INTERNATIONAL CONSORTIUM FOR CELL THERAPY AND IMMUNOTHERAPY

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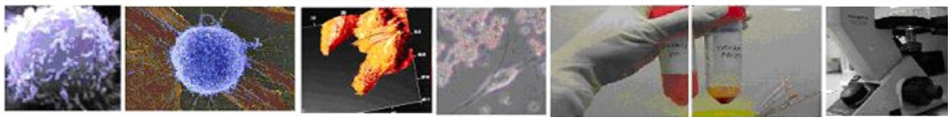

ICCTI ANNUAL CONFERENCE 5th

May 15-16, 2015
Hotel Karel V, Utrecht, The Netherlands

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INTERNATIONAL CONSORTIUM FOR CELL THERAPY AND IMMUNOTHERAPY

5-th ICCTI ANNUAL CONFERENCE

Dear Colleagues,
I would like to cordially invite you to the 5-th ICCTI ANNUAL CONFERENCE which will be held in a beautiful city of Utrecht full of tulips in May. As you may know, ICCTI propagates cell therapy and immunotherapy worldwide and this year we would like to point out 4 main hot topics that reflect the state-of-art in cell therapies and immunotherapies.
In the name of organizing committee I am looking forward to see you in Utrecht.
With warmest regards

Jaroslav Michalek, MD PhD
ICCTI President

Brno 2015

5-th ICCTI ANNUAL CONFERENCE

Program topics:

1. **Stem cells – from biology to clinical application**
2. **Cell-therapies and regulations**
3. **Adipose tissue in regenerative medicine**
4. **Cell-based immunotherapy**

Conference proceedings of abstracts

Editor: Jaroslav Michalek, MD PhD

Conference Program

Conference Day 1, Friday, May 15, 2015

13:00-13:30 Registration

Block 1: Stem cells – from biology to clinical application

Chair: J. Michalek

13:30- 14:00 **Dr. A. Darinskas** (National Cancer Institute, Lithuania)
Stem cells in regenerative medicine

14:00- 14:30 **Dr. T.K. Malan** (Okyanos Heart Institute, USA)
Understanding the role of Adipose Stem Cells in the spectrum of Degenerative Diseases

14:30- 15:00 **Dr. G. Grisendi** (University Hospital of Modena and Reggio Emilia, Italy)
Engineering Adipose Mesenchymal Progenitors for Cancer Therapy

15:00- 15:30 **Prof. E. Chernykh** (Institute of Fundamental and Clinical Immunology, Russia)
Macrophages as new candidate cells for stroke treatment

15:30- 16:00 **Prof. N. Zakaria** (University of Antwerp, Belgium)
Corneal Regeneration – From the Lab to the Clinic and Back

16.00- 16.30 Coffee break

Block 2: Cell-therapies and regulations

Chair: T. K. Malan

16:30- 17:00 **Prof. J. Michalek** (ICCTI, Brno, Czech Republic)
Legal issues in cell therapies and EU Regulations + ICCTI Letter to EMA

17:00- 17:30 **T. Stasch, MRCS (Ed.), F EBOPRAS, MD** (Valentis Beauty, Clinic for Plastic Surgery and Lifestyle Medicine, Cologne, Germany)
The DEALTH Method - How we can improve Wound Healing of Chronic Ulcers of the Lower Limb and Diabetic Feet using Autologous Lipotransfer

17:30- 18:00 **Dr. K. Freitag** (President of the Spanish society for schockwave SETOC, Spain)
Biological effects of Shockwave therapy

18:00- 18:30 **Dr. N. Cools** (University of Antwerp, Belgium)
The pursuit of tolerance: Immunomodulation of dendritic cells for therapeutic intervention in multiple sclerosis

19:00 Dinner and networking

Conference Day 2, Saturday, May 16, 2015

Block 3: Adipose tissue in regenerative medicine

Chair: K. Ueberreiter

9:00- 9:30	**Dr. HJ Duckers** (University Medical Center, Netherlands) *Hype or Hope: The future of adipose-derived stem cells in cardiovascular disease*
9:30- 10:00	**Prof. M. Harmsen** (PI Cardiovascular Regenerative Medicine Group, Netherlands) and **Dr. M. Spiekmann** (Univ. Groningen, Netherlands) *Adipose-derived stromal cells for soft tissue reconstruction and regeneration: future or fiction?*
10:00- 10:30	**Prof. J. Michálek** (ICCTI, Brno, Czech Republic) *Treatment of osteoarthritis with freshly isolated stromal vascular fraction cells from adipose and connective tissue*
10:30- 11:00	**Prof. D. Garcia-Olmo** (Fundacion Jimenez Diaz University Hospital, Spain) *Adipose Derived Stem cells in Crohn Disease*
11:00- 11:30	**Prof. O. Tapparo** (Private day hospital for aesthetics and interdisciplinary dentistry, Germany) *Risk factor oral cavity, treatment with autologous blood factors (plasma-, growth factors and stem cells)*
11:30- 12:00	**Dr. E.A. Blinova** (Institute of Fundamental and Clinical Immunology, Russia) *T-cell vaccines in the treatment of atopic dermatitis and multiple sclerosis*
12:00- 12:55	Lunch break

Block 4: Cell-based immunotherapy

Chair: V. Schijns

12:55- 13:00	**Prof. V. Schijns** *Introduction note*
13:00- 13:30	**Dr. M. Strioga** (National Cancer Institute, Lithuania) *Dendritic cell-based vaccines for the treatment of recurrent glioblastoma multiforme*
13:30- 14:00	**Dr. E. Smits** (Antwerpen University, Belgium) *Vaccination with WT1 mRNA-Electroporated Dendritic Cells: Report of 66 Cancer Patients*
14:00- 14:30	**Dr. M. Glassy** (Nascent Biotech Inc., USA) *Pritumumab – a clue to using the natural human immune response for drug development*
14:30- 15:00	**Dr. H. Dolstra** (Nijmegen Radboud University, Netherlands) *Cancer immunotherapy with ex vivo-generated NK cells from hematopoietic progenitor cells*
15:00- 15:30	**Dr. E.Ya. Shevela** (Institute of Fundamental and Clinical Immunology, Russia) *Immunomodulatory effects of mesenchymal stromal cells in lymphoma patients following HSC transplantation*
16:00	End of Conference

Stem cells in regenerative medicine

Dr. A. Darinskas

National Cancer Institute, Lithuania

Stem cells has been known for a long time in the field of regenerative medicine, plenty of sources has been mentioned and described in literature worldwide. Starting from embryonic and ending with adult stem cells plenty of formulations and applications could be done. In the last few years our team worked on adipose tissue stromal vascular (SVF) cells and their practical applications to treat conditions where active regeneration is needed. After successful collaborative start (Cellthera) with orthopedic applications we managed to apply this technology in the field of angiosurgery. We have treated 15 patients with autologous SVF cell injections with critical limb conditions. 13 patients clinically improved and were stable for up to 12 months after procedure. Collateral neovascularization of the limbs was observed in all the cases. Only two patients didn't respond to the treatment and their limbs should have been amputated. Nevertheless, this technology seems to be promising tool to treat critical limb ischemia in different groups of ischemic patients.

Understanding the role of Adipose Stem Cells in the spectrum of Degenerative Diseases

Dr. T.K. Malan

Okyanos Heart Institute, USA

Adipose derived mesenchymal stem cells (ASC's) or Stromal Vascular Fraction (SVF) has been shown to be a rich source of bone marrow derived mesenchymal stem cells (BMSC's) like $CD34^+$ stem cells. Autologous adipose tissue derived SVF is easily harvested from the patient utilizing minimally invasive techniques with the ability to achieve superior concentrations of mesenchymal stem cells (MSC's) and MSC like cells as compared to any other tissue source without the use of culture expansion. More importantly, adipose ASC's or SVF has demonstrated potential therapeutic benefits in a variety of pre-clinical and clinical models not previously demonstrated when utilizing expanded BMSC's or Peripheral Blood mesenchymal stem cells (PBMSC's). There is a plethora of anecdotal and more recently, evidence based information to suggest various source MSCs may have significant beneficial use for a large variety of tissue ischemia, auto-immune, inflammatory and degenerative conditions. In addition to simply replacing damaged tissue by direct differentiation, ASC's have demonstrated immunoprivilege as well as the ability to; both up and down regulate the immune response, enhance cell to cell communication via paracrine and microvesicle formation essential in blocking harmful inflammatory and apoptotic responses, as well as providing trophic factors known to regulate angiogenesis. I will discuss our experience at Okyanos in developing protocols for a variety of degenerative, ischemic, inflammatory, and autoimmune disease states based on an understanding of the cycle of disease and the ability of ASC's to interrupt that cycle. I will discuss our preferred methods for adipose cell harvesting and processing as well as the novel use of new non-enzymatic cell processing that excels at the treatment of soft tissue injuries.

Engineering Adipose Mesenchymal Progenitors for Cancer Therapy

Giulia Grisendi, Carlotta Spano, Massimo Dominici

Laboratory of Cellular Therapy, Department of Medical and Surgical Sciences for Children & Adults, University Hospital of Modena and Reggio Emilia, Modena, Italy

Despite significant advances in the field of cancer gene therapy, the lack of a specific tumor tropism of viral vectors and the possible stimulation of an immune cells limit the clinical potential of a direct delivery of genes into the cancer cells. The use of mesenchymal stromal/ stem cells (MSC), as cellular vehicles, represents an attractive option to overcome these barriers supporting a targeted delivery of the desired gene or therapeutic protein to the tumor site. Thanks to their biological and immunological features, including easy accessibility from different source, rapid proliferation in culture and poor immunogenicity, MSC represent a powerful weapon to develop novel strategy to fight cancer. In particular, the tropism displayed by MSC for tumor sites generating a growing interest among researchers. Several studies on solid tumor microenvironment have indicated that MSC take active part in the generation of a supportive stroma, becoming structural components of tumor architecture and releasing cytokines and chemokines at the tumor site.

Based on this knowledge, we conceived to use modified adipose MSC as “trojan horse” to deliver the anticancer molecule TNF-related apoptosis-inducing ligand (TRAIL) against different tumours. TRAIL is a potent cytotoxic molecule physiologically produced by immune cells and it plays a pivotal role in immunosurvelliance, inducing apoptosis in a wide variety of human cancers sparing normal tissues. As previously demonstrated, we gene modified adipose derived (AD) MSC generating a cellular reservoir for a lasting TRAIL production.

In this paper we are presenting data on how AD-MSC armed with TRAIL are able to induce apoptosis in a variety of tumor cell lines including cervical adenocarcinoma; pancreatic cancer and colon cancer. A specific attention is provided to different sarcoma histotypes, as still deathly tumor types. Moreover, data on antitumor effectiveness of AD-MSC TRAIL is showed on primary lung and Ewing’s sarcoma tumor cells .

In vivo findings are then shared demonstrating that, when intravenously or sub-cutaneously injected into mice, AD-MSC TRAIL localized into tumor microenvironment counteracting cancer development both causing massive malignant cells apoptosis and exerting potent anti-angiogenic functions. Collectively, these results suggest that MSC are valid cellular vehicles for TRAIL and could open novel therapeutic opportunities for tumours still characterized by poor prognosis.

This work has been possible thanks to AIRC and ASEOP contributions.

M2-macrophages as alternative to stem cell transplantation for stroke treatment

Chernykh E.R., Shevela E.Ya., Morozov S.A., Davydova M.N., Starostina N.M., Ostanin A.A.

Institute of Fundamental and Clinical Immunology, Novosibirsk, Russia

Introduction To assess the safety and clinical efficacy of autologous M2 macrophages in non-acute stroke patients, a phase I/II non-randomized open-labeled clinical study has been conducted. We also evaluated whether intrathecal administration of macrophages influences the production of cytokines by blood cells and if the levels of cytokines correlate with stroke severity and responsiveness to cell-therapy.

Materials and Methods Sixteen patients diagnosed with ischemic (n=12) or hemorrhagic (n=4) stroke have been subjected to cell transplantation therapy (study group). On average 21.9 x 10^6 of autologous M2-like macrophages were injected via intrathecal introduction. Ten matched case-control stroke patients formed the control group. A primary outcome measure was safety. A secondary outcome measure was functional improvement in a 6-months follow-up. The levels of cytokines in blood cell cultures were evaluated in ten cell-treated patients using 26-Bioplex analysis.

Results Intrathecal administration of M2 macrophages was generally well tolerated. One patient in the study group and two patients in the control group died during the 6-mo follow-up period due to recurrent stroke. In a 6 mo follow-up the study group NIHSS score decreased from 11.5 to 6 ($p < 0.01$) Patients in the control group showed a less pronounced neurological improvement which manifested as a trend. The obvious positive response (improvement of NIHSS score ≥ 3) in the study group was observed in 73% versus 25% in the control group ($p_{EFT} = 0.039$). Intrathecal introduction of M2 macrophages did not significantly affect the production of various cytokines. Nevertheless, pretreated levels of IL-8, IL-10 and IL-4 correlated with stroke severity. Moreover, responder patients were differed from non-responders by lower spontaneous production of IL-10, FGF-β, PDGF, VEGF and higher stimulation indexes of IL-1β, TNF-α, IFN-γ and IL-6.

Conclusions Intrathecal delivery of M2-macrophages in non-acute stroke patients is safe, and is accompanied by more pronounced neurological improvement as compared with the control group. Pretreatment levels of cytokines correlate with stroke severity and responsiveness to cell therapy.

Keywords Macrophages, cell therapy, stroke

Corneal Regeneration – From the Lab to the Clinic and Back

Prof. Dr. Nadia Zakaria

Center for Cell Therapy and Regenerative Medicine, University of Antwerp, Belgium

Purpose: To describe the results of a phase I/II clinical trial for standardized, non-xenogenic, cultivation and "no touch" surgical transplantation of limbal stem cell grafts.

Methods: 18 eyes of 18 patients were transplanted with either autologous (n=15) or allogenic (n=3) limbo-amnion composite grafts that were generated using a standardized culture protocol free of xenogenic culture products and transplanted using a standardized "no touch" surgical technique. In vitro cellular outgrowth and phenotype of the limbo-amnion composite graft was assessed prior to transplantation. The clinical outcome measures investigated were: corneal neovascularization, central corneal opacity, pain, photophobia and visual acuity pre and post transplantation.

Results: Limbal epithelial cells showed an average outgrowth of 14.2mm ±3.7mm by day 14. The majority of the cells displayed a progenitor phenotype: p63 bright, CK14, desmoglein, ABCG2 bright and CK3/12 dim protein expression. The transplant recipients were followed up for a mean of 22 months (range 4-43 months). 12 out of the 18 transplant recipients were graded successful (12 had anatomical success and 7 also attained some degree of functional success), giving an overall success rate of 67%. We did not see a significant reduction in pain, photophobia or central corneal opacity for the patient group post transplant. However, the ocular surface photographs for pre- and post stem cell transplantation, showed a significant (p=0.007) reduction in the percentage area of corneal neovascularization.

Conclusions: We have been able to show that our standardized, xenogenic free culture system and "no touch" surgical technique has outcome measures comparable to other clinical studies. This technique has the added advantage of being free from animal contaminants such as mouse feeder layers and foetal bovine serum. Improved functional success is attained once penetrating keratoplasty is performed following successful stem cell grafting.

Legal issues in cell therapies and EU Regulations + ICCTI Letter to EMA

M. Vasicek, Prof. J. Michalek

International Consortium for Cell Therapy and Immunotherapy (ICCTI), Brno, Czech Republic

Recently, cell therapies are discussed across EU and abroad. European Medicinal Agency (EMA) regulates this growing field by EU Directives 2001/83/ES and 1394/2007 dealing with advanced therapy medicinal products (ATMPs). Despite the fact that both Directives regulate only industrial and mass production of ATMPs, both national and central EU responsible authorities and regulators try to regulate even cell products that are not ATMPs (minimally manipulated cells used in homologous setting) or are based on non-industrial production of ATMPs. This approach can be abused by bureaucratic machineries in EU member states leading to slow-down of progress in clinical research and clinical application of cell therapies in EU, thus supporting medical tourism to third countries where less regulation is applied. Furthermore, expensive randomized, double-blinded phase III clinical trials may be unethical when autologous tissue or cells are taken from patients where significant amount of patients receives only placebo instead of autologous cell therapy. Such phase III studies rarely leads to EMA registration due to difficulties in financing. If finally registered, their introduction into the clinical practice in EU member states is complicated by extremely high costs for healthcare system in comparison to alternative treatments. If such approach is further supported as the only Evidence-Based Medicine alternative, it may soon lead to a collapse of health care systems in EU member states. At ICCTI Consortium we believe, the hospital exemption rule in case of ATMPs or homologous use of autologous cells can serve as an excellent examples of safe, clinically effective and economic alternative to highly expensive model of phase III randomized, double-blind studies in this growing field of medicine.

The DEALTH Method - How we can improve Wound Healing of Chronic Ulcers of the Lower Limb and Diabetic Feet using Autologous Lipotransfer

Tilman Stasch, MRCS (Ed.), F EBOPRAS, MD

Valentis Beauty, Clinic for Plastic Surgery and Lifestyle Medicine, Cologne, Germany

Introduction and Objectives: Aim of this prospective cohort study was to assess the healing progress of chronic, non-healing lower-limb wounds and diabetic ulcers in patients following peri-lesional autologous fat grafting.

Materials and Methods: 26 patients with non-healing wounds were treated with surgical debridement and autologous lipotransfer (using the DEALT Method). The mean age of the wounds before intervention was 16.7 months. Wound size after debridement averaged 5.1 +/- 2.6 cm2. On average, 7.1 +/- 3.3 cc of lipoaspirate was transferred into the wound area.

Results: 22 / 25 wounds (88 percent) of wounds healed completely within a mean of 68.0 +/- 33.0 days. A reduction of wound size by 50 percent was achieved after an average of 4 weeks. In one patient with an ulcer within particularly scarred tissues on the lower limb, a repeat session of lipotransfer lead to complete wound healing after another 4 weeks.

Conclusions: The authors describe a simple and useful technique to improve wound healing in diabetic feet and chronic lower limb ulcers with a background of peripheral vascular disease, where other interventional options to achieve wound healing have failed.

Biological effects of Shockwavetherapy

Dra. Karin Freitag

President of the Spanish society for schockwave SETOC, Spain

The new concept of "mecanotransduction" is based on intranuclear changes with neoangiogenesis through the liberation of vegf in tendinopathies. The shockwavetherapy induces the synthesis of colagen typ2 and the activation of tenocites.

In bone pathology the studies confirm activation of osteoblasts and a notilcabe elevation of bmp2.
In skin lesions like ulcers and burns the significant elevation of fibroblasts helps healing.
The new studies involved reclutation of stem cells in the treatment focus.

The pursuit of tolerance: Immunomodulation of dendritic cells for therapeutic intervention in multiple sclerosis

Dr. N. Cools
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Many studies have demonstrated the role of the adaptive immune system in general and T cells in particular in the pathogenesis of multiple sclerosis (MS). Recent data suggest that dendritic cells (DC), which are innate immune cells, also contribute to the pathogenesis of MS. Due to their specialized antigen-presenting capacity, DC play an important role in polarizing and controlling the T cell response, regulating the balance between immunity and tolerance. Our recent observations indicate that both circulating as well as in vitro generated DC of MS patients display an altered phenotype and function as compared to those of healthy controls and that they have a pro-inflammatory influence on T cells. Of interest, we also observed that migratory patterns of DC in MS are altered, as evidenced by aberrant expression of the migration-associated molecules CD62L, CCR5 and CCR7 by DC of MS patients as compared to healthy controls. This could account for the accumulation of DC observed in MS brain lesions and cerebrospinal fluid. Literature data indicate that current immune-modulating therapies of MS (eg. interferon beta, glatiramer acetate) decrease the pro-inflammatory DC features of MS patients, further underscoring the potentially pivotal role of DC in MS pathogenesis and therapy. In light of these observations, we also examined whether it is possible to modulate DC function in order to restore the immunological imbalance in MS. We demonstrated that in vitro treatment of DC from MS patients with immunomodulatory cytokines (such as vitamin D3) results in a more stable tolerogenic DC phenotype. Indeed, modulation of DC by vitamin D3 treatment arrests DC in a semi-mature state and prevents the up-regulation of costimulatory molecules as well as the secretion of pro-inflammatory cytokines. Moreover, tolerogenic DC generated from MS patients are capable of suppressing myelin-specific T cell responses. These data suggest that DC play an important role in MS and that it can be envisaged to develop a new form of immunotherapy for this disease, using tolerogenic DC.

Hype or Hope: The future of adipose-derived stem cells in cardiovascular disease

Dr. HJ Duckers

University Medical Center, Netherlands

Adipose derived stem cells can be easily extracted from lipoaspirate in therapeutically amounts within 1,5 hrs. These adipose derived stem cells (ASC) consist of mesenchyme-like stem cells, as well as endothelial progenitor cells and immune-competent cells. These adipose-derived mesenchymal-like stem cells have assessed in various small and large animal models of acute myocardial infarction and (ischemic) congestive heart failure. In these models, ASC resulted in improved myocardial perfusion with increased capillary density, resulting in improved or preserved cardiac contractility. These beneficial effects prompted the first cardiovascular clinical trials to assess the safety, feasibility and efficacy of autologous ASCs in the treatment of acute myocardial infarction and congestive heart failure.

The APOLLO was the first-in-men multi center, randomized placebo-controlled clinical study of ASC in the treatment of patients with a ST-elevation myocardial infarction (STEMI). STEMI patients treated with ASC, showed a significant reduction of infarct size (-51,3%) with improved myocardial perfusion, resulting in a improved cardiac contractility (+5.7% LVEF) and reduced adverse cardiac remodeling on long term follow-up. The ADVANCE was a phase IIb, multi center placebo-controlled trial in 275 STEMI patients. In the first STEMI patients, ASC therapy showed a significant reduction in infarct size.

The PRECISE study sought to define the safety and therapeutic effect of intramyocardial injection of ASCs in patients with NYHA class II-III ischemic congestive heart failure. ASC therapy resulted in a significant improvement of VO_2 max in these heart failure patients.

The ATHENA I and II have recently been initiated as phase IIB randomized placebo-controlled trials to assess the effect of im ASC therapy in patients with advanced congestive heart failure using two doses of aSCs (0.4 and 0.8 x 10^6 cells/kg body weight).

Thus far, therapy with adipose-derived stem cells resulted in consist improvement of cardiac function and myocardial perfusion in animals models of cardiac ischemia, as well as in patients with acute ST elevation myocardial infarction and ischemic heart failure.

Adipose-derived stromal cells for soft tissue reconstruction and regeneration: future or fiction?

Maroesjka Spiekman (BSc); Martin C. Harmsen (PhD)

Cardiovascular Regenerative Medicine Group, Dept. of Pathology and Medical Biology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands

Lipografting or lipofilling comprises the use of autologous adipose tissue as a natural filler of soft tissue defects. This technique of autologous tissue transfer has been used as a treatment modality in plastic and reconstructive surgery since the early 1900's. Interestingly, lipografting causes reduction of pre-existing scars, while it also improves wound healing. It is still unknown, which molecular or cellular component(s) of adipose tissue are responsible for these remarkable effects, yet attention is focused on the mesenchymal stromal/stem cells – also known as ADSC (Adipose-Derived Stromal/Stem Cells).

ADSC are highly proliferative and harbour a typical trilineage differentiation potential (fat, bone, cartilage), while these also easily differentiate in vascular and connective tissue cell types such as (myo)fibroblasts, pericytes and smooth muscle cells.

Our previous work showed that ADSC harbour a potent capacity to influence their local microenvironment through paracrine secretion of signalling factors such as growth factors, cyto/chemokines and non-proteinaceous factors such as prostaglandins. Together, these stimulate vascularization and proliferation of tissue parenchyme, while reducing apoptosis and excessive inflammation. In other words: ADSC contribute to regeneration of damaged tissue in multiple ways.

We set out to test the hypothesis that ADSC suppress fibrosis. In our in vitro approach we showed that spent culture medium of ADSC (conditioned medium, cmADSC) suppressed the TGFbeta-stimulated proliferation and differentiation of human dermal fibroblasts from healthy donors as well as from keloid scars. Furthermore, cmADSC strongly reduced the secretion of fibrosis-associated extracellular matrix molecules such as collagens type I and III. Finally, cmADSC also suppressed the contraction of myofibroblasts. Taken together we provided evidence that the function and differentiation of the culprit cell in fibrosis, i.e. the fibroblast, can be modulated by factors secrete by ADSC.

Treatment of osteoarthritis with freshly isolated stromal vascular fraction cells from adipose and connective tissue

Prof. J. Michalek

International Consortium for Cell Therapy and Immunotherapy and Cellthera Ltd., Brno, Czech Republic

Therapy of osteoarthritis relies on non-steroid analgesics, chondroprotectives and in late stages total joint replacement is considered a standard of care. We performed a pilot study using novel stem cell therapy approach that was performed during one surgical procedure. It relies on abdominal lipoaspiration and processing of connective tissue to stromal vascular fraction (SVF) cells that typically contain relatively large amounts of mesenchymal stromal and stem cells. SVF cells are injected immediately to the target joint or to the connective tissue of the target joint. Since 2011, total of 1128 patients have been recruited and followed for up to 42 months to demonstrate the therapeutical potential of freshly isolated SVF cells. At the same time, one to four joints (knees and hips) were injected with SVF cells per patient. A total number of 1856 joints were treated. Clinical scale evaluation including pain, non-steroid analgesic usage, limping, extent of joint movement and stiffness was used as measurement of the clinical effect. All patients were diagnosed with stage II-IV osteoarthritis using clinical examination and X-ray, in some cases MRI was also performed to monitor the changes before and after stem cell therapy. After 12 months from SVF therapy, at least 50% clinical improvement was recognized in 91%, and at least 75% clinical improvement in 63% of patients, respectively. Within 1-2 weeks from SVF therapy 72% of patients were off the non-steroid analgesics and most of them remain such for at least 12 months. No serious side effects, infection or cancer was associated with SVF cell therapy. In conclusion, here we report a novel and promising therapeutical approach that is safe, cost effective, and relying only on autologous cells.

This work was supported in part by the International Consortium for Cell Therapy and Immunotherapy (www.iccti.eu) and Czech Ministry of Education Grant No. CZ.1.07/2.3.00/20.0012.

Adipose Derived Stem cells in Crohn Disease

Prof. D. Garcia-Olmo

Fundacion Jimenez Diaz University Hospital, Madrid, Spain.

Crohn's related fistula still remains to be a real challenge. Many different therapeutic approaches have been described, both clinical and surgical, with variable success rates. Limited surgery may result in recurrence while aggressive surgery is associated with faecal incontinence. Application of adipose-derived stem cells (ASCs) represents a novel approach for enhancing regeneration and/or repair of damaged tissues in an environment particularly unfavorable for wound healing. It has been hypothesized that the immunoregulatory and anti-inflammatory properties of ASCs may work together to accelerate healing. ASCs are purified from subcutaneous fat, which can readily and safely be obtained by liposuction.

A proof-of-concept phase I clinical trial in fistulizing Crohn's disease, a phase II clinical trial in fistulas of cryptoglandular origin and associated with Crohn´s disease, and a multicenter phase III trial in cryptoglandular complex fistula-in-ano found ASCs to be safe and effective. A healing rate of 71% in the phase II and up to 83.33% in the phase III (when the therapy was applied by selected medical teams) with almost no risk of incontinence and a low recurrence rate was achieved. We found that adverse events were absent and consequently, as other authors published before, a stem cells (e-ASC) were considered to be safe and also feasible. Based on these data we will try to develop the most appropriate pattern to apply the stem cell therapy. In this way is important to point out that a Phase III Clinical Trial to assess efficacy and safety of expanded allogenic adipose-derived stem cells (eAscs) for the treatment of perianal fistulising Crohn's disease (ADMIRE Study) is now ongoing.

Risk factor oral cavity, treatment with autologous blood factors (plasma-, growth factors and stem cells)

Prof. Ottaviano Tapparo

Private day hospital for aesthetics and interdisciplinary dentistry, Germany

Oral risk factors are bacterias (caries-, periodontal bacteria) and funghi (candida) in an variable ph at different temperatures. Heavy metals, ceramics, resins and their corrosion products from dental restoration or implants are in direct contact 24 hours a day to jaw bone and intestinal system. Furthermore mercury vapour from Amalgam fillings after chewing is produced and inhaled in the lung or nose. Gut bacteria can transform it into methylmercury the more toxic organic form. Secondary amines and cancerogenic products are found or produced in the periodontium and in the root of the tooth. Oral foci or teeth can influence organs and vice versa. Treatment with autologous blood factors in a bedside procedure without interfering or stopping the therapy of the patient. After drawing 20-80 ml of venous blood and centrifugating it at different speeds and times we get in 13 minutes from the patient own blood plasma factors, platelet concentrate and CD 34^+ stem cells in different forms i.e liquid gel or as a clot to form a membrane to cover wounds.

T-cell vaccines for the treatment of atopic dermatitis and multiple sclerosis

Blinova E.A.

RIFCI, Novosibirsk, Russian Federation

It is believed that autoimmune Th1 cells specific for encephalitogenic myelin antigens play a major role in the pathogenesis of multiple sclerosis (MS). These findings provide the rationale for using T-cell vaccination (TCV) to induce anti-idiotype immune response that are capable inactivate idiotype self-reactive lymphocytes in MS. Patients received attenuated by irradiation myelin-specific T-cells. During 24 month TCV number of exacerbation per year decreased from 1,7 to 0,2 in patients with remittent course of MS. 54% patients with progressive course of MS demonstrated a stable state: EDSS index don't increase, whereas 27% patients in group without TCV achieved stability.

Atopic dermatitis (AD) as other allergic diseases characterized deficiency of T-regulatory cells and huge number of activated lymphocytes. Induction of anti-ergotopic response was occurred during TCV and enlarged T-cells specific against activation markers. It was suggest that TCV leads to changing the functional activity of Tregs and correct the clinical manifestations of AD. For treatment we used autologous polyclonal anti-CD3 activated T-lymphocytes, because the allergen is quite difficult to identify in AD. High clinical efficacy and safety of this immunotherapy has been shown for patients with allergic and nonallergic form of the disease. It was observed more rapid positive changes (SCORAD, DLQI) in the allergic form: one month after beginning TCV versus 6 months in opposite group. In patients with allergic form of AD CD8+ cells increased and IgE level declined during TCV, whereas in patients with nonallergic form the number of CD8+25+ cells and the activity of delayed-type hypersensitivity effectors enlarged.

Dendritic cell-based vaccines for the treatment of recurrent glioblastoma multiforme

Dr. M. Strioga

National Cancer Institute, Lithuania

Specific active immunotherapy or therapeutic cancer vaccination emerges as an attractive treatment option for patients with glioblastoma multiforme (GBM). It targets dendritic cells (DCs), which have a unique capacity of inducing naive and central memory T cell immune responses most efficiently. DCs can be therapeutically targeted either in vivo (in situ vaccination) or by their ex vivo manipulations and subsequent re-injection back into the same patient. Here we provide data about compassionate-use therapeutic DC vaccination of recurrent GBM patients, treated with autologous, monocyte-derived, mature DCs, loaded with autologous tumor lysate.

Patients: we treated 20 patients with the first recurrence of GBM. Ten patients received DC vaccination (6 doses; 5 million DCs per dose) plus standard treatment, including reoperation plus + irinotecan (ST+DC group). Control group included clinically-matched patients (n=10), who received standard treatment alone.

Results: There was no significant difference in progression-free survival (PFS) between the ST+DC and control groups (mPFS was 7 versus 6 months, p=0.2, respectively). However we observed a significant increase in overall survival (OS) of DC-treated patients compared with controls (mOS was 16.5 versus 10 months, p=0.014, respectively). One-year OS rate was 70% in the st+DC group versus 20% in controls; 24-month OS rate was 30% in the st+DC group versus 0% in controls.

Comment: Therapeutic vaccination works by reprogramming the cancer-dysbalanced immune system from the state of tumor tolerance to the state of immune-mediated cancer control. This dynamic process requires time (about 6-9 months) to achieve a clinical effect. Furthermore immunotherapy may increase the sensitivity of cancer cells to subsequent chemotherapy. These arguments explain why the addition of therapeutic DC vaccination to postoperative chemotherapy did not prolong PFS, but significantly increased mOS in our patients with recurrent GBM.

Conclusions:

1. Therapeutic DC vaccination can be safely combined with chemotherapy.
2. Postoperative chemoimmunotherapy seems to be a more effective treatment approach for recurrent GBM compared with postoperative chemotherapy alone.

Vaccination with WT1 mRNA-electroporated dendritic cells: report of 66 cancer patients

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In order to control or prevent recurrence of malignant disease, we performed phase I/II Wilms' tumor (WT1)-targeted dendritic cell (DC) vaccination studies as adjuvant therapy in 30 patients with acute myeloid leukemia (AML) in remission and in 10 patients with unresectable malignant pleural mesothelioma (MPM).

No major DC-related systemic toxicity was observed. Overall 8 of 30 AML patients at high risk of relapse have not relapsed yet, with a median follow-up from diagnosis and start of DC vaccination of respectively 70 months and 63 months. In MPM, 7 patients had stable and 3 progressive disease. Median overall survival (OS) from start of chemotherapy was 32 months. In summary, WT1-targeted DC vaccination is feasible, safe and immunogenic. In AML there is evidence of objective response. In MPM OS data compare favorably with the best data reported so far for similar cohorts of patients, suggesting that adjuvant WT1/DC-based immunotherapy provides clinical benefit. Results of DC vaccination in other tumor types will also be discussed.

Pritumumab – a clue to using the natural human immune response for drug development

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Pritumumab is a natural human IgG1kappa antibody derived from a regional draining lymph node of a cancer patient. The human hybridoma derived antibody has been used to treat 249 brain cancer patients through a Phase II trial in Japan. Overall, there was a 9-fold benefit compared to standard of care. To make the antibody more economical a recombinant version was made in CHO cells. Comparable analysis of sequence, binding, specificity, and antigen recognition show the similarity to both the original hybridoma and the recombinant versions. The antigen recognized by pritumumab is a novel neo-epitope expressed on the surface of cancer cells. The intelligence of the natural human immune response in a regional draining lymph node of a cancer patient defined a unique surface antigen that may be useful in drug development.

Cancer immunotherapy with ex vivo-generated NK cells from hematopoietic progenitor cells.

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Adoptive transfer of allogeneic natural killer (NK) cells is a relatively non-toxic approach which is gaining interest to combat cancer. We reported previously a GMP-compliant cytokine-based culture system for the generation NK cell products from umbilical cord blood $CD34^+$ hematopoietic progenitor cells (HPC) with high cell numbers, purity and functionality, and importantly absence of T cell contaminants. Pre-clinical studies conducted in mice demonstrated that these HPC-NK cells have bone marrow homing capacity and potently prolong survival of K562-bearing mice. Currently, this HPC-NK cell product is being investigated in a phase I clinical trial in older acute myeloid leukemia (AML) patients who are not eligible for allo-SCT. In this study, escalating doses of allogeneic HPC-NK cells, ranging from $3x10^6$ up to $100x10^6$ NK cells/kg, are infused in cohorts of 3 patients following fludarabine/ cyclophosphamide conditioning. In addition to the non-transplant cancer setting, it would be highly valuable to exploit HPC-NK cell products for adoptive immunotherapy after allo-SCT. However, isolation of high numbers of functional NK cells from donors is challenging. Hence, we adapted the cytokine-based ex vivo culture protocol to generate high numbers of functional NK cells from G-CSF mobilized $CD34^+$ HPC that are devoid of T cell impurity. We demonstrated that addition of aryl hydrocarbon receptor (AhR) antagonist StemRegenin-1 (SR1) to the culture protocol potently enhances expansion of G-CSF mobilized $CD34^+$ HPC, and induces expression of NK cell associated transcription factors promoting NK cell differentiation. Moreover, we observed that combining IL-15 with IL-12 drives the generation of more mature and highly functional HPC-NK cells. Collectively, these data show that adaptation of the culture protocol using the AhR antagonist SR1 and IL15/IL12 cytokine combination can lead to improved next-generation HPC-NK cell products from various $CD34^+$ cell sources for adoptive cancer immunotherapy.

Immunomodulatory effect of MSCs in lymphoma patients following HSC transplantation

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Introduction: The recent evidence has shown that the immunomodulatory effects of mesenchymal stem cells (MSC) are not limited by immunosuppression, and, in certain settings, MSC can also be immunostimulatory. Previously we have demonstrated that MSC from lymphoma patients are able to stimulate lymphocyte proliferation in vitro and improve early recovery of lymphocytes when transplanted with hematopoietic stem cells (HSCT). In the present study, we evaluated the possible mechanisms by which MSCs can effect T cell reconstitution.

Materials and Methods: Co-transplantation of autologous MSCs in a mean dose of $0.2x10^6$/kg was conducted in 29 patients with HSCT; the control group included 38 patients with standard HSCT. Cell cycle parameters of T-lymphocytes were studied in peripheral blood of patients in both groups.

Results: MSCs transplanted at low doses with HSC improved early lymphocyte recovery of both memory and naive T cells with more prominent effect on naive $CD4^+$ T cells. At the day of lymphocyte recovery, T-lymphocytes in MSC-administered patients were characterized by decreased apoptosis of naïve $CD4^+$ T cells. Concurrently, there was a significant decrease in the number of resting cells and a strong tendency to an increase of the proliferating cells in naive $CD8^+$ T-lymphocytes. Moreover, MSC co-transplantation was accompanied by a significant increase in the number of proliferating cells with simultaneous reduction in the number of quiescent cells within $CD8^+$ memory T cells. In the later post-transplantation period patients with MSC co-transplantation showed more effective reconstitution of $CD31^+$ naive T cells.

Conclusions: A positive impact of MSCs on early lymphocyte recovery in vivo is due to a higher proliferaion of naive $CD8^+$ T cells and a higher survival rate - due to the reduction of apoptosis - of naive $CD4^+$ T cells, whereas in the later post-transplantation period MSC promote more effective reconstitution of recent thymic emigrants.

Keywords: Mesenchymal stromal cell; autologous hematopoietic stem cell transplantation; immune reconstitution; homeostatic proliferation; thymopoiesis.

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