EACTS - PACT Joint Symposium:

Regenerative Medicine:
Taking the Science to the Patient

30th November and 1st December 2017
Vienna, Austria

Abstract Book

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Michael Comer – PACT Chairman

November 2017

Dear Delegates, Speakers and Colleagues,

it is with great pleasure and sincere good wishes that I welcome you on behalf of the Organising Committee, to the first Joint Symposium of the European Association for Cardio-Thoracic Surgery (**EACTS**) and the Platform for Advanced Cellular Therapies (**PACT**) entitled:-

"Regenerative Medicine: Taking the Science to the Patient"

In these days where it would seem that people, ethnic and religious communities, political parties, indeed countries are becoming more and more egotistic, internalising and yet outwardly aggressive whilst simultaneously withdrawing from agreements and collaborations. It is therefore, the more refreshing that our carefully chosen Symposium title would suggest an entirely different and contrary approach to the aforementioned undesirable current global trends. In "medicine" the patient is of paramount importance but as we know to bring an idea for a potential treatment from it's concept to a product and the eventual application in the clinic may not only take several years but as well involve the efforts of some thousand or more people in addition to their very many and specialist skills. Furthermore, where the cost of developing health giving, innovative and novel treatments are reaching astronomical figures and sums of one billion dollars are common. Therefore, the combining of efforts where the sum of the whole can be greater than the sum of the individual parts is not only desirable but also essential that is, in cooperation, where synergies can be created and also socio-economic optimisation can be realised. We may agree through our associations to establish a "P.L.E.A.S.E." concept where our young people can inherit a better world in "P" for peace, "L" for liberty through an "E" for education in the "A" for the arts, "S" for the Sciences and "E" for the economic disciplines, bound by these subjects with a respect for "all" as a principal "principle" and a means of working together irrespective of prejudices in gender, religion, belief, creed or politics. We hope at our symposium you will not only find interesting presentations but we also expect you will experience an inspiring atmosphere with stimulating ideas for future research and companionships that will come to the benefit of patient care and alleviate pain and suffering to create a better world for all life and life sciences. Please reach out for P.L.E.A.S.E.! You are very welcome!

INFARCT HEALING AS A THERAPEUTIC TARGET

Kai Christoph Wollert

Molecular and Translational Cardiology, Department of cardiology and Angiology, Hannover Medical School

Improved reperfusion strategies and health care logistics have shortened ischemic times and enhanced myocardial salvage in most patients with acute myocardial infarction (MI). Despite these achievements, patients with extensive myocardial injury during the acute phase remain at risk of developing heart failure and continue to have increased mortality.
MI triggers an inflammatory response that replaces the necrotic area with vascularized granulation tissue and eventually a collagen-rich scar. The heart can undergo deleterious changes in left ventricular (LV) geometry and function during this vulnerable period before scar formation has stabilized the infarct area. Therapeutic modulation of infarct healing may therefore hold promise for preventing postinfarction heart failure.
In patients, intracoronary infusion of autologous bone marrow cells (BMCs) during this critical time window has been explored as an adjunctive therapeutic strategy to improve heart function after MI. Transcriptome and proteome analyses indicate that various BMC populations release a broad repertoire of cytokines and growth factors that may enhance tissue repair in a paracrine manner, thus providing a conceptual framework for this approach. Clinical trials investigating the efficacy of BMC therapy after MI have produced mostly neutral results, however, and a recent meta-analysis by the Cochrane Collaboration concluded that there is insufficient evidence for a beneficial effect. Indeed, several issues complicate the therapeutic use of autologous BMCs. The approach is limited by low cell retention rates after intracoronary or intramyocardial delivery and by interindividual variations in cell functionality. Moreover, the therapeutically active cell population(s) and/or paracrine factor(s) in heterogeneous BMC preparations remain ill defined. Systemic application of specific BMC-derived secreted proteins may be an alternative biologic approach to improving tissue repair and heart function after MI. We recently performed a bioinformatic secretome analysis in BMCs from patients with acute MI who were enrolled in the Bone Marrow Transfer to Enhance ST-elevation Infarct Regeneration 2 (BOOST-2) cell therapy trial. From this analysis, we identified several poorly characterized secreted proteins that promote tissue repair after acute MI mice. These proteins and the translational implications will be discussed.

SECRETOME AND REGENERATION: FROM INITIAL TO CURRENT WORK

Massimiliano Gnecchi

University of Pavia, Italy

SECRETOME-BASED REGENERATION FROM SKIN TO HEART

Hendrik Jan Ankersmit

Medical University of Vienna, Austria

For almost two decades, cell-based therapies have been tested in modern regenerative medicine to either replace or regenerate human cells, tissues, or organs and restore normal function. Secreted paracrine factors are increasingly accepted to exert beneficial biological effects that promote tissue regeneration. These factors are called the cell secretome and include a variety of proteins, lipids, microRNAs, and extracellular vesicles, such as exosomes and microparticles. The stem cell secretome has most commonly been investigated in pre-clinical settings. However, a growing body of evidence indicates that other cell types, such as peripheral blood mononuclear cells (PBMCs), are capable of releasing significant amounts of biologically active paracrine factors that exert beneficial regenerative effects. The apoptotic PBMC secretome has been successfully used pre-clinically for the treatment of acute myocardial infarction, chronic heart failure, spinal cord injury, stroke, and wound healing.
In this review we describe the benefits of choosing PBMCs instead of stem cells in regenerative medicine and characterize the factors released from apoptotic PBMCs. We also discuss pre-clinical studies with apoptotic cell-based therapies and regulatory issues that have to be considered when conducting clinical trials using cell secretome-based products. This should allow the reader to envision PBMC secretome-based therapies as alternatives to all other forms of cell-based therapies.

SECRETOME AND MOA

Michael Mildner

Medical University of Vienna

The development of new therapeutics requires accurate analysis of the mode of action (MOA) of the drug substance. However, this becomes much more difficult, if not even impossible, when the drug substance consists of paracrine factors of cultivated human cells.
Our laboratory has been working for many years on the elucidation of the MOA of a cell-secretome derived from apoptotic peripheral blood mononuclear cells (PBMC). We could recently show that this secretome (Aposec) display strong tissue regenerative properties in several experimental settings, including myocardial infarction, wound healing and stroke. As

the secretome shows a complex composition, consisting of proteins, lipids, nucleic acids and extracellular vesicles, its biologic function is multifaceted. The major MOAs of Aposec include a strong anti-inflammatory, pro-angiogenic and cytoprotective activity. Besides, Aposec induces vasodilatation and inhibits platelet aggregation, and shows a strong anti-microbial activity. All these effects are well desired in tissue regenerative processes, and elucidation of the exact underlying mechanisms is the main goal of our laboratory. We were able to identify subfractions of Aposec responsible for individual effects. However, the full spectrum of activity is only achieved when the whole secretome is applied.
Cell secretomes may revolutionize regenerative medicine in the future when safety and effectivity are proven in appropriate clinical trials in humans.

BRINGING CELL AND TISSUE BASED THERAPIES TO THE MARKET

D.Niese

Regulatory Affairs Consultant, Germany

A brilliant scientific discovery or idea as such is a necessary but not a sufficient condition for giving patients access to an innovative therapeutic concept. Rather, it has to be proven based on stringent scientific data that the new therapy offers a positive benefit-risk balance to patients, and a justifiable cost-benefit ratio to payers. While the European pharmaceutical legislation covering approval of medicines and medical devices was just recently celebrating its 50th anniversary, the regulatory framework governing the authorisation of cell and tissue based therapies in Europe was entirely national and highly diverse until 10 years ago: European Member States regulated such products as medicines, as devices, as transplants, or left them completely unregulated. Factually, this blocked patients in need in Europe from access to innovative therapies which were already available to some patients in individual European hospitals, in the United States of America and beyond. While e.g. the first industrially produced bi-layered skin product for treatment of chronic ulcers and burns developed, manufactured and marketed by a US based company received approval by the FDA in 1998, it took until 2015 that this product received approval from Swissmedic, the Swiss regulatory authority. Approval by European authorities is still pending.
In 2007, following an intensive consultation and discussion process with stakeholders all over Europe, the European institutions, patient organisations, industry associations, large and small pharmaceutical companies and producing hospitals celebrated the first reading agreement by the European Parliament and the Council on the Regulation (EU) No. 1394/2007 on “Advanced Therapy Medicinal Products for Human Use”. This approval also marked the creation of a new specialised scientific committee at the European Medicines Agency: The Committee for Advanced Therapies (CAT).
Yet, until today only a very small number of products reached the market based on this regulatory pathway. This unsatisfactory situation has its roots in both, the complexity of the products themselves but also in the way the legislation emerged and how it is being handled by National European governments. One major issue remains the diverse implementation of the so called “hospital exemption” according to article 28. The developments until today, the European Commission and EMA action plan of 2016/17, as well as consequences for product developers will be discussed.

REGULATORY AFFAIRS - FROM SCIENCE TO TRANSLATION

Tobias Ostler

Regenold, Germany

Advanced therapy medicinal products (ATMP) are a new medicinal product category comprising gene therapy and cell-based medicinal products as well as tissue engineered medicinal products. ATMP development opens novel avenues for therapeutic approaches for many diseases and a number of the products currently under development address unmet medical needs. Over the past years, the European Medicines Agency (EMA) and its scientific

committees have been working on a number of initiatives aimed at further supporting development with a view to accelerate patients' access to medicines based on ATMPs. However, there are important bottlenecks for their development due to the complexity of the products. Principles of the quality development in the „accelerated development environment" will be presented and discussed in context of the regulatory framework.

SAFETY TESTING OF BIOLOGICS AND ADVANCED THERAPIES

Monika Chabicovsky and Alexandra Günzl

MC Toxicology Consulting

Biotechnology-derived pharmaceuticals and especially cell-based, gene and gene editing therapies hold great promise for a wide range of indications. Currently, a significant number of drug candidates are heading towards clinical trial applications (CTAs) and / or investigational new drug applications (INDs). Many of these drug therapies have the potential to be curative. And many of these products are currently in the research pipelines of pharma companies and academic institutions.
The novelty and uniqueness of some of these approaches are a potential challenge for all, scientists, product developers and regulators, because there is an increasing misalignment between scientific development rationale and standard regulatory requirements for (non-clinical) safety testing.
The overriding goal of non-clinical drug development is to ensure patient safety at the highest level of confidence, something which has been reasonably well established for small molecules, a drug class which is tightly regulated. In contrast, advanced therapies, but also biologics, require a more product-tailored approach based on a sound science-driven risk assessment. Thus, companies are forced to a "tightrope walk" between science, development feasibility considerations and regulatory requirements.
This presentation provides insights into non-clinical safety testing strategies for a cell-based product and a biologic intended for a wound healing indication. It is concluded that there is a clear benefit of early authority interactions to seek support for innovative non-clinical development strategies, which in many cases need to deviate from classical guidance.

POTENCY ASSAYS TO ASSESS ACTIVITY AND QUALITY OF BIOLOGICS

Michael Erb

Synlab, Germany

Potency assays are used to measure the ability of a drug (e.g. a therapeutic antibody) to elicit a specific response at a certain dose in a relevant biological system. The selected test system should recapitulate the mode of action of the drug. Potency assays can be one of two types: ligand-binding assays measure the interaction of the drug with it's target (e.g. ELISA) whereas functional assays measure a biological response (e.g. cell-based reporter gene assay).
Dose-response data generated from a drug sample and a well-characterized reference standard are evaluated to determine the potency (activity) of the drug sample relative to the

reference standard. Potency assays are usually required by regulatory agencies for release of drug product under GMP (good manufacturing practice). Taken together, potency assays are used to measure product attributes associated with drug product quality and manufacturing controls and are performed to assure identity, purity, activity (potency) and stability of drug products used during all phases of clinical studies.

REGULATORY UPDATE 2017

Ilona Reischl

AGES, Vienna, Austria:

Global development of ATMPs is gaining momentum with a number of products approaching or gaining marketing authorization. New products have highlighted the need for guidance and issues that need to be resolved to improve the regulatory framework. The presentation will provide a summary of recent European activities and outline future directions.

GMP PRODUCTION: REGULATORY PREREQUISITES

Anja Peterbauer-Scherb

Red Cross Blood Transfusion Service of Upper Austria, Linz

Within the EU medicinal products for human use have to ensure a high level of public health protection. Therefore they are subject to the granting of marketing authorisation by the competent authorities. Aiming for providing safe medicinal products a multitude of legislation has been developed. Volume 1 of “The Rules Governing Medicinal Products in the European Union” contains all EC legislation regarding medicinal products for human use while other volumes have been adopted to give guidance on the application. One of them is volume 4 that covers the legal requirements concerning good manufacturing practice (GMP) for medicinal products for human and veterinary use. It is also used to assess applications for manufacturing authorisation and as a basis for inspection of manufacturers of medicinal products by competent authorities. The guideline is divided in three parts and supplemented by several annexes addressing specific areas of activity.
Besides introducing to basic GMP requirements several annexes that apply to the manufacturing of biologicals will be highlighted in the talk while demonstrating their implementation in a not-for profit manufacturing unit for blood and tissue products as well as biological (investigational) medicinal products. Finally, the Austrian legislative situation demanding GMP for producing medicinal products and cell/tissue products will be presented.

DELIVERING THE PRODUCT TO THE PATIENT IN A CLINICAL TRIAL CENTER

Ulrich Jäger

Medical University of Vienna

Cell therapy offers vast new perspectives for the treatment of patients. On the other hand, preparation of clinical trials necessitates several additional steps compared to conventional drug therapy studies:

1. Most cell therapy studies will involve several cooperation partners within a hospital
2. Hospital approval is specific including accreditation procedures
3. The facility may require special preparations including Bioafety approval

4. Regulations are different and special government approval is needed
5. Product handling and study conduct are more complicated
6. The clinical and research staff needs special information and training
7. Patients are usually in later treatment lines and must be informed about the special nature of the product.

These facts require a higher level clinical research facility (e.g. University Hospital, Transplant center). Practical issues using the example of CAR-T cell therapy will be discussed.

NEW REGULATION FOR ANCILLIARY/RAW MATERIALS FOR CELL THERAPYS

Katharina Schallmoser

Paracelsus Medical University, Salzburg, Austria

CELL- OR PARACRINE- MEDIATED REPARATIVE EFFECTS IN THE INFARCT HEART

Gemma Vilahur

Cardiovascular Research Center (CSIC-ICCC), IIB-HSCSP, CIBERCV Instituto Salud Carlos III, Barcelona, Spain

Despite recent advances in medical therapy and interventional techniques, ischemic heart disease remains one of the major causes of morbidity and mortality in developed countries. Over the last several years, adult stem cells have appeared as one of the novel promising therapeutic approaches for the treatment of ischemic heart disease. However, key questions remain on the best cell-based preparation, delivery method, dosage and timing. The ideal stem cell type should be able of differentiating into functional cardiomyocytes and of forming new vessels to support the damaged myocardium. Recent studies have shown that adipose tissue contains multipotent stem cells, the so-called adipose-tissue derived stem cells or ASC. ASCs have become an attractive stem cell source since they are easy to harvest in large numbers. The true *in vivo* differentiation capacity of ASCs and their possible contribution to cardiac tissue regeneration remain controversial. In fact, ASCs therapeutic potential is mainly explained by the production and release of bioactive molecules that mediate neovascularization, cell survival and proliferation (paracrine effects). We have demonstrate in an animal model with high translatability and by system biology approaches that co-administration of ASC and their secretome synergistically contribute to the neovascularization of the infarcted myocardium through a coordinated upregulation of the pro-angiogenic protein interactome, eventually leading to improved cardiac perfusion. Autologous use of ASC may have, however, important limitations. As such, the number and/or function of ASC may be affected by patients' concomitant co-morbid conditions or cardiovascular risk factors. Yet, their low immunogenic potential makes them a good candidate for allogeneic use. Results of on-going human trials will provide an understanding of the real safety and efficacy or these cells in patients with ischemic heart disease, giving new insights into the possible role of ASC in the treatment of cardiovascular disease. However, before moving to the clinical setting, further basic and translational research seems warranted in order to better characterize the surviving cells, and to answer questions like - how long these injected cells can function in the heart and whether ASCs transplantation is "regenerative cell therapy" or "cell-based paracrine therapy"

DIFFERENCES IN STEM CELL PROCESSING LEAD TO DISTINCT SECRETOMES SECRETION — IMPLICATIONS FOR DIFFERENTIAL RESULTS OF PREVIOUS CLINICAL TRIALS OF STEM CELL THERAPY FOR MYOCARDIAL INFARCTION

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INTRODUCTION

Ischemic heart disease (IHD) following acute myocardial infarction (AMI) still constitutes the major cause of premature death and disability in Western countries. Stem cell therapy for acute myocardial infarction (AMI) seemed to be a promising therapy, however large clinical trials brought differential outcome. It has been shown that paracrine effects of secretomes of stem cells rather than cell therapy might play a fundamental role.

AIM

The present study sought to compare cell processing protocols of clinical trials and investigated effects of differential cell culture conditions on chemokine secretion and functional effects.

METHODS

Different secretomes were compared regarding IL-8, VEGF, MCP-1 and TNF-alpha secretion. Secretome mediated effects were evaluated on endothelial cell (HUVEC) tube formation and migration. Cardioprotective signalling kinases in human cardiomyocytes were determined by Western Immunoblotting.

RESULTS

Cells processed according to the REPAIR-AMI protocol secreted significantly higher amounts of IL-8 (487.3±1231.1pg/mL vs 9.1±8.2 pg/mL; $p<0.05$). REAPIR-AMI supernatants led to significantly pronounced migration on HUVECs. Tube formation assays using HUVECs were performed to investigate effects of supernatants on tube formation or inhibition. Tube length was significantly higher under REPAIR-AMI conditions (166%±9% of control) compared to cells incubated with supernatant from ASTAMI-conditioned PBMCs (90%±3% of control; $p<0.05$). HUVECs incubated with REPAIR-AMI-supernatant induced tube formation comparably to positive controls whereas ASTAMI-supernatant was not effective in stimulation of tube formation. The effects of supernatants on intracellular pathways were investigated by Western Immunoblotting. REPAIR-AMI supernatant gradually enhanced phosphorylation of ERK1/2 (5.2-fold; $p<0.01$), Akt (3-fold; $p<0.05$) and CREB (2.2-fold; $p<0.05$) compared to REPAIR-AMI supernatant.

CONCLUSION

Cell processing conditions had a major impact on the composition of the secretome. In conclusion, we found that (i) IL-8 might probably play an important role in the secretome of stem cells administered after AMI, (ii) propose with induction of angiogenesis and inhibition of cellular death via phosphorylation of ERK1/2, Akt and CREB two possible mechanisms via which IL-8 might mediate favourable effects on cardiomyocytes/endothelial cells. Of importance, our study clearly pointed out the importance of temperature, therefore we (iii) suggest with increasing incubation temperature to body temperature, i.e., 37° Celsius, and adding autologous serum instead of plasma, two possible tweaks to induce a more favourable secretomes regarding cardioprotection. This might be of importance for further *in vitro* and *in*

vivo studies, which we certainly think are worth conducting and could improve lives of patients suffering from ischemic heart failure.

EXTRACELLULAR VESICLES PLAY A RELEVANT ROLE IN THE REGENERATIVE ACTIVITIES OF THE SECRETOME OF APOPTOTIC PERIPHERAL BLOOD MONONUCLEAR CELLS

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INTRODUCTION
The secretome of g-irradiated peripheral blood mononuclear cells (PBMCs) has been shown to have tissue protective and regenerative capacity *in vitro* and *in vivo*. We could show previously that it strongly enhanced wound healing and tissue regeneration after myocardial infarction. However, the exact mode of action is still not fully understood and the exact composition of the released paracrine factors remains to be elucidated.

AIM
In order to gain more information about the composition and function of the PBMC-derived secretome, we wanted to subdivide it into several fractions [proteins, lipids and extracellular vesicles (EVs) comprising microparticles and exosomes] and characterize their biological functions. Since the regenerative properties of EVs have been already reported the main focus of this study was on the in depth characterization of the molecular components of the EVs, including their mRNAs, micro-RNAs, protein and lipid content.

METHODS
PBMCs isolated by Ficoll-Paque density gradient centrifugation were gamma irradiated (60 Gy) and cultivated for 20 hours in serum-free CellGro culture medium. After incubation the cell culture supernatant (secretome) was harvested and the extracellular vesicles were purified by ultracentrifugation. The number and size of isolated exosomes and microparticles were evaluated with NanoSight. A pool of exosomes and microparticles from different PBMC donors were used for protein, RNA and lipid isolation. Transcriptome-, proteome- and lipidome-analyses were performed. For biological assays, keratinocytes stimulated with the secretome and the extracellular vesicles were used for the preparation of a 3D *in vitro* skin model.

RESULTS
Irradiation of PBMCs induced the release of exosomes and microparticles. The size distribution profile was typical for the respective EVs. Analysis of the transcriptome of extracellular vesicles showed that miRNAs were differently expressed in exosomes and microparticles and

significantly changed after g-irradiation. Lipid analysis by thin layer chromatography revealed that exosomes and microparticles from irradiated PBMCs contained higher concentrations of phospholipids (PL) and cholesterol compared to the extracellular vesicles from non-irradiated PBMCs. In detail analysis of phospholipids by high pressure lipid chromatography-tandem mass spectrometry indicated that the extracellular vesicles contained a variety of bioactive phospholipids at levels up to 40 times higher than in the culture medium. The highest lipid levels were observed for hydroperoxides (-OOH) and hydroxides (-OH). To investigate to biological function of the EVs we cultivated *in vitro* skin models in the presence or absence of the whole secretome as well as exosomes and microparticles. Interestingly, both, the whole secretome and the exosomes, led to a significantly enhanced production of proteins, associated with epidermal barrier formation.

CONCLUSIONS

Our data show that especially exosomes released from g-irradiated PBMCs are biologically active important components of the PBMC-derived secretome.

IN SITU TISSUE ENGINEERING OF HEART VALVES: FROM BENCH TO BEDSIDE

Jolanda Kluin

Academic Medical Center, Netherlands

We investigate and design *in situ* heart valve tissue engineering technologies using cell-free biodegradable synthetic scaffolds as a novel approach to create living valves inside the human body. While this would constitute a simple procedure, starting from the implantation of a biomaterial 'device', it requires the development of advanced materials and a detailed understanding of the complex interactions between (circulating) cells, scaffold, and tissue formation under *in vivo* hemodynamic conditions.
The focus of the talk is on our systematic approach from biomimetic *in vitro* models and computational analyses to *in vivo* small-animal experiments and finally large-animal experiments.
The regulatory affairs concerning this new products will also be addressed.

NERVAL REGENERATION IN RECONSTRUCTIVE THERAPY

Christine Radtke

Medical University of Vienna, Austria

HEART - STEM CELL TRANSPLANTATION: REALITY CHECK AND FUTURE

Mariann Gyöngyösi

Medical University of Vienna, Austria

Due to the limited propensity for the heart to repair itself following injury, numerous strategies to regenerate the ischemic damaged tissue have been proposed and tested in pre-clinical models and small to medium sized phase I and phase II clinical trials. One of the most promising strategies to repair or regenerate the damaged myocardium involves the use of cell-based therapies, aiming to regenerate and re-juvenile the cardiomyocytes as a causative therapy, by revaluation of the hibernated cells, or to replace them with new ones, or to reactivate the local cardiac progenitor cells, or to improve the cell-to-cell interaction exerted by exosomes or coding or non-coding RNAs. Currently, approximately 5000 patients with ischemic heart disease were treated in randomized or non-randomized trials with different types of cells, mostly with bone-marrow origin mononuclear cells. Other cell types, such as mesenchymal stem cells of different origin (bone marrow, adipose tissue-derived, etc), bone-marrow and peripheral blood progenitor cells, cardiac progenitor cells (cardiospheres) or myoblasts were also used.

After almost 20-year intensive biological, or pre-clinical and clinical research, human cardiac regenerative therapy could not become a breakthrough treatment mode. Even if previous small randomized or cohort clinical trials led to hopeful results, the number of cell-based cardiac regenerative trials with neutral outcome is increasing. Recent meta-analysis of the cell-therapy studies revealed also questionable benefit of intracoronary cell application in patients with acute myocardial infarction. Patients with therapy refractory heart failure or angina pectoris still might have benefit from the paracrine effect of the cells, as recent reviews and meta-analyses suggest. However, the current statement of the Working Group on cardiac regeneration of the ESC, published recently in global position paper (Fernandez-Aviles 2017 EHJ) is, that even if the cell-based therapy in patients proved to be safe, that results are rather inconsistent. Currently, the utilization of the paracrine effect of the reparative cells, and cell-to-cell interaction via extracellular vesicles (eg. exosomes), or coding or non-coding RNAs are considered to be a new option for cardiac regeneration.

TRACHEAL TRANSPLANTATION - CURRENT STATE - FUTURE ASPECTS

Konrad Hoetzenecker

Division of Thoracic Surgery, Medical University of Vienna

INTRODUCTION
A number of attempts have been made to bridge long-segment circumferential airway defects, which are not eligible for primary anastomosis. An ideal tracheal substitute has to be radially rigid but longitudinally flexible. It should be covered by respiratory epithelium and needs a vascular supply.

AIMS AND METHODS
This lecture will give an overview on different techniques of airway replacements. It will cover a variety of approaches including tissue engineering, allotransplantation, indirect revascularization, aortic homografts, and the use of composite grafts as airway substitutes.

RESULTS
There are five principal concepts of airway replacement: synthetic prostheses, bioprostheses, allografts, autografts and bioengineered conduits. Although numerous experimental studies have been published on each of these principles, to date only three techniques have been transferred from bench to bedside. Deleare and colleagues (Leuven) utilized indirect revascularization of allografts by implanting a donor trachea into patients' forearms. This allograft was later successfully transferred to the neck to cover partial tracheal defects. However, a circumferential airway replacement is not possible using this technique. Partial tracheal defects were also successfully bridged using aortic grafts. This technique has been suggested by the FREnch Group for Airway Transplantation (FREGAT) and by the airway unit from Massachusetts General Hospital. Although results were mixed, aortic homografts represent a viable option for management of large partial airway defects. Currently, the only successful circumferential airway replacement has been published by the surgeons from Marie Lannelongue Hospital, Paris. Over a time period of 8 years, 12 full tracheal replacements have been performed by constructing a tube from a forearm free fasciocutaneous flap reinforced by rib cartilages.

CONCLUSIONS

Despite three decades of research, replacement of the human trachea still remains an unsolved problem. Techniques of bioengineering and tissue regeneration are novel concepts, which are currently tested in preclinical and clinical trials*t*

INTERACTION NETWORKS BETWEEN CELLULAR COMPONENTS OF THE SKELETAL MUSCLE ENVIRONMENT DURING HOMEOSTASIS, PHYSIOLOGICAL AND PATHOLOGICAL REPAIR

Pier Lorenzo Puri

Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA & Fondazione Santa Lucia, Rome, Italy.

Regeneration of injured or diseases skeletal muscles entails a coordinated activation of and interplay between different cell types, to ultimately instruct the direct effectors of myofiber formation – the skeletal muscle stem cells (otherwise indicated as satellite cells – SC). Among the cell types that contribute to this regenerative environment are components of the inflammatory infiltrate (i.e. macrophages) and muscle interstitial cells otherwise referred to as fibro-adipogenic progenitors (FAPs). Emerging evidence indicates that altered interactions between these cell types contribute to pathogenesis and progression of an increasing number of muscular diseases. Here I will present recent discoveries from my lab that reveal functional interactions between these cell types, and in particular the identification of genome-wide transcriptional profiles that reveal the existence of specific sub-cellular populations that contribute to maintenance of skeletal muscle homeostasis, and physiological or pathological repair upon homeostatic perturbations. Moreover, it will be presented the contribution of extracellular vescicles in coordinating these cellular interactions.

CELLULAR PLASTICITY AND TISSUE REGENERATION: WHAT CAN WE LEARN FROM TUMOR BIOLOGY

Thomas M. Marti

Division of Thoracic Surgery, Inselspital, University Hospital Bern, Switzerland

Lung cancer is the most common cause of cancer-related mortality worldwide. This is mainly due to the difficulty of early detection and lack of effective treatment methods, thus more effective treatment options are desperately needed.
It was postulated that tumor initiation and propagation are mediated by so called tumor-initiating cells (TICs), which can self-renew and spawn differentiated progeny. Initially, it was proposed that the generation of bulk cancer cells from TICs is a unidirectional process. However, recent advances in the field indicate that more differentiated cancer cells might be able to dedifferentiate thereby giving rise to TICs. The recent progresses in this area will be briefly discussed.
For basic and translational lung cancer research, cell lines are an essential tools to characterize patient-derived mutations and to standardize and compare experimental findings within the research community. Hence, it is generally assumed that cells within a cell line are phenotypically and functionally homogenous. However, we identified and comprehensively characterized morphologically distinct subpopulations in the non-small cell lung cancer cell line A549. Exploiting the A549 cell line as a model system, the correlation, respectively plasticity, between stemness, tumor initiation capacity, invasion and metastasis potential and chemotherapy resistance will be briefly discussed.

OPTIMIZATION OF A HUMAN LUNG ORGANOID MODEL FOR LUNG REGENERATION

Sean Hall

University Hospital Inselspital Bern, Switzerland

INFLAMMATION AND HEART - MYOCARDITIS AND AMI

Urs Eriksson

University of Zurich, GZO Regional Health Center, Switzerland

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HUMORAL IMMUNITY IN THE INFLAMED HEART

Christoph Binder

CEMM, Medical University of Vienna, Austria

ENHANCED REGENERATIVE EFFECT OF CARDIOSPHERE-DERIVED CELLS BY PRE-TREATMENT WITH PARACRINE FACTOR APOSEC IN PORCINE CLOSED-CHEST REPERFUSED MYOCARDIAL INFARCTION PROVED BY QUANTITATIVE CARDIAC PET-MRI

Ljubica Mandic MSc, Julia Mester-Tonczar BSc, Andras Jakab MD PhD, Katrin Zlabinger MSc, Dominika Lukovic PhD, Alfred Gugerell PhD, Noemi Pavo MD PhD, Eduardo Marbán MD, Johannes Winkler PhD, Mariann Gyöngyösi MD PhD

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BACKGROUND

In animal models and the clinical trial CADUCEUS, cardiosphere-derived cells (CDCs) have shown promise for treatment of ischemic left ventricular (LV) dysfunction after myocardial infarction (AMI). Recent data indicates that paracrine effects are important for the efficacy of cardiac cell-based therapy. APOSEC, a secretom of apoptotic peripheral blood mononuclear cells (PBMC) is a mixture of paracrine factors with cell protective, angiogenic and antiapoptotic effects. We hypothesized that pretreatment of CDCs with APOSEC enhances the paracrine cardiac regenerative effect of CDCs in a pig model of closed-chest AMI.

METHODS

CDCs were isolated from porcine heart tissue (kind donation of E. Marban). Allogeneic porcine CDCs were incubated in vitro with APOSEC in different doses and incubation times and the effects on CDC phenotype and transcriptome were assessed for selection of the optimal pre-treatment mode (CDC-Apo). Domestic pigs underwent AMI via percutaneous balloon occlusion of the mid-LAD for 90 min followed by balloon deflation. Fifteen min after reperfusion the pigs were randomized and received intracoronary infusion (3 ml/min, stop-flow technique) of either

10^7 CDCs (n=5) or 10^7 CDC-Apo (n=5) or phys. saline (placebo) (n=4). Cardiac 18F-FDG-PET-MRI images with late enhancement were performed at 30-day follow-up. Quantitative comparative 17-segment analysis of PET-MRI of the groups was performed with in-house developed software.

RESULTS
After selection of the optimal APOSEC dose (secretome derived from 10^7 PBMC) and incubation time (48h) both VEGF secretion (63.0±11.7 vs 10.7±10.0 pg/mL) and SDF-1/CXCL12 expression of CDC-Apo (2.3±0.03 vs 1.0±0.1 fold relative expression) were significantly increased as compared to CDCs cultured in standard cell culture medium. Intracoronary infusion of CDC-Apo led to significant ($p<0.05$) increase in LV ejection fraction (43.6±6.1% vs 37.8±4.4% and 36.3±4.1%), decrease in infarct scar (12.5±4.1% vs 16.5±4.0% and 22.7±2.2% of the LV) as compared to CDC and placebo groups. Relative quantitative ^{18}F-FDG-uptake of the MRI-derived infarcted area (defined as transmurality over 50%) was significantly higher in CDC-Apo vs CDC and placebo groups (67±2% vs 53±3%, and 55±7%), indicating more preserved viability within the infarcted area in CDC-Apo group (Figure).

CONCLUSION
In vitro treatment of CDCs with APOSEC induces enhanced expression of factors (SDF-1/CXCL12, VEGF) that have been shown to play a role in the regenerative effect of cell-based therapy. CDC-Apo-treated pigs suffered from less severe infarct scars. In summary, pretreatment of CDCs with APOSEC enhanced the therapeutic potential of CDCs for treatment of ischemic LV dysfunction.

CHARACTERISATION OF ENGINEERED VASCULAR NETWORKS DERIVED FROM ENDOTHELIAL CELLS AND ADIPOSE-DERIVED STEM CELLS IN A FIBRIN MATRIX

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INTRODUCTION
Co-cultures of endothelial cells with mesenchymal stem cells currently represent one of the most promising approaches in providing oxygen and nutrient supply for microvascular tissue engineering.

AIM
To translate this model into clinics several *in vitro* parameters including growth medium and scaffold degradation need to be fine-tuned.

METHODS
We recently described the co-culture of adipose-derived stem cells with endothelial cells in fibrin, resulting in capillary formation *in vitro* as well as their perfusion *in vivo*. Here, we aimed

to further characterise microvascular tube formation in fibrin by determining the role of scaffold degradation, thrombin concentration and culture conditions on vascularisation.

RESULTS

We observed that inhibition of cell-mediated fibrin degradation by the commonly used inhibitor aprotinin resulted in impaired vascular network formation. Aprotinin had no effect on laminin and collagen type IV deposition or formation of tube-like structures in plasminogen-free fibrin or scaffold-free co-culture, indicating that poor vascularisation of fibrin clots is primarily caused by inhibition of fibrinolysis. Furthermore, we demonstrate that thrombin negatively affects vascular network density at high concentrations. However, only transient activation of incorporated endothelial cells by thrombin could be observed, thus excluding a long-term inflammatory response in tissue-engineered micro-capillaries. Finally, we show that vascularisation of fibrin scaffolds in basal medium is undermined because of increased fibrinolytic activity leading to scaffold destabilisation without aprotinin.

CONCLUSIONS

Taken together, our data reveal a critical role of fibrinolysis inhibition in microvascular tissue engineering as it reduces cell-mediated vascularisation of fibrin scaffolds but is required for culture in basal medium.

THERAPEUTIC OF MSC EVS

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CORRELATION OF BONE STRUCTURE AND miRNA PLASMA LEVELS

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miRNA LEVELS IN A MODEL OF OSTEOPOROSIS AND THERAPY

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ENDOTHELIAL EXTRACELLULAR VESICLES - PROMISES AND CHALLENGES

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Extracellular vesicles, including exosomes, microparticles, and apoptotic bodies, are phospholipid bilayer-enclosed vesicles that have once been considered as cell debris lacking biological functions. However, they have recently gained immense interest in the scientific community due to their role in intercellular communication, immunity, tissue regeneration as well as in the onset, and progression of various pathologic conditions.
Extracellular vesicles of endothelial origin have been found to play a versatile role in the human body, since they are on the one hand known to contribute to cardiovascular diseases, but on the other hand have also been reported to promote endothelial cell survival. Hence, endothelial extracellular vesicles hold promising therapeutic potential to be used as a new tool to detect as well as treat a great number of diseases. This calls for clinically approved, standardized, and efficient isolation and characterization protocols to harvest and purify endothelial

extracellular vesicles. However, such methods and techniques to fulfill stringent requirements for clinical trials have yet to be developed or are not harmonized internationally. In this review, recent advances and challenges in the field of endothelial extracellular vesicle research are discussed and current problems.and limitations regarding isolation and characterization are pointed out.

PLACENTAL STROMAL CELLS FOR REGENERATION OF INJURED GLUTEAL MUSCLE AFTER HIP ARTHROPLASTY

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To date, there is no effective therapy to address skeletal muscle injuries. Conservative approaches use different substances without scientific proof of their efficacy. Surgical approaches aim at adapting traumatized muscle tissue and are dependent on the intrinsic healing capabilities of the muscle. Our studies have explored therapy with mesenchymal stromal cells (MSC) for skeletal muscle injuries and transferred this therapy from preclinical tests to the patient.
First, we established an animal model, mimicking a clinically relevant crush trauma of the soleus muscle, and tested several applications of autologous MSC transplantation, among them immediate versus delayed, intraarterial versus local and therapy of male versus female animals. Following this, we tested the efficacy of an allogeneic approach in the same trauma model, using human placenta-derived mesenchymal like adherent stromal cells (PLX-PAD). We then translated this therapy into the clinics by using acute iatrogenic muscle damage after total hip arthroplasty (THA) as a model system and conducted a prospective, randomized, double blind, placebo-controlled phase I/II study. 20 patients undergoing THA via lateral approach were included and received a transplantation of $300x10^6$ (300M), $150x10^6$ (150M) PLX-PAD or placebo into the injured gluteus medius muscles (GM). Follow-up included safety, function, MRI and muscle biopsies.
Preclinical experiments showed improved muscle healing with increased force generation after autologous and allogeneic MSC therapy versus placebo. Patients of the phase I/II study were followed for 2 years. No relevant AEs have been observed during this period The primary efficacy endpoint, change of GM strength after 6 months, showed a significant increase in the 150M group ($p=0.0067$) compared to placebo, which was accompanied by an increase in muscle volume ($p = 0.004$). The change of strength and volume in the 300M group showed a similar pattern as in the 150M group but was not statistically significant. Histology indicated faster healing after PLX-PAD therapy.
Our data showed consistent positive results of MSC therapy for skeletal muscle trauma in different application modes and finally in patients. Treatment with allogeneic cells could be a game changer not only in the treatment of the analyzed injuries but also for other traumatic or iatrogenically induced muscle injuries.

BACK TO THE FUTURE - NEW STRATEGIES IN GENE THERAPY

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Gene therapy has been successfully used as a treatment option for a number of inherited diseases including diseases of the blood forming system like X-linked severe combined immunodeficiency (X-SCID), adenosine deaminase-deficient SCID (ADA-SCID), Wiskott–Aldrich syndrome (WAS), chronic granulomatous disease (CGD) and β-hemoglobinopathies

to mention only some of the most advanced HSC gene therapies. Though demonstrating sustained clinical benefit for the patients, initial clinical trials were associated with insertional mutagenesis resulting in leukemogenesis in a number of patients. The development of safer vector systems and the advances made in the last decade ultimately lead to market approval of the first gene therapies in the western world. The advent of gene editing technologies - the precise modification of genes and genomic loci - in recent years is revolutionizing both basic as well as translational research. Designer nucleases like clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas9), transcription activator-like effector nucleases (TALEN) or zinc-finger nucleases (ZFN) enable to correct disease causing mutations in situ thus eliminating the risk of insertional mutagenesis and allowing the corrected gene to be expressed under endogenous gene regulation. However, biosafety of gene edited cell products is an issue that needs careful evaluation. To make these technologies as safe as possible for the patients, it is crucial to assess off-target activity in modified cells from the patients to minimize the risk of transforming therapeutic blood stem cells.

WHAT IF WE COULD GIVE THE BODY INSTRUCTIONS TO PRODUCE ITS OWN DRUGS?

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Advancements in mRNA technology in recent years have opened up a completely new treatment approach, focusing on overcoming the challenges and limitations of traditional pharmaceuticals by employing mRNA. mRNA is the intermediate that nature uses to translate DNA's genetic information into proteins. The sequence of its building blocks determines the amount of protein to be produced. Modifying this sequence can optimize mRNA expression to achieve a desired therapeutic effect. We are using modified mRNA to produce proteins on-demand as a treatment for local skin conditions. Skin offers various opportunities with regard to development of mRNA-based therapeutics: diseases with validated molecular targets/attractive markets and direct access facilitating quantification of mRNA expression/clinical activity. We systematically modified specific IVT-mRNAs and tested the most interesting ones in skin explant systems varying formulation and delivery.

WHY WE DEVELOP iPSC THERAPY STRATEGIES

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HIPPO EFFECTOR PROTEIN YAP AS THE MASTER REGULATOR OF CELL-MATRIX INTERACTION IN HEALTH AND DISEASE

Giancarlo Forte

FNUSA-ICRC/St. Anne's University Hospital, Brno, Czech Republic

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MECHANICAL STIMULATION BY SHOCKWAVE THERAPY INDUCES MYOCARDIAL REGENERATION

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THE IMPORTANCE OF MECHANOTRANSDUCTION IN MYOGENESIS

Philipp Heher

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EFFECTS OF SHOCKWAVE ON ADIPOSE DERIVED STEM CELLS

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INTRODUCTION
A highly interesting source for adult stem cells is adipose tissue, from which the stromal vascular fraction (SVF) - a heterogeneous cell population including the adipose-derived stromal/stem cells (ASC) - can be obtained. In the last decades, cell-based therapies with autologous adipose tissue derived cells have shown great potential in several clinical studies.

AIM
The aim of this study is to enhance stem cell properties and functionality of freshly isolated SVF cells using extracorporeal shock wave therapy (ESWT).

METHODS
In our three approaches, ESWT was applied on freshly isolated SVF cells (ESWT in vitro), on freshly obtained human adipose tissue (ESWT ex vivo) and on the adipose tissue harvest site before liposuction (ESWT in situ). Cells derived from all three approaches were analyzed regarding cell phenotype, yield, viability, adenosine triphosphate (ATP) content, but also proliferative capacity, surface marker profile, differentiation potential and secretory protein profile.

RESULTS
After ESWT ex vivo we could achieve higher cellular ATP levels compared with ESWT in vitro as well as the untreated control. ESWT ex vivo resulted in a significantly higher expression of single mesenchymal and vascular marker compared with the untreated control. Similarly, after ESWT in situ ATP concentration and also viability of freshly isolated cells were significantly increased compared to the untreated group. Likewise, cells expressing mesenchymal and endothelial/pericytic markers were significantly elevated after ESWT in situ concomitant with an improved differentiation capacity towards the adipogenic lineage and enhancement in specific angiogenic proteins.

CONCLUSION
ESWT potentially provides a more regenerative cell population. Since the effectiveness of autologous cell therapy is dependent on the therapeutic potency of the patient's cells, this technology might raise the number of patients eligible for autologous cell transplantation.

CALCIFICATION OF THE HEART VALVE BY TLR3

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P01 TITLE

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INTRODUCTION
Hemorrhagic traumatic shock (HTS) followed by reperfusion is typically accompanied by inflammation cell/organ injury and frequent death. Contemporary literature indicates that beneficial effects of mesenchymal stem cells (MSCs) may be attributed to the mediators they release.

AIM
Rats were subjected to HTS and a resuscitation protocol that mimics pre-hospital restrictive reperfusion followed by an adequate reperfusion phase. 20 minutes into the restrictive reperfusion, animals received an intravenous bolus of $2x10^6$ cells (ASC group) or the secretome produced by $2x10^6$ ASCs/24h (ASC-Secretome group). Controls received the vehicle (Vehicle group). All rats were observed for 28-day survival.

RESULTS
HTS-induced inflammation represented by IL-6 was inhibited in the ASC (80%, $p<0.001$) and in ASC-Secretome (59%, $p<0.01$) group at 48h compared to Vehicle group. At 24h, HTS-induced liver injury reflected in plasma alanine aminotransferase was ameliorated by 36% ($p<0.001$) in both the ASC and ASC-Secretome groups when compared to the Vehicle. There was no effect on kidney function and/or general cell injury markers. HTS-induced a moderate 28-day mortality (18%) that was prevented ($p = 0.08$) in the ASC but not in the ASC-Secretome group (12%).

CONCLUSION
Our data suggest that the ASC-secretome supplemented resuscitation following HTS, in absence of the stem cells, exerts anti-inflammatory and liver protective effects. Given its ease of preparation, storage, availability and application (in contrast to the stem cells) we believe that the cell free secretome has a better therapeutic potential in the early phase of an acute hemorrhagic shock scenario.*at*

P02 BARIATRIC SURGERY ALTERS THE INTRINSIC COAGULATION CASCADE

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INTRODUCTION
Obesity is associated with a prothrombotic milieu and increased risk for thrombotic events. Bariatric surgery is the most effective treatment for obesity resulting in dramatic weight loss and reduced inflammation and extrinsic coagulation pathway activation.

METHODS
Blood samples were drawn from 76 patients undergoing Roux-en-Y gastric bypass surgery before and 1 year after surgery. Activated partial thromboplastin time (APTT), total and active protein c (PC), and soluble thrombomodulin (sThromb) were evaluated.

RESULTS
APTT was increased one year after bariatric surgery from 28.9±4.5 sec. to 31±4.5 sec. ($p<0.001$). Changes in APTT were not due to increased levels of PC as total PC (187.5±63% before surgery versus 121.7±54.2% after bariatric surgery, $p<0.001$) and active PC (134±58% before surgery versus 67.8±63.9% after bariatric surgery, $p<0.001$) were similarly reduced one year after surgery. sThromb was significantly reduced (5.8±2.5 ng/ml before surgery versus 3.3±1.6 ng/ml after surgery) indicating reduced activation of endothelial cells.

DISCUSSION
Bariatric surgery induced weight loss is associated with an amelioration of coagulation risk including previously reported reduction in tissue factor and PAI-1. However, bariatric surgery is also associated with deep vein thrombosis (DVT). We propose that the inhibition of the intrinsic coagulation cascade is fully activated in morbidly obese patients partially via increased thrombomodulin. After bariatric surgery this protective effort is no longer necessary as indicated by reduced sThromb and hence leads to a reduction in PC and aPC without negatively affecting coagulation time.

P03 ADIPOSE-DERIVED MICROTISSUES

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INTRODUCTION
Human adipose tissue is an attractive and abundantly available source of adult stem cells applicable in regenerative medicine and tissue engineering. The process to obtain the contained stromal vascular fraction (SVF) reveals, as intermediate step after reduction of fat and oil, a microtissue with a heterogeneous cell population including the adipose-derived stromal/stem cells (ASC), endothelial (precursor) cells and fibroblasts. Hence, prerequisite for the translation into clinics is a suitable production protocol for SVF. Current isolation methods for cells from adipose tissue depend on enzymes such as collagenase, resulting in single cell suspensions dissociated from their natural microenvironment.

AIM
The aim of this study was to develop a closed, sterile and safe isolation method with reduced enzyme concentration to obtain adipose-derived microtissues comprising therapeutic cells without dissociation from adipose tissue extracellular matrix.

METHODS
Microtissue was isolated from adipose tissue with a low concentration of collagenase (GMP quality) under sterile and safe conditions. The isolated microtissue was characterized regarding identity and functionality by analyzing cellular composition, surface marker profile, adipogenic and vasculogenic differentiation potential as well as graft integration in nude mice after intramuscular and subcutaneous injection.

RESULTS
The isolated microtissue comprised connective tissue (collagen, fibroblasts) and intact vessels (CD31+, smooth muscle actin+) detected by histological stainings. The contained but not isolated or substantially manipulated mesenchymal stem cells were verified by flow cytometry analysis, as defined by the guidelines from the IFATS/ISCT. Moreover, the microtissue demonstrated a high adipogenic differentiation potential, indicated by enhanced expression of adipogenic marker genes (PPARG, FABP4, Leptin) after induction, and a strong potential to form tube-like structures (CD31+), which is a prerequisite for neovascularization. In vivo analyses demonstrated successful integration of transplanted grafts composed of microtissue alone or combined with fat tissue in immunocompromised mice.

CONCLUSIONS
In summary, we developed a closed, safe and sterile isolation protocol with a low enzyme concentration to obtain an adipose-derived microtissue enriched with regenerative cells. The essential structures of the microtissue were maintained and enabled a high adipogenic and vasculogenic differentiation potential, which is obligatory for successful graft integration and survival. This procedure provides a microtissue for structural restoration of tissue defects with therapeutic cells for potential autologous clinical applications in regenerative medicine.

P04 THE INFLUENCE OF CELL-CELL COMMUNICATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS ON CELL DEATH AND CYTOKINE SECRETION

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INTRODUCTION
The secretome of g-irradiated peripheral blood mononuclear cells (PBMC) is well known for its pleiotropic effects on tissue protection and regeneration. Recently, we were able to show, that

γ-irradiation of peripheral blood mononuclear cells (PBMC) with 60Gy induced, in addition to apoptosis, also necroptosis, a recently discovered new form of cell death. However, the impact of necroptosis on the composition of the PBMC-secretome and its function is still not known.

AIM

We therefore wanted to analyse the effects of apoptotic and necroptotic cell death on the secretome-composition and its biological function. Furthermore, we wanted to investigate whether PBMC-subsets behave different, when they were irradiated in the tissue context or separately.

METHODS

Natural killer cells (CD56), monocytes (CD14), CD8 and CD4 positive T-cells and B-cells (CD19) were separated with the autoMacs technology. One half of the cells was irradiated with 60Gy and the other half was cultivated without irradiation for 24h. The form of cell death was analysed via Image stream analysis. The secretomes of the subsets, as well as apoptosis associated protein expression were analysed with proteome profiler, western blot analysis and multiplex assays.

RESULTS

Cell death was detected in 10% of the non-irradiated and 50% of the g-irradiated PBMC. 2/3 of the cells died via apoptosis and 1/3 via necroptosis in both conditions. Interestingly, the type and the extent of cell death changed dramatically in the purified PBMC-subsets. Much more cells died already under non-irradiating conditions. Whereas the majority, CD4 and CD8 positive T-cells, NK-cells as well as B-cells were driven into apoptosis without irradiation, irradiation switched the mode of cell-death to necroptosis. By contrast, monocytes showed a high percentage of necroptotic cells under both, irradiated and non-irradiated conditions. This switch from apoptosis to necroptosis was accompanied by a strong deregulation of pro- and anti-apoptotic gene-expression. We also detected major differences of the secretome-composition of PBMCs and the purified subsets. Most interesting was the observation that certain cytokines (e.g. ENA-78) were only present in the PBMC-derived secretome but not in the secretome of any of the cell-subsets, suggesting that interactions of the different subsets are essential for the composition of the secretome. In addition, we could demonstrate that inhibition of apoptosis by caspase-3 inhibitors, led to a strong deregulation of a variety of cytokines, including a massive increase of IL-8 release.

CONCLUSION

Our data suggest that PBMCs in the context of the tissue are able to compensate high levels of cell death triggers by inducing anti-apoptotic survival pathways. In contrast, the major cell death pathway in single blood cell-populations is necroptosis, which cannot be rescued by the anti-apoptotic machinery of the cells. Therefore cell-cell communication and paracrine effects seem to play a far more crucial role in immune response and regenerative capacity of the PBMC-derived secretome than suspected.

P05 DYNAMICS OF PLASMA-EXOSOMAL MIR-1, MIR-133 AND MIR-208 CONCENTRATION DURING ACUTE MYOCARDIAL INFARCTION IN A PORCINE MODEL

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Affiliation

BACKGROUND
Exosomes are small (30-100nm) extracellular membrane vesicles. They are important carriers of stable microRNAs (miRs) in plasma. The levels of several miRs related to acute myocardial infarction (AMI) have been shown to change several hours after AMI. We have investigated the change of exosomal levels of miR-1, miR-133 and miR-208 during the ischemic period of an AMI.

METHODS
3 fully anesthetized pigs underwent 90-min percutaneous occlusion of the mid LAD followed by reperfusion. EDTA blood samples were taken at baseline and at 10, 30, 60 and 90 minutes of occlusion. Plasma was prepared for exosome isolation by progressive centrifugation steps of 1200 x g for 10 minutes, 1800 x g for 10minutes and 10 000 x g for 20 minutes. The resulting plasma was then filtered through 0.2µm syringe filters. One mL of this prepared plasma was suspended in 9mL of PBS and ultra-centrifugated at 100 000 x g for 120 minutes, followed by a washing step and another round of centrifugation at 100 000 x g for 120 minutes. Isolation of exosomes was verified by Nanoparticle Tracking and Western Blot of CD63 and CD9. miRs were isolated using QIAGEN miRNeasy Serum / Plasma kits, reverse transcribed using QIAGEN miScript RT kit and qPCR was performed using miScript SYBR® Green PCR Kit. Fold changes were normalized using ce-miR-39 Spike-in-Control of the QIAGEN Serum / Plasma kit. Relative fold changes of miR-1, miR-133 and miR-208 at baseline, 10, 30, 60 and 90 minutes after begin of reperfusion were evaluated.

RESULTS
Relative fold changes are shown in boxplots. All 3 miRs were detected in the isolated plasma exosomes. However, no significant differences of miR-1, miR-133 and miR-208 levels could be detected at the different time points during and shortly after reperfusion of acute myocardial infarction.

CONCLUSION
While all investigated miRs could be detected in plasma exosomes, no significant relative changes in miR-1, miR-133 and miR-208 concentration were observed in the present study. It is likely that 90 minutes of ischemia are too short to significantly change the already minute levels of plasma-exosomal miRNAs.

P06 EFFECT OF PROTEIN TEMPLATED THREE-DIMENSIONAL HYDROXYAPATITE MICROSPHERES ON THE MYOBLAST CELL LINE C2C12

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INTRODUCTION
There have been many attempts to produce three-dimensional (3D) assembled hydroxyapatite (HAP) microspheres due to their great potential in different applications such as drug delivery, dental implants, bone repair, tissue engineering, heavy metal ions adsorption and catalysis. Recently, different molecules such as polymers, DNA and proteins including hemoglobin (Hb) have been used as templates to control the size and morphology of HAP particles.

AIM
The purpose of this study was first to synthesize and characterize 3D HAP microspheres using hemoglobin in a protein assisted assembly process via the hydrothermal method, and then investigate their biological properties by culturing with the mouse myoblast cell line C2C12.

METHODS
HAP microspheres were synthesized through the template mediated hydrothermal method. Briefly, aqueous solutions of $Ca(CH_3COO)_2$, Hb and NaH_2PO_4 were mixed under stirring. The hydroxyapatite suspensions were transferred to a stainless steel autoclave. The hydrothermal treatment was set up at 120°C for 8h. HAP microspheres were characterized by Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy analyses to evaluate particles, chemical functional groups, crystal structure and morphology, respectively. The myoblast cell line C2C12 was cultured with HAP particles (direct assay) and HAP extract (indirect assay) for 72h. Cell proliferation and biocompatibility of HAP microspheres was tested by LDH assay.

RESULTS
FT-IR spectrum showed characteristic bands representing apatitic PO_4^{-3} groups at 570, 601 cm^{-1} and strong bond around 1040 cm^{-1}. Also there are peaks around 1635 and 3440 cm^{-1} reflecting the presence of water molecules in the structure. XRD analysis by X'pert highscore software revealed successful synthesis of HAP particles. SEM images confirmed the formation of HAP 3D structures. *In vitro* experiments demonstrated nontoxic behaviour of HAP microspheres on C2C12 cells up to a concentration of 1mg/ml when cultured in direct contact and 30mg/ml when exposed to particle-conditioned medium).

CONCLUSIONS
In summary, we confirmed the formation of 3D assembled HAP microspheres and demonstrated good cellular compatibility properties of the produced HAP microspheres.

P07 DENTAL PULP-DERIVED CELL SPHEROIDS FORM IN THE PRESENCE OF HYPOXIA AND HYPOXIA MIMETIC AGENTS

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INTRODUCTION
In regenerative endodontics, scaffold-free transplantation of dental pulp-derived cell (DPC) spheroids represents a novel experimental approach for pulp regeneration. Conditioning cells with hypoxia enhances engraftment and their pro-angiogenic capacity. It is unclear if this pre-conditioning approach has an influence on DPC spheroid formation.

AIM
This study was designed to reveal the impact of hypoxia and hypoxia mimetic agents on the formation and activity of DPC spheroids.

METHODS
Spheroids of DPC were produced using agarose wells. During this process DPCs were exposed to hypoxia and the hypoxia mimetic agents Dimethyloxalylglycine (DMOG), Desferrioxamine (DFO), L-mimosine (L-MIM) or combinations of hypoxia and hypoxia mimetic agents. Images were taken after seeding, 6 and 24 hours. Based on these images spheroid size was measured with ImageJ. Viability of DPC was assessed using the resazurin-based toxicity assay, Live-Dead staining and MTT staining. The protein levels of vascular endothelial growth factor (VEGF), interleukin(IL)-8, stromal cell-derived factor(SDF)-1, angiogenin (ANG) and angiopoietin-like 4 (ANGPTL 4) were evaluated with enzyme-linked immunosorbent assays.

RESULTS
We observed formation of spheroids also in the presence of hypoxia, hypoxia mimetic agents and their combination. DPC were vital and no significant changes in spheroid sizes compared to normoxic DPC were found. VEGF, IL-8 and ANGPTL 4 protein levels in the supernatants of DPC spheroids were increased by hypoxia and hypoxia mimetic agents. However, no significant change of SDF-1 was found. ANG protein levels were increased by hypoxia mimetic agents, but no significant modulation by hypoxia was observed. The combination of hypoxia with HMA did not further boost VEGF and IL-8 protein levels significantly.

CONCLUSIONS
Overall our results show that hypoxia and hypoxia mimetic agents do not interfere with DPC spheroid formation and that an increase in the pro-angiogenic capacity was found. Pre-clinical studies will assess if this pre-conditioning strategy supports regenerative approaches in endodontics.

P08 CD4^{+}CD28^{NULL} T LYMPHOCYTES ARE ASSOCIATED WITH THE DEVELOPMENT OF ATRIAL FIBRILLATION AFTER ELECTIVE CARDIAC SURGERY

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INTRODUCTION
Post-operative atrial fibrillation (POAF) is postulated as a complex interaction of different pathogenic factors, suggesting inflammatory processes as a main trigger of this particular type of atrial fibrillation.

AIM
Therefore, the study sought to assess the impact of cellular immunity on the development of POAF.

METHODS
Fluorescein-activated cell sorting was performed in 129 patients undergoing elective cardia valve and/or coronary-artery-bypass-graft surgery.

RESULTS
Comparing patients developing POAF to individuals free of POAF the fraction of CD4^{+}CD28^{null} T Lymphocytes was significantly higher in individuals developing POAF (11.1% [POAF] vs. 1.9% [non-POAF];p<0.001). Moreover, there was a strong correlation of CD4^{+}CD28^{null} cells and post-operative maximum C-reactive Protein values (r=0.216;p=0.041). CD4^{+}CD28^{null} cells were independently associated with the development of POAF with an adjusted odds ratio per one standard deviation of 3.91 (95% CI: 2.09-7.31;p<0.001). An area under the curve of 0.812 indicated a strong discriminatory power of CD4^{+}CD28^{null} cells for the occurrence of POAF. Compared to N-terminal Pro-Brain Natriuretic Peptide, the fraction of CD4^{+}CD28^{null} cells demonstrated an increased discriminatory power for the development of POAF (NRI: 87.9%, p<0.001; IDI: 30.9%, p<0.001). Interestingly, a pre-operative statin-therapy was associated with a lower fraction of CD4^{+}CD28^{null} cells (p<0.001) and showed an inverse association with the development of POAF (p<0.001).

CONCLUSION
CD4^{+}CD28^{null} cells proved to be predictive for the development of POAF after cardiac surgery. Our results potentially indicate an auto-immune impact of this preexisting, highly cytotoxic T cell subset in the pathogenesis of POAF, which might be modified via the anti-inflammatory potential of a pre-operative statin-therapy.

P09 INFLAMMATORY MODULATION OF HAEMOSTASIS IN TRAUMA-INDUCED COAGULOPATHY

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INTRODUCTION

Trauma-induced coagulopathy (TIC) represents a severe dysregulation of haemostasis that often occurs in the aftermath of a major traumatic incident. Hyperfibrinolysis (HF), platelet dysfunction and reduced coagulation factor activity are vital features of TIC, which is still associated with a high mortality. Many of the underlying mechanisms are involved in both haemostasis and inflammation and are equally affected by dysregulation. For instance, Neutrophils are key players of innate immunity, are capable of forming platelet-leukocyte aggregates (PLA) and are known to release serine proteases that interfere with mediators of coagulation and fibrinolysis.

AIM

We investigated whether some of the features of TIC, like platelet dysfunction and HF were associated with the formation of PLA or the activation of other pro-inflammatory pathways.

METHODS

To study PLA formation in vitro, whole blood of 10 healthy human donors was stimulated with selective and general platelet and leukocyte agonists and analyzed by flow cytometry. Leukocyte subsets were identified by morphology and specific antibodies. Platelet-mediated haemostatic function in whole blood was measured by thromboelastometry (ROTEM) and impedance aggregometry (Multiplate). Complete blood counts (CBC) were performed to determine changes in platelet numbers. In addition blood samples of patients who suffered from non-traumatic out-of hospital cardiac arrest were drawn on scene by an emergency physician and were analysed by thromboelastometry (ROTEM). Plasma levels of t-PA, PAI-1, sTM, t-PA-PAI-1-complex and APC-PCI-complex were determined with ELISAs. PMN elastase alpha1-PI and histonylated (h)DNA fragments were measured as an indicator for NET formation. HF was diagnosed with a ROTEM (maximum lysis, ML >15%).

RESULTS

When states of cellular activation were simulated in vitro, platelet aggregation with monocytes was associated with a reduced blood clot firmness (MCF) in ROTEM and impaired platelet responsiveness to TRAP and ADP in the Multiplate assay. Aggregation with monocytes occurred also when only platelets were activated, whereas the interaction with granulocytes required a profound co-activation of both cell types. None of the observed phenomena was paralleled by a reduction of measurable platelet counts. Of the 31 patients enrolled, 19 presented with a ML>15% (HF). Interestingly t-PA activity but not antigen levels were significantly higher in HF. This was neither accompanied by an increased formation of t-PA-PAI-1 complex nor with elevated PAI-1 levels. There was a highly significant correlation between elastase and hDNA levels but no association with HF. APC-PCI complex level were significantly higher in HF.

CONCLUSIONS

The aggregation of leukocytes and platelets was associated with signs of platelet dysfunction in ex vivo experiments. Similar to the situation in trauma patients, this was not associated with reduced platelet counts in CBCs. Both plasmin and the protein C pathway are tightly interwoven in the pathophysiology of HF. Ischemia and reperfusion appears to result in signs of NETosis but there was no association with HF. TIC occurs at the interface between haemostasis and inflammation.

P10 INFLUENCE OF MATRIX METALLOPROTEINASE-2 ON COMPROMISED HOMING OF INTRACORONARY DELIVERY OF MESENCHYMAL STEM CELL IN A PORCINE REPERFUSED MYOCARDIAL INFARCTION: COMPARISON WITH INTRAMYOCARDIAL CELL DELIVERY

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BACKGROUND
Intracoronary injection of mesenchymal stem cells (MSC) results in a prompt decrease of absolute myocardial blood flow (AMF) with late and incomplete recovery of myocardial tissue perfusion, therefore it leads to less homing with consequent diminished regenerative effect of the ischemic injured heart tissue compared to intramyocardial (IM) cell implantation. We investigated the effect of AMF on the fate and homing of MSC after intracoronary or intramyocardial cell delivery in a closed-chest reperfused myocardial infarction (MI) in pigs.

METHODS
One week after myocardial infarction, porcine GFP-Luc-MSCs were injected either intracoronary (group IC) or intramyocardially (group IM). AMF was measured before, immediately after, and 24h post cell delivery. In vitro bioluminescence signal was used to identify tissue samples containing GFP-Luc-MSCs. Myocardial tissue matrix metalloproteinase 2 (MMP2) (index of ischemic/oxidative stress) and CXCR4 receptor expression (index of homing signal) were measured in bioluminescence positive and negative myocardial areas one day post cell transfer. Biodistribution of the implanted cells was quantified by using Luciferase assay and confirmed by fluorescence immunochemistry. Global left ventricular ejection fraction (LVEF) was measured at baseline and one month post cell therapy using MRI.

RESULTS
AMF decreased immediately after intracoronary cell delivery, while no change in tissue perfusion was found in the IM group. Intracoronary delivery led to a significant increase in myocardial MMP2 expression and decreased expression of CXCR4. Fluorescence immunochemistry indicated a higher expression level of a variety of homing (tenascin and cadherin) and angiogenic factor (FGF-2 and VEGF) in the IM group. LVEF increase was also significantly higher in IM group at the 1-month follow up.
CONCLUSION
Intracoronary stem cell delivery decreased AMF, increased myocardial expression of MMP2, and lead to reduced CXCR4 expression with enhanced biodistribution and diminished functional recovery post-infarction

P11 CHARACTERIZE THE FUNCTION OF MITOCHONDRIAL ACTIVITY IN LUNG CANCER CHEMOTHERAPY RESISTANCE

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INTRODUCTION

Lung cancer is the most common cause of cancer-related mortality worldwide. This is mainly due to the difficulty of early detection and lack of effective treatment methods, thus more effective treatment options are desperately needed. More than 80% of lung tumors are non-small-cell lung cancers (NSCLC). It was postulated that tumor initiation and propagation are mediated by so called tumor-initiating cells (TICs), which can self-renew, spawn differentiated progeny and are associated with chemotherapy resistance. In NSCLC, high mitochondrial activity correlates with sphere formation capacity and increased tumor growth, which is in agreement with the findings in breast and pancreatic cancer.
A549 is one of, if not, the most frequently studied human NSCLC cell line with more than 17'000 citations up to date. Our previous study revealed that, the parental A549 cell line consist of three phenotypically distinct subpopulations. Holoclone cells are characterized by an epithelial phenotype and an increased tumor initiation capacity. Paraclone cells are characterized by a mesenchymal phenotype and increased chemotherapy resistance whereas meroclone cells are characterized by an intermediate phenotype.

AIM

The aim of this study is to further characterize the role of the mitochondrial activity in lung cancer chemotherapy resistance.

METHODS

The cell line A549 cells were stained with MitoTracker, metabolically fractionated into mito-high (5%) and mito-low (5%) subpopulations according to the mitochondrial activity. Sorted subpopulations were further examined by *in vitro* colony formation assay. Pemetrexed resistance was assessed by colony formation assay. Mitochondrial activity of A549 after pemetrexed treatment was analyzed by muti-color flow cytometry.

RESULTS

Colony formation capacity was not dependent on mitochondrial activity. However, pemetrexed resistance correlated with mitochondrial activity. Interestingly, pemetrexed treatment increased the average mitochondrial activity over time. In detail, treatment with pemetrexed for 72 hours increased mitochondrial activity 5-fold compared to untreated control.

CONCLUSIONS

In summary, our results indicate that mitochondrial activity might affect chemotherapy resistance. Further experiments will be required to confirm these results. We speculate that inhibition of the mitochondrial activity might potentiate the efficiency of current lung cancer therapy.

P12 HEMOCOMPATIBILITY OF STROMAL CELLS DEPENDS ON EXPRESSION OF TISSUE FACTOR AND IS INFLUENCED BY ORGAN ORIGIN AND CULTURE CONDITIONS

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INTRODUCTION
Mesenchymal stromal cells (MSC) are promising candidates for regenerative medicine. Optimal cell source and expansion protocols using fetal bovine serum (FBS) or human platelet lysate (HPL) are still under debate. After systemic application long-term engraftment is low or absent in recipients and it was recently shown that MSC hemocompatibility depends on intrinsic coagulation-activating properties. An instant blood-mediated inflammatory reaction (IBMIR) can activate complement and coagulation cascades resulting in hyper-acute cell clearance. IBMIR has been observed in various cell therapy settings and was related to cellular tissue factor (TF) expression.

AIM
We hypothesized that TF expression may be a surrogate safety marker prior to clinical application and compared TF surface expression and hemocompatibility of human MSC derived from bone marrow (BM), white adipose tissue (WAT) and umbilical cord (UC).

METHODS
MSC were cultured in media with HPL or FBS. Proliferation, clonogenicity, trilineage differentiation capacity and characteristic surface markers were analyzed. TF expression levels were tested by qRT-PCR, flow cytometry (FC) and immunocytochemistry. Pro-coagulant activity of MSC was analyzed by rotational thromboelastometry (ROTEM). Clotting time (CT) and maximum clot firmness (MCF) of MSC from different sources were tested. The impact of TF was confirmed by using factor VII-deficient plasma and by sort-depleting TF/CD142$^+$ MSC.

RESULTS
MSC proliferation was significantly increased in HPL- compared to FBS-media, trilineage potential was maintained. FC revealed CD14$^-$/19$^-$/34$^-$/45$^-$/MHCII$^-$ and CD73$^+$/90$^+$/105$^+$ phenotype whereas TF expression differed depending on cell source and culture conditions. UC-MSC in HPL showed the highest expression of TF (median 99%; range 87-99), compared to UC-MSC in FBS (59%; 31-78). In contrast, TF expression of WAT-MSC in FBS was higher (80%; 71-97) than in HPL (56%; 47-72). Significantly lower TF expression was only observed in BM-MSC independent of media supplements (<7%). These distinct expression levels were confirmed by immunocytochemistry and qRT-PCR.
In ROTEM increasing cell amounts of BM-MSC and UC-MSC shortened the CT significantly. UC-MSC in HPL showed the shortest CT (58s, 51-76) followed by WAT-MSC in HPL (105s, 74-171). In FBS, WAT-MSC (83s, 53-113) displayed more pro-coagulant activity than UC-MSC (91s, 67-126). BM-MSC activated coagulation just weakly (HPL 412s, 247-492; FBS 345s, 159-489). All MSC showed an elongated CT in factor VII deficient plasma, independent of

source and culture conditions. After sort-purification the TF-deficient BM-MSC lacked pro-coagulant activity in ROTEM showing no difference in the CT compared to cell-free plasma.

CONCLUSIONS
We detected high variations of *in vitro* hemocompatibility of MSC of different sources and culture conditions. BM-MSC had the lowest TF expression and the weakest pro-coagulant activity presumably favoring BM-MSC for intravenous therapy. Due to our results, we recommend TF expression analysis for surrogate safety testing. New strategies for selecting TF-deficient BM-MSCs or engineering additional TF-deficient stromal cells can contribute to improving cell therapy applicability at reduced IBMIR risk.

P13 LASER INCISIONS PROMOTE THE REPOPULATION OF DECELLULARIZED ARTICULAR CARTILAGE

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INTRODUCTION
Although decellularized allogenic cartilage is considered to be a promising biomaterial for the treatment of large chondral defects, poor cell infiltration due to the exceptionally dense matrix of articular cartilage remains an issue.

AIM
In our approach we used lasers to create incisions into the scaffold in order to enhance its surface area and promote cell migration into deeper tissue regions.

METHODS
Grid patterns were engraved into human articular cartilage biopsies using either a CO_2 or a femtosecond laser. Scaffolds were then either devitalized or treated with a protocol leading to full decellularization and glycosaminoglycan depletion (“decell-deGAG”) and seeded with adipose-derived stromal cells (ASC), chondrocytes or a co-culture of both to evaluate their effect on cell adhesion, chondrogenic differentiation and *in vivo* performance in a mouse model.

RESULTS
Both lasers were found to be a suitable method for engraving articular cartilage in a reproducible, precise and fast way. In scaffolds of either pretreatment cells and newly produced matrix filled up the incisions, yet the decell-deGAG protocol was shown to be highly beneficial for cell adhesion. Newly synthesized collagen type II fibers were well connected to the scaffold and showed a parallel alignment resembling the structure in native cartilage. *In vivo* implanted scaffolds were well integrated in all cases and chondrocytes as well as cells in co-culture deposited cartilage-like matrix.

CONCLUSIONS
In our study we demonstrated that laser engraved cartilage allografts can successfully be repopulated with cells to achieve a well-integrated tissue. The homologous matrix architecture

and mechanical stability allow earlier joint loading for patients, thus contributing to improved regeneration.

P14 TUMOR-ASSOCIATED CD90+ MESENCHYMAL PRECURSOR CELLS ARE PART OF AN IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT IN EARLY-STAGE NON-SMALL CELL LUNG CANCER

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INTRODUCTION AND AIM

Blockade of immune-checkpoint PD-1/PD-L1 pathway using antibodies has shown durable antitumor responses in patients with advanced non-small cell lung cancer (NSCLC). Despite this initial success, immune checkpoint blockade remains ineffective in the majority of NSCLC patients with documented PD-L1 expression.

METHODS

To analyze the functional orientation of tumor infiltrating lymphocytes (TILs) using a multiparametric flow cytometric approach. In parallel, we interrogated the tumor mesenchymal compartment within the tumor microenvironment in early-stage NSCLC specimens and matched nonadjacent normal lung tissue.

RESULTS

$CD4^+$ and $CD8^+$ TILs with increased PD-1 expression showed a lack of degranulation while also downregulating CD127 expression in bothpulmonary adenocarcinoma (AC) and squamous cell carcinoma (SQCC) patients. The majority of CD4 and CD8 PD-1^{hi} TILs were found in the central memory and effector memory compartment with increased TIM3 expression, with diminished or absent CD127 expression. We found coexpression of PD-L1 and CD47 in tumor-associated $CD90^+$ mesenchymal precursor cells, despite elevated PD-L1 and CD47 also being coexpressed in the tumor epithelial fraction. Using machine learning we were able to identify a combination of features that were predictive of a smoking signature, tumor recurrence and survival. Last, TNFα/IFN-γ immune primed tumor-associated $CD90^+$ mesenchymal precursor cells display an immunosuppressive secretory phenotype and target T cells for immunosuppression, irrespective of PD-L1 expression.

CONCLUSION

Our observations suggest that an immune reactive tumor microenvironment may fine tune and enhance the immunosuppressive signaling in constituent mesenchymal precursor cells in early-stage NSCLC. Therefore, a multi-targeted approach may be necessary to overcome the immunological barrier in early-stage NSCLC.

P15 INCREASED SENSITIVITY TO APOPTOSIS UPON ER STRESS-INDUCED UPR IN CHEMOTHERAPY-RESISTANT MALIGNANT PLEURAL MESOTHELIOMA

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INTRODUCTION
Malignant pleural mesothelioma (MPM) is a rare but aggressive cancer, notoriously known for lack of therapeutic options and for extremely poor prognosis. The present standard of care for patients with advanced MPM is the dual chemotherapeutic regimen that combines cisplatin and pemetrexed (MTA), which, however, is frequently confronted by intrinsic and/or acquired drug resistance, leading inevitably to tumor relapse.

AIM
To identify molecular mechanisms of chemoresistance and to uncover novel therapeutic strategies to treat patients with relapsed, chemotherapy-resistant MPM.

METHODS
In vitro three-dimensional (3D) cultivation of MPM cell lines and *ex vivo* organotypic culture of patient-derived xenograft (PDX) tumors were used to enrich chemotherapy-resistant MPM cells and to mimic *in vivo* MPM environment, respectively. Quantitative real-time polymerase chain reaction (qRT-PCR), Western blots, immunohistochemistry (IHC) and flow cytometry (FACS) were used to probe endoplasmic reticulum (ER) stress, the unfolded protein response (UPR) and apoptotic cell death. Cell viability was determined by a colorimetric approach based on the activity of acid phosphatase (APH assay). Clonogenic assay was used to assess growth inhibition elicited by chemotherapeutics and other pharmacological agents that induce ER stress.

RESULTS
MPM cells resistant to standard chemotherapy (cisplatin plus MTA) displayed deregulated UPR, which renders the cells hypersensitive to agents that induce ER stress. Bortezomib, a clinically approved drug that targets proteasome activity, preferentially impaired chemotherapy-resistant MPM cells. Mechanistically, hyperactivation of the PERK/ATF4/CHOP pathway is important for bortezomib-induced UPR and apoptosis, as genetic perturbation of the pathway by CHOP depletion attenuated the effectiveness of bortezomib. These results reveal that deregulated UPR confers chemotherapy resistance in MPM and preferentially sensitizes chemotherapy-resistant MPM cells to ER stress. Thus, perturbation of the UPR pathway by altering ER stress constitutes a promising strategy to treat patients with chemotherapy-refractory or relapsed MPM.

CONCLUSION
Our study provides a mechanistic link between UPR signaling and chemoresistance in MPM, and shows for the first time that altering ER stress might be a rationale to treat patients with relapsed chemoresistant MPM.

P16 INHIBITION OF HSP90 OVERCOMES ACQUIRED CHEMOTHERAPEUTIC RESISTANCE IN *KRAS*-MUTNAT LUNG CANCER BY ABROGATING EIF4E-MYC SIGNALING AXIS

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INTRODUCTION
KRAS mutation is one of the most common oncogenic drivers in non-small cell lung cancer (NSCLC). Currently, the standard therapy for *KRAS*-mutant NSCLCs remains cytotoxic chemotherapy due to lack of effective targeted therapies. Patients experience recurrence and ultimately death because of chemotherapeutic resistance, which is a significant clinical challenge. Therefore, strategies to overcome chemotherapeutic resistance are urgently needed.

AIM
To identify the molecular mechanism driving chemoresistance in *KRAS*-mutant lung cancer and develop novel therapeutic strategies to treat *KRAS*-mutant lung cancer.

METHODS
KRAS-mutant NSCLC cell lines were chronically exposed to clinically relevant chemotherapeutic agents to generate chemotherapy resistant cells. Pharmacological screens were then conducted to identify the pathway whose inhibition specifically reverts chemotherapy resistance in *KRAS*-mutant NSCLC cells.

RESULTS
Heat shock protein 90 (HSP90) outstood as one of the top hits in our screen. Further analyses showed that Hsp90 regulates a key survival pathway through eukaryotic translation initiation factor 4E (eIF4E)-Myc axis, which is essential for chemotherapy resistance in *KRAS*-mutant NSCLC cells.

CONCLUSIONS
Our studies identified a novel signaling axis involving eIF4E-Myc that confers acquired chemotherapy resistance, and validates a rational strategy by targeting Hsp90 in reverting chemotherapy resistance in *KRAS*-mutant lung cancer.