Current clinical applications of stem cells in Norway

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Norwegian Center for Stem Cell Research
Oslo University Hospital Rikshospitalet
and University of Oslo
The stem cell hierarchy

**Totipotent stem cell (zygote)** → **Inner cell mass of a blastocyst** → **Pluripotent stem cell** → **Embryonic stem cell (ES-cell)** → **Adult, or Multipotent stem cells**:

- CNS
- PNS
- Hema.
- Liver
- Skin
- Mesen.
- etc.

Candidates for cell therapy
Embryonic stem cells

- Proliferates indefinitely
- Always pluripotent (teratoma assay)
- Can differentiate to cells typical of all three germ layers (ectoderm, mesoderm, endoderm)
- But: we can not yet fully control the differentiation
- Teratogenesis
- Always allogeneic
Cells from different people are different

Can stem cells from one individual still be used to treat another individual?
Somatic cell nuclear transfer

Unfertilized egg → Somatic cell

Blastocyst

Differentiated cells
- Haematopoietic
- Neuronal
- Myogenic
- Cardiac
- Pancreatic

Derive pluripotent stem cells

Transfer for development in vivo

Pluripotent stem cells med pasientens HLA
Background:
Reprogramming of differentiated cells has been shown to be possible:
• Somatic cell nuclear transfer (Wilmut et al., 1997)
• cell fusion with embryonic stem cells (Cowan et al., 2005; Tada et al., 2001)

Is it possible to induce pluripotency in end differentiated cells by introducing a limited number of genes?
Unsolved issues for the clinical use of hIPCs

• If gene transduction is to be used: random insertion of transgene?
• If the cells need to be reprogrammed to pluripotency: malignancy, neodifferentiation strategy
• If transdifferentiation is possible: complete transdifferentiation?
Hematopoietic stem cell transplantation has been used in the clinic for more than 40 years.
Hematopoietic stem cell transplantations

• Autologous: From the patient herself

• Allogeneic: From another individual
  » Family (including umbilical cord blood)
  » Bone marrow donor registries
  » Umbilical cord biobanks
  » For all these: HLA compatibility very important
Organization of stem cell transplants in Norway:

Autologous (høydosebehandling med autolog stamcellestøtte: HMAS)

- All University hospitals in Norway
- Oslo Universitetssykehus:
  - Ullevål: Lymphomas and multiple myelomas
  - Rikshospitalet: Multiple myelomas, solid tumors (children)
  - Radiumhospitalet: Lymphomas, some solid tumors

Lorentz Brinch, Department of Blood Diseases, OUS
High dose chemotherapy followed by autologous bone marrow transplantation is an option for patients with lymphomas

<table>
<thead>
<tr>
<th>Histology</th>
<th>1.line</th>
<th>First chemosensitive relapse</th>
<th>Later chemosensitive relapse</th>
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</thead>
<tbody>
<tr>
<td>Hodgkin's lymphoma</td>
<td>Not recommended</td>
<td>Clinical option</td>
<td>Clinical option</td>
</tr>
<tr>
<td>T/B lymphoblastic lymphoma</td>
<td>Clinical option</td>
<td>Not recommended</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Aggressive B cell NHL</td>
<td>Not recommended</td>
<td>Clinical option</td>
<td>Clinical option</td>
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<tr>
<td>Transformed NHL</td>
<td>Not recommended</td>
<td>Clinical option</td>
<td>Clinical option</td>
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<tr>
<td>Follicular NHL</td>
<td>Not recommended</td>
<td>Not recommended</td>
<td>Clinical option</td>
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<tr>
<td>Mantle cell NHL</td>
<td>Clinical option</td>
<td>Not recommended</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Aggressive T cell NHL</td>
<td>ACT-1 randomised study Clinical option</td>
<td>Clinical option</td>
<td>Clinical option</td>
</tr>
</tbody>
</table>

Arne Kolstad, Norwegian Radium Hospital OUS
Allogeneic stem cell transplantation: bone marrow depletion

Bu: Busulfan: 16 mg/kg in total
Cy: Cyclofosfamid: 120 mg/kg in total

Stem cell infusion: From bone marrow or blood

Day -8 -7 -6 -5 -4 -3 -2 -1 0 +1
## Difference between autologous and allogeneic HSC transplantation

<table>
<thead>
<tr>
<th></th>
<th>Autologous</th>
<th>Allogeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy stem cells</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>HLA compatibility</td>
<td>Yes</td>
<td>Very important</td>
</tr>
<tr>
<td>Transplant rejection</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Need for treatment against rejections</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Transplant versus malignancy effect</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Diseases treated with allogeneic stem cell transplantation
Allogeneic stem cell transplantation in Norway: only performed at Rikshospitalet
Tissue engineering

Elements:
- Cells
- Biomaterials
- Imaging
- Advanced surgery

In the clinic:
- Heart
- Cartilage
- Bone
- Eye
Stem/progenitor cells in the bone marrow

- MSC
- HSC
- EPC
- MAPC
- SP
Repair of Infarcted Myocardium by Autologous Intracoronary Mononuclear Bone Marrow Cell Transplantation in Humans

Bodo E. Strauer, MD; Michael Brehm, MD; Tobias Zeus, MD; Matthias Köstering, MD; Anna Hernandez, PhD; Rüdiger V. Sorg, PhD; Gesine Kögler, PhD; Peter Wernet, MD

Background—Experimental data suggest that bone marrow–derived cells may contribute to the healing of myocardial infarction (MI). For this reason, we analyzed 10 patients who were treated by intracoronary transplantation of autologous, mononuclear bone marrow cells (BMCs) in addition to standard therapy after MI.

Methods and Results—After standard therapy for acute MI, 10 patients were transplanted with autologous mononuclear BMCs via a balloon catheter placed into the infarct-related artery during balloon dilatation (percutaneous transluminal coronary angioplasty). Another 10 patients with acute MI were treated by standard therapy alone. After 3 months of follow-up, the infarct region (determined by left ventriculography) had decreased significantly within the cell therapy group (from 30±13 to 12±7%, P=0.005) and was also significantly smaller compared with the standard therapy group (P=0.04). Likewise, infarction wall movement velocity increased significantly only in the cell therapy group (from 2.0±1.1 to 4.0±2.6 cm/s, P=0.028). Further cardiac examinations (dobutamine stress echocardiography, radionuclide ventriculography, and catheterization of the right heart) were performed for the cell therapy group and showed significant improvement in stroke volume index, left ventricular end-systolic volume and contractility (ratio of systolic pressure and end-systolic volume), and myocardial perfusion of the infarct region.

Conclusions—These results demonstrate for the first time that selective intracoronary transplantation of autologous, mononuclear BMCs is safe and seems to be effective under clinical conditions. The marked therapeutic effect may be attributed to BMC-associated myocardial regeneration and neovascularization. (Circulation. 2002;106:1913-1918.)
Cardiac repair: can bone marrow cells improve myocardial function in patients with acute myocardial infarction (AMI)?

a) Blood is aspirated to get serum
b) Bone marrow aspiration day 4 - 5

Injection into the affected coronary artery or into the myocardium
Expected improvement in LVEF after AMI by routine treatment

$\Delta$LVEF = 7%
P < 0.01

Baks et al, Eur Heart J 2005;26:1070
Results on LVEF in clinical trials with Bone Marrow Cells in AMI

**BOOST**  
n=60  
P = 0.27

**Leuven**  
n=67  
P = 0.36

**ASTAMI**  
n=100  
P = 0.77

**REPAIR-AMI**  
n=204  
P = 0.01

Meyer et al  
*Circulation* 2006;113:1287-1294

Janssens et al  
*Lancet* 2006;367:113-21

Lunde et al  
*NEJM* 2006;355:1199-209

Schächinger et al  
*NEJM* 2006;355:1210-21
What is the reason for the limited success?

The human left ventricle contains
\sim 4-5 \times 10^9 \text{ cardiomyocytes}

25\% \text{ MI destroys } \sim 1 \times 10^9 \text{ cardiomyocytes}

Approximately 1\% \text{ HSC in BM-MNC}

Injection of 150 \times 10^6 \text{ BM-MNC} \rightarrow 1.5 \times 10^6 \text{ HSC}
Very few of the injected cells home to or remain in the myocardium

Analysed 1 hr after injection

Hou et al
Circulation 2005;112[suppl I]:I-150-I-156
Bone marrow–derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation

Jens M Nygren1, Stefan Jovinge2,3, Martin Heitbruch4, Bettina Sauer1, Wilhelm Boll1, Ulrich Heuck3,4, Jahal Taneera1, Bernd K Fleischmann1,8, St

Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium

Leora B. Balsam1, Amy Theo Kofidis1, Irving L. Weissman2

Nature 2004;428:668-73

Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

Charles E. Murry1, Mark H. Soonpaa1, Hans Reinecke1, Hidehiro Nakajima2, Hisako O. Nakajima2, Michael Rubart3, Kishore B. S. Pasumarthi4, Jitka Ismail Virag1, Stephen H. Bartelmez1, Veronica Poppa1, Gillian E. Desdouits6, David A. Williams2,7 & Louis W. Pearlman1

Nature 2004;428:664-8

Bone marrow cells adopt the cardiomyogenic fate in vivo


*Cardiovascular Research Institute, Department of Medicine, New York Medical College, Valhalla, NY 10595; Institute of Molecular Cardiology, University of Louisville, Louisville, KY 40292; and Heart Institute and Department of Biology, San Diego State University, San Diego, CA 92182

Edited by Andrew R. Marks, Columbia University College of Physicians and Surgeons, New York, NY, and approved September 7, 2007 (received for review June 14, 2007)

PNAS 2007;104:17783-8
Results of Intracoronary Stem Cell Therapy After Acute Myocardial Infarction

Jochen Wöhrle, MD\textsuperscript{a,*}, Nico Merkle, MD\textsuperscript{a}, Volker Mailänder, MD\textsuperscript{b}, Thorsten Nusser, MD\textsuperscript{a}, Peter Schauwecker, MD\textsuperscript{b}, Fabian von Scheidt\textsuperscript{a}, Klaus Schwarz, MD\textsuperscript{b}, Martin Bommer, MD\textsuperscript{c}, Markus Wiesneth, MD\textsuperscript{b}, Hubert Schrezenmeier, MD\textsuperscript{b}, and Vinzenz Hombach, MD\textsuperscript{a}

or LV end-diastolic and end-systolic volume indexes. In conclusion, in this rigorous double-blind, randomized, placebo-controlled trial, we did not observe an evidence for a positive effect for intracoronary BMC versus placebo therapy with respect to LV ejection fraction, LV volume indexes, or infarct size. © 2010 Elsevier Inc. All rights reserved. (Am J Cardiol 2010;105:804–812)
Is it possible to improve myocardial function using cell therapy or tissue engineering following AMI? **Probably**

Should this be offered to patients in acute stage MI? **Unlikely, the cells need to be expanded in vitro, and should be autologous**

Which are the best cells to use? **Not known, animal studies are ongoing**

What would be the most likely mechanism for the effect of cell therapy?
• Transdifferentiation transplanted cells → cardiomyocytes? **Perhaps, but unlikely**
• Stimulation of endogenous repair mechanisms? **More likely**
• Improvement of local blood supply? **Important, may need to include cells specifically for this purpose**
Can adult stem cells be used to treat focal lesions of hyaline cartilage?
In vitro expanded chondrocytes is used for regeneration of hyaline cartilage, but the result is frequently fibrocartilage.
Mesenchymal stem cell

- Bone marrow
- Adipose tissue
- Synovium
- Skeletal muscle?
- Skin fibroblasts?

- Myocyte
- Osteocyte
- Chondrocyte
- Fibroblast
- Adipocyte
- Tenocyte
Alginate as a scaffold for chondrogenic differentiation of MSC

The scaffold can be made to shape of choice
• Cells are quite evenly distributed
• The alginate can be easily removed
• Alginate may be made biodegradable?

3 mm = thickness of hyaline cartilage of knee

Size of the lesion
Expression of proteins of importance for chondrogenesis after 21 days of differentiation in alginate discs

A

COL2
SOX9
day 7

COL2
SOX9
day 14

COL2
SOX9
day 21

B

COL1

SOX5

ACAN

COL10

SOX6

VCAN
MSC may exert immunosuppressive effects

- NK cells
  - ↓ proliferation
  - ↓ cytotoxicity
  - ↓ IFN-γ
  - ↑ IDO

- B cells
  - ↓ proliferation
  - ↓ differentiation to plasma cells
  - + PGE2

- MSC
  - ↓ proliferation
  - ↓ CTL formation
  - ↓ IFN-γ, ↑ IL-4
  - ↑ IDO

- T cells
  - ↓ activation
  - ↑ IL-10

- Monocytes
- iDCs
- mDCs

- Treg
Diseases of the cornea may be treated with stem cell therapy

• The first corneal transplant was performed in Norway in 1933.
• Corneas are kept in a tissue bank at the Center for Eye Research, Ullevål
• Can be stored for up to 4 weeks before the operation.

Challenges:
• Some corneas must be discarded before the operation due to poor quality tissue.
• Some transplanted corneas become non-translucent
• There is a lack of corneas, many are bought from USA, expensive
Strategy

• The different layers of the cornea have their own stem cells

• In patients with damage to only one of the corneal layers, stem cell therapy may be sufficient
Transplantation of autologous limbal stem cells to a patient with stem cell failure

Morten C Moe, Department of Eye Diseases, Ullevål
Corrosion damage


Dag 1

Dag 7

Dag 30

Dag 450

Morten C Moe, Department of Eye Diseases, Ullevål
Can expressed genes from glioblastoma stem cells be used in a therapeutic vaccination?
Tumor biopsy

Leukapheresis

Tumor stem cells

mRNA amplification and purification

hTERT and survivin mRNA

mRNA loading by electroporation

Immature DCs

Maturation of DCs

Monocytes

Tumor biopsy

Leukapheresis
The Ex vivo cell laboratory is a GMP regulated production facility for cells for therapeutic trials.
Patient Handbook on Stem Cell Therapies

Appendix I of the Guidelines for the Clinical Translation of Stem Cells

December 3, 2008
Home

The XCell-Center is a private clinic group and institute for regenerative medicine located in Düsseldorf and Cologne, Germany. Bringing together therapeutical use of autologous adult stem cells and medical research, it is our mission to:

- Provide therapeutic application of autologous adult stem cells to patients at the highest medical standard;
- Extend existing knowledge on the effects of autologous adult stem cells by supporting pre-clinical and clinical research.

We offer patients with degenerative diseases the opportunity to undergo an innovative and promising stem cell treatment.

Since the start in January 2007, more than 2500 patients have safely undergone our various stem cell treatments.
Therapeutic use

The XCell-Center treats patients with their own autologous adult stem cells. It is the first private clinic worldwide to hold an official license for the extraction and approval of stem cell material for autologous treatment.

Therapy focuses on the treatment of cerebral palsy, spinal cord injuries, diabetes mellitus (types 1 and 2 as well as sequelae) and neurological diseases/disorders such as Parkinson's and stroke. Further indications include multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and Alzheimer's as well as arthritis, heart disease, and eye diseases such as macular degeneration.

Advisory board

Learn more about the XCell-Center's Scientific Partners.
Overview of our stem cell treatment

As a patient or the friend or relative of a patient, you have likely consulted this website to learn some basic facts about our stem cell treatment offerings. Therefore, we have carefully compiled relevant information on these pages that we hope will help you.

We would like to point out from the start that there are still some questions concerning the function of stem cells that science has not yet been able to answer, and that despite the advances that have been made recently, there is no guarantee for the success of stem cell therapy. Nevertheless, every week we see this new "medicine" helping a lot of people. Therefore, we offer therapies with adult stem cells whenever classical treatment does not yield the type of results that are satisfactory for the patient.

After evaluating important information from each prospective patient's medical history, our medical team decides whether the prospective patient is a suitable candidate for therapy.

Related topics
- Healing potential
- Methods of use
- Physiological mechanism
- Limits of therapy
- No Tumor risk
- Treatment process
Methods of use - adult stem cells

The use of endogenous adult stem cells is ethical and legally straightforward. Under German law, the extracted stem cells are categorized as drugs. Because they are exclusively for personal use, they are individual drugs, and under German law do not require the same governmental approval as other drugs. Despite this, the clinic still has to obtain a manufacturing license from the surveillance authority. At the XCell-Center, it is guaranteed that the processes of extraction, cleaning and transplantation are all carried out in compliance with Good Manufacturing Practice (GMP) standards, thus guaranteeing maximum quality and safety for the patient.

For the last few years, attempts at therapy with adult stem cells from bone marrow have been carried out at university hospitals. This means that unlike animal testing with embryonic stem cells, adult stem cells are in-part, already being clinically tested. The well-documented success of the cardiologist Prof. Dr. Bodo Strauer from Düsseldorf can be seen as an example. He treated a patient suffering from a series of heart attacks for whom common therapies could not assure any chance of survival with the patient's own bone marrow stem cells. Nine days after the stem cells had been injected into the diseased area, the patient was able to leave the intensive care unit. Up to now, more than 300 patients have been treated in Düsseldorf using this procedure - most of them successfully.

The XCell-Center’s treatment is based on the therapy experiences of more than 2500 patients treated both in the XCell-Center directly and in cooperation with other hospitals.
Stem cells carry a lot of promise for the development of new therapeutic options, but they should be introduced into the clinic with great caution.