (homotypic) Exosomes

Uninduced + UC-ECFC (heterotypic) Exosomes

Alizarin Red S staining at day 14 post osteogenic induction
However, exosomes modulate the speed and magnitude of differentiation during osteogenic induction.
Exosomes & Adipogenic Lineage Induction

Sudan 3 staining at day 14 post osteogenic induction

- Uninduced
- Induced
- Adipogenic induction + BM-MSPC Exosomes (homotypic)
- Adipogenic induction + UC-ECFC Exosomes (heterotypic)

- noninduced
- induced
- induced + BM-MSPC exosomes
- induced + UC-ECFC exosomes
Development of Human Cell-based Medicinal Products for Regenerative Therapies Needs:

- Personell Practising Pharmazeutikal QM
- Production Facilities / Clinical Environment
- Basic Science Facilities / Brain Power

To Cover the Complex Requirements of:

- Quality and Manufacturing Aspects
- Non-Clinical Development
- Clinical Development

Precondition for Clinical Studies to Evaluate Cell-based Therapies – GMP for RegMed Needs Much Ressources

**Transfusion Medicine Provides the Perfect Link!**
Transfusion Medicine:

M. Gimona

M. Öller

K. Schallmoser

D. Streif

T. Lener

S. Laner-Plamberger

D. Peckl-Schmid
Thank You For Your Attention!

We Are Also Grateful to:

Paracelsus Medical University Salzburg

Salzburger Landeskliniken

„Wings For Life“ for Catalyzing the SCI-TReCS Project

And: The SCI-TReCS Sponsor!
Contractile vSMC / pericyte markers in MSCs

Calponin & Actin at 20x magnification

Calponin & Actin at 100x magnification

M. Gimona, Transfusion Medicine Salzburg
STEM CELL RESEARCH UNIT GRAZ
Osteogenic lineage induction of BM-MSPC

Induced + BM-MSPC Exosomes

Induced + UC-ECFC Exosomes

Sustained matrix deposition of induced & replated BM-MSPCs

Alizarin Red S staining at day 14 post osteogenic induction
Mechanosensation & - Response

Paxillin (top), pSrcY416 (bottom) & Actin

Cortactin (top), ILK (bottom) & Actin

Mechanosensory molecules and mecanoresponse in UC-MSCs

M. Gimona
DDR2 in BM-MSCs at P1

Phalloidin

Rb anti DDR2 extracellular discoidin domain

Mo anti DDR2 intracellular domain

M. Gimona
GMP-requirements (facility)
Potential GMP-Labor

Ermöglicht Translationale Forschung: Klinische Anwendung Zelltherapie

Industrie-Kooperationen

Intellectual Property Verwertung

Herstellung innovativer Therapien

PMU

GMP-Labor SISCIC

Kräftiger Impuls für R & D „Klinische Zelltherapie“ in Salzburg

Zelluläre Toxizitätstests Pharmakologische Entwicklung

Effizienz der Verwendung und Focus?
Potential use for a GMP-Lab?

Effizienz der Verwendung und Focus?

Knochen/Sehnen

Haut

Augen/Netzhaut

Muskel

SCI ~ 70%

SCI  Neuro  Muskel  Augen  Haut  Knochen/Sehnen