GSCN non-PI Meeting,

Heidelberg, 18 Sept. 2018, 8:30 – 18:00 h

Marsilius Kolleg, seminar room 2 (1. Floor)

Organizers: *Georgios Kalamakis* (Heidelberg), *Magdalena Laugsch* (Cologne), *Adele Marthaler* (Copenhagen), *Sina Stäble* (Heidelberg)

Tuesday, 18. September

8:30 – 09:00	**Welcome and set up**
09:00 – 10:30	**Session 1** (chaired by *Sina Stäble)*
09:00 – 09:15	N01 - Hematoma components orchestrate immunomodulatory effects of human bone marrow mesenchymal stromal cells *Drenka Trivanović*, Würzburg, Germany
09:15 – 09:30	N02 - Quiescence regulates the number of neural stem cells and their regenerative capacity in the aging brain *Georgios Kalamakis*, Heidelberg, Germany
09:30 – 09:45	N03 - PURA syndrome: elucidating the neurodevelopmental mechanism using patient specific PUR-alpha interactome data *Lena Molitor*, München, Germany
09:45 – 10:00	N04 - Evidence for neuronal plasticity in bioengineered neuronal organoids (BENOs) *Maria Patapia Zafeiriou*, Göttingen, Germany
10:00 – 10:15	N05 - Functional consequences of a novel mutation in CHMP2B, causing frontotemporal dementia linked to chromosome 3 (FTD3) in a German patient *Adele Marthaler*, Copenhagen, Denmark
10:15 – 11:00	Coffee break
11:00 – 12:30	**Session 2** (chaired by *Georgios Kalamakis)*
11:00 – 11:15	N06 - The detection and minimisation of genetically variant stem cells in human pluripotent stem cell cultures *Jason Halliwell*, Sheffield, UK
11:15 – 11:30	N07 - Steps towards standarized assessment and use of immunomodulatory equine MSC *Olivia Lee*, Guelph, Canada
11:30 – 11:45	N08 - Spatially controlled differentiation of human mesenchymal stem cells *Smita Patil*, Delhi, India

11:45 – 12:00	N09 - Guided epicardial cell transfer of induced cardiomyocyte progenitors (iCMP) by human cardiac ECM hydrogel coated biological scaffold upon infarction *Matthias Becker*, Berlin, Germany
12:00 – 12:15	N10 - Mechanism of bFGF-regulated pluripotency maintenance in human induced pluripotent stem cells *Fereshteh Haghighi*, Düsseldorf, Germany
12:15 – 12:30	N11 - Adipose-derived stem cells as vehicel for epothilon B *Annemarie Baaße*, Rostock, Germany
12:30 – 13:45	Lunch break
13:45 – 15:00	**Session 3** (chaired by *Magdalena Laugsch)*
13:45 – 14:00	N12 - Modeling Canavan's disease in vitro *Julia Fischer*, Bonn, Germany
14:00 – 14:15	N13 - A chemically defined differentiation protocol independent from dual SMAD inhibition generates cortical neurons from hiPSCs by avoiding endogenous propensity towards neural crest *Alexandra Kowalski*, Ludwigshafen, Germany
14:15 – 14:30	N14 - A web application for predicting cell-fate determinants in cell differentiation *Andras Hartmann*, Luxembourg
14:30 – 14:45	N15 - Deciphering the role of the stem cell niche in human acute myeloid leukemia - insights from mathematical modeling *Thomas Stiehl*, Heidelberg, Germany
14:45 – 15:00	N16 - Unidirectional and progressive DNA methylation changes define hematopoietic lineage commitment *Sina Stäble*, Heidelberg, Germany
15:00 – 15:30	Coffee break
15:30 – 16:45	**Session 4** (chaired by *Adele Marthaler)*
15:30 – 15:45	N17 - Cell-extrinsic and -intrinsic regulation of PU.1 in HSC lineage choice *Nouraiz Ahmed*, Basel, Switzerland
15:45 – 16:00	N18 - Human HSCs show cell polarity and division kinetic changes upon aging *Amanda Amoah*, Ulm, Germany
16:00 – 16:15	N19 - Identification of novel signaling molecules involved in neuronal differentiation of iPS cells from schizophrenic patients with CHRNA7 copy number variants *Mohsen Moslem*, Stockholm, Sweden

16:15 – 16:30 N20 - Development of novel CRISPR/Cas9-based gene therapeutic approaches for osteopetrosis

Anna Floriane Hennig, Berlin, Germany

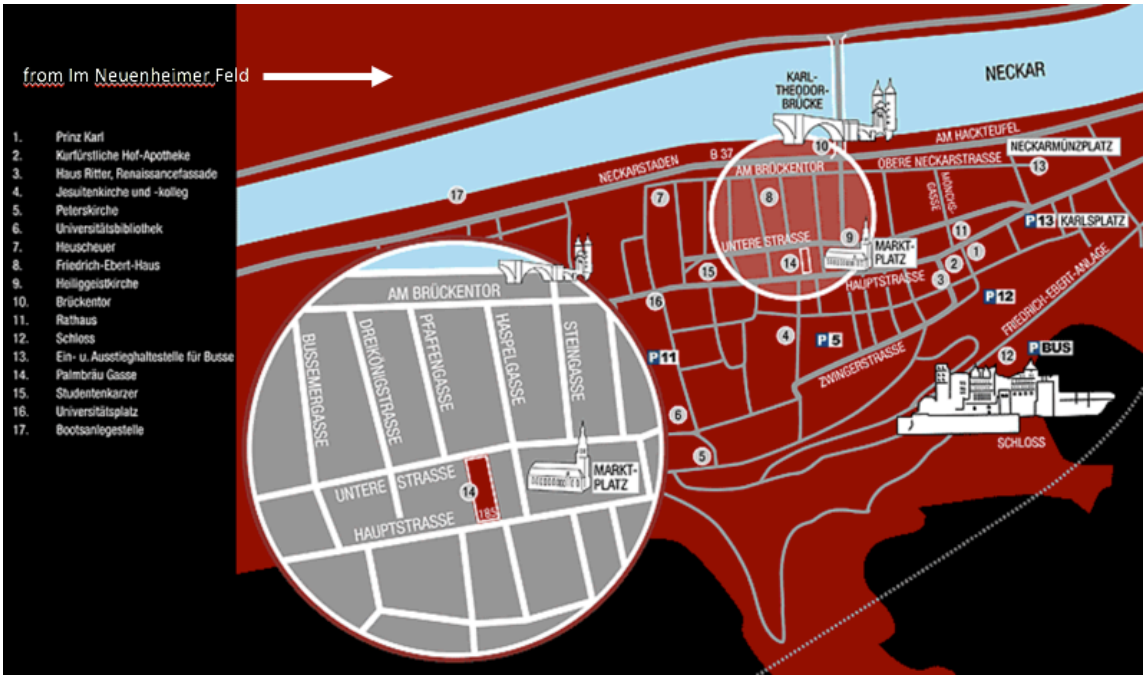
16:30 – 16:45 N21 - Hematopoietic progenitors selectively maintain the pSTAT5 self-renewal response to Interleukin-3 (IL-3) in low energy environments

Waseem Nasr, Leipzig, Germany

16:45 – 18:00 Poster session, one-on-one discussions, beer

19:00 – late **Dinner**

at Restaurant Palmbräugasse, Hauptstrasse 185, 69117 Heidelberg (https://palmbraeugasse.de/en/)



Non-PI meeting: N01 – N23

N01 Hematoma components orchestrate immunomodulatory effects of human bone marrow mesenchymal stromal cells
Drenka Trivanovic

N02 Quiescence regulates the number of neural stem cells and their regenerative capacity in the aging brain
Georgios Kalamakis

N03 PURA Syndrome: Elucidating the neurodevelopmental mechanism using patient specific PUR-alpha interactome data
Lena Molitor

N04 Evidence for neuronal plasticity in bioengineered neuronal organoids (BENOs)
Maria Patapia Zafeiriou

N05 Functional consequences of a novel mutation in CHMP2B, causing frontotemporal dementia linked to chromosome 3 (FTD3) in a German patient
Adele Marthaler

N06 The detection and minimisation of genetically variant stem cells in human pluripotent stem cell cultures
Jason Halliwell

N07 Steps towards standardized assessment and use of immunomodulatory equine MSC
Olivia Lee

N08 Spatially controlled differentiation of human mesenchymal stem cells
Smita Patil

N09 Guided epicardial cell transfer of induced cardiomyocyte progenitors (iCMP) by human cardiac ECM hydrogel coated biological scaffold upon infarction
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N10 Mechanism of bFGF-regulated pluripotency maintenance in human induced pluripotent stem cells
Fereshteh Haghighi

N11 Adipose-derived stem cells as vehicle for epothilon B
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N12 Modeling Canavan's disease in vitro
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N13 A chemically defined differentiation protocol independent from dual SMAD inhibition generates cortical neurons from hiPSCs by avoiding endogenous propensity towards neural crest
Alexandra Kowalski

N14 A web application for predicting cell-fate determinants in cell differentiation
Andras Hartmann

N15 Deciphering the role of the stem cell niche in human acute myeloid leukemia - insights from mathematical modeling
Thomas Stiehl

N16 Unidirectional and progressive DNA methylation changes define hematopoietic lineage commitment
Sina Stäble

N17 Cell-extrinsic and -intrinsic regulation of PU.1 in HSC lineage choice
Nouraiz Ahmed

N18 Human HSCs show cell polarity and division kinetic changes upon aging
Amanda Amoah

N19 Identification of novel signaling molecules involved in neuronal differentiation of iPS cells from schizophrenic patients with CHRNA7 copy number variants
Mohsen Moslem

N20 Development of novel CRISPR/Cas9-based gene therapeutic approaches for osteopetrosis
Anna Floriane Hennig

N21 Hematopoietic progenitors selectively maintain the pSTAT5 self-renewal response to Interleukin-3 (IL-3) in low energy environments
Waseem Nasr

Abstract No. N01

Hematoma components orchestrate immunomodulatory effects of human bone marrow mesenchymal stromal cells

Drenka Trivanovic[*] , *Theresa Kreuzahler*, and *Marietta Herrmann*

IZKF Group Tissue Regeneration in Musculoskeletal Diseases, University Clinics, Wuerzburg

*Presenting author

Formation of hematoma in the early phase of bone healing precedes the generation of a pro-regenerative microenvironment. To study immunomodulatory roles of bone marrow mesenchymal stromal cells (BM-MSC) in inflammatory hematoma, we cultivated BM-MSC in hematoma-simulating hydrogels containing platelet-rich plasma (PRP) or fibrin control gels.

BM-MSC isolated from patients undergoing hip endoprosthesis surgery (n=5) were embedded in 3D PRP and fibrin gels, where PRP was prepared from pooled apheresis-derived thrombocyte concentrates (n=4) containing 10-fold increased platelet concentration. BM-MSC gels were co-cultured with peripheral blood (PBMC, pool, n=5) or bone marrow mononuclear cells (BMC, n=4). After 3-day indirect co-culture, the lymphocyte phenotype was analyzed in polyclonal mitogen-activated PBMC, while the myeloid cell population was investigated by colony forming cell (CFC) assay. Cell marker expression was analyzed by multi-color flow cytometry. Values are given as mean ± standard error. Significance was analyzed by ANOVA.

We found that the frequency of cultured BM-MSC (cBM-MSC) co-expressing CD73+CD90+CD44+CD105+CD13+ (79.2±6.4) decreased after cultivation in PRP (56.9±6.8%) and at lesser extent in fibrin gel (63.0±8.3%). Cultivation of cBM-MSC in PRP gel increased their CD146 expression (from 1.7±0.7% to 10.1±3.4%; p=0.027) and CD146 MFI, while fibrin gel did not alter it. In addition, PRP supported the capacity of cBM-MSC to elevate the frequency of FOXP3+ cells in CD3+CD4+CD8-CD25+ (CD4+ Treg, from 14.3 to 22.5±0.5%; p=0.003) and sustained induction of FOXP3 in CD3+CD4-CD8+CD25+ PBMC (CD8+ Treg, from 8.2±0.1 to 18.2±2.2; p=0.009). Incubation in PRP gel impaired cBM-MSC hematopoietic-supporting activity, decreasing the CFC efficiency of BMC to 0.14±0.03%, in comparison with cBM-MSC alone and fibrin gel groups (0.16±0.03% and 0.17±0.03%), respectively.

Observed insights in the significance of hematoma cues for the interplay of BM-MSC with circulating lymphoid and bone marrow myeloid cells, indicate necessary further investigations elucidating the immunomodulatory role of BM-MSC in the course of bone healing.

Abstract No. N02

Quiescence regulates the number of neural stem cells and their regenerative capacity in the aging brain

Georgios Kalamakis[1,*], *Daniel Brüne*[1], *Srikanth Ravichandran*[2], *Jan Bolz*[1], *Wenqiang Fan*[3], *Frederick Ziebell*[1], *Francisco Catalá Martinez*[1], *Janina Kupke*[1], *Sheng Zhao*[1], *Enric Llorens Bobadilla*[1], *Katharina Bauer*[4], *Stefanie Limpert*[1], *Birgit Berger*[1], *Urs Christen*[5], *Peter Schmezer*[1], *Jan Phillip Malm*[6], *Benedikt Berninger*[3], *Simon Anders*[7], *Antonio Del Sol*[2], *Anna Marciniak-Czochra*[8], and *Ana Martin Villalba*[1]

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[3]Institute of Physiological Chemistry, University Medical Center Johannes Gutenberg, Mainz
[4]Heidelberg Center for Personalised Oncology, DKFZ
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[6]Single Cell Open Lab, DKFZ
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*Presenting author

Neurogenesis in the brain of rodents is known to persist throughout life. Neural stem cells (NSCs) residing in the ventricular-subventricular zone (V-SVZ) of the lateral ventricle generate neurons involved in fine odor discrimination. However, the ability to discriminate similar odors is drastically compromised in old mice. Whether this functional deficit is due to a lower amount and/or compromised function of stem cells in the aging V-SVZ is not clear. Examination of 2 and 22 month-old mice revealed that the number of V-SVZ-NSCs significantly decline but their ability to generate differentiated neurons is comparable to their younger counterparts. Using mathematical modeling of V-SVZ dynamics, we found that aging-induced decline of stem cells is counteracted by increasing the number of stem cells in a quiescent state. This allows retention of a small pool of stem cells even at very old ages but also prevents stem cell activation by injury in the old brain. Using single-cell RNA sequencing we identified increased inflammation and increased expression of the Wnt inhibitor sFRP5 as the main drivers of increased quiescence. Inhibition of either of these features prevented aging-induced quiescent and allowed injury-induced activation of stem cells for repair of the old brain.

Abstract No. N03

PURA Syndrome: Elucidating the neurodevelopmental mechanism using patient specific PUR-alpha interactome data

Lena Molitor[1,*] , *Ejona Rusha*[1], *Marcel Proske*[1], *Robert Janowski*[1], *Micha Drukker*[1], and *Dierk Niessing*[2]

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*Presenting author

PURA Syndrome is a rare disease caused by diverse mutations in the PURA gene (5q31.2). It encodes the protein Pur-alpha, which is expressed ubiquitously. This protein is involved in major cellular processes including transcription, mRNA transport and translation during neuronal development. Defects in the gene cause neurodevelopmental delays, resulting in a lack of speech, difficulties with motion and epileptic seizures.

A first approach to understand the structural effect of these mutations was to solve the crystal structures of Pur-alpha and its homolog Pur-beta. Additionally, we generated patient specific induced pluripotent stem cells (iPSCs). Based on the reported plethora of neuronal phenotypes in various patients and studies in mouse models we expect to see a delay or a reduced differentiation potential in the neuroectoderm. We would like to perform a time course study focusing on pluripotency exit and lineage identity acquisition in PURA patients. Using individual-nucleotide resolution Cross-Linking and ImmunoPrecipitation (iCLIP) and proximity-dependent biotin identification (BioID) approaches at different time points we intend to identify RNA and protein interaction partners respectively. This will eventually lead to a better understanding of the mechanisms that cause the disorders associated with PURA syndrome. Ultimately, we want to provide information that can lead to the development of treatment strategies for PURA syndrome patients.

Abstract No. N04

Evidence for neuronal plasticity in bioengineered neuronal organoids (BENOs)

Maria Patapia Zafeiriou[1,*], *Guobin Bao*[2], *Rashi Halder*[3], *Andreas Fischer*[3], *Detlev Schild*[2], and *Wolfram-Hubertus Zimmermann*[1]

[1]Institute of Pharmacology and Toxicology, University Medical Center, Georg-August-University-Göttingen; Germany
[2]Institute of Neurophysiology and Cellular Biophysics, University of Göttingen, Germany
[3]Research Group for Epigenetics in Neurodegenerative Diseases, German Center for Neurodegenerative Diseases, Göttingen, Germany

*Presenting author

3D modelling of human brain in combination with genome editing technologies represent powerful tools for understanding and treating human neurological diseases. Despite the advances in these technologies there is still little known about neuronal network function and plasticity in brain organoids. To study human neuronal network function, we developed a defined, Matrigel-free 3D cell culture system termed human bioengineered neuronal organoids (BENOs). Neural differentiation of pluripotent stem cells (iPSCs) embedded in a collagen matrix was directed under defined serum-free conditions. RNA-sequencing at different time points of BENO-development (n=3-6/time-point) provided evidence for neurogenesis (d30-40) with subsequent gliogenesis (d50-60), similar to developmental patterns observed in human brain. Whole mount immunofluorescence microscopy revealed the presence of glutamatergic (VGLUT) and gabaergic (GABA) neurons. By day 90, organoids contained increasing numbers of oligodendrocyteswe and myelinated axons. Calcium imaging revealed tetradotoxin (1 µM)-sensitive neuronal activity by d30. To test spontaneous neuronal network activity, BENOs (day 30-60) were subjected to calcium imaging under GABAergic (picrotoxin, 58 µM; saclofen, 330 µM) inhibition. Interestingly, spontaneous Ca^{2+} signals of synchronized neurons became asynchronous upon GABAR inhibition (2 independent experiments). Antagonist washout restored synchronicity suggesting the presence of functional GABAergic networks. Stimulation (injected current: 20-100 µA)-evoked Ca^{2+} influx in remote regions (distance from electrode 0.5 to 1.5 mm) suggested a strong neuronal network that extends throughout the organoid. Multi-pulse stimulation demonstrated a Ca^{2+} influx pattern similar to paired pulse depression (PPD). The PPD-like Ca^{2+} signal pattern was alleviated by a GABA-A inhibition (picrotoxin 58 µM) and was restored upon washout (2 independent experiments). Field potential measurements by multielectrode array systems revealed high frequency induced long-term potentiation (n=3) as observed in neurons suggesting neuronal plasticity. In conclusion, BENOs from human pluripotent stem cells contain electrically active neuronal networks that exhibit typical forms of plasticity observed in the human brain.

Abstract No. N05

Functional consequences of a novel mutation in CHMP2B, causing frontotemporal dementia linked to chromosome 3 (FTD3) in a German patient

Adele Marthaler[1,*] , *Rojan Demirtas*[1], *Hans H. Klünemann*[2], *Jørgen E. Nielsen*[1], *Thomas H. Braunstein*[3], and *Troels T. Nielsen*[1]

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*Presenting author

Frontotemporal dementia (FTD) is a group of heterogeneous neurodegenerative diseases primarily affecting the frontal and temporal lobes of the brain. The clinical presentation of the disease is extremely diverse, including changes in personality, behavior, and language. It is also amongst the main causes for early onset dementia.

A mutation in the *CHMP2B* gene, located on chromosome 3, was described to cause FTD in a large Danish pedigree and was termed FTD linked to chromosome 3 (FTD3). In this study, we describe a distinct mutation in *CHMP2B*, found in an unrelated German patient. *CHMP2B* is a component of the endosomal complex required for transport-III (ESCRTIII) and plays a crucial role in vesicle formation and scission in autophagy and endolysosomal trafficking.

The aim of this project is to compare the newly identified *CHMP2B* mutation, and its disease pathology, to the already characterized Danish FTD3 patients. To this end, we are comparing patient-derived fibroblasts in terms of CHMP2B transcript and protein variants. Furthermore, we are investigating presence of an enlarged endosomal phenotype, already described in Danish FTD3. Hereby, we put special focus on visualization and, more importantly, quantification of endosomal size and localization. Automated segmentation of individual endosomes is difficult with confocal microscopy, and even more so in conventional fluorescence microscopy, due to limitations in resolution resulting in bulk fusion of single endosomes. Thus, meaningful quantification is almost impossible. We, therefore, chose to image the endosomes using structured illumination microscopy (SIM, a super resolution technique) to achieve best possible acquisition of the endosomes, in order to be able to obtain reliable and unprecedented information on endosomes in FTD3 patients.

Abstract No. N06

The detection and minimization of genetically variant stem cells in human pluripotent stem cell cultures

Jason Halliwell[*], *Peter Andrews*, and *Ivana Barbaric*

The University of Sheffield, United Kingdom

*Presenting author

A key goal in achieving the translational promise of human pluripotent stem cells (hPSCs) is ensuring the safety of the cells transplanted. The safety of hPSC-based therapies could be compromised by the presence of genetic changes in the transplanted cells. It is now well documented that hPSC acquire genetic changes as a consequence of prolonged *in vitro* culture. If the acquired changes provide a growth advantage to the variant cell, they are subsequently selected for in ensuing passages. Retrospective analysis has revealed a commonly occurring panel of genetic changes harbored by hPSCs, including amplifications of chromosome 1, 12, 17, 20 and deletions to 10, 18 and 22.

It has been well documented that these genetic changes affect the behavior of the undifferentiated cells, although it is largely unknown how they may affect their differentiated derivatives. However, as many hPSC based therapies move closer to clinical applications it is important that we address this issue. One concerning observation is the similarity of the common genetic changes in hPSC to those that occur in embryonal carcinoma cells, the malignant stem cells of testicular, germ cell tumors.

Our work is focusing on understanding the mechanisms by which genetic changes arise in hPSCs as a pre-requisite to optimizing the culture conditions to minimize the mutational load on hPSCs and/or eliminating the selective advantage of the variant cells. Additionally, we have developed new approaches that can sensitively and robustly detect the common genetic changes in hPSC cultures.

Abstract No. N07

Steps towards standardized assessment and use of immunomodulatory equine MSC

*Olivia Lee** , *Sarah LePage*, *Rames Salcedo*, and *Thomas Koch*

University of Guelph, Canada

*Presenting author

Background: Inflammation-associated disorders are significant causes of morbidity in horses. Equine mesenchymal stromal cells(MSCs) hold promise as cell-therapy candidates due to their secretory non-progenitor functions[1]. MSC derived from umbilical cord blood(eUCB) and cord tissue(eCT) are of clinical interest due to ease of procurement, multipotency, and immunomodulatory ability[2]. Using mononuclear cell suppression assay(MSA), we have shown that eUCB-MSCs and eCT-MSCs are mononuclear cells(MNCs) suppressive *in vitro*. Due to the inherent heterogeneity of MSCs and varied culture expansion protocols, the development of methods to circumvent donor-to-donor heterogeneity, as well as robust and easily deployable methods of potency assessment may be beneficial for improving MSCs' predictability in treating inflammatory diseases. This study focuses on the development of a robust *in vitro* MSC potency assay and assessment of potential MSC therapeutic end-products generated from pooled MSCs (pMSCs).

Hypothesis: MSA using pooled MNCs(pMNCs) is a robust *in vitro* assay for the evaluation of MSCs` immunosuppressive potency; pMSCs are able to suppress MNC proliferation.

Approach: MSC cultures were assessed with pMNCs MSA using Brdu ELISA and CFSE. MSCs were stimulated with TNF-a, IFN-y, LPS, poly(I:C) and electrohydraulic shockwave and their potencies were compared. pMSC cultures were generated by pooling MSCs from multiple unrelated donors in equal ratios.

Results: MSA using MNCs from single donors demonstrated MNC donor variability. The pMNCs MSA of eUCB-MSCs and eCT-MSCs revealed MSCs from both sources are capable of suppressing MNCs to a similar extent. pMSCs are able to suppress MNC proliferation under all conditions tested.

Conclusion: Using MSA based on pMNCs to minimize individual immune response variability, we have developed a consistent and reproducible equine MSC potency assay. Utilizing this assay, we have also demonstrated that pMSCs have immune suppressive properties. This knowledge could be used in production monitoring of cellular potency and as release criteria prior to clinical use.

[1] Tessier L, Bienzle D, Williams LB, Koch TG. Phenotypic and Immunomodulatory Properties of Equine Cord Blood-Derived Mesenchymal Stromal Cells. Plos one. 2015;1–19.

[2] Carrade DD, Lame MW, Kent MS, Clark KC, Walker NJ, and Borjesson DL. Comparative Analysis of the Immunomodulatory Properties of Equine Adult-Derived Mesenchymal Stem Cells. Cell Medicine. 2012.

Abstract No. N08

Spatially controlled differentiation of human mesenchymal stem cells

Smita Patil[*] , and *Neetu Singh*

Indian Institute of Technology Delhi

*Presenting author

Fabrication of appropriate biomaterials which not only support cellular adhesion and proliferation but also direct lineage-specific differentiation of stem cells is crucial for developing tissue engineering construct. Mesenchymal Stem Cells (MSC) are being extensively studied in preclinical and clinical trials and are considered the most important cell type for tissue engineering due to their easy isolation, high yield, high plasticity, and anti-inflammatory properties. In recent years, natural materials such as silk have been used for MSC culture because of its superior mechanical strength, biocompatibility, and biodegradability. It is widely reported in literature that biomaterial surface chemistry modulates hMSC differentiation in a lineage dependent manner. In this study, functional groups have been used to direct differentiation of hMSCs, since they are defined by precise chemistry, provide more control over modification and patterning of surface. Our group has recently reported a plasma induced graft polymerization method to enhance the cell adhesion efficacy of *Bombyx mori* silk fibroin films (SF). This method can be used to tune the hydrophilicity of SFs, it also provides functional groups for bioconjugation.

Against this background, we report here a simple strategy for differentiating hMSCs into two different lineages on the same surface. Chemical groups (COOH and PO_4) were grafted onto SF and this causes hMSC differentiation into chondrogenic and osteogenic lineage, respectively on the same surface. Unlike other, our strategy does not require any external growth factors or chemokines in the culture medium to trigger the hMSC differentiation. Here the differentiation is initiated due to the difference of functional groups present on the surface. This approach can be used in osteochondral tissue engineering where composite tissue structure made up of chondrocytes and osteocytes is desired. We believe this study will foster the development of effective silk-based tissue engineered constructs.

Patil S, Singh N, Mat Sci Engg: C, 91, 796–805, 2018

Abstract No. N09

Guided epicardial cell transfer of induced cardiomyocyte progenitors (iCMP) by human cardiac ECM hydrogel coated biological scaffold upon infarction

Matthias Becker[1,*] , *Dipthi Bachamanda Somesh*[1], *Kristin Klose*[1], *Aarón X. Herrera Martin*[1], *Olivia Reidell*[2], *Oliver Klein*[1], *Karsten Jürchott*[1], *Manfred Gossen*[3], *Volkmar Falk*[4], and *Christof Stamm*[4]

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*Presenting author

Upon cardiac infarction, mammals, in contrast to lower vertebrates or neonatal mice have little or no ability to regenerate the necrotic tissue.

Therefore, we sought to design an epicardial hybrid patch system composed of an extracellular matrix (ECM) based scaffold enhanced with induced cardiomyocyte precursors (iCMPs) – a novel tool for epicardial regeneration.

A cardiac hydrogel (gECM) was produced upon decellularization of human cardiac tissue and processing via pulverization and Pepsin digestion. Cell-free human amniotic membrane (DeAM) was subsequently coated by dry coating protocol with gECM (DeAM+E).

Surface analysis of scaffolds by electron microscopy (SEM) revealed solid coating and an additional new nano-scaffold onto DeAM+E. Uniaxial pulling test confirmed stability for epicardial transplantation. Protein analysis by mass spectrometry (MS) identified cardiac specific proteins relevant for viability under hypoxic conditions. Determination of immune reaction by cytokine secretion of monocytes as well as macrophage-polarization and T cell proliferation by flow cytometry displayed no pro-inflammatory activation.

Enriched population of proliferative iCMPs were generated from cardiac fibroblasts (CFs) by forced expression of cardiac transcription factors Gata4, Mef2c, Tbx5 and Myocd.

Immunocytology revealed expression if troponin T, α-actinin and myosin heavy chain (MHC) proteins in iCMPs. RNA sequencing of iCMPs showed upregulation of genes associated with cardiac development, differentiation and morphogenesis while they showed downregulation of genes associated to cell-proliferation in comparison to their parental CFs and cardiomyocytes.

When cultured on gECM, iCMPs showed promising effects with regards to cell adherence, proliferation and maturation. The iCMPs maintained expression of cardiomyocyte specific markers and exhibited higher cardiac marker expression and decreased levels of endothelial marker expression.

In conclusion, an epicardial hybrid patch system was tailored for cardiac regeneration. The patch system specifically supports the viability of cardiac cells and enhances iCMP maturation.

Abstract No. N10

Mechanism of bFGF-regulated pluripotency maintenance in human induced pluripotent stem cells

Fereshteh Haghighi[1,*] , *Julia Dallmann*[2], *Saeideh Nakhaei-Rad*[1], *Alexander Lang*[1], *Roland P. Piekorz*[1], *Martin Zenker*[3], *George Kensah*[2], and *Mohammad Reza Ahmadian*[1]

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*Presenting author

The development of human embryonic stem cells opens new windows for basic research and regenerative medicine due to their two remarkable properties, self-renewal and pluripotency. A key goal in stem cell research is to identify the factors, which keeps human pluripotent stem cells (hPSCs) undifferentiated *in vitro* and differentiating later to mature functional derivatives. However, obtaining a clear and detailed view of how signaling pathways maintain pluripotency *in vitro* has been difficult to achieve due to some limiting factors including; disparate culture conditions, tools for evaluation of signal transduction pathways, their crosstalk and feedback loops. Basic fibroblast growth factor (bFGF) was the first factor found to be crucial for the maintenance of hPSCs *in vitro*. It promotes hPSCs self-renewal and pluripotency in two ways by directly activating RAS-MAPK and RAS-PI3K pathways and by indirectly stimulating autocrine effects. Here, we investigated the effect of bFGF and its potential downstream signaling in human induced pluripotent stem cells. Our results revealed that by withdrawing bFGF, iPSCs were differentiated to ectoderm lineage. MAPKs (ERK, JNK and p38), PI3K and JAK/STAT pathways, were reciprocally regulated during the bFGF starvation and stem cell differentiation. Interestingly, we identified the specific RAS isoform which activates MAPK downstream of bFGF. Our data provide additional insights into the mechanisms of pluripotency, which will be presented and discussed in details.

Abstract No. N11

Adipose-derived stem cells as vehicle for epothilon B

Annemarie Baaße, *Guido Hildebrandt*, and *Katrin Manda*

University Medical Centre Rostock

Introduction: The use of mesenchymal stem cells as a vehicle for chemotherapeutic agents is a promising tool for tumor therapy. Due to tumor-associated recruitment processes of mesenchymal stem cells, the drug could be transportet directly to the tumor cells and enhance its effect by portionwise and long-lasting release of the therapeutic agent. This effect was previously detected of bone-marrow derived mesenchymal stem cells. The aim of this study is to demonstrate this capability in adipose-derived stem cells (ADSCs), whose isolation from adipose tissue is less invasive for patients and results in a higher yield of cells.

Material and Methods: ADSCs were isolated from human reduction mammoplasties of healthy female donors and analyzed for their multilineage capacity and expression panel of specific surface proteins. To exclude donor-specific effects, ADSCs of 10 donors were pooled and used for the experiments. First, the cytotoxic effects of various chemotherapeutic agents on pADSCs were analyzed and tolerated concentrations determined. By using cell culture inserts, the effect of ADSCs incubated with the chemotherapeutic agents on the clonogenic survival of tumor cells was investigated. The most promising therapeutic was selected and analyzed for effects after incubation with ADSCs and co-culture on tumor cell growth as well as apoptosis/necrosis.

Results: Isolated ADSCs exhibited multilineage capacity and showed the expression of the surface marker CD90 and CD29 as well the lack of CD31, CD34, CD45 and CD106. Adipose-derived stem cells isolated from the breast are robust to the treatment with taxol, epothilone B and doxorubicin. After incubation with epothilone B they mediate a toxic effect on breast cancer cells, which significantly reduces cell survival through reduced cell growth and increased rates of apoptosis and necrosis.

Conclusion: On the basis of our results we assign ADSCs to be a good vehicle for chemotherapeutics to destroy cancer cells.

Abstract No. N12

Modeling Canavan's disease in vitro

Julia Fischer[1,*] , *Jonas Saal*[1], *Wolfram Kunz*[2], *Vivian Kaps*[1], *Marija Mizhorova*[1], *Miriam Reisenhofer*[1], *Melanie Bloschies*[1], *Jörn Oliver Sass*[3], and *Oliver Brüstle*[1]

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Canavan's Disease (CD) is an early onset, autosomal-recessive disorder characterized by massive demyelination and spongiform appearance of the brain parenchyma. Mutations in the aspartoacylase gene (*ASPA)* have been identified as the main genetic cause of CD. This gene codes for an enzyme that cleaves N-acetylaspartate (NAA), yielding aspartate and acetate. The loss of ASPA activity leads to elevated systemic levels of NAA. Ultrastructural analyses of CD patient brain samples not only show extensive lack of myelin and vacuolization but also astrocytes containing extremely elongated mitochondria with abnormally structured cristae. Early studies have therefore suggested that spongy degeneration is due to mitochondria dysfunction (Adachi et al., 1973).

The objective of our research is to establish an *in vitro* model to further explore this astrocytic phenotype. To meet this aim we initially reprogrammed fibroblasts from three CD patients with mutations in the *ASPA* gene into human induced pluripotent stem cells (hiPSCs) using Sendai viral vectors. The resulting CD-hiPSCs were fully validated for virus inactivation, pluripotency marker expression (Tra1-60, Tra1-81, and SSEA4) and their ability to differentiate into all three germ layers *in vitro* and *in vivo*. CD-hiPSCs were then differentiated into proliferative radial glia-like neural stem cells (RGL-NPCs) according to an established protocol (Gorris et al., 2015). CD-RGL-NPCs could be successfully differentiated into astrocytes. A detailed analysis by immunofluorescence and electron microscopy revealed that CD-RGL-NPC-derived astrocytes contain significantly larger mitochondria with an abnormally structured cristae compared to control cells. First data suggest a mitochondrial complex I deficiency. Exploiting this *in vitro* phenotype we are currently extending our studies towards the role of astrocytes in the pathogenesis of Canavan's Disease. From a medical perspective, CD-hiPS-derived astrocytes could also represent a versatile tool for studying regenerative approaches *in vitro*.

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Abstract No. N13

A chemically defined differentiation protocol independent from dual SMAD inhibition generates cortical neurons from hiPSCs by avoiding endogenous propensity towards neural crest

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Human induced pluripotent stem cells (hiPSCs) play an important role in disease modeling as they allow the generation of patient-specific somatic cells. In case of Alzheimer's Disease (AD) cortical neurons are affected and thus, differentiating cortical neurons from hiPSCs is essential for disease modeling that recapitulates important disease pathology. Typically, to generate cortical neurons hiPSCs are directed into the direction of neuroectoderm. Commonly, inhibition of BMP and TGF/Activin signaling which is referred to as dual SMAD inhibition (dSMADi) is applied or at least included for the generation of the neuroectoderm from hiPSCs.

In this study we investigate alternative signaling pathways that are crucial for neuroectoderm induction. We identified combinations of signaling pathway inhibitors that greatly accelerate formation of telencephalic neural progenitor cells. Nevertheless, we observed that some hiPSC lines might have a propensity towards neural crest formation. This allowed us to identify signaling pathways involved in this unwanted differentiation. These findings could help to develop the protocol further into a more robust one as it can also be applied for lines susceptible for neural crest formation.

With these findings we developed a neural induction protocol towards the neuroectoderm which is not dependent on dSMADi and is widely applicable upon different hiPSC lines even if some of the lines have an endogenous propensity towards neural crest formation. The developed protocol might help to derive cortical neurons from patient-derived hiPSCs to evaluate AD-relevant phenotypes.

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Abstract No. N14

A web application for predicting cell-fate determinants in cell differentiation

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Differentiation is a complex process, where pluripotent, multipotent or progenitor cells evolve into more specialized cells. Although the detailed mechanisms and the pathways involved are not fully understood, it is generally accepted that a few transcription factors play a crucial role in determining cell fates (cell-fate determinants). Several valuable data-driven approaches have been proposed for the inference of cell-fate determinants [1]. However, these tools either require comprehensive training or background data, prevent the user from inputting new transcriptomic data, or have no publicly accessible implementation.

Here, we have developed SeesawPred, a web application that can systematically predict cell-fate determinants from transcriptomics data based on a computational model of cell differentiation. The model relies on the assumption that the progenitor cell phenotype is maintained in a metastable state by the opposing cell-fate determinants, which are part of interconnected feedback loops [2]. During binary cell-fate decisions, the equilibrium is shifted towards either of the two cell-fate determinants and the gene expression profile stabilizes in the corresponding daughter cell type.

Unlike previous approaches, SeesawPred computes the prediction from gene expression data uploaded directly by the user, enabling its application to novel differentiation systems. The validity of SeesawPred was confirmed on various binary cell differentiation examples in both human and mouse, where known cell-fate determinants were recapitulated. Moreover, the comparison with already published methods showed a superior performance on the example data set. Further validation both computationally and experimentally will reinforce the generalizability of the method. SeesawPred is made publicly available free of charge for academic non-profit use in order to guide differentiation experiments in stem cell research and regenerative medicine.

The web application containing the example data set is available at http://seesaw.lcsb.uni.lu/

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Abstract No. N15

Deciphering the role of the stem cell niche in human acute myeloid leukemia - insights from mathematical modeling

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Hijacking of the stem cell niche seems to play a crucial role in many cancers. However, in humans, experimental limitations make it difficult to observe this process directly. Using acute myeloid leukemia (AML) as an example, we show how mathematical models can provide insights into the role of the stem cell niche in human cancers. AML is a stem cell-driven malignant disease of the hematopoietic (blood forming) system. The interaction of leukemic stem cells (LSC) and hematopoietic stem cells (HSC) is of crucial importance to understand disease dynamics and treatment failure. There is evidence from model organisms that LSC out-compete HSC from their protective bone marrow niche. Due to experimental limitations it is challenging to determine whether a similar mechanism exists in humans. Using a combination of mathematical modelling, experiments and patient data we provide evidence that human HSC and LSC compete for spaces in a joined bone marrow niche and that LSC can dislodge HSC from that niche. We consider the following questions: (i) How can we use patient data to study competition of LSC and HSC in the human bone marrow niche? (ii) How can we quantify LSC-HSC competition? What is its impact on disease dynamics? How does it differ between patients? (iii) Does the cell cycle status of the HSC have an impact on HSC-LSC competition?The developed models include various feedback signals and interactions of stem cells with the niche. Comparison of computer simulations and longitudinal observations of CD34+CD38-ALDH+ and leukemic cell counts in individual patients provides insights into niche processes. We use the model to predict different prognostic subgroups and we confirm our predictions based on clinical data.

Abstract No. N16

Unidirectional and progressive DNA methylation changes define hematopoietic lineage commitment

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Hematopoietic stem cells (HSC) facilitate the lifelong production of most mature blood cell types. Recently, the classical hierarchical differentiation tree of hematopoiesis has been challenged based on single-cell RNA sequencing (RNA-seq) approaches. These data suggest a continuous rather than a step-wise differentiation process with lineage priming occurring in the hematopoietic stem and progenitor cell (HSPC) compartment. However, while differentiation trajectories can be inferred from such an approach, it is not possible to discern points whether discreet lineage commitment decisions occur at specific points along these trajectories. The existence of such commitment points could still be compatible with a step-wise differentiation model and their characterization at the molecular level would be essential for the accurate modeling of the hematopoietic hierarchy.

Based on our previous data, we hypothesized that whole genome DNA methylation analysis would facilitate the identification of such molecular commitment marks due to the progressive and irreversible nature of how this mark is programmed during differentiation. We therefore generated a comprehensive DNA methylation map encompassing 27 murine hematopoietic cell populations. We identified differentially methylated segments (DMS) between HSCs and the most differentiated hematopoietic cell populations, which reveal progressive and unidirectional DNA methylation dynamics throughout the hematopoietic system. Depending on the dynamic of methylation changes, DMS could be subdivided into pan-hematopoietic, lineage-specific and cell-type specific DMS. Based on the unidirectionality of DNA methylation changes observed, we were able to infer a phylogenetic tree of the hematopoietic system, which is solely based on DNA methylation patterns and recapitulates known lineage differentiation trajectories. By the application of non-negative matrix factorization lineage-specific programs could be identified within the primitive HSPC compartment. Further analysis confirmed that DMS identified within HSPCs were sufficient to separate and terminally differentiated hematopoietic cell lineages, indicating that commitment decisions are made within the primitive multipotent progenitor cell compartment.

Abstract No. N17

Cell-extrinsic and -intrinsic regulation of PU.1 in HSC lineage choice

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Transcription factors (TFs) play a crucial role in regulating the lineage choice of hematopoietic stem cells (HSCs). Previous studies suggested functional antagonism between the core hematopoietic TFs PU.1 and Gata1, their putative involvement in myeloid lineage choice of HSCs, autoregulation of PU.1 and M-CSF as a cell-extrinsic direct regulator of PU.1 in HSCs. Here, using continuous long-term quantitative time-lapse imaging of HSCs from the PU.1eYFP/Gata1mCherry reporter mouse line, we showed that a random PU.1 and Gata1 switch does not initiate myeloid lineage choice. To further analyze the cell-intrinsic regulation of PU.1 in HSCs, we generated a novel mouse line with an inducible PU.1-ERT^2 allele crossed into the PU.1eYFP/Gata1mCherry reporter mouse line. Using single-cell reprogramming assays, microfluidics facilitated reversible PU.1 induction with TNFα and quantification of endogenous PU.1eYFP protein dynamics in response to transgenic PU.1-ERT^2 activation, we observe no direct autoregulation of PU.1 in 9 different HSPC types. We find PU.1 autoregulation not to be involved in initiating differentiation, but to be an indirect consequence of it. To address the possible cell extrinsic regulation of PU.1, we treated HSCs from PU.1eYFP reporter mice with a panel of cytokines followed by quantification of PU.1 dynamics in single HSCs and identify TNFα as an essential cell-extrinsic regulator of PU.1, both in vitro and in vivo. TNF$^{-/-}$ and M-CSF$^{-/-}$ animals combined with transplantation experiments and PU.1 specific immunostainings show that M-CSF and LPS regulate PU.1 expression via niche derived TNFα in vivo, thus abolishing the notion of direct effects of M-CSF on PU.1 in HSCs. These data disprove several previous assumptions about the regulation of PU.1 and HSPC fate control, identify novel regulators, and demonstrate vacancy for other cell-extrinsic and -intrinsic players in regulating PU.1 specifically and lineage choice of HSCs in general.

Abstract No. N18

Human HSCs show cell polarity and division kinetic changes upon aging

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The adult human bone marrow is known to produce around 10^{12} cells daily in a process known as hematopoiesis. Hematopoiesis is driven by hematopoietic stem cells (HSC) and is crucial for maintaining blood homeostasis throughout life. HSCs are multipotent cells with the capacity to self-renew, thereby sustaining the stem cell pool, and to differentiate into any mature blood cell. Recent findings using mouse models, however, show distinct functional and phenotypic changes that occur in HSCs upon aging, which are reversible. To better explore the prospects of rejuvenation, we sought to characterize aging-induced changes in human HSCs isolated from the bone marrow. Our results show that although the number of total bone marrow-mononuclear cells (BM-MNCs) remain unchanged, the number of multipotent hematopoietic stem and progenitor cells (HSPC) ($CD34^+CD38^-$) and the number of HSCs ($CD34^+CD38^-CD90^+$) increase upon aging. Furthermore, we observed a delay in aged HSCs in their response to cytokine stimulation *ex vivo* irrespective of the cytokine cocktail and oxygen conditions. Similar to murine HSCs, aged human HSCs show a significant decrease in the frequency of cells that present with a polar distribution of tubulin and the small RhoGTPase Cdc42 in the cytoplasm and Ac-H_4K_{16} in the nucleus. Additionally, we observe an increase in the amount of active Cdc42 (ie. the GTP bound form) relative to the total Cdc42 protein, in low-density bone marrow (LDBM) cells upon aging.

Collectively these findings show that the percentage of human HSCs within the $CD34^+$ BM population increases with age, which correlates with higher Cdc42 activity and a reduced stem cell polarity. Our data also reveals an intrinsic delay in replication time of human aged HSCs during *ex vivo* culture.

Abstract No. N19

Identification of novel signaling molecules involved in neuronal differentiation of iPS cells from schizophrenic patients with CHRNA7 copy number variants

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Schizophrenia (SCZ) is a sever psychiatric disorder with a median lifetime prevalence of 4 per 1000 with a huge economic burden on patient and society. SCZ linked to more than 100 loci or chromosomal abnormalities. Among them, 15q13.3 microdeletion leads to haplo-insufficiency of a small number of genes including the α7 neuronal nicotinic acetylcholine receptor (CHRNA7) that shown to be significantly decreased in post-mortem brain of schizophrenics. Although our knowledge about biological mechanisms behind development of SCZ is incomplete, recent studies have suggested a number of hypotheses, including dysregulation of synaptic plasticity and pruning and WNT/β-catenin signaling during early brain development. We have developed induced pluripotent stem cells (iPSCs) models of SCZ patients with full and partial deletion of CHRNA7 (SCZ#1 and SCZ#2 respectively) and investigated altered Wnt signaling in these cell lines. We derived Neuroepithelial stem (NES) cells from iPSCs with rosette-shape structures, expressing NESCs markers such as Nestin, ZOI, PLZF and SOX2. SCZ#1 cells have significantly higher Wnt baseline and their response to overexpression and inhibition is skewed during 12 days of dual-SMAD inhibition protocol of neural induction. While SCZ#2 cells have Wnt baseline and response alteration in a lower extent when compared to healthy control cells. Proliferation rate of SCZ#1 NES cells was significantly lower and Taqman array data indicated significantly higher expression of Wnt3A, Wnt1, Wnt10B, Wnt8B and FZD10 as well as significantly lower expression of DKK2 and Wnt16 compared to control cells. Furthermore, 28 days of neural differentiation indicated significantly higher glutamatergic neurotransmitters expression and significantly lower expression of GABAergic neurotransmitters in SCZ#1 neurons. These data indicates CHRNA7 full deletion is causing Wnt signaling alterations in early neural development and this alteration is affecting cell identity in long-term neural differentiation.

Abstract No. N20

Development of novel CRISPR/Cas9-based gene therapeutic approaches for osteopetrosis

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Autosomal recessive osteopetrosis (ARO) is a severe hereditary bone disease characterized by dense and fragile bone leading to bone marrow failure in early childhood. ARO is caused by a lack of osteoclast-mediated bone resorption that can be caused by mutations in the *CLCN7* gene coding for the chloride channel ClC-7. As osteoclasts develop from hematopoietic stem cells (HSCs), allogeneic HSC transplantation is a curative treatment for ARO. However, this therapy is associated with high mortality and often no suitable donors are available. Somatic gene therapy by *ex vivo* CRISPR/Cas9-based genome editing in patient-derived HSCs followed by autologous stem cell transplantation could offer novel treatment options.

To this end, we developed a general additive gene transfer strategy that is applicable to all types of *CLCN7* mutations. By using recombinant adeno-associated virus serotype 6 donor delivery and Cas9 ribonucleoproteins with synthetic guide RNAs, wild type *CLCN7* cDNA can be introduced into the safe harbour region *AAVS1*. In cord blood HSCs, targeted integration of the donor construct into the *AAVS1* locus was achieved in over 65% of the treated cells. For a more physiological rescue, we are also working on patient-specific mutation correction. Therefore, we generated induced pluripotent stem cells (iPSCs) from a *CLCN7*-related ARO patient. Single base pair editing was performed by delivering Cas9 ribonucleoproteins and single-stranded donor oligonucleotides to the cells. Genomic analysis of both edited iPSC clones and bulk cultures revealed 30-40% mutation correction. Currently, we are investigating the rescue efficiencies of our gene therapeutic approaches in our established iPSC-based osteopetrosis disease model. Collectively, we showed highly efficient CRISPR/Cas9-based genome editing in HSCs and iPSCs forming the basis for new gene therapeutic treatments of ARO.

Abstract No. N21

Hematopoietic progenitors selectively maintain the pSTAT5 self-renewal response to Interleukin-3 (IL-3) in low energy environments

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Hematopoietic bone marrow contains a range of stromal environments that differ in terms of both signaling and metabolism. It is likely that the self-renewal and lineage specific differentiation of hematopoietic cells is organized and contained within niches that offer appropriate support. We are interested in how metabolic parameters may influence the balance between self-renewal and differentiation in a given signaling environment and are using Phospflow techniques to study the IL-3 response of self-renewing and differentiating FDCP-Mix cells under different metabolic conditions, focusing on the STAT5 signaling pathway associated with self-renewal.

Consistent with previous observations in primary cells, we demonstrate by using metabolic inhibitors that self-renewing FDCP-Mix derive their energy primarily from oxidative phosphorylation, but switch to glycolysis during commitment and differentiation. Decreasing the glucose concentration over the range (5 – 0.1) mM progressively decreases the pErk1/2 response to IL-3 while pSTAT5 levels actually increase under low glucose. A subpopulation of cells maintains a strong pSTAT5 response to IL-3 in the absence of glucose, and even in the presence of inhibitors of both glycolysis and oxidative phosphorylation.

Our results demonstrate that the JAK-STAT pathway has a surprisingly low requirement for free ATP and that the pSTAT5 self-renewal response dominates in very low energy environment. This suggests one way in which self-renewal may be supported and contained by a metabolic niche in vivo.