



ITERA Life-Sciences Forum

On behalf of the steering committee, the Scientific Board of Advisors, The Ethical committee and myself we would like to thank all of you to attend this Workshop and wish you a fruitful meeting and a very interesting staying.

HISTORIC

The ITERA Life-Sciences Forum is an international consortium, founded in September 2004, located in Aachen (D) Eisenhutte nr 21, in which different European, North-American and Asian Universities, University Hospitals and Research Institutes are participating in both, fundamental stem cell research as well as in research on dendritic cells, stroma cells and other cells. In addition biotechnical companies that with the help of innovative technologies focus there research and knowledge on different scientific areas are taking part in this forum.

The ITERA Life-Sciences Forum intends an international cooperation in the field of the development of cellular lines starting from the stem cells. The scientific advisory board of the ITERA Life-Sciences Forum exists of scientists and physicians as well as experts in ethical questions as lawyers.

Since June 2006 the ITERA Life-Sciences Forum cooperates closely together with the ITERTAL Klinik in Aachen and this cooperation has led to the idea to create a Life-Sciences Centre of Research and Novel Therapeutical Applications in cooperation with different extern small and middle sized Biotechnology Companies like Innovacell, Strategus, Cryo-Save and finally the Life-Sciences Group NV.

ACTIVITIES

In June 2005 the ITERA Life-Sciences Forum (LSF) has organized the 1st ITERA LSF Workshop in the Congress Hotel Vaeshartelt. Under the impulse of the steering committee and the scientific board of advisors a FP6 EU (CRYSTAL) project has been submitted. In this project the following groups are involved: 5 Universities, 1 Research Institute and 1 SME, objectively and critically evaluated and selected by the scientific board as well as by an international independent organism Matrix45 from N-America. The steering committee, the scientific board of advisors as well as the ethical committee of ITERA LSF are proud about the score and the ranking that we obtained.

Hereby I would like to express my sincerest feelings of thanks to the participating groups but also to Arttic France for the excellent righting contribution.

On the 7th of June 2006, ITERA LSF has organized together with the "Berufsverband der Frauenärzte e.V". Nordrhein, with the GSZ (Deutsche Gesellschaft für Stammzell - Forschung e.V.), with Grünenthal - Pharma, with Cryo-Save GmbH and the Life-Sciences Group NV a symposium on „Stammzellen in der Gynaekologie“

On the 13th of June 2006, The ITERA LSF and SIEMENS Belgium has organized a symposium on " In vitro molecular imaging on stem cells" in the Erasmushuis Antwerp.

Several ITERA Life-Sciences members are frequently invited as speakers or moderators on different international and national symposia, workshops as well as congresses.

For the end of the year a Website will be finished, actually there is a ITERA Life-Sciences Forum provisional Website that you can find by GOOGLE ITERA Life-Sciences Forum.

From the 23rd until the 24th of October the 2nd ITERA Life-Sciences Forum Workshop will take place in the Congress Hotel Vaeshartelt, a very picturesque place in the near of Maastricht.

The Workshop will be pointed to the Regenerative Medicine in general but particularly dealing with actual existing therapies with autologous cells, and stem cells. Therapies with autologous cells and stem cells will be discussed in a panel of experts in the field. During the second day the scientist from different disciplines will present research data and perspectives for the future.

Last but not least, on behalf of the Steering committee of the ITERA, we would particularly address to the **Life-sciences Group NV** (CEO Waeterschoot) our sincere feelings of gratitude and thanks for the fuel sponsoring of this 2nd workshop.

RAMON Albert
ITERA Life-Sciences Forum
Chairman



ITERA
International Tissue Engineering Research Association
ITERA 2nd Workshop
23 – 24 October 2006



Program – 23rd of October 2006

**Autologous Stem cells, perspectives,
features and future of Regenerative Medicine
Clinical Approaches**

Chairmen: Imhof M. , Strasser H., Ebbesen P., Ramon A.

08.30 – 09.00	Registration
09.00 – 9.15	Welcome Introduction Tissue Engineering, Regenerative Medicine Ramon A. President ITERA – Life-Sciences Forum University Hospital of Antwerp, Belgium Research & Therapeutic Centre Aachen (ARTZ), Germany
09.15 – 09.45	European Research in Regenerative Medicine Gwennaël Joliff-Botrel, Principal Scientific Administrator Assistant to the Health Research Director European Commission, Brussels
09.45 – 10.30	State of the art Lecture Autologous versus allogeneic stem cell transplantation: engraftment studies using nanoparticles. Marc Ramael - University of Hasselt, Belgium
10.30 – 11.00	Diabetic foot: therapeutical perspectives with autologous stem cells. Kristien Van Acker St. Jozef Ziekenhuis Bornem, Belgium
11.00 -11.15	Coffee break
11.15 – 11.45	Transurethral ultrasound guided application of autologous myo- and fibroblasts in treatment of incontinence Hannes Strasser University Hospital of Innsbruck, Austria
11.45 – 12.30	Umbilical stem cells for autologous use - Facts and fictions, an overview of possibilities. Martin Imhof - University Hospital of Vienna, Austria
12.30 – 14.00	Lunch



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Program: 23rd of October 2006

**Liver - Heart
Clinical Approaches**

Chairmen: Muraca M., Gehling U., Michielsen P., Ysebaert D., Pirenne J.

14.00 – 14.45	State of the art Lecture Stemcells for the treatment of inherited Hepatic metabolic disorders. Maurizio Muraca – University Hospital of Rome, Italy IRCCS Ospedale Bambino Gesù
14.45 – 15.15	Mobilization of unique populations of hematopoietic progenitor cells in response to clinical situations associated with liver regeneration. Ursula Gehling – University of Hamburg , Germany
15.15 – 15.45	NASH and liver regeneration. Sven Francque – University Hospital of Antwerp, Belgium
15.45 – 16.00	Coffee break
16.00 – 16.30	Engraftment of mesenchymal stem cells after in-utero transplantation into immunocompetent mice. Carolyn Troeger – University of Basel, Switzerland
16.30 – 17.00	Pathological assessment of stem cell therapy in acute and chronic myocardial ischemia Max Buja – University of Texas, U.S.A
17.00 – 17.30	Update on the Advanced Therapy Medicinal Products Regulatory Framework in Europe, illustrated with a practical example. Peter De Waele, Life-Science Group
17.30 – 18.00	Meet the experts, moderated by Peter Ebbesen Stemcell Institute of Aalborg, Denmark Eric Mehuys , Life – Sciences Group , Europe Hannes Strasser – Univ. Hospital Innsbruck, Austria Martin Imhof , Univ. Hospital of Vienna, Austria Andreas Zisch , Univ. of Zürich, Switzerland
19.30	Dinner



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Program: 24th of October 2006

Research and Development (R & D)

Chairmen: Ebbesen P., Ponsaerts P., Spitkovsky D., Zisch A.

09.00 – 09.15	Registration 2 nd day
09.15 – 10.00	State of the art Lecture Regenerative Medicine in Aalborg, GMP facilities, first results and plans. Peter Ebbesen – Stemcell Institute of Aalborg, Denmark
10.00 – 10.30	Non-hematopoietic stem/progenitor cells from human cord-blood-account of a Zürich experience. Andreas Zisch – University of Zürich, Switzerland
10.30 – 11.00	Bone-Marrow-derived stromal cells as vehicles for neurotrophic factors. Peter Ponsaerts –Experimental Haematology University of Antwerp, Belgium
11.00 – 11.15	Coffee Break
11.15 – 11.45	Biological limitations in regenerative medicine relevant properties of autologous adult stem cells. Dimitry Spitkovsky – University of Cologne, Germany
11.45 – 12.15	Transition Of A Mesenchymal Progenitor Cell Line To Study Bone, Cartilage And Fat Development. Alan Chan – Percuros, Leiden – The Netherlands
12.15 – 12.45	Therapeutic stem cell approaches for Multiple Sclerosis Niels Hellings –University of Hasselt, Belgium
12.45 – 14.15	Lunch



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Research and Development (R & D)

Chairmen: Ebbesen P., Ponsaerts P., Spitkovsky D., Zisch A

- 14.15 – 14.45 | **Dendritic Cells - DC – based immunotherapy of cancer**
Viggo van Tendeloo
Laboratory of experimental Haematology
University of Antwerp, Belgium
- 14.45 – 15.15 | **Ex vivo expansion of red blood cells.**
Andrea Kolbus – University of Vienna, Austria
- 15.15 – 15.30 | **Coffee Break**
- 15.30 – 16.00 | **Pamchip Technology**
Rinnie van Beuningen – Pamgene, The Netherlands
- 16.00 – 16.30 | **High performance polymers and carriers for drug delivery, cryopreservation, tissue engineering, tumor cell screening**
Detlef Müller – Schulte, Albert Ramon
R.I.D.I.S Innovation – Aachen – Germany
- 16.30 – 17.00 | **Conclusions / Questions**
Panel: Imhof M. – Zisch A. – Spitkovsky D. – Ebbesen P. –
Ponsaerts P. – Van Tendeloo V. – Muraca M.
- 17.00 – 17.30 | **TissueFAXS: A New Solution for Multicolor Tissue Cytometry**
Georg E. Steiner
TissueGnostics GmbH, R&D,
Institute for Computer Graphics and Vision, Technical University, Graz
Department of Urology, Medical University of Vienna, Austria
- 19.30 | **Candle-light dinner**



Tissue engineering – Regenerative Medicine.

Ramon Albert, Chairman of ITERA
University Hospital of Antwerp, dept. Gastroenterohepatology.

Tissue Engineering – Regenerative Medicine

Tissue engineering is an interdisciplinary field in which the principles and methods of engineering combine with those of biological science for the fundamental understanding of structure-function relationships in normal and pathological tissues and organs, as well as for the development of biologic substitutes that can restore, maintain, or improve tissue or organ function. More recently, the expression “regenerative medicine” has been often used either as a synonym or as an all-embracing branch of medical science that would include tissue engineering per se.

In the current context of tissue engineering, as defined above, new tissues or organs can be created through three general strategies, or a combination of them as follows:

1. Infusion of isolated cells, i.e. cells are isolated from donors, expanded and/or modified in vitro and re-implanted for the supply of a specific function.
2. Tissue-inducing substances. In this process, appropriate signal molecules (e.g. growth factors) are delivered to specific targets for the stimulation or control of tissue growth or maturation.
3. Cells placed on or within performed scaffold (matrices). This methodology consists of culturing the cells on or within a natural or synthetic but degradable scaffold matrix in vitro and transplantation of the cell-matrix composite at the site where regeneration is required; the scaffold material provides initial mechanical support and a template for three-dimensional organization.

All of these strategies can be supplied in autologous, heterogenous, or xenologous manner. They all carry potential problems, enclosing immunologic rejection, growth limitations, differentiation and function restrictions, incorporation barriers and cell or tissue delivery difficulties. Despite the limited clinical experience to date, efforts at engineering almost every mammalian tissue have already occurred.

Acute wound healing: an example for tissue regeneration.

Healing of an acute wound follows a predictable chain of events. This chain of events occurs in a carefully regulated manner that is reproducible from wound to wound. The phases of wound healing are overlapping, but are described in a linear fashion for the purpose of clarity. The five phases that characterize wound healing include (1) hemostasis, (2) inflammation, (3) cellular migration and proliferation, (4) protein synthesis and wound contraction, and (5) remodelling (figure next site)

Figure Wound healing.

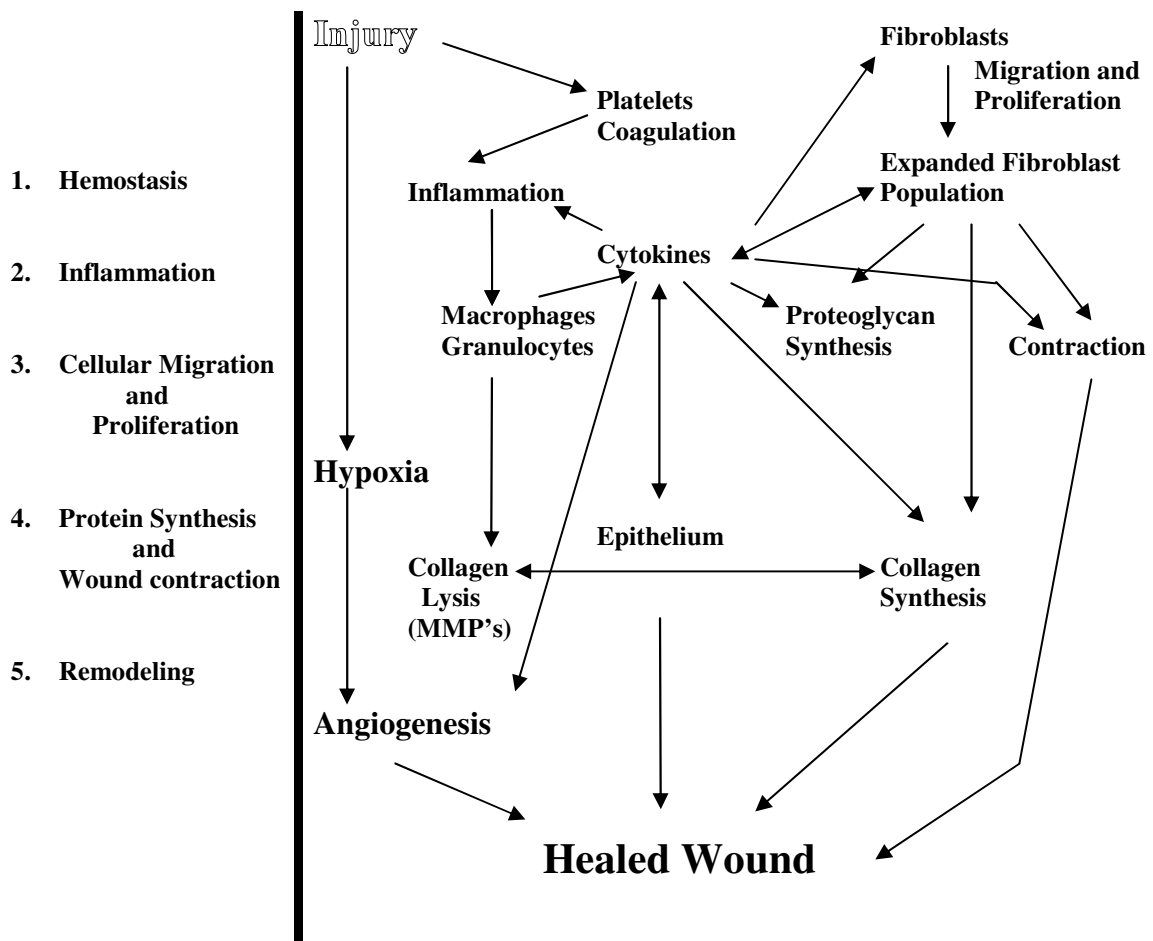


Figure.

The wound-healing cascade of an acute trauma. The progression of acute wound healing from haemostasis to the final phases of matrix remodelling is dependent on a complex interplay of varied acute wound-healing events. Cytokines play a central role in wound healing and serve as a central signal for various cell types and healing events. Taken from Monaco et al. (2003)



European Research in Regenerative Medicine

Gwennaël Joliff-Botrel, Principicpal Scientific Administrator
 Assistant to the Health Research Director
 European Commission Brussels

- What will be funded at EU level?
- Stem cell Research at EU level
- What is the future policy of the EU?

Type of projects funding / Previous programs

- Fundamental research to differentiation
 - neural SC, mesodermal SC, insulin-producing Islets
- Tissue engineering, cartilage, bone marrow
- Haematopoietic SC therapy, bone marrow & cord blood transplantation
- Ethical, legal and social research aspects.

Difference between FP5 – FP6 (Stem Cell Research)

- Change in size
 - Significant increase of SC research funded at EU level ($\pm 6x$)
- Change in nature
 - FP6 new instruments help to :
 (± 25 partners / $\pm \text{€ } 10$ million / ie $\pm \text{€ } 400.000$ partner / 4 years)
 - Comparison of SC from different origins
 - Translational research (together)
- Stem cell research is a field where EU added value is clear.

Total Budgets of the EU Framework Programmes

- | | | |
|---------------|--|---------------------------|
| • 1984 – 1987 | 1 st Framework programme, | € 3.27 billion / 4 yr |
| • 1987 – 1991 | 2 nd Framework programme | € 5.36 billion / 4 yr |
| | ◦ Science becomes a community responsibility | |
| • 1995 – 1964 | 3 rd Framework programme | € 6.60 billion / 4 yr |
| • 1964 – 1968 | 4 th Framework programme | € 13.12 billion/ 4 yr |
| • 1968 – 2002 | 5 th Framework programme | € 14.95 billion/ 4 yr |
| • 2002 – 2006 | 6 th Framework programme | € 17.50 billion/ 4 yr |
| • 2007 – 2013 | 7 th Framework programme | € 53.3* billion / 7 yr !! |
| | ◦ Including 2.75 billion for Euratom for 2007 - 2011 | |

The EU budget is splitted in:

For 2006 € 123.5 billion

Expenditure (commitments; payments 1.08% of GNI)(GNI = gross national income)

42,6%	agriculture
36,1%	structural functions, economics etc..
5,3%	
2,9%	
4,3%	research
4,5%	
4,3%	

Europe is still much lower than Japan, than U.S.A by investing in research.

More information:

General information on research
General information on the 6th FP

<http://europa.eu.int/comm/research>
<http://europa.eu.int/comm/research/fp6/>
<http://cordis.europa.eu/fp6/stepbystep/home.html>

Information on research programmes and projects

<http://www.cordis.lu>

Information requests

research@cec.eu.int

Information on 7th FP and Health

http://cordis.europa.eu/fp7/cooperation/home_en.html

Stem cell research at EU Level

hES – cell research, the point of discussion

- average impact is very high (6.03): outstanding interest
- ± 414 hESC lines from at least 20 countries
- Only 43% in peer-reviewed
- H9 the most frequently used, then H1 (in 2005 big change)
- Less than 10% free of animal feeder, animal derived serum but not “animal free” or “xerofree”
- 27 hESC lines with genetic defects

Creation of a European hESC registry:

- to gather the information on all the European lines
- in order to maximise the use of existing ones and
- to disseminate the information to all scientific community
- to favour the comparability of results

Consortium with a representative from all EU Member States where this research is performed (including Israel and Switzerland)

Citizens Opinion

Extracted from Eurobarometer surveys on biotechnology published in June 2006 (ref. Eurobarometer 64.3)

⇒ 59% of the European Citizens are supportive provided it is regulated

- 23% approved
- 36% approved , more tightly regulated

Regulation worldwide

Europe uses at most the eggs from rest embryos from the IVF

hESC research EU Policy

- EU has no legal competency to regulate in this sector of ethics.
- The commission has the responsibility to implement the EU research programs even where some areas of research raise important ethical issues.
- Respect of national rules is a fundamental principle ⇒ no research forbidden in a Member State supported by EU funds there.

Ethical framework in FP6 (2002 -2006)

3 areas are excluded from funding:

- Human reproductive cloning
- Intentional Germ Line modification
- Creation of human embryos for research

⇒ same ethical framework expected in FP7 (2006 – 2013) with a revision for 2010 -2013

Process in FP6 (hESC research EU policy)

- Case by case evaluation
 - Must be scientific justification, strict ethical rules
- Systemic ethical review at EU level
 - Respect of national law
 - Source
 - Informant consent
 - Protection of personal data
 - Nature of financial inducements, if any
- Approval by the Regulatory Committee
- Approval by the relevant national or local ethics committees (double ethical review)

The future EU policy – FP7

How will the money be splitted in specific programmes?

- Cooperation – collaborative research.
Cooperation between industry and academic in key technology areas.
- Ideas – support Frontier Research.
Novelties, competition on excellence.
- People – Human Potention
support for mobility and career development of researchers, scientific young people.
- Capacities – Research capacity.
enhance research and innovation capacities.
- JRC – Non nuclear actions by the Joint Research Centre
- Euratom- Civil nuclear actions by the European Atomic Energy Community (Euratom)

Priorities & Budgets.

- Information 1st place, 9.1%
- Health 2nd place, 6.1%
- Ideas EU Council, 7.5%

Core Business is the Cooperation

- Collaborative research
- Joint Technology Initiatives
- Coordination non-community research programmes (ERA-NET)
- International cooperation

Health Collaborative Research

Activities ⇒ 3 Pillars:

1st Pillar : Biotechnology

- High-throughput research: to develop new research tools for modern biology ⇒ to enhance data generation & improve data & specimen (biobanks) standardisation, acquisition & analysis.
- Detection, diagnosis and monitoring – to develop visualisation, imaging, detection & analytical tools & technologies for biomedical research, for prediction diagnosis, monitoring & prognosis of diseases & for the support & guidance of therapeutic interventions. Emphasis on no- or invasive & quantitative methods & quality assurance aspects.
- Innovative therapeutic approaches and interventions: to consolidate & ensure further developments in advanced therapies & technologies with broad potential application (gene & cell therapy, regenerative medicine, transplantation, immunotherapy & vaccines and other).

- Predicting suitability, safety and efficacy of therapies: to develop & validate the parameters, tools, methods & standards needed for bringing to the patient safe & effective new biomedical.

2nd Pillar: Translating research for human health

- Integrating biological data & processes. Large-scale data gathering to use high throughput technologies for genomics, proteomics, population genetics, comparative & functional genomics.
- System biology to understand and model biological processes
- Research on the brain and related diseases, human development & ageing.
 - Brain & brain related diseases to better understand the integrated structure & dynamics of the brain, to study brain diseases, disorders & search for new therapies.
 - Human development & aging: to better understand the process of life-long development & healthy ageing.
- Translational research in major infectious diseases
 - Anti-microbial drug resistance: to combine basic research with clinical research towards new interventions.
 - HIV/AIDS, malaria & tuberculosis ⇒ emphasis on preclinical & early clinical research.
 - Emerging epidemics: to SARS & highly pathogen Influenza

3rd Pillar: Optimising the delivery of health care to European Citizens.

- Enhanced health promoting and disease prevention ⇒ to provide evidence for the best public health measures in terms of life styles and interventions – different levels & different contexts (mental health will be addressed).
- Translational clinical research outcome into clinical practice: Better use of medicines, appropriate use of behavioural and organisational interventions, health therapies & technologies. Special attention paid to patient safety (e.g. benchmarking of strategies, investigating outcomes of different interventions including medicines)
- Quality, solidarity and sustainability of health systems. Basis for countries to adopt their health systems taking into account national contexts and population characteristics
 - Organizational, financial & regulatory aspects
 - Implementation, best practice
 - Outcomes – effectiveness, efficiency and equity
 - Special attention on investment and human resources

Health Collaborative Research.

Activities ⇒ 3 Pillars

- **Biotechnology**, generic tools and technologies for human health, producing knowledge that will be applied in the area of health and medicine.
- **Translating research for human health** – making sure that basic discoveries have practical benefits and improve the quality of life.
- **Optimising the delivery of health care to European citizens** – ensuring that the results of biomedical research will ultimately reach the citizens.

Two other strategic activities that will be addressed across activities:

- Child health
- The health of the ageing population.

Regenerative medicine

Collaborative research:

- Health
- Nanosciences, nanotechnologies, materials, new production technologies (NMP)

European Technology Platform on Nanomedicines established s strategic research agenda on regenerative medicine.

European Research Council (basic research where collaborative is needed).

From FP6 to FP7

- Continuity in the research activities.
- Focus on genomics has disappeared
- Emphasis put on translational research
- Biomedical technology & engineering (rich in SMEs) is re-introduced.
- New: Emerging epidemics
- Health-policy-driven research (public health) is strongly reinforced.



Autologous versus allogeneic stem cell transplant: novel engraftment studies by using nanoparticles.

M. Ramael* A. Ramon**

* Biomedical Research Institute (Biomed) Hasselt University, Belgium, Dept. Pathology, General Hospital St. Elisabeth Herentals, Belgium

** Life Science group, Mechelen, Belgium

Umbilical cord blood is one of three sources for the blood-forming cells used in transplants. The other two sources are bone marrow and peripheral (circulating) blood. The first cord blood transplant was done in 1988. Cord blood plays an important role in transplant today. The donated cord blood is tested, frozen and stored at a cord blood bank for future use. A close match between the patient and the donor or cord blood unit can improve a patient's outcome after transplant. Even though a closely matched cord blood unit is preferred, clinical studies suggest the match may not have to be as close as is needed for marrow or peripheral blood transplants. If the patient has an uncommon tissue type, it may be not possible to find a closely matched adult donor. However, a cord blood unit may be an option. Cord blood units are stored and ready to use. Graft-versus-host disease (GVHD) is a common side effect of an allogeneic graft. An allogeneic transplant uses cells donated by a family member, unrelated donor or cord blood unit. In GVHD, the immune cells from the donated marrow or cord blood (the graft) attack the body of the transplant patient (the host). GVHD can range from mild to life-threatening. Graft-versus-host disease (GVHD) is a common complication after an allogeneic transplant. GVHD can range from mild to life-threatening. Acute GVHD appears within the first 100 days after transplant. Acute GVHD can range from mild to life-threatening. Chronic GVHD can begin anytime during or after the third month post-transplant. Transplant patients who get acute GVHD are more likely to also get chronic GVHD, but it can also appear in patients who did not get acute GVHD. Chronic GVHD can range from mild to life-threatening. Some transplant survivors have problems with chronic GVHD for many years. Studies have found that after a cord blood transplant, fewer patients get GVHD than after marrow or peripheral blood transplants. Patients in the studies who did get GVHD after a cord blood transplant tended to get less severe cases. Cord blood transplants also have all the same risks as marrow and peripheral blood transplants. The risk of infection may be higher after a cord blood transplant because of the longer time to engraft. The risk of GVHD may be lower, but the risk is still there. Another well known complication is the engraftment syndrome which can be seen after autologous stem cell treatment. The symptoms are similar to GVHD but the clinical course is more benign and self limiting. Evaluation of engraftment after stemcell transplant is a very important item both from a research point of view as well as from a clinical point of view. Genetic manipulation of stem cells has been carried out so that these cells express e.g. green fluorescent protein as a marker. Such methods can be used in basic research but are not useful in a clinical setting. Ideal marking methods in vivo should be based on small biologically inert marker molecules that can be visualised in vivo as well as in vitro. Nanoparticles are potential candidates that can be used for labelling specific membrane proteins, cytoplasmic proteins and nuclear targets.



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Diabetic Footulcers: Current Therapie and New Insigths.

Dr Kristien Van Acker, Md, PhD
Sint Jozefkliniek Bornem
Coordinator of national diabetic foot projects

In a millennium where diabetes becomes a pandemic disease we are aware that foot ulcers and amputation will be one of the greatest problems in diabetes care all over the world.

Since the last decades much more attention is taken for the diabetic foot problems.

Mike Edmonds demonstrated the importance of multidisciplinary working in the first publication in this field in 1986.

The aetiology of foot ulcers is complex and multifactor with a vascular and neuropathic component. This means that some diabetic patients have foot at risk and due to a trauma, in most cases by unadapted shoes, they develop a ulcer. Uncontrolled diabetes plays an important role in developing infections. These patients are optimal hosts for infections. In a short time an ulcer ends in a deep infection and especially in cases of bad peripheral arterial blood supply it will end in an amputation.

Curative and urgent care of such ulcer is necessary. Standard care exists of off loading, revascularisation if possible and treat the infection as good as possible with surgical debridement and with antibiotics.

If ulcer treatment is delayed these ulcers become chronic and healing gets more difficult. A mean healing time of 3 to 11 months is often the case in those patients. The cost is tremendously for health care organisations. For this reason chronic wounds became a hot topic of research. Mechanisms of healing were examined. This was the start of the tissue engineering in wound healing. Clinical trials with all kind of growth factors were developed. After a while even artificial skin techniques were taken into account.

During the meeting a review of studies will be presented. On the other hand it must be stressed that good clinical trials are very difficult to organise because of the complex standard therapy. We will discuss some of the pitfalls, which are obligatory to take care of if we think on new developments, as stem cell techniques in this field.



Transurethral ultrasound guided application of autologous myo- and fibroblasts in treatment of incontinence

Hannes Strasser, M.D., Rainer Marksteiner, Ph.D., Eva Margreiter, Ph.D., Martin Fussenegger, M.D., Ferdinand Frauscher, M.D., Michael Mitterberger, M.D., Germar M. Pinggera, M.D., Steffen Hering, M.D., and Georg Bartsch, M.D.

Introduction: In the present study accuracy as well as the morphological and functional effects of transurethral ultrasound guided injections of autologous myo- and fibroblasts in treatment of urinary incontinence have been evaluated.

Methods: Starting from September 2002, 186 incontinent patients (age: 36-85 years; 123 women, 63 men) have been treated so far with ultrasound guided injections of autologous myoblasts and fibroblasts. The patients suffered from stress or mixed urinary incontinence. Small skeletal muscle biopsies were taken from the upper arm under local anaesthesia. The cells were then grown in a GMP-laboratory fulfilling strict clean room environment regulations. The fibroblasts were eventually mixed with a small amount of collagen as carrier material (about 2.5 ml). Using a transurethral ultrasound probe and a specially designed injection device, the fibroblasts were injected into the urethral sub mucosa to treat atrophies of the mucosa. The myoblasts were directly injected into the rhabdosphincter to reconstruct the muscle. In all patients application of cells was documented. Pre- and postoperatively transurethral ultrasound was performed to investigate the postoperative changes in thickness of urethra and rhabdosphincter as well as contractility of the rhabdosphincter. For quantification of contractility the distance between the transducer and the inner aspect of the muscle was measured at rest and during voluntary contraction of the muscle. The difference then served as parameter for contractility of the rhabdosphincter

Results: In all patients transurethral ultrasound guided application of cells could be performed without problems. The rhabdosphincter, the urethra, the injection needle and the injected cell depots could be visualized in all patients, which has not been possible with endoscopic techniques so far. Postoperatively, thickness of urethra and rhabdosphincter as well as contractility of the rhabdosphincter were significantly increased. No side effects occurred. In 156 patients incontinence was completely cured after therapy, and in 30 patients incontinence was markedly improved postoperatively. One year postoperatively 114/141 patients were continent.

Conclusion: The present data indicate that transurethral ultrasound guidance represents the state of the art application technique for transurethral injection of autologous stem cells.



Umbilical Stem Cells for Autologous Use - Facts and Fictions, an Overview of Possibilities

Martin Imhof

Department for Gynecological Endocrinology and Reproductive Medicine University Hospital Vienna, Waehringer Gürtel 18-20, A-1090 Vienna, Austria
Head: Prof. Johannes C. Huber M.D. Ph.D.

In gynaecology and obstetrics, umbilical stem cells are a point of very intense discussion. The main topic is the question of if the obstetrician should inform and convince his pregnant patients to store the umbilical stem cells of her unborn child. Obstetricians usually are not familiar with stem cell medicine and are therefore very dependent on information from independent organisations. In many cases, autologous collections are criticized by competing organisations. For example, haematologists prefer bone marrow stem cells and have a very critical opinion towards stem cells received from the umbilical cord.

To be able to discuss the sense of cord blood storage, it is important to divide the medical possibilities into two groups in order to identify and discuss unanswered questions. On the one hand, there is tumour therapy as an alternative to donor received bone marrow transplantation. Autologous cord blood is used in cases of the reduced production of erythrocytes (hereditary erythrocytopenia). On the other hand, there is the very important and auspicious field of tissue engineering. It seems that this may be the most promising way of application of umbilical cord stem cells. Scientists realize that the future of tissue engineering is related to a source of young and vital stem cells as umbilical stem cells. Unanswered questions are mainly the number of stem cells and what type of stem cells can be found in umbilical cord blood. Techniques of expansion seem to show progress with some groups claiming to have solved the problem. Differentiation in various cell types seems to get practicable various tissues could already be grown. As recent literature shows, fiction slowly seems to become a reality.



State of the art lecture:

STEM CELLS FOR THE TREATMENT OF INHERITED HEPATIC METABOLIC DISORDERS

Maurizio Muraca – IRCCS Ospedale Bambino Gesù – Rome (Italy)

The pioneering work of Lagasse et al.¹ on the FAH ^{-/-} mouse, an animal model of Tyrosinemia type I, has shown that bone marrow transplantation with purified haematopoietic stem cells from wild type animals could repopulate the liver and cure the inherited metabolic disorder. This observation originated the hypothesis of obtaining “liver from bone marrow”, i.e. use bone marrow-derived cells for the treatment of liver disease. Enthusiasm for this therapeutic approach has been more recently tempered by the finding that cell fusion, rather than true differentiation, was responsible for liver repopulation in Lagasse’s model with cells bearing normal enzyme activity²⁻³. Moreover, several studies demonstrated insignificant liver parenchymal repopulation with BMDCs following either bone marrow transplantation or the intravenous systemic delivery of a selected cell phenotype⁴⁻⁸. Evidence of hepatocytic differentiation of BMDCs was indeed observed by Jang et al⁹ in liver-injured mice transplanted with *in vivo*-activated hematopoietic stem cells. However, several Authors currently believe that liver repopulation with bone marrow-derived cells is a stochastic phenomenon with little biological significance, unfeasible for therapeutic application. Possible reasons for lack of reproducible results, leading to the current attitude, include the model and timing of liver injury, the route of cell administration and the selection or activation of the transplanted cell population.

We used reversible ischemia/reperfusion liver injury to induce engraftment and hepatic parenchymal differentiation of exogenous β 2-microglobulin ^{-/} Thy1⁺ bone marrow-derived cells¹⁰. This method of hepatic parenchymal repopulation, theoretically applicable to clinical practice, was further tested to correct the metabolic disorder in a rat model of congenital hyperbilirubinemia.

Analysis by confocal laser microscopy of fluorescence-labelled cells and by immunohistochemistry for β 2-microglobulin 72 hours after intraportal delivery demonstrated engraftment of infused cells in liver parenchyma of rats with ischemia/reperfusion, but not in control animals with non-injured liver. Transplantation of bone marrow-derived cells obtained from GFP-transgenic rats into Lewis rats resulted in the presence of up to 20% of GFP⁺ hepatocytes in ischemia/reperfusion liver lobes after one month. The repopulation rate was proportional to the number of transplanted cells. Infusion of GFP⁻ bone marrow-derived cells into GFP-transgenic rats resulted in the appearance of GFP⁻ hepatocytes, suggesting that the main mechanism underlying parenchymal repopulation was differentiation rather than cell fusion. Transplantation of wild-type bone marrow-derived cells into hyperbilirubinemic Gunn rats with deficient bilirubin conjugation after ischemia/reperfusion damage resulted in 30% decrease of serum bilirubin, in the appearance of bilirubin conjugates in bile and in the expression of normal UDP-glucuronyltransferase enzyme evaluated by PCR analysis.

In conclusion, ischemia/reperfusion injury induced hepatic parenchymal engraftment and differentiation into mature hepatocytes of bone marrow-derived cells. Transplantation of bone marrow-derived cells from non affected animals resulted in the partial correction of hyperbilirubinemia in the Gunn rat, suggesting that this procedure could potentially be used for the treatment of inherited metabolic liver diseases.

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Mobilization of unique populations of hematopoietic progenitor cells in response to clinical situations associated with liver regeneration.

Gehling Ursula

A series of transplantation studies suggests that bone marrow-derived hematopoietic stem and progenitor cells, in particular precursor cells committed to the myelomonocytic lineage, have the capacity to migrate into the liver and to contribute to liver regeneration. In this study, we hypothesized that liver injury induces recruitment of stem and progenitor cells from the bone marrow. In order to detect liver injury-mobilized precursor cells, pre- and postoperative peripheral blood samples from 11 living liver donors as well as steady-state blood samples from 72 patients with liver cirrhosis were analyzed by flow cytometry using antibodies against the stem cell markers CD133 and CD34. Stem cell marker-positive cells were further characterized by using antibodies to CD45, CD14, c-kit, and Bcrp-1. Immunomagnetic separation was performed to select putative progenitor cells for functional assays in vitro. In all liver donors studied, a significant increase in the percentage of CD133-positive (+) cells could be observed 12 hours after partial hepatectomy (PH). Following a decrease over the next two days, levels of CD133⁺ cells rose again at day 4 post operational. This oscillatory pattern recurred during the next ten days with repeated increases at days 7, 10, and 14, respectively. In contrast, no postoperative increase in the percentage of stem cell marker-positive was found in patients undergoing gynecological or traumatological surgery. Further phenotypic characterization of PH-induced circulating CD133⁺ cells revealed that virtually all cells co expressed the pan-leukocyte antigen CD45 and the monocytic marker CD14. Functionally, these cells possessed hematopoietic and hepatic differentiation potential in vitro. Circulating progenitor cells with identical phenotype and functional properties could also be observed in more than 50 % of patients with liver cirrhosis. Unexpectedly, the percentages of circulating CD133⁺ cells varied from day to day and did not correlate with any clinical parameter, such as etiology, disease stage, liver enzymes, and leukocyte counts, respectively. In addition, two other populations of circulating precursor cells were identified in these patients. The first population was characterized by expression of c-kit and was found in more than 90 % of the patients. The second expressed the stem cell marker Bcrp-1 and was present in 17 % of the patients. Functionally, both populations showed multilineage hematopoietic potential. When cultured under the same growth conditions, which supported hepatic differentiation of CD133⁺ cells, c-kit⁺ cells as well as Bcrp-1⁺ cells developed a fibroblastic morphology. Again, no correlation was found between the population of progenitor cell and any clinical parameter. Interestingly, all three populations expressed CXCR4, the receptor for stromal cell-derived factor-1 (SDF-1), indicating that SDF-1/CXCR4 interactions might play a role in progenitor cell recruitment to the liver. In line with this interpretation, elevated levels of SDF-1 could be demonstrated in all patients with liver cirrhosis. In summary, we have identified three distinct populations of hematopoietic progenitor cells, which might play different roles in regeneration. The observation that all three populations co expressed CXCR4 suggests that they might be released from the bone marrow and that hypoxia-inducible factor-1 triggers this process. In vivo studies are underway to investigate this assumption and to determine their exact role in liver repair.



Abstract 2nd ITERA Workshop

NASH and liver regeneration

S. Francque, MD

University Hospital Antwerp
Department of Gastroenterology and Hepatology
University Hospital Antwerp, Edegem, Belgium

Non-alcoholic Liver Disease (NAFLD) and Non-alcoholic Steatohepatitis (NASH) are increasingly recognised as an important source of liver related morbidity and mortality. It is not only a cause of progressive fibrosis and cirrhosis, but also complicates liver surgery and liver transplantation. The pathophysiology is complex and only partially understood. The steatosis related problems in liver surgery and liver transplantation (primary graft non-function and delayed graft function) are associated with a reduced liver regeneration capacity that could be demonstrated in several animal models, although not in all. The impaired regeneration capacity has a multifactorial aetiology. Changes in liver blood flow, different cytokines (e.g. TNF) and different intracellular pathways (e.g. PPAR γ) seem to be implicated. Hepatocyte transfusion and stem cell transfusion are of potential interest in treating steatosis related regeneration impairment.



Engraftment of mesenchymal stem cells after in utero transplantation into immunocompetent mice.

Troeger C, Holzgreve W, Dudler, Perahud I, Hahn S
Laboratory for Prenatal Medicine, Women's Hospital, University Basel, Switzerland

Introduction: Early prenatal diagnosis and in utero therapy of certain fetal diseases have the potential to reduce fetal morbidity and mortality. The intrauterine application of stem cells for regenerative purposes would probably provide a therapeutic option before definitive organ failure has occurred. We have established an in utero mouse model for the transplantation of mesenchymal stem cells (MSC) to test this hypothesis.

Methods: GFP-expressing murine fetal liver MSC were derived from culture passage 9 and 10^5 MSC in 5 μ l were injected into the peritoneal cavity of C57BL/6 Pups on day 13.5 of pregnancy. Newborn mice were sacrificed and analyzed 2 resp. 4 weeks after delivery using FACS and IHC. Quantification of engraftment by PCR will be performed in the near future.

Results: Significant engraftment of MSC could be observed in the liver, lymph nodes, spleen and kidney.

Conclusion: Further work will clarify the optimal time point and dosage of IUT as well as morphology and function resp. expression pattern of engrafted cells.



Pathological Assessment of Stem Cell Therapy In Acute And Chronic Myocardial Ischemia

L. Maximilian Buja, MD; Deborah Vela, MD; Guilherme V. Silva, MD; Yong J. Geng, MD, PhD; James T. Willerson, MD; Emerson C. Perin, MD, PhD

Despite promising results for the use of stem cells (SC) in heart disease, there remain major questions without a definitive answer that range over several aspects of stem cells for their use in man such as: the optimal types and doses of SC, the optimal delivery methods or routes, and the biosafety and efficacy of the treatment.

We have been addressing these issues via large animal experimental models in which we have produced both acute and chronic myocardial ischemia, particularly in the canine and swine. The acute ischemia is usually produced by acute coronary occlusion and the chronic ischemia by the ameroid constrictor method. Types of SC utilized in cardiac cell therapy include: embryonic SC, skeletal myoblasts, various types of bone marrow SC, and cardiac SC. Our experience has been centered around bone marrow SC (particularly its mononuclear subfraction and mesenchymal SC) and fetal cardiomyocytes (with and without overexpression of certain genes).

Our pathologic approach in evaluating the efficacy of these different treatments has included: infarct size methodology; fibrosis quantification, vascular density assessment, SC retention rate, SC distribution, immunohistochemistry, identification of the transplanted SCs (DAPI-, DiI- or GFP-labeled) and colocalization with adult phenotypical markers.

Among our major findings we have successfully identified transplanted SC in the myocardium and determined their selective patterns regarding their retention and distribution with regards to different delivery routes. In some models we have observed increased healing and vascular density. Colocalization has been mostly with vascular cell markers (smooth muscle and endothelial cells) and rarely with cardiomyocytes. Certain issues remain, however, in the interpretation of such colocalization, such as paracrine effects versus transdifferentiation and fusion. Improvement in regional circulation and cardiac function has been observed in some models.

As a major and general conclusion, there is evidence that SC therapy can lead to cardiac recovery, both clinically and pathologically, but the responsible mechanisms are still under active investigation.



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Update on the Advanced Therapy Medicinal Products Regulatory Framework in Europe, illustrated with a practical example.

Dr. Peter De Waele, Ph.D., Director Regulatory & Clinical Affairs, Life-Science Group

An update on the current situation concerning the harmonisation of the regulatory framework (patchwork) within Europe will be given, focusing particularly on the somatic cell therapy and tissue engineered products which, together with gene therapy products, belong to the Advanced Therapy Medicinal Products. Recommendations towards somatic cell therapy products particularly will be given and illustrated with a practical example from own experience.



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" Regenerative medicine in Aalborg. GMP facilities, first results and plans"

Peter Ebbesen. Trine Fink. Helle Lysdahl, Vladimir Zachar (Aalborg University); Anette Gabrielsen Ciconia Co.

Our new laboratory building prepared for GMP work was used for establishing human adult and embryonic stem cells lines. Focusing on the effect of peri cellular oxygen tension we have developed a method for controlling this parameter. The first results indicate the importance of adjusting oxygen tension to the differentiation stage of the cells. during chondrogenesis. Work in progress deals with prevention of differentiation of the stem cells during culturing. Animal experiments on the long term effect of stem cells transplantation has been initiated.



Non-hematopoietic stem/progenitor cells from human cord blood-account of a Zurich experience

Andreas H. Zisch

Dept. of Obstetrics, University Hospital Zurich, Switzerland; andreas.zisch@usz.ch

Hematopoietic stem cells from cord blood have proven life saving in treating blood disorders and specific cancers. Recently, non-hematopoietic type stem/progenitor cells isolates from cord blood have entered the scene as developing therapy. Claims are that cord blood may provide an almost unlimited autologous supply of true progenitors to vascular endothelial cells and mesenchymal stem cells for potential application as cellular substrates for replacement tissues, i.e. blood vessels, bone, cartilage. These claims deserve careful scrutiny. I will give an account of our laboratory's experience with non-hematopoietic cell types grown from human cord blood. I will discuss (1) Great biological disparity between cord blood samples in frequency of outgrowing stem cells (2) Limited capacity for self renewal of progenitors to endothelial cells (OECs) (3) The plasticity of mesenchymal stem cells and cell death accompanying differentiation (4) Specific molecular signature of cord blood-derived endothelial cells as determined by comparative proteomics versus mature vessel wall-derived endothelial cells. Our still preliminary experience with cultured cord blood-derived non-hematopoietic cells suggests that their practicality and utility for creating tissue replacements may be not as wide-ranging as claimed. A more effective route of exploiting these cells in tissue regeneration could be to genetically engineer these cells for delivery of curative biological agents, but this remains to be demonstrated.



Bone marrow-derived stromal cells as vehicles for neurotrophic factors

Peter Ponsaerts¹, Mark Ronsyn², Jasmijn Daans¹, Shyama Chatterjee³, Dirk Ysebaert⁴,
Eric Van Marck³, Philippe Jorens², Zwi Bememan¹

- (1) Laboratory of Experimental Hematology, Antwerp University, Belgium.
- (2) Division of Pharmacotherapy, Antwerp University Hospital, Belgium.
- (3) Laboratory of Pathology, Antwerp University, Belgium.
- (4) Laboratory of Experimental Surgery, Antwerp University, Belgium.

Stem cells, both adult and embryonic, are potentially attractive targets for *ex vivo* gene therapy in a variety of diseases. In this context, we aim to investigate the use of genetically modified bone marrow-derived stromal cells (MSC) as vehicles for neurotrophic factors (BDNF and NT3) in spinal cord.

Using traditional plasmid DNA construction, DNA electroporation and combined antibiotics selection + FACS sorting, we have constructed three MSC lines designated as: (i) hMSC/EGFP, a human MSC line expressing the enhanced green fluorescent protein (EGFP), (ii) hMSC/BDNF-IRES-EGFP, a human MSC line co-expressing brain-derived neurotrophic factor (BDNF) and EGFP, and (iii) hMSC/NT3-IRES-EGFP, a human MSC line co-expressing neurotrophin-3 (NT3) and EGFP. During *in vitro* culture (up to 3 months) all three lines remained highly positive for their transgene expression.

Next, in order to reproducibly identify transplanted hMSC, we first optimized several histological and molecular techniques. Upon transplantation of hMSC-EGFP into healthy rat spinal cord, at several time points after transplantation, engrafted hMSC-EGFP could be (up to 3 weeks post-transplantation) positively identified: (i) by histological analysis showing direct EGFP fluorescence and positive antibody staining for EGFP, human nuclear antigen and human mitochondrial antigen, and (ii) by PCR/RT-PCR analysis showing the presence of EGFP DNA and mRNA in dissected tissue samples.

When hMSC/BDNF-IRES-EGFP and hMSC/NT3-IRES-EGFP were transplanted into healthy rat spinal cord we observed the same degree of cell survival as compared to transplanted hMSC-EGFP. However, transgene expression of transplanted hMSC/BDNF-IRES-EGFP and hMSC/NT3-IRES-EGFP rapidly declined during the first week *in vivo* as demonstrated by histological analysis. Currently, we do not yet know why this transgene silencing occurred and several strategies (epigenetic modification / novel DNA constructs) to circumvent this problem are under investigation.

In conclusion, we demonstrate that hMSC transplanted in healthy rat spinal cord survive well and persist for at least 3 weeks post-transplantation. However, novel gene therapy and/or epigenetic strategies need to be developed and tested in order to prevent transgene expression.



Biological limitations in regenerative medicine relevant properties of autologous adult stem cells

Dimitry Spitkovsky

Institute of Neurophysiology, University of Cologne

Stem cells are persisting through entire life of individuals in almost all adult tissues, including bone marrow, adipose tissue, heart and central nervous system. Stem cells in therapeutic applications may be combined and therefore increase the value of existing therapies or may be a basis for entirely new therapies and providing cure where current medicine reaches its limits.

However optimal stem cells are not defined yet and several stem cell sources are being currently under intense investigation. It is still possible that different stem cells would be required for different indications and necessity to combine several types of the stem cells for single medical indication could not be ruled out. Presently stem cell accessibility and number are among major factors considered for their potential use in clinical settings. Stem cells from several tissues were already successfully isolated and processed to significant numbers as for example in case of mesenchymal stem cells isolated from either bone marrow or adipose tissue. Application of autologous stem cells in regenerative medicine represents least controversial approach because of their accessibility and because immune rejection after their transplantation could be excluded. However expansion of autologous stem cells to sufficient therapeutic dose may be time consuming and currently it is taken at least 2-4 weeks to expand the cells to desired amount. This time may not be acceptable in some acute indications and life threatening diseases as for example in case of myocardial infarction. It is also considered that there is a decline in stem cell number/performance, which is associated with age and degenerative diseases. Such decline in stem cell function could lead to decline in regenerative capacities of the stem cells after transplantation. In this regard it is important to consider how biological properties of the stem cells could affect their performance in clinical settings. For future stem cell application it would be necessary to bypass limitations associated with adult stem cells isolated from sick or aged donors. Such goal could be probably only achieved after stem cell transplantation under allogenic settings. In such case stem cells could be isolated from presently discarded surplus tissue sources as placenta/umbilical cord or from umbilical cord blood or alternatively from healthy donors. The stem cells should be expanded, quality controlled and stored as "ready of shelf" cell product. Significant research efforts still required defining feasibility of such approach.



The Rapid Transition of A Mesenchymal Progenitor Cell Line From In Vitro To In Vivo As A Model System For Studying Bone Development

Alan Chan, Chief Executive Officer, Percuros B.V.

Differentiated cell lines, by nature of their immortality and ability to represent various cell types, have proven to be valuable tools for drug screening, for studying pathophysiological mechanisms and to understanding issues of clinical relevance. However, to obtain such a ready access of cells, special considerations must be addressed to establish in vitro function. These same considerations, paradoxically, can often result in discordancy between the in vivo effects, in which the cell lines seek to represent, and their in vitro function.

When the in vitro assay is put into the context of a simple model system, these cell lines can address many aspects of cellular mechanisms. For example, the Caco-2 cell line is widely used to predict candidate drug compounds across the intestinal epithelial barrier. In contrast, cell lines for studying hepatotoxicity have often been unconvincing, primarily being unable to mimic the complexities of liver function. Furthermore, with the human genome sequencing and mapping projects revealing more about genes and pathway activities, the limitations of using differentiated cell lines for gene function are becoming more and more apparent.

Percuros, based in Leiden, has genetically modified cell lines, both differentiated and progenitor in nature, to tackle some of these issues. More specifically, we have developed a mesenchymal progenitor cell line that differentiates into several mesoderm-type tissues (bone, cartilage and fat) for our studies of bone metabolic diseases and associated therapeutic areas such as diabetes and obesity. After the discovery of the bone mass gene, SOST (protein: sclerostin), by two of the founders of Percuros, the KS483 progenitor cell line was developed to allow us to study gene function and especially the pathways around sclerostin.

The advantage of such cells, whether they are pluripotent (like stem cells) or progenitor in nature, allows much better differentiation of cells for relevant representation of tissues in vivo. Furthermore, we have genetically modified the cells with an open insertion site to allow genes to be over-expressed or silenced with a shRNA construct slotted in. The cell line is stable without the typical problems of clonal variation. Lineage specific gene reporters and cell signalling reporters with luciferase expression allow the cells to be monitored under high content screening and for gene functional assays. This adaptation of the cells has also allowed us to extrapolate such systems very rapidly into pre-clinical testing. The same cells are transplanted into the mouse in either bone marrow or under the skin of the mouse to study for ectopic bone formation (molecular imaging).



Therapeutic stem cell approaches in Multiple sclerosis –

N. Hellings, Biomed – University of Hasselt, Belgium

Multiple sclerosis is an auto-immune mediated inflammatory disease of the central nervous system (CNS) ultimately leading to myelin breakdown and damage to both oligodendrocytes and axons. While the inflammatory component is prominent at early stages of the disease, neurodegenerative processes are more important in later stages. Several reports illustrate the importance of axonal loss and neuronal damage in determining disease burden in MS. One of the challenges in MS research is to devise strategies that will promote remyelination and neuroregeneration in the CNS, in addition to therapeutic approaches that limit inflammatory responses. Based on current knowledge of CNS remyelination, two approaches could be adopted. In the first, methods of *in vivo* manipulation of the patient's own neural progenitor population can be applied to promote repair (e.g. by local delivery of differentiation or migration promoting agents). In the second approach, the strategy would be to replace the absent or inhibited oligodendrocytes and neurons in or adjacent to MS plaques by transplanting progenitor cells. Brain derived adult or fetal neural progenitor/stem cells are likely to be the best candidates to promote neural tissue repair as was recently demonstrated by Pluchino et al in an animal model for MS. However, from a clinical point of view, a more readily accessible source of stem cells is needed. Moreover, the use of fetal or embryonic derived stem cells is highly debated based on ethical arguments. In this light, the identification of pluripotent/multipotent progenitor/stem cells (e.g. derived from human bone marrow or umbilical cord tissue) is of incremental importance. We will evaluate whether these human multipotent stem cell populations have therapeutic value in an animal model for MS and devise strategies to enhance their migration and therapeutic effects.



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DC-based immunotherapy of cancer

Van Tendeloo (ITERA, Maastricht)
Laboratory of experimental Hematology
University of Antwerp, Belgium

The aim of cancer vaccination is to place an antigen within the body of a cancer patient so that the immune system can be provoked to unleash the wrath of the killer T cells on the patient's tumor cells. Armed with the knowledge of how T cells interact with antigens at the molecular level, scientists are able to design potent antigen-presenting cells (APC) such as dendritic cells (DC) that can selectively activate specific T cells to eradicate cancer cells. In general, the success of DC-based vaccine strategies depends on multiple factors, such as the mode of antigen delivery, the choice of DC, administration route and frequency. Also, circumventing T cell tolerance present in the tumor-bearing host warrants further investigation. I will present some of our *in vitro* and preclinical work on the design of DC-based cancer vaccines based on delivery of the antigen via mRNA gene transfer. I will also discuss a recently started clinical DC vaccination trial in leukemia patients at the Antwerp University Hospital.



Ex vivo expansion of red blood cells.

Kolbus Andrea, University of Vienna, Austria

Haematopoiesis is a complex process regulated by multiple growth factors and hormones supporting the renewal, differentiation and survival of haematopoietic progenitors at different stages of maturation. Pluripotent, multipotent and committed precursors can undergo a number of “renewal divisions”. During terminal differentiation, committed progenitors obey a fixed program which directs both the residual number of cell divisions and the specific timing of differentiation. The correct balance between renewal and terminal differentiation is essential for homeostasis of the haematopoietic system (maintaining adequate numbers of precursors as well as of mature cells) and lost under pathological conditions such as leukemia and anemia

The ex vivo expansion of human umbilical cord blood derived haematopoietic cells may be an ideal source for reconstitution of human bone marrow. The generation of erythroid progenitors and the differentiation of these progenitors into mature erythrocytes would be a potentially powerful erythrocyte sources for blood transfusion.

Erythroid progenitor cells isolated from umbilical cord blood can be expanded in vitro under serum free culture conditions and can be differentiated into mature erythrocytes in the presence of high concentrations of Erythropoietin. The differentiation of these erythroid progenitors into mature erythrocytes is documented by the three parameters: proliferation during differentiation, reduction of cell volume and increase of hemoglobin accumulation.

The here described ex vivo expanded red blood cells could be a new source for red blood cells which can be helpful in supplying additional material for transfusions. In addition, the expansion under controlled and defined conditions will reduce inherent risks of transfusions like viral infections. Thus, the limited supply of human blood products that often causes shortages for patients can be overcome by such ex vivo expanded red blood cells.



PamChip® Technology

Rinnie van Beuningen
Pamgene

We present here the applications of PamChip® Technology, a three dimensional microarray system suitable for the simultaneous analysis of up to 400 peptides or oligonucleotides.

Kinase PamChip® microarrays consist of 140+ putative tyrosine kinase peptide substrates or 240+ putative serine/threonine substrates which can be used to monitor activity of kinases in purified samples as well as cell-lysates derived from cell-lines or tissue samples. Applications include unraveling of cell-signalling pathways, new biomarker development, simultaneous potency and selectivity determination of kinase compounds and basic proteomic research.

Nuclear Hormone Receptor PamChip® microarrays consist of 50+ coregulators and enable testing of thousands of conditions in one experimental run. Compound classification based on efficacy and potency and understanding the fundamentals of nuclear hormone receptor biology are the two main applications of this technology.

Genomic applications such as gene expression, gene copy number analysis, SNP detection and biomarker development will also be discussed.



Synergistic Polymertechnologies for Cryopreservation, (Stem)cell Technology and Tumor-Therapy/ Diagnostics

D. Müller-Schulte
DMS-Technologies

A novel approach using a combinatorial polymer technology is described to circumvent the drawbacks with the current cryopreservation using e.g. high salt concentration, toxic agents (DMSO) or ineffective substances. This enables a basis to procure optimal protocols for cryopreservation and in parallel to facilitate adaptation of the optimal protocols to specific cells, e.g. keratinocytes for tissue replacement, hepatocytes for transplantation or stem cells.

The novel approach comprises the following technologies which can be combined in a synergistical way:

- **layer-by-layer technique using radiation grafted support matrix** (beads, tubes, flat membranes) for immobilization of cells which is finally covered by a top layer consisting of a cryopreservant
- **Encapsulation into biocompatible polymer matrices (inverse suspension technique):**
gelatin, silica gel, alginate, agarose in which simultaneously e.g. electrolytes, growth factors and cryopreservants are encapsulated
- **Usage of surface active substances** selected in a combinatorial manner according to:
 - Biocompatibility
 - Solubility parameter
 - Partition coefficient; aim: substitution of DMSO
 - Viscosity
 - Hydrophilic-Lipophilic Balance (HLB-concept)

Based on novel inverse suspension techniques DMS-Technologies has synthesized a variety of polymer spherical polymer carriers (beads). The spectrum of these carriers comprises the following polymers:

- ⇒ Gelatine
- ⇒ Acrylates
- ⇒ Alginates
- ⇒ Chitosane
- ⇒ Polyvinylalcohol
- ⇒ Agarose
- ⇒ Polysaccharides

The key aspect of the new suspension technique is highlighted by the fact that the synthesis of the spherical particles can be accomplished **in minutes**. This represents a time saving factor in comparison to established procedures e.g. used by Dynal, Estapor, Merck, Promega, MicroMod of >100 thus saving time, manpower and costs.

Due to the large product and polymer portfolio, a parallel spectrum of functionalities can be covered which opens up a broad spectrum of diverse applications. Important examples are:

- Hemoperfusion for blood detoxification
- Drug carrier
- Cell cultivation / Stem cell cultivation
- Scaffold for tissue engineering
- Cell separation

An optimal adaptation to the targeted application is enabled by the possibility to select between a large variety of diverse polymers in combination with a adjustable size range from 1 to 3000 μm . **All polymer carriers can also be rendered magnetic.**



TissueFAXS: A New Solution for Multicolor Tissue Cytometry

Georg E. Steiner, Radu Rogojanu, Katja Oesterreicher and Rupert Ecker

*TissueGnostics GmbH, R&D,
Institute for Computer Graphics and Vision, Technical University, Graz
Department of Urology, Medical University of Vienna,
Vienna, Austria*

The necessity of a standard for automated evaluation of cell stainings in immunohistology has been increasingly emphasized.

Based on AF-CLSM (Automated Fluorescence Confocal Laser Scanning Microscopy), we developed the TissueFAXS as the microscopic equivalent to flow cytometry. The technique consists of a fully automated microscope as well as advanced image processing software (*HistoQuest/TissueQuest*). It can be used with immunohistochemical and immunofluorescence staining to quantify tissues on the single cell level. A variety of versatile identification strategies for automated recognition of individual cells, covering color separation for brightfield images, fluorescence-based pattern recognition, single cell identification by combination of fluorescent or chromogen signals, and separate data evaluation for nuclear, cytoplasmic and surface membrane structures is provided. Complex interactions on the cellular as well as subcellular level can be addressed with respect to how many of which cells are where and what is their (functional) status. In histology phenotypic characteristics can be associated with localization and morphologic features in a quantitative manner.

Its versatile functionality makes TissueFAXS appropriate for the search for predictive markers in cancer diagnosis as well as post transplantation monitoring based on solid tissue sections and biopsy material, respectively. The composition and state of activation of tissue infiltrating leukocytes can be determined in situ. This technique is a versatile tool for histopathology, immunology, single cell cytometry and cytomics.