Symposium of BMBF projects: Innovative technologies in stem cell research for individualized medicine

Tuesday, 18 September

Marsilius-Kolleg, Heidelberg

Despite the broad spectrum of established medical procedures, for many diseases no suitable treatment option is available. A particular challenge is the increasing number of degenerative diseases for which there are hardly any causal therapies due to the lack of donor organs. Innovative stem cell technologies offer tremendous potential for the development of new, customized cell-based therapies as well as for the creation of human disease models based on in vitro generated tissues and organoids. Such in vitro models can be of great value for both molecular pathology research and drug discovery. The efficacy and undesirable side effects of drugs can be tested in advance for specific groups of patients or individuals, contributing to the much-needed increase in drug development. The projects funded in this BMBF initiative are intended to help overcome existing barriers to the medical use of innovative stem cell technologies, thereby unlocking the potential of new stem cell technologies for individualized medicine.

13 research consortia with 49 projects will present their strategies and newest results in this symposium.

Lecture hall

14:00 – 14:10 **Welcome** BMBF/PT; GSCN

Model- & Testsystems I (Chairs: *Jan Georg Hengstler / Thomas Moritz*)

14:10 – 14:30 **Neuro2D3:** Standardized systems for modeling late-onset neurological disorders and compound screening in 2D and 3D culture
Oliver Brüstle, University Hospital Bonn

14:30 – 14:50 **HIT-Tau:** High Throughput Approaches for the Individualized Therapy of Tau-Related Diseases
Günter Höglinger, DZNE & TU Munich

14:50 – 15:10 **PDdementia:** Identification of compounds preventing cognitive decline in Parkinson's disease patients using clinically correlated iPS cell models
Jared Sterneckert, TU Dresden

15:10 – 15:30 **MAIV:** Modelling ALS disease in vitro
Sergio Gascón, Complutense University of Madrid

15:30 – 15:45 Coffee break

Model- & Testsystems II (Chairs: *Marius Ader / Jared Sterneckert*)

15:45 – 16:05 **HiPSTAR:** Establishment, validation and standardization of individualized hiPS-based blood brain barrier models for Alzheimer drug development and testing in vitro
Ole Pleß, Fraunhofer IME ScreeningPort Hamburg

16:05 – 16:25 **Micro-iPS-Profiler:** Individualized Microfluidic-Multiorgan-Chip Multimode Profiling Platform based on iPS-Technology (kidney)
Juliane Schnabel, microfluidic ChipShop GmbH

16:25 – 16:45 **PancChip:** Pancreas development and disease modelling on an iPSC chip platform
Matthias Meier, Helmholtz Zentrum München

16:45 – 17:05 **StemNet:** iPS cell derived human hepatocytes: improved reprogramming and development of in vitro disease models
Jan Georg Hengstler, IfADo &TU Dortmund

17:05 – 17:25 **Stabil-Ice:** Stabilizing an efficient workflow for drug discovery and patient-specific disease modelling by introducing a novel multi-usage disposable, capable of the generation, cultivation, ice-free cryopreservation, and ultra-fast recovery of neuronal cell systems
Ina Meiser, Fraunhofer IBMT

17:25 – 17:40 Coffee break

Therapy (Chairs: *Oliver Brüstle / Claudia Gärtner*)

17:40 – 18:00 **i-MACnet:** Genetically corrected iPSC-derived macrophages (i-MAC) for innovative gene therapeutic strategies
Thomas Moritz, Hannover Medical School Therapy

18:00 – 18:20 **iCARE:** Induced pluripotent stem cells (iPSCs) for Clinically Applicable heart REpair
Ulrich Martin, Hannover Medical School

18:20 – 18:40 **ReSight:** Generation of transplantable hiPSC-derived retinal cells for treatment of retinal degeneration
Marius Ader, TU Dresden

18:40 – 19:00 **iPStemRNA:** Preparation of clinical grade mRNA iPSC lines from a panel of universal donors according to federal permission §13AMG
Micha Drukker, Helmholtz Zentrum München

19:00 – 20:00 **Poster session** (please find the abstracts on page 283)

20:00 – 21:00 **Get-together**

Federal Ministry of Education and Research

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Abstracts for the BMBF symposium

6th Annual Conference

German Stem Cell Network

18 September 2018

Innovative technologies in stem cell research for individualized medicine: B01 – B17

B01 A stably self-renewing adult blood-derived induced neural stem cell exhibiting patternability and epigenetic rejuvenation
Lea Flitsch, Neuro2D3

B02 A human iPSC-based 3D system for high content imaging of Alzheimer disease-specific pathology and drug discovery
Matthias Hebisch, Neuro2D3

B03 High throughput approaches for the individualized therapy of tau-related diseases
Tabea Strauss, HIT-Tau

B04 Identification of compounds preventing cognitive decline in Parkinson's disease patients using clinically correlated iPS cell models
Jared Sterneckert, PDdementia

B05 Using reprogramming to model human amyotrophic lateral sclerosis disease in vitro
Sergio Gascón, MAIV

B06 HiPSTAR: Humane iPS-zellbasierte Blut-Hirn-Schranken Technologie in der Alzheimerforschung
Ole Pleß, HiPSTAR

B07 Individualisierter Mikrofluidik-Multiorgan-Chip Multimode Profiling Plattform basierend auf iPS-Technologie
Juliane Schnabel, Micro-iPS-Profiler

B08 Medikamentenscreening sichtbar gemacht: ein humanes iPSC-basiertes, mikrophysiologisches Model der Leber und der Niere
Martin Raasch, Micro-iPS-Profiler

B09 PancChip – Optimizing a microfluidics/iPSC system to model endocrine and exocrine development
Matthias Meier, PancChip

B10 StemNet: a gene network modeling approach to engineer stem cell-derived hepatocyte-like cells
David Feuerborn, StemNet

B11 Enabling large-scale cryopreservation by sterile vitrification of adherent human induced pluripotent stem cells and their neural derivates (Project "Stabil-Ice")
Ina Meiser, Stabil-Ice

B12 Genetically corrected iPSC-derived macrophages (i-MAC) for innovative gene therapeutic strategies (iMACnet Consortium –Subprojects 2, 3, and 4, Hannover Medical School)
Thomas Moritz, iMACnet

B13 Genetically corrected iPSC-derived macrophages (i-MAC) for innovative gene therapeutic strategies (iMACnet Consortium – Subprojects 1 and 5, Medical Center-University Freiburg)
Thomas Moritz, iMACnet

B14 Induced pluripotent stem cells for Clinically Applicable heart REpair (iCARE): cell production incl. regulatory issues
Ina Gruh, iCARE

B15 Induced pluripotent stem cells for Clinically Applicable heart REpair (iCARE): in vivo experiments and roadmap for clinical application and commercialisation
Ina Gruh, iCARE

B16 ReSIGHT - Generation of transplantable hiPSC-derived retinal cells for treatment of retinal degeneration
Marius Ader, ReSIGHT

B17 Translation of human induced pluripotent stem cell (hiPSC) production to GMP
Thure Roman Adler, iPStem-RNA

B18 Zooming in on non-invasive cryopreservation of hiPSCs and neural derivatives: a dual center study using adherent vitrification
Johanna Kaindl, Stabil-Ice

Abstract No. B01: Neuro2D3

A stably self-renewing adult blood-derived induced neural stem cell exhibiting patternability and epigenetic rejuvenation

Chao Sheng[1,2], Lea Flitsch[1,], Johannes Jungverdorben[1,2], Hendrik Wiethoff[1], Qiong Lin[3], Daniela Eckert[1], Matthias Hebisch[1,2], Jaideep Kesavan[1], Julia Fischer[1], Beatrice Weykopf[1], Linda Schneider[1], Dominik Holtkamp[4], Heinz Beck[4], Andreas Till[1], Ullrich Wüllner[2,5], Michael Ziller[6], Wolfgang Wagner[3], Michael Peitz[1,2],* and *Oliver Brüstle[1]*

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Recent reports suggest that somatic cell-derived induced neurons (iNs), but not induced pluripotent stem cell (iPSC)-derived neurons largely preserve age-associated traits such as age-specific DNA methylation patterns, transcriptomic aging signatures and nuclear lamina-associated changes. One key question to be addressed in our Neuro2D3 consortium is whether and to what extent age-associated epigenetic and transcriptional signatures are conserved upon direct conversion of adult human peripheral blood cells (PBCs) into stably proliferating induced neural stem cells (iNSCs). Employing restricted and integration-free Sendai virus-mediated expression of SOX2 and c-MYC we generated a fully functional, bona fide NSC population from PBCs, which remains highly responsive to regional patterning cues and is capable of differentiating into functional neurons and glia. We then studied DNA methylation of CpG sites known to reflect age-associated changes of the methylome. Interestingly, newly converted low passage iNSCs display a profound loss of age-related DNA methylation signatures, which further erodes across extended passaging, thereby approximating the DNA methylation age of isogenic iPSC-derived neural precursors (NPCs). This remarkable epigenetic rejuvenation is accompanied by a lack of age-associated transcriptional signatures and absence of cellular aging hallmarks. Thus, despite the lack of a pluripotency transit, iNSCs exhibit reprogramming of age-associated traits to a degree similar to that observed in iPSC-derived NPCs. This demarcates iNSCs from iNs and makes them equally suitable for disease modeling and regenerative applications – prospects we underpin with first data on the use of iNSCs for studying protein aggregation in a polyglutamine disorder and for neural transplantation in the adult mammalian brain.

Supported by Bundesministerium für Bildung und Forschung (BMBF), Innovative Stammzelltechnologien für die individualisierte Medizin, Förderkennzeichen: 01EK1603

Abstract No. B02: Neuro2D3

A human iPSC-based 3D system for high content imaging of Alzheimer disease-specific pathology and drug discovery

Matthias Hebisch[1,], Sven Fengler[2,*], Vesselina Semkova[3], Simone Haupt[3], Beatrice Weykopf[3], Kevin J. Washicosky[4], Doo Yeon Kim[4], Philip Denner[2], Eugenio Fava[2], Michael Peitz[1],* and *Oliver Brüstle[1]*

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* Presenting authors

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease in the aged population. So far, no suitable treatment for AD patients exists. Major hallmarks of AD are deposition of amyloid β (Aβ) plaques in the extracellular space and the formation of intraneuronal neurofibrillary tangles composed of hyperphosphorylated aggregated tau. Since these pathognomonic alterations encompass both intra- and extracellular compartments, they are difficult to model in conventional 2D iPSC models. For that reason, a key aim of the Neuro2D3 consortium is to facilitate AD drug candidate discovery by combining 3D matrix cultures with multiparametric high content and high throughput screening tools. To approach this goal, we first modified the safe-harbor locus AAVS1 of human iPSCs to conditionally overexpress familial AD (FAD) mutations in the genes encoding the amyloid precursor protein (APPSwe/Lon) and Presenilin-1 (PS-1ΔE9). Transgenic human iPSCs were differentiated into stably expandable neuroepithelial stem cells and embedded in a 3D matrix for long-term differentiation. After 8 weeks, induced 3D cultures displayed Aβ deposits that strongly resemble amyloid plaques in size and structure that can be visualized by specific autofluorescence and amyloid dyes such as Thioflavin T. Furthermore, induced cultures display an intracellular accumulation of p-tau, dystrophic neurites and an increased fraction of apoptotic cells. In order to adapt our 3D model to multi-parametric high-content analysis (HCA), a prerequisite for preclinical drug discovery, we currently transfer the system to 384-well microtiter plates and establish automated protocols for sample preparation, image acquisition as well as image analysis pipelines. We anticipate that HCA combined with an innovative chemogenomic screening approach will enhance the understanding of molecular mechanisms underlying AD disease pathology and provide an avenue for the identification of AD drug candidates.

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Abstract No. B03: HIT-Tau

High Throughput Approaches for the Individualized Therapy of Tau-Related Diseases (HIT-Tau)

Tabea Strauss[1,2,], Amir Tayaranian Marvian[1,2], Ashutosh Dhingra[3], Sigrid C. Schwarz[1], Peter Heutink[3],* and *Günter Höglinger[1,2]*

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Proteinaceous deposits of the microtubule-associated protein tau (MAPT) are the main hallmark of neurodegenerative diseases called tauopathies. Genome-wide association studies (GWAS) have identified homozygosity for the *MAPT* haplotype H1 as an important risk factor in tauopathies such as PSP (odds ratio 5.5), CBD (odds ratio 3.7) and the synucleinopathy Parkinson's disease (odds ratio 0.8), as opposed to the protective haplotype H2. With a frequency in the Caucasian population between 50% - 70%, the *MAPT* haplotype H1/H1 is a highly relevant genetic predisposition.

We aim to study the possibilities to modify the molecular consequences of this MAPT-dependent genetic predisposition by high throughput approaches. Therefore, we identified 12 induced pluripotent stem cells (iPSC) lines with the respective haplotypes (H1/H1, H1/H2, H2/H2) to generate a highly relevant model of tau-dependent neurodegeneration.

To differentiate the iPSCs into neurons, we employed a recently developed cell model that uses NGN-2 overexpression to induce neuronal differentiation. Immunoblot and immunocytochemical characterization of this cell model revealed that they express neuronal markers including tau protein after eight days of differentiation. Tau expression increased over a prolonged differentiation period of 30 days. Further analysis of pathological aspects such as tau uptake, release, and aggregation, as well as neurodegeneration is ongoing. In order to investigate the impact of this haplotype on tau uptake and aggregation, we generated recombinant 2N4R tau in a bacterial system and produced fibrillar tau-aggregates that will be employed as seeds for uptake and intracellular aggregation.

Establishing this model, we will be able to identify screenable MAPT-dependent phenotypes to perform high-throughput screens (CrisprCas9, shRNA, compound libraries) for druggable targets and drug candidates to prevent tau-dependent neurodegeneration.

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Abstract No. B04: PDdementia

Identification of compounds preventing cognitive decline in Parkinson's disease patients using clinically correlated iPS cell models

Lydia Reinhardt[1], Arun Pal[2], Amayra Hernández-Vega[3], Susanne Kordes[4], Kathrin Brockmann[5,6], Jessica Bellmann[1], David Schöndorf[5,6], Andrea Meixner[7], Benjamin Schmid[5,6], Peter Reinhardt[1†], Hannes C. A. Drexler[8], Santiago Tena[1], Marius Ueffing[7], Hans R. Schöler[9,10], Michela Deleidi[5,6], Christian Johannes Gloeckner[6,7], Matthias Baumann[4], Bert Klebl[4], Anthony Hyman[3], Simon Alberti[3], Thomas Gasser[5,6], Andreas Hermann[1,2,11], and *Jared Sterneckert[1,*]*

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Although Parkinson's disease (PD) is best known as a motoric disorder due to the loss of dopaminergic neurons, PD is actually a progressive disorder with an end stage often marked by dementia and cognitive decline. No treatments are available to prevent the progression of PD. Human genetics studies have identified that polymorphisms in Tau, α-Synuclein, and LRRK2 are risk alleles and promote PD progression. To better understand PD pathology, we studied induced pluripotent stem cell-derived neurons with LRRK2 G2019S and isogenic gene-corrected controls. We demonstrated that human neurons with the *LRRK2* G2019S mutation show reduced organelle trafficking in the distal axon, which is rescued by LRRK2 kinase inhibitors. Phospho-proteomics reveals that phosphorylation of multiple microtubule-associated proteins (MAPs), including Tau, is regulated by *LRRK2* G2019S and associated with degradation of microtubules. Abolishing a Tau phospho-site ameliorated axonal trafficking and reduced the levels of α-Synuclein protein. We also found that neurons with *LRRK2* G2019S were more susceptible to seeding with fibrils of α-Synuclein protein. Significantly, we found that this spreading was dependent on Tau-phosphorylation. Since α-Synuclein aggregates correlate in time and space with clinical phenotypes, this assay was transferred to the Lead Discovery Center to identify compounds that inhibit α-Synuclein spreading using a high-throughput screening (HTS) campaign. The LDC has optimized multiple parameters of this assay and, as a next step, a pilot screen with several thousands of compounds will be performed in preparation for a subsequent HTS campaign. The Alberti team is developing biophysical models of PD pathology – particularly using Tau phase separation – to identify PD risk factors, validate hit compounds, and enable hit-to-lead generation. Finally, using samples from patient cohorts, the Thomas Gasser's department is using genomic markers and biomarkers to stratify patients and identify the optimal time to administer the compounds that we identify.

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Abstract No. B05: MAIV

Using reprogramming to model human amyotrophic lateral sclerosis disease in vitro

Amel Falco[1,2], María Demestre[3], Marcus Conrad[4], Wolfgang Wurst[4], Tobias M. Böckers[3], Magdalena Götz[2,5], and *Sergio Gascón[1,2,*]*

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An important limitation to study the pathology of the CNS is that living human neural cells are not easy to obtain. To overcome this problem techniques based on iPSCs reprogramming and direct reprograming have recently emerged. In this study we used different reprogramming approaches of patient derived keratinocytes and fibroblasts to model human ALS in vitro. As ALS is a neurodegenerative condition that typically affects motor neurons and muscle cells, both cell types are desired in this study. In the first approach we reprogrammed keratinocytes into iPSCs that thereafter differentiate into interacting motor neurons and muscle cells, and analyze several properties of the neuromuscular junction. However, the acquisition of pluripotency implies resetting of cellular age, which is particularly adverse to explore cellular age in the context of ALS. Therefore, we also accessed to neuronal and muscle cells through direct reprogramming strategies that do not imply cell rejuvenation. For neuronal direct reprogramming, we use a method based on the retroviral-mediated expression of Neurog2, Bcl-2 and Islt1. This approach yielded a pure population of motor neurons from human fibroblasts. In addition, reprogramming of fibroblasts into myoblasts is accomplished by co-expression of Myc and MyoD. We will compare our results from cultures obtained through iPSCs and direct reprogramming approaches. Differences in phenotypes might reveal the extent to which age determines the penetrance of ALS.

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Abstract No. B06: HiPSTAR

HiPSTAR: Humane iPS-zellbasierte Blut-Hirn-Schranken Technologie in der Alzheimerforschung

Antje Appelt-Menzel[1], Winfried Neuhaus[2], Undine Haferkamp[3], Matthias Jung[4], Uwe Marx[5], Ursula Müller-Vieira[6], Julia Susewind[6], Manuela Meyer[6], Lothar Terfloth[7], Dan Rujescu[4], Ole Pleß[3,],* and *Marco Metzger[1]*

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Im Verbundprojekt HiPSTAR (Förderkennzeichen 01EK1608A-F) ist das übergeordnete Ziel, ein neues *in vitro* Modell der humanen Blut-Hirn-Schranke (BHS) zu entwickeln, welches aus induziert pluripotenten Stammzellen (iPS) abgeleitet ist.

Dieses Modell soll als Forschungswerkzeug eingesetzt werden, um Entstehungsmechanismen sowie neuartige Diagnose- und Therapiekonzepte für Morbus Alzheimer zu untersuchen. Diese Erkrankung zählt derzeit zu den häufigsten Demenzerkrankungen mit steigenden Prognosen.

Innerhalb des Förderzeitraumes ist es Aufgabe des interdisziplinären Verbundes, neben einem „gesunden“ BHS-Modell ein neuartiges Alzheimer BHS-Modell basierend auf Zellen mit funktionellen Mutationen in Alzheimer-relevanten Genen aufzubauen. Die Modelleigenschaften sollen durch dynamische Kultivierung, z.B. in Mikrofluidik-Chips, und Stimulation der Zellen beispielsweise durch Aβ-Oligomere beeinflusst werden. Mit Hilfe ausgewählter Referenzsubstanzen und Alzheimer-relevanter Medikamente werden die Modelle prävalidiert und mit *in vivo* Daten korreliert. Diese Ringstudien sollen den Weg für eine Zertifizierung der Technologie für die breite Anwendung in der Wirkstoffforschung legen. Weiterhin wird ein *in silico* Modell in Form eines mechanistischen Netzwerkes entwickelt, um zelluläre Targets zu identifizieren und zukünftig die BHS-Permeabilität von Medikamenten vorhersagen zu können.

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Abstract No. B07: Micro-iPS-Profiler

Individualisierter Mikrofluidik-Multiorgan-Chip Multimode Profiling Plattform basierend auf iPS-Technologie

Juliane Schnabel[1,], Nadine Hlawatsch[1], Stefan Wölfl[2], Jannick Theobald[2], Xinlai Cheng[2], Alexander S. Mosig[3], Martin Raasch[3], Ralf Mrowka[4], Stefanie Reuter[4], Susanne Ackermann[4], Anika Westphal[4], Andreas Kurtz[5], Krithika Hariharan[5],* and *Claudia Gärtner[1]*

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Derzeit beruht die Vorhersage der therapeutischen Wirksamkeit und der Sicherheit von Medikamenten auf Untersuchungen im Zellkultur- und Tiermodell. Mit dem Micro-iPS-Profiler wird die Entwicklung Organ-ähnlicher Strukturen aus Derivaten induzierter pluripotenter Stammzellen (iPS) in einem mikrofluidischen Chip ermöglicht, um damit Wirkstoffforschung automatisiert und individualisiert durchführen zu können. Der mikrofluidische Chip wird durch Implementierung entsprechender Module eine präzise steuerbare Medienzuführung und eine Abführung der Zellstoffwechselprodukte ermöglichen. Die Integration der entsprechenden Sensorik für die Analyse der entscheidenden Parameter wie Sauerstoffgehalt und pH-Wert erlaubt ein optimiertes Prozessmonitoring während der Analysen. Durch die Entwicklung eines entsprechenden iPS-Profiler-Betriebsgerätes soll ein weitestgehend nutzerunabhängiges System bereitgestellt werden.

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Abstract No. B08: Micro-iPS-Profiler

Medikamentenscreening sichtbar gemacht: ein humanes iPSC-basiertes, mikrophysiologisches Model der Leber und der Niere

Martin Raasch[1,], Stefanie Reuter[2], Susanne Ackermann[2], Anika Westphal[2], Enrico Fritsche[3], Krithika Hariharan[3], Jannick Theobald[4], Xinlai Cheng[4], Juliane Schnabel[5], Nadine Hlawatsch[5], Claudia Gärtner[5], Stefan Wölfl[2], Andreas Kurt[4], Ralf Mrowka[2], Alexander S. Mosig[1]*

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In den letzten Jahren wurden Organ-on-Chip- (OoC) Modelle entwickelt, um die Grundlagenforschung und das Medikamentenscreening in präklinischen Studien zu verbessern. Humane induzierte pluripotente Stammzellen (hiPSCs) tragen zu diesen Modellen bei, da sie aus patientenspezifischen Zelltypen generiert werden und nicht die bei immortalisierten Zelllinien bekannten Beeinträchtigungen aufweisen. In unserer Studie werden hiPSC-basierte OoC-Modelle der Leber und Niere erstellt, validiert und zu einer Leber-Nieren-Achse gekoppelt. Modifikationen von Reportergenen in den Zielzellen erlauben eine Echtzeit-Bewertung des Zellverhaltens und Medikamenten-vermittelter Toxizität. Die Leber ist durch CYP-Enzyme für etwa 75% des Medikamenten-Metaboliten-Umsatzes verantwortlich und somit das zentrale Organ im menschlichen Medikamenten-Metabolismus. Bisher wird die präklinische Medikamententestung mit Hilfe von Tierexperimenten durchgeführt. Diese Ergebnisse sind jedoch nicht vollständig auf den Menschen übertragbar. Daher stellen humane OoC-Leber-Modelle eine vielversprechende Alternative dar, um auf zellulärer Ebene toxische Nebenwirkungen im individuellen genetischen Hintergrund zu analysieren. Verglichen mit immortalisierten Zelltypen verfügen hiPSCs über stabile Karyotypen sowie potentiell unlimitierte Verfügbarkeit. HiPSCs wurden bereits verwendet, um Organoide der Leber und Niere zu generieren [1, 2]. Bisher reflektiert aber keines dieser Modelle die Kommunikation zwischen den Organen zum Zwecke eines effizienten Medikamentenscreening-Prozesses. Unser funktionelles hiPSC-basiertes OoC-Leber-Modell exprimiert verschiedene CYP-Enzyme, sekretiert adäquate Mengen an Albumin und Harnstoff und weist Glykogen- und Triglycerit-Metabolismus auf. Dieses Lebermodell wird für ein effizientes Medikamentenscreening weiterhin mit hiPSC-basierten Nierenmodellen auf einer Biochip-Plattform kombiniert. Zudem werden geeignete Reportergene, die ungewünschte Nebenwirkungen von Medikamenten aufzeigen, mittels Crispr/ Cas-9-Technololgie mit einer Luciferase im Genom markiert. Diese Modifikation ermöglicht die Echtzeit-Messung der Aktivität der Reportergene als Antwort auf die Medikamente in einem Screen und gibt somit Aufschluss auf eine mögliche Toxizität. Die generierten iPSC-basierten OoC-Modelle weisen alle Hauptzelltypen der Leber sowie deren Funktionalität auf. Weiterhin werden Nierenkompartimente, wie Glomerulus, proximaler und distaler Tubulus generiert und charakterisiert und spezifische Reportergene für Zellschädigungen in den Nierenzellen mit einer Luciferase versehen, um individuelle Medikamentenscreens zu realisieren.

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Abstract No. B09: PancChip

PancChip – Optimizing a microfluidics/iPSC system to model endocrine and exocrine development

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The pancreas is both an exocrine and endocrine organ. It plays a pivotal role in digestion of food and regulation of blood glucose. While the destruction of the endocrine part of the pancreas causes diabetes mellitus, destruction of the exocrine part by chronic pancreatitis leads to pancreatic insufficiency and a dramatically increased risk for pancreas cancer. In this presentation we will report on the achievements of the three partners in the PancChip consortium.

For the endocrine research field we show how the CRIPSR/Cas9 technology can be used to generate iPSC reporter cell lines to improve cell differentiation protocols for obtaining functional mature like beta cells. In particular, we demonstrate the generation of two fluorescent iPSC reporter lines, one for the expression of the regulatory protein Flattop and another for the expression of the hormone insulin. Flattop is a Wnt/planar cell polarity pathway gene known to be expressed in a fraction of beta-cells with high insulin secretory function and low expression in proliferating beta-cells. Combining the single reporter iPSC lines to a double insulin-Flattop iPSC reporter line we reached one major goal of the PancChip project, to create a tool to discern mature and immature beta-cells. We are now able to optimize the differentiation protocol to control embryonic lineage fate bifurcations in order to efficiently yield acinar and ductal cell types. Upon addition of small biomolecular signaling molecules and growth factors it is also possible to specifically induce in a 3D tissue model acinar or ductal development, while inhibiting the respective other lineage with inhibitors.

A major goal for the exocrine research field of the PancChip project was to generate iPSC lines from patients with hereditary chronic pancreatitis (HCP) to enable first disease models. First patient derived iPSC with mutations in the cationic trypsinogen and Spink1 genes were used to prove an increased autodigestion or disrupted protein folding finally resulting in chronic pancreatitis.

In the overarching microsystem technology project, we managed long term culture of iPSC on a microfluidic chip, an important step in the implementation of automated cell differentiation protocols with the goal to derive insulin responsive beta-cells. For standardization and testing of 3D cell culture systems increasing the maturation rates of iPSC towards mature exocrine and/or endocrine cell lineages we developed a new microfluidic large scale integration chip platform. For the production of the chip a new 3D printing process was applied and will be presented. An outlook on the combination of the microfluidic and the respective iPSC systems is given.

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Abstract No. B10: StemNet

StemNet: a gene network modeling approach to engineer stem cell-derived hepatocyte-like cells

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The differentiation of stem cells to hepatocyte-like cells (HLC) offers the perspective of unlimited supply of human hepatocytes. In our previous work we demonstrated that the gene expression profile of HLCs still includes prominent features of liver, intestine, fibroblast and stemness that negatively affect the degree of differentiation in comparison to primary human hepatocytes. However, due to the limitations of bulk transcriptomic analysis it remains unclear whether HLCs represent homogenous populations of cells with chimeric identities or include subpopulations that arise from uncontrolled signaling dynamics in the differentiation program. We now employ an approach of single cell transcriptomics, epigenetics and phospho-proteomics combined with advanced statistical analysis to identify key components of non-liver associated differentiation that currently prevent full hepatic maturation. In the BMBF network StemNet we will make use of our insights to improve terminal differentiation of HLCs through targeted interventions.

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Abstract No. B11: Stabil-Ice

Enabling large-scale cryopreservation by sterile vitrification of adherent human induced pluripotent stem cells and their neural derivates (Project "Stabil-Ice")

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State of the art in cryopreservation of stem cells and their derivates is slow freezing as dissociated single cells or small clusters in suspension. This method is usually efficient enough for stock keeping cells like human induced pluripotent stem cells (hiPSCs) but has to be followed by time-consuming re-expansion steps to generate sufficient cell numbers for application. The loss of cells after cryopreservation of hiPSCs in general is much higher than 50%, and the cells cannot be immediately applied in experimental studies since they need time to recover or being manipulated into desired derivates. However, adherent cells or multicellular systems are increasingly relevant for biomedical research but are even more sensitive to the damaging mechanisms resulting from ice crystal formation (e.g. osmotic shock, mechanical ruptures). To prevent these damages, a second cryopreservation regime, the so-called vitrification, can be applied that avoids ice crystallization and thus enables preservation of adherent cell systems. However, it requires skilled handling and small sample sizes and is therefore considered as not suitable for bulk storage. To overcome these limitations and enable a sterile vitrification for large cell numbers in an adherent state, we introduce a multi-usage cell culture disposable covering all relevant steps in stem cell workflows: cultivation, differentiation/manipulation, cryopreservation, storage and immediate application. This novel disposable is validated by a comparative multi-centre study, examining the enabled adherent vitrification against standard suspension-based slow freezing of six hiPSCs lines and their neural derivates. Our data shows superior performance of vitrification over slow freezing of both cell systems especially regarding the recovered cell number. Together with the option to parallelize the disposable into a standardized multi-well format, a sterile vitrification of adherent multi-cellular systems is enabled and can provide ready-to-use cell products for a variety of biomedical purposes.

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Abstract No. B12/13: iMACnet

Genetically corrected iPSC-derived macrophages (i-MAC) for innovative gene therapeutic strategies (iMACnet Consortium –Subprojects 2, 3, and 4, Hannover Medical School)

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Genetically corrected iPSC-derived macrophages (i-MAC) for innovative gene therapeutic strategies (iMACnet Consortium –Subprojects 1 and 5, Medical Center-University Freiburg)

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iMACnet is a collaborative research project that assesses the clinical potential of genetically corrected induced pluripotent stem cell derived macrophages (i-MACs) as an easy, reliable, safe, and highly standardized cell source for innovative gene and cell therapies. The project aims to demonstrate a proof of concept in two severe congenital diseases affecting the myeloid cell compartment: hereditary Pulmonary Alveolar Proteinosis (herPAP) and Chronic Granulomatous Disease associated colitis (CGD-Col). To this point, iMACnet (i) establishes the GMP-compliant generation, gene editing and quality control of patient derived iPSCs, (ii) develops genetic switches to ensure the safety of iPSC and thereof derived progeny, (iii) establishes the large scale and GMP compliant generation of functional macrophages from genetically corrected iPSCs using bioreactor technology and (iv) explores the therapeutic potential of i-MACs in a humanized mouse model of herPAP and *in vivo* models of CGD-col. Data on the GMP compliant gene correction of iPSCs and large scale cell production as well as first transplant data in a humanized herPAP model will be presented.

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Abstract No. B14/15: iCARE

Induced pluripotent stem cells for Clinically Applicable heart REpair (iCARE): cell production incl. regulatory issues

Induced pluripotent stem cells for Clinically Applicable heart REpair (iCARE): in vivo experiments and roadmap for clinical application and commercialisation

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Myocardial infarction and cardiac insufficiencies are a leading cause of pre-mature death worldwide. Cellular therapies based on induced pluripotent stem cells (iPSCs), for the first time, offer a potent cell source for the *de novo* generation of human cardiomyocytes, which might serve to replace lost heart muscle. These are considered innovative but complex, multi-layered therapeutic concepts. Addressing the elaborate requirements, the iCARE team is composed of basic scientists with leading expertise in hiPSC technology, experts in clinical process scale up and toxicology testing, veterinarians and clinician scientists experienced in preclinical large animal models, imaging and clinical translation of innovative therapies. Furthermore, a leading Biotech company with wide experience in the production of GMP-compliant cell culture and processing products, an SME qualified in the production of biological implants, and experts in ethical and legal aspects of life sciences are involved. This unique consortium closely cooperates towards developing clinical scale up of the respective cell products including numerous GMP-compliant components such as culture media and other life science products. Moreover, clinical and commercial translation of hiPSC-based heart repair strategies are spearheaded applying the most relevant pre-clinical model, i.e. non-human primates, to assess hiPSC-cardiomyocyte function and safety. Importantly, the planned project aims at answering the question, whether the injection of small iPSC-derived muscle aggregates can lead to substantial recovery of heart function without elimination of existing scar tissue.

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Abstract No. B16: ReSIGHT

ReSIGHT - Generation of transplantable hiPSC-derived retinal cells for treatment of retinal degeneration

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Degeneration of the light-sensing photoreceptors results in vision impairment and eventually complete blindness and represents one of the main causes for disability in industrialized societies. Conditions include age-dependent macular degeneration (AMD) and inherited forms of retinal degeneration like retinitis pigmentosa (RP). With more than 2 million affected individuals in Germany alone, retinopathies represent a massive burden for patients and their relatives besides having a significant socio-economic impact. Currently, no established therapies for the replacement of photoreceptors and the supporting retinal pigment epithelium (RPE) are available. Within the 'ReSIGHT' consortium the generation of human iPSC-derived photoreceptors and RPE cells for transplantation into animal models of late-stage retinal degeneration will be evaluated. An update of our studies will be presented including approaches for the optimization of iPSC-retinal organoid generation, identification of cell surface markers for target cell enrichment and quality control besides histological and functional assessment of donor cells following pre-clinical transplantation.

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Abstract No. B17: iPStemRNA

Translation of human induced pluripotent stem cell (hiPSC) production to GMP

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Translation of hiPSC research to therapy requires hiPSC lines manufactured under authorization regarding §13AMG in compliance with guidelines for Good Manufacturing Practice (GMP).

The BMBF grant consortium *iPStemRNA* is aiming at developing a process to generate hiPSCS using the modified mRNA technique employing certified reagents and processes that are in compliance with GMP. As a starting material, we will use fibroblasts from donors harboring HLA class I haplotype homozygous alleles that provide MHC-I immunocompatibility with a large part of the population in Germany. The process implies challenging tasks that go beyond hiPSC generation, from donor screening, assessment of donor suitability, donor consent, material qualification, vendor assessment, and protocol establishment to process evaluation and quality control. Our final goal will be to deliver the community universal donor iPSC lines as starting material for Advanced Therapy Medicinal Products (ATMP).

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Abstract No. B18: Stabile-Ice

Zooming in on non-invasive cryopreservation of hiPSCs and neural derivatives: a dual center study using adherent vitrification

Johanna Kaindl[1,], Julia Majer[2], Ina Meiser[2], Annika Sommer[1], Judith Feldewerth[1], Jürgen Winkler[3], Beate Winner[1], Julia Neubauer[2,4]*, and *Heiko Zimmermann[2,4,5,6]*

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