# 7th
# SCRM PhD Students Retreat

## Gurten Park
## 4 September 2020

SCRM
Stem Cell Research
Regenerative Medicine
Bern
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| | | |
|---|---|---|
| 09:10 | 09:25 | Welcome coffee |
| 09:25 | 09:30 | Welcome by the Organizing Committee |
| **Morning Session Chairs: Cristina/Viviana** | | |
| **Morning Session Mentor: PD Dr. Amiq Gazdhar** | | |
| 09:30 | 09:50 | Fatemeh Safari |
| 09:50 | 10:10 | Zhang Yang |
| 10:10 | 10:30 | Viviana Rubino |
| 10:30 | 10:50 | Chantal Bachmann |
| 10:50 | 11:20 | **Coffee Break** |
| 11:20 | 11:40 | Patricia Renz |
| 11:40 | 12:00 | Stefan Forster |
| 12:00 | 13:00 | Mentor Talk: Dr. Roland Leathers |
| 13:00 | 14:00 | **Lunch Break** |
| **Afternoon Session Chairs: João/Chantal** | | |
| **Afternoon Session Mentor: Prof. Deborah Keogh-Stroka** | | |
| 14:00 | 14:20 | Laura Jahnke |
| 14:20 | 14:40 | Franziska Silvia Strunz |
| 14:40 | 15:00 | Haibin Deng |
| 15:00 | 15:30 | **Coffee Break** |
| 15:30 | 15:50 | Xingshuo Zhang |
| 15:50 | 16:10 | Cristina Kalbermatter |
| 16:10 | 17:10 | Mentor Talk: Prof. Catherine Verfaillie |
| 17:10 | 17:20 | **Conclusive Remarks and Thanks from the SCRM Steering Committee** |
| 17:30 | | **Apéro** |



This event was made possible with the generous support of:

SCRM
Stem Cell Research Regenerative Medicine Bern
www.stemcellsbern.ch

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Graduate School for Cellular and Biomedical Sciences

We want to thank you for your support, which made this event possible for the seventh time already.

*4th Retreat, Gurten Park, 1st September 2017*

*5th Retreat, Paul Klee Center, 31th August 2018*

*6th Retreat, Gurten Park, 30th August 2019*



Dear participants,

Welcome to the 7th SCRM PhD Students Retreat!

We are happy to continue with you this successful history of PhD students retreats, which started in 2014, initiated by our colleague Dr. Luca Tamò.

The day starts with a coffee and a short welcome from the organizing committee. The program will continue with a morning and an afternoon session of PhD project presentations and two coffee breaks for networking and discussion.

We are also excited to attend two interesting keynote lectures, which will be given by this year's mentors **Dr. Roland Leathers** from ThermoFisher and **Prof. Catherine Verfaillie** from the University of Leuven, Belgium. We are very grateful to them for being our mentors for a day.

The retreat will be concluded with brief remarks and thanks by the SCRM Steering Committee, followed by the Apéro.

We are looking forward to meeting you and we wish you a fruitful and pleasant time during the retreat.

Sincerely yours,
The organizing committee

Viviana Rubino
Chantal Bachmann
Cristina Kalbermatter
João Marques

In order to prevent any risk of SARS-Cov2 infections spreading, we will keep official safety measures in place during the whole day.

Social distancing will be maintained as a limited number of people are participating at the retreat.

In agreement with the GCB, hand disinfectant and masks will be provided to be used at your own discretion.

We thank you all for your understanding and compliance with the rules.

- Prof. Catherine Verfaillie, KU Leuven
- Dr. Roland Leathers, Thermo Fisher Scientific
- PD Dr. Amiq Gazdhar, DBMR, Pulmonary Medicine
- Prof. Dr. Deborah Stroka, DBMR, Visceral Surgery

## Guests

The SCRM Steering Committee:

- Prof. Eliane Müller
- Prof. Volker Enzmann
- Prof. Daniel Surbek
- Prof. Benjamin Gantenbein
- Dr. Amiq Gazdhar
- PD Dr. Carsten Riether
- Prof. em. Thomas Krause
- PD Dr. Marianna Kruithof-de Julio
- Prof. Dr. Deborah Keogh-Stroka

- Principal Investigators of the PhD students
- Dr. Felix Baier
- Rene Aeberhard



### Fatemeh Safari

**Noggin as a regulator of bone remodelling Bone Morphogenetic Protein 2 (BMP2) is used in orthopaedic surgery to promote bone healing**

The endogenous synthesis of BMP-2 antagonist family members, however, may limit the efficacy of exogenous BMP2. Noggin is one of these inhibitors that blocks the effects of BMP on the differentiation and activation of osteoblast (OB) in vitro and in vivo and inhibits OB-mediated osteoclast (OC) development. Furthermore, Noggin was found to modulate osteoclastogenesis through a direct effect on OC lineage cells. The present study aimed at elucidating the underlying mechanisms of these effects. Direct (conventional culture dishes) and indirect (transwell culture dishes) co-cultures of murine OB/OPC (Osteoclast Progenitor Cells) and cultures of OPC alone were supplemented with combinations of Noggin, BMP2, L51P (engineered, inactive variant of BMP2) and DMH1 (BMP receptor 1 inhibitor). In cultures of OPC, Noggin but not DMH1 caused an increase in the number of OC by a factor of 3 ($p<0.01$). This e ffect could not be reversed by BMP2 and L51P, respectively. In contrast, in co-cultures of OB/OPC, exposure to Noggin attenuated OC development. In direct co-cultures, this inhibitory effect of Noggin was blocked by BMP2 and L51P. In both direct and indirect co-culture systems, exposure to Noggin induced the release of GM-CSF, a potent inhibitor of osteoclastogenesis, by a factor of 6 and 4, respectively ($p<0.01$). Treatment of the cultures with Î±GM-CSF Ab, however, restored OC development in the indirect co-culture system only. The data suggests a previously unknown function of Noggin directly acting pro-differentiation on OC lineage cells independently of BMP signalling. In co-cultures, besides GM-CSF, cell-cell contact between OB and OPC is required for mediation of the maximal inhibitory effects of Noggin on OC development. The nature of potential interaction partners for Noggin, however, remains to be elucidated.

## Zhang Yang

**A Kinome CRISPR screen in FGFR-amplified lung cancer**

Background: Oncogenic alterations in fibroblast growth factor receptor (FGFR) are frequent, but therapeutic targeting of FGFR-driven cancers remains an unmet challenge in clinical oncology in that FGFR inhibitors as a single agent are inevitably limited by poor responsiveness and/or compensatory activation of various acquired resistance mechanisms.

Methods: We performed kinome CRISPR/Cas9 screens to identify genetic determinants that limit efficacy of FGFR-targeted therapy in FGFR-mutant squamous lung carcinoma (SQLC).

Results: We identify several kinases including polo-like kinase I (PLK1) as key determinants of sensitivity to AZD4547, a selective FGFR inhibitor currently investigated in phase III clinical trials. We provided evidence showing that genetic and pharmacological inhibition of PLK1 synergistically enhances the effect of FGFR inhibitors in FGFR-amplified lung SQLC.

Conclusions: CRISPR/Cas9 screening is a powerful tool to uncover genetic determinants underlying drug sensitivity in cancer.



- Ya Lu, PhD student
- Adrienne Vancura, PhD student
- Liang Zhao, PhD student
- Elisa Rodrigues, PhD student
- Martina Minoli, PhD student
- Andreas Croft, PhD student
- Vedat Burak Ozan, PhD student
- Chaonan Jin, PhD student

**Meng Tian**

**Treatment of Macular Degeneration Using Embryonic Stem Cell-Derived Retinal Pigment**

Epithelium Embryonic stem cells hold great promise for various diseases because of their unlimited capacity for self-renewal and ability to differentiate into any cell type in the body. However, despite over 3 decades of research, there have been no reports on the safety and potential efficacy of pluripotent stem cell progeny. Here, we report the safety and tolerability of subretinal transplantation of human embryonic-stem-cell (hESC)-derived retinal pigment epithelium. They were followed for 1 year. There was no evidence of adverse proliferation, tumorigenicity, ectopic tissue formation, or other serious safety issues related to the transplanted cells. Visual acuity improved 9â€“19 letters in three patients and remained stable (+1 letter) in one patient. The results confirmed that hESC-derived cells could serve as a potentially safe new source for regenerative medicine.

## Haibin Deng

**Lactate utilization is a metabolic dependency of tumor-initiating cells in non-small cell lung cancer**

**Background**

Lung cancer is the most common cause of cancer-related mortality worldwide. It was postulated that tumor initiation and propagation are mediated by so-called cancer stem cells (CSCs) or tumor-initiating cells(TICs). In various solid tumors, it has been shown that CSCs are highly dependent on oxidative phosphorylation as indicated by a high mitochondrial activity whereas bulk tumor cells are dependent on glycolysis. Recent studies revealed that lactate is the dominant carbon source for the TCA cycle providing substrate and electrons to oxidative phosphorylation. Lactate utilization is mainly mediated by Lactate dehydrogenases (LDHB). Despite decades of cancer research in lactate metabolism, it is still unknown if lactate utilization is essential for tumor initiation. Our goal of this project is to discover whether lactate utilization contributes to tumor initiation and if so, study the underlying molecular mechanisms.

**Methods**

1. Three-dimensional (3D) sphere formation of A549, H460, H358 cell lines, and primary tumor cells will be implemented as an in vitro surrogate assay to quantify the tumor initiation capacity.
2. In vivo, xenograft mouse models will be applied to investigate the tumor initiation.
2. Western blotting and flow cytometry (FACS) will be used to study the underlying molecular mechanisms after modulating lactate metabolism.
3. siRNA and shRNA knockdown are used to modulating lactate metabolism.
4. Mass spectrometry and isotope tracking are implemented to identify metabolic characteristics of TICs before and after modulating lactate metabolism.
5. A genetically engineered mouse model will be established to investigate how lactate metabolism affects orthotopic tumorigenesis of lung cancer.

**Preliminary results**

1. Transient LDHB knockdown dramatically inhibited the sphere formation capacity and proliferation in vitro.
2. LDHB knockdown diminishes the tumor-initiating cells in A549, H460, H358 cell lines.
3. LDHB knockdown induced DNA damage and S phase arrest.
4. Long-term LDHB knockdown with shRNA inhibited sphere formation capacity but not proliferation.



## Viviana Rubino

**IL-21 secreted by CD4 T cells reduces leukemia stem cell function in human and murine acute myeloid leukemia**

Leukemia stem cells (LSCs) are resistant to standard treatment and to elimination by the immune system, representing the main reason for disease relapse. The IL-21 receptor (IL-21R) is widely expressed by hematopoietic cell populations and can modulate their function, upon binding of interleukin 21 (IL-21). The role of IL-21/IL-21R signalling in leukemia is unknown.

In this study, we show that IL-21R is heterogeneously expressed on leukemic stem/progenitor cells (LSPCs) of newly diagnosed acute myeloid leukemia (AML) patients and that IL-21 is produced by CD4 T cells. IL-21 is significantly increased in serum of AML patients compared to healthy controls and acts as a positive prognostic marker for overall survival. Functionally, IL-21 significantly reduced colony-forming capacity of primary LSPCs ex vivo, while not affecting hematopoietic stem/progenitor cells from healthy bone marrow (BM) donors.
Thus, we hypothesized that IL-21/IL-21R signalling restricts the functionality of AML LSCs. We used syngeneic murine AML models to study how this signalling regulates LSCs function in vivo. IL-21R -proficient and -deficient lineage- Sca-1+ c-kit+ cells were transduced with three different AML oncogenes followed by transplantation into immuno-competent recipient mice. IL-21R deficiency on LSCs resulted in increased LSCs number in the BM of AML mice, leading to faster disease progression and reduced survival in all models. Similarly, treatment of human AML LSCs with recombinant IL-21 reduced their frequency in patient-derived xenografts.

In summary, IL-21 secreted by CD4 T cells regulates LSCs function and contributes to leukemia control. Further investigations will focus on the underlying cellular and molecular mechanisms

## Chantal Bachmann

**Immune-Checkpoints in the Regulation of Leukemia and Cancer Stem Cells**

Chronic myeloid leukemia (CML) shows many characteristics also present in its healthy counterpart, normal hematopoiesis: a rare (cancer) stem cell on top of the hierarchy, which divides only infrequently, shows self-renewal and can give rise to the different lineages present in the healthy tissue or, in case of CML, in the cancer. As chemotherapy mainly targets bulk cancer cells but spares the more resistant cancer stem cells (CSCs), CSC-targeting therapies are needed. Immune-Checkpoints like PD1, LAG3 or TIM3 are receptors regulating T-cell activation, but it has been shown that they can also be expressed on subsets of cancer cells in different types of cancer. For example, TIM3 is upregulated on AML CSCs where it drives their self-renewal via an autocrine loop with its ligand. To screen for immune checkpoint receptors and ligands of importance for CML and CML CSCs, we will set up a pooled small-scale in vivo CRISPR-KO screening. We are generating a small pooled library of single-guide RNAs targeting 25 different genes of interest, and clone it into a lentiviral vector to transduce isolated Cas9-expressing CML stem cells. The mixed cell population will then be used to generate a secondary CML in non-irradiated recipients. Positive and negative effects of KO can be assessed by measurement of the abundance of the respective sgRNAs by NGS. Effects of KO can also be analysed in vitro by colony formation. Once this system is established it will be a versatile tool to study different cancer models and genes.



## Laura Jahnke

**Cross-species comparison of multicellular response during retinal gliosis**

The main goal of the project is to characterize the timeline of the gliotic/fibrotic response of Müller cells and how those glial cells are involved in the extracellular matrix (ECM) remodeling during an hypothized “transient” gliosis in zebrafish, able to regenerate the retina, and a previously observed chronic gliosis in mouse. The main components of scar are collagen fibers, which lead to a pathological proliferation of connective tissue trough all retina areas. Therefore, the focus of the project will be on fibrinolysis -like processes as the transient glial scar dissolution in the zebrafish shows similar characteristics.

## Darya Karatkevich

**Schedule-dependent treatment increases chemotherapy efficacy in malignant pleural mesothelioma**

Introduction Malignant pleural mesothelioma (MPM) is a type of neoplasm of the mesothelium that lines the pleural cavity. Mesotheliomas can arise from the mesothelium that lines the chest wall and abdomen, MPMs account for 80% of all mesotheliomas.
MPMs are rare and aggressive, they are characterised by a poor prognosis, with a median survival time of only 9-12 months after first presentation. The aetiology of MPM is heavily linked with exposure to carcinogenic mineral fibres, most importantly asbestos. Three histological subtypes of MPM can be distinguished: sarcomatoid, biphasic and epithelioid. The latter is associated with a better prognosis.
Treatment options for MPM are currently very limited. Surgery is mostly ineffective leading to high recurrence rates. This is mostly due to advanced disease at diagnosis in the majority of patients. Radiation therapy (RT) is limited by the requirement to treat large volumes of tissue while sparing the radiation sensitive organs that surround the pleura (heart, lung, esophagus and spinal cord).
The current standard for first-line systemic therapy in MPM patients is a combination of pemetrexed and cisplatin.
Pemetrexed is chemically similar to folic acid and is in the class of chemotherapy drugs called folate antimetabolites. It works by inhibiting three enzymes used in purine and pyrimidine synthesisâ€"thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase. By inhibiting the formation of precursor purine and pyrimidine nucleotides, pemetrexed prevents the formation of DNA and RNA, which are required for the growth and survival of both normal cells and cancer cells.
Cisplatin belongs to the anticancer class of alkylating agents and is used in a wide variety of human cancers including lung cancer, ovarian cancer, breast cancer and brain cancer. Cisplatin makes intra- and interstrand breaks in DNA. This lessions induce permanent proliferative arrest (senescence) and apoptosis.
Treatment with both pemetrexed and cisplatin results in higher response rates, time to progression and overall survival time compared to monotherapy with cisplatin. Since the toxicity profiles of cisplatin (gastrointestinal, neurological, renal) and pemetrexed (mucositis, neutropenia, leukopenia) do not overlap, their use in combination is beneficial. The toxicity of pemetrexed and cisplatin combination chemotherapy can be reduced by the supplementation with vitamin B12 and folic acid, without reducing the overall survival time.
However, currently there are no second-line chemotherapy options for MPM patients. Previous studies in several cancer types have demonstrated that both pemetrexed and cisplatin show schedule-dependent effects in combination with other drugs.



## Cristina Kalbermatter

**The role of maternal microbiota in durably shaping intestinal immunity and gene expression in the offspring through epigenetic mechanisms**

Postnatally acquired microbes shape host immune development during a critical window of opportunity. However, recent findings reveal that already microbial signals of the maternal microbiota during pregnancy and metabolites in the mother's milk remarkably shape neonatal immunity. Using an auxotrophic E. coli strain, it is possible to reversibly colonise pregnant dams during gestation and hence distinguish between the contribution of the maternal microbiota from the influence of postnatal colonisation. Since long-lasting effects on the offspring immune system were observed after gestational colonisation, we hypothesise that epigenetic alterations are the consequence of microbe-host interactions during pregnancy. We assessed differences in chromatin accessibility, DNA methylation and histone modifications in pups born to germ-free dams compared to pups born to gestation-only colonised mice. To attain these results we performed ATAC-Seq (Assay for Transposase-Accessible Chromatin-Seq) and whole-genome sequencing subsequent to bisulphite conversion or chromatin immunoprecipitation. In addition, numerous microbial metabolites originating from the maternal microbiota have been identified from the mother's milk. It is our goal to characterise their epigenetic potential using in vitro intestinal organoids and to then analyse the effects of those metabolites during gestation on the neonatal immune development in vivo. One metabolite which gained our interest was 4-hydroxybenzoic acid (4-HBA), enriched in the milk of dams treated with the auxotrophic E. coli HA107: small intestinal organoids from germ-free pups treated with 4-HBA in vitro showed changes in gene expression comparable to the changes observed in the neonatal intestine following gestational colonisation. In addition, administration of 4-HBA to pregnant dams could recapitulate several of these effects. Our data reveal essential insights into epigenetic mechanisms as a result of interactions between maternal microbiota and the neonate. It will increase the knowledge about the importance of maternal colonisation during pregnancy and lactation for neonatal health and display its durable consequences.

## Xingshuo Zhang

**Spheroid-like Cultures for Expanding Angiopoietin Receptor-1 (a.k.a Tie2) Positive Cells from the Human Intervertebral Disc**

Low back pain is a leading cause of disability worldwide. The recovery of nucleus pulposus (NP) progenitor cells (NPPCs) from the intervertebral disc (IVD) holds high promise for future cell therapy. NPPCs are positive for the angiopoietin-1 receptor (Tie2), and possess stemness capacity[1-3]. However, the limited Tie2+ NPC yield has been a challenge for their use in regenerative medicine[2]. In this study, we attempted to expand NPPCs from the whole NP cell population by spheroid-formation-assay. Flow cytometry was used to quantify the percentage of NPPCs with Tie2-antibody in human primary IVD cells. Cell proliferation was accessed by population doublings level (PDL). Presence of extracellular matrix (ECM) of NP cell (NPC) spheroids was confirmed by qPCR and immunostaining. Compared with monolayer, spheroid-formation assay enriched the Tie2+ population of NPCs from ~5% to ~25%. Spheroid-formation assay also inhibited the Tie2- NPCs from proliferation with nearly no prolif eration. After one additional passage of spheroid-formation assay, NPC spheroids increased the Tie2+ NPC population even further from about 25% to 40%. The detection of ECM major components, i.e., aggrecan and collagen type 2, showed the potency of defining NPC spheroids as organoids. Our spheroid culture system could be successfully applied to culture and expand tissue-specific progenitors in the context of cell therapy for IVD regeneration.

1. Sakai, D. et al 2012
2. Tekari, A. et al 2016,
3. Frauchiger, D.A. et al 2019



## Patricia Renz

**Neuroprotection in preterm birth by modification of astrocyte polarization**

**Introduction**
White matter injury (WMI) is the most common form of brain injury in preterm infants. WMI is characterized by reactive microgliosis and astrocytosis, delayed oligodendrocyte differentiation, and in severe cases, neuronal death. Two different types of reactive astrocytes are recognized in brain injury, A1 astrocytes, which promote neurodegeneration and A2 astrocytes, which are neuroprotective. Given recent findings that A1 formation is induced by activated microglia and that these astrocytes delay oligodendrocyte differentiation and promote neuronal death, we hypothesize that A1 play a central role in WMI and may be an exciting therapeutic target for this disease.

**Materials and Methods**
Several rat models of WMI involving prenatal and postnatal inflammatory and hypoxic-ischemic insults were tested. In situ hybridization (ISH) with probes for A1-specific mRNA transcripts was performed on brain tissue from injured and control neonatal rat brains at multiple post-injury timepoints. Immunohistochemistry (IHC) was performed on injured and control postnatal day 11 brains. Immunopanning was used to purify astrocytes from rat brains for an in vitro model to investigate therapeutic treatments.

**Results**
ISH experiments demonstrate a significant increase in the prevalence of A1 astrocytes in subcortical white matter tracts after WMI in all of the rodent models studied. IHC showed the severity of the WMI. An immunopanning protocol optimized for our disease model yields acutely purified viable primary astrocytes, which so far were isolated from control brains.

**Conclusion**
We demonstrate the formation of A1 astrocytes across multiple rodent models of WMI and make steps towards understanding astrocyte polarity in the neonatal brain over the course of the disease. This result opens the door to experiments investigating whether prevention of A1 formation ameliorates WMI disease outcomes.

## Stefan Forster

**The functional role of CD70/CD27/TNIK signaling in multiple myeloma**

Although multiple myeloma (MM) is generally considered incurable, an improved understanding of its genetic architecture has recently led to improved treatments, and long-term remissions are achieved in a fraction of patients. However, around 20% of MM patients do not benefit from new therapy approaches and show a dismal prognosis with overall survival (OS) rates of less than two years after initial diagnosis. This group of MM patients is often categorized as high-risk. Thereby, risk stratification is performed by a combination of clinical staging systems, cytogenetic aberrations and tumor biology markers (i.e. enhanced cell proliferation, extramedullary survival) helping to identify high-aggressive disease states that correlate with worse prognosis. However, to date there is no single pathogenic mechanism that can explain high-risk MM sufficiently. Previous work from the Ochsenbein laboratory indicated that the TRAF2-and NCK interacting kinase (TNIK) is involved in the CD70/CD27 sign aling pathway in leukemic stem cells of chronic and acute myeloid leukemia. However, whether CD70/CD27/TNIK signaling plays a role in MM progression and disease outcome has not been addressed so far. In first preliminary data, we show that CD70/CD27/TNIK is co-expressed in a subset of MM patients and high CD70/TNIK levels correlate with worse overall survival. Moreover, small hairpin (sh) RNA induced TNIK knockdown in MM cell lines leads to reduced cell proliferation in-vitro and delays disease progression in-vivo. In summary, the proposed project will help to understand the functional role of CD70/CD27/TNIK signaling in MM and enlighten its potential as a target for novel treatment approaches.



## Franziska Silvia Strunz

**Repair of a critical size defect in osteoporotic mice**

Osteoporosis is a major health issue for our aging society. Mostly women suffer from reduced bone stability due to menopausal changes in hormone levels. Bisphosphonates (BP) are a common treatment to prevent osteoporotic bone loss. Uptake of BP by osteoclasts leads to a block of cellular activity and bone resorption. Consequentially, BP therapy effectively reduces fracture risk in osteoporosis. But still, patients under BP medication suffer from bone fractures and large defects due to trauma or tumor resection. Currently, critical-size defects are filled with natural or synthetic bone grafts, often in combination with the osteoinductive bone morphogenetic protein-2 (BMP-2) to improve bone healing. It is still debated, whether prolonged therapy with BP interferes with biomaterial turnover during repair of defects fitted with β-tricalcium phosphate (βTCP) ceramics. To investigate the effect of BP on bone healing and turnover of biomaterials, a murine model for post-menopausal osteopo
rosis was used to study the healing process of a critical-size defect. Eight weeks after induction of osteoporosis by ovariectomy, and after detection of bone loss, treatment with alendronate (ALN), a commonly used BP, commenced. Five weeks later, a critical-size defect was applied in the left femur, filled with βTCP cylinders that were coated with BMP-2 and L51P. L51P has high binding affinity to BMP2 antagonists, blocking their activity and thereby potentially reducing the required amounts of BMP-2 stimulating bone formation and repair. The implantation site was rigidly fixed, using an osteosynthesis system with an internal fixateur. Femora were collected six and twelve weeks post-surgery to assess implant turnover and bone healing processes by micro-computer tomography, histology and by transcriptome analysis. The performed pilot study demonstrated the suitability of our animal model to study bone repair and revealed sufficient induction of bone healing by high dosage of BMP-2 in comparison to the control group.