



GermanStemCellNetwork

12th GSCN Conference

25 – 27 September 2024

Friedrich Schiller University
Jena

www.gscn.org



Program & Abstracts

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GermanStemCellNetwork

12th GSCN Conference

25 – 27 September 2024

Friedrich Schiller University
Jena

Organizer:

German Stem Cell Network (GSCN)

c/o Charité Universitätsmedizin
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10117 Berlin

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Welcome address



Dear colleagues and friends, partners and participants,

It is our greatest pleasure to welcome you to the 12th Conference of the German Stem Cell Network (GSCN) at the Friedrich Schiller University in Jena, one of the oldest universities in Germany. Life, light, and liberty, the three profile areas of our university highlight the importance of excellent science in Jena to understand how life is preserved and nurtured.

Jena is a lively and young city that receives every year a boost of energy from young people who come to the city by the river Saale to study. Thinking without boundaries spans from Friedrich Schiller, who taught in Jena, to the Zeiss-Planetarium, the oldest continuously operating

planetarium in the world that fascinates with digital full-dome projections. Embrace the rich history and knowledge this vibrant city offers during the conference. Maybe you will even discover the “Seven Wonders of Jena” during your stay?

The conference will cover the latest findings in stem cell biology and foster scientific exchange. There will be new and exciting findings and intense discussions in the oral sessions, during the poster sessions, and during the social get together events! Come and share our enthusiasm for stem cells in all flavors: pluripotent stem cells, adult stem cells, stem cells in development, cancer stem cells, or stem cells in the aging organism.

We feature many oral presentations that were selected from the large number of excellent abstracts that have been submitted, and we welcome our outstanding keynote speakers that are leaders in the field.

An interesting and broad industry exhibition will offer latest technologies and personal contact in combination with industry sessions of GSCN industry partners. There will also be the non-PI meeting, as well as the GSCN mentoring program workshop and the ELSA keynote lecture and symposium. Finally, we will enjoy two social events to continue networking after working hours. Check out the whole program on our online platform (Program & Speakers 2024 (gscn-conferences.org)).

The GSCN is a network of scientists enthusiastic about stem cells. Here, colleagues become friends, and we invite you to become part of this vibrant community, and to feel the joy of meeting other stem cell scientists. We welcome you to this conference and to the City of Light, Jena.

A handwritten signature in blue ink, appearing to read 'C. Waskow'.

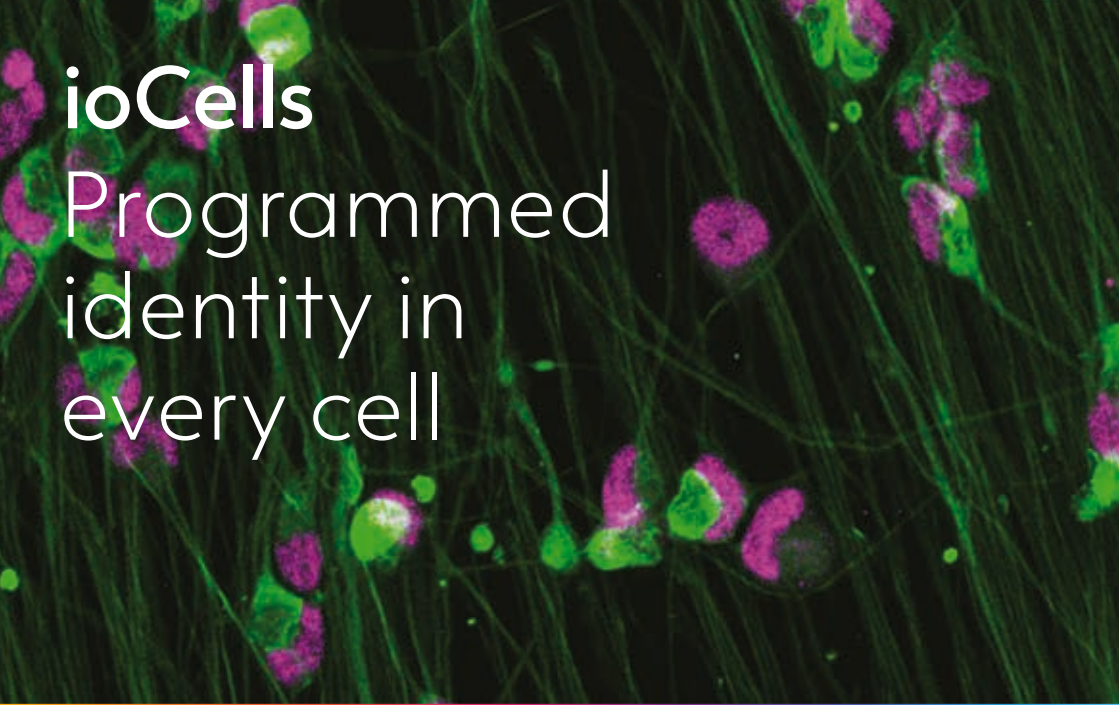
Prof. Dr. Claudia Waskow (*Jena*), *GSCN president*

For the program committee

Sina Bartfeld (Berlin) • Daniel Besser (Berlin) • Michael Cross (Leipzig) • Anne Grapin-Botton (Dresden) • Florian Heidel (Hannover) • Heiko Lickert (Munich) • Helen Morrison (Jena) • Hans Schöler (Münster) • Claudia Waskow (Jena)

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Conference Information

Venue

Friedrich Schiller University
Ernst-Abbe-Platz
Carl-Zeiss-Str. 3
07743 Jena

Date

Wednesday, 25 September to
Friday, 27 September 2024

Registration

Regular fee	€ 620,–	Student fee	€ 310,–
Regular member fee	€ 400,–	Student member fee	€ 200,–

The registration fee includes the admission to all scientific sessions, additional scientific events, poster and commercial exhibition, the get-together on 25 September and the networking evening on 26 September, lunch and coffee breaks, free internet access, conference documents including badge, final program (incl. abstracts) book.

Internet

Internet access via Wireless LAN is free of charge.

Poster exhibition

Posters will be displayed during the conference in two sessions. Authors are asked to be present at their poster during the poster session. Even numbers will be presented on Wednesday, 25 September from 17:30 – 18:30 h and odd numbers will be presented 18:30 – 19:30 h. On Thursday, 26 September even numbers will be presented from 16:30 – 17:30 h and odd numbers from 17:30 – 18:30 h.

You will find the number of your poster in this abstract volume. Please prepare the size DIN A 0 portrait for the poster. Otherwise, there are no further specifications. Posters in poster session I should be mounted on Wednesday 12:00 – 16:30 h and removed at latest on Thursday, 12:00 h. Poster in poster session II should be mounted on Thursday 12:00 – 16:30 h and removed latest on Friday at 14:00 h.

Poster session I (Poster number P001 – P064)

Wednesday, 25 September 2024, 17:30 – 19:30 h

Stem cells in disease modeling &

drug development: P001 – P038

Hematopoietic stem cells: P039 – P054

Pluripotency & reprogramming: P055 – P064

Poster session II (Poster number P065 – P126)

Thursday, 26 September 2024, 16:30 – 18:30 h

Stem cells in aging & disease: P65 – P077

Computational stem cell biology &

systems biology: P078 – P082

Stem cell organoids: P083 – P105

Stem cells in regenerative therapies: P106 – P121

Somatic stem cells & development: P122 – P126

The poster session I is supported by
PeptiGrowth



The poster session II is supported by
Bio-Technie



GSCN Awards

Travel awards

The following participants have been selected for the GSCN travel awards.

- *Eleonora Clemente* (CRTD, TU Dresden)
- *Júlia Csátári* (University Hospital Münster)
- *Bruno Ederer* (University of Leipzig)
- *Fatima Efendic* (University Medical Center Rostock)
- *Ian Ghezzi* (DKFZ Heidelberg)
- *Kimberly Hartl* (Charité Berlin)
- *Clara Hayn* (University of Bonn)

Poster awards

There will be two poster awards for each poster session. Members of the poster jury have a red dot on their badge – please present them your poster with due attention.

The awardees will receive € 500 each. Authors are asked to be present at the poster award ceremony on Friday, 27 September 2024 at 15:30 – 15:50 h in Hall 1.

The poster awards are supported by Thermo Fisher Scientific Germany

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Scientific awards 2024

A top-class commission supported the GSCN in choosing this year's awardees. The three awardees will give a presentation in the Presidential Symposium on Thursday, 26 September 2024 at 14:00 – 16:15 h.

GSCN 2024 Young Investigator Award: *Claudia Gerri*, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

GSCN 2024 Hilde Mangold Award: *Mina Gouti*, Max Delbrück Center, Berlin

GSCN 2024 Publication of the Year Award: *Jorge Lázaro Farré*, EMBL Barcelona ... *Miki Ebisuya*, TU Dresden for the publication "A stem cell zoo uncovers intracellular scaling of developmental tempo across mammals" in the journal *Cell Stem Cell*, 30: 938-949, 2023.

ELSA Symposium

What makes it conscious?

The topic of consciousness holds significant relevance for ethical discussions surrounding neuro-organoids, advanced human embryo models, and, from a distinct perspective, the use of artificial intelligence in analyzing complex biomedical and clinical data. At this year's conference, we aim to foster a dialogue on these important issues, and we are pleased to welcome bioethicist Garðar Ágúst Árnason from Akureyri, Iceland, as the keynote speaker for Wednesday evening. During the ELSA Symposium on Thursday morning, he will join us at the ELSA Symposium when we explore the latest advancements in the rapidly growing fields of neuro-organoid research, embryo models, and AI algorithms. This will serve as a foundation for discussing potential biological correlates that could be used to assess the "consciousness" of these entities. While the scope of this symposium will not be extensive enough to provide comprehensive answers, we rather expect an enlightening start of such a debate.

Social Events

Get-together

On Wednesday, 25 September 2024, 20:30 – 22:00 h, all participants and exhibitors are invited to the get-together with dinner buffet in the Mensa Philosophenweg 20, which is a 10-minute walk from the Friedrich Schiller University.

• • • •

Address: Mensa Philosophenweg
Philosophenweg 20 · 07743 Jena

Networking Event

The GSCN Networking Event with dinner buffet and DJ is open for all participants and exhibitors and will take place on Thursday, 26 September, 19:30 – 01:00 h at the Volksbad Jena. It is a 10-minute walk from the Friedrich Schiller University.

• • • •

Address: Volksbad Jena
Knebelstr. 10 · 07743 Jena





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Public outreach event (in German)

Aktuelle Forschung, neue Therapien und alte Medikamente: Stammzellen – die Alleskönner unter den Zellen

Wann: **Freitag, 27. September 2024, 18:30 – 20:00 Uhr**

Podiumsdiskussion mit Stammzellforscher*innen

Programm: 18:30 Uhr: Podiumsdiskussion & Kurzfilme
20:00 – 20:30 Uhr: Ausklang mit Gesprächen, Brezeln und Getränken

Ort: Volksbad Jena, Knebelstr. 10, 07743 Jena, Eintritt frei

Auf dem Podium sind:

- Prof. Dr. Claudia Waskow, Leibniz-Institut für Altersforschung & Uni Jena, Expertin für Blutstammzellen
 - Dr. Ole Pless, Fraunhofer ITMP Hamburg, Expertin für Wirkstofftestung und seltene Erkrankungen
 - Prof. Dr. Sina Bartfeld, TU Berlin, Expertin für Organoiden
 - Prof. Dr. Heiko Lickert, Helmholtz München, Expertin für Diabetes
- Moderation: *Stefanie Mahler*

Haare wachsen ein Leben lang. Wunden heilen wieder. Zellen im Blut erneuern sich alle drei Monate. Verantwortlich für diese Wunder sind Stammzellen, eine ganz besondere Zellart in unserem Körper: Ein Leben lang können sie sich teilen, vermehren und in alle verschiedenen Körperzellen entwickeln. Wenn bei diesen fundamentalen Vorgängen Fehler passieren, können Krankheiten entstehen. Daher liefert die Erforschung von Stammzellen wichtige Erkenntnisse für die Medizin.

Bei der Podiumsdiskussion berichten die eingeladenen Wissenschaftler*innen, welche Rolle Stammzellen bei der Erforschung von Krebs, Erkrankungen des Magen-Darm-Trakts, der Modellierung von Organoiden, bei Therapieansätzen für Diabetes oder bei neu verwendeten, „alten“ Medikamenten für seltene Erkrankungen spielen.

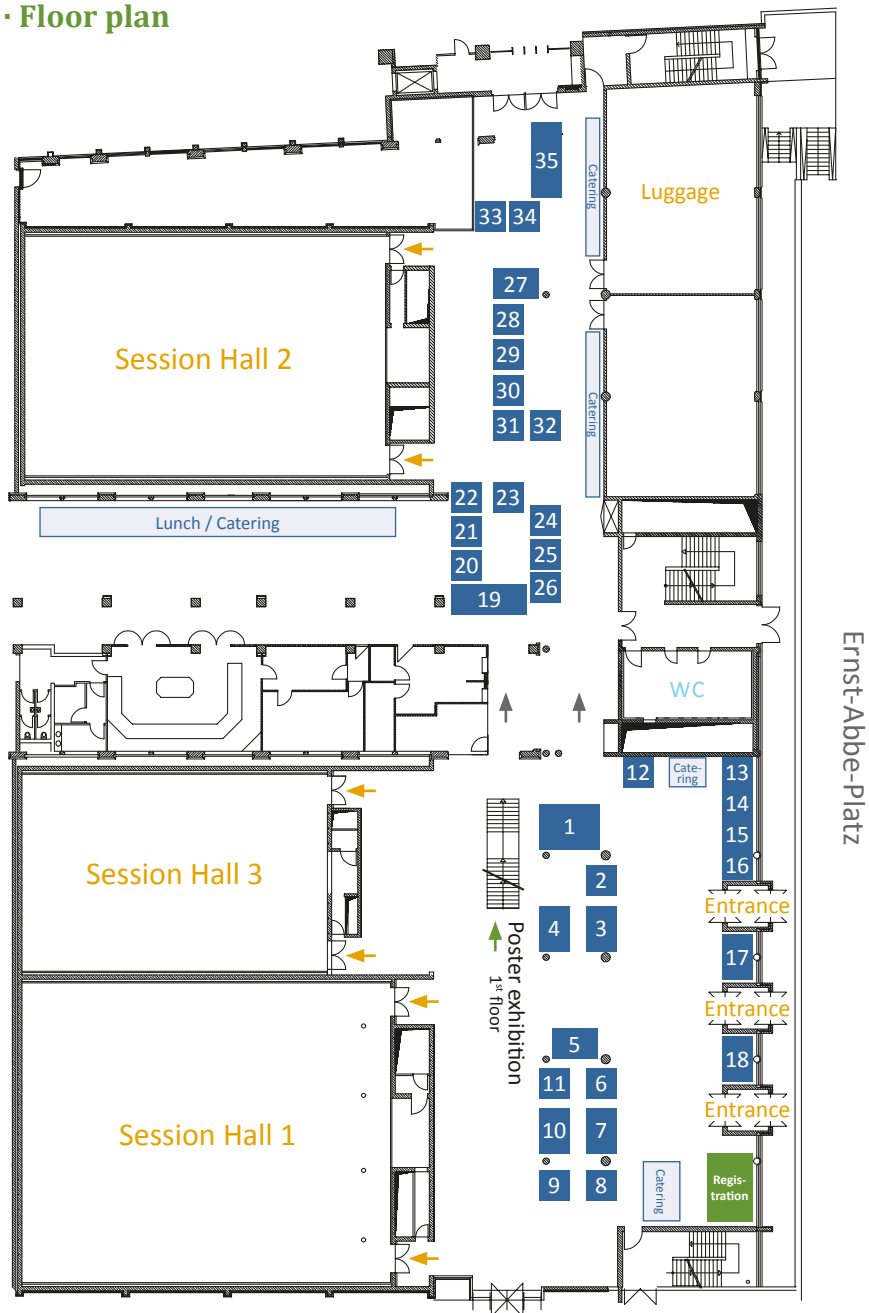
Mithilfe von Stammzellen lassen sich zukünftig neue Therapien entwickeln: Man kann aus ihnen kleine Gewebestückchen und Organoiden züchten und an diesen Medikamenten auf ihre Wirkung testen oder sie als Ersatz für verloren gegangenes Gewebe verwenden. Kurzfilme stellen die Forschung der Wissenschaftler*innen vor, in der Diskussion dreht es sich um Stand und Zukunft der Stammzellforschung sowie neue Behandlungsmöglichkeiten.

Floor plan

Sorted in alphabetical order		Sorted by booth number	
COMPANY	NUMBER		COMPANY
*Active Bioscience	11	1	*STEMCELL Technologies
*AMSBIO	2	2	*AMSBIO
Aseptic Technologies	9	3	*Thermo Fisher Scientific
*Axion BioSystems	6	4	*Sartorius
Becton Dickinson GmbH	28	5	*LIFE & BRAIN GmbH
BioLamina AB	13	6	*Axion BioSystems
*Bio-Techne	27	7	*bit.bio
BioThrust GmbH	15	8	PL BioScience GmbH
*BIOTREND Chemikalien GmbH	14	9	Aseptic Technologies
*bit.bio	7	10	*PeptiGrowth Inc.
*Cenibra GmbH	16	11	*Active Bioscience
*Consarctic	26	12	*Miltenyi Biotec B.V. & Co. KG
*Eppendorf	18	13	BioLamina AB
GCT – Netze rk büro	32	14	*BIOTREND Chemikalien GmbH
Getinge Deutschland	17	15	BioThrust GmbH
*ibidi GmbH	24	16	*Cenibra GmbH
*IDT Germany GmbH	21	17	Getinge Deutschland
INTEGRA Biosciences	20	18	*Eppendorf
*iotaSciences	22	19	TAKARA BIO EUROPE
*LIFE & BRAIN GmbH	5	20	INTEGRA Biosciences
MedChemExpress	35	21	*IDT Germany GmbH
*Miltenyi Biotec B.V. & Co. KG	12	22	*iotaSciences
New England Biolabs	23	23	New England Biolabs
Novogene Europe	29	24	*ibidi GmbH
*PeptiGrowth Inc.	10	26	*Consarctic
PL BioScience GmbH	8	27	*Bio-Techne
Proteintech	30	28	Becton Dickinson GmbH
*Sartorius	4	29	Novogene Europe
Scale Biosciences	34	30	Proteintech
*STEMCELL Technologies	1	31	Telight
TAKARA BIO EUROPE	19	32	GCT – Netze rk büro
Telight	31	33	Xceltis GmbH
*Thermo Fisher Scientific	3	34	Scale Biosciences
Xceltis GmbH	33	35	MedChemExpress

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Foyer · Floor plan



Program overview

Wednesday, 25 September 2024

	Friedrich Schiller University			
09:00 – 19:00	Registration			
	Hall 1			
11:00 – 11:30	Opening			
11:30 – 12:15	Keynote lecture I <i>Amander Clark</i>			
12:15 – 13:00	Keynote lecture II <i>Cantas Alev</i>			
13:00 – 14:30	Lunch break / Industry exhibition			
	Hall 1	Hall 2	Hall 3	
	Concurrent scientific working group session I			
14:30 – 16:00	Stem cells organoids	Stem cells in aging & diseases	Computational stem cell biology	
16:00 – 16:30	Break time / Industry exhibition			
			Hall 3	
16:30 – 17:30			GSCN Members Meeting	
17:30 – 19:30				Poster session I (P001 – P064)
17:30 – 18:30				posters with even numbers
18:30 – 19:30				posters with odd numbers
	Hall 1			
19:45 – 20:30	Keynote lecture III (on ELSA topics) <i>Gardar Agust Arnason</i>			
20:30 – 22:00	Informal GSCN get-together with dinner buffet at the Mensa Philosophenweg			

Thursday, 26 September 2024

	Friedrich Schiller University			
08:00 – 18:00	Registration			
	Hall 1	Hall 2	Hall 3	SR114
	Concurrent scientific working group session II			
09:00 – 10:30	Pluripotency, somatic stem cells & development	HEAL Symposium		ELSA Symposium
10:30 – 11:00	Break time / Industry exhibition			
	Hall 1	Hall 2	Hall 3	
11:00 – 12:30	Industry session: Technologies from GSCN industry partners			
	LIFE & BRAIN GmbH Sartorius Stedim Biotech STEMCELL Technologies UK bit.bio	Bio-Techne Getinge Thermo Fisher Scientific Eppendorf SE PeptiGrowth		
12:30 – 14:00	Lunch break / Industry exhibition			

	Hall 1		
14:00 – 16:15	Presidential Symposium		
14:00 – 14:45	Presidential Invite <i>Hans-Reimer Rodewald</i>		
14:45 – 15:15	Young Investigator Award <i>Claudia Gerri</i>		
15:15 – 15:45	Hilde Mangold Award <i>Mina Gouti</i>		
15:45 – 16:15	Publication of the Year Award <i>Jorge Lázaro Farré</i>		
16:15 – 16:30	Break time / Industry exhibition		
16:30 – 18:30			Poster session II (P065 – P126)
16:30 – 17:30			posters with even numbers
17:30 – 18:30			posters with odd numbers
19:00 – 01:00	GSCN Networking Event with dinner buffet & DJ at Volksbad Jena		

Friday, 27 September 2024

	Friedrich Schiller University		
08:00 – 16:00	Registration		
	Hall 1	Hall 2	Hall 3
	Concurrent scientific working group session III		
09:00 – 10:30	Stem cells in disease modeling & drug development	Hematopoietic stem cells	Stem cells in regenerative therapies
10:30 – 11:00	Break time / Industry exhibition		
11:00 – 12:30	Concurrent strategic working group session		
	Clinical trials & regulatory affairs	Career development	Technologies in stem cell research
12:30 – 14:00	Lunch break / Industry exhibition		
	Hall 1		
14:00 – 14:45	Keynote lecture IV <i>Camilla Forsberg</i>		
14:45 – 15:30	Keynote lecture V <i>Thomas Zwaka</i>		
15:30 – 16:00	Poster Award Ceremony and closing remarks		
	End of GSCN Conference 2024		
18:30 – 20:30	GSCN public outreach event with panelists Volksbad Jena (in German) Aktuelle Forschung, neue Therapien und alte Medikamente: Stammzellen – die Alleskönner unter den Zellen		



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Program

Wednesday, 25 September 2024

- 11:00 – 11:30 **Opening**
H1 **GSCN Conference 2024**
- 11:00 – 11:08 Welcome to Jena University
Lars-Oliver Klotz, Dean of the Faculty of Biological Sciences, Friedrich-Schiller University, Jena
- 11:08 – 11:16 Welcome by the Medical Faculty
Thomas Kamradt, Scientific Director and Dean of the Faculty of Medicine, University Hospital Jena
- 11:16 – 11:24 Welcome to Jena Science Campus
Dario Valenzano, Scientific Director, Leibniz Institute on Aging/FLI
- 11:24 – 11:30 Opening of GSCN Conference 2024
Claudia Waskow, President GSCN
- 11:30 – 12:15 **Keynote lecture I**
K1 – Building stem cell models for women's health research
Amander Clark, Broad Stem Cell Research Center, UCLA, Los Angeles, US
Chair: *Florian Heidel*
- 12:15 – 13:00 **Keynote lecture II**
K2 – Towards reconstituting human & non-human primate axial development in vitro
Cantas Alev, ASHBi Kyoto University, JP
Chair: *Sina Bartfeld*
- 13:00 – 14:30 **Lunch break / Industry exhibition**
- 14:30 – 16:00 **Concurrent scientific working group session I**
- 14:30 – 16:00 **Stem cells organoids**
H1 (Chairs: *Sina Bartfeld* & *Mina Gouti*)
- 14:30 – 15:00 T01– Infection and host cell tropism in the gut – organoids as model
Sina Bartfeld, TU Berlin
- 15:00 – 15:15 T02 – Human liver ductal cell heterogeneity and cell-state transitions in an organoid system
Javier Bregante Boixg, MPI-CBG Dresden
- 15:15 – 15:30 T03 – P53 activation in epithelial cells terminates the fetal-like regenerative state after colitis-associated injury
Kimberly Hartl, Charité Berlin

- 15:30 – 15:45 T04 – Cell type-specific epigenome profiling identifies H3K27me3 dynamics in the human developing neocortex
Mareike Albert, CRTD, TU Dresden
- 15:45 – 16:00 T05 – Assessing the impact of prenatal paracetamol exposure on early human forebrain development using cerebral organoids
Clara Hayn, University of Bonn
- 14:30 – 16:00 **Stem cells in aging & diseases**
H2 (Chairs: *Hartmut Geiger & Michael Rieger*)
- 14:30 – 15:00 T06 – The role of leukemic stem cells in patients treated with the BCL-2 inhibitor venetoclax
Andreas Trumpp, Heidelberg Institute for Stem Cell Technology HI-STEM
- 15:00 – 15:15 T07 – High fat diet impacts intestinal stem cell niche and causes hyperproliferation in the pig intestinal epithelium
Falk Farkas, Helmholtz Center Munich
- 15:15 – 15:30 T08 – Relevance of dormant cancer stem cells in disease propagation and therapy resistance in JAK2V617F-driven myeloproliferative neoplasms
Ian Ghezzi, DKFZ Heidelberg
- 15:30 – 15:45 T09 – Targeting SRSF2 mutations in leukemia with RKI-1447: a method to disrupt HSC division and alter nuclear formation
Natalie Kaushansky, Weizman Institute, Rehovot, IL
- 15:45 – 16:00 T10 – The inflammatory connection: insights from spatial mapping of human hematopoiesis in aging and blood malignancies
Borhane Guezguez, University of Mainz
- 14:30 – 16:00 **Computational stem cell biology**
H3 (Chairs: *Ingmar Glauche & Carsten Marr*)
- 14:30 – 15:00 T11 – Hematopoiesis by the numbers: advances in cellular barcoding and counting cell division
Leila Perié, Curie Institute, Paris, FR
- 15:00 – 15:15 T12/P – A scalable computational framework for annotation-independent combinatorial target identification in scRNA-seq databases
Maximilian Buser, Helmholtz Center Munich
- 15:15 – 15:30 T13 – Tracking bone marrow regeneration after CAR-T cell therapy in myeloma at spatial single-cell resolution
Jennifer Wing Lam Cheung, University Würzburg
- 15:30 – 15:45 T14 – Inferring the clonal dynamics of development from holistic barcoding: theory and application to tissue macrophages
Maurice Langhinrichs, DKFZ Heidelberg

15:45 – 16:00 T15/P – Unveiling the mechanisms of macrophage-mediated cytokine release syndrome in CAR-T cell therapy with mathematical modeling
Artur César Fassoni, TU Dresden

16:00 – 16:30 Break time / Industry exhibition

16:30 – 17:30 **General Meeting**

H3 Executive Board & Managing Director
Claudia Waskow, Hans Schöler, Heiko Lickert, Michael Cross, Andreas Trumpp, Daniel Besser

17:30 – 19:30 **Poster session I** (Poster number P001 – P064)

Foyer + H 6 Stem cells in disease & drug development: P001 – P038

(1st floor) Hematopoietic stem cells: P039 – P054

Pluripotency & reprogramming: P055 – P064

Even numbers: 17:30 – 18:30

Odd numbers: 18:30 – 19:30

19:45 – 20:30 **Keynote lecture III (on ELSA topics)**

H1 K3 – Consciousness as an ethical issue in neural organoid research
Garðar Ágúst Árnason, University of Akureyri, IS
Chair: *Tobias Cantz*

20:30 – 22:00 GSCN Get-together for all participants

Mensa Dinner buffet at the Mensa Philosophenweg 20

Philosophenweg (10-minute walk from the Friedrich Schiller University)

Thursday, 26 September 2024

09:00 – 10:30 **Concurrent scientific working group session II**

09:00 – 10:30 **Pluripotency, somatic stem cells & development**

H1 (Chairs: *Thomas Braun & Hans Schöler*)

09:00 – 09:30 T16 – Capture of human axial stem cells

Micha Drukker, Stem Cell Technology for Microphysiological Modeling, Leiden University, NL

09:30 – 09:45 T17 – The homeobox transcription factor DUXBL controls toti- to pluripotent transition

Felipe Lüttmann, TRON Mainz

09:45 – 10:00 T18 – SOX and POU transcription factors are older than stem cells and animals

Ralf Jauch, University of Hong Kong, HK

10:00 – 10:15 T19/P – Lifelong persistence of nuclear RNAs in the rodent brain

Sara Zocher, DZNE Dresden

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- 10:15 – 10:30 T20 – Epithelial-stromal communication via BMP signaling is a critical regulator of site specificity in the mouse oral mucosa
Kai Kretzschmar, Uni Würzburg
- 09:00 – 10:30 **HEAL Symposium**
H2 (Chairs: *Ulrich Martin & Robert Zweigerdt*)
- 09:00 – 09:30 T21 – Advancing cardiac therapeutics from animals to patients
Eldad Tzahor, Weizmann Institute of Science, Rehovot, IL
- 09:30 – 09:45 T22 – Lineage-specific cancer-related mutations in human pluripotent and adult stem cells
Jonathan Jung, Hebrew University of Israel, Jerusalem, IL
- 09:45 – 10:00 T23 – HLA-homozygous GMP iPSCs show efficient cardiac induction on a new differentiation platform featuring scalability and enhanced robustness
Soraia Martins, Catalent, Langenfeld
- 10:00 – 10:15 T24 – hPSC-cardiomyocyte production: standardization and protein-free upscaling to 2000 mL
Niels Kriedemann, MH Hannover
- 10:15 – 10:30 T25/P – Optimization of cardiomyocyte cryopreservation and quality control aspects for potency and residual iPSC contaminations
Fabienne Becker, University Düsseldorf
- 09:00 – 10:30 **ELSA symposium:**
SR114 (Chairs: *Stefan Hoffmann & Tobias Cantz*)
Panelists: *Garðar Ágúst Árnason & others*
- 10:30 – 11:00 Break time / Industry exhibition
- 11:00 – 12:30 **Industry session: Technologies from GSCN industry partners**
- 11:00 – 12:30 **Supporter I**
H1 (Chair: *Nina Cabezas-Wallscheid / Harald Stachelscheid*)
- 11:00 – 11:15 C01 – Assay-ready human microglia for automated image-based phenotypic subgrouping and targeted compound screening
Michael Peitz, LIFE & BRAIN GmbH (Bronze Supporter)
- 11:15 – 11:30 C02 – Advancing stem cell research with automated detection and isolation of stem cells and colonies
Jurij Kintz, Sartorius Stedim Biotech GmbH (Bronze Supporter)
- 11:30 – 12:00 C03 – Improved genetic stability of human pluripotent stem cell cultures passaged as single cells using eTeSR™
Adam Hirst, STEMCELL Technologies UK Ltd. (Gold Supporter)

- 12:00 – 12:20 C04 – Harnessing CRISPR-ready human iPSC derived cells for disease modelling and drug discovery in neurodegenerative diseases
Rebecca Northeast, bit.bio (Silver Supporter)
- 11:00 – 12:30 **Supporter II**
H2 (Chair: *Hartmut Geiger / Anne Grapin-Botton*)
- 11:00 – 11:15 C05 – Take your research translational the easy way: streamline your cell culture with our ready-to-use products and automated solutions
Volker Arps, Bio-Techne (Bronze Supporter)
- 11:15 – 11:35 C06 – Bioreactor systems | cell & gene therapy applications | state-of-the-art
Sascha Gandert, Getinge (Silver Supporter)
- 11:35 – 11:55 C07 – NK cell generation from iPSC suspension cultures
Sara Pérez Muñoz, Thermo Fisher Scientific (Silver Supporter)
- 11:55 – 12:15 C08 – From R&D to manufacturing: the cell as a product
Philipp Nold, Eppendorf SE (Silver Supporter)
- 12:15 – 12:30 C09 – Innovative synthetic peptide-based growth factor: overcoming challenges in stem cell medicine production
Robert Brownlee, PeptiGrowth (Bronze Supporter)
- 12:30 – 14:00 **Lunch break / Industry exhibition**
- 14:00 – 16:15 **Presidential Symposium**
H1 Chair: *Claudia Waskow*
- 14:00 – 14:45 **Presidential Invite**
PS1 – Deconvolution of stem and progenitor cell fates and immune lineages by polylox barcoding
Hans-Reimer Rodewald, German Cancer Research Center (DKFZ), Heidelberg
- 14:45 – 15:15 **GSCN 2024 Young Investigator Award**
PS2 – Fetal-maternal interface across species
Claudia Gerri, MPI-CBG Dresden
- 15:15 – 15:45 **GSCN 2024 Hilde Mangold Award**
PS3 – Building the next generation of neuromuscular models to study disease
Mina Gouti, MDC Berlin
- 15:45 – 16:15 **GSCN Publication of the Year 2023 – 2024 Award**
PS4 – A stem cell zoo uncovers intracellular scaling of developmental tempo across mammals
Jorge Lázaro Farré, TU Dresden & EMBL Barcelona, ES
- 16:15 – 16:30 **Break time / Industry exhibition**

- 16:30 – 18:30 **Poster session II** (Poster number P065 – P126)
Foyer + H 6 (with beverages)
(1st floor) Stem cells in aging & disease: P065 – P077
Computational stem cell biology & systems biology: P078 – P082
Stem cell organoids: P083 – P105
Stem cells in regenerative therapies: P106 – P121
Somatic stem cells & development: P122 – P126
- Even numbers: 16:30 – 17:30
Odd number: 17:30 – 18:30
- 19:00 – 01:00 **GSCN networking event for all participants**
The entrance fee is collected on site.
Event location: Volksbad Jena · Knebelstraße 10 · 07743 Jena

Friday, 27 September 2024

- 09:00 – 10:30 **Concurrent scientific working group session III**
- 09:00 – 10:30 **Stem cell in disease modeling & drug development**
H1 (Chairs: *Oliver Brüstle & Karl-Ludwig Laugwitz*)
- 09:00 – 09:30 T26 – iPSCs in drug discovery – from a user's and a personal perspective
Bert Klebl, Lead Discovery Center (LDC), Dortmund
- 09:30 – 09:45 T27 – Patient-specific iPSC-derived hepatic organoids as model for drug dosing and mRNA-based gene therapy
Malte Sgodda, Hannover Medical School (MHH)
- 09:45 – 10:00 T28 – Human multi-tissue organoids as an advanced teratogenicity model
Liam Wilson, Hannover Medical School (MHH)
- 10:00 – 10:15 T29 – Cellular metabolism senses exogenous fatty acids to reprogram dormant tumour cells into aggressive oncogenic cell states
Patricia Inacio, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden
- 10:15 – 10:30 T30 – iPSC modeling of bipolar disorder 1 based on polygenic risk scores
Tamara Krutenko, University of Bonn
- 09:00 – 10:30 **Hematopoietic stem cells**
H2 (Chairs: *Michaela Frye & Mick Milsom*)
- 09:00 – 09:30 T31 – The bonemarrow niche under stress
Marieke Essers, DKFZ & HI-Stem, Heidelberg

- 09:30 – 09:45 T32 – Apelin signaling restricts the formation of the blood stem cell-forming hemogenic endothelium
Jean Eberlein, University of Marburg
- 09:45 – 10:00 T33/P – Intrinsic reconstitution potential of developmentally restricted hematopoietic stem cells in non-conditioned immunocompromised mice
Marcel G.E. Rommel, UC Santa Cruz
- 10:00 – 10:15 T34 – Metabolic atlas of human bone marrow hematopoietic stem cells upon differentiation, aging and leukemia
Maria Eleni Lalioti, MPI-IE, Freiburg
- 10:15 – 10:30 T35 – The function of PD-L2 expression on human hematopoietic stem cells in allogeneic immune regulation at single cell resolution
Tessa Schmachtel, University Hospital Frankfurt
- 09:00 – 10:30 **Stem cells in regenerative therapies**
H3 (Chairs: *Michael Cross & Ulrich Martin*)
- 09:00 – 09:30 T36 – Tracking of early cell fate decisions of iPSCs based on DNA methylation
Wolfgang Wagner, RWTH Aachen
- 09:30 – 09:45 T37 – Pooled mesenchymal stromal cells fail to represent average donor cell characteristics
Janina Burk, vetmeduni, Vienna, AT
- 09:45 – 10:00 T38/P – Humanized osteoclasts: an interspecies chimeric immune cell
Gülce Percin, Leibniz Institute on Aging/FLL, Jena
- 10:00 – 10:15 T39 – Resolving human alpha versus beta cell fate allocation for the generation of stem cell-derived islets
Corinna Cozzitorto, Helmholtz Center Munich
- 10:15 – 10:30 T40 – Generation of designer stem cell-derived islets (SC-islets) to improve safety and efficacy of SC-based islet replacement therapy
Eunike Setyono, Helmholtz Center Munich
- 10:30 – 11:00 **Break time / Industry exhibition**
- 11:00 – 12:30 **Concurrent strategic working group session**
- 11:00 – 12:30 **Clinical trials & regulatory affairs**
H1 (Chairs: *Zoltán Ivics & Andreas Kurtz*)
- 11:00 – 11:30 S1 – Exploring public and government funding opportunities
Bettina Otto, Berlin Institute of Health (BIH)
- 11:30 – 12:00 S2 – High-Tech Gründerfonds – early stage seed investments
Christian Kannemeier, High-Tech Gründerfonds, Berlin

12:00 – 12:30 S3 – The National Office for Gene- and Cell Therapies (GCT): updates on activities and future impact
Elke Luger, GCT National Network Office, Berlin

11:00 – 12:30 **Career development**
H2 (Chairs: *Marieke Essers & Insa Schroeder*)
S4 – Publish or perish – feed back from a journal editor
Achim Breiling, EMBO Reports, Heidelberg

11:00 – 12:30 **Technologies in stem cell research**
H3 (Chairs: *Andreas Bosio & Sebastian Diecke*)

11:00 – 11:30 S5 – Cellular microenvironments fabricated with fluid walls for disease modeling
Edmond Walsh, University of Oxford, UK

11:30 – 12:00 S6 – Combining stem cell and device engineering for in vitro models of human physiology
Anna Herland, KTH Royal Institute of Technology, Solna, SE

12:00 – 12:30 S7 – Stem cell-derived vessels-on-chip for cardiovascular disease modeling
Matthias Meier, Helmholtz Center Munich

12:30 – 14:00 **Lunch break / Industry exhibition**

14:00 – 14:45 **Keynote lecture IV**
H1 K4 – Colorful aging? An aging-specific platelet differentiation path from hematopoietic stem cells
Camilla Forsberg, UC Santa Cruz, US
Chair: *Meritxell Huch*

14:45 – 15:30 **Keynote lecture V**
H1 K5 – Bat induced pluripotent stem cells as a model for viral tolerance and immunity
Thomas Zwaka, Icahn School of Medicine at Mount Sinai, New York, US
Chair: *Helen Morrison*

15:30 – 15:50 **Poster award ceremony**
H1 *Daniel Besser*
Poster awards
Travel awards

15:50 – 16:00 **Closing remarks by the GSCN president**
H1 *Heiko Lickert*

End of GSCN Conference 2024

Speaker abstracts

12th GSCN Conference
German Stem Cell Network

25 – 27 September 2024
Friedrich Schiller University
Jena

Keynote lectures: K1 – K5

- K1** Building stem cell models for women's health research
Amander Clark
- K2** Towards reconstituting human & non-human primate axial development in vitro
Cantas Alev
- K3** Consciousness as an ethical issue in neural organoid research
Garðar Ágúst Árnason
- K4** Colorful aging? an aging-specific platelet differentiation path from hematopoietic stem cells
Camilla Forsberg
- K5** Bat induced pluripotent stem cells as a model for viral tolerance and immunity
Thomas Zwaka

Abstract No. K1**Building stem cell models for women's health research***Amander Clark*

University of California, Los Angeles, Molecular Cell and Developmental Biology, USA

Ovarian function is a key factor in the reproductive health and overall wellness of women and girls. Major disorders and dysfunction of the ovary include primary ovarian insufficiency (POI), ovarian cancer and polycystic ovarian syndrome (PCOS), which collectively affect tens of millions of people around the world. Even in the absence of disease, ovarian function declines with age, and as a consequence, risk for cardiovascular disease, immune dysfunction, cognitive decline and osteoporosis increases. Therefore, models that recapitulate human ovarian biology are needed for biomedical innovation in order to understand and prevent ovarian disease and dysfunction, reverse ovarian aging and promote ovarian health and wellness for women and girls. In this talk, I will discuss our work building cells and models of the ovary using pluripotent stem cells, and demonstrate the importance of integrating knowledge from genetics, genomics, developmental biology and reproductive biology to achieve this goal.

Abstract No. K2**Towards reconstituting human & non-human primate axial development in vitro***Cantas Alev*

Kyoto University, Institute for the Advanced Study of Human Biology (ASHBi), Japan

Recent efforts to reconstruct complex developmental processes in vitro from stem cells open up exciting new opportunities for studying the biological principles, which govern the emergence of form and function during embryonic development in humans and other animals. Early embryonic developmental events including somitogenesis, during which the metameric body plan of vertebrates is laid out, have been extensively studied using model organisms such as mouse or chick but remain largely elusive and poorly understood when it comes to human and other primates. Using induced pluripotent stem cell (iPSC)-derived presomitic mesoderm (PSM), we previously succeeded to quantify oscillatory activity of the segmentation clock, a molecular oscillator believed to control the segmentation process [1]. Interestingly, these in vitro models of the segmentation clock did not show any sign of segmentation or somitogenesis despite the presence of oscillatory activity of clock genes such as HES7. Extending on these earlier findings we then asked whether we could recapitulate not only the clock but also the actual process of segmentation and epithelial somite formation in vitro. Utilizing again pluripotent stem cells as starting material we succeeded to establish a 3D in vitro model of human somitogenesis, which exhibited periodic formation of properly patterned epithelial somites in synchrony with the segmentation clock [2]. Our selforganizing ‘axioids’ reconstituted various morphological and molecular features of the emerging human embryonic axis and could be also used to study the pathogenesis of human congenital diseases of the spine. Currently, we are continuing to utilize axioids and other in vitro models of early embryonic development to study and increase our still limited understanding of development and disease in human and other primates.

[1] Matsuda M, Yamanaka Y,... Nakamura M, Matsumoto T, Yoshitomi H, Ikeya M, Kawakami N, Yamamoto T, Woltjen K, Ebisuya M, Toguchida J, Alev C*. *Recapitulating the human segmentation clock with pluripotent stem cells. Nature (2020) Apr1;580(7801):124-129*

[2] Yamanaka Y, Hamidi S,... Kurokawa Y, Mieda A, Eriksson R, Thompson JL, Kerwin J, Lisgo S, Yamamoto T, Moris N, Martinez-Arias A, Tsujimura T, Alev C*. *Reconstituting human somitogenesis in vitro. Nature (2023) Feb16;614(7948):509-520*

Abstract No. K3**Consciousness as an ethical issue in neural organoid research***Garðar Ágúst Árnason*

University of Akureyri, School of Humanities and Social Sciences-Faculty of Education, Iceland

There seems to be a near consensus about two things: first, that human neural organoids are far from having the possibility of developing consciousness, and second, that it would be a major ethical issue if human neural organoids could develop consciousness. A common recommendation is that the situation should be carefully monitored, and appropriate regulation and ethical guidance be put in place if there is any chance that neural organoids could develop consciousness. At first glance, it may seem that there is an agreement about what consciousness is, that it can be measured or observed, and that consciousness has a certain moral value (that consciousness matters morally). Unfortunately, there is no agreement on these issues. I will discuss some of the main concepts and theories of consciousness relevant for neural organoid research from a philosophical perspective, the implications of these views for measuring or identifying consciousness, and why it would be an ethical problem if neural organoids develop or may develop the capacity for the sort of consciousness in question. I focus on recent work from two opposite camps. One concerns what may be called “marginal sentience”, which has been combined with a precautionary ethical approach. This work suggests neural organoids might become candidates for sentience, and hence ethically problematic, even without developing the capacity for full human consciousness. The other camp suggests that neural organoids may not be able to develop the capacity for consciousness at all. Rather than attempting to give definitive answers to the questions whether or when neural organoids may be conscious and how to respond with regulation, I argue that we need to be sensitive to diverse approaches to consciousness with different ethical implications, and I hope to encourage reflection on what aspects of consciousness have moral value and why.

Abstract No. K4**Colorful aging? an aging-specific platelet differentiation path from hematopoietic stem cells***Camilla Forsberg*

University of California Santa Cruz, USA

Platelet dysregulation is drastically increased with advanced age and contributes to making cardiovascular disorders the leading cause of death of elderly humans. Here we reveal a direct differentiation pathway from hematopoietic stem cells into platelets that is progressively propagated upon aging. Remarkably, the aging-specific platelet path is decoupled from all other hematopoietic lineages, including erythropoiesis, and operates as an additional layer in parallel with canonical platelet production. This results in two molecularly and functionally distinct populations of megakaryocyte progenitors. The age-induced megakaryocyte progenitors have profoundly enhanced capacity to engraft, expand, restore, and reconstitute platelets in situ and upon transplantation, and produce an additional platelet population in old mice. The two pools of co-existing platelets cause age-related thrombocytosis and dramatically increased thrombosis in vivo. Strikingly, aging-specific platelets are functionally hyper-reactive compared to the canonical platelet populations. These findings reveal stem cell-based aging as a mechanism for platelet dysregulation and age-induced thrombosis.

Poscablo, D.M. et al. Forsberg, C. (2024). An age-progressive platelet differentiation path from hematopoietic stem cells causes exacerbated thrombosis. Cell 187, 3090 – 3107; <https://pubmed.ncbi.nlm.nih.gov/38749423/>

Abstract No. K5**Bat induced pluripotent stem cells as a model for viral tolerance and immunity***Thomas Zwaka*

Icahn School of Medicine at Mount Sinai, Black Family Stem Cell Institute, USA

Bats possess extraordinary traits, including unique immune responses that enable them to tolerate numerous deadly viruses. To explore these abilities, we generated induced pluripotent stem cells (iPSCs) from two bat species using a modified Yamanaka protocol. These bat iPSCs exhibit a core pluripotency program that mimics cells under viral attack and reveal a rich repertoire of endogenized viral sequences. Additionally, we discovered new enzymes in these cells that contribute to their immune defense. Our findings suggest a deep evolutionary entanglement between bats and viruses, offering new insights into disease resistance and surveillance potential.

Presidential Symposium lectures: PS1 – PS4

GSCN Presidential Invite

- PS1** Deconvolution of stem and progenitor cell fates and immune lineages by polylox barcoding
Hans-Reimer Rodewald

GSCN 2024 Young Investigator Award

- PS2** Fetal-maternal interface across species
Claudia Gerri

GSCN 2024 Hilde Mangold Award

- PS3** Building the next generation of neuromuscular models to study disease
Mina Gouti

GSCN Publication of the Year 2023 - 2024 Award

- PS4** A stem cell zoo uncovers intracellular scaling of developmental tempo across mammals
Jorge Lázaro Farré

Abstract No. PS1: GSCN Presidential Invite**Deconvolution of stem and progenitor cell fates and immune lineages by polylox barcoding**

Fuwei Shang¹, Larissa Frank¹, Branko Cirovic¹, Daniel Postrach¹, Maurice Langhinrichs², Tamar Nizharadze², Katrin Busch¹, Ann-Kathrin Fanti¹, Thorsten B. Feyerabend¹, Xi Wang³, Thomas Höfer², and Hans-Reimer Rodewald^{1,*}

¹German Cancer Research Center (DKFZ), Division of Cellular Immunology

²German Cancer Research Center (DKFZ), Division of Theoretical Systems Biology

³Nanjing Medical University, State Key Laboratory of Reproductive Medicine, China

*Presenting author

The development of non-invasive fate mapping techniques and high-resolution endogenous DNA barcoding ('Polylox') has yielded insights into the physiology of the blood and immune system. We measured hematopoietic stem cell (HSC) differentiation rates (HSC output) for native hematopoiesis and under challenges including sepsis, and identified major HSC clone fate categories. We found that individual HSC clones are endowed with stable fates over time, suggesting that fates may be 'imprinted' as soon as HSC arise in the embryo. RNA-barcoding ('PolyloxExpress') directly linked fate measurements with transcriptome reading in HSC clones and single HSC, which yielded transcriptional signatures associated with fate patterns. We are applying these approaches to deconvolute embryonic and adult hematopoiesis, including the development of tissue resident macrophages and innate lymphocytes. Finally, we identified fates, markers and transcriptomes distinguishing HSC-independent (embryonic) from HSC-derived multipotent progenitors (MPP). Unexpectedly, these experiments revealed that only in the order of 15% of adult HSC clones possess multilineage fates.

Pei W, Feyerabend TB, Rössler J, Wang X, Postrach D, Busch K, Rode I, Klapproth K, Dietlein N, Quedenau C, Chen W, Sauer S, Wolf S, Höfer T, Rodewald HR. (2017) Polylox barcoding reveals haematopoietic stem cell fates realized in vivo. Nature 548:456-460

Pei W, Wang X, Rössler J, Feyerabend TB, Höfer T, Rodewald HR. (2019) Using Cre-recombinase-driven Polylox barcoding for in vivo fate mapping in mice. Nature Protocols 14:1820-1840

Abstract No. PS2: GSCN 2024 Young Investigator Award**Fetal-maternal interface across species***Claudia Gerri*

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

The tissue microenvironment cooperates with intrinsic molecular regulators to model cell identity and plasticity. In our lab, we are interested in studying how cell lineages are established, and how they communicate with each other and the surrounding microenvironment to build an organ. Our goal is to understand how the environmental cues and the neighbouring tissues influence early cell fate decisions, and how progenitor cells interpret these signals and react, thereby affecting their surroundings. To tackle these questions, we aim to study a very enigmatic process of mammalian reproduction: the development of the placenta. Mammals evolved divergent architectures of placenta to receive nourishment from the mother during embryogenesis. I will explain our approaches to study how the uterine microenvironment shapes the fetal-placental interface of various animals, by using early embryos and placental organoids. Our ultimate goal is to investigate how complex and diverse structures emerge to achieve similar functions.

Abstract No. PS3: GSCN 2024 Hilde Mangold Award**Building the next generation of neuromuscular models to study disease***Mina Gouti*

Max Delbrück Center, Berlin

Locomotion results from the interaction between muscles and the nervous system. Dysfunctions in these cellular systems are linked to severe neuromuscular diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). The regional selectivity observed in these diseases remains poorly understood, mainly due to the absence of an appropriate human model. To address this gap, we have developed advanced human neuromuscular organoids (NMOs) from pluripotent stem cell-derived neuromesodermal progenitor cells. NMOs self-organize into three-dimensional complex structures that exhibit functional neuromuscular junctions supported by terminal Schwann cells and demonstrate spontaneous contractions and the formation of central pattern generator-like neuronal circuits. To enhance the reproducibility and throughput of our research, we have integrated automated systems for the culturing and analysis of NMOs. This automation enables the high-throughput generation of NMOs, significantly advancing our ability to study the early development of the human neuromuscular system and to model neuromuscular diseases. By incorporating these technologies, our approach not only provides deeper insights into the sequential events leading to specific neuromuscular disorders but also opens avenues for exploring targeted therapies.

Abstract No. PS4: GSCN Publication of the Year 2023 - 2024 Award**A stem cell zoo uncovers intracellular scaling of developmental tempo across mammals**

Jorge Lázaro Farré^{1,*}, Maria Costanzo¹, Marina Sanaki-Matsumiya¹, Charles Girardot², Masafumi Hayashi³, Katsuhiko Hayashi³, Sebastian Diecke⁴, Thomas Hildebrandt⁵, Giovanna Lazzari⁶, Jun Wu⁷, Stoyan Petkov⁸, Rüdiger Behr⁸, Mitsuhiro Matsuda¹, Vikas Trivedi¹, and Miki Ebisuya^{1,9}

¹EMBL Barcelona, Spain

²EMBL Heidelberg

³Osaka University, Department of Genome Biology, Japan

⁴Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Technology Platform Pluripotent Stem Cells

⁵Leibniz Institute for Zoo and Wildlife Research, Berlin

⁶Avantea & Avantea Foundation, Cremona, Italy

⁷University of Texas Southwestern Medical Center, Department of Molecular Biology, USA

⁸German Center for Cardiovascular Research, Platform Degenerative Diseases, Göttingen

⁹Cluster of Excellence Physics of Life, TU Dresden

*Presenting author

The rate of embryonic development varies considerably across mammalian species. These differences in the tempo or duration of developmental processes are known to influence animal size and morphology, being an important mechanism of evolutionary change. The goal of this project is to investigate how different mammalian species, despite using conserved molecular toolkits, develop at different speeds. For this, we have focused on investigating the segmentation clock, the oscillatory gene expression regulating the sequential formation of body segments.

Using in vitro differentiation of pluripotent stem cells, we recapitulated the segmentation clocks of diverse mammalian species varying in body weight and taxa: marmoset, rabbit, cattle, and rhinoceros. Together with mouse and human, the segmentation clock periods of the six species did not scale with the animal body weight, but with the embryogenesis length. The biochemical kinetics of the core clock gene HES7 displayed clear scaling with the species-specific segmentation clock period. However, the cellular metabolic rates did not show an evident correlation. Instead, genes involving biochemical reactions showed an expression pattern that scales with the segmentation clock period. Altogether, our stem cell zoo uncovered general scaling laws governing species-specific developmental tempo.

[https://www.cell.com/cell-stem-cell/pdf/S1934-5909\(23\)00210-2.pdf](https://www.cell.com/cell-stem-cell/pdf/S1934-5909(23)00210-2.pdf)

Selected presentations

**12th GSCN Conference
German Stem Cell Network
25 – 27 September 2024**

Selected presentations: T01 – T40

(the PXXX number indicates an accompanying poster presentation)

- T01** Infection and host cell tropism in the gut - organoids as model
Sina Bartfeld
- T02** Human liver ductal cell heterogeneity and cell-state transitions in an organoid system
Javier Bregante Boix
- T03** P53 activation in epithelial cells terminates the fetal-like regenerative state after colitis-associated injury
Kimberly Hartl
- T04/P083** Cell type-specific epigenome profiling identifies H3K27me3 dynamics in the human developing neocortex
Mareike Albert
- T05/P094** Assessing the impact of prenatal paracetamol exposure on early human forebrain development using cerebral organoids
Clara Hayn
- T06** The role of leukemic stem cells in patients treated with the BCL-2 inhibitor venetoclax
Andreas Trumpp
- T07** High fat diet impacts intestinal stem cell niche and causes hyperproliferation in the pig intestinal epithelium
Falk Janos Farkas
- T08/P067** Relevance of dormant cancer stem cells in disease propagation and therapy resistance in JAK2V617F-driven myeloproliferative neoplasms
Ian Ghezzi
- T09** Targeting SRSF2 mutations in leukemia with RKI-1447: a method to disrupt HSC division and alter nuclear formation
Nathali kaushansky
- T10** The inflammatory connection: insights from spatial mapping of human hematopoiesis in aging and blood malignancies
Borhane Guezguez
- T11** Hematopoiesis by the numbers: advances in cellular barcoding and counting cell division
Leïla Perié
- T12/P079** A scalable computational framework for annotation-independent combinatorial target identification in scRNA-seq databases
Maximilian Buser

- T13/P107** Tracking bone marrow regeneration after CAR-T cell therapy in myeloma at spatial single-cell resolution
Jennifer Wing Lam Cheung
- T14** Inferring the clonal dynamics of development from holistic barcoding: theory and application to tissue macrophages
Maurice Langhinrichs
- T15/P081** Unveiling the mechanisms of macrophage-mediated cytokine release syndrome in CAR-T cell therapy with mathematical modeling
Artur C. Fassoni
- T16** Capture of human axial stem cells
Dolunay Kelle
- T17/P062** The homeobox transcription factor DUXBL controls toti- to pluripotent transition
Felipe Lüttmann
- T18** SOX and POU transcription factors are older than stem cells and animals
Ralf Jauch
- T19/P126** Lifelong persistence of nuclear RNAs in the rodent brain
Sara Zocher
- T20/P124** Epithelial-stromal communication via BMP signaling is a critical regulator of site specificity in the mouse oral mucosa
Kai Kretzschmar
- T21** Advancing cardiac therapeutics from animals to patients
Eldad Tzahor
- T22** Lineage-specific cancer-related mutations in human pluripotent and adult stem cells
Jonathan Jung
- T23** HLA-homozygous GMP iPSCs show efficient cardiac induction on a New differentiation platform featuring scalability and enhanced robustness
Soraia Martins
- T24** hPSC-cardiomyocyte production: standardization and protein-free upscaling to 2000 mL
Nils Kriedemann
- T25/P106** Optimization of cardiomyocyte cryopreservation and quality control aspects for potency and residual iPSC contaminations
Fabienne Becker
- T26** iPSCs in drug discovery – from a user's and a personal perspective
Bert Klebl

- T27** Patient-specific iPSC-derived hepatic organoids as model for drug dosing and mRNA-based gene therapy
Malte Sgodda
- T28** Human multi-tissue organoids as an advanced teratogenicity model
Liam Wilson
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- T32/P041** Apelin signaling restricts the formation of the blood stem cell-forming hemogenic endothelium
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- T33/P052** Intrinsic reconstitution potential of developmentally restricted hematopoietic stem cells in non-conditioned immunocompromised mice
Marcel GE Rommel
- T34** Metabolic atlas of human bone marrow hematopoietic stem cells upon differentiation, aging and leukemia
Maria Eleni Lalioti
- T35** The function of PD-L2 expression on human hematopoietic stem cells in allogeneic immune regulation at single cell resolution
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- T36** Tracking of early cell fate decisions of iPSCs based on DNA methylation
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- T37** Pooled mesenchymal stromal cells fail to represent average donor cell characteristics
Janina Burk
- T38/P115** Humanized osteoclasts: an interspecies chimeric immune cell
Gülce Perçin
- T39** Resolving human alpha versus beta cell fate allocation for the generation of stem cell-derived islets
Corinna Cozzitorto
- T40/P118** Generation of designer stem cell-derived islets (SC-islets) to improve safety and efficacy of SC-based islet replacement therapy
Eunike Sawitning Ayu Setyono

Abstract No. T01**Infection and host cell tropism in the gut - organoids as model***Sina Bartfeld*

Der Simulierte Mensch (Si-M), TU Berlin

Infections are still a major health threat worldwide. Organoids have been used to study many diseases, including genetic disease, inflammatory disease and infections. In my lab, we use organoids to better understand host pathogen-interactions in the gastrointestinal tract.

We generated a biobank of human and murine organoids covering 6 sites from stomach to colon. RNA-sequencing showed that the tissue identity is conserved in the adult stem cells. Moreover, components of the epithelial innate immune sensing, such as toll like receptors, are part of the tissue identity and highly organized along the gastrointestinal tract. This organization is for a large part determined by developmental processes rather than by environmental factors.

For infection, monolayers derived from organoids have the advantage that bacteria or viruses can easily be added to the cells. However, single-cell RNA-seq of gastric organoids and organoid-derived monolayers under standard growth conditions show that they are not identical, but gastric organoid-derived monolayers recapitulate the pit region, while organoids capture the gland region of the gastric units. In both models, cells respond to directed differentiation.

- Infection with *Helicobacter pylori* shows a tropism for highly differentiated pit cells and this depends on urea chemotaxis.
- SARS-CoV-2 can infect the intestine but not the stomach epithelium and this depends on expression of ACE2.
- Enteropathogenic *E. coli* show a preference for small intestinal epithelium depending on cellular differentiation.

For infection biology, organoids provide an unprecedented tool, because pathogens exhibit tropism for organ systems, organs, cells or subtypes of cells and organoids are a resource to produce these target cells. Understanding host pathogen-interaction in turn informs about host cell differentiation.

Abstract No. T02**Human liver ductal cell heterogeneity and cell-state transitions in an organoid system**

Javier Bregante Boix^{1,*}, Flaminia Kaluthantrige Don¹, André Gohr¹, Germán Belenguer¹, Dylan Liabeuf², Daniel E. Stange², and Meritxell Huch^{1,3}

¹Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

²University Hospital Carl Gustav Carus, Department of Visceral, Thoracic and Vascular Surgery, Dresden

³Center for Systems Biology (CSBD), Dresden, Cluster of Excellence Physics of Life, TU Dresden, Dresden

*Presenting author

The human liver ductal epithelium is morphologically, functionally and transcriptionally heterogeneous, with populations of cells showing distinct differentiation states from more mature to progenitor or even bi-potent states. However, understanding the dynamics of the ductal cell states and their contribution to human liver regeneration and disease has been hampered by the absence of in vitro systems that fully mimic this heterogeneity. Hitherto, ductal organoid systems solely recapitulate the proliferative counterpart of ductal epithelium. Similarly, a system that faithfully recapitulates cholangiocyte-to-hepatocyte differentiation occurring upon extensive hepatocellular injury has been limited by the low yield of current differentiation approaches. Here, we developed a niche-inspired organoid system that allows long-term expansion of human ductal cell organoids, acquiring a bi-potent state and resembling transcriptional heterogeneity observed in vitro. To dissect the transcriptional heterogeneity of this refined organoid system, we employed single-cell RNA sequencing. We described five transcriptionally distinct cell states (ductal progenitor, bipotent ductal progenitor, proliferative ductal, mature ductal and mature hepatocyte) that mimic the transcriptional heterogeneity described in vivo. Next, we unveiled that human ductal cell organoids arise from a non-proliferative ductal progenitor population, which is capable of transitioning to either a proliferative state or to a mature state, via an intermediate bipotent state. We found that the acquisition of mature ductal cell states is accompanied by a reduction of Wnt signaling, thus we combined Wnt inhibition with our refined organoid system to improve the efficiency of current cholangiocyte-to-hepatocyte differentiation protocols. Overall, our refined liver ductal organoid system will enable the investigation of ductal cell states in homeostasis, regeneration and disease, and could be used for high-throughput approaches to understand mechanisms of ductal cell state dynamics and ductal cell plasticity.

Abstract No. T03**P53 activation in epithelial cells terminates the fetal-like regenerative state after colitis-associated injury**

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Cell clones that lack p53 signaling frequently occur in ulcerative colitis (UC) and are considered early drivers in UC-associated colorectal cancer (CRC) but not in spontaneous CRC. Colitis is associated with epithelial stem cell reprogramming from the adult, homeostatic to a highly proliferative, “fetal-like” regenerative state. Yet, it is unclear if and how loss of p53 signaling in the context of colitis contributes to cell state reprogramming.

Here, we used proteome and transcriptome analyses of inducible murine and organoid-based models to study the role of Trp53 during epithelial reprogramming. We find that p53 signaling is silent during homeostasis and that loss of Trp53 does not disrupt homeostatic crypt architecture. Upon dextran sodium sulfate (DSS)-induced chemical colitis, epithelial reprogramming into the regenerative state is linked to strong activation of p53 signaling. While in WT cells this causes termination of the regenerative state and restoration of homeostasis, crypts that lack Trp53 remain locked in the highly proliferative, regenerative state long-term and fail to re-establish normal crypt architecture and functional mature cell types. The impaired differentiation into secretory and absorptive cells results in a loss of the mucus barrier and promotes inflammation. We mimic regeneration and return to homeostasis by adjusting niche factors in organoid cultures and our data reveal that the highly proliferative regenerative state depends on elevated levels of glycolysis, which in WT cells requires exogenous induction of Wnt signaling. Trp53 knockout cells fail to return to homeostatic levels of proliferation and as a result, become locked in the regenerative state.

In our study, we reveal the context-dependent relevance of p53 signaling in the injury-induced regenerative state, explaining the high abundance of clones lacking p53 signaling in UC and UC-associated CRC.

Abstract No. T04/P083**Cell type-specific epigenome profiling identifies H3K27me3 dynamics in the human developing neocortex**

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During neocortex development, neural progenitor cells proliferate and undergo neuronal differentiation. This process is driven by temporal and spatial gene expression changes that are in turn guided by dynamic epigenetic mechanisms, including post-translational histone modifications. Intriguingly, mutations in various epigenetic players cause neurodevelopmental disorders. Currently, we lack a comprehensive understanding of the complex epigenetic remodelling that occurs during human neurogenesis. Here, we perform an exhaustive characterization of the epigenetic landscape in the human developing neocortex. We leverage cytometry by time of flight for the simultaneous single cell analysis of more than 30 histone modifications (Epi-CyTOF). We find tri-methylation of lysine 27 of histone 3 (H3K27me3), a repressive epigenetic mark deposited by the Polycomb repressive complex 2 (PRC2), to display global cell type-specific distribution with enrichment in postmitotic neurons, despite ubiquitous distribution of PRC2 subunits. By applying cell type-specific CUT&Tag, we profile histone modifications in neural progenitor cells and neurons isolated from cortical tissue. To functionally investigate the role of H3K27me3 in human cortical development, we apply pharmacological inhibition of PRC2 over five weeks of cortical organoid development, resulting in ablation of H3K27me3. We show that impaired PRC2 activity results in reduced proliferation of different neural progenitor cell types as well as in premature differentiation. Taken together, this study provides comprehensive insights into the complex epigenomic landscape in the human developing neocortex and reveals a role for H3K27me3 in human neural fate specification.

Abstract No. T05/P094**Assessing the impact of prenatal paracetamol exposure on early human forebrain development using cerebral organoids**

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Recent epidemiological studies suggest that prenatal Paracetamol (N-Acetyl-p-aminophenol; APAP) exposure can impair fetal neurodevelopment in a time- and dose-dependent manner, leading to autism and attention-deficit-hyperactivity disorder. The mechanisms underlying this phenomenon remain largely unexplored. It also remains unclear which developmental stages are particularly vulnerable and whether Paracetamol itself or its metabolites elicit these effects.

Here, a novel 3D cell culture workflow, involving human iPSC-derived forebrain organoids was employed to assess the impact of APAP exposure on human brain development. Immediately following neural induction, when neural precursors start proliferation and differentiation, we initiated an eight-week treatment, applying the mean maximal plasma concentration reported in females upon oral administration. As APAP readily crosses the placental and blood-brain-barrier, our setup is suited to simulate prenatal exposure in vivo and to evaluate its effects on forebrain development.

For phenotypic assessment of whole forebrain organoids, we used light-sheet fluorescence expansion microscopy (LSFEM), which enables intact imaging from meso- to nanoscale resolution within a single imaging session. LSFEM fully exploits the potential of brain organoids by portraying intricate cell-cell interactions and the impact of surrounding tissue architecture on individual cells in a 3D context. We found that APAP exposure reduces the organoids' cross-sectional area by 20% and disrupts their overall structure. Via LSFEM, we detected a 33% reduction in organoid volume and a 10% increase in neural rosette density, with individual rosettes exhibiting a 33% reduced volume. Ongoing analysis, including 3D assessment of cell proliferation and survival as well as mRNA sequencing and miRNA profiling, are expected to elucidate the mechanisms underlying these effects.

Our observations point to a profound impact of APAP on early human neural development. Further, our experimental set-up provides a robust and adaptable platform for studying prenatal neurotoxic effects of pharmaceutical compounds on human brain development in a controlled in vitro scenario.

Abstract No. T06**The role of leukemic stem cells in patients treated with the BCL-2 inhibitor venetoclax***Andreas Trumpp*

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The BCL-2 inhibitor Venetoclax (VEN) in combination with 5-AZA is currently transforming Acute Myeloid Leukemia (AML) therapy. We now identified leukemic stem cells (LSC) as primary targets of 5-AZA/VEN whose elimination determines therapy outcome. We developed and validated a flow cytometry-based “MAC-Score” linking the ratio of protein expression of BCL-2, BCL-xL, and MCL-1 in LSCs. MAC-Scoring predicts initial response with a positive predictive-value of >97% associated to increased event-free survival. In essence, combinatorial levels of BCL-2-family members in AML-LSCs are a key denominator of response and MAC-Scoring reliably predicts individual patient response to 5-AZA/VEN. MAC scoring outperforms genetic predictors of poor VEN response such as TP53 mutations or complex karyotype. Although mutations do influence the MAC score of LSCs, additional factors such as differentiation plasticity can mediate secondary VEN resistance and relapse. Along these lines we recently identified four distinct AML-LSC types. First, LSCs mirroring quiescent hematopoietic stem cells (HSC-LSCs) exhibit low apoptotic-priming, contributing to chemotherapy resistance. Conversely, lineage-primed LSCs maintain stemness while exploiting differentiation programs. For example, LSCs during BCL-2 inhibition, can shift lineage-priming from BCL-2 dependent Lymphoid-Myeloid-Primed-Progenitor-LSCs (LMPP-LSCs) to Megakaryocytic-Erythrocytic-Progenitor-LSCs (MEP-LSCs). The latter are primed towards MEP-lineage output and show increased BCL-xL expression leading to Venetoclax escape. In rare, aggressive cases, patients possess LSCs outside the classical stem-progenitor state, resembling lineage-committed Monocytic/Dendritic precursors (MoDe-LSCs) expressing high levels of BCL-1. Together, AML encompasses heterogeneous LSC types beyond the canonical HSPC-myeloid state. The lineage priming of these LSCs can adapt plastically under therapeutic pressure, mediating resistance.

Abstract No. T07**High fat diet impacts intestinal stem cell niche and causes hyperproliferation in the pig intestinal epithelium**

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Diet-induced obesity elevates the risk of type 2 diabetes (T2D) and cancer, imposing economic and social burdens. The intestine, as the body's largest endocrine and highly proliferative organ, is strongly affected by diet, paving the way for T2D and colorectal cancer. Recent studies suggest both, epithelial cells and the subepithelial mesenchyme, to influence the regulation of ISC maintenance and differentiation in homeostasis and disease. However, despite extensive studies in rodents, it is not clear how a HFD affects the stem cell niche and stem cell function and causes hormonal imbalance due to conflicting results.

Insights into the hyperproliferation in HFD models are needed to unify the field and elucidate the role of the subepithelial niche. In this project, we utilized the pig as a large animal model, with physiology closely resembling humans, to gain insights into the subepithelial niche composition, its molecular function, and its alterations after a HFD challenge. Overall, we aim to investigate how these changes in the niche impact on HFD-induced intestinal dysfunction.

Mouse organoid co-cultures with isolated subepithelial niche cells from HFD-fed animals revealed increased organoid formation, suggesting contributions of subepithelial signaling to diet-induced ISC hyperproliferation. Investigating this effect in vivo, we developed a novel dissection technique to isolate ISCs and their surrounding niche. Proteomic and single-nucleus RNA sequencing (snRNAseq) experiments were conducted to generate the first comprehensive resource for the ISC microenvironment in the porcine small and large intestine. Initial analyzes showed distinct changes in the niche composition and ISC transcriptome in HFD-fed animals. These findings demonstrate early changes in ISCs and their subepithelial niche upon HFD in large animals, advancing our understanding of the molecular mechanisms involved in the process, and potentially opening new avenues for research and therapy to prevent pathogenic changes in the intestine related to diabetes or colorectal cancer development.

Aliluev, A., [...] Lickert, H., & Böttcher, A. (2021). Diet-induced alteration of intestinal stem cell function underlies obesity and prediabetes in mice. Nature Metabolism 2021

Beyaz, S., Mana, M. D., Roper, J.,[...] Yilmaz, Ö. H. (2016). High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. Nature

Abstract No. T08/P067**Relevance of dormant cancer stem cells in disease propagation and therapy resistance in JAK2V617F-driven myeloproliferative neoplasms**

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Long-term dormancy is proposed to mediate therapy resistance of cancer stem cells. However, the biological relevance of dormancy in hematologic malignancies has not been formally addressed. Using a novel mouse model that allows identification and isolation of dormant label retaining cells (LRCs) in the setting of JAK2V617F-driven Polycythemia Vera (PV), we could identify LRCs that maintained dormancy for at least 20 weeks in vivo in PV mice, despite harboring the pro-proliferative JAK2 mutation. Isolation and transplantation of mutant LRCs and nonLRCs unambiguously demonstrated that only LRCs were capable of propagating the PV phenotype. Treatment of primary PV mice with a JAK2 inhibitor (Fedratinib, FED) failed to target mutant LRCs, while pegylated interferon- α (IFN α) reduced their number, in line with the clinical observation that IFN α can facilitate molecular remission while FED cannot. After therapy, purified residual LRCs were still uniquely able to propagate PV, demonstrating their role as disease-reinitiating cells and that their level of depletion is a biomarker of PV eradication. Side effects of IFN α treatment limit its clinical effectiveness in PV. To circumvent this, we explored using low dose IFN α (IFN α LD) alone or in concert with FED. IFN α LD alone had no impact on mutant LRC numbers, while IFN α LD plus FED resulted in a synergistic depletion of mutant LRCs and correction of PV phenotype, suggesting that IFN α LD renders LRCs dependent upon JAK2 signalling. In summary, we provide definitive evidence of the importance of dormancy in propagation and therapy resistance of PV; create a model for pre-clinical evaluation of novel therapies for PV; and demonstrate that a combination of IFN α LD and FED may be effective at eliminating mutant LRCs while limiting side effects of standard IFN α treatment.

Abstract No. T09**Targeting SRSF2 mutations in leukemia with RKI-1447: a method to disrupt HSC division and alter nuclear formation**

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In the quest to develop targeted therapies for myeloid malignancies, spliceosome machinery mutations, particularly those affecting the *SRSF2* gene, represent a significant yet challenging target. This study identifies RKI-1447, a Rho-associated protein kinase inhibitor, as a potent agent against *SRSF2* mutant hematopoietic stem cells (HSCs), proposing a novel therapeutic strategy that could transform treatment paradigms for leukemia characterized by spliceosomal mutations.

Utilizing a high-throughput screening approach across various isogenic cell lines, RKI-1447 emerged as uniquely effective in selectively impairing the viability of *SRSF2* mutant HSCs. The inhibitor's mechanism of action involves inducing mitotic catastrophe, reorganizing the cytoskeleton, and causing pronounced nuclear deformation, thereby preventing successful cell division. Significantly, in xenograft models using primary human leukemia samples, RKI-1447 demonstrated remarkable efficacy, highlighting its potential clinical applicability.

This research uncovers the critical role of RKI-1447 in disrupting the cytoskeletal and nuclear architecture of *SRSF2* mutant HSCs. Detailed analyses, including transmission electron microscopy and 3D light microscopy, revealed that *SRSF2* mutations result in severe nuclear indentation and segmentation. These nuclear deformations are exacerbated by RKI-1447 treatment and are attributed to microtubule-rich cytoplasmic intrusions, suggesting a novel interplay between microtubules and nuclear integrity in the presence of *SRSF2* mutations.

The findings from this study open new avenues for the targeted treatment of leukemias with *SRSF2* mutations. By elucidating the unique vulnerabilities of *SRSF2* mutant HSCs to RKI-1447, this work not only advances our understanding of the pathogenic mechanisms underlying these malignancies but also lays the groundwork for the development of innovative therapeutic strategies. The potential of RKI-1447 to selectively target mutant HSCs while sparing healthy cells offers a promising direction for future research and clinical applications in the treatment of myeloid leukemias.

Abstract No. T10**The inflammatory connection: insights from spatial mapping of human hematopoiesis in aging and blood malignancies**

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Hematopoietic stem cells (HSC) are fundamental units that regulate hematopoiesis throughout life. Upon Aging, HSC can accumulate somatic mutations (DNMT3A, TET2, SF3B1...), leading to clonal expansion and lineage bias, known as clonal hematopoiesis (CH). Moreover, aging alters the function and composition of the bone marrow (BM) niche, including HSC-supportive cues from the mesenchymal stromal cells (MSC). These intrinsic and extrinsic defects may contribute to the progression of CH to hematopoietic malignancies such as myelodysplastic syndromes (MDS). However, the detailed cellular interactions causing BM niche remodeling in CH and associated MDS remain to be fully explored. Here, we conducted a comprehensive study utilizing single cell multi-omics, proteomics, and multiplexed imaging to explore the pathological interactions between HSCs, MSCs, and T cells in matched BM liquid and bone biopsies from a longitudinal CH/MDS study (NCT02867085). Bone imaging revealed significant niche remodeling in CH compared to healthy aged donors, marked by emergent inflammatory MSCs and a noticeably expanded vasculature. These changes were amplified in MDS through accumulation of exhausted regulatory T cells. ScRNA-seq supported these observations and identified specific inflammatory subsets in CH/MDS including cytokine-producing HSCs, stress-induced MSC subpopulations, and increased IFN-responsive cytotoxic T cells. Moreover, we used single-cell long-read sequencing (RaCH-seq) to identify mutated HSCs, allowing us to computationally unravel CH/MDS-specific inflammatory BM networks. Lastly, cytokine profiling assessed CH/MDS specific inflammatory signatures, while proteomic analysis from BM-MDS co-culture assays pinpointed pro-angiogenic processes, emphasizing the importance of these cues for stroma remodeling. Overall, our findings describe age dependent inflammatory remodeling of the BM niche causing malignant changes in HSC, which can be further investigated to define a possible risk predictor for the emergence of CH and MDS.

Abstract No. T11**Hematopoiesis by the numbers: advances in cellular barcoding and counting cell division***Leïla Perié*

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In this talk, I will discuss our latest advancements in both experimental and computational cellular barcoding. Firstly, I will introduce our novel computational tools designed for both biologists and bioinformaticians to study cell lineages using artificial and natural barcodes. Next, I will present the in situ DRAG barcoding system, which enables the study of hematopoietic stem and progenitor cells (HSPCs) in their native niche. Combining DRAG barcoding with single-cell transcriptomics and metabolomics, we revealed that a subset of multipotent progenitors that have higher activity of the pentose phosphate pathway are myeloid-biased. These results show that HSPCs undergo significant metabolic changes to facilitate the bioenergetic and epigenetic demands of myeloid lineage specification.

Finally, I will present a new method we have developed to count division in vivo, the DivisionCounter. While approximately 44 divisions are needed to produce without death all 10^{13} cells in the human body, current methods are limited to count 10 cell divisions in vivo across diverse mammalian cell types. The DivisionCounter, counts cell division in vivo over large division ranges (~70 divisions) using an easy fluorescence readout. We demonstrate that the DivisionCounter accurately measures the average cell division count of cells both in vitro and in vivo. Its use revealed that leukemia tumor division rates are independent of the organ's specific microenvironment and CAR-T cell treatment, providing an estimate of tumor death rates in vivo. The DivisionCounter method holds unique potential for quantifying contributions of cell division, death, and migration to the growth of healthy and pathological mammalian tissues.

Abstract No. T12/P079**A scalable computational framework for annotation-independent combinatorial target identification in scRNA-seq databases***Maximilian Buser*, and Carsten Marr*

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The applicability of novel targeted cell therapies is constrained by on-target off-tumor toxicities, posing significant challenges for safe and effective treatment. To develop strategies for mitigating these toxicities, a comprehensive understanding of the distribution of single-cell profiles across human tissues is essential. Single-cell RNA sequencing (scRNA-seq) provides detailed insights into cellular diversity and function. However, as data collection expands and RNA expression atlases comprise tens of millions of cells, effectively querying these databases to identify cell populations matching specific gene expression patterns becomes challenging. Key challenges include managing data volume, handling batch effects, navigating study-dependent data curation and annotation, and addressing the combinatorial complexity of possible target patterns. Userfriendly screening tools that allow for complex query target patterns without relying on existing annotations are currently missing. Here we present *otopia*, a computational framework that enables fast queries of large scRNA-seq databases, enhancing the precision of cell population identification. Using precomputed neighborhood graphs and boolean logic for expression patterns, *otopia* enables efficient queries addressing arbitrary combinatorial target patterns. *otopia* does not depend on cell type annotations and by exploiting unbiased expression pattern matching scores, it uncovers critical populations that annotation based methods overlook. We exemplify *otopia*'s capabilities by identifying the largest B cell populations in the CELLxGENE census data using a CD19+CD22+CD5-query target pattern. Moreover, we show that *otopia* can provide a concise overview of cell populations matching hypothetical acute myeloid leukemia (AML) dual targets, realizing logic AND gates, across different tissues. *otopia* enables the estimation of ontarget off-tumor toxicities for drugs targeting both single markers and complex marker combinations, surpassing previous methods that focus mainly on single markers. With the expanding availability of scRNA-seq data, *otopia* represents a scalable, unbiased, and automated approach to refine putative therapeutic targets in order to minimize toxicities.

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Abstract No. T13/P107**Tracking bone marrow regeneration after CAR-T cell therapy in myeloma at spatial single-cell resolution**

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Anti-BCMA CAR-T cell therapy is a widely used treatment strategy of relapsed/refractory multiple myeloma. Despite its notable efficacy and substantial overall response rate, this treatment is frequently accompanied by severe undesirable side effects. In particular, the pathophysiology of impaired bone marrow (BM) regeneration post therapy, which is critical for patient recovery, is not understood. This study investigates BM regeneration after anti-BCMA CAR-T cell therapy in multiple myeloma patients, focusing on hematopoietic stem cells (HSCs) and their potential niche communications. We analyzed BM biopsies from 16 patients, integrating single-cell RNA-sequencing and single cell resolution spatial transcriptomic technologies. This approach allowed spatial mapping of >20 cell types in the post-CAR-T BM, and revealed significant inter-patient variability in cell states and BM architecture. Importantly, elevation of mesenchymal cell inflammation is prevalent in patients experiencing prolonged cytopenia, a severe side effect of CAR-T therapy, characterized by a lower-than-normal density of erythroid, platelet or neutrophil blood count. Ligand-receptor interaction analysis and spatial niche information suggested that these mesenchymal cells act as signaling hubs, relaying inflammatory signals to myeloid cells, thereby maintaining an inflammatory state. In vitro culture systems showed that this persistent inflammation impaired functional outputs of hematopoietic stem and progenitor cells (HSPCs), potentially causing prolonged cytopenia. Gene set enrichment analysis indicated a senescence signature in HSCs from cytopenic patients, suggesting that HSPC dysfunction might be linked to their senescence state. In conclusion, our study showed that the inflammatory microenvironment in BM may induce HSPC senescence, contributing to the prolonged cytopenia observed in some patients post-CAR-T therapy. This research provides insights into the mechanisms of BM regeneration and the challenges associated with cytopenia in multiple myeloma treatment.

Abstract No. T14**Inferring the clonal dynamics of development from holistic barcoding: theory and application to tissue macrophages**

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Adult tissues originate from progenitor cells in the embryo, yet the types, differentiation pathways, and quantitative contributions of progenitors have been difficult to assess. Here we show that barcoding the cells of the embryo at successive developmental stages, followed by adult barcode readout, enables the quantitative inference of progenitor fate combinations and downstream pathways of fate restriction. Our approach is based on the stochastic modeling of progenitor clone dynamics linked with Bayesian model selection. Importantly, this allows us to address the sampling problem inherent in barcoding analyzes and identify true fate restriction based on experimental samples. We used this method to infer the development of tissue-resident macrophages from experimental data obtained with holistic, time-resolved Polylox barcoding of the mouse embryo from embryonic day (E) 6.5 to E10.5. Using information from several thousand clones, emerging from progenitors at different developmental stages, we identified the progenitor fates and differentiation routes of the major tissue macrophage populations, microglia, Kupffer cells, alveolar macrophages in the lung and red pulp macrophages in the spleen. Our findings are consistent with an early developmental origin of all these tissue-specific macrophage populations, emerging at the onset of organogenesis. Moreover, we obtained testable predictions of progenitor numbers and clone sizes. In summary, our findings elucidate the clonal lineage dynamics of tissue macrophage development and provide a general computational framework to use unbiased and quantitative fate mapping for developmental and stem cell biology.

Abstract No. T15/P081**Unveiling the mechanisms of macrophage-mediated cytokine release syndrome in CAR-T cell therapy with mathematical modeling**

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Chimeric Antigen Receptor (CAR) T-cell therapy has emerged as a promising avenue for cancer treatment. However, challenges such as recurrence and cytokine release syndrome (CRS) necessitate a deeper understanding of the underlying dynamics to optimize therapeutic outcomes.

Preclinical studies suggest that CRS results from complex interactions between CAR-T and host cells, with macrophages identified as a primary source of cytokines. Three primary mechanisms of macrophage activation were identified. First, upon antigen-binding-mediated activation, CAR-T cells release inflammatory signals that activate macrophages. Second, following tumor cell targeting by CAR-T cells, damage-associated molecular patterns (DAMPs) released by pyroptotic tumor cells enhance macrophage activation. Third, the interaction of CD40 on activated macrophages with CD40L on CAR-T cells further promotes macrophage activation in a contact-dependent manner.

To better understand the underlying mechanisms and temporal dynamics during CAR-T cell therapy response, we developed a novel multi-layer mathematical model that accurately describes the time courses of 25 patients with diverse responses and IL-6 cytokine kinetics. We successfully linked the dynamic shape of the response to interpretable model parameters and studied the influence of CAR-T cell dose and initial tumor burden on CRS occurrence and treatment outcomes.

By accounting for the three established inflammation mechanisms and reconstructing the timeline of macrophage activation, we found that each activation mechanism governs a different phase of CRS. Damage-associated molecules released by dying tumor cells drive early CRS, antigen-binding-mediated inflammation occurs parallel to CAR-T cell expansion, followed by CD40 contact. Moreover, we identified the CD40-CD40L axis as the main driver of CRS and a clinically feasible target to control the activation process and modulate IL-6 peak height.

Our study underscores the utility of mechanistic modeling in deciphering therapy dynamics and guiding clinical interventions, emphasizing the importance of close integration with clinical data.

Abstract No. T16**Capture of human axial stem cells**

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The spinal cord, nerves, and skeletal muscles arise from neuromesodermal progenitors (NMPs). We have developed a growth-factor screening strategy, utilizing ES and iPS cells, facilitating the indefinite self-renewal of two types of human axial stem cells (AxSCs), closely resembling mouse NMPs (NM-AxSCs) and posterior neural tube progenitors (N-AxSCs). Under specific regimens these AxSCs self-renew and sustain telomeres. Single cell transcriptomics and proteomics have revealed expression of posterior growth-zone and dorsoventral neural tube markers in NM-AxSCs, and correspondingly, differentiation to a wide spectrum of neural tube neurons and myocytes. N-AxSCs rapidly matured into dorsal sensory subsets and neural crest. Crucially, neither AxSC type produces teratomas, and analogous mouse NM-AxSCs integrated successfully into the neural tube and somites. Capturing of AxSCs from patient and GMP ES / iPS cells without transgenesis unveils ontogeny and promises modeling and therapy in neuropathies.

Abstract No. T17/P062**The homeobox transcription factor DUXBL controls toti- to pluripotent transition**

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The first transcriptional event in life results in thousands of genes being transiently upregulated at the 2-Cell (2C) stage in mice. Shortly after the 2C-stage, this transcriptional burst must be decommissioned to allow development to progress post the totipotent stage. However, the mechanisms that enable the exit from totipotency remain elusive. We discovered that the 2C-specific double homeobox transcription factor (DUX) drives an essential negative feedback loop by activating its paralog DUXBL. We show that DUXBL-deficient embryos display upregulation of the 2C-specific program and fail to develop further. Conversely, precocious overexpression of DUXBL before the 2C-stage is sufficient to impede the 2C-transcriptional program in vivo. Mechanistically, we show that DUXBL occupies 2C-associated genomic sites that were opened and bound by DUX in mouse embryonic stem cells, repressing transcription from those sites. In DUX-induced nuclear foci, DUXBL interacts with transcription-silencing members from the TRIM superfamily. We identify DUXBL as a key regulator of early transcriptional control, enabling toti-to-pluripotent transition and cell fate divergence. Our study highlights the importance of antagonistic interplays within transcription factor families for development. These findings pave the way to better understand developmental processes and may lead to ways to control them in the future.

Vega-Sendino, M., Lüttmann, F.F., Olbrich, T. et al. The homeobox transcription factor DUXBL controls exit from totipotency. *Nat Genet* 56, 697–709 (2024). <https://doi.org/10.1038/s41588-024-01692-z>

Abstract No. T18**SOX and POU transcription factors are older than stem cells and animals***Ralf Jauch*

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Pluripotent stem cells are a hallmark of animal multicellularity, and, consistently, stem cell-associated Sox and POU transcription factors were considered animal innovations. However, we discovered Sox and POU members in the closest unicellular relatives of animals. We show that unicellular Sox binds DNA like mammalian Sox2 and can induce pluripotent stem cells. The capacity to form stem cells is shared by the ancestrally reconstructed Ur-Sox factors. In contrast, unicellular POU proteins bind DNA differently than their animal counterparts and cannot induce pluripotency. Our findings imply that the evolution of stem cells exploited a pre-existing set of transcription factors, entailing critical changes in DNA specificity of POU and the exaptation of the ancestral capacity to interact with Sox transcription factors.

Abstract No. T19/P126**Lifelong persistence of nuclear RNAs in the rodent brain**

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Genomic DNA that resides in the nuclei of mammalian neurons can be as old as the organism itself. Also some nuclear proteins are known to be long-lived and contribute to life-long epigenetic regulation. However, the life span of nuclear RNAs, which are critical for proper chromatin architecture and transcription regulation, has not been determined in adult tissues. In this work, we identified and characterized nuclear RNAs that do not turn over for at least 2 years in the mouse brain. These long-lived RNAs were stably retained in nuclei in a neural cell type-specific manner, with postnatally generated granule cells and quiescent neural stem cells in the hippocampus showing high capacity for the RNA long-term retention. Using next generation sequencing, we revealed the molecular identity of long-retained RNAs in quiescent neural stem cells and identified many non-coding RNAs, predominantly repetitive RNAs such as major satellite RNAs, as particularly stable RNAs. Knock-down and overexpression of major satellite RNAs confirmed their long-term retention in quiescent neural stem cells. Perturbation experiments further demonstrated that major satellite RNAs are required for heterochromatin maintenance and for the reactivation of quiescent neural stem cells into proliferation, indicating their functional significance for the maintenance of neural stem cells. These results suggest that the life span of neural cells may depend not only on the longevity of DNA for the storage of genetic information and on long-lived proteins for epigenetic regulation, but also on the extreme stability of RNA for the functional organization of chromatin.

<https://www.science.org/doi/10.1126/science.adf3481>

Abstract No. T20/P124**Epithelial-stromal communication via BMP signaling is a critical regulator of site specificity in the mouse oral mucosa**

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*Presenting author

The mouth or oral cavity consists of the tongue, cheeks, gums, and palate. Without these specialized tissues, eating, tasting, and speaking would not be possible. As a multifunctional organ, the oral cavity has a high degree of structural heterogeneity. Each oral site is unique and contains distinct niches for oral epithelial stem cells. Despite their importance, our understanding of oral stem cell niches remains limited. Here, we use single-cell and spatial transcriptomics, and organoid technology to define the molecular mechanisms governing oral site-specific stemness. Using a Ki67 knock-in mouse model, we found that proliferation in the epithelial basal layer is site-specific, with the dorsal tongue being the most proliferative tissue and the buccal mucosa being the least proliferative. We then generated a comprehensive single-cell atlas of major oral sites in the mouse. Our atlas comprises 100,000 cells from all major cell lineages. Sub-clustering of epithelial cells confirmed site-specific molecular signatures of stemness and differentiation. Ligand-receptor analysis revealed robust communication between epithelial basal cells and stromal cells. Subsequent sub-clustering of stromal cells revealed that oral fibroblasts have site-specific gene expression profiles, including expression of bone morphogenetic protein (BMP) signaling molecules. The BMP antagonist Noggin was robustly expressed in all oral sites except the buccal mucosa. BMP ligands likewise showed oral-site-specific expression. For instance, BMP5 transcripts were enriched in tongue fibroblasts, whereas BMP7 robustly expressed by palatal fibroblasts. To functionally assess BMP signaling, we established site-specific epithelial organoid cultures. Organoid-forming efficiency assays showed that tongue and palate organoids were sensitive to BMP inhibition, whereas buccal mucosa organoids grew independently of BMP modulation. Our comprehensive cell atlas of the mouse oral mucosa revealed a critical role of epithelial-stromal communication via BMP signaling for site-specific stemness. It represents a rich resource for dissecting other key modulators of site specificity in the mouse oral mucosa.

Abstract No. T21**Advancing cardiac therapeutics from animals to patients***Eldad Tzahor*

Weizmann Institute of Science, Rehovot, Israel

I will introduce novel mechanisms of mammalian cardiac regeneration and repair following injury, a major challenge in current biomedical research using mouse, rats and pigs' models (Tzahor and Poss, Science 2017, Tzahor and Dimmeler, Science 2022). We focused on the role of NRG1-ErbB2 signaling pathway in cardiac regeneration (D'Uva NCB 2015; Aharonov NCB 2020, Shakked NCVR 2023). We have revealed that the proteoglycan Agrin can serve as an inducer of mammalian heart regeneration, with a great therapeutic potential for the treatment of ischemic heart disease (Bassat et al., Nature 2017; Sarig et al., Circulation 2019; Baehr et al., Circulation 2020; Zhang NCVR 2024). I will describe the novel aspect of regenerative senescence in cardiac injuries (Zhang NCVR 2024) and our efforts to repurpose Copaxone, a prescribed drug for multiple sclerosis, for the treatment of heart disease patients (Aviel, Elkahal NCVR 2024 in press).

Abstract No. T22**Lineage-specific cancer-related mutations in human pluripotent and adult stem cells**

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*Presenting author

Human pluripotent stem cells (hPSCs) are known to acquire genetic aberrations in culture, affecting their growth as well as their tumorigenic potential. To survey cancer-related mutations in hPSCs and their differentiated derivatives, we analyzed over 2,200 transcriptomes from about 140 independent lines. About 20% of the samples had at least one cancer-related mutation in either undifferentiated cells or in their ectodermal, mesodermal, and endodermal progeny cells. We have identified mutations in over 25 tier-1 tumor-related genes, and by far the most mutated gene is *TP53* gene. Cells with *TP53* mutations showed a pronounced selective advantage, perturbed target gene expression and altered cellular differentiation.

To compare the rate and the type of mutations in pluripotent versus adult stem cells, we have analyzed over 500 samples from mesenchymal stem cells (MSCs) derived from fetal and adult tissues. We found that about 18% of the samples had a cancer-related mutation in a total of 24 genes, 13 of which were mutated in different studies. All tumor-related genes identified in MSCs are interconnected, and show dramatically different prevalence from those identified in hPSCs. We thus demonstrate a lineage-specific mutation profile in pluripotent versus adult stem cells, attributed to their origin, growth conditions and expression profiles. These results may enable us to better understand the genetic stability of stem cells, and the forces that direct their aberrations in culture. These findings should have significant implications on the need for surveillance of cancer-related mutations in stem cells, especially in clinical applications.

Lezmi, E., Jung, J. & Benvenisty, N. High prevalence of acquired cancer-related mutations in 146 human pluripotent stem cell lines and their differentiated derivatives. Nat Biotechnol (2024). <https://doi.org/10.1038/s41587-023-02090-2>

Abstract No. T23**HLA-homozygous GMP iPSCs show efficient cardiac induction on a New differentiation platform featuring scalability and enhanced robustness***Soraia Martins*, Melanie Hühne, and Boris Greber*

Catalent Düsseldorf GmbH

*Presenting author

iPSC-based therapies hold significant promise for treating various diseases, including heart failure. However stringent quality and consistency requirements imposed by GMP regulations challenges their implementation. To address these, we have established a GMP-compliant workflow for generating iPSCs using episomal reprogramming from eligible donors in the EU and US. These iPSC lines met comprehensive release specifications, show no critical cancer-associated gene lesions, and exhibited a low global mutation load, reflecting their neonatal origin - cord blood.

Beyond the generation of this critical starting material, we have developed a GMP-compatible workflow for converting HLA-homozygous GMP iPSCs into cardiomyocytes (CMs). Applying a co-stimulation strategy involving both WNT and BMP signaling to induce cardiac mesoderm resulted in improved differentiation efficiencies. However, to enhance the robustness of this methodology, the co-stimulation strategy was further extended to the FGF and TGF β signalling pathways. Moreover, inter-experimental variation was critically reduced via media change optimization, with counterintuitive results. This optimized methodology effectively eliminated bulk cell density as a critical process parameter and significantly increased consistency: these critical improvements now allow for multiple sequential differentiation runs at high efficiency – crucial for GMP translation. The workflow is scalable to larger culture volumes with a CM yields of 2-3x10⁶ cells/ml at high purity (<95% cTnT+), which facilitates the generation of therapeutic doses. Furthermore, we observed no induction of cancer-related gene mutations - neither in iPSC expansion nor in CM differentiation - and negligible residual iPSCs in the product indicating a satisfactory safety profile. Technically, our weekend-free workflow is compatible with different 3D platforms like vertical-wheel bioreactors and can be combined with prior expansion of the iPSCs in an independent system, thus enabling a flexible therapeutic platform.

In summary, our findings and systematic optimization solve critical bottlenecks in the generation of therapeutic iPSC-CMs, which will enable a facilitated path to clinic.

Abstract No. T24**hPSC-cardiomyocyte production: standardization and protein-free upscaling to 2000 mL**

Nils Kriedemann^{1,*}, Felix Manstein¹, Kevin Ullmann¹, Wiebke Triebert¹, Carlos A. Hernandez-Bautista¹, Jana Teske¹, Mira Mertens¹, Annika Franke¹, Lika Drakhlis¹, Alexander Haase¹, Caroline Halloin¹, Ines Pombeiro Stein², Andreas Leffler³, Merlin Witte¹, Tamari Askurava¹, Veronika Fricke¹, Birgit Piep⁴, Kathrin Kowalski⁴, Theresia Kraft⁴, Ulrich Martin¹, and Robert Zweigerdt¹

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To fully unlock the potential of human pluripotent stem cell-derived cardiomyocytes (CMs) for regenerative medicine, cost-effective large-scale production is essential. This includes the development of standardized processes utilizing chemically defined, low-cost media of consistent quality.

Recently, we have addressed the challenge of hPSC-CM mass production from two perspectives:

I) Aiming at the simplified GMP-compliant production, single-use stirred spinner flasks were applied. A novel process based on SOP-like handling was established, including prolonged maintenance of the differentiated hPSC-CM aggregates, enabling the uncoupling of cardiac differentiation and quality control measures from the cells' application. Distinct quality control steps were established at different stages emphasizing GMP compliance. The process allowed for the production of $\geq 100 \times 10^6$ CMs per 300 mL scale batch at ≥ 90 % CM purity within 10 days of differentiation. Live-dead staining on intact aggregates, flow cytometry for CMs and "bystander lineages", and confocal microscopy of the 3D aggregate structure promoted the in-depth analysis of the generated cell product.

II) Further, we have focused on differentiation media-related challenges, particularly high protein concentrations that hamper the secretome analysis and lead to batch-dependent alterations in media quality. This has led to the development of entirely protein-free media formulations (PFMFs). These formulations, which include components such as polyvinylalcohol to replace albumin, allow the production of CM yields and purity equivalent to established, protein-containing differentiation media. The applicability of our novel media formulations is demonstrated across numerous culture platforms, including Erlenmeyer flasks (20 mL volume), followed by process upscaling to 150 mL in stirred tank bioreactors (STBRs) and ultimately into 2000 mL STBR scale. At the 2000 mL scale, we achieved the production of $\geq 1.3 \times 10^9$ CMs per process batch. Our extensive quality control measures, including assessing electrophysiological and contractile characteristics, provide a robust assurance of the sustained quality of CMs generated in PFMFs.

Abstract No. T25/P106**Optimization of cardiomyocyte cryopreservation and quality control aspects for potency and residual iPSC contaminations**

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*Presenting author

Screening of Cord blood units (CBUs) for most frequent HLA haplotypes allows the selection of corresponding HLA homozygous (HLA^h) CBUs. After isolation of CD34⁺ hematopoietic stem cells (HSC) from these HLA^h CBUs, cells are reprogrammed towards iPSC.

Within the HEAL Project (101056712), the HLA^h iPSC were differentiated into cardiomyocyte-aggregates (CMAs) via 3D-suspension culture. For possible applications of the CMAs in a clinical setting, certain quality-assessments /-controls (QA/QC) need to be established to ensure the final safety of the product. Cryopreservation is a good option for the optimal clinical process to allow time for QA/QC assays.

CMA differentiation was achieved by WNT pathway modulation with an optimized protocol (Terheyden-Keighley et al. 2024, accepted in SCTM). The CMAs were analyzed for their phenotype and residual cell contaminations applying flow cytometry and qPCR. Detection of potency/function was conducted by video-analysis. CMs were cryopreserved either as aggregates or as single cells. In case of single cells, re-aggregation after thaw was performed for a maximum of 5 days.

Cardiomyocytes with >85% cTnT⁺ were used for downstream analysis. Spontaneous contraction, as an analysis for the function, was monitored as early as differentiation-day 7, resembling immature cardiomyocytes, with low beating frequencies (<20 bmp). Cryopreservation resulted in an overall recovery of <60% CMAs after 5d of culture and recuperation of spontaneous contraction (~10 bmp). Cryopreservation of single cell cardiomyocytes was associated with a greater loss of cells (overall recovery <40%). For Screening of residual iPSC contaminations, qPCR could detect contaminations of <0.1% iPSC with specific markers, e.g. miR-302.

Cryopreservation is a useful step of processing, however both the freezing of aggregate and single cell cardiomyocytes need further optimization. Although <0.1% iPSC contamination can be detected by qPCR, *in vivo* animal studies are a mandatory requirement in the development of a save product (Tamura et al. 2024 SCTM).

Terheyden-Keighley et al. GMP-compliant iPS cell lines show widespread plasticity in a new set of differentiation workflows for cell replacement and cancer immunotherapy, accepted in Stem Cells Translational Medicine, 2024

Tamura et al. Study on the Extrapolability of Current Tumorigenicity Test With Mice by Comparing the Syngeneic or Allogeneic Mouse Transplantation Model, Stem Cells Translational Medicine, 2024, <https://doi.org/10.1093/stcltm/szae019>

Abstract No. T26**iPSCs in drug discovery – from a user's and a personal perspective***Bert Klebl*

Lead Discovery Center GmbH, Dortmund

The iPSC community claims iPSCs differentiated into specific cell types to be superior translational tools/solutions for drug discovery and development:

- for regenerative therapy, which I cannot really much comment on
- as human disease models to a, identify novel targets and b, (randomly) test different development stage compounds for efficacy (in high throughput – whatever that means!?)
- as human models for toxicity monitoring to profile different development candidate compounds with the aim to reduce animal testing
- to grow organoids or to implement co-culture systems, also for compound testing

Generally, I agree with some of these bold statements, but there are also several issues from a drug discovery (and development) perspective, which warrant further optimization and development of these iPSC-based models, largely based on economic, but also on pharmacological considerations.

This short impulse talk aims at triggering a discussion amongst iPSC and drug discovery experts to optimize the use of human-disease models in translational medicine and pharmacology.

Abstract No. T27**Patient-specific iPSC-derived hepatic organoids as model for drug dosing and mRNA-based gene therapy**

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*Presenting author

Progressive familial intrahepatic cholestasis (PFIC) is characterized by defective bile formation and account for 10-15% of neonatal cholestasis. The underlying mechanism of intrahepatic cholestasis can be associated with mutations in hepatobiliary transport proteins or in nuclear receptors responsible for proper transcriptional control of these transporters.

In this study, we investigated, if iPSC-derived organoids could serve as model to predict drug dosing in patients or would provide a preview on effective mRNA-based gene therapy. First, we exploited hepatic organoids from a patient carrying a compound heterozygous variation in the ABCB11 gene encoding the bile salt export to (BSEP). Applying different drugs, we were able to point out the most suitable patient-specific dose for each drug and the impact of combinatorial drug treatment based on the restored BSEP mediated hepatobiliary transport. In a next set of experiments, we studied organoids from two patients suffering from BSEP deficiency caused by mutations in the farnesoid-X receptor (FXR) gene NR1H4. The NR1H4 gene encodes four different FXR variants whereas two FXR α 1 variant are most abundant in the liver. One cell line carries a homozygous variation in exon7, resulting in a complete abrogation of downstream signal transduction. Another cell line carries a homozygous variation resulting in an exon-skipping and loss of FXR α 1 variants, while FXR α 2 variants are unaffected. We generated lipid nanoparticles (LNP) carrying the mRNA of the four different FXR variants and BSEP as positive control. After treatment of the hepatic organoids with the different LNP, we were able to restore BSEP transport with all FXR variants. This indicates that even the gut-associated FXR α 2 variants are able to restore hepatic function and might be targetable to induce BSEP function in FXR α 1 deficient patients. However, as the different FXR variants are involved in several metabolic pathways, the global impact of this alternative activation needs further investigation.

Abstract No. T28**Human multi-tissue organoids as an advanced teratogenicity model**

Liam Wilson^{1,*}, Lika Drakhlis¹, Julia Dreger¹, Dorina Lang¹, Jana Teske¹, Felix Kleemiß¹, Miriana Dardano¹, and Robert Zweigerdt²

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Human pluripotent stem cell (hPSC)-based organoids recapitulate development in a dish, allowing for teratogenic assessment without the ethical issues and inter species variation associated with animal studies. However, current models lack developmental complexity. We recently described a hPSC-derived multi-tissue organoid termed “heart-forming organoids” (HFOs), that mimics early heart, vasculature, anterior and posterior foregut development in an axis reminiscent of early organogenesis. We therefore sought to understand HFOs capability to assay teratogenicity, identify specific teratogenic cell type susceptibility and the affects these have on HFO developmental patterning. To this aim, HFOs were exposed to FDA-categorized teratogens Valproic acid (V) and Thalidomide (T). Flow cytometry (FC) and immunofluorescence staining (IF) revealed the expected anti-angiogenic properties of T and V as well as the cardio-toxic effect of T, suggesting HFOs are capable of replicating previously described in vivo teratogenic phenotypes. Moreover, FC revealed a decrease in the endodermal (EPCAM+/CD117+) population of V and T HFOs. Notably, following whole-mount IF, V HFOs showed a relative increase of AFE (FOXA2+/SOX2+) over PFE (FOXA2+/HNF4α+) and a concomitant increase in cardiac progenitors (NKX2.5+/FOXA2+) suggesting a coupled delay in development and an anterior bias. In contrast, T treatment resulted in disruption of the endodermal patterning, juxtaposing AFE and PFE seemingly disrupting developmental axis establishment. These results highlight HFOs potential to shed light on cell type specific teratogenic mechanisms and there affects in a morphological context, an ability previously difficult with in vitro models. Furthermore, this provides a proof-of-concept for further studies to understand the mechanisms behind commercially successful teratogenic pharmaceuticals in the hope of reducing teratogenic affects with foresight to increase the throughput of the system to assay developing drugs.

Abstract No. T29**Cellular metabolism senses exogenous fatty acids to reprogram dormant tumor cells into aggressive oncogenic cell states**

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During uncontrolled growth, cancer cells exhibit a spectrum of cellular states from highly oncogenic/stem cell-like to more quiescent/dormant states. However, it remains unknown, especially in human cancer, how diet and metabolites regulate these heterogeneous cancer cell states. Here, we demonstrate that dietary, long-chain fatty acids induce an epigenetic and transcriptional reprogramming on quiescent/dormant tumor cells resulting in an aggressive tumor cell state. Using patient-derived liver cancer organoids and upon modulation of fatty acids metabolism we show that exogenous supplementation of dietary long-chain fatty acids is necessary and sufficient to reprogram quiescent (non-tumor competent) cells into highly aggressive tumor-promoting cells, both in vitro and in vivo. Using metabolomics, proteomics and transcriptomic analysis we delineate the timeline of events whereby the phenotypic and transcriptional reprogramming are driven by mitochondria-derived acetyl-CoA levels followed by promotion of histone acetylation in loci of key oncogenic pathways, which ultimately leads to the cancer cell fate change. Metabolic flux data from a cohort of liver cancer patients confirmed our findings, with enhanced fatty acid metabolism in the more aggressive oncogenic subpopulations compared to quiescent/dormant ones. Our results show that quiescent/dormant cancer cells can be reprogrammed into aggressive tumor-competent cells following exposure to dietary fatty acids. With the rising levels of fat intake and the ensuing fat accumulation in liver cells caused by the Western diet, this novel paradigm has significant implications for the management of patients with cancer and for the development of therapeutic strategies to fight tumor resistance from quiescent/dormant tumor cells.

Abstract No. T30**iPSC modeling of bipolar disorder 1 based on polygenic risk scores**

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Bipolar I Disorder (BD1) is a prevalent neuropsychiatric condition affecting up to 1% of the population that remains poorly understood and inadequately treated. Studies on patient-derived neuronal progenitor cells (NPCs) and neurons have revealed altered transcriptomic profiles, changes in proliferation and differentiation, and disruptions in cell fate specification and functionality, primarily linked to calcium metabolism. Genome-wide association studies have identified over 60 common genetic variants associated with BD1 contributing to its high heritability. However, the molecular changes mediated by these variants and their translation into disease-associated pathophenotypes remain unclear. To address this void, our induced pluripotent stem cell (iPSC)-based model approach focused on donor selection using polygenic risk score (PRS) with exclusion of high penetrance variants. We generated multiple iPSC lines from three BD1 patients with high PRS and three healthy controls with low PRS, followed by extensive quality control. Our analysis revealed a complex copy number variation (CNV) architecture consisting of blood-born and reprogramming-associated CNVs. Given the neurodevelopmental nature of BD1, we focused on the early developmental stages. Patient and control-derived iPSCs were differentiated into NPCs using a highly standardized small molecule-based protocol. EdU assay and quantification of TBR1-positive cells showed homogeneous proliferation and differentiation across all cell lines. RNAseq performed at four time points identified a number of differentially expressed genes, including *NBPF3*, a gene regulating vitamin B clearance, and *IFITM2* that has been associated with the innate immune response and found upregulated in schizophrenia. Ongoing analyzes are addressing potential differences in store-operated calcium entry, a pathway previously shown to be downregulated in BD1 patient-derived NPCs. Our study provides an experimental workflow for PRS-based pathophenotyping in BD1 including donor selection based on PRS combined with variant filtering and high-resolution SNP analysis for *de novo* CNV detection – a workflow that can now be extended to larger donor cohorts.

Abstract No. T31**The bone marrow niche under stress***Marieke Essers*

German Cancer Research Center (DKFZ), Heidelberg & HI-STEM, Heidelberg

Inflammation is a key component in a complex biological response of the body to harmful stimuli. In the context of the bone marrow, inflammation is an overarching process central to most if not all forms of stress, challenges and disease settings including leukemia and immunodeficiencies. Often descriptive analysis of the stress response of the niche falls short in translating it to functional applications confounded by a single marker approach of classifying the niche cell diversity. Here, we have used the power of single-cell sequencing to investigate the heterogeneity and dynamics of the response of the bone marrow niche after treatment with the pro-inflammatory cytokine IFN α . We have identified and described a novel bone-lining MSC sub-population, which unlike its stromal counterpart responded directly and dynamically to IFN α stimulation (iMSCs). Using ligand-receptor mapping, we further identified pivotal inflammation specific interactions between iMSCs and HSCs within the bone marrow. To further investigate these interactions, we have developed a long-term murine and human co-culture system with MSCs and HSCs, which allows us to maintain and expand functional HSCs ex vivo over several weeks. In conclusion, using single cell sequencing and an ex vivo culture system, we have been able to unbiasedly study the dynamic bone marrow response and crosstalk between HSCs and MSCs to inflammatory stress.

Abstract No. T32/P041**Apelin signaling restricts the formation of the blood stem cell-forming hemogenic endothelium**

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During definitive hematopoiesis hemogenic endothelial cells in the aorta-gonad-mesonephros region differentiate into multipotent hematopoietic stem cells in a process called endothelial-to-hematopoietic-transition. Although only a small pool of hematopoietic stem cells is formed during embryonic development, these hematopoietic stem cells generate all blood lineages throughout life. Previous work revealed that hemogenic endothelial cells and vascular endothelial cells share a common lineage. Since the process of hematopoiesis is highly conserved among vertebrates, we are using the zebrafish as an in vivo model. Here, we show that the embryonic dorsal aorta is zonated into apelin receptor (aplnr) expressing and aplanr non-expressing endothelial cells by live confocal imaging. Of interest, aplanr non-expressing endothelial cells are exclusively located in the ventral part of the dorsal aorta, where hematopoietic stem cells arise. The G-protein-coupled receptor Apelin Receptor has been shown to play an important role in vasculogenesis and angiogenesis. However, little is known about the role of Apelin signaling in hematopoiesis. Our cellular and molecular analysis demonstrated that these aplanr-negative endothelial cells give rise to hematopoietic stem cells. Genetic analysis revealed that the predefined hemogenic endothelium by the zonation of aplanr expression is upstream of Runx1 function. Furthermore, zonation by aplanr expression in the dorsal aorta functionally restricts the hemogenic endothelium. Loss of Apelin signaling leads to an extension of the hemogenic endothelium and subsequently to an increased formation of hematopoietic stem cells within the dorsal aorta. Conversely, forced activation of Apelin signaling in the vasculature leads to a complete loss of hematopoietic stem cell development in the dorsal aorta. Our results suggest that Apelin signaling restricts the zonation of the hemogenic endothelium and thus regulates the number of hematopoietic stem cells emerging during embryonic development. This provides new insights into the process of hematopoiesis, enabling a better understanding of hematopoietic disorders to improve treatment possibilities.

Abstract No. T33/P052**Intrinsic reconstitution potential of developmentally restricted hematopoietic stem cells in non-conditioned immunocompromised mice**

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*Presenting author

New insights are challenging the traditional view of strictly hierarchical hematopoietic specifications during development and into adulthood. Instead, evidence is bolstering a model where hematopoietic stem and progenitor cells (HSPCs) arise in parallel; however, to what extent perinatally derived HSCs contribute to adult hematopoiesis is not well known.

Using an unequivocal genetic lineage tracing mouse model (FlkSwitch), we revealed the co-existence of developmentally restricted HSCs (drHSCs) alongside fetal HSCs (fHSCs), which do not persist as HSCs into adulthood *in situ*. In contrast, drHSCs are capable of long-term multilineage reconstitution when transplanted into irradiated hosts, possibly because of their high responsiveness to inflammatory signals. To test this, we isolated drHSCs from neonatal livers of FlkSwitch mice and transplanted them into adult unconditioned immunocompromised recipient mice with a c-Kit mutation (NBSGW mice). Strikingly, drHSCs exhibited robust long-term reconstitution (20 weeks) of peripheral blood cells and HSPCs in the bone marrow. The phenotypic HSC pool established by drHSCs in the bone marrow of NBSGW recipients were notably smaller compared to that established by fHSCs, potentially indicating limited self-renewing expansion capacity of drHSCs, despite their long-term persistence. Quantitative cell analysis revealed that drHSCs are superior in reconstituting B and T cells in the peripheral blood, tissue-resident innate-like B1a cells in the peritoneal cavity, and marginal zone B cells in the spleen in recipient mice compared to fHSC. This emphasizes the importance of drHSC in immune cell generation during development and potentially over the lifespan. As early developmental lymphoid cells play essential roles in establishing the immune system, self-recognition, and tolerance, these findings are critical for understanding the development of disease susceptibility and autoimmune diseases in adulthood.

Abstract No. T34**Metabolic atlas of human bone marrow hematopoietic stem cells upon differentiation, aging and leukemia**

Maria Eleni Lalioti^{1,*}, Mari Carmen Romero-Mulero¹, Noémie Karabacz¹, Julian Mess¹, Jasmin Rettkowski¹, Helen Demollin¹, Karin Jäcklein¹, Konrad Schuldes¹, Michael Mitterer¹, Katharina Schönberger¹, Nikolaos Karantzelis², Carolin Wadle¹, Mirijam Egg¹, Michael-Jason Jones¹, Beatriz Silva-Rego¹, Simon Renders³, Darja Karpova⁴, Khalid Shoumariyeh², Aurélien Dugourd⁵, Lukas Braun², Julio Saez-Rodriguez⁵, Robert Zeiser², Hind Medyouf⁶, Sascha Gravius⁷, Sebastian Gottfried Walter⁸, Joerg Buescher¹, and Nina Cabezas-Wallscheid¹

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Hematopoietic stem cells (HSCs) represent a rare cell population in the bone marrow (BM) that lies at the top of the hematopoietic hierarchy, uniquely capable of replenishing the entire blood system. Metabolic cues are crucial for regulating HSCs, and advances in genetic models and -omics techniques have significantly increased our understanding of these important regulatory mechanisms. However, most studies have been conducted in mice and the metabolic profile of human HSCs remains largely unexplored due to the limited number of cells and the scarcity of healthy BM samples. In this study, we present an integrated analysis of the metabolome, lipidome, and transcriptome of human adult BM HSCs during differentiation, aging, and leukemia (Lalioti*, Romero-Mulero*, et al., under review in *Nature Cell Biology*). Utilizing our newly established low-input targeted metabolomics protocol (Schönberger et al., *Cell Stem Cell* 2022) and by establishing a novel untargeted lipidomics method, we detected up to 175 metabolites and lipids from as few as 3,000 and 5,000 cells, respectively. Importantly, we found specific metabolic and lipid patterns upon aging and in the context of leukemia which we functionally validated using molecular, metabolic tracing, in vitro and in vivo experiments. Together, this study represents the first metabolic atlas of human BM HSCs which we extend in the context of physiological stress (aging) and disease (leukemia). Our findings highlight promising regulators for HSC maintenance and metabolic alterations that could be targeted to maintain human HSC function.

Abstract No. T35**The function of PD-L2 expression on human hematopoietic stem cells in allogeneic immune regulation at single cell resolution**

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*Presenting author

Identifying precise and dependable markers for human hematopoietic stem cells (HSCs) is essential for their prospective isolation, facilitating the study of their distinct physiological functions within the blood cell hierarchy. Despite notable progress in surface marker identification for isolating HSCs from human bone marrow, the resulting populations remain heterogeneous. Here, we utilized single-cell CITE-Seq on FACS-enriched hematopoietic stem/progenitor cells (HSPCs) resulting in the identification of Programmed Death Ligand 2 (PD-L2/CD273), an immune checkpoint inhibitor protein selectively upregulated in the most immature HSCs. Flow cytometry confirmed PD-L2 surface expression on a subset of HSCs, prompting further exploration of its immune-regulatory function.

Transcriptomic analysis revealed a distinct gene expression profile in PD-L2 expressing HSCs, characterized by upregulated stem cell genes (MPL, Thy1, DLK1). In vitro assays demonstrated that PD-L2hi HSCs exhibit delayed cell cycle entry, reduced differentiation, and increased protein expression of stem cell markers.

To explore the immunomodulatory functions of PD-L2hi HSPCs we developed an innovative allogeneic reaction system combining multi-omics scCITE-seq with cytokine profiling, functional readout and a bioinformatic analysis pipeline. Our technology relies on co-culture of HSPCs with T-cells from different healthy donors and characterized PD-L2's remarkable role for HSPC immune surveillance at single-cell resolution. We discovered decreased T cell proliferation, heightened secretion of anti-inflammatory cytokines, increased apoptosis, and reduced activation of CD8+ cytotoxic T cells (lower CD69 expression) upon co-culture. PD-L2 checkpoint blockade reversed these effects, enhancing inflammatory responses and decreasing regulatory T cell populations. Single-cell sequencing validated these findings, showing increased expression of T-cell activation markers upon PD-L2 blockade, an increase of CD8 proliferative T cells and alterations in HSPC profiles under allogeneic conditions.

Our system combines single-cell proteo-transcriptomic profiling with functional readouts and thereby identified PD-L2 as a critical surface marker on immature HSPCs, pivotal in shielding from adverse immune reactions through its immunoregulatory functions.

Abstract No. T36**Tracking of early cell fate decisions of iPSCs based on DNA methylation**

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*Presenting author

Quality control of induced pluripotent stem cells (iPSC) remains a challenge, particularly for assessment of pluripotency and differentiation potential into the three germ layers: endoderm, mesoderm, and ectoderm. Conventional quality control methods rely on analysis of morphological criteria, surface markers, gene expression profiles, or teratoma formation in mouse models. However, these techniques are often difficult to standardize and quantify. To address the growing need for precise characterization of iPSCs and their derivatives, we developed PluripotencyScreen, an epigenetic biomarker platform that monitors early cell fate decisions by analyzing DNA methylation at specific CpG sites. Using the computational framework CimpleG, we identified three characteristic CpG at pluripotent state and after differentiation into endoderm, mesoderm, and ectoderm. From these signatures, we established scores that not only track reprogramming of iPSC but also monitor both, the directed differentiation of iPSC and multilineage differentiation in embryoid bodies (EBs). Validation through targeted pyrosequencing assays in many iPSC lines and DNA samples confirmed the reliability of site-specific DNA methylation for characterization of iPSC. For standardized and scalable multi-lineage differentiation in EBs we developed a new method that uses geometrically confined iPSC colonies, which spontaneously detach after 5-7 days of culture. This technique was further refined by ultraviolet (UV) micropatterning of circular vitronectin-coated areas. PluripotencyScreen successfully identified iPSC lines with impaired ectodermal differentiation capacity within EBs, and it detected reduced ectodermal cell fractions in PRDM8, YAP1, and CTCF knockout lines. We are continually optimizing and validating this approach. Overall, PluripotencyScreen offers a reliable and reproducible tool for iPSC quality control by quantifying pluripotency and early lineage-specific commitment.

Abstract No. T37**Pooled mesenchymal stromal cells fail to represent average donor cell characteristics**

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*Presenting author

Pooling cells from different donors is a common practice when investigating primary mesenchymal stromal cell (MSC) characteristics and potency. This approach overcomes limitations regarding available cell numbers and reduces data variability as compared to experiments with separate biological replicates, while it is claimed that the different donors are still reflected in the results. However, there are not sufficient data supporting this assumption. In this study, we compared functional properties of MSC pools to those of their corresponding biological replicates, and monitored the composition of MSC pools over time.

Human MSC from 9 donors were characterized in terms of CFU and proliferative potential and accordingly assigned to low, medium and high fitness groups. Different MSC pools, each consisting of MSC from 3 donors, were then composed (low fitness, medium fitness, high fitness and mixed fitness pool). Functional MSC characterization assays were then performed with the individual MSC and with the MSC pools. Furthermore, MSC from the individual donors were labeled with cell tracer dyes before pooling, and the composition of the cell pools was monitored by microscopy and flow cytometry.

High fitness biological replicates showed shorter population doubling times and higher metabolic rates ($p < 0.05$), as anticipated. However, while population doubling times from cell pools and their corresponding biological replicates were in line, large discrepancies between pooled cells and biological replicates were observed in metabolic rates, trilineage differentiation and wound closure potential. This was not only observed for the mixed fitness pool, but also for the pool consisting of donors with high fitness only. Moreover, cell tracing results confirmed that the cell pool compositions changed during the cultivation period.

The current data are alarming as they demonstrate that pooled cells are not representing the average characteristics of the included donors, which is likely to entail misleading preclinical predictions of cellular potency.

Abstract No. T38/P115**Humanized osteoclasts: an interspecies chimeric immune cell***Gülce Perçin^{1,*} and Claudia Waskow^{2,3}*¹Leibniz Aging Institute/FLI, Jena²Leibniz Institute on Aging, Immunology of Aging, Jena³Friedrich Schiller University Jena, Faculty of Biological Sciences

*Presenting author

Osteoclasts are multinucleated immune cells that are critical for bone remodelling to ensure space for adult hematopoiesis. Initially, because of their dependence of Csf1r signalling, osteoclasts were suggested to be of macrophage/monocyte origin that are constantly generated from Hematopoietic Stem Cells (HSCs), laying the foundation of the current clinical treatment for osteopetrosis. However, mouse models that specifically inhibited Csf1r signalling in embryonic or adult hematopoiesis revealed that osteoclasts have an embryonic origin. Adult Csf1r depletion revealed that embryo-derived osteoclasts are maintained in adults by fusing with HSC-derived monocytes, forming a syncytium with genetic material from two ontogenically distinct immune cells. Taking it a step further, we are interested in understanding the mechanism of osteoclast maintenance in xenograft models. In comparison to other commercially available models, NSGW41 was chosen because of the increased and stable human hematopoietic cell engraftment over time with increased myeloid cell output. Thus, we discovered mouse osteoclasts with one or more human nuclei in humanized NSGW41 bones, indicating an interspecies chimeric immune cell. We isolated distinct blood monocytes from cord blood or adults to find the human donor cell that can fuse with pre-existing osteoclasts. We discovered that osteoclastogenesis in the blood monocyte compartment was limited to CD14⁺ inflammatory blood monocytes. As a result of this *in vitro* finding, CD14⁺ blood monocytes potentially be the target cells that can fuse with pre-existing mouse osteoclasts in xenograft models to maintain osteoclasts. By adoptive transfer experiments to the humanizable NSGW41oc/oc model, we hope to demonstrate the osteoclast potential of human CD14⁺ blood monocytes *in vivo* in the near future.

Abstract No. T39**Resolving human alpha versus beta cell fate allocation for the generation of stem cell-derived islets**

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Generating stem cell-derived glucagon-producing alpha (SC-alpha cells) and insulin-producing beta cells (SC-beta cells) allows to engineer an *in vitro* biomimetic of the islet of Langerhans, the micro-organ controlling blood glucose, however, there is still a major knowledge gap in the mode and mechanism by which human SC-alpha and beta cells are specified. Mouse studies postulated that Aristaless Related homeobox (Arx) and Paired box 4 (Pax4) transcription factors cross-inhibit each other in endocrine progenitors to promote alpha or beta cell fate allocation, respectively. To test this model in human, we generated an ARX^{CFP/CFP}; PAX4^{mCherry/mCherry} double knock-in reporter induced pluripotent stem cell (iPSC) line to combine time-resolved cell lineage labeling with high-resolution single cell multiomic analysis. Strikingly, lineage labelling and tracing, proteomic and gene regulatory network (GRN) analysis together with potency assays revealed a human specific regulation of alpha *versus* beta cell fate allocation. Importantly, pharmacological perturbation using drugs previously proposed to trigger alpha-to-beta cell transdifferentiation or identified via our GRN analysis led to enhanced endocrine induction and directed alpha versus beta cell fate commitment. Thus, shedding light on basic mechanisms of endocrine induction and fate segregation not only paves the way to engineer islets from pluripotent stem cells, but also has broader implications for cell-replacement therapy, disease modelling and drug screening.

Abstract No. T40/P118**Generation of designer stem cell-derived islets (SC-islets) to improve safety and efficacy of SC-based islet replacement therapy**

Eunike Sawitning Ayu Setyono^{1, 2,*}, Nicole K. Rogers¹, Katharina Scheibner¹, Silvia Schirge¹, Michael Sterr¹, Timucin Öztürk¹, Ingo Bartscher^{1, 2}, and Heiko Lickert^{1, 2}

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*Presenting author

Stem cell (SC)-derived pancreatic islets are an alternative source of islets for Type 1 Diabetes replacement therapy. Despite their great potential as an unlimited cell source, SC-islets still have limitations due to current protocol efficiency to generate large numbers of mature endocrine cells. Infact, unspecified non-endocrine cell types generated from this method could give rise to cysts and unwanted outgrowths, posing a potential safety risk for patients. Additionally, differentiation protocols towards endocrine pancreatic lineages have been focused on SC-beta-cells and overlooking other islet cell types, despite their importance. In particular SC-alpha-cells, for their important role in regulating SC-beta-cells functionality. For these reasons, with this project, we aim to generate a superior SC-islets with improved purity, functionality, and safety.

To generate more functional SC-islets with defined composition and depletion of unwanted cell types, we have established sorting strategies for enrichment of SC-beta-cells and SC-alpha-cells. We generated an ARX-CFP x C-Peptide-mCherry reporter iPSC cell line and utilized the reporter expression to enrich for SC-beta-cells (C-Peptide⁺) and SC-alpha-cells (ARX⁺), thus allowing the generation of SC-islets with a defined ratio of SC-beta-cells and SC-alpha-cells. Here we showed that enriched SC-beta-islets and SC-beta/alpha-islets showed an improved insulin secretion upon glucose challenge compared to the non-enriched SC-islets.

With this result, we confirmed our initial hypothesis that the presence of SC-alpha-cells are crucial for SC-beta-cells function. Next, we aim to do a comprehensive transcriptomic analysis of our SC-islet product before and after in vivo transplantation. This would provide a novel insight on SC-derived product efficacy and safety upon transplantation and pave the way for future clinical trials.

Strategic sessions presentations

**12th GSCN Conference
German Stem Cell Network
25 – 27 September 2024**

Strategic sessions presentations: S1 – S7

- S1** Exploring public and government funding opportunities
Bettina Otto
- S2** High-Tech Gründerfonds - early stage seed investments
Christian Kannemeier
- S3** The National Office for Gene- and Cell Therapies (GCT): updates on activities and future impact
Elke Luger
- S4** Publish or perish - feedback from a journal editor
Achim Breiling
- S5** Cellular microenvironments fabricated with fluid walls for disease modeling
Edmond Walsh
- S6** Combining stem cell and device engineering for in vitro models of human physiology
Anna Herland
- S7** Stem cell-derived vessels-on-chip for cardiovascular disease modeling
Caroline Remmert

Abstract No. S1**Exploring public and government funding opportunities***Bettina Otto*

Charité Universitätsmedizin Berlin, Charité BIH Innovation

The path from research to marketable products is often challenging and requires financial support from various sources. Public and government funding can be a valuable option for researchers and entrepreneurs who want to develop their ideas into marketable solutions. However, navigating the complex landscape of funding programs and application processes can be challenging. In this talk I will provide an overview of the main public and government funding opportunities for startups and R&D collaborations with small and medium-sized companies in Germany and Europe.

Abstract No. S2**High-Tech Gründerfonds - early stage seed investments***Christian Kannemeier*

High-Tech Gründerfonds, Life Sciences

HTGF is one of the leading and most active early-stage investors in Germany and Europe, financing start-ups in the fields of Deep Tech, Industrial Tech, Climate Tech, Digital Tech, Life Sciences and Chemistry. With its experienced investment team, HTGF supports start-ups in all phases of their development into international market leaders. HTGF invests in pre-seed and seed phases and can participate significantly in later-stage financing rounds. Across its funds, HTGF has over 2 billion euros under management. Since its inception in 2005, HTGF has financed more than 750 start-ups and successfully sold shares in more than 180 companies.

Fund investors in the public-private partnership include the German Federal Ministry of Economics and Climate Action, KfW Capital and 45 companies.

Abstract No. S3**The National Office for Gene- and Cell Therapies (GCT): updates on activities and future impact**

Elke Luger

Berlin Institute of Health (BIH), National Office for Gene and Cell Therapies

In this presentation, the National Office for Gene and Cell Therapies (Netzwerkbüro, NWB) will introduce itself and provide an update on current projects and developments. The NWB was established in October 2023 as part of the National Strategy for Gene and Cell Therapies (GCT). It operates under the mandate of the BMBF to the BIH, to bring together relevant stakeholders to develop and implement the National Strategy for GCT.

The mission of the NWB is to build a national GCT community, GCT Germany, through independent, cross-regional information sharing and networking, welcoming and considering all stakeholder groups. These stakeholders include representatives from science, industry, politics, and society, as well as authorities, foundations, and patient organizations.

I will present GeneNovate, the first nationwide entrepreneurship program in Germany focused on GCT (GeneNovate: Empowering Innovators and Entrepreneurs in Gene and Cell Therapies). This six-month program targets early-career individuals, such as doctoral students and postdocs, as well as experienced researchers and physicians. The program's goal is to provide comprehensive knowledge in entrepreneurship and to mentor participants in the development of therapies.

The presentation will also cover two new funding calls as part of the National Strategy for GCT. These calls invite applications for individual or collaborative translational projects that focus on product and/or process development of therapeutic and diagnostic approaches in GCT. The funding aims to improve and accelerate the translation of innovative GCTs and associated diagnostics into marketable and clinically applicable products and processes. Support includes financing, mentoring, coaching, and training based on the SPARK concept, with funding available for up to two years. A second call is planned for 2025.

<https://www.bihealth.org/de/translation/nationales-netzwerkburo-fuer-gen-und-zelltherapien>

LinkedIn: Netzwerkbüro GCT Germany

Abstract No. S4**Publish or perish - feedback from a journal editor***Achim Breiling*

EMBO Reports, Heidelberg

You know the pressure: either you publish in (preferably high ranking) journals or your scientific career is potentially at an end before you even really started. But what to do about it? Scrambling together whatever data you can manage to increase the number of your publications and your visibility (aka lab-book cleaning) rather than taking the time to develop significant research? And anyway, what is a significant publication and how does my research get published? Get insights into these questions and into the work of a journal editor in this session.

URL EMBO Reports: <https://www.embopress.org/journal/14693178>

Abstract No. S5**Cellular microenvironments fabricated with fluid walls for disease modeling***Edmond Walsh*

University of Oxford, Engineering Science, United Kingdom

We present a new method to fabricate cellular microenvironments within standard tissue cultureware, with a focus on the development of neuronal microenvironments for disease modelling. In the brain the cortex and striatum, together with the basal ganglia and thalamus, play an important role in regulating voluntary movement, learning, executive function, and emotion. Cortical neurons (CNs) project axons toward the striatum where medium spiny neurons (MSNs) constitute up to 95% of striatal subtypes. Connectivity between CNs and MSNs is directional and monosynaptic, whilst MSNs communicate with CNs indirectly via downstream circuits, particularly the basal ganglia. This oriented arrangement of cortico-striatal projections is critical for their functioning but is often oversimplified by in vitro co-cultures. However, the use of fluid-walls provides a solution to this challenge by enabling the fabrication of microenvironments to investigate the directional connectivity between cortex and striatum within standard 6cm Petri-dishes. Additionally, we developed a localized non-contact axotomy assay that can be performed within the fluid-walled microenvironments where connecting axons are severed by a microjet of liquid medium. In summary this talk will address the fabrication technique of neuronal microenvironments, spatial cell confinement, passive axon cutting and experimental validation with preliminary data. Further details of the method are reported in Nebuloni et al. 2024.

F. Nebuloni, Q.B. Do, P.R. Cook; E.J. Walsh, R. Wade-Martins, 2024, "A fluid-walled microfluidic platform for human neuron microcircuits and directed axotomy", Lab Chip, 2024, 24, 3252–3264

Abstract No. S6**Combining stem cell and device engineering for in vitro models of human physiology**

Anna Herland

KTH Royal Institute of Technology, Protein Science & Karolinska Institute, Neuroscience, Solna, Sweden

In vitro studies have a poor translation to human in vivo processes and treatment outcomes. This translational gap originates from the low resemblance between cell culture and the dynamic, three-dimensional, and multicellular in vivo environment. However, the demand to model human physiology has risen with insights into inter-species differences and human-specific treatments.

Human stem cells hold the potential to be the basis of human-relevant models. To meet the demand for a physiological environment, microfluidic Organ-on-Chip models have emerged. In Organ-on-Chips, cells are cultured in microcompartments with perfusion. We are developing these systems from three perspectives: first, to increase human physiological relevance through stem cell engineering and relevant 3D microenvironment; second, non-disruptive real-time monitoring; and third, user-friendliness and cost-effectiveness. Our focus is the neurovascular unit (NVU). We have established protocols to generate vascular and neural cells from human pluripotent stem cells. We have developed new materials and sensors to study barrier penetration of small drugs and biopharmaceuticals, cellular interactions, and inflammatory responses in real time. We furthermore developed methods to study astrocytes and neurons with micrometer precision.

Organoids have demonstrated a remarkable resemblance with human tissue. Unfortunately, self-assembled cellular structures typically lack perfusable vasculature. To study vascular-organoid interactions and physiological drug exposure, we have developed a patterning method called cavitation molding. Cavitation molding allows us to pattern 10-20 μ m wide lumens in ECM around an organoid without impairing its viability. These lumens formed a defined vascular network around the organoid. We are now designing our NVU-chips as a combination of top-down and bottom-up approaches with integrated sensing. We aim to understand metabolic interactions and disturbances in the neurovascular unit, as we previously studied in primary cells. We are investigating NVU interactions in patients with inborn metabolic diseases as well as models of radiation damage and hemorrhage.

Buchmann, S., Enrico, A.,..., Herland, A. (2023) Probabilistic cell seeding and non-autofluorescent 3D-printed structures as scalable approach for multi-level co-culture modeling. Materials Today Bio, 2023, 100706, doi: 10.1016/j.mtbio.2023.100706

Enrico, A.,...,Herland, A. & Stemme, G. (2022), 3D Microvascularized Tissue Models by Laser-Based Cavitation Molding of Collagen. Adv. Mater. 2022,2109823. doi: 10.1002/adma.202109823

Abstract No. S7**Stem cell-derived vessels-on-chip for cardiovascular disease modeling**

Caroline Remmert¹, Maren Marder¹, and Matthias Meier^{1,2}

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²Leipzig University

Cardiovascular diseases are associated with atherosclerosis, a process in which lipids, mainly cholesterol-containing low-density lipoproteins (LDL), accumulate in arterial endothelial cells (ECs). The subsequent formation of reactive oxygen species (ROS) leads to EC dysfunction, followed by the formation of vascular lesions, impaired vasodilation due to reduced nitric oxide (NO) synthesis, flow disturbances, and local inflammation. Chronic metabolic syndromes such as obesity, diabetes, and aging are associated with atherosclerosis, but our understanding of disease onset remains imperfect. Thus, reliable human *in vitro* models for endothelial tissues and EC dysfunction are in urgent demand, given the complexity of the human vascular system and the proven metabolic distance of rodent *in vivo* models to human biology.

Here¹, we have developed a robust and easy-to-use microfluidic vascular chip platform that allows multiomic analysis of vessels in a healthy state and under metabolic stress. The chip enables the culture of human induced pluripotent stem cell-derived ECs (SC-ECs) in an organotypic microchannel under gravity-induced shear stress. The chip platform enables seamless integration with state-of-the-art analytical technologies, whereby small sample volumes are sufficient. As demonstrated by single-cell transcriptomics, high-resolution imaging, and functional assays, gravity-driven flow induced SC-EC maturation, reflected by barrier formation, high levels of NO synthesis, and arterial toning. Beyond the characterization of the organotypic EC culture on-chip, we validated the general functional relevance of the chip platform by modeling the pathogenesis of atherosclerosis. Upon individual stimulation of SC-ECs with oxidized LDL (oxLDL) and free fatty acids (FFA), proteome and secretome analysis revealed the early appearance of atherosclerosis hallmarks. In summary, we report a human stem cell-based vessel-on-chip model that facilitates the screening of EC function at the single-cell level and high resolution. Our approach serves the urgent need to replace animal experiments by developing a functionally reliable and easily customizable vascularized human organ-on-chip platform.

¹ Marder, M.; and Remmert et al. *Stem Cell-Derived Vessels-on-Chip for Cardiovascular Disease Modeling*. *Cell Rep.* 2024, 43 (4), 114008. <https://doi.org/10.1016/j.celrep.2024.114008>.

Company presentations

**12th GSCN Conference
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Company presentations: C01 – C09

(the PXXX number indicates an accompanying poster presentation)

- C01** Assay-ready human microglia for automated image-based phenotypic subgrouping and targeted compound screening
Michael Peitz, Life & Brain GmbH
- C02** Advancing stem cell research with automated detection and isolation of stem cells and colonies
Jurij Kintz, Sartorius Stedim Biotech GmbH
- C03/P057** Improved genetic stability of human pluripotent stem cell cultures passaged as single cells using eTeSR™
Adam Hirst, STEMCELL Technologies GmbH
- C04** Harnessing CRISPR-ready human iPSC derived cells for disease modelling and drug discovery in neurodegenerative diseases
Rebecca Northeast, bit.bio
- C05** Take your research translational the easy way: streamline your cell culture with our ready-to-use products and automated solutions
Volker Arps, Bio-Techne
- C06** Bioreactor systems | cell & gene therapy applications | state-of-the-art
Sascha Gandert, Getinge
- C07** NK cell generation from iPSC suspension cultures
Sara Perez Munoz, Thermo Fisher Scientific
- C08** From R&D to manufacturing: the cell as a product
Philipp Nold, Eppendorf SE
- C09** Innovative synthetic peptide growth factor alternative peptides: overcoming challenges in stem cell medicine
Robert Brownlee, PeptiGrowth

Abstract No. C01**Assay-ready human microglia for automated image-based phenotypic subgrouping and targeted compound screening**

Michael Peitz^{1,*}, and Johannes Wilbertz²

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*Presenting author

LIFE & BRAIN is a premium provider of omics analyzes and stem cell-based R&D services in Europe, specializing in advanced cell culture systems and neurological disease modeling. As pioneers in stem cell-based models, we prioritize authenticity and standardization to ensure the highest quality.

In this talk, we introduce **LB:MiGs**—highly authentic, functional human microglia derived from hiPSCs. Our proprietary protocol replicates the microglial development process in vivo from the yolk sac through final priming upon invasion of the central nervous system. This results in microglia that are ready for functional assays within just 24 hours, including those related to phagocytosis, redox states, and the NLRP3 inflammasome pathway. Moreover, **LB:MiGs** are integral to the phenotyping platform *PhenoMiG*, developed with our strategic partner Ksilink. *PhenoMiG* automates high-throughput microglia characterization through microscopic profiling and data analysis. The platform's robotic system handles microglia seeding and chemical treatments in 384-well plates, followed by automated imaging with a confocal microscope. A deep learning algorithm extracts diverse image features, while image segmentation quantifies biologically relevant characteristics. This approach detects subtle phenotypic effects that sequencing methods may miss, enhances reproducibility, and offers a comprehensive view of microglial behavior.

The fidelity of the **LB:MiGs**-based *PhenoMiG* platform will be showcased for the first time with proof-of-concept data from a recent study involving over 200 substances, underscoring its potential to streamline drug development.

Abstract No. C02**Advancing stem cell research with automated detection and isolation of stem cells and colonies***Jurij Kintz*

Sartorius, Göttingen

The CellCelector Flex streamlines stem cell research, particularly in stem cell colony picking and monoclonal iPSC cell line development by automating the selection and isolation of single cells and colonies. This advanced system eliminates the need for manual identification and selection, thereby reducing user error and time, while enhancing selection precision and cell viability post-isolation.

Utilizing phase contrast, brightfield, or fluorescence imaging, the CellCelector Flex identifies cellular structures based on user-defined parameters such as size and fluorescence intensity. The instrument's robotic arm is equipped with precise coordinates of the structures of interest, gently aspirates the targeted cell or colony from the source plate and transfers it to a variety of culture vessels or PCR tubes. The process is validated by imaging before and after picking, ensuring that the desired structure is accurately isolated.

Interchangeable picking modules, including high-precision glass capillaries, autoclavable stainless-steel tips, or disposable plastic tips, enable the isolation of a diverse range of cellular structures. This versatility positions the CellCelector Flex as a valuable tool for applications in iPSC cell line development, stem cell colony picking as well as organoid research.

The presentation will show the automated workflow of the CellCelector Flex and emphasise its application in stem cell research, addressing the specific needs of this field.

Abstract No. C03/P057**Improved genetic stability of human pluripotent stem cell cultures passaged as single cells using eTeSR™**

Adam Hirst^{1,*}, Kimberly Snyder², Vicky Wang², Darielle Lim², Helen Vo², Thuy Hoang², Christopher Price², Aaron Ang², Ryan Bailey², Eloi Mercer², Mark Hills², Allen Eaves², Sharon Louis², and Arwen Hunter²

¹STEMCELL Technologies UK Ltd., United Kingdom

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*Presenting author

Genetic instability has been widely documented in human pluripotent stem cells (hPSCs). Recurrent genetic abnormalities arise throughout culture and confer a selective advantage to genetically variant cells through mechanisms including resistance to cell death and reduced differentiation capacity. We have previously shown that routine single-cell passaging of hPSCs can result in a high incidence of de novo genetic abnormalities and that eTeSR™, a novel hPSC maintenance medium developed to better support single-cell passaging, can significantly reduce the appearance of recurrent abnormalities. To further demonstrate the increased genetic stability observed when routine single-cell passaging hPSCs in eTeSR™, 72 clonal sublines were derived from three hPSC lines (H1, H9 and SCTi003-A) using single-cell deposition. Each clone was then independently maintained for 20 weeks in either two commercially available control media (A & B) or eTeSR™. Genomic DNA was extracted and analyzed using single nucleotide polymorphism microarray. Of the 136 samples maintained in control media, 93 (68%) had acquired at least one de novo abnormality compared to only 34 (25%) of the 135 samples maintained in eTeSR™. This can be attributed to fewer small copy number variants (CNVs) (<10,000 kb) observed in subclones maintained in eTeSR™, with only six detected across 135 samples compared to 94 detected in 136 samples maintained in control media. Strikingly, 75 of 136 (55%) samples maintained in control media harbored a gain of 20q, with 68 instances presenting as small CNVs that are often undetectable using G-band karyotyping. Conversely, only four 20q gains were detected in eTeSR™-maintained samples, a significant finding due to the prevalence of 20q11 duplications reported in large-scale studies that describe recurrent karyotypic changes in hPSCs 1,2. This study provides a better understanding of culture-acquired genetic aberrations in hPSCs and demonstrates that innovative media formulations, like eTeSR™, can address major challenges in the hPSC field.

International Stem Cell Initiative; Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Biotechnol. 2011 Nov 27;29(12):1132-44.

Taapken SM, Nisler BS, Newton MA, Sampsell-Barron TL, Leonhard KA, McIntire EM, Montgomery KD. Karyotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. Nat Biotechnol. 2011 Apr;29(4):313-4.

Abstract No. C04**Harnessing CRISPR-ready human iPSC derived cells for disease modelling and drug discovery in neurodegenerative diseases***Rebecca Northeast^{1,*}, and Sejla Salic-Hainzl²*¹bit.bio, Product Management, Cambridge, United Kingdom²bit.bio discovery, Research and Development, Cambridge, United Kingdom

*Presenting author

The emergence of CRISPR-Cas9 technology has opened a new horizon in functional genomics, reshaping our approach to studying ties between genes and disease. Many scientists are looking to harness this technology to advance both basic and translational research through CRISPR-Cas9 based knockout screening. Human iPSC-derived neuronal cells offer researchers a functional platform for disease modelling, target identification and compound screening that is scalable and reproducible. However, genetic manipulation of these cells has historically been difficult to perform, leaving researchers to pursue less physiologically relevant options such as immortalized cell lines or animal models. In this talk, we present a solution with CRISPR-Ready ioCells, specifically ioGlutamatergic Neurons and ioMicroglia engineered with constitutive expression of cas9, built for the routine generation of genetic knockouts and CRISPR screening workflows. We performed a scCRISPR screen in these cells, revealing disease-associated genes whose knockout caused significantly altered transcriptomic signatures. In ioMicroglia, the same scCRISPR screening workflow highlighted genes that influenced the response to LPS stimulation, shedding light on microglial activation dynamics. Taken together, CRISPR-Ready ioCells provide a unique platform for comprehensive exploration of disease-relevant cell states, laying the foundation for advanced disease modelling, target discovery, and validation.

Abstract No. C05**Take your research translational the easy way: streamline your cell culture with our ready-to-use products and automated solutions***Volker Arps*

Bio-Techne, Cell & Gene Therapy Specialist, Wiesbaden

Bio-Techne offers a range of high quality, consistent reagents for organoid culture, as well as innovative technologies for spatial biology and fully automated protein analysis. In this presentation we will highlight Bio-Techne's contributions to organoid, stem cell and cell and gene therapy: Our products can help you prepare your research for translational approaches by setting up your workflow using certified animal/serum-free reagents and switching to GMP equivalents when necessary. Simplify your immune cell therapy manufacturing with ready-to-use reagents such as liquid cytokines, fully licensable gene transfer methods and expand, differentiate and sort your cells under sterile conditions. Once your final product is ready for testing, characterize it with our automated solutions for ELISA, Western blots, CE-SDS and cIEF and monitor biodistribution, efficacy and immune response in vivo with our spatial transcriptomics and analytical technologies.

Abstract No. C06**Bioreactor systems | cell & gene therapy applications | state-of-the-art***Sascha Gandert*

Getinge Deutschland GmbH, Key Account Management Bioprocessing, Rastatt

The field of cell and gene therapy has seen unprecedented advancements in recent years, promising transformative treatments for a wide range of diseases. Central to the success of these therapies is the development and optimization of bioreactor systems, which play a crucial role in the cultivation and expansion of therapeutic cells. This talk will explore the state-of-the-art in bioreactor technology, focusing on its applications in cell and gene therapy. We will discuss the latest innovations in bioreactor design, including scalable systems that ensure consistency and quality in cell production. Key topics will include the integration of automation and real-time monitoring to enhance process control, the use of novel materials and configurations to support diverse cell types, and strategies to overcome current challenges such as shear stress and nutrient distribution. Case studies will illustrate the application of advanced bioreactor systems in the production of CAR-T cells, stem cells, and other genetically modified cell therapies. By providing a comprehensive overview of current bioreactor technologies and their applications, this talk aims to highlight the critical role of bioreactors in advancing cell and gene therapy and to inspire further innovation in this dynamic field.

Abstract No. C07**NK cell generation from iPSC suspension cultures***Sara Pérez Muñoz*

Thermo Fisher Scientific, FAS - Scientific support, Madrid, Spain

Natural killer (NK) cells have emerged as a crucial component in the field of allogeneic cell therapy due to their cytotoxic ability and unneeded HLA matching. Yet requirements such as donor sourcing and successful expansion hamper the ability to efficiently generate large quantities of functional NK cells. Here we present an approach for the generation of iPSC-derived NK cells from suspension cultures grown in CTS StemScale in a scalable format that allows to produce a highly enriched and functional NK population. Phenotypic characterization, expansion capabilities and cell viability pre- and post-thaw will be discussed, as well as functional data on NK cell activation and cytolytic potential to eliminate patient-derived cancer cells in a 3D-culture setting.

Abstract No. C08**From R&D to manufacturing: the cell as a product***Philipp Nold*

Eppendorf SE, Bioprocess Center, Jülich

Cell and gene therapies represent groundbreaking advancements in personalized medicine and regenerative therapies, employing "living" medications derived from genes, tissues, or cells to combat various diseases. Within this innovative domain, the progress in the development of cell therapies necessitates the need of high quantities and qualities of cells manufactured through precisely monitored and traceable bioprocesses.

As the industry moves more and more in the direction of allogeneic cell therapies, single-use stirred-tank bioreactors have emerged as the tool of choice for the scalable production required in the transition from autologous to allogeneic cell therapies. This paradigm shift underscores a future where the scalability and control afforded by cutting-edge bioprocessing technologies are central to harnessing the full potential of cell therapies, marking a significant milestone in the journey from laboratory research to robust manufacturing frameworks.

In this talk, we will highlight the importance of process understanding and control, the implementation of automation and digital solutions significantly improves process performance.

Abstract No. C09**Innovative synthetic peptide growth factor alternative peptides: overcoming challenges in stem cell medicine***Robert Brownlee*

PeptiGrowth Inc., Market Harborough, United Kingdom

Traditional recombinant protein-based growth factors and cytokines used in regenerative and cell therapy products face several challenges, including lot-to-lot quality variation, contamination with animal-derived components, low stability, and extremely high costs. To address these issues, we have developed a novel class of **"Growth Factor Alternative Peptides"** with comparable or superior activity to traditional growth factors and cytokines, utilizing our proprietary Peptide Discovery Platform System (PDPS).

Our growth factor alternative peptides are cyclic peptides **synthesized through complete chemical reaction**, allowing for high-quality, cost-effective production and exceptional stability.

To date, we have successfully developed peptides that have equivalent or superior activity to the functions of HGF, BDNF, Noggin, VEGF, Wnt3a, EGF, and TPO. This presentation will provide an overview of our development of these growth factor alternative peptides and will include new data on the recently completed development of an FGF2 (bFGF) alternative peptide.

Poster presentations

12th GSCN Conference German Stem Cell Network 25 - 27 September 2024

Poster session: P001 – P064

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Poster session I: P001 – P064

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- P001** Modeling CNTNAP2 deficiency in human iPSC-derived neuronal cortical networks
Jasmin Aicher
- P002** Influence of titin variants on myocardial function in a 3D disease model of Peripartum Cardiomyopathy using human induced pluripotent stem cells
Tamari Askurava
- P003** Human induced pluripotent stem cell-derived microglia-like cells to investigate therapeutic strategies targeting multiple sclerosis progression
Alica Blenkle
- P004** Strategies for personalized medicine exploiting primary cilia dynamics in glioma Stem cells.
Tejas Vandeo Borkar
- P005** Exposing iPSC-derived brain pericytes-like cells to itaconate supports metabolic treatment approach for inflammatory conditions and oxidative stress
Franziska Buck
- P006** Identifying new targets that protect against the cell-to-cell propagation of tauopathy
Eleonora Clemente
- P007** Primary functional effects caused by a HCM-associated mutation in MYBPC3 on long-term cultured hiPSC-CMs
Ferdinand Conrad
- P008** Neuronal injury in autoimmune encephalitis – human disease biomarkers and direct antibody-neuron interaction in a human in vitro human disease model
Júlia Csátri
- P009** In vitro disease modeling and pathophysiological characterization of fatty acid hydroxylase-associated neurodegeneration
Fatima Efendic
- P010** Exploring the role of BCL11A in human pluripotent stem cell-derived cortical projection neurons and midbrain dopaminergic neurons
Lena Fahrbach
- P011** Investigation of mitochondrial function in hiPSC-derived neurovascular in vitro models of PARK2 copy number variation carriers with adult ADHD
Markus Glaser

- P012** Human skin organoids: a new frontier in melanoma research
Dieter Groneberg
- P013** Towards personalized CRISPR/Cas9-based gene therapy for Duchenne muscular dystrophy
Óscar Gutiérrez-Gutiérrez
- P014** Induction of DNA methylation by transcription in an in vitro iPS cell model
Josephine Haake
- P015** Brain delivery of PROTACs: systematic exploration in human iPSC-based blood-brain barrier models
Undine Haferkamp
- P016** Deciphering collective oncogenic GNAS and KRAS signaling in an organoid model for cystic pancreatic neoplasms
Meike Hohwieler
- P017** Modeling Leigh syndrome and sildenafil treatment with patient-derived neurons
Caleb Jerred
- P018** Disease modeling using co-cultures of iPSC-derived neural cell types
Martin Kriebel
- P019** A human iPSC-derived brain metastasis model to test efficacy and toxicity of drug candidates
Kim Krieg
- P020** The impact of deficiency in GPM6A, a schizophrenia-associated gene, on synapse formation and function of NGN2-based forward programmed neurons
Yevheniia Kyriachenko
- P021** Application of single cell RNA sequencing to characterize transient cell populations and molecular signalling during respiratory differentiation of human pluripotent stem cells
Anna Kuleshova
- P022** A complete workflow for high-efficiency crispr/cas9-mediated gene editing in pluripotent stem cells
Florian Paasch
- P023** The role of GLIS3 in human pancreas development and diabetes mellitus
Jifeng Liu
- P024** Reduced potassium current density is underlying sensory neuron hyperactivity in a human model of CNTNAP2-deficiency
Lisa Mainardi
- P025** A ONECUT1 regulatory, non-coding region in pancreatic development and diabetes
Sarah Merz

- P026** CRISPR/Cas9 editing workflows in human iPSCs
Katrin Neumann
- P027** Understanding mechanisms of biliary fibrosis using periportal liver assembloids
Armin Niksic
- P028** A toolbox of human iPSC-derived microglia in different genetic backgrounds and disease models for neurodegeneration drug discovery
Rebecca Northeast
- P029** Development of a novel CF lung disease model based on CF patient specific human induced pluripotent stem cells
Ruth Olmer
- P030** Development of a novel human induced pluripotent stem cell (hiPSC)-based in vitro model for mechanistic studies of motile cilia in the context of Primary Ciliary Dyskinesia
Ruth Olmer
- P031** Development of chemically defined, animal-origin free (CD-AOF) supplement for hPSC derived $\alpha\beta$ T cell differentiation
Maja Petkovic
- P032** Inducing a pseudounipolar morphology in human sensory neurons
Pascal Röderer
- P033** A luminescent insulin secretion reporter in hESC-derived islets for high-throughput screening of inhibitors of IAPP-induced beta-cell failure
Alexis Zi Le Shih
- P034** Novel bioartificial cardiac tissue model for the investigation of cross-talk between pluripotent stem cell-derived cardiac cell types involved in fibrosis
Sára Szádocka
- P035** Investigating the neuronal phenotype of type 2 diabetes in induced pluripotent stem cell derived neuronal progenitors and neurons
Katalin Vincze
- P036** Combined approach for target-centric screening and validation of molecular degraders in neuronal in vitro models
Arjen Weller
- P037** High-throughput screen using patient-derived neuronal precursor cells (NPCs) identifies small molecule mitochondrial uncouplers targeting neurodegenerative diseases
Annika Wittich
- P038** New methods in melanoma research: employing stem cell-generated human skin organoids and cell types for in vitro skin test systems
Saskia Zöphel

Abstract No. P001**Modeling CNTNAP2 deficiency in human iPSC-derived neuronal cortical networks**

Jasmin Aicher^{1,*}, Luzia Heidrich^{1,2}, Lydia Fischer³, Pascal Röderer¹, Martin K. Schwarz³, Lea Berg¹, and Oliver Brüstle¹

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²LIFE & BRAIN GmbH, Bonn

³Institute of Experimental Epileptology and Cognition Research (IEECR), University of Bonn & University Hospital Bonn

*Presenting author

Contactin Associated Protein 2 (CNTNAP2) deficiency is a rare disorder, displaying four core phenotypes: intellectual disability, autism spectrum disorder, specific language impairment and epilepsy. Previous studies in mice revealed a network asynchrony in the medial prefrontal cortex, which could be rescued by optogenetic inhibition of the excitatory neurons or the optogenetic activation of the parvalbumin positive interneurons, emphasizing the relevance of both compartments for the investigation of the disease. In the healthy human cortex CNTNAP2 expression is noticeably higher even compared to other human primates, underlining the need for human models to study its function.

Human induced pluripotent stem cell (iPSC) reprogramming and subsequent differentiation by forced expression of transcription factors represents a robust approach to derive standardized cultures of human neurons. To study the effect of a CNTNAP2 knock-out in excitatory and inhibitory forebrain neurons CNTNAP2^{-/-} iPSCs were edited to stably express inducible NGN2 or ASCL1/DLX2 cassettes from the AAVS1 safe harbor locus. Upon doxycycline-mediated transgene induction, CNTNAP2^{-/-} iPSCs displayed regular transition into primarily glutamatergic and GABAergic neurons, respectively. To study the functional impact of CNTNAP2 deficiency, excitatory and inhibitory neuronal populations were co-cultured in defined ratios and subjected to functional recordings on multi-electrode arrays. First data suggest that knock-out of CNTNAP2 in either the NGN2 or the ASCL1/DLX2 neurons results in a reduced weighted mean firing rate and reduced synchronicity and that a knock-out in both co-cultured populations further decreases burst duration and the number of spikes per burst, while the number of bursts is increased. We are currently in the process of consolidating these functional alterations and aim at extending our read-out portfolio towards AAV-based tools such as calcium indicators, optogenetics or rabies tracing to further dissect the mechanisms underlying the pathophenotype caused by CNTNAP2 loss and to facilitate the investigation of potential therapeutic options.

Abstract No. P002**Influence of titin variants on myocardial function in a 3D disease model of Peripartum Cardiomyopathy using human induced pluripotent stem cells**

Tamari Askurava^{1,*}, Melanie Ricke-Hoch², Alexandra Haase¹, Evelyn Gebel¹, Carina Pawlow¹, Denise Hilfiker-Kleiner², Anna Melchert^{1, 3}, Gudrun Göhring⁴, Denise Hilfiker-Kleiner², Ulrich Martin¹, and Ina Gruh^{1, 3}

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⁴Hannover Medical School, Institute for Human Genetics

*Presenting author

Peripartum cardiomyopathy (PPCM) is a complex, multifactorial disease that typically affects women without prior structural heart disease, emerging in late pregnancy or the early postpartum period. The "two-hit" model suggests that a combination of a toxic vasculo-hormonal environment and genetic predisposition underlies the development of PPCM¹. However, the specific genetic alterations and mechanisms contributing to this predisposition remain poorly understood. Notably, around 15% of PPCM patients have variants in sarcomeric genes, with 10% carrying truncating variants in the titin gene (TTNtvs). The role of these variants in PPCM pathogenesis is still unclear, highlighting the need for further investigation.

To address this, human induced pluripotent stem cell (hiPSC) technology was employed for the creation of the first 3D disease model of PPCM. Multiple clonal hiPSC lines were generated and fully characterized from three patients carrying previously unstudied heterozygous TTN variants, including TTNtvs. Additionally, scalable differentiation protocols were optimized to produce high-purity, spontaneously beating hiPSC-derived cardiomyocytes (hiPSC-CMs), with over 95% of cells expressing key cardiac markers such as ACTN2, MHC, and cTnT. These patient-specific hiPSC-CMs were utilized to construct bioartificial cardiac tissues (BCTs)², which successfully formed, remodeled, and exhibited spontaneous contractions.

The patient-specific BCTs were stimulated with disease-related factors to assess contractile forces and tissue stiffness using multimodal bioreactors. Most applied stimuli, such as pregnancy hormone prolactin, β -adrenergic stimulation, and cyclic stretch did not significantly alter these properties. However, initial experimental data suggest, that combined stimulation with several factors significantly reduces contractile forces in patient tissues, a change not observed in wild-type tissues. Additionally, bulk-RNA sequencing conducted following the stimulation offers the first insights into the underlying mechanisms of this reduction.

Taken together, our findings suggest a potential partial recapitulation of the *in vitro* PPCM disease phenotype, providing a foundation for further exploration of the TTN variants in PPCM pathogenesis.

1. Ricke-Hoch M, Pfeiffer TJ, Hilfiker-Kleiner D. Peripartum cardiomyopathy: basic mechanisms and hope for new therapies. *Cardiovasc Res* 2020; 116(3):520-31.

2. Kensah G, Roa Lara A, Dahmann J, et al. Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue *in vitro*. *Eur Heart J*. 2013 Apr 14;34(15):1134-46.

Abstract No. P003**Human induced pluripotent stem cell-derived microglia-like cells to investigate therapeutic strategies targeting multiple sclerosis progression**

Alica Blenkle^{1, 2,*}, Anastasia Geladaris^{1, 2}, Lukas Cyganek^{1, 3}, Maria-Patapia Zafeiriou^{4, 5}, and Martin S. Weber^{1, 6}

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*Presenting author

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS), which typically results in neurological disability. The onset and in particular progression of MS are characterized by continuous microglia reactivity and microglia-mediated inflammation in the CNS, suggesting microglia as a key driver of disease progression. Therefore, a prime therapeutic target are microglia functions and pathways that regulate them. In this regard, the enzyme Bruton's tyrosine kinase (BTK) is centrally involved in the activation of microglia. Consequently, inhibition of BTK is under investigation to be a promising therapeutic strategy diminishing MS progression.

Accordingly, we aim to establish a human in vitro model based on human induced pluripotent stem cell (iPSC)-derived microglia-like cells (i-microglia) to identify modulatory effects of various BTK inhibitors.

Thus, we generated i-microglia by optimizing a protocol developed by Guttikonda et al. (2021, Nature Neuroscience). The differentiation conditions promoted the generation of KDR+CD235A/B+ mesoderm, defining the induction of in vitro yolk sac hematopoiesis, the origin of microglia. From this mesoderm population CD43+CD34+ yolk sac-derived hematopoietic progenitors emerged and matured into CD11b+CD45+ yolk sac-derived macrophages. The latter differentiated into i-microglia expressing key microglial markers IBA1, CX3CR1, TMEM119 and P2RY12. Additionally, the i-microglia expressed Fcγ receptors and molecules involved in antigen presentation and activation. Importantly, they also expressed BTK.

This successful generation of human i-microglia will serve as a tool to assess the activity of BTK inhibitors currently in clinical trials for MS and future compounds targeting MS progression. Thereby this will complement pre-clinical studies in evaluating new therapeutic agents and pave the way for their investigation in clinical studies.

Abstract No. P004**Strategies for personalized medicine exploiting primary cilia dynamics in glioma Stem cells.**

Tejas Vandeo Borkar^{1, 2,*}, *Jay Gopalakrishnan*³, *Michael Sabel*⁴, *Marion Rapp*⁵, *Julia Steinmann*⁴, and *Glio-Org Glioma-PerMed*³

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⁴Neurosurgery Clinic, University Hospital, Heinrich-Heine-Universität, Düsseldorf

⁵Universitätsklinikum Düsseldorf

*Presenting author

Glioblastoma (GBM) is a highly aggressive tumor harboring Glioma Stem Cells (GSC), which have a block in differentiation with uncontrolled self-renewal properties. GSCs have high neuroinvasive properties in the brain, which are unpredictable. Analyzing primary cilia, we notice a vast heterogeneity in cilia frequency and length across xxx GBM patients. During the cell cycle, the assembly and disassembly of cilia play a crucial role in regulating the cell cycle. Assembly of cilia occurs after mitosis in the G1 phase, and ciliated cells enter the G0 or quiescent phase; disassembly of cilia acts as a checkpoint for re-entry of the cell into the cell cycle. As cilia act as sensors for mechanical and chemical stimuli that control cellular differentiation or division, we hypothesize disrupting the cilium disassembly would arrest the GBM cells in a quiescent ciliated state and reduce their high proliferative behavior. To this end, we target the NEK2-KIF24 axis. KIF24 is minus end microtubule depolymerase, following activation by NEK2 involved in disassembling primary cilium during the G2/M phase. Inhibiting the NEK2-mediated phosphorylation of KIF24 can restore the ciliation in GBM cells, thereby reducing proliferation and loss of stemness, leading to differentiation. We noticed high levels of SOX2 in GBM-derived cell lines, which could be used as a reported for loss of stemness in GBM. We would be using different strategies: i) where we use hGLuc downstream to SOX2 promotor enabling us to do high throughput drug screening through Luciferase assay, ii) use GFP downstream to SOX2 promotor in secondary screening enabling us to quantify the changes in SOX2 levels. We will then screen for the small molecules, perturbing NEK2-KIF24 interaction leading to loss in stemness of the GBM cells.

Abstract No. P005**Exposing iPSC-derived brain pericytes-like cells to itaconate supports metabolic treatment approach for inflammatory conditions and oxidative stress**

Franziska Buck^{1,*}, Gabriel Marzari², Fangfang Chen^{3,4}, Thekla Cordes^{3,4}, and Thomas Winkler^{1,5}

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³Technische Universität Braunschweig, Department of Bioinformatics and Biochemistry & Braunschweig Integrated Centre of Systems Biology (BRICS)

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⁵KTH Royal Institute of Technology, Stockholm, Sweden, Division of Micro- and Nanosystems

*Presenting author

While surmounting the high-risk state of limited supply of oxygen and nutrients to brain tissue, post-ischemia reperfusion triggers inflammatory responses, oxidative stress, and metabolic reprogramming, aggravating tissue damage. We have previously shown that the myeloid “immunometabolite” itaconate can mitigate these adverse effects of reoxygenation in mouse models by preventing a sudden increase in cellular respiration [1]. To date, however, the roles of pericytes in the critical metabolic cascades at the neurovascular interface remain opaque.

Here, we analyzed how human iPSC-derived brain pericyte-like cells [2] alter their metabolic state in response to exogenous itaconate. Differentiation of iPSC via neural crest induction resulted in cells showing upregulation of NG2 and FOXF2 (i.e., brain pericyte markers). For our metabolic study, we exposed them to 10 mM itaconate in [U-13C] glucose-supplemented media, and after 24 hours subjected supernatant and cell lysates to metabolomic analysis (via GC-MS). In particular, we found levels of succinate elevated over 10-fold compared to negative controls. This is in line with our previously-described results from rat astrocyte/neuron co-cultures and indicative of an impairment of succinate dehydrogenase (SDH) activity in the TCA cycle. Our metabolic flux studies also revealed that glucose utilization for TCA cycle metabolism was not significantly affected. Cell proliferation and cell morphology, meanwhile, remained unaffected.

These results suggest that a potential treatment approach with itaconate would be further supported by metabolic adaptations in brain pericytes, which tightly interact with other cell types of the neurovascular unit (NVU). Considering the unmet medical need, therapeutically targeting metabolism to alleviate reperfusion injury effects can be a promising approach and our first insights into the reaction of human pericytes to itaconate further reinforces this idea.

[1] Cordes et al., *Mol. Metab.* 32, 122-135 (2020)

[2] Faal et al., *Stem Cell Rep.* 12, 451-460 (2019)

Abstract No. P006**Identifying new targets that protect against the cell-to-cell propagation of tauopathy**

Eleonora Clemente^{1,*}, Susanne Kordes², Matthias Baumann², Axel Choidas², Jan Eickhoff², Bert Klebl², and Jared Sternecker¹

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*Presenting author

Tauopathies are a group of neurodegenerative disorders associated with cognitive decline that have in common the abnormal aggregation of tau protein in the brain. The cell-to-cell propagation of tau pathology between neurons is an important event in the clinical progression of these disorders. In collaboration with the Lead Discovery Center GmbH, we performed a high-throughput screening campaign to identify small molecules that can reduce tau spreading. For this purpose, we used tau biosensor cells, a genetically engineered cell line that generates intracellular aggregates in response to tau conformations with seeding potential. We identified a novel key regulator of tau internalization, and we validated this target using additional small molecule inhibitors as well as siRNA-mediated knockdown. We recently established a model of tau pathologic spreading using induced pluripotent stem cell-derived neurons, which we are using to assess the target's role in tau pathology. In addition, we are testing antisense oligonucleotides (ASOs) for specific reduction of the target as a potential therapeutic approach. Unlike small molecules, directly injecting ASOs into the cerebrospinal fluid should allow us to control the level and localization of the target. Through this approach, we hope to lower the putative side effects stemming from systemic drug administration. Results from this study will enable development of therapies to protect patients against tau spreading and tau-related cognitive decline and dementia.

Abstract No. P007**Primary functional effects caused by a HCM-associated mutation in MYBPC3 on long-term cultured hiPSC-CMs**

Ferdinand Conrad^{1,*}, Karina Ivaskevica¹, Birgit Piep¹, Sarah Konze¹, Jana Teske², Robert Zweigerdt², Theresia Kraft¹, and Bogdan Iorga¹

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Hypertrophic cardiomyopathy (HCM) is a genetic disorder affecting cardiomyocytes (CMs) and it is characterized by left ventricular wall thickening of the heart. Most HCM-causing mutations can be found in genes encoding for sarcomeric proteins such as cardiac myosin binding protein C (*MYBPC3*, cMyBP-C) or beta myosin heavy chain (*MYH7*, beta-MyHC). Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with introduced heterozygous *MYBPC3*-truncation mutation can represent a cellular disease model for HCM. Here we investigated and compared force-related parameters of heterozygous *MYBPC3*^{c.927-2A>G, p.309fsX} mutant (MT) and isogenic (wild-type, WT) cardiac myofibrils within demembranated hiPSC-CMs cultivated on stiff substrate for two different long-term cultivation periods to understand the early primary functional effects caused by the *MYBPC3* (c.927-2A>G, p.309fsX) mutation at subcellular (myofibrillar) level.

Subcellular myofibrils within demembranated hiPSC-CM were mounted in a micromechanical setup (15°C) designed to assess myofibrillar isometric force and force-related kinetic parameters at saturating or intermediate Ca²⁺ concentrations and following Ca²⁺ removal. Our results indicate that, after 35+ days post differentiation, there were no significant differences in contractile function between MT and WT myofibrils of hiPSC-CMs regarding maximal isometric force ($F_{act,max}$), rate of force redevelopment ($k_{tr,max}$), force-pCa relationships and relaxation kinetics. However, after 56+ days post differentiation, functional investigations revealed significant differences between MT and WT myofibrils of hiPSC-CMs: higher Ca²⁺-sensitivity of force, lower cooperativity index of Ca²⁺-regulation and overall slower relaxation kinetics in myofibrils of MT *MYBPC3* hiPSC-CMs. In conclusion, the effect of the *MYBPC3*-mutation is only observed in longer cultured cells, suggesting a progressive developing of the HCM-cellular phenotype and worsening of the contractile function of mutant *MYBPC3* hiPSC-CMs during cell maturation.

Abstract No. P008**Neuronal injury in autoimmune encephalitis – human disease biomarkers and direct antibody-neuron interaction in a human in vitro human disease model**

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Autoimmune encephalitides (AIE) associated with antibodies against the neuronal surface or synaptic antigens are often underdiagnosed as a driving force of neurological diseases. A key concept regarding their pathobiology is the binding of the neuronal autoantibodies to extracellular epitopes, thereby exerting direct effects. AIE presents with a spectrum of symptoms including epileptic episodes, cognitive decline, and psychiatric disturbances all of which are consequences or causes of neuronal injury.

Our previous research has highlighted the pivotal role of B-cells and specific antibodies produced by plasma cells in AIE disease mechanism. Most of these antibodies targeted the hippocampal area in the brain but intriguingly diverse clinical outcomes and treatment responses are seen in different antibodies. Based on this, we aim to investigate how anti-IgLON5, anti-Lgi1 as well as anti-Caspr2 antibodies directly affect brain cells - as these paradigmatic antibodies cause different hippocampal damage patterns and in varying intensity. We have established a toolkit to study these interactions in the lab using a human iPSC-derived disease model and patients IgG. Initial findings suggest that exposure to IgLON5 antibodies reduces neuronal activity and increases cell death, shedding light on direct antibody mediated brain damage.

Finally, we seek to identify potential new disease biomarkers from the study of direct antibody-neuron interactions, and we aim to examine these in the blood/CSF of patients. Moreover, by analysing molecular interactions and change on a cellular level, we aim to identify diagnostic markers for AIE and potential treatment targets which can be tested on patients with AIE at the early clinical trial unit.

Overall, our project aims to unravel the complex mechanisms underlying neuronal injury in AIE, offering insights that may lead to more effective and earlier diagnosis, treatment, and management of this debilitating neurological condition.

Abstract No. P009**In vitro disease modeling and pathophysiological characterization of fatty acid hydroxylase-associated neurodegeneration***Fatima Efendic^{1,*}, Andreas Hermann^{1,2}, and Moritz J. Frech^{1,3}*

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Demyelination and degeneration are the most common pathophysiological hallmarks of neurodegenerative disorders. Fatty acid hydroxylase-associated neurodegeneration (FAHN) is a rare neurodegenerative disorder caused by mutations in the *FA2H* gene. This mutation results in a reduction in enzymatic activity, which in turn leads to instability of the myelin sheath, demyelination, and axonal degradation. In this study, we generated FAHN patient iPSCs-derived neurons and oligodendrocytes and employed the combination of these two cell types to investigate studies on myelination and myelin sheath in vitro. Immunocytochemistry and Western blot demonstrated a significant impairment in the colocalisation and a markedly reduced expression of myelin proteins in single OPCs and in coculture. The examination of impaired myelination in FAHN was accompanied by the observation of reduced colocalisation, with a notable decrease in the amount of MBP and PLP protein. The balance of myelin proteins in myelin membrane synthesis is essential for the formation of a compact myelin sheath. The results were corroborated by a significant variation in the myelinated axon structure, including axon length, internode length, and the number of Ranvier nodes. Another common finding in neurodegenerative disorders is a defect in the recycling process. Consequently, we sought to ascertain whether this defect in autophagy was applicable to FAHN. The results demonstrated a reduction in p62 expression and progression, indicated by elevated LC3BII levels and a compromised fusion of autophagosomes and lysosomes. The generation of a human iPSC-derived myelin model for FAHN disorder has not only highlighted the pathophysiological phenotype of the FAHN cell model but also provided important directions for further investigation. Consequently, it is postulated that a mutation in *FA2H* affects the formation of a compact and stable myelin sheath and impairs the functionality of the autophagy recycling machinery in neuronal cells.

Abstract No. P010**Exploring the role of BCL11A in human pluripotent stem cell-derived cortical projection neurons and midbrain dopaminergic neurons**

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BCL11A (B-cell lymphoma/leukemia 11A) is a zinc finger transcription factor expressed in neurons in various brain regions, including the midbrain and cortex. BCL11A-haploinsufficiency in humans is associated with intellectual disability and autism spectrum disorder, suggesting an important neurodevelopmental role for BCL11A.

In the cortex, mouse models have shown that BCL11A plays a role in deep layer cortical projection neuron (CPN) subtype specification and upper layer CPN migration. To study the role of BCL11A in human cortical development, we used a CRISPR-Cas9 approach to generate human induced pluripotent stem cells (hiPSCs) with a *BCL11A* null allele. hiPSCs homozygous for the null allele (*BCL11A* ko) or wild-type (wt) hiPSCs were differentiated into CPN in both 2D and 3D cultures. In wt hiPSCs, BCL11A was expressed in differentiated CPN but not in cortical progenitors, consistent with BCL11A expression in the mouse cortex. Ongoing analyzes using stage- and subtype-specific markers are focusing on the effect of *BCL11A*-inactivation on deep layer CPN fate and differentiation.

In addition to its role in brain development, data from mice suggest that BCL11A may also play a role in neurodegeneration by contributing to the vulnerability of midbrain dopaminergic neurons (mDANs) in the substantia nigra. The progressive loss of these neurons is characteristic of Parkinson's disease. To investigate the role of BCL11A in human mDANs, *BCL11A*-ko and wt hiPSCs were differentiated into mDANs. Approximately 10% of wt mDANs expressed BCL11A. In *BCL11A*-ko mDANs, subtype specification was altered with fewer mDANs expressing ventral tegmental area markers. Upon rotenone treatment, *BCL11A*-ko mDANs were less susceptible to degeneration than wt mDANs, suggesting that *BCL11A*-deficiency may provide protection against oxidative stress.

In conclusion, *BCL11A*-ko hiPSCs are a useful tool to gain insight into developmental and potentially disease predisposing alterations induced by *BCL11A*-deficiency in human neurons from different brain regions.

Abstract No. P011**Investigation of mitochondrial function in hiPSC-derived neurovascular in vitro models of PARK2 copy number variation carriers with adult ADHD**

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Copy number variations (CNVs) in the *PARK2* gene have been shown to be associated with attention deficit hyperactivity disorder (ADHD). Parkin is involved in mitochondrial quality control, indicating that *PARK2* CNVs might impair mitochondrial energy metabolism. Blood-brain barrier (BBB) disruption has been implicated in ADHD, since changes in BBB functionality and metabolic activity can lead to barrier opening and a disrupted microenvironment, disturbing neuronal function.

While treatment with stimulant medication can alleviate symptoms of ADHD, about 50% of adult patients are non- or only partial-responders to pharmacological therapy, demonstrating that development of novel medication is inevitable. Therefore, an isogenic microfluidic *in vitro* co-culture model of the neurovascular unit (NVU) based on human induced pluripotent stem cell (hiPSC)-derived brain capillary endothelial-like cells (BCECs) and cortical neurons was established to provide a standardized platform for drug screening in ADHD treatment.

To investigate *PARK2* CNV-associated functional and metabolic changes at the NVU, we analyzed BCECs derived from hiPSC lines of ADHD patients with *PARK2* CNVs compared to wildtype carriers with (and without) ADHD. Mono-cultured BCECs of *PARK2* CNV carriers with ADHD did not display signs of disrupted barrier integrity and impaired cellular functionality under baseline conditions. However, Seahorse extracellular flux analysis revealed that both mitochondrial-related and glycolytic ATP production of BCECs with *PARK2* CNVs was slightly diminished compared to wildtype controls. Within BCECs, mitochondrial oxidative phosphorylation accounted for just over a third of total ATP production, suggesting that BCECs utilize primarily glycolysis for ATP synthesis. Furthermore, co-culture and microfluidic culture conditions were established for future studies.

Our work will promote drug screening in ADHD treatment, thereby improving drug-related therapy approaches at the BBB and NVU. Additionally, novel therapeutic approaches will be validated to restore cellular and mitochondrial function.

Abstract No. P012**Human skin organoids: a new frontier in melanoma research**

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Malignant melanoma is the deadliest form of skin cancer, necessitating a deep understanding of its pathogenesis and the exploration of new treatments. Traditional model systems like animal models and in vitro human melanoma cell cultures have limitations in accurately replicating human pathology and the microenvironment. To overcome these challenges, we use hair-bearing skin organoids from human pluripotent stem cells, which closely resemble native human skin. These organoids provide a relevant platform for studying melanoma progression.

Our goal is to create a comprehensive in vitro melanoma model by co-cultivating melanoma cells with these organoids. We tested eight melanoma cell lines with different genetic defects for their ability to form tumor spheroids, which were then co-cultured with skin organoids. Immunofluorescence staining and microenvironmental analysis, focusing on cancer-associated fibroblasts, were performed to assess tumor marker expression.

This novel approach using stem cell-generated skin organoids offers a physiologically relevant melanoma model, addressing the limitations of existing models. Our findings are expected to advance melanoma research and aid in the development of more effective therapies through preclinical evaluation of medicinal compounds.

Abstract No. P013**Towards personalized CRISPR/Cas9-based gene therapy for Duchenne muscular dystrophy**

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Duchenne muscular dystrophy (DMD) is a rare disease characterized by progressive muscle loss, which leads to muscle weakness, respiratory disabilities and cardiomyopathies in adulthood, and ultimately premature death. This X-linked recessive disorder has an incidence of 1 in 5,000-6,000 males and is caused by mutations in the dystrophin gene. Dystrophin is a large protein primarily expressed in muscle cells, where its function is to stabilize muscle fibers by connecting the cytoskeleton to the extracellular matrix. More than 5,000 different DMD mutations have been reported, most of which are out-of-frame deletions of at least one exon. Consequently, dystrophin expression is impaired. Interestingly, in-frame deletions resulting in truncated but functional dystrophins can lead to milder forms of muscular dystrophy or even asymptomatic clinical phenotypes.

In our study, we aimed to develop personalized CRISPR/Cas9-based gene therapy strategies for different DMD patients by converting out-of-frame deletions into in-frame deletions through the skipping of single or multiple exons. To achieve this, patient-specific iPSCs were generated, differentiated into cardiac and skeletal muscle cells, phenotyped at the cellular and tissue levels, and then subjected to CRISPR editing. Depending on which exon was skipped, stable or unstable dystrophin peptides were generated. Remarkably, robust dystrophin re-expression and restoration of contractile function was achieved by treating engineered cardiac (EHMs) and skeletal muscle (ESMs) with all-in-one AAVs containing smaller Cas9 variants. Altogether, the use of patient-specific iPSC-engineered muscle tissues and all-in-one AAVs represents an excellent platform to test different clinically translatable CRISPR/Cas9-based exon skipping strategies for treating DMD.

Abstract No. P014**Induction of DNA methylation by transcription in an in vitro iPS cell model***Josephine Haake*, Maren Kaufmann, and Laura Steenpass*

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Imprinted genes are controlled by differentially methylated imprinting centers resulting in allele specific gene expression. The imprinted Prader-Willi / Angelman syndrome locus (PWS/AS) on human chromosome 15 has a bipartite imprinting center, consisting of two functional elements: the PWS-SRO, which becomes methylated on the maternal allele and the AS-SRO promoter, located 35kb upstream to the PWS-SRO (SRO: shortest region of overlap). To test the assumption that transcription initiating at the AS-SRO promoter causes DNA methylation at the PWS-SRO, an iPSC-based human in vitro system was developed. By replacing the AS-SRO with an inducible promoter that is regulated by a doxycycline-responsive transactivator protein (rtTA), transcription initiation at the AS-SRO can be manipulated.

To study if cellular differentiation could play a role in induction of DNA methylation the experiments were performed in undifferentiated iPS cells, during embryoid body differentiation and after targeted differentiation into derivatives of the three germ layers. Transcription across the PWS-SRO was successfully induced by addition of doxycycline in all three experimental settings. Transcripts spliced onto exon 2 of the SNRPN gene, as observed for known oocyte-specific transcripts. Acquisition of DNA methylation at the PWS-SRO was analyzed by targeted deep bisulfite sequencing and was only observed in the endoderm lineage with an increase of 10%. This might indicate that differentiation is needed as additional stimulus for induction of de novo DNA methylation.

The expression of SNRPN could hinder the acquisition of DNA methylation, leading to small methylation levels during endodermal differentiation. Expression of SNRPN is lowest in liver tissue, which is derived from the endodermal lineage. Differentiation of the modified cell lines into hepatic cells could be a good opportunity to achieve higher levels of DNA methylation due to a longer differentiation period and decreased SNRPN expression. Further differentiation experiments are ongoing, and results will be presented.

Abstract No. P015**Brain delivery of PROTACs: systematic exploration in human iPSC-based blood-brain barrier models**

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The rising global burden of central nervous system (CNS) diseases highlights the urgent need for effective treatments. In neurodegenerative diseases, classical drug development strategies based on small molecule inhibitors encounter limitations as disease-causing agents involve misfolded proteins and other toxic aggregates. Proteolysis-targeting chimeras (PROTACs) have emerged as a promising therapeutic approach. Unlike classical enzyme function inhibitors, PROTACs harness the ubiquitin-proteasome system to facilitate the degradation of target proteins, thus targeting previously undruggable proteins. However, the development of effective CNS therapies using PROTACs faces a significant obstacle: the selective blood-brain barrier (BBB). The complex physicochemical properties and higher molecular weight of PROTACs make BBB permeation particularly challenging.

To address this, we established an in vitro platform composed of human-induced pluripotent stem cell-derived brain capillary endothelial cells (hiPSC-derived BCECs). Unlike conventional BBB models, these cells exhibit in vivo-like barrier properties, characterized by the expression of a broad spectrum of relevant marker proteins, limited transcellular transport, and high transendothelial electrical resistance.

We developed a robust workflow to systematically assess the BBB permeability of various PROTACs by comparing them to reference substances and data obtained from conventional Caco-2 and MDCK-MDR1 permeability assays. Our model also enables the exploration of novel strategies to improve brain delivery of PROTACs, such as encapsulation with cyclodextrin- or lipid-based nanoparticles. In conclusion, our hiPSC-based platform and systematic evaluation of BBB transport not only advances our understanding of PROTACs' BBB permeability but also contributes valuable insights for optimizing their delivery to the brain, offering new possibilities for the treatment of CNS diseases.

Abstract No. P016**Deciphering collective oncogenic GNAS and KRAS signaling in an organoid model for cystic pancreatic neoplasms**

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Intraductal Papillary Mucinous Neoplasms (IPMN) are cystic lesions of the pancreatic ducts that typically manifest at an advanced age and can progress to invasive adenocarcinoma. While certain morphological and spatial features can be indicative of progression, a mutation pattern defining this risk remains to be identified. IPMNs frequently harbor activating mutations in either GNAS or KRAS, or both; however, interaction and crosstalk between these oncogenes are poorly understood.

Here, we engineered iPSCs derived from a McCune-Albright syndrome patient harboring wildtype or mutant GNAS^{R201C} along with an inducible KRAS^{G12D} cassette. iPSCs were differentiated towards the pancreatic lineage, resulting in the formation of pancreatic duct-like organoids (PDLO). Subsequently, these organoids were induced with doxycycline to activate oncogenic KRAS, after which they underwent molecular characterization through RNA-sequencing and proteomics analysis. Additionally, pancreatic progenitor cells derived from the iPSCs were transplanted onto an ex vivo scaffold known as the porcine urinary bladder (PUB) model for non-disruptive long-term culture.

The engraftments on the PUB indicated that GNAS^{R201C} drives cyst formation, dysplasia progression and increased proliferation, while also reducing oncogene-induced senescence. Furthermore, both dysplasia progression and senescence were boosted by additional KRAS induction. At RNA and protein level, the effects were classified as either additive, combinatorial or synergistic. This classification facilitated the identification and validation of potential pathways through which GNAS affects oncogene-induced senescence and early dysplasia formation.

Using our iPSC-derived PDLO model, we can closely observe and identify key targets and players in early IPMN formation and progression. This model system is particularly valuable given the challenge associated with culturing patient-derived IPMN organoids and the elusive nature of risk factors for progression from IPMNs to PDAC. In our study, we detected a reduction in oncogene-induced senescence driven by GNAS^{R201C}, along with an increased inflammatory and reduced genomic stability signature.

Abstract No. P017**Modeling Leigh syndrome and sildenafil treatment with patient-derived neurons**

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Leigh Syndrome (LS) is a rare pediatric metabolic disease without any cure. More than 75 mutations have been identified contributing to the disease in both the nuclear and mitochondrial genome. One of the primary diagnostic features of LS is bilateral lesions in the basal ganglia. Previous work from our lab has identified the PDE5 inhibitor avanafil as a LS treatment candidate based on its ability to ameliorate the elevated mitochondrial membrane potential phenotype seen in MT-ATP6 mutant LS neural progenitor cells (NPCs). Sildenafil was selected for treatment testing as it shares the PDE5i mechanism of avanafil and has existing approval for the treatment of pulmonary hypertension in children. To model LS basal ganglia pathology, midbrain dopaminergic neurons (mDANs) were generated from NPCs made using patient derived iPSCs. MT-ATP6 LS patient mDANs were treated with sildenafil and examined for the effect on neuronal outgrowth using high-content image analysis. Upon analysis, it was determined that sildenafil treatment increased the mean neurite length of LS mDANs with a greater effect seen in the top 50 percentile of neurites. Further testing was performed in mDANs generated from NDUFS4 LS CRISPR engineered NPCs with a consistent increase to neurite length observed. The sildenafil increased neurite length was exclusive to the LS mDANs, which suggests that sildenafil treatment is a viable option for supporting the vulnerable cell populations of the basal ganglia.

Lorenz et al. Human iPSC-Derived Neural Progenitors Are an Effective Drug Discovery Model for Neurological mtDNA Disorders. Cell Stem Cell. 2017 May 4;20(5):659-674.e9. doi: 10.1016/j.stem.2016.12.013. Epub 2017 Jan 26. PMID: 28132834.

Abstract No. P018**Disease modeling using co-cultures of iPSC-derived neural cell types**

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iPSC-derived neural cell types have proven to be invaluable tools for modeling central nervous system (CNS) diseases due to their accurate representation of the human genetic background. Common protocols involve either undirected differentiation into a heterogeneous mix of neural cell types or the generation of specific cell types through controlled expression of transcription factors. Although the latter approach, which focuses on defined cell types, has its benefits, it fails to capture the complex intricacies of the nervous system in single-cell type cultures. To create more accurate disease models, we developed a series of co-culture models using iPSC-derived neural cells to represent different endophenotypes of schizophrenia. These models included: (1) microglia with neurons to study neuroinflammation, (2) glutamatergic and GABAergic neurons to mimic disrupted microcircuitry, and (3) glutamatergic and dopaminergic neurons to examine dysfunctions in mesocortical connections. Our findings revealed significant insights, such as increased inflammasome activation in microglia and synaptic damage in neuron-microglia co-cultures. Additionally, co-cultures of glutamatergic and GABAergic neurons exhibited phenotypes related to synaptic connectivity and transmission that were not apparent in separate cultures of these neuronal subtypes. Lastly, co-cultures of glutamatergic and dopaminergic neurons showed increased neuronal activity, a phenomenon not observed in cultures of either cell type alone. This study illustrates the advantages of using complex co-culture models over simpler ones, using the example of the neurodevelopmental disorder schizophrenia and its multifaceted aspects.

Psychiatr Genet. Zamanpoor et al.; 2020; 30(1):1-9

Biological Psychiatry Weinstein et al.; 2017; 81(1): 31–42

Abstract No. P019**A human iPSC-derived brain metastasis model to test efficacy and toxicity of drug candidates**

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Cancer metastasis results from the spread of disseminated cancer cells (DCCs) from primary tumors to distant organs and constitutes the primary cause of cancer-related death. Approximately 25% of cancer patients are affected by brain metastasis with severe neurological symptoms but face limited therapeutic options. Consequently, there is an unmet clinical need to specifically explore therapeutic vulnerabilities of brain metastases rather than of the corresponding primary tumor cells. For the identification of such prospective pharmacological strategies, scalable human preclinical in vitro models predicting the interplay between metastatic cancer cells and the brain niche microenvironment are crucial. We have therefore established a production workflow of human induced pluripotent stem cell (iPSC)-derived cortical organoids in a screening-compatible 96-well format. In order to mimic brain metastasis, cortical organoids were exposed to cancer cells of different primary tumor origin. Metastatic colonization of a fluorophore-labeled melanoma cell line was established and visualized by advanced imaging. This workflow was extended to breast cancer cell lines and melanoma patient-derived DCCs. Further, comprehensive characterization of metastasis signatures has thoroughly been conducted on mRNA expression and protein levels. For the establishment of a robust screening assay, we challenged these metastasis models with rationally selected and blood-brain barrier permeable tool compounds and could establish assay windows for efficacy and toxicity assessment. Finally, we utilized our melanoma brain metastasis platform to confirm and prioritize hit compounds identified in a drug screen with patient-derived DCCs. In summary, our brain metastasis platform presents a complementary tool to validate and profile drug candidates with specific on-target efficacy on metastatic cancer cells and minimal off-target cytotoxicity on the niche forming brain tissue.

Birey et al., *Nature*, 2017 May 4;545(7652):54-59. doi: 10.1038/nature22330

Abstract No. P020**The impact of deficiency in GPM6A, a schizophrenia-associated gene, on synapse formation and function of NGN2-based forward programmed neurons**

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The neuronal glycoprotein M6A (GPM6A) is highly expressed in the brain and is abundant in synapses of mature neurons. It is involved in neurodevelopmental processes such as neurite outgrowth and synapse formation. GPM6A is also implicated in synaptic vesicle release and postsynaptic density complex formation, possibly affecting long-term potentiation. Studies have linked GPM6A to schizophrenia, depression, bipolar disorder, and intellectual disability. The exact mechanisms of M6A are unresolved. Most studies have employed animal models, pointing towards a role of GPM6A in the regulation of synapse formation in neurons, with a potential reduction upon GPM6A depletion. However, until now, there has been no data on potential impact of GPM6A deficiency on neuronal function, neither in rodents, nor in human models.

Here, we aimed to address this question in an isogenic GPM6A knock out (KO) model utilizing induced neurons (iNs) derived from human pluripotent stem cells (hPSCs). We generated two isogenic GPM6A KO and two wild type (WT) hPSC clones and forward programmed them into iNs by NGN2 overexpression from a cassette integrated into the AAVS1 safe harbor locus. The iNs were further matured in co-culture with primary mouse astrocytes for up to eight weeks. Synaptic density was evaluated at weeks four and eight by assessing Synapsin 1 and PDS95 co-localization puncta, while multielectrode array was employed to measure network functional activity. Results revealed reduced synapse counts in GPM6A-deficient iNs after eight weeks of maturation. We also observed reduced burst duration and numbers of spikes per network burst in GPM6A-deficient networks while the weighted mean firing rate remained unchanged. These preliminary findings indicate that GPM6A impacts synapse formation and functional activity in human neurons derived from PSCs while our further experiments focus on the investigation of the potential influence of GPM6A deficiency on homeostatic synaptic plasticity.

Abstract No. P021**Application of single cell RNA sequencing to characterize transient cell populations and molecular signalling during respiratory differentiation of human pluripotent stem cells**

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Our research group has developed protocols to differentiate human induced pluripotent stem cells (hiPSCs) into mature proximal and distal respiratory epithelia through stages of endoderm, foregut, and early respiratory progenitors marked by NKX2.1 expression. While these protocols generally succeed, they only partially mimic human development, displaying limited efficiency and robustness. Additionally, the protocols often require adaptation for each hiPSC line. Although key signaling pathways such as FGF, BMP, and Wnt have been identified as crucial, their timing needs optimization, and the role of other pathways remains unclear. Furthermore, the impact of cell cycle characteristics, epigenetic features of individual lines, and paracrine factors released by contaminating cell types on differentiation outcomes is yet to be fully understood.

To address these issues, we conducted further investigations into the cellular composition and molecular signaling of cells differentiated according to our established protocol. By selecting a deliberately inefficient differentiation scenario, yielding only 20.8% NKX2.1-positive cells, we aimed to highlight dysregulated pathways and contaminating non-target cell populations. These samples were subsequently analyzed using single-cell RNA sequencing (scRNA-seq) to characterize the molecular phenotypes of the contaminating cell populations.

At day 12 of differentiation, scRNA-seq analysis identified two distinct cell populations: NKX2.1-expressing lung progenitors and CDX2-expressing cells, the latter likely representing a contaminating posterior endoderm population. Further cluster annotation revealed the presence of cells expressing markers associated with pancreatic, neuroendocrine, proliferative, apoptotic, respiratory, and intestinal lineages. These findings provided critical insights into the heterogeneity within the differentiated cell population. Based on this knowledge, experiments aimed at improving the differentiation protocol are currently being successfully conducted, with candidate factors that may influence respiratory differentiation now under evaluation.

Abstract No. P022**A complete workflow for high-efficiency CRISPR/Cas9-mediated gene editing in pluripotent stem cells**

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Disease-specific human induced pluripotent stem cells (hiPSCs) can be generated directly from individuals with known disease characteristics. Alternatively, healthy hiPSCs can be modified using genome editing approaches to introduce disease causing genetic mutations to study the biological response of those mutations. The emergence of CRISPR/Cas9 technology has made gene editing easier to achieve; yet, a vital step to get homogenous edited populations, producing single-cell clones of hiPSCs is still a major challenge in the field. In contrast to colony culture of hiPSCs, the specific composition of Cellartis® DEF-CS™ Culture System allows culture of hiPSCs in a non-colony, monolayer fashion, enabling very high rates of single-cell survival and clone expansion.

Here we introduce a complete workflow that achieves a high number of edited and expandable hiPSC clones that maintain the hallmarks of pluripotency. Cas9/sgRNA ribonucleoprotein complex was delivered into hiPSCs either via electroporation or via vesicles, followed by successful single cell cloning of edited hiPSCs. Endogenous gene knockout efficiencies of up to 65% were achieved for the membrane protein CD81. Seeding of single edited hiPSCs into a 96-well plate either by limiting dilution or by fluorescence activated cell sorting, resulted in a survival rate >50% using both methods. Notably, edited hiPSC clones maintained their expression of markers characteristic to stem cells even after expansion (>90% positive for Oct4, Tra-1-60 and SSEA-4).

The described workflow implementing the DEF-CS™ culture system results in a high number of edited and expandable hiPSC clones, making it highly suitable for disease modelling.

Abstract No. P023**The role of GLIS3 in human pancreas development and diabetes mellitus***Jifeng Liu^{1,*}, Michael Larsen², Yung Hae Kim¹, and Anne Grapin-Botton¹*¹Max Planck Institute of Molecular Cell Biology and Genetics²Novo Nordisk, Plainsboro, NY, USA

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The Krüppel-like zinc finger transcription factor Gli-similar 3 (GLIS3) has been identified as a diabetes-related gene, since patients with homozygous or compound heterozygous mutations in GLIS3 exhibit a syndrome of neonatal diabetes and congenital hypothyroidism (NDH). In addition, patients carrying GLIS3 mutations also display clinical features in multiple organs, such as a cystic change in the head of the pancreas, cysts in the kidney, or fibrosis in the liver. To elucidate the mechanisms underlying the association of GLIS3 mutations with neonatal diabetes, studies in mice have found that Glis3 regulates pancreatic β cell differentiation, protects β cells from apoptosis, and maintains β -cell function. Building on these findings in mice, a monolayer culture study in humans has revealed that GLIS3 can protect pancreatic progenitors and β -like cells from apoptosis. To further understand GLIS3's role, we employed various 3D in vitro models, including stem cell-derived islets (SC-islets) and human embryonic stem cell-derived 3D pancreatic spheres. Our findings from the SC-islets model indicate that GLIS3 knockout does not significantly impact the differentiation processes of pancreatic endocrine progenitor cells or β -like cells. However, in the pancreatic sphere model, we found that GLIS3 inactivation promotes cyst formation, which recapitulates the cystic phenotype observed in Glis3 mutant mice and some GLIS3 mutant patients. In addition, transcriptome analysis has revealed that the reduced ductal differentiation, increased proliferation, and decreased cilia program may contribute to this phenotype. Next, we will conduct experiments to further elucidate the mechanisms by which GLIS3 mutations promote cyst formation and to clarify GLIS3's role in regulating ductal differentiation.

Abstract No. P024**Reduced potassium current density is underlying sensory neuron hyperactivity in a human model of CNTNAP2-deficiency**

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Abnormal sensitivity and chronic pain are common comorbidities in autism spectrum disorder (ASD), with varying severity of pain responses due to its genetic heterogeneous presentation. CNTNAP2, an autism candidate gene, encodes CASPR2, a neuronal adhesion molecule essential for the development and function of the nervous system. Induced pluripotent stem cells (iPSC) present a valuable opportunity to study pathophysiological mechanisms and model neurodevelopmental disorders in vitro, making ASD an ideal candidate for stem cell-based disease modeling. We generated human-induced sensory neurons (iSNs) by inducible overexpression of NGN1, BRN3A, and ISLET1 in iPSC to study the role of CNTNAP2 in pain sensation. iSNs express characteristic markers such as PRPH, NTRK1, Nav1.7, Nav1.8, and TRPV1. CNTNAP2-deficient iSNs displayed increased firing rates and bursts with shorter durations in multi-electrode array recordings. Consistent with previous reports indicating that CNTNAP2-deficiency can disrupt membrane localization of the voltage-gated potassium channel Kv1.2 in rodent sensory neurons, voltage clamp analysis of iSNs revealed a significant reduction in voltage-gated potassium currents in mutant cells. Despite this reduction in potassium channel activity, Western blot analyzes revealed no differences in Kv1.2 protein expression levels, implying that the observed hyperactivity is due to aberrant channel localization or reduced membrane stability rather than changes in protein level. Furthermore, a preliminary gene expression analysis of CASPR2 interacting partners (Kv1.1, Kv1.2, CNTN2, NLGN3, NLGN4X, LGI1, and ADAM22) revealed that CASPR2-deficient iSNs downregulate ADAM22, a transcript encoding a non-catalytic metalloprotease-like protein involved in the trafficking and stabilization of potassium channels by recruiting and clustering of scaffolding proteins and cell adhesion molecules. Taken together, our data provide first evidence that CNTNAP2-deficiency in human sensory neurons impairs Kv channel function, resulting in hyperactivity. We expect our iSN platform to further unravel pathomechanisms connecting CNTNAP2 deficiency and hyperactivity and to serve as a tool for drug discovery for affected patients.

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Abstract No. P025**A ONECUT1 regulatory, non-coding region in pancreatic development and diabetes**

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Diabetes mellitus has become a global health burden with considerable clinical and etiological diversity. While type 1 (T1D) and type 2 diabetes (T2D) have multifactorial, polygenic causes, some cases result from single-gene mutations. Numerous genes linked to monogenic diabetes have been identified, revealing various mechanisms that disrupt β -cell integrity. Interestingly, common variants in several of these genes have also been associated with polygenic predisposition to diabetes, suggesting shared disease mechanisms across different forms of diabetes. However, many diabetic characteristics remain unexplained by typical genetic alterations, emphasizing the underestimated role of the non-coding genome.

Recently, we identified ONECUT1 coding mutations as cause of a monogenic, recessively inherited syndromic form of diabetes with neonatal or very early onset. Functional studies using human embryonic stem cell (hESC) differentiations show that ONECUT1 plays a crucial role in pancreatic progenitor (PP) formation and endocrine development, affecting transcriptional regulation and epigenetic processes across various forms of diabetes [1,2].

In a follow-up project, we studied a child with neonatal syndromic diabetes and a family history of T2D whose clinical presentation was very similar to cases with biallelic ONECUT1 truncating mutations. Genetic and genomic studies of the patient and his family followed by functional assays in genome-edited hESC forming stem cell-derived islets (SC-islets) identified a novel ONECUT1 regulatory element, characterized the disease-causing mechanism and experimentally underpinned clinical relevance of our findings.

In summary, our findings reveal the pathomechanism leading to neonatal diabetes in a patient with a compound heterozygous ONECUT1 truncating variant and a deletion affecting key regulatory elements. This discovery highlights essential ONECUT1 regulatory regions involved both in monogenic diabetes and in common T2D, emphasizing the transcription factor's role in endocrine pancreas development. Variation in this locus may contribute to the specific T2D subtype characterized by low insulin secretion.

[1] Philippi, A. et al. *Mutations and variants of ONECUT1 in diabetes. Nature Medicine* 27, 1928-1940 (2021).

[2] Heller, S., et al., *Transcriptional changes and the role of ONECUT1 in hPSC pancreatic differentiation. Communications biology*, 2021. 4(1): p. 1-12.

Abstract No. P026**CRISPR/Cas9 editing workflows in human iPSCs**

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The **CRTD Stem Cell Engineering facility in Dresden** offers diverse established workflows for gene editing in human induced pluripotent stem cells (iPSCs) using CRISPR/Cas9.

In order to generate **fluorescent reporters** or introduce other **functional transgenes** under a gene-specific promoter we recommend using the endogenous locus of the gene of interest instead of artificial or randomly integrated promoters that might not function properly outside of their natural genomic context. By employing a T2A-based strategy for co-translational cleavage most genes can be targeted at the C-terminal end of one allele keeping the expression of the gene itself largely intact. We reach heterozygous editing efficiencies of 5-30% even with big inserts (10 kb). An example shows the introduction of mRuby-NTR2.0, a dual fluorescent reporter and prodrug converting enzyme (Sharrock *et al.*, 2022) into the Insulin locus for gene-specific expression of mRuby and functional ablation of cells expressing Insulin.

Besides, **large deletions** were introduced to remove whole genes or large regions from the genome. One example shows the removal of NR1H4 exons 2-4 (32 kb) to phenocopy the deletion found in two patients suffering from progressive familial intrahepatic cholestasis. The editing efficiency is increased by providing a single stranded oligonucleotide (ssODN) as homology-directed repair (HDR) template that bridges the deletion site and by treating the iPSCs with small molecule inhibitors of DNA-PK and Polθ.

A third workflow is set up to **correct or introduce small mutations** in iPSCs for modelling genetic diseases. We utilize a specific gRNA cutting very close to the editing site and supply ssODN containing the desired edit as HDR template. Simultaneous introduction of silent PAM mutations can increase the editing efficiency. Examples show the correction of a frame-shifting heterozygous mutation in the KMT2D gene in Kabuki patient iPSCs and the introduction of heterozygous EZH2 mutations to model Weaver syndrome.

Reference: Sharrock AV, Mulligan TS, Hall KR, et al. NTR 2.0: a rationally engineered prodrug-converting enzyme with substantially enhanced efficacy for targeted cell ablation. Nat Methods. 2022;19(2):205-215. doi:10.1038/s41592-021-01364-4

Abstract No. P027**Understanding mechanisms of biliary fibrosis using periportal liver assembloids**

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Chronic liver disease, including fibrosis, cirrhosis and cancer affects millions world-wide. Biliary fibrosis is a form of liver fibrosis that affects the peri-portal region of the liver lobule. At the tissue level, it is characterized by extracellular matrix deposition as well as aberrant numbers and cellular interactions between the different liver cell populations, namely periportal mesenchyme, cholangiocytes and hepatocytes. Despite this knowledge, the molecular and cellular mechanisms that lead to this pathological state are not completely understood. This is in part because these are highly dynamic processes that are difficult to study in vivo. Here, we aimed to obtain a reductionist system to investigate the dynamics of fibrogenesis. We developed a multicellular liver organoid system containing cholangiocytes, hepatocytes and portal mesenchymal cells, which recapitulates the architecture and cell-cell interactions of the native peri-portal region of the liver lobule. The periportal liver assembloids faithfully mimics liver peri-portal architecture and functional interactions between cholangiocytes, portal fibroblasts and hepatocytes. Through manipulation of the portal mesenchyme cell numbers, we induce a biliary-fibrosis-like state in vitro. Single-cell RNA sequencing and immunofluorescent imaging analysis indicate that our assembloids recapitulate features of in vivo biliary fibrosis both, at the molecular and protein level, respectively. We demonstrate that excessive number of portal fibroblasts is sufficient to induce biliary fibrosis-like state. By abrogating homotypic contacts between portal fibroblasts we prevent occurrence of biliary fibrosis. Moreover, creating assembloids with the Mdr2 ^{-/-} hepatocyte organoids results in ductular proliferation, showing the importance of the interaction between two main epithelial cell types of liver in biliary pathogenesis. In conclusion, our novel periportal liver assembloid model recapitulates liver periportal cell-cell interactions and provides an invaluable platform to elucidate the intricate cellular dynamics involved in biliary fibrosis.

Abstract No. P028**A toolbox of human iPSC-derived microglia in different genetic backgrounds and disease models for neurodegeneration drug discovery**

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Microglia are the tissue-resident macrophages of the brain. They survey neuronal function, play roles in neurogenesis and synaptic remodeling, and are the first responders to infection. Microglia have been implicated in a number of neurodegenerative diseases including Alzheimer's disease (AD). Due to the importance of microglia during development and disease, new models that can replicate the human phenotype are crucial to advance drug discovery. Using opti-ox, we have generated human induced pluripotent stem cells (iPSCs)-derived microglia from both male and female backgrounds in a consistent manner, with the derived microglia expressing CD45, P2RY12, CD11b, CD14, IBA1, and TREM2. Transcriptomically, male and female-derived ioMicroglia show high similarity, and with a signature similar to primary microglia. Functionally, whilst both male and female-derived microglia demonstrate capacity to phagocytose various particles and exhibit a cytokine response to LPS and INF γ stimulation, background specific responses can be observed. We have also demonstrated that ioMicroglia from both male and female-backgrounds can be co-cultured with excitatory neurons (ioGlutamatergic Neurons), highlighting their ability to be used to form more complex cellular model systems. With an aim to provide a platform for investigation into mechanisms involved in neurodegeneration, we employed CRISPR/Cas-9 gene editing to introduce specific point mutations in TREM2 (R47H), and APOE (4/3 C112R) into the ioMicroglia male background; both genetic risk factors associated with late onset AD. The derived ioMicroglia disease model cells maintain a microglia phenotype and remain functionally active, providing a platform for further phenotypic characterisation. In conclusion, we have demonstrated that opti-ox deterministic cell programming generates highly consistent microglia from human iPSCs that show key microglia marker expression and ramified morphology by day 10, demonstrate key microglia functions, form co-cultures with ioGlutamatergic Neurons, and can be engineered into disease model cells that are genetically matched to the wild type cells.

Abstract No. P029**Development of a novel CF lung disease model based on CF patient specific human induced pluripotent stem cells**

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Cystic Fibrosis (CF) is a rare genetic disorder and is caused by mutations of the chloride channel CFTR. CF results in severe lung disease by mucus accumulation, chronic infection and inflammation of the airways. Most in vitro studies of CF lung disease rely on the utilisation of primary respiratory cells and immortalised cell lines despite limited accessibility of patient specific cells and inadequate recapitulation of the human pathophysiology, respectively. However, induced pluripotent stem cells (hiPSCs) are emerging as an alternative model system. hiPSCs possess a virtually unlimited proliferation capacity, can easily be generated from healthy (WT) and diseased donors, can be differentiated into all CF affected cell lineages and qualify for complex genome engineering on a clonal basis. In our study, we developed a novel CF lung disease model based on CF patient specific hiPSCs and demonstrated its utility in CF research.

A multistep differentiation protocol was applied to WT and CF hiPSCs to generate respiratory epithelial cells in air-liquid-interface cultures (iALI cultures) that contained a structured epithelium comprised of ciliated, goblet and basal cells. Molecular and functional analyzes verified the airway specification of iALI cultures and revealed high similarities compared to primary respiratory epithelial cells (pALI cultures). Moreover, analyzes confirmed the manifestation of a CF disease phenotype in CF iALI cultures that was characterised by: (I) an impaired CFTR protein expression, (II) reduced transepithelial chloride conductance, (III) reduced ciliary beating and (IV) an altered mucous layer ultrastructure shown by electron microscopy. Furthermore, the application of CFTR modulator drugs (ETI) enabled to modulate and partially rescue the disease phenotype of CF iALI cultures.

Our hiPSC-based CF lung disease model will represent a valuable tool to advance the field of CF research by facilitating novel study approaches to investigate alternative therapeutic targets in CF and to improve drug and therapy development.

Abstract No. P030**Development of a novel human induced pluripotent stem cell (hiPSC)-based in vitro model for mechanistic studies of motile cilia in the context of Primary Ciliary Dyskinesia**

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Primary ciliary dyskinesia (PCD) is a rare genetic disorder affecting the function of motile cilia. In the respiratory tract, an impairment in the mucociliary clearance (MCC) leads to progressive lung disease. To date, mutations in over 50 genes involved in cilia genesis, structure or function are known to cause PCD. This vast genetic and phenotypic heterogeneity, together with limited understanding of the complex cilia machinery, and the lack of convenient models have hindered the unveiling of underlying pathomechanisms and the development of curative drugs.

Human induced pluripotent stem cells (hiPSCs) represent a promising cell source for disease modeling due to their unlimited self-renewal and multi-lineage differentiation potential. Via directed differentiation, we are able to derive pseudostratified airway epithelium on air-liquid-interface (ALI) cultures, which closely resemble primary bronchial epithelial cell-derived counterparts regarding (I) epithelial cell type composition, (II) molecular expression profiles, (III) axoneme ultrastructure (IV) ciliary beating pattern (CPB) and frequency (CBF), and (V) MCC potential. Further, airway epithelia derived from PCD patient-specific hiPSCs carrying pathogenic mutations in the genes DNAH5, NME5, and CCNO, respectively, accurately recapitulate distinct molecular and functional diseased phenotypes. Moreover, the model enabled the identification of a novel interaction between NME5 and RSPH1. The potential of this model for mechanistic studies can be further advanced by utilizing the CRISPR/Cas9 genome editing machinery to introduce mutations or reporter systems within loci of interest. Lately, we successfully generated a fluorescent DNAI1 reporter hiPSC line that allows straightforward monitoring of ciliogenesis, investigation of protein dynamics, and readout for drug screening

Abstract No. P031**Development of chemically defined, animal-origin free (CD-AOF) supplement for hPSC derived $\alpha\beta$ T cell differentiation***Maja Petkovic*

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Differentiation medium for hPSC often contain animal-derived components such as serum or extracted materials from animal tissues. However, differentiation with animal-derived components have some obstacles to develop cell culture process for clinical application because of their lot-to-lot variation of components or risks of viral contamination. Therefore, it is desirable to use chemically defined and animal origin free (CD-AOF) media for developing safe and stable cell differentiation process. In this study, we developed novel CD-AOF supplement to make $\alpha\beta$ T cells from hiPSC. We expect our novel CD-AOF T cell differentiation supplement (T- diff) will support development of safe and stable cell culture process for T cell therapy using CD- AOF media for iPSC to T cell differentiation.

Abstract No. P032**Inducing a pseudounipolar morphology in human sensory neurons**

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Neural crest cells develop into bipolar peripheral sensory neurons (SNs), during early embryogenesis, which later transform into mature pseudounipolar neurons. This specialized morphology allows collection of sensory signals in the periphery and transmission towards the central nervous system. While human induced pluripotent stem cell (iPSC) derived SNs have become an important tool in current pain research, they typically present with a multi- or bipolar morphology. As the underlying mechanisms and time scale of SN pseudounipolarization are not fully understood we aimed to induce and visualize this morphological maturation and to evaluate its functional impact.

iPSCs were equipped with a doxycycline-inducible expression cassette encoding the fate specifying transcription factors NGN1, BRN3A and ISLET1 ('NBI'), driving their differentiation into induced SNs (iSNs) within seven days. iSNs express key markers of peripheral neurons such as PRPH, NTRK1, SCN9A, as well as TRPV1 and transcriptome analysis revealed time-dependent maturation. The cells are electrophysiologically functional and more than 98% of iSNs showed TTX-resistant sodium currents. Similarly, RNAscope experiments showed an increasing co-expression of the peripheral sodium ion channels SCN9A, SCN10A and SCN11A over time. Direct co-culture with non-neuronal cells from rat DRGs triggered pseudounipolarization in about 20% of iSNs. Live cell imaging showed that this process can occur within 24 hours and might be reversible at early stages. Preliminary HD-MEA recordings of iSNs, and tracking of their axonal action potential propagation, also indicated pseudounipolarized electro-morphic properties in these cells, with a faster conduction velocity in the thicker presumptive peripheral axon branch.

Together our data suggests that forward programmed iSNs express molecular and functional markers of nociceptors and can transition into a pseudounipolar morphology. We expect our iSNs to provide further valuable insights into the mechanisms underlying peripheral sensory neuron development, as well as pathomechanisms involved in neurological and neurodevelopmental disorders.

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Abstract No. P033**A luminescent insulin secretion reporter in hESC-derived islets for high-throughput screening of inhibitors of IAPP-induced beta-cell failure**

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Type 2 diabetes (T2D) is a metabolic disorder driven, in part, by the failure or loss of insulin-producing beta cells, resulting in sustained elevated blood glucose levels. A hallmark of T2D is the aggregation of the beta-cell hormone islet amyloid polypeptide (IAPP) into islet amyloid. IAPP oligomers and amyloid fibrils promote beta-cell death by disrupting the plasma membrane. Identifying inhibitors of IAPP aggregation is therefore of clinical importance. Current methods of drug discovery rely on synthetic human IAPP peptides and lack consideration of physiologically relevant levels of endogenous IAPP. We aim to utilize human pluripotent stem cell-derived beta-cells (SC-beta cells) to model islet amyloid.

To establish a high-throughput screening platform for small molecules that can revert IAPP/amyloid-induced beta-cell failure, we developed a luciferase-based insulin secretion reporter line. Using CRISPR-Cas9, we inserted the HiBiT sequence, which encodes a fragment of NanoLuc luciferase, into the C-peptide portion of the proinsulin gene, allowing HiBiT to be co-secreted with insulin. Upon the addition of luciferase assay reagents, luminescence is produced and measured as a proxy for insulin. We validated that the presence of HiBiT does not alter the development and maturation of SC-beta cells and that HiBiT reliably reflects glucose-stimulated insulin secretion. This newly established human pluripotent stem cell line offers the ability to assay beta-cell function at scale.

Building on this reporter line, we aim to examine the impact of islet amyloid by generating beta-cells carrying a highly amyloidogenic variant of IAPP, where serine at position 20 is substituted with glycine (S20G). This naturally occurring missense mutation is associated with early-onset T2D. Ongoing work is underway to introduce S20G mutations to the luciferase-based reporter line.

This project offers a human cell model to study islet amyloid and a high-throughput platform to identify therapeutics for the treatment of T2D.

Abstract No. P034**Novel bioartificial cardiac tissue model for the investigation of cross-talk between pluripotent stem cell-derived cardiac cell types involved in fibrosis**

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Cardiovascular diseases (CVD) are among the leading causes of morbidity and mortality worldwide, emphasising the need for their early detection and management with already established, as well as novel therapeutic strategies. The majority of CVD albeit differing in the original pathology and disease mechanism, in one way or another, lead to the development of cardiac fibrosis. The increased extracellular matrix (ECM) deposition by activated cardiac fibroblasts can alter the tissue architecture, resulting in systolic, diastolic, and electrophysiological disturbances 1. Three-dimensional engineered heart tissue models derived from hiPSCs (human induced pluripotent stem cells) can mimic the characteristics of the native myocardium, and therefore have tremendous potential in disease modelling, and regenerative strategies and might facilitate the advancement of preclinical drug testing.

Here we report on combining iPSC-derived cardiomyocytes, stromal cells and/or epicardial cells in the already established bioartificial cardiac tissue (BCT) model 2. Characterisation of the cardiac resident cell types involved in the disease and their cross-talk in a three-dimensional environment was performed. Since the exploration of novel therapeutic strategies and understanding of the pathophysiology of cardiac fibrosis is dependent on the implementation of appropriate experimental models, in this study, we introduce a new bioreactor system. The newly established mini-BCT model facilitates a simplified functional assessment of cardiac tissue constructs with different compositions and properties, and increases the available experimental throughput, while still allowing for in-depth analysis of contraction kinetics of engineered heart tissues.

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Abstract No. P035**Investigating the neuronal phenotype of type 2 diabetes in induced pluripotent stem cell derived neuronal progenitors and neurons**

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Recent studies highlighted an increased comorbidity between type 2 diabetes (DM2) and certain neuropsychiatric disorders. To explore the potential association between DM2 and neuronal phenotypes, we established a human induced pluripotent stem cell (hiPSC)-based model. The hiPSCs were generated from peripheral blood mononuclear cells (PBMCs) obtained from two monozygotic twins diagnosed with DM2 (referred to as DM2-A and DM2-B). The generated lines were characterized and published¹. The DM2 cell lines and the age- and sex-matched control cell line were differentiated into neuronal progenitor cells (NPCs).

To assess functionality, the effects of metformin, a common diabetes medication, were tested on the NPCs. Metformin was applied at various concentrations (100-1000 µM) over short-term periods (2 to 48 hours). The number of cells and multiple process parameters were assessed. The results demonstrated that DM2-A-1 exhibits significantly shorter elongations compared to DM2-B-2 and the control cell lines. In response to treatment with both metformin and a ROCK inhibitor, we observed that the DM2-A-1 line exhibited reduced responsiveness in terms of process length and the number of processes within the 2 and 4-hour windows. Moreover, during paraquat treatment, (oxidative stress assay) the DM2-A-1 line showed decreased cell viability after 48 hours in comparison to the other cell lines.

Further differentiation of NPCs into a neuronal direction, it was observed that all neuronal cultures exhibited spontaneous synchronised electrical activity and Ca-signals in response to glutamate. Following a four-week period of neuronal differentiation, DM2 neuronal cultures exhibited a significantly higher fluorescence intensity as a response to glutamate compared to the control line (N=3). This trend persisted after six weeks. However, at this point, we observed that diabetic lines from different donors were also significantly different from each other suggesting that the differences observed are dependent not only on the diagnosis but also on the specific cell line.

Szabó E et al. Generation of iPSC lines from peripheral blood mononuclear cells of identical twins both suffering from type 2 diabetes mellitus and one of them additionally diagnosed with atherosclerosis. Stem Cell Res. 2020 Dec;49: PMID: 33099106.

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Abstract No. P036**Combined approach for target-centric screening and validation of molecular degraders in neuronal in vitro models**

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Neurodegenerative disease is characterized by the toxic accumulation of specific proteins, which are classically considered hard to drug. Molecular degraders act via non-classical mechanism and may present a promising therapeutic approach in this context. One class of molecular degraders are Proteolysis-targeting chimeras (PROTACs), which facilitate the interaction between target protein and E3 ligase, ultimately leading to the degradation of the target protein via the ubiquitin-proteasome system (UPS). The potential of PROTAC-type molecular degraders in the context of preventing or slowing neurodegenerative disease is currently being explored by, among others, Arvinas, Inc., who have reported preclinical development of a degrader of pathogenic Tau. Along these lines, we have generated novel neuronal in vitro models including hiPSC-derived neurons to elucidate the mode-of-action of potential molecular degraders. As an initial step, a screening workflow was established to identify potential molecular degraders for protein targets associated with neurodegenerative disease with a focus on relevant cellular models, higher throughput assay formats and robust read-outs. We generated genetically modified cells expressing different proteins of interest, which were then used to identify hits from our in-house small molecule libraries. Prototypical degraders typically function through the ubiquitin-proteasome system (UPS) by recruiting active, i.e., NEDDylated Cullin-RING E3 Ubiquitin ligases. Consequently, NEDDylation-deficient models can be employed to mechanistically validate and confirm these hits. For this purpose, we developed hiPSC-derived neuronal models for subsequent hit validation and mechanistic profiling.

Abstract No. P037**High-throughput screen using patient-derived neuronal precursor cells (NPCs) identifies small molecule mitochondrial uncouplers targeting neurodegenerative diseases**

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Neurodegenerative diseases are progressive conditions that involve the gradual demise of nerve cells. These diseases can vary in time of onset, severity, and number of affected patients, but seem to be based on common disease hallmarks like mitochondrial dysfunction and oxidative stress as a consequence of specific genetic mutations. Leigh syndrome (LS) is a severe genetic disorder, which is caused by mutations in genes encoding proteins related to the mitochondrial energy metabolism. Currently, there are no available treatments that can cure or alleviate the symptoms of this disease. To address the need for new therapeutic options, particularly for rare diseases like LS, drug repurposing has emerged as a promising strategy. This approach leverages existing drugs for new medical applications, significantly reducing the time and cost associated with developing new treatments.

We made use of induced pluripotent stem cell (iPSC)-derived neuronal precursor cells (NPCs) from LS patients carrying a mitochondrially-encoded mutation in the *MT-ATP6* gene (complex V) and screened a well-annotated small molecule repurposing library consisting of 5,632 compounds. Employing advanced machine learning neuronal network methods alongside high-throughput image-based analysis, we identified Tyrphostin 879 and Tyrphostin A9 as lead compounds. Both demonstrated the ability to reduce the disease-specific elevated mitochondrial membrane potential ($\Delta\Psi_m$) in LS NPCs in a dose-dependent manner.

Following these findings, we identified compounds of chemical similarity and established initial structure-activity relationship data with four analogues. We confirmed the $\Delta\Psi_m$ normalizing effect in neuronal models of monogenic Parkinson's disease (alpha-synuclein triplication). Additionally, we investigated the drug-mediated effect on reactive oxygen species. In brief, we have implemented a hit identification and validation pipeline using a combination of patient-derived NPCs, phenotypic screening and machine learning algorithms. This process has led to the identification of small molecule mitochondrial uncouplers with the potential to serve as general treatment options for neurodegenerative diseases.

Abstract No. P038**New methods in melanoma research: employing stem cell-generated human skin organoids and cell types for in vitro skin test systems**

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Malignant melanoma is the primary cause of skin cancer-related deaths. Therefore, it is essential to understand its pathogenesis and explore new therapeutic approaches. Model systems like animal models and human in vitro melanoma cell cultures have significantly contributed to understanding this disease's complexities. However, translating findings from animal models to human pathology and reproducing an appropriate microenvironment in human in vitro models remains challenging.

To address these challenges, we have generated skin organoids (SO) from human pluripotent stem cells that closely mimic native human skin. These hair-bearing SO provide a relevant basis for studying melanoma progression. We aim to establish a thorough in vitro melanoma model by co-culturing melanoma cells to capture the nuances of the tumor microenvironment and study melanoma progression and interactions between tumor and stroma.

Eight different melanoma cell lines were tested for their spheroid-forming ability and then co-cultured with 80-day-old organoids to generate tumor-SO. Immunofluorescence staining was used to analyze tumor markers and assess changes in the microenvironment, focusing on the extracellular matrix and cancer-associated fibroblasts. This method offers valuable insights into the interaction between melanoma cells and their environment.

Additionally, keratinocytes and fibroblasts are isolated from SO to form a full-thickness skin equivalent (FTSE). Fibroblasts were successfully inserted into a collagen matrix to create a dermis equivalent, and a reconstructed human epidermis (RHE) was established with keratinocytes. In the future, melanoma cells will also be inserted into these stem cell-based FTSE to investigate the tumor and stroma in these structured in vitro models.

In conclusion, this research introduces novel approaches to melanoma studies by utilizing human skin organoids to develop a physiologically relevant melanoma model. This model addresses existing system limitations and facilitates the exploration of tumor-stroma interactions. The findings will help advance more effective melanoma therapies through preclinical drug testing.

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Abstract No. P039**NAD-supplementation enables late-life dietary restriction to improve proteostasis, stem cells, and lifespan**

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Dietary restriction (DR) is the best-proven intervention increasing health and lifespan across species. For unknown reason, the capacity of DR to induce health benefits diminishes in late life. Here, we show that NAD-supplementation via nicotinamide riboside (NR) enables DR-mediated improvements of maintaining proteostasis when the intervention is started in aged mice. These synergistic effects of late-life NR plus DR (NRDR) treatment on the enhancement of proteostasis occur in different strains of mice. The combined treatment enhances the capacity of late-life DR to reduce protein synthesis and translation error rates as well to decrease protein aggregates in hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs). Proteomics analysis reveals a complete rescue of the aging-related accumulation of ribosomal proteins and activation of proteostasis stress responses by NRDR treatment, which is associated with improvement in the capacity of selective autophagy on ribosomes in aged hematopoietic stem and progenitor cells. Additionally, NR is required for late-life DR to reduce the expansion of myeloid-biased HSCs and improve HSC function in aged mice and doubles DR's effect on lifespan extension to 14.3% as compared to mice with Ad libitum diet. Together, these results identify NRDR cotreatment as a dietary intervention showing promising potential to improve health and lifespan when started at old age.

Abstract No. P040**NSGW41 mice are optimal 'tools' to study the human immune system in a surrogate host**

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Generating all human blood lineages efficiently in immunodeficient mice is still challenging. This study introduces NSGW41 humanized mouse models, utilizing Kit-mutant (W41) mice, to enhance the engraftment of human hematopoietic stem and progenitor cells (HSPCs) by exploiting the functional deficiencies of murine hematopoietic stem cells (HSCs). This advancement results in substantial reconstitution of mature human blood cells, including myeloid cells and advanced stages of red blood cells and platelets, which are usually underrepresented in other mouse models. Our findings show that mature human monocytes, neutrophils, and dendritic cells derived from NSGW41 mice are effectively activated upon stimulation, demonstrating full functionality of innate human immune cells developed in mice. We also focus on the differentiation and function of human T cells (Tc), enhanced through the expression of human interleukin 7 in NSGW41 mice. The resulting NSGW41hIL7 mice exhibit improved functional Tc reconstitution, including regulatory T cells, and display a "human-like" distribution of peripheral Tc subsets. These T cells are efficiently activated both in vitro and in vivo, as shown by the enhanced development of secondary lymphoid tissues (SLTs) in a steady state. Overall, our novel humanized mouse models significantly improve the engraftment, reconstitution, and function of various subsets of both the adaptive and innate immune systems. These models offer valuable tools for investigating complex human immune responses.

Abstract No. P041/T32**Apelin signaling restricts the formation of the blood stem cell-forming hemogenic endothelium**

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During definitive hematopoiesis hemogenic endothelial cells in the aorta-gonad-mesonephros region differentiate into multipotent hematopoietic stem cells in a process called endothelial-to-hematopoietic-transition. Although only a small pool of hematopoietic stem cells is formed during embryonic development, these hematopoietic stem cells generate all blood lineages throughout life. Previous work revealed that hemogenic endothelial cells and vascular endothelial cells share a common lineage. Since the process of hematopoiesis is highly conserved among vertebrates, we are using the zebrafish as an in vivo model. Here, we show that the embryonic dorsal aorta is zoned into apelin receptor (aplnr) expressing and aplanr non-expressing endothelial cells by live confocal imaging. Of interest, aplanr non-expressing endothelial cells are exclusively located in the ventral part of the dorsal aorta, where hematopoietic stem cells arise. The G-protein-coupled receptor Apelin Receptor has been shown to play an important role in vasculogenesis and angiogenesis. However, little is known about the role of Apelin signaling in hematopoiesis. Our cellular and molecular analysis demonstrated that these aplanr-negative endothelial cells give rise to hematopoietic stem cells. Genetic analysis revealed that the predefined hemogenic endothelium by the zonation of aplanr expression is upstream of Runx1 function. Furthermore, zonation by aplanr expression in the dorsal aorta functionally restricts the hemogenic endothelium. Loss of Apelin signaling leads to an extension of the hemogenic endothelium and subsequently to an increased formation of hematopoietic stem cells within the dorsal aorta. Conversely, forced activation of Apelin signaling in the vasculature leads to a complete loss of hematopoietic stem cell development in the dorsal aorta. Our results suggest that Apelin signaling restricts the zonation of the hemogenic endothelium and thus regulates the number of hematopoietic stem cells emerging during embryonic development. This provides new insights into the process of hematopoiesis, enabling a better understanding of hematopoietic disorders to improve treatment possibilities.

Abstract No. P042**An ex vivo culture model for normal and neoplastic hematopoiesis**

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The applications of culture models of hematopoiesis range from basic research through preclinical drug screening to functional diagnostics and treatment stratification for personalised therapy.

For the latter applications in particular, it is important to reproduce as far as possible the stromal interactions, signalling and metabolic environment of endogenous hematopoietic marrow. While this is possible in animal models, these are resource intensive and unsuitable for large-scale comparative studies. Two-dimensional long-term bone marrow cultures can be established in multi-well plates and maintain stroma-supported hematopoiesis for long periods. However, stroma are variable and slow to form, hematopoietic activity is unpredictable and the success rate with neoplastic marrow is low.

To provide a more reliable and reproducible culture system for drug screening, we have established a 3D spheroid model using either bone marrow mononuclear cells or purified stem and progenitor cells combined with pre-cultured stroma cell types (endothelial-, mesenchymal stromal- and osteo-progenitor cells). In both cases, aggregates form in ultra-low adhesion 96-well plates with high efficiency in the absence of extracellular matrix and begin to produce hematopoietic progeny within 1 week. High level activity is maintained for at least 4-5 weeks from all samples, including those from donors with myelodysplastic neoplasia (MDS) or acute myeloid leukemia (AML). Hematopoietic progeny harvested from the well retain the genetic markers of the input neoplasm. Furthermore, while the default hematopoietic activity of the system is myeloid, erythropoiesis can be recruited by the addition of erythropoietin to the medium.

This simplicity, reliability and accessibility of this system makes it particularly attractive for screening drug effects on myeloid neoplasms using patient cells in a niche-like environment. Initial tests have confirmed drug responses. We are currently validating the system by examining the response of myelodysplastic cells to clinically relevant hypomethylating agents.

Abstract No. P043**A proteome-centric view on blood aging: insights from GSK-3 dysregulation in hematopoietic stem cells***Eric Schmitt^{1, 2}, Petra Bell^{1, 3}, and Borhane Guezguez^{4,*}*¹Institute of Molecular Biology (IMB), Mainz²University Medical Center Mainz, Department of Hematology and Oncology³Institute of Developmental Biology and Neurobiology (IDN), Johannes Gutenberg-University Mainz⁴University Medical Center Mainz/DKTK, Department of Hematology and Oncology

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Proteostasis in hematopoietic stem cells (HSCs) encompasses the regulation of protein synthesis, folding, and degradation, which is vital for their differentiation and function. Effective proteostasis facilitates proper HSC differentiation into various blood cell lineages and sustains their self-renewal capacity. Aging HSCs exhibit altered protein synthesis rates and diminished efficiency in protein degradation, leading to the accumulation of misfolded proteins, thereby contributing to a functional decline in HSCs and a skewed hematopoietic lineage toward myeloid-biased output. Elucidating and addressing these age-associated changes in proteostasis is crucial for maintaining HSC function and enhancing hematopoietic health in the aging population. Glycogen synthase kinase -3 (GSK-3), known for its multifaceted role in various cellular processes, has been implicated in aging of different organs. Our group has shown that HSCs deficient in GSK-3 exhibit a myeloid biased output and phenotypic features reminiscent of age-related blood disease, highlighting the significance of GSK-3 in maintaining HSC homeostasis.

Here, we present our ex-vivo approach to model HSC aging by isolating and culturing highly purified HSC populations, followed by chemical inhibition of GSK3 kinase activity (CHIR99021). This workflow enables us to explore the functional consequences of GSK-3 inhibition on HSC behavior and cell fate decision. To assess the impact of GSK-3 inhibition on HSC translational regulation, we employed highly sensitive mass spectrometry-based omics, which allow us to comprehensively analyze low-input protein expression profiles and signaling networks of HSCs, providing insights into the molecular mechanisms underlying GSK-3 dependent HSC aging.

Preliminary screening of our proteomic data revealed that GSK-3 Inhibition increases translation in HSC and acquires characteristics of Myeloid Skewing by dysregulating significant proteins associated with ribosomal/RNA biogenesis, myeloid differentiation and cellular senescence. Our data suggest a potential link between GSK-3 inhibition, myeloid lineage-skewing, and cellular senescence, potentially shedding some light on the molecular mechanisms underlying HSC aging.

Abstract No. P044**Enhanced in vitro proliferation of adult human long term hematopoietic stem cells induced by dimerization of intracellular Mpl and c-Kit**

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Long Term-Hematopoietic stem cells (LT-HSC) can self-renew and differentiate into all lineages of the hematopoietic system. In fact, HSCs are used for autologous and allogeneic transplantations (HSCT) to treat several hematologic diseases such as rare inherited disorders and leukemias. However, HSCT is often accompanied by complications including graft rejection, graft vs host disease (GVHD) and impaired immune activity. Therefore, novel therapies to enhance engraftment of transplanted HSCs and accelerate immune recovery after HSCT, are necessary to minimize these risks.

Towards developing a strategy that gives transplanted HSCs a competitive growth advantage over existing HSCs in the bone marrow niche and potentially over cancer stem cells, we use the constitutive expression of a fusion protein which consists of the ligand-binding domain F36V fused to the signaling domain of Mpl or c-Kit (1). To improve expansion and clonogenic capacity of CD34+ cells, a chemical inducer of dimerization (CID) called Rimiducid, is added to cells which triggers the homodimerization of F36VMpl or F36VKit monomers and induces their signaling pathways.

To this purpose, CD34+ cells from adult donors were transduced with lentiviral vectors containing F36VMpl or F36VKit with or without addition of Rimiducid (100 nM) and transduction enhancer Protamine sulfate (8 ng/mL). The transduced cells were cultured in TexMACS™ GMP media with 0.5 % HSA in presence of hFLT3 and hSCF or hTPO (100 ng/ml) for 48 h, 72 h and 96 h and analyzed via Flow and CFU analysis.

Our preliminary results showed that Rimiducid treatment significantly increased the proliferation of LT-HSCs after 48 h and 72 h with F36VMpl and showed an enhanced trend for F36VKit after 48 h in combination with Protamine sulfate. Similar results were observed also at the CFU level, specifically for 48 h after Rimiducid and Protamine sulfate treatment for F36VKit.

1. Parekh C et al., Novel pathways to erythropoiesis induced by dimerization of intracellular C-Mpl in human hematopoietic progenitors. *Stem Cells*. 2012 Apr;30(4):697-708. doi: 10.1002/stem.1046.

Abstract No. P045**Epigenetic regulation of DNA damage response in hematopoiesis of mice and humans**

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SETD1A is the key histone methyltransferase for lysine residue 4 of histone 3 in murine hematopoietic stem and progenitor cells (HSPCs). We have previously shown that inactivation of SETD1A in the hematopoietic compartment led to an impairment of blood cell formation from definitive hematopoietic stem cells (HSCs) and to a decrease in expression of DNA damage signaling and repair pathways. As the transcript levels of SETD1A decreases over time in murine and human HSCs, we questioned its role on the human HSPCs as well as its contribution to the ageing of the hematopoietic stem cells.

The deletion of SETD1A in human HSPCs revealed blunted responses to growth factor mediated proliferation, both, in terms of frequency of responding cells as well as division rate of responders, suggesting a conserved function of SETD1A in murine and human hematopoiesis. The loss of SETD1A in human HSCs leads to an accumulation of a higher fraction of DNA damage after irradiation, highlighting that this histone methyl transferase affects the ability that the HSPCs have to resolve DNA damage. Furthermore, interferon alpha receptor expression was elevated on human and murine HSPCs upon depletion of SETD1A, suggesting that SETD1A-deficient HSPCs could be more sensitive to inflammatory signaling, especially the Type 1 interferon pathway. In addition, loss of SETD1A in the HSCs leads to a progressive reduction of common lymphoid progenitors, while the number of the myeloid progenitors do not change over time. These features have also been implicated to mediate the ageing process of the hematopoietic system. Finally, we could show that the phenotype on both human and murine HSPCs lacking SETD1A can be rescued via the ablation of p53. Overall, our study suggests the mechanism behind the loss of SETD1A in HSCs and that the deficiency of SETD1A is a potent driver of stem cell ageing.

Abstract No. P046**Acute myeloid leukemia classification using self-supervised pre-trained multiple instance learning**

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Automated disease diagnosis using medical image analysis relies on deep learning, often requiring large labeled datasets for supervised model training. Diseases like Acute Myeloid Leukemia (AML) pose challenges due to scarce and costly annotations on a single-cell level. Multiple Instance Learning (MIL) addresses weakly labeled scenarios but necessitates a powerful feature extractor typically trained with cell-level labeled data ¹. In this study, we explore Self-Supervised Learning (SSL) as a pre-training approach for MIL-based AML subtype classification from blood smears, removing the need for cell-level labeling.

We investigate three state-of-the-art SSL methods—SimCLR, SwAV, and DINO—each employing distinct strategies to extract the cell features necessary for AML subtype classification within the MIL framework. SimCLR represents a contrastive learning approach, SwAV employs a clustering-based technique, and DINO utilizes self-distillation-based SSL. These methods have demonstrated strong performance on widely recognized computer vision benchmark datasets ². By leveraging these SSL approaches, we pre-train the feature extractor without requiring cell-level labels. Subsequently, we embed the pre-trained feature extractor within a MIL architecture and train the entire model using patient-level annotations to classify AML subtypes.

Our findings show that SSL-pre-trained feature extractors perform comparable to supervised pre-training, with an F1-macro score of 0.86. Furthermore, our framework provides cell-level predictions, allowing biomedical experts to review and validate the most relevant cells for AML subtype classification. Given that AML originates in hematopoietic stem cells (HSCs), our approach could also aid in identifying key cellular features associated with disease initiation and progression, potentially offering deeper insights into the role of HSCs in AML. This validation highlights the efficacy and promise of leveraging SSL techniques for accurate and data-efficient AML subtype classification within the MIL framework without the need for labor-intensive single-cell annotations.

1. Matthias Hehr, Ario Sadafi, Christian Matek, Peter Lienemann, Christian Pohlkamp, Torsten Haferlach, Karsten Spiekermann, and Carsten Marr. *Explainable ai identifies diagnostic cells of genetic aml subtypes*. PLOS Digital Health

2. Bin Li, Yin Li, and Kevin W Eliceiri. *Dual-stream multiple instance learning network for whole slide image classification with self-supervised contrastive learning*. In *Proceedings of the IEEE/CVF conference on computer vision and pattern recognition*

Abstract No. P047**Tracking codons from genome to proteome along the hematopoietic hierarchy**

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Transfer RNAs (tRNAs) deliver amino acids to the protein translation machinery and thereby link the coding transcriptome with the proteome of a cell. Differential regulation of tRNA expression to control translational efficiency is mostly unappreciated by the research community, raising the question whether tRNA abundance and codon usage (CU) regulate cell states in stem-cell driven systems. To address this point, we investigate tRNA expression, CU and translation at single-codon level in the organization of hematopoiesis.

We established an integrative approach to calculate the demand and supply of tRNAs using matched mRNA- and tRNA-sequencing and quantitative proteomics of cell populations at different stages of hematopoiesis. Hematopoietic stem/progenitor cells (HSPCs), oligopotent progenitors, and T-cells of healthy donors were FACS-sorted, and low-input mRNA- and tRNA-sequencing was performed. Strikingly, principal component analysis solely based on tRNA gene expression enabled HSPC and T-cell cluster separation, suggesting the tRNA profile to be indicative for cell identity.

Calculating the transcriptional CU (tCU) as a unit describing codon abundance per million mRNA transcripts, we found that HSPCs and T-cells strongly differ in their global tCU/tRNA ratio exclusively for weakly expressed proteins. In contrast, intermediate and high abundant proteins confer a similar tCU/tRNA correlation, suggesting cell-type-specific synchronization of tRNA and codon abundance depends on translational fidelity.

At single-codon level, we identified the Lysine-codon AAG to be increasingly used in T-cell mRNAs of intermediately/highly translated genes, despite decreased tRNA expression relative to HSPCs. In both cell types, elevated AAG usage correlates with high protein abundance, defining AAG as a differentiation-dependent bottleneck between tRNA demand and supply.

In summary, our original study describes differential tRNA expression within the hematopoietic hierarchy, presents a novel approach to track codons from transcription to translation, and sheds light on previously unrecognized mechanisms of translational control potentially driving cell state and specification.

Abstract No. P048**Immune regulation of preleukemic mutations in hematopoietic stem cells**

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Clonal hematopoiesis (CHIP) refers to a set of mutations in hematopoietic stem cells that have been demonstrated to lead to an increased probability of developing a leukemia and are thus considered a preleukemic disease. In this study we argue that our natural regulatory mechanisms can detect CHIP clones and suppress them. We demonstrate that using several publicly available longitudinal and single-time-point data sets. We compare several stochastic models and demonstrate that models involving immune regulation are more successful at describing the real-world data than models involving proliferation and clone competition only. This suggests the importance of better understanding the role of the immune system in leukemia development and suggests at an interesting possibility of employing the immune system in preventive measures.

Abstract No. P049**Unraveling leukemia subtypes through AI-driven integration of clinical data***Ilaria Looser^{1,*}, Carsten Marr², and Matthias Heinig¹*¹Helmholtz Center Munich, Institute for Computational Biology²Helmholtz Munich, Institute of AI for Health

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Leukemia is initiated in the bone marrow by oncogenic lesions in hematopoietic stem and progenitor cells. In the process termed “clonal evolution”, these transformed cells with self-renewal capacity give rise to a variety of leukemic sub-populations, which may acquire additional genetic alterations. Distinct combinations genetic aberrations have been shown to determine a heterogeneous pathobiology and treatment response, making the distinction of different leukemia types a primary concern for diagnostics and targeted treatment prescription. Currently, leukemia diagnosis relies on a hierarchical framework which classifies the disease into entities and subtypes. Classification criteria are based on the integration of cell morphology, immunophenotype, and genetics, as defined by the World Health Organization (WHO).

Our study aims to leverage AI for integrating multi-view diagnostic information from a clinical dataset of over 100'000 patients and uncover biologically defined clusters within leukemia types. Focusing on deviations from the current WHO classification, we seek to elucidate the relevance of potential new mutation patterns. Moreover, we aim to capture genetic similarity across different subtypes and define mutation-based progression trajectories.

The model architecture will furthermore allow the integration of other diagnostic modalities and thus capture their impact on subtype definition.

Major implications of a data-driven and explainable subtype-defining model are the improvement of treatment selection and refinement of patient risk stratification. On a subtype-specific level, our research will provide insights into putative biological mechanisms resulting in genetic interdependencies and phenotypic manifestations. Additionally, by exploring similarities and potential progressions across subtypes we aim to contribute to a nuanced understanding of leukemia heterogeneity.

Abstract No. P050**Ex-vivo expansion of human hematopoietic stem cells using a cytokine-free approach and HOXB4 overexpression**

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Hematopoietic stem cells (HSCs), with their unique ability for self-renewal and differentiation into various blood lineages, are essential for blood and immune system regeneration. Expanding these rare transplantable HSCs ex-vivo is crucial for treating inherited hematopoietic disorders. To refine the ex vivo expansion protocol for adult human HSCs, we introduced two significant modifications to the existing culture systems. First, human recombinant cytokines were replaced with synthetic small molecule agonists, specifically utilizing the phosphoinositide 3-kinase (PI3K) activator 740Y-P in combination with the thrombopoietin receptor agonist butyzamide. This effectively facilitates HSCs expansion, while preserving their intrinsic stemness characteristics. Second, we sought to promote HSC expansion ex vivo by transient overexpression of the transcription factor HOXB4. HOXB4 overexpression studies across various species have demonstrated its significant impact on HSC biology. We transfected hematopoietic stem and progenitor cells (HSPCs) cells with HOXB4 mRNA via electroporation. Notably, peak expression of the HOXB4 protein occurred 4 hours post-transfection, followed by a gradual decline over time. In response to the limited half-life of HOXB4 protein, we implemented a lipid nanoparticle (LNP)-based delivery system for transfecting HSCs with HOXB4 mRNA. This approach allows for controlled, transient release and the possibility of redosing, thereby extending the duration of HOXB4. Next, we transfected HSPCs with LNPs encapsulating HOXB4 mRNA and the expression kinetics demonstrated peak HOXB4 protein levels at 12 hours post-transfection, with expression dissipating by 48 hours. Ongoing research efforts are focused on combining cytokine free approach with HOXB4 overexpression for long-term ex-vivo expansion of HSCs. The aim is to thoroughly understand how HOXB4 overexpression influences human HSCs expansion and the maintenance of their stemness properties. Ultimately, this research is poised to pave the way for establishing a clinically viable culture system capable of fostering the expansion of long-term repopulating adult human HSCs.

Abstract No. P051**EPCR/PAR1 signaling in hematopoietic stem and progenitor cells**

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Hematopoietic stem cells (HSCs) are the definitive source of all blood cells and replenish them lifelong. A tight regulation of HSC differentiation, self-renewal and quiescence is necessary for hematopoiesis. The endothelial protein C receptor (EPCR) is expressed on a subpopulation of highly quiescent and stress resistant HSCs with high engraftment potential after transplantation. However, the signaling of EPCR and its coreceptor protease activated receptor 1 (PAR1) in HSCs is not well understood. On endothelial cells, thrombin activation of PAR1 causes proinflammatory signaling, whereas EPCR-bound activated protein C (aPC) conveys cytoprotective PAR1 signaling.

We studied EPCR/PAR1 signaling in human CD34⁺ cells, where PAR1 is expressed on a subset of EPCR⁺CD90⁺ HSPCs, by phosphoflow after in vitro stimulation with thrombin and aPC. Thrombin caused PAR1 activation and internalization in CD34⁺ HSPCs, alongside ERK1/2 and Akt phosphorylation and rapid mobilization of Ca²⁺ into the cytosol of expanded CD34⁺CD45RA⁺CD90⁺ cells with subsequent phosphorylation of the transcription factor CREB shortly after stimulation, whereas aPC only led to Akt phosphorylation. EPCR⁺ HSC are protected from activation by inflammation. Exposure of murine HSPCs to TNF α , IL1 α (100ng/mL) and thrombin (1U/mL) in vitro led to EPCR shedding after 24 hours, reducing EPCR cell surface presentation to less than half. Addition of the sheddase inhibitor TAPI-0 (30 μ M) partially prevented loss of EPCR. Chronic inflammation in the bone marrow is described in patients suffering from primary immune deficiencies, like chronic granulomatous disease (CGD). In CGD mouse models, EPCR⁻ HSCs (Lin-Sca-1+cKit+CD150+CD48⁻) are less quiescent (55% in G0) compared to EPCR⁻ in wildtype and EPCR⁺ HSCs in CGD mouse models (75% in G0).

Our study reveals differential EPCR/PAR1 signaling in HSCs after activation with thrombin or aPC. In an inflamed bone marrow environment, EPCR expression protects HSCs from activation and EPCR/PAR1 activation may be explored during in vitro HSC-modification for gene therapy.

Abstract No. P052/T33**Intrinsic reconstitution potential of developmentally restricted hematopoietic stem cells in non-conditioned immunocompromised mice**

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New insights are challenging the traditional view of strictly hierarchical hematopoietic specifications during development and into adulthood. Instead, evidence is bolstering a model where hematopoietic stem and progenitor cells (HSPCs) arise in parallel; however, to what extent perinatally derived HSCs contribute to adult hematopoiesis is not well known.

Using an unequivocal genetic lineage tracing mouse model (FlkSwitch), we revealed the co-existence of developmentally restricted HSCs (drHSCs) alongside fetal HSCs (fHSCs), which do not persist as HSCs into adulthood *in situ*. In contrast, drHSCs are capable of long-term multilineage reconstitution when transplanted into irradiated hosts, possibly because of their high responsiveness to inflammatory signals. To test this, we isolated drHSCs from neonatal livers of FlkSwitch mice and transplanted them into adult unconditioned immunocompromised recipient mice with a c-Kit mutation (NBSGW mice). Strikingly, drHSCs exhibited robust long-term reconstitution (20 weeks) of peripheral blood cells and HSPCs in the bone marrow. The phenotypic HSC pool established by drHSCs in the bone marrow of NBSGW recipients were notably smaller compared to that established by fHSCs, potentially indicating limited self-renewing expansion capacity of drHSCs, despite their long-term persistence. Quantitative cell analysis revealed that drHSCs are superior in reconstituting B and T cells in the peripheral blood, tissue-resident innate-like B1a cells in the peritoneal cavity, and marginal zone B cells in the spleen in recipient mice compared to fHSC. This emphasizes the importance of drHSC in immune cell generation during development and potentially over the lifespan. As early developmental lymphoid cells play essential roles in establishing the immune system, self-recognition, and tolerance, these findings are critical for understanding the development of disease susceptibility and autoimmune diseases in adulthood.

Abstract No. P053**Deciphering clonal hematopoiesis of indeterminate potential via single-cell multiome analysis**

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Clonal Hematopoiesis of Indeterminate Potential (CHIP) outlines an age-related, incremental acquisition of leukemogenic mutations within hematopoietic stem and progenitor cells (HSPCs). This accrual of somatic mutations fosters a clonal proliferation of blood cells, heightening the risk of malignancy in the myeloid compartment. Predominantly influenced by alterations in two key epigenetic regulators, DNMT3A and TET2, these mutations - when co-occurring or possessing high variant allele frequencies (VAF) - confer a selective fitness advantage to mutated HSPCs over their mutation-free or neutrally mutated counterparts.

To elucidate the mechanisms underpinning clonal expansion associated with CHIP, we performed single-cell multiome analyzes, complemented by genotyping, of bone marrow samples from 17 individuals undergoing hip replacement surgery.

Our comparative analysis integrates single-cell RNA-seq and ATAC-seq data. It allows us to delineate the variations in cell type composition but also unveils distinct transcriptomic, epigenomic, and interactome signatures across cell lineages. Furthermore, it sheds light on the clonal hierarchies and trajectories unique to each group, providing deeper insights into the dynamics of CHIP.

Steensma, D. P., Bejar, R., Jaiswal, S., et al. (2015). Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood, 126(1), 9-16.

Buck, M. C., Bast, L., Hecker, J. S., et al. (2023). Progressive disruption of hematopoietic architecture from clonal hematopoiesis to MDS. iScience 26, 8.

Abstract No. P054**Refinement of FDCP-Mix cell cultivation and promoter optimization for hematopoietic stem cell research**

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FDCP-Mix cells are non-leukemic, murine, multipotent hematopoietic progenitor cells that respond to self-renewal and differentiation signals. They share many features of normal stem/progenitor cells. The ability to generate large numbers of cells at defined stages of differentiation makes them particularly useful for metabolic studies, where they are providing new insights into connections between metabolism and signaling pathways. Our present goal is to characterize the role of the nucleoside diphosphate kinase “Non-metastatic 2” (Nme2) protein in mediating the cellular response to low energy niche conditions through direct interaction with signaling proteins. On the one hand, we are refining FDCP-Mix culture conditions to better reflect and support stem cell metabolism and have identified branched chain amino acids to be of relevance. On the other hand, we are developing viral vectors with which to modulate the expression of wild type and tagged Nme2. To this end, we have focused on Serpin 2A, a stem cell specific gene originally described in the FDCP-Mix system and since confirmed in primary cells. We revisited these original findings and extrapolated the minimal promoter sequence using the mouse mm39 genome annotation, which is now recognized as part of the Serpina3g gene promoter. We cloned this fragment into a lentiviral backbone, replacing the original CMV promoter and driving the expression of an EGFP transgene. Undifferentiated FDCP-Mix cells were transduced with this new construct and with a range of other established viral vectors for comparison. The new minimal Serpin promoter directs a level of expression 3-4-fold higher than that from the CMV promoter. We are currently assessing the activity in differentiating cells, in which we expect the expression to be low or absent, providing us with a new tool with which to direct stem cell specific expression of Nme2 and to investigate protein/protein interactions specific to low-energy conditions.

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Boissan, M., Schlattner, U. & Lacombe, M.-L. The NDPK/NME superfamily: state of the art. Lab Invest, 2018 98, 164–174; 10.1038/labinvest.2017.137.

P055 – P064 Pluripotency & reprogramming

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Abstract No. P055**Calcium imaging in human pluripotent stem cells for characterizing pluripotent state and disease modeling**

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Intracellular calcium signaling pathways are crucial for regulating cellular processes such as proliferation, differentiation, and apoptosis. Human pluripotent stem cells (hPSCs) offer unparalleled opportunities to study the development and differentiation of various human cell types. Despite their significance, intracellular calcium responses to different ligands and the associated signaling pathways have not been extensively explored in hPSCs. We previously demonstrated that using the fluorescent calcium indicator Fluo-4 with confocal microscopy allows for sensitive and reliable measurements of calcium modulation in hPSCs and stem cell-derived cardiomyocytes, mesenchymal stem cells, and neural cell types. Building on this work, we developed hPSC lines stably expressing genetically encoded Ca²⁺ indicators (GCaMP2 and GCaMP6fast) via a transposon-based gene delivery system. Our findings show that the differentiation properties of the GCaMP-expressing hPSC lines are fully preserved, enabling Ca²⁺ imaging without toxic dye-loading. In undifferentiated hPSCs, calcium signals induced by various ligands, including ATP, LPA, trypsin, and angiotensin II, were comparable to those observed in Fluo-4 loaded cells. Consistent with previous studies, no calcium signals were elicited by thrombin, histamine, or GABA. Cardiomyocyte colonies derived from hPSC-GCaMP2 cells were identifiable by spontaneous contractions and Ca²⁺ oscillations (1). GCaMP6fast-expressing neural cells were characterized based on their morphological and immunostaining properties, and their Ca²⁺ signals were thoroughly examined. Additionally, spontaneous and ligand-induced Ca²⁺ signals, as well as their pharmacological modulation, were successfully analyzed in hPSC-derived disease models using fluorescence imaging (2). This study highlights the efficacy of calcium signaling as a functional assay in both pluripotent and differentiated cell types and in the context of disease modeling.

1: Apati et al. *Cell Signal*. 2013 PMID: 23305950

2: Tordai et al. *Schizophr Res* 2024 PMID: 38290943

Abstract No. P056**Exploring G-quadruplex and Yamanaka factor interactions in stem cell pluripotency and cancer progression**

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DNA G-quadruplexes (G4s) are non-canonical secondary structures highly abundant in embryonic stem cells (ESCs) but tend to diminish during lineage specification, indicating a potential role in maintaining pluripotency¹. Many transcription factors preferentially bind to G4s over double-stranded (ds) or single-stranded (ss) DNA², implying that G4s might play a critical role in regulating gene expression. Our preliminary research has revealed that all Yamanaka Factors (YFs), except SOX2, prefer binding to synthetic G4 relative to ss and dsDNA structures, suggesting these factors interact with G4s. To investigate this further, we employ *in situ* proximity ligation to identify proteins that potentially mediate YF-G4 interactions. Disrupting these mediators, through chemical targeting, knockout or knockdown, may destabilize YF-G4 interactions, thereby affecting the pluripotency of both normal and cancer stem cells. Our findings could provide critical insights into the molecular mechanisms underlying cellular reprogramming and cancer transformation, which share many similarities. This research has the potential to lead to novel therapeutic strategies targeting these interactions, offering new approaches to enhance stem cell biology and improve cancer therapies.

1. Zyner, K. G., Simeone, A., Flynn, S. M., Doyle, C., Marsico, G., Adhikari, S.,... & Balasubramanian, S. (2022). G-quadruplex DNA structures in human stem cells and differentiation. *Nature Communications*, 13(1), 142.

2. Spiegel, J., Cuesta, S. M., Adhikari, S., Hänsel-Hertsch, R., Tannahill, D., & Balasubramanian, S. (2021). G-quadruplexes are transcription factor binding hubs in human chromatin. *Genome biology*, 22(1), 117.

Abstract No. P057/C03**Improved genetic stability of human pluripotent stem cell cultures passaged as single cells using eTeSR™**

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Genetic instability has been widely documented in human pluripotent stem cells (hPSCs). Recurrent genetic abnormalities arise throughout culture and confer a selective advantage to genetically variant cells through mechanisms including resistance to cell death and reduced differentiation capacity. We have previously shown that routine single-cell passaging of hPSCs can result in a high incidence of de novo genetic abnormalities and that eTeSR™, a novel hPSC maintenance medium developed to better support single-cell passaging, can significantly reduce the appearance of recurrent abnormalities. To further demonstrate the increased genetic stability observed when routine single-cell passaging hPSCs in eTeSR™, 72 clonal sublines were derived from three hPSC lines (H1, H9 and SCTi003-A) using single-cell deposition. Each clone was then independently maintained for 20 weeks in either two commercially available control media (A & B) or eTeSR™. Genomic DNA was extracted and analyzed using single nucleotide polymorphism microarray. Of the 136 samples maintained in control media, 93 (68%) had acquired at least one de novo abnormality compared to only 34 (25%) of the 135 samples maintained in eTeSR™. This can be attributed to fewer small copy number variants (CNVs) (<10,000 kb) observed in subclones maintained in eTeSR™, with only six detected across 135 samples compared to 94 detected in 136 samples maintained in control media. Strikingly, 75 of 136 (55%) samples maintained in control media harbored a gain of 20q, with 68 instances presenting as small CNVs that are often undetectable using G-band karyotyping. Conversely, only four 20q gains were detected in eTeSR™-maintained samples, a significant finding due to the prevalence of 20q11 duplications reported in large-scale studies that describe recurrent karyotypic changes in hPSCs 1,2. This study provides a better understanding of culture-acquired genetic aberrations in hPSCs and demonstrates that innovative media formulations, like eTeSR™, can address major challenges in the hPSC field.

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Taapken SM, Nisler BS, Newton MA, Sampsell-Barron TL, Leonhard KA, McIntire EM, Montgomery KD. Karyotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. Nat Biotechnol. 2011 Apr;29(4):313-4.

Abstract No. P058**Impact of a hydrogel-based 3D environment and externally applied mechanical stress on iPSCs**

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Cells embedded in hydrogels exhibit an abundance of mechanical cues that play a role in driving cellular differentiation. Hydrogels, which are hydrophilic 3D substrates of polymer fibres, are embedded with cells to mimic the in vivo microenvironment. In this study, we want to better understand the influence of the mechanical cues of hydrogels on iPSCs and whether the cells remain pluripotent or differentiate towards one of the three lineages namely endoderm, mesoderm, and ectoderm. For this purpose, we selected collagen and polyethylene glycol (PEG) hydrogels of varying stiffnesses to decipher whether properties of the gel impact the iPSCs. On culturing iPSCs in collagen hydrogels, we observed the expression of endodermal marker GATA6 along with pluripotency marker OCT4 in the cellular aggregates, indicating the onset of differentiation. A preliminary analysis of the apical marker ZO-1 also indicated the formation of luminal structures when cells were embedded in collagen. When iPSCs were embedded in PEG hydrogels, a rapid decline in viability was observed unless they were embedded as 3D aggregates known as embryoid bodies. Subsequently, undirected differentiation of the embryoid bodies indicated endodermal differentiation in PEG hydrogels of intermediate stiffness, suggesting that hydrogel stiffness guides cellular differentiation. Furthermore, we investigated the effect of externally applied shear stress on the differentiation patterns in embryoid bodies embedded in geltrex for a period of 5 days. The embryoid bodies were exposed to a shear stress resulting from a parallel movement forward and backward at an amplitude of 10 mm with a frequency of 0.1 Hz. At the end of the stress exposure, the embryoid bodies depicted increased expression of mesodermal marker (Brachyury) in comparison to the control embryoid bodies during an initial assessment. Taken together, our results support the notion that mechanical cues, both emerging from the microenvironment and applied externally, can direct cellular differentiation.

Friedland, F., Babu, S., Springer, R., Konrad, J., Herfs, Y., Gerlach, S.,... & Noetzel, E. (2022). ECM-transmitted shear stress induces apoptotic cell extrusion in early breast gland development. Frontiers in Cell and Developmental Biology, 10, 947430.

Abstract No. P059**Induced pluripotent stem cells from human skin biopsies within three weeks via non-integrative reprogramming**

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Human induced pluripotent stem cells with their potential to differentiate into countless different cell types hold great potential for allogenic as well as autologous cellular therapy. However, many traditional techniques used for generation of iPSCs entail the risk for genomic integration and therefore limit their use in a clinical setting. In addition, the established protocols are often lengthy and require the subsequent screening for unwanted vector integration. Transient mRNA-based reprogramming eliminates this risk and is one of the fastest and most efficient reprogramming technologies available today.

We hereby present a fast and robust protocol to generate monoclonal iPSCs from human skin biopsies within four weeks. Fibroblasts from five healthy donors (age 50 – 62) were isolated from abdominal skin via combined mechanical and enzymatic dissociation. The cells were expanded for only one passage before starting the reprogramming routine, eliminating the need for extensive upstream expansion of fibroblast and thus minimizing the risk for cultivation induced aberrations. For reprogramming, cells were transfected for five consecutive days with an mRNA cocktail containing the transcription factors OCT3/4, SOX2, KLF4, MYC, NANOG and LIN28A. During a short growth phase iPSC colonies formed with high efficiency and could be further expanded into stable monoclonal cell lines.

We derived and phenotyped 36 iPSC lines with low intra- and inter-donor variability. Generated iPSCs showed characteristic stem cell morphology and typical pluripotency-associated markers expressed at high levels. Moreover, the vast majority of generated iPSCs exhibited intact genomic integrity as assessed by digital PCR and all tested lines successfully differentiated into the three germ layers endoderm, ectoderm and mesoderm.

In summary, the described protocol allows for efficient and reliable integration-free generation of high quality monoclonal iPSCs from human skin punch biopsies within only four weeks and will therefore facilitate the generation of iPSCs from individual donors.

Abstract No. P060**Ensuring GMP Standards: a closed-system approach to expand human pluripotent stem cells**

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Human pluripotent stem cells (hPSCs) hold great promise for disease modeling, drug discovery and clinical applications. Therefore, working with highly pluripotent, well characterized and quality-controlled cell stocks during development is crucial to ensure reproducible experimental conditions.

Here, we established a workflow encompassing stable expansion of hPSCs using a xeno-free and GMP compliant cultivation medium. For quality control of cultivated hPSCs the pluripotency was assessed using a defined flow-based marker cocktail, functional pluripotency was identified after differentiation in all three germ layers by multicolor flow cytometric analysis. Furthermore, genetic stability was evaluated.

Following this workflow, hPSCs could be stably expanded for 20 passages with persistent, high expression of pluripotency markers TRA-1-60, SSEA-4, Oct3/4 and Nanog as well as almost no expression of differentiation marker SSEA-1 (CD15). PSCs revealed a homogenous morphology and retained genetic stability. Quantitative, flow cytometry-based analysis reproducibly proved their differentiation potential into ectoderm, mesoderm, and endoderm. Thus, the workflow shown here assures a standardized, robust expansion of hPSCs and includes characterization as well as quality control of the expanded cells.

The flow-based QC strategy was also successfully applied for characterization of cells cultivated under standardized, automated, closed system conditions using the CliniMACS Prodigy Instrument which is of major relevance for generation of master cell banks (MCB) and working cell banks (WCB) for future clinical cell manufacture.

Abstract No. P061**GMP compliant generation and expansion of iPS derived MSCs and characterization of their extracellular vesicles in a closed cultivation system using xeno-free MSC-Brew GMP Medium**

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Human mesenchymal stem cells (MSCs) hold great promise for clinical use and cell therapy applications and can be isolated from multiple tissue, e.g. bone marrow (BM), umbilical cord (UC) or adipose tissue (AT). To overcome limitations in proliferation and the risk of senescence induced pluripotent stem cell (iPS) have become a promising source of MSCs. Recent studies of extracellular vesicles (EVs) derived from MSCs have indicated high potential in clinical applications for many diseases. This brings also iPS-derived MSC (iMSC) EVs into the focus of preclinical research.

To increase the level of process standardization and product safety we developed the CliniMACS Prodigy® Adherent Cell Culture System for GMP compliant cultivation of adherent cells using a closed single used tubing set. Here we show that iMSCs can be generated and expanded using the CliniMACS Prodigy® Adherent Cell Culture System. Stable iMSC expansion was achieved by using the xeno-free MSC-Brew GMP Medium. After iMSCs reached 80-90% confluency cell culture medium was changed using a modified version of the MSC-Brew GMP Medium to produce iMSC-EVs.

Resulting iMSCs displayed MSC specific characteristic as defined by the ISCT consortium. iMSC-EVs from the cell culture supernatant were characterized using the MACSPlex Exosome Kit, optimized for MSC-derived EVs.

We show that iPS derived MSCs (iMSCs) can be generated and expanded in an efficient, standardized, closed and GMP compliant process. Furthermore, iMSC-EVs were isolated within this process and characterized using a multiplex flow-based assay.

Abstract No. P062/T17**The homeobox transcription factor DUXBL controls toti- to pluripotent transition**

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The first transcriptional event in life results in thousands of genes being transiently upregulated at the 2-Cell (2C) stage in mice. Shortly after the 2C-stage, this transcriptional burst must be decommissioned to allow development to progress post the totipotent stage. However, the mechanisms that enable the exit from totipotency remain elusive. We discovered that the 2C-specific double homeobox transcription factor (DUX) drives an essential negative feedback loop by activating its paralog DUXBL. We show that DUXBL-deficient embryos display upregulation of the 2C-specific program and fail to develop further. Conversely, precocious overexpression of DUXBL before the 2C-stage is sufficient to impede the 2C-transcriptional program in vivo. Mechanistically, we show that DUXBL occupies 2C-associated genomic sites that were opened and bound by DUX in mouse embryonic stem cells, repressing transcription from those sites. In DUX-induced nuclear foci, DUXBL interacts with transcription-silencing members from the TRIM superfamily. We identify DUXBL as a key regulator of early transcriptional control, enabling toti-to-pluripotent transition and cell fate divergence. Our study highlights the importance of antagonistic interplays within transcription factor families for development. These findings pave the way to better understand developmental processes and may lead to ways to control them in the future.

Vega-Sendino, M., Lüttmann, F.F., Olbrich, T. et al. The homeobox transcription factor DUXBL controls exit from totipotency. *Nat Genet* 56, 697–709 (2024). <https://doi.org/10.1038/s41588-024-01692-z>

Abstract No. P063**iPSC banking at the DSMZ - establishment of a workflow**

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The German Collection of Microorganisms and Cell Cultures (DSMZ) is home to almost 90.000 bioresources, including bacteria, archaea, fungi, bacteriophages, plant viruses and human and animal cell lines. All bioresources are subject to extensive quality control, characterization, documentation and are supplied to researchers world-wide. Currently, iPSCs are not included in the DSMZ open cell line collection but are used in several research projects. We applied our long-standing experience in cell banking to set up a workflow for banking the research iPSC lines ensuring their quality.

Sources of iPSC lines are collaborators, EBiSC or lines reprogrammed in-house. Upon receipt, authentication via STR genotyping and testing for mycoplasma will be performed first. If both assays are passed, expansion for the master cell bank is initiated. All iPSC lines are passaged three to five times before the final expansion step to three 6-well plates. Cells of two plates are frozen at a density of 1 million cells per ampoule in single-cell solution using serum-free Cryostor CS10 medium. Cells of the third plate are used for 1) preparation of metaphases, 2) seeding for immunostaining of pluripotency markers, 3) collection of DNA for authentication, SNP array and virus testing, 4) seeding for trilineage differentiation and 5) flow cytometry of surface pluripotency markers, and 6) supernatant was collected for mycoplasma testing. When all tests are passed, the master cell bank is released, and one vial of the master cell bank is thawed and expanded for the working cell bank. At freezing of the working cell bank, a reduced quality control regimen is performed, only including STR authentication, mycoplasma testing and flow cytometry of surface pluripotency markers. With tests passed, the vials of the working cell bank are released for use in experiments. The question of a central iPSC repository in Germany can be discussed.

Abstract No. P064**Stem cell associated techniques to rescue the northern white rhino from extinction**

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With only two non-reproductive females remaining, the northern white rhino (NWR) is functionally extinct. To save the charismatic animals from extinction, the BioRescue consortium combines advanced assisted reproductive technologies and stem cell associated techniques.

Natural oocytes can be collected from Fatu, the younger NWR individual, and subsequently in vitro fertilized by intracytoplasmic sperm injection (ICSI) with cryopreserved sperm of already deceased NWR bulls. Thereby, we generated already more than 30 NWR embryos, which are awaiting transfer into surrogate mothers of the closely related, less threatened southern white rhino (SWR). Recently, we demonstrated the first successful embryo transfer in rhinos, which paved the way for NWR calves to be born from SWR mothers in the near future.

However, one genetic mother is not sufficient to found a healthy, self-sustaining NWR population. To increase the genetic variation, we aim to differentiate gametes from induced pluripotent stem cells (iPSCs). Towards this aim, we developed a robust reprogramming protocol (1), which enabled integration-free generation of iPSCs from five NWR individuals and one S/NWR hybrid. The protocol was also suitable to reprogram fibroblasts from the critically endangered Sumatran rhino (2). By developing a primordial germ cell-like cell (PGCLC) induction protocol for SWR-ESCs and NWR-iPSCs (1), we successfully made the first step towards generating NWR oocytes and sperm. To produce NWR PGCLCs from wild type iPSCs of multiple NWR individuals in high quality and quantity, we currently focus on optimizing and automating the PGCLC induction protocol.

(1) Hayashi et al. 2022: Robust induction of primordial germ cells of white rhinoceros on the brink of extinction. *SCI ADV* 8, eabp9683.

(2) Zywitzka et al. 2022: Induced pluripotent stem cells and cerebral organoids from the critically endangered Sumatran rhinoceros. *iScience*. 25:105414.

Poster session II: P065 – P126

(the TXX number indicates an accompanying oral presentation)

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P077 Gastric cancer immuno-peptidome
Gabriela Vallejo Flores

Abstract No. P065**Dental pulp stem cells ameliorate D-galactose-induced cardiac ageing in rats**

Gehan El-Akabawy^{1,*}, Sherif Othman Fathy El-Kersh², Ahmed Othman Fathy Othman El-Kersh³, Shaimaa Nasr Amin⁴, Laila Ahmed Rashed⁵, Noha Samir Abdel Latif⁶, Ahmed Abdelmagied Abdelgawad Elshamey⁷, Mohamed Abdallah Abd El Megied Abdallah⁸, Ibrahim G. Saleh⁹, Zaw Myo Hein¹, Ibrahim El-Serafi¹, and Nabil Eid¹⁰

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Background. Ageing is a key risk factor for cardiovascular disease and is linked to several alterations in cardiac structure and function, including left ventricular hypertrophy and increased cardiomyocyte volume, as well as a decline in the number of cardiomyocytes and ventricular dysfunction, emphasizing the pathological impacts of cardiomyocyte ageing. Dental pulp stem. cells (DPSCs) are promising as a cellular therapeutic source due to their minimally invasive surgical approach and remarkable proliferative ability. This study is the first to investigate the outcomes of the systemic transplantation of DPSCs in a D-galactose (D-gal)-induced rat model of cardiac ageing.

Study design. Thirty 9-week-old Sprague-Dawley male rats were randomly assigned into three groups: control, ageing (D-gal), and transplanted groups (D-gal + DPSCs). D-gal (300 mg/kg/day) was administered intraperitoneally daily for 8 weeks. The rats in the transplantation group were intravenously injected with DPSCs at a dose of 1×10^6 once every 2 weeks.

Results. The transplanted cells migrated to the heart, differentiated into cardiomyocytes, improved cardiac function, upregulated Sirt1 expression, exerted antioxidative effects, modulated connexin-43 expression, attenuated cardiac histopathological alterations, and had anti-senescent and anti-apoptotic effects.

Conclusion. Our results reveal the beneficial effects of DPSC transplantation in a cardiac ageing rat model, suggesting their potential as a viable cell therapy for ageing hearts.

Abstract No. P066**Tracing brain infiltrating acute lymphoblastic leukemia stem cells using single-cell barcoding**

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Central nervous system (CNS) involvement is a major concern in B-cell precursor ALL (BCP-ALL). Approximately 10% of patients are diagnosed with lymphoblasts present in their cerebrospinal fluid with a shorter overall survival compared to those without CNS involvement. However, the exact rate of CNS disease development in adult BCP-ALL remains unclear and represents a major therapeutic challenge. Despite remission rates exceeding 90% after primary therapy, the prognosis after relapse, particularly in the brain, is poor. As organ/tissue (or brain)-residing therapy-resistant leukemia-initiating cells (LICs) are suspected to be the source of relapse, the identification of molecular resistance mechanisms of LICs is of high medical need. However, the identity, functional role, and presence of LICs in the brain in BCP-ALL remain controversial.

To investigate the clonal organ distribution and brain infiltration potential in BCP-ALL, we developed lentiviral barcoding technologies to track LICs and their fate at clonal and single-cell resolution in vivo.

By transplanting barcoded patient-derived BCP-ALL cells (PDLTCs) from several donors into NSG mice we showed that most cells homed into bone marrow, spleen, and the leptomeninges, thereby modeling the neurological involvement of the disease including hind limb impairment. Brain infiltration correlated with disease aggressiveness but the clonal composition between organs varied substantially, implying a selective pressure exerted by the local microenvironment.

Additionally, we performed scCITE-Seq of freshly barcoded leukemic cells retrieved from different organs, including the brain, to decipher the clonal relationship of single cells together with their molecular phenotype. These data revealed a strong separation of leukemic cells collected from the brain compared to all other organs, and the upregulation of markers involved in cell migration and metastasis, which will be used for further prospective isolation. Our findings provide valuable insights into the understanding of brain infiltration potential in B-ALL and new perspectives for redefined and risk-stratified treatment strategies.

Abstract No. P067/T08**Relevance of dormant cancer stem cells in disease propagation and therapy resistance in JAK2V617F-driven myeloproliferative neoplasms**

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*Presenting author

Long-term dormancy is proposed to mediate therapy resistance of cancer stem cells. However, the biological relevance of dormancy in hematologic malignancies has not been formally addressed. Using a novel mouse model that allows identification and isolation of dormant label retaining cells (LRCs) in the setting of JAK2V617F-driven Polycythemia Vera (PV), we could identify LRCs that maintained dormancy for at least 20 weeks in vivo in PV mice, despite harboring the pro-proliferative JAK2 mutation. Isolation and transplantation of mutant LRCs and nonLRCs unambiguously demonstrated that only LRCs were capable of propagating the PV phenotype. Treatment of primary PV mice with a JAK2 inhibitor (Fedratinib, FED) failed to target mutant LRCs, while pegylated interferon- α (IFN α) reduced their number, in line with the clinical observation that IFN α can facilitate molecular remission while FED cannot. After therapy, purified residual LRCs were still uniquely able to propagate PV, demonstrating their role as disease-reinitiating cells and that their level of depletion is a biomarker of PV eradication. Side effects of IFN α treatment limit its clinical effectiveness in PV. To circumvent this, we explored using low dose IFN α (IFN α LD) alone or in concert with FED. IFN α LD alone had no impact on mutant LRC numbers, while IFN α LD plus FED resulted in a synergistic depletion of mutant LRCs and correction of PV phenotype, suggesting that IFN α LD renders LRCs dependent upon JAK2 signalling. In summary, we provide definitive evidence of the importance of dormancy in propagation and therapy resistance of PV; create a model for pre-clinical evaluation of novel therapies for PV; and demonstrate that a combination of IFN α LD and FED may be effective at eliminating mutant LRCs while limiting side effects of standard IFN α treatment.

Abstract No. P068**Progressive niche remodeling shapes the dynamics of leukemic expansion***Artur C. Fassoni¹, and Ingmar Glauche^{2,*}*¹Universidade Federal de Itajubá, Brasil²TU Dresden

*Presenting author

The progression of Acute Myeloid Leukemia (AML) involves a history of clonal evolution culminating in the accumulation of mutations and clonal heterogeneity at diagnosis. Whole genome single cell sequencing revealed that the preleukemic phase often originates from mutations very early in life. The question remains how this intrinsically slow clonal expansion suddenly transitions into the excessive overproduction of leukemic cells. Although it is well recognized that clonal hematopoiesis is intervened with a remodeling of the bone marrow microenvironment and the deterioration of immunological control functions, it remains elusive to which extend this remodeling can actually shape the dynamics of leukemic expansion.

We aim to investigate how clonal hematopoiesis can slowly alter a supportive microenvironment and how this progressive loss of function can lead to a rapid overproduction of leukemic cells. We developed an ODE-based mathematical model of leukemia-niche interactions, where cellular functions of leukemic cells, such as niche binding affinity or proliferation capacity, are regulated by an immunologically competent microenvironment.

Our results indicate that a small growth advantage of the leukemic clone triggers a slow clonal expansion and deteriorates the control ability of the bone marrow. Under certain conditions, this progressively deteriorated immune control eventually fails to suppress the leukemic clone, triggering accelerated clonal growth associated with acute leukemia. The findings suggest that inducing sustained remission necessitates reducing leukemic cells and re-modeling the bone marrow microenvironment to reestablish immunological control.

We conclude that the transition from preleukemic, clonal hematopoiesis to AML is driven by both intrinsic genetic factors and extrinsic environmental influences. Mathematical models highlight the importance of the bone marrow microenvironment in controlling preleukemic clones and suggest that its deterioration is a key factor in the sudden progression to AML. Effective treatment strategies should consider both clonal dynamics and the bone marrow niche state to achieve sustained remission.

Abstract No. P069**Replenishment of the bone marrow macrophage pool drives HSC aging***Nina Henning^{1,*}, Gülce Percin¹, Stephan Culemann¹, and Claudia Waskow^{1, 2}*¹Leibniz Institute on Aging, Immunology of Aging²Friedrich-Schiller University Jena, Faculty of Biological Sciences

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Hematopoietic stem cells (HSCs) are the key reservoir for lifelong blood and immune cell formation, but stem cells from aged mice display functional alterations compared to younger age. Stem cell function is regulated through interactions with their surrounding niche, and important components of the niche space include non-hematopoietic and hematopoietic cells.

Tissue-resident macrophages are heterogeneous with respect to their developmental origin and can be of embryonic or adult origin. Using a lineage-tracing mouse model we could reveal that also the bone marrow harbors embryonic macrophages and their relative contribution to the bone marrow macrophage pool inversely correlates with frequencies of megakaryocyte-biased CD41⁺ HSCs during aging. Age-associated hematopoietic stem cell expansion is exclusively based on increasing numbers of megakaryocyte-biased CD41⁺ stem cells in old mice, a finding consistent with increased megakaryocyte progenitors and platelet production in old animals. Co-culturing HSCs ex vivo with adult or embryonic macrophages proved embryonic macrophages to be essential for maintaining the young HSC phenotype. Experimental depletion of all macrophages in the mouse leads to the replenishment of the pool by adult-derived macrophages. This macrophage turnover increases CD41⁺ HSCs and elevates megakaryopoiesis, suggesting accelerated aging in the stem cell compartment through changes in the composition of the macrophage pool. Moreover, transplantation approaches only reveal reduced engraftment of old HSCs when they are transplanted into myeloablated recipients, while an undisturbed bone marrow environment evenly supports young and old HSC engraftment. We hypothesize that macrophages are important niche cells in the bone marrow and that heterogeneity within the macrophage pool regulates aging-associated changes within the hematopoietic stem cell compartment either directly or indirectly via alternative niche-communication.

Abstract No. P070**Comparative analysis of PURG isoforms within human neural stem cells***Estera Jeruzalska*

Institute of Pharmaceutical Biotechnology at Ulm University

In vertebrates, the highly conserved PUR family of sequence-specific nucleic acid-binding proteins consists of the paralogs PURA, PURB, and PURG, which are present at a high level in brain tissues. PURA plays a role as an RNA-dependent transcription factor and participates in RNA transport and translational control, and PURB plays a role as a transcriptional regulator. On the other hand, little is known about the last PUR paralog, PURG, which, due to the high sequence similarity, especially within functional domains, is predicted to function as a DNA/RNA binding protein.

PURG, contrary to other PUR paralogs, consists of two nearly identical isoforms, which differ on the C-terminal end, where the changes influence the fold of the domain responsible for protein dimerization. On the other hand, the nucleic acid-binding domain remains the same in both isoforms. Preliminary experiments in our lab showed that PURG isoforms tend to localize differentially in cells, suggesting that these little changes in protein structure have an impact on protein functions. Therefore, to investigate the differences in these isoforms' functions, we have performed in parallel the Cut&Run experiment for DNA-binding motif analysis and iCLIP for RNA-binding motif screen.

Abstract No. P071**Nifuroxazide induces DNA damage, G1 arrest and cell death via perturbing centrosome integrity in patient-derived glioblastoma stem cells**

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Glioblastoma is the most malignant and aggressive form of primary brain tumor in adults that constitutes self-renewing and highly tumorigenic glioblastoma stem cells (GSCs). Glioblastoma inevitably recur as therapy-resilient tumors due to their heterogeneous nature. Higher heterogeneity of GBM contributes to tumor progression and recurrence. Thus, identifying GSC vulnerabilities is critical, which may provide previously unexplored therapeutic targets for treating glioblastoma. Centrosomes are crucial for accurate cell division and viability, whose numerical amplification is a hallmark of several cancer types, offering an attractive intervening point. Here, we reveal that patient-derived GSCs, do not display aberrant centrosome numbers. However, when we genetically abolished CPAP-SIL interaction, bonafide centrosome duplication factors upregulated in GSCs, GSCs underwent G1 arrest, eventually leading to cell death. Intervening CPAP-SIL interaction significantly induced centrosome fragmentation and centrosome loss in GSCs. Furthermore, applying biochemical compound screening, we identified Nifuroxazide as a potent inhibitor of CPAP-SIL interaction which induced DNA damage that further triggered G1 arrest and cell death in GSCs. GSCs have efficient DNA repair machinery and DNA repair proteins are also found to be localized to Centrosomes. Hence, perturbing Centrosomes by Nifuroxazide can prevent the DNA repair capacity of GSCs. Nifuroxazide also significantly perturbed 3D GSC spheroids growth and GSC invasion in human brain organoids and mouse xenografts. These findings underscore the importance of targeting centrosomes in GSCs, although they do not exhibit amplified centrosomes.

Abstract No. P072**Aging-induced alteration of proteome dynamics in mouse intestinal tissue upon regeneration**

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Aging reduces the regenerative capacity of the intestinal epithelium in different species including humans. The causes of delayed regeneration in the elderly remain unclear. Here, we used proteomic and metabolomic profiling of intestinal tissues together with functional assays to characterize the dynamics of regeneration following injury induced by 5-fluorouracil, a commonly used chemotherapeutic agent. Comparison of regeneration dynamics in mice of different ages revealed emergence of a proteostasis stress signature and increased levels of polyamines following injury exclusively in old epithelia. Mechanistically, we show that delayed regeneration is a cell-intrinsic feature of old epithelial cells that display reduced protein synthesis and accumulation of ubiquitylated proteins. We demonstrate that an intervention based on dietary restriction followed by re-feeding prior to injury elevates polyamine levels, alleviates proteostasis stress and restores the regenerative capacity of the old intestines. Our work provides novel targets and strategies to improve intestinal regeneration in old organisms.

Abstract No. P073**Targeting CDC42 restores osteoprogenitor cell function after hematopoietic stem cell transplantation**

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Osteopenia and osteoporosis are common long-term complications of the cytotoxic conditioning regimen for hematopoietic stem cell transplantation (HSCT). We examined mesenchymal stem and progenitor cells (MSPCs) that include skeletal progenitors from mice undergoing HSCT. Such MSPCs showed reduced CFU-F frequency, increased DNA damage and enhanced occurrence of cellular senescence, while there was a reduced bone volume in animals that underwent HSCT. The reduced MSPC function correlated with elevated activation of the small RhoGTPase Cdc42, disorganized F-actin distribution, mitochondrial abnormalities and impaired mitophagy in MSPCs. Changes and defects like those in mice were also observed in MSPCs from patients undergoing HSCT. A pharmacological treatment that attenuated the elevated activation of CDC42 restored F-actin fiber alignment, mitochondrial function, and mitophagy in MSPCs in vitro. Finally, targeting CDC42 activity in vivo in animals undergoing transplants improved MSPC quality to increase both bone volume and trabecular bone thickness. Our study shows that attenuation of CDC42 activity is sufficient to restore function of MSPCs in a BM transplant setting.

See also: Blood Adv. 2024. <https://doi.org/10.1182/bloodadvances.2024012879>

Abstract No. P074**Single cell landscape of the stromal bone marrow microenvironment in human clonal hematopoiesis**

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Clonal hematopoiesis (CH) is a preleukemic condition mainly observed in aged individuals, in which blood stem cells (HSCs) acquire somatic mutations conferring a clonal advantage. CH increases the risk of Acute Myeloid Leukemia and cardiovascular disease. It remains unclear how CH clones gain a selective advantage, leading to clonal expansion. An age-related inflammatory environment has been linked to expansion of mutant clones in mouse models. However, to date, the role of the stromal bone marrow microenvironment (BM niche) in human CH has hardly been studied.

To identify potential alterations of the BM niche in human CH, we established a protocol to generate high-quality scRNAseq data of non-hematopoietic cells derived from BM and solid bone of donors undergoing total hip arthroplasty. A total of 28,339 cells from 7 individuals with DNMT3A- or TET2-mutated CH and 5 healthy age-matched controls were analyzed. We identified 2 endothelial cell and 9 stromal cell populations in all human donors. All stromal populations showed enriched stress and inflammatory signatures in CH compared to controls. We identified one CXCL12+ stromal population that was significantly expanded in CH individuals and showed high expression of the HSC quiescence regulator Netrin1. Receptor-ligand analysis of BM niche data and TARGET-seq+ data of hematopoietic stem and progenitor cells from the same donors ⁽¹⁾ identified candidate receptor-ligand interactions with differential expression between CH and controls. Differentially expressed ligands included known HSC regulators such as CXCL12 and VEGFB as well as adhesion molecules and inflammatory cytokines, among others. Ongoing functional studies will reveal if these interactions play a role in regulating clonal expansion of mutant HSCs in CH and thus may represent potential therapeutic targets for early intervention.

(1) Jakobsen et al.: Selective advantage of mutant stem cells in human clonal hematopoiesis is associated with attenuated response to inflammation and aging. *Cell Stem Cell*. 2024 Jun 19:S1934-5909(24)00207-8. doi: 10.1016/j.stem.2024.05.010

Abstract No. P075**Effect of the NOS inhibitor L-NAME on senescence development and regenerative capacity in Human adipose stem cells derived from different age groups**

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Background

Human adipose-derived stem cells (hASCs) have a promising therapeutic potential in regenerative medicine due to their immunomodulatory effects and ability to differentiate into multiple cell lineages. However, advanced patient age induces cellular senescence in ASCs, diminishing their regenerative abilities. Previous studies state that oxidative stress and Ca²⁺ signaling determine senescence, and that Ca²⁺-oscillations affect stem cell plasticity. Our previous research demonstrated that NOS inhibition synchronized Ca²⁺-oscillations through gap junctional coupling.

Aim

This study was performed to investigate if the NO-level and Ca²⁺-oscillations can determine differentiation and senescence of hASCs, using L-NAME to evaluate the influence of NO on Ca²⁺-oscillations and oxidative stress. We hypothesized that this mechanism could potentially improve coordinated differentiation and functional integration of stem cells.

Methods

hASCs from young (20-35 years) and old donors (70-90 years) were analyzed for oxidative stress (H₂DCFDA), Ca²⁺-oscillations (Fluo4-AM), senescence markers (p21^{Cip1}, p27^{Kip1}, Lamin B1) and proinflammatory markers (IL-6, TNF- α). All parameters were evaluated under both physiological conditions and following a seven-day treatment with the NOS inhibitor L-NAME. Additionally, adipogenic (Oil Red) and osteogenic (Alizarin Red) differentiation were assessed in both conditions.

Results

Our results demonstrated that L-NAME treatment reversed the age-related increase in oxidative stress and decrease in number and frequency of Ca²⁺-oscillations. Although hASCs responded gradually different, but in a consistent way, individual donor analysis revealed enhanced adipogenic and osteogenic differentiation potential following L-NAME treatment. In addition, the expression of proinflammatory cytokines IL-6 and TNF α was reduced by L-NAME. In contrast, the expression of senescence markers p21, p27 and Lamin B1 remained unaltered.

Conclusion

The functionality and therapeutic potential of hASCs regarding differentiation capacities and cytokine expression can be improved by pre-incubation with L-NAME. However, we postulate that the individual donor history must be considered to classify the therapeutic benefit of ASCs in alignment with personalized medicine.

Sauer H, Sharifpanah F, Hatry M, et al. NOS inhibition synchronizes calcium oscillations in human adipose tissue-derived mesenchymal stem cells by increasing gap-junctional coupling. J Cell Physiol. 2011;226(6):1642-1650. doi:10.1002/jcp.22495

Abstract No. P076**Human iPSC-derived blood brain barrier model to study late onset Alzheimer's disease**

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Late-onset Alzheimer's disease (LOAD) is an age-related disorder that affects 24 million people worldwide. However, there is currently no effective treatment. The neuropathological features include extracellular amyloid-beta (A β) and intracellular neurofibrillary tangles. Not only the neurons but the entire neurovascular unit (NVU) is disrupted in LOAD, reflecting the complex pathogenesis of LOAD. Risk factors for LOAD include key genes for the ubiquitin-proteasome system (UPS) and autophagy, which regulate the degradation of misfolded proteins such as A β . We aimed to investigate these mechanisms in more detail using our Alzheimer-specific human in vitro model of the NVU, which consists of brain capillary cells (BCECs) and neighboring brain cells (pericytes, astrocytes, microglia, neurons). For this, Brain cells derived from iPSCs were characterized by transcription, flow cytometry and immunofluorescence analysis. We evaluated the blood-brain barrier (BBB) formed by BCECs by measuring trans-endothelial resistance (TEER), impedance (ECIS) and transport of sodium fluorescein. The functionality of neurons was investigated by electrophysiological analysis (MicroElectrode Arrays). The functionality of astrocytes was demonstrated by a glutamate uptake test and a corresponding ELISA. In terms of protein degradation study, the autophagic activity of healthy and LOAD neurons was analyzed by comparing changes in autophagosomes. For A β transport studies on BCECs, we used A β 1-42 and a corresponding ELISA. Healthy and LOAD BCECs, neurons, astrocytes and microglia expressed cell-specific markers. Functional analyzes confirmed their maturity. In co-cultures, neurons and astrocytes promoted the barrier formed by BCECs while microglia had no effect on the barrier. Neuronal autophagy assay and A β transport by BCECs revealed differences between the analyzed groups. We established a human in vitro model suitable for the analysis of A β clearance at the NVU with regard to proteasomal and autophagic processes.

Abstract No. P077**Gastric cancer immunopeptidome**

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Gastric cancer is a complex and heterogeneous disease representing a global health challenge, with high mortality and limited treatment options. Recently, targeted therapies and immunotherapy have improved the outcome of many cancers. Understanding the cancer immunopeptidome has allowed the identification of tumor antigens as immunotherapy candidates. Therefore, the goal of this project was to identify MHC-presented tumor-associated antigens in primary gastric cancer cells. **Methods:** We expanded three cancer and non-cancer primary epithelial cells derived from gastric cancer biopsies in stemness conditions medium on feeder cells. We subjected 1×10^8 cells from each sample to mass spectrometry analysis of HLA-bound peptides. Whole exome sequencing was performed for each tumor to identify tumor-specific somatic mutations that would give rise to neoepitopes. **Results:** We identified around 10-2500 HLA-bound peptides from in vitro expanded epithelial gastric cancer cells. Besides, we observed an enrichment of peptides from proteins belonging to ER membranes and those associated with DNA replication in the gastric cancer immunopeptidome.

Furthermore, we detected unique immunopeptidome profiles related to gastric cancer subtypes. No tumor neoepitopes were identified among the presented peptides. **Conclusions:** Primary gastric cancer cells are a key source for deciphering immunopeptidome and generating systematic insights into gastric cancer-presented antigens. This research will bring the opportunity of generating vaccines or targeted immunotherapies for better patient treatment.

P078 – P082 Computational stem cell biology & systems biology

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Abstract No. P078**NiCo identifies extrinsic drivers of cell state modulation by niche covariation analysis**

Ankit Agrawal^{*}, Stefan Thomann, Sukanya Basu, and Dominic Grün

Würzburg Institute of Systems Immunology, Computational Biology of Spatial Biomedical Systems

*Presenting author

Cell states are modulated by intrinsic driving forces such as gene expression noise and extrinsic signals from the tissue microenvironment. The distinction between intrinsic and extrinsic cell state determinants is essential for understanding the regulation of cell fate in tissues during development, homeostasis and disease. The rapidly growing availability of single-cell resolution spatial transcriptomics makes it possible to meet this challenge. However, available computational methods to infer topological tissue domains, spatially variable gene expression, or ligand-receptor interactions are limited in capturing cell state changes driven by crosstalk between individual cell types within the same niche. We present NiCo (Niche Covariation), a computational framework for integrating single-cell resolution spatial transcriptomics with matched single-cell RNA-sequencing reference data to infer the influence of the spatial niche on the cell state. By applying NiCo to mouse embryogenesis, adult small intestine and liver data, we demonstrate the capacity to predict novel niche interactions that govern cell state variation underlying tissue development and homeostasis. In particular, NiCo predicts a feedback mechanism between Kupffer cells and neighboring stellate cells limiting stellate cell activation in the normal liver. NiCo provides a powerful tool to elucidate tissue architecture and to identify drivers of cellular states in local niches.

Ankit Agrawal, Stefan Thomann, Sukanya Basu, Dominic Grün NiCo Identifies Extrinsic Drivers of Cell State Modulation by Niche Covariation Analysis (under consideration at Nature Genetics)

Abstract No. P079/T12**A scalable computational framework for annotation-independent combinatorial target identification in scRNA-seq databases***Maximilian Buser*, and Carsten Marr*

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*Presenting author

The applicability of novel targeted cell therapies is constrained by on-target off-tumor toxicities, posing significant challenges for safe and effective treatment. To develop strategies for mitigating these toxicities, a comprehensive understanding of the distribution of single-cell profiles across human tissues is essential. Single-cell RNA sequencing (scRNA-seq) provides detailed insights into cellular diversity and function. However, as data collection expands and RNA expression atlases comprise tens of millions of cells, effectively querying these databases to identify cell populations matching specific gene expression patterns becomes challenging. Key challenges include managing data volume, handling batch effects, navigating study-dependent data curation and annotation, and addressing the combinatorial complexity of possible target patterns. Userfriendly screening tools that allow for complex query target patterns without relying on existing annotations are currently missing. Here we present *otopia*, a computational framework that enables fast queries of large scRNA-seq databases, enhancing the precision of cell population identification. Using precomputed neighborhood graphs and boolean logic for expression patterns, *otopia* enables efficient queries addressing arbitrary combinatorial target patterns. *otopia* does not depend on cell type annotations and by exploiting unbiased expression pattern matching scores, it uncovers critical populations that annotation based methods overlook. We exemplify *otopia*'s capabilities by identifying the largest B cell populations in the CELLxGENE census data using a CD19+CD22+CD5- query target pattern. Moreover, we show that *otopia* can provide a concise overview of cell populations matching hypothetical acute myeloid leukemia (AML) dual targets, realizing logic AND gates, across different tissues. *otopia* enables the estimation of ontarget off-tumor toxicities for drugs targeting both single markers and complex marker combinations, surpassing previous methods that focus mainly on single markers. With the expanding availability of scRNA-seq data, *otopia* represents a scalable, unbiased, and automated approach to refine putative therapeutic targets in order to minimize toxicities.

<https://doi.org/10.1101/2024.06.24.600275>

Abstract No. P080**Identifying niche determinants of cardiomyocyte dedifferentiation by spatio-temporal analysis of myocardial infarction**

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The adult human heart is one of the least regenerative organs, due to the absence of cardiac stem cells and the inability of cardiomyocytes (CMs) to regenerate after injury. This often results in fibrotic scar formation and permanent loss of cardiac function following myocardial infarction. To enhance healing outcomes, understanding the dynamics of wound tissue architecture over time and space is essential. By combining single-cell RNA sequencing (scRNA-seq) with single-cell resolution spatial transcriptomics, we reconstructed the spatio-temporal dynamics of lesion repair in infarcted adult mouse hearts from 1 to 56 days post-injury. This integration revealed heterocellular interactions that drive fibrotic scar formation. Using ligand-receptor predictions from scRNA-seq data and spatially multiplexed in situ sequencing of 475 genes via the 10x Xenium platform, our study demonstrated dynamic regulation of local cell communication niches across different lesion zones post-injury. Pseudotime analysis identified a rare population of partially dedifferentiating CMs emerging as early as day 1 post-lesion. Signals from endothelial and immune cells in the border zone and endocardial region were predicted to support their emergence. Notably, innate lymphoid cell 2 (ILC2) promotes CM dedifferentiation through BMP-7 signaling, rather than hypertrophy. Culturing non-regenerative CMs from P7 mice with BMP-7 together with other niche factors synergistically reactivated progenitor gene expression and cell cycle activity. Similarly, ex vivo culture of human cardiac tissue slices with these ligands led to loss of striated sarcomeric structures and increased cell cycle activity, indicating conserved pathways that enhance CM plasticity between mouse and human. In summary, this spatio-temporal cell type atlas of lesion repair post-cardiac injury provides valuable insights into heterocellular communications that drive CM plasticity and fibrotic scar formation, offering novel perspectives for improving cardiac repair and regenerative medicine.

Herman, J. S., Sagar & Grun, D. FateID infers cell fate bias in multipotent progenitors from single-cell RNA-seq data. Nat Methods 15, 379-386 (2018)

Abstract No. P081/T15**Unveiling the mechanisms of macrophage-mediated cytokine release syndrome in CAR-T cell therapy with mathematical modeling**

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Chimeric Antigen Receptor (CAR) T-cell therapy has emerged as a promising avenue for cancer treatment. However, challenges such as recurrence and cytokine release syndrome (CRS) necessitate a deeper understanding of the underlying dynamics to optimize therapeutic outcomes.

Preclinical studies suggest that CRS results from complex interactions between CAR-T and host cells, with macrophages identified as a primary source of cytokines. Three primary mechanisms of macrophage activation were identified. First, upon antigen-binding-mediated activation, CAR-T cells release inflammatory signals that activate macrophages. Second, following tumor cell targeting by CAR-T cells, damage-associated molecular patterns (DAMPs) released by pyroptotic tumor cells enhance macrophage activation. Third, the interaction of CD40 on activated macrophages with CD40L on CAR-T cells further promotes macrophage activation in a contact-dependent manner.

To better understand the underlying mechanisms and temporal dynamics during CAR-T cell therapy response, we developed a novel multi-layer mathematical model that accurately describes the time courses of 25 patients with diverse responses and IL-6 cytokine kinetics. We successfully linked the dynamic shape of the response to interpretable model parameters and studied the influence of CAR-T cell dose and initial tumor burden on CRS occurrence and treatment outcomes.

By accounting for the three established inflammation mechanisms and reconstructing the timeline of macrophage activation, we found that each activation mechanism governs a different phase of CRS. Damage-associated molecules released by dying tumor cells drive early CRS, antigen-binding-mediated inflammation occurs parallel to CAR-T cell expansion, followed by CD40 contact. Moreover, we identified the CD40-CD40L axis as the main driver of CRS and a clinically feasible target to control the activation process and modulate IL-6 peak height.

Our study underscores the utility of mechanistic modeling in deciphering therapy dynamics and guiding clinical interventions, emphasizing the importance of close integration with clinical data.

Abstract No. P082**Machine-learning based detection of measurable residual disease in acute myeloid leukemia – an update**

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Detecting Measurable Residual Disease (MRD) in Acute Myeloid Leukemia (AML) is crucial for identifying leukemia persistence or recurrence and enabling personalized treatment strategies. Multiparametric Flow Cytometry (MFC) in bone marrow has emerged as a cost-efficient, fast method for MRD monitoring, complementing molecular techniques. However, manual gating and analysis is labor-intensive, subjective, and requires expert knowledge.

Our study explores machine learning (ML) approaches for MFC-based MRD monitoring to speed-up and support clinical decision-making. So far, AML has proven more difficult to access for ML methods than other hematologic diseases due to its large heterogeneity and complex diagnosis. By focusing on spatial features of a reference latent space, we aim to overcome this complexity. We apply Residual Neural Networks to a learned reference latent space mapping of representative aggregated patient data, predicting individual MRD statuses via the analysis of embedded cell populations. This method captures characteristics of healthy individuals versus AML patients as well as subtle differences between MRD positive and negative status. Unlike manual approaches using sequential 2D plots, our method covers higher dimensional relations and patterns in the data.

In this update we present current results of our pipeline and newly incorporated data for training and testing. For model training and validation, we use data from 989 patients to classify “healthy vs diagnosed” patients, and 503 for MRD status classification. This includes 90 leukemia-free controls, some with prior chemotherapy exposition, and 410 post-intensive induction chemotherapy patients. Data labeling was done using a clinically validated, standardized MFC MRD classification and diagnosis (Röhnert et al., Leukemia 2022).

Benchmarking our automated classification against the validated MRD approach, we show that our ML pipeline achieves high accuracies, particularly in distinguishing healthy references from AML patients. More importantly, MRD status prediction accuracy exceeds 80%, highlighting our approach's potential to support patient monitoring and treatment decision-making.

Röhnert MA, Kramer M, Schadt J, et al. Reproducible measurable residual disease detection by multiparametric flow cytometry in acute myeloid leukemia. Leukemia. 2022;36(9):2208-2217. doi:10.1038/s41375-022-01647-5

P083 – P105 Stem cell organoids

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Abstract No. P083/T04**Cell type-specific epigenome profiling identifies H3K27me3 dynamics in the human developing neocortex**

Nora Ditzer¹, Ezgi Senoglu¹, Theresa Schütze¹, Aikaterina Nikolaidi¹, Sevina Dietz¹, Razvan P. Derihaci², Cahit Birdir², Anne Eugster¹, Mike Karl¹, Pauline Wimberger^{2,3}, Franziska Baenke⁴, Claudia Peitzsch¹, and Mareike Albert^{1,*}

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During neocortex development, neural progenitor cells proliferate and undergo neuronal differentiation. This process is driven by temporal and spatial gene expression changes that are in turn guided by dynamic epigenetic mechanisms, including post-translational histone modifications. Intriguingly, mutations in various epigenetic players cause neurodevelopmental disorders. Currently, we lack a comprehensive understanding of the complex epigenetic remodelling that occurs during human neurogenesis. Here, we perform an exhaustive characterization of the epigenetic landscape in the human developing neocortex. We leverage cytometry by time of flight for the simultaneous single cell analysis of more than 30 histone modifications (Epi-CyTOF). We find tri-methylation of lysine 27 of histone 3 (H3K27me3), a repressive epigenetic mark deposited by the Polycomb repressive complex 2 (PRC2), to display global cell type-specific distribution with enrichment in postmitotic neurons, despite ubiquitous distribution of PRC2 subunits. By applying cell type-specific CUT&Tag, we profile histone modifications in neural progenitor cells and neurons isolated from cortical tissue. To functionally investigate the role of H3K27me3 in human cortical development, we apply pharmacological inhibition of PRC2 over five weeks of cortical organoid development, resulting in ablation of H3K27me3. We show that impaired PRC2 activity results in reduced proliferation of different neural progenitor cell types as well as in premature differentiation. Taken together, this study provides comprehensive insights into the complex epigenomic landscape in the human developing neocortex and reveals a role for H3K27me3 in human neural fate specification.

Abstract No. P084**Direct scale-up of iPSC organoid cultivation from petri-dish into 2L-scale achieving >100.000 EB at once with a novel bionic bioreactor system.***Patrick Bongartz*

BioThrust GmbH, Neuss

While more and more allogeneic stem cell therapies emerge from the lab into (pre-)clinical studies and find their way into medical application, large-scale production of sensitive stem cells is still an unsolved challenge, as single cell, on Micro-carriers or as agglomerates. In this talk, two examples of different Organoid cultivations with a novel stirred-tank bioreactor technology are presented: iPSC derived Cardiomyocyte-Organoids (COs) and MSC-derived Lung-Organoids. Both examples serve as showcases for the universal applicability of the novel, bionic bioreactor system. The core part of this system is the unique Membrane Stirrer for gentle mixing, bubble-free aeration and close DO & pH control.

Together with our partner Prof. Kurt Pfannkuche of the UK Köln, we directly scaled-up from petri dish to 2L-scale and cultivated more than 100.000 Embryoid Bodies at once receiving over 90% of functional, differentiated beating COs within only 7 days. We present the hurdles in classical cultivation and the development made in this cooperation.

In the second cultivation, Prof. Diana Klein and her team at the UK Essen seeded their Lung Organoids in our 2L reactor for more than 5 weeks and received highly differentiated organoids with close to *in-vivo* histological and mRNA profiles.

In addition to these examples, the functionality, construction and operation of the bionic bioreactor in different scales is presented and further applications (together with the audience) are discussed. We highlight flow regimes, local shear areas and gas introduction via a dense membrane from the medical technology sector. We show a mode of operation for retrofitting existing bioreactors with the Membrane Stirrer system (as in the two bioprocess examples) and concepts up to 200 L for future industrialization of therapy production.

Abstract No. P085**Primary cilia dynamics in determining neural progenitor cell maintenance in brain development***Enes Cicek* and Jay Gopalakrishna*

Institute for Human Genetics, Jena

*Presenting author

Self-renewal of Neural Progenitor Cells (NPCs) and their differentiation influences neuroepithelium development. Primary cilia is a microtubule based non motile organelle which elongates from basal body and present on the surface of the cells. Cilia contains receptors that are part of developmental signaling pathways on its membrane. Dynamic property of primary cilia by its timely assembly and disassembly during cell cycle is a factor over stem cell proliferation and differentiation. Cilium disassembly in NPCs is required for cell cycle progression to S and G2. Without proper disassembly, lengthened cell cycle progression causes the premature differentiation of NPCs which leads to their depletion. Therefore, even a minor change on the cilia dynamics could result in a significant developmental defect on human brain development. To study the effect of cilia and its dynamics on the developing brain, 3D brain organoids generated from stem cells are being used. These brain organoids mimic the early stages of brain development. Studying dynamic localization of cilia disassembly complex in brain organoids could reveal the components to be used for a controlled manipulation of cilium disassembly during brain development.

Abstract No. P086**Blood-generating heart-forming organoids (BG-HFOs) recapitulate co-development of the human hematopoietic system and the embryonic heart**

Miriana Dardano^{1,*}, Felix Kleemiß¹, Maike Kosanke², Dorina Lang³, Liam Wilson¹, Annika Franke¹, Jana Teske¹, Akshatha Shivaraj³, Jeanne De la Roche³, Martin Fischer³, Lucas Lange³, Axel Schambach³, Lika Drakhlis¹, and Robert Zweigerdt¹

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Deriving in vitro hematopoietic stem and progenitor cells (HSCs/ HPCs) is fundamental for therapeutic purposes yet challenging since their niche-like induction and stabilization in a proper developmental context is poorly understood. This is due to the limited knowledge of signals controlling these processes and the ethical concerns, since human embryogenesis is not easily investigable in vivo. Thus, a human in vitro approach properly resembling cellular and molecular aspects of hematopoietic development with neighboring tissues is of interest.

Our recently established human pluripotent stem cell (hPSC)-derived heart-forming organoids (HFOs) represent a complex, well-patterned multi-tissue model recapitulating key aspects of the interconnected heart, vasculature and foregut development. Modulating HFO differentiation by the stage-specific supplementation of hemato-endothelial factors, we here report the generation of blood-generating-HFOs (BG-HFOs). While maintaining their heart anlagen, the organoids comprise specific, morphologically distinct endothelial subtypes including a mesenchyme-embedded endothelial layer giving rise to hematopoietic cells putatively via endothelial-to-hematopoietic transition (EHT). The in vitro model thus reflects central aspects of the intra-embryonic region that gives rise to hematopoiesis in vivo. Single-cell RNA sequencing of BG-HFOs revealed gene expression signatures resembling HPCs as well as hematopoietic derivatives including erythroid, megakaryocytic and myeloid cells. Functional assays revealed that BG-HFO-derived hemato-endothelial cells display erythroid, myeloid and lymphoid potential. Together, the data suggest that our model simultaneously presents aspects of both primitive and definitive hematopoiesis. The study reveals the first human model of self-organized, morphologically structured co-development of cardiac, endothelial and multipotent-hematopoietic tissues, providing a valid tool for pharmacological assessments, promoting research on hematopoiesis development in vitro, and overcoming limitations of such studies in the human embryo.

Dardano et al., Nature Cell Biology (accepted, 2024).

Drakhlis et al., Nature Biotechnology 39, 737–746 (2021).

Abstract No. P087**Assay development for functional analysis of iPSC-derived neural organoids**

Ben Streeter, Monica De Maria, Denise Sullivan, Parker Ellingson, Stacie Chvatal, and Daniel Millard*

Axion Biosystems

*Presenting author

The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Advanced cell preparations, such as spheroids or organoids, are under intense investigation with aims toward establishing mature physiologically relevant cell models in vitro. The objective of this work is to develop and optimize a functional high throughput assay of neural organoids in vitro. A customized multiwell microplate was developed to ensure optimal placement of organoids on a multiwell microelectrode array (MEA) by utilizing a unique funnel design. Impedance measurements were used to quantify the attachment and electrode coverage of the neural organoids to the surface of the plate, while functional activity was quantified via electrophysiological measurements. Acute (no surface coating) and chronic (surface coating-mediated attachment) recording protocols were evaluated and compared. Specifically, the use of surface coatings (no coating, PEI, Matrigel) and centrifugation were evaluated for chronic recording protocols with regular measurements of attachment and network function over 4 weeks of culture. PEIcoated wells exhibited the best performance, with neural organoid attachment in 100% of wells (38.7 ± 4.2 Ohms for weighted mean resistance), 5.4 ± 2.7 active electrodes (exhibiting neural spiking) per well and the development of synchronous network activity within 2 weeks of culture. Matrigel and no coating conditions displayed fewer active electrodes overall, but consistent results across wells due to the funnel design. Together, these results support the continued development of assays that investigate in vitro 3D neural models.

Abstract No. P088**Label-free functional analysis for the characterization of iPSC-derived neural organoid development and maturation**

Denise Sullivan, Monica De Maria, Svenja Meiler, Ben Streeter, Stacie Chvatal, and Daniel Millard*

Axion Biosystems

*Presenting author

Rapid advances in stem cell technology have led to widespread adoption for the development of in vitro neuron electrophysiology models for drug discovery and safety screening. Advanced cell preparations, such as spheroids or organoids, are intensively studied with aims to establish mature human phenotypes in vitro. The objective of this work is to develop and validate a live-cell analysis workflow for the characterization of neural organoids. First, whole-vessel live-cell imaging with the Omni was used to monitor iPSC colony formation and growth in real-time. The iPSC module was used to measure iPSC colony size and coverage to ensure consistent iPSC passaging, while iPSC stemness was confirmed with fluorescence staining of well-known pluripotency markers, TRA-1-60 and SSEA4-1. Next, iPSC derived embryoid bodies (EBs) were differentiated towards neural organoids. Real-time live-cell imaging was used to track the size and shape of embryoid bodies during the neural differentiation process. Lastly, neural organoids were transferred at day 50+ and allowed to attach to a multiwell microelectrode array (MEA) plate. Impedance measurements were used to quantify the attachment of the organoids over the array, as a measure of cell viability and electrode coverage. The emergence and maturation of neural organoid electrophysiological activity was tracked via measurements of spiking activity and low frequency oscillations. Broadband (1 – 5000 Hz) electrophysiological data was acquired and then separately processed for action potential detection (200 – 5000 Hz) and low frequency oscillations (1 – 50 Hz). This new iPSC to neural organoid workflow, that couples live-cell imaging and MEA measurements, supports the continued development of in vitro 3D models of neural function.

Abstract No. P089**Novel workflow for matrix-free generation and fast-track, high-throughput characterization of human brain organoids by light sheet microscopy**

Jens-Peter Derks, Johanna Werheid, Manuela Adrian, Kevin Bigott, Daniel Barleben, Patrick McCormick, Martin Ruoff, Marc Teckentrup, Sara Kheireddine, Matthias Grunwald, Simon Merz, Kathrin Olschok, Lea Bornemann, Philip Dannhauser, Lena Nolte, and Dominik Eckardt*

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*Presenting author

Due to its complexity, the processes of human brain development and its function are incompletely understood. Development of new in vitro models - in particular disease models - as well as implementation of state-of-the-art, high-throughput analytical workflows are urgently required to enable a better understanding of neurological diseases and to advance therapeutic developments.

To overcome these limitations, we developed a novel and standardized workflow including matrix-free generation of human pluripotent stem cell (hPSC)-derived brain organoids followed by detailed characterization of the cellular composition using 3D light-sheet microscopy.

First, we established a xeno- and Matrigel-free differentiation protocol for highly reproducible generation of hPSC-derived cerebral organoids, successfully recapitulating early brain development in 3D suspension culture. Next, we optimized the sample preparation procedure of organoids for light sheet microscopy by developing an efficient and fast, non-toxic clearing protocol, allowing as well for preservation of endogenous fluorescence markers, like GFP. Multiplex labeling of organoids with recombinant antibody conjugates was optimized for light sheet microscopy.

In order to facilitate high-throughput imaging characterization of up to 48 organoids in a single imaging run we developed a dedicated multi-sample holder, the MACS® UltraMount 48. This imaging holder for the UltraMicroscope Blaze™ is also suited for parallelized sample preparation, thereby minimizing sample handling times.

Our data indicate development of a straightforward differentiation protocol allowing for standardized generation of hPSC-derived cerebral organoids with high efficiency.

Specific whole mount staining using REAfinity antibody conjugates was optimized for brain organoids at different developmental stages and combined with a non-toxic tissue clearing procedure. Parallelized organoid sample processing followed by light sheet imaging revealed the formation of multiple ventricular zones and cortical plate-like regions, indicative of early cortical development.

Next, this workflow will be used for characterization of patient-derived organoid models and in vitro evaluation of cellular therapeutics.

Abstract No. P090**Functional periportal liver assembloids recapitulate the mesoscale hepatic architecture and model biliary fibrosis in vitro**

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Tissue-derived organoid models are reductionist and easy to manipulate cellular systems, which provide basic biological understanding of the specific organ they derive from. For the liver, both cholangiocyte and hepatocyte organoids have been described, however, they lack any of the supporting stromal cells present in the tissue. Here, we present a next generation organoid model composed of adult murine hepatocytes, cholangiocytes and primary liver mesenchymal cells. First, we modified the previously published hepatocyte organoid models, to allow the formation of a bile canaliculi network that recapitulates in vivo murine bile canaliculi size and architecture. Then, by combining the three cell types, we obtained multicellular organoid structures, where bile duct cells surrounded by mesenchyme are embedded within the hepatocyte parenchyma, thus recapitulating in vitro the in vivo architecture of the periportal region of the liver lobule. The assembloid organoid structures present functional bile duct-bile canaliculi connection, where bile canaliculi formed by hepatocytes drain bile acid into the lumen of bile ducts. Finally, by manipulating the number of portal mesenchymal cells we describe that non-physiological mesenchymal cell numbers result in induction of pro-fibrotic state, independent of an immune compartment, suggesting that tissue fibrosis is actively driven by aberrant mesenchyme-hepatocyte interactions. The pro-fibrotic organoids mimic collagen deposition, hepatocyte apoptosis and gene expression of cholestatic liver fibrosis mouse models. Our results open up opportunities to better understand the role of the periportal cell populations in cholestasis and liver fibrosis initiation. This proof-of-concept study opens an avenue for more complex liver tissue-derived organoid models, which better recapitulate liver complexity, as well as physiological and pathological liver features in vitro.

Abstract No. P091**Gastruloids as a model for pregastrulation cell competition in mammals**

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Cell competition (CC) is a comparison of fitness among neighboring cells, in which suboptimal but otherwise viable cells undergo apoptosis when faced with ‘fitter’ cells. Due to its central role in fitness perception, loss of p53 results in the emergence of “supercompetitor” cells, which stand at the apex of cell competition and induce apoptosis in neighboring wild-type (WT) cells. Developmental cell competition has been described in the **early mammalian embryo**, where it can result in the death of up to 35% of cells within the epiblast, likely as a **quality control checkpoint** to eliminate suboptimal cells prior to gastrulation. Currently, our understanding of mammalian cell competition remains limited due to the inaccessibility of the embryo and insufficiency of 2D cell cultures.

Here, I use **gastruloids**, an embryonic stem cell (ESC)-based model of early mammalian development, to study CC in a real time 3D context with high-throughput capacity. Mosaic gastruloids consisting of fluorescently distinct WT cell populations develop normally and contribute equally to gastruloid development. However, mosaics of WT and p53 knock-out (p53KO) cells undergo strong competition, with even small p53KO populations being able to outcompete WT cells. In gastruloids, CC takes place in between 48-96h after cell aggregation, which developmentally corresponds to E5.5-E7.5 in the mouse embryo and mirrors the developmental timing of CC in vivo. CC is **independent of gastruloid size and nutrient availability and is not explained by differences in proliferation rates**. Inhibiting apoptosis by overexpressing Bcl2 between 48-96h after aggregation entirely abolishes CC, confirming that it is executed through apoptosis. Neither pluripotent mosaic 3D aggregates, nor 3D Epi-gastruloids, which model more advanced developmental processes, demonstrate any competition, supporting the hypothesis that **developmental CC is specific to pre-gastrulation stages**. Competition gastruloids are thus a useful system to study the mechanisms that mediate CC in mammalian embryos.

Abstract No. P092**Reliability of high-quantity human brain organoids for modeling microcephaly, glioma invasion, and drug screening***Anand Ramani, and Jay Gopalakrishnan**

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Brain organoids offer unprecedented insights into brain development and disease modeling and hold promise for drug screening. Significant hindrances, however, are morphological and cellular heterogeneity, inter-organoid size differences, cellular stress, and poor reproducibility. Here, we describe a method that reproducibly generates thousands of organoids across multiple iPSC lines. These High Quantity brain organoids (Hi-Q brain organoids) exhibit reproducible cytoarchitecture, cell diversity, and functionality, are free from ectopically active cellular stress pathways, and allow cryopreservation and re-culturing. Patient-derived Hi-Q brain organoids recapitulate distinct forms of developmental defects: primary microcephaly due to a mutation in CDK5RAP2 and progeria-associated defects of Cockayne syndrome. When modeling glioma invasion, Hi-Q brain organoids displayed a similar invasion pattern for a given patient-derived glioma cell line. This enabled a medium-throughput drug screen to identify Selumetinib and Fulvestrant, which also perturbed glioma invasion in vivo. Thus, the Hi-Q approach can easily be adapted to reliably harness brain organoids' application for personalized neurogenetic disease modeling and drug discovery.

Abstract No. P093**ARHGAP11A is a key player in maintaining the apical progenitor pool in human cortical organoids**

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During corticogenesis, apical progenitor cells (APs) are found in the ventricular zone (VZ). As brain development progresses, APs delaminate from the ventricular surface to give rise to basal progenitors that reside in the more basal subventricular zone, where they give rise to cortical neurons and glia. This process of delamination can involve symmetric or asymmetric cell division, influenced by the orientation of the AP division plane. However, the precise mechanisms regulating delamination remain poorly understood. Here, we show that ARHGAP11A, a conserved Rho GTPase-activating protein that is highly expressed in APs, plays a key role in delamination. Here, we generated human induced pluripotent stem cell (iPSC) ARHGAP11A-knockout (KO) lines by applying CRISPR/Cas9-based gene editing. After validation, we applied a standardised forebrain-type organoid protocol. Analysing early KO organoids and isogenic controls, we observed enlarged and flattened ventricles, together with altered expression patterns of cytoskeletal and extracellular matrix (ECM)-related genes in the KO organoids. Furthermore, ARHGAP11A-KO APs delaminated pre-maturely from the VZ and settled basally, as observed in hybrid organoids composed of EGFP-labelled KO and isogenic control cells. This was associated with a mis-orientated cleavage plane of dividing APs in the KO. Examining more mature organoids, we observed a significantly reduced abundance of S100 β -positive cells, suggesting a reduced production of late-born astroglia. Hence, our data suggest that ARHGAP11A plays a key role in maintaining the AP pool, by preventing pre-mature delamination of APs, through regulating cleavage plane orientation and ECM composition in the VZ.

Y.H. and N.E. contributed equally

A.J., M.H., and J.L. are corresponding authors

Abstract No. P094/T05**Assessing the impact of prenatal paracetamol exposure on early human forebrain development using cerebral organoids**

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Recent epidemiological studies suggest that prenatal Paracetamol (N-Acetyl-p-aminophenol; APAP) exposure can impair fetal neurodevelopment in a time- and dose-dependent manner, leading to autism and attention-deficit-hyperactivity disorder. The mechanisms underlying this phenomenon remain largely unexplored. It also remains unclear which developmental stages are particularly vulnerable and whether Paracetamol itself or its metabolites elicit these effects.

Here, a novel 3D cell culture workflow, involving human iPSC-derived forebrain organoids was employed to assess the impact of APAP exposure on human brain development. Immediately following neural induction, when neural precursors start proliferation and differentiation, we initiated an eight-week treatment, applying the mean maximal plasma concentration reported in females upon oral administration. As APAP readily crosses the placental and blood-brain-barrier, our setup is suited to simulate prenatal exposure in vivo and to evaluate its effects on forebrain development.

For phenotypic assessment of whole forebrain organoids, we used light-sheet fluorescence expansion microscopy (LSFEM), which enables intact imaging from meso- to nanoscale resolution within a single imaging session. LSFEM fully exploits the potential of brain organoids by portraying intricate cell-cell interactions and the impact of surrounding tissue architecture on individual cells in a 3D context. We found that APAP exposure reduces the organoids' cross-sectional area by 20% and disrupts their overall structure. Via LSFEM, we detected a 33% reduction in organoid volume and a 10% increase in neural rosette density, with individual rosettes exhibiting a 33% reduced volume. Ongoing analysis, including 3D assessment of cell proliferation and survival as well as mRNA sequencing and miRNA profiling, are expected to elucidate the mechanisms underlying these effects.

Our observations point to a profound impact of APAP on early human neural development. Further, our experimental set-up provides a robust and adaptable platform for studying prenatal neurotoxic effects of pharmaceutical compounds on human brain development in a controlled in vitro scenario.

Abstract No. P095**Leigh syndrome patient-derived cortical brain organoids as a model system for the study of pathomechanisms and gene therapy approaches**

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Leigh syndrome (LS) is a neurodevelopmental metabolic disorder caused by mutations in genes of the nuclear or mitochondrial DNA that encode for proteins in the mitochondrial respiratory chain. LS manifests in multiple neurological impairments including an early death in most of the affected children. However, the pathomechanism of LS is poorly understood due to a lack of effective model systems. Patient-derived induced pluripotent stem cells (iPSCs) were used to generate cortical brain organoids (cBO)¹ as a promising model system to shed light on LS pathology and eventually assess treatment approaches.

cBO were differentiated from patient iPSC lines carrying mutations in the mitochondrial gene MT-ATP6. Developmental characteristics regarding gene expression and cytoarchitecture were investigated. By qPCR and RNA sequencing, pathways affected by the LS mutation were observed. For further maturation, cBO were sliced and cultivated as cortical brain organoid slices (cBOS)² at the air-liquid interface to detect calcium signals. Finally, cBO and cBOS were transduced with mCherry-linked adeno-associated virus 9 (AAV9) expressed under the synapsin 1 promotor.

cBO showed developmental features similar to the human brain, as neurogenic zones, expression of neural progenitor and neuronal markers, and occurrence of markers typical for astrocytes, oligodendrocytes and synapses in later stages. Compared to control cBO, patient-derived cBO showed decreased expression of PRKG1, a downstream regulator of cGMP-mediated pathway, and altered pathways related to synapses. After chemically induced metabolic stress, patient cBOS manifested an increased calcium response. cBOS and cBO exhibited AAV9-induced transduction after reaching enough maturity. The success of transduction was dependent on viral concentration, tissue accessibility and incubation time, shown by fluorescence imaging and FACS analysis.

The results indicate some differences between control and patient cBO, and functional assays are necessary to further elucidate patient-specific disease phenotypes. The successful AAV9 transduction enables testing AAV-mediated gene therapies in cBO and cBOS.

[1] Le S, Petersilie L, Inak G, Menacho-Pando C, Kafitz KW, Rybak-Wolf A, Rajewsky N, Rose CR, Prigione A. Generation of Human Brain Organoids for Mitochondrial Disease Modeling. *J Vis Exp.* 2021 Jun 21;(172)

[2] Petersilie L, Heiduschka S, Nelson JSE, Neu LA, Le S, Anand R, Kafitz KW, Prigione A, Rose CR. Cortical brain organoid slices (cBOS) for the study of human neural cells in minimal networks. *iScience.* 2024 Mar 4;27(4):109415

Abstract No. P096**An optimized and versatile small molecule-based cell supplement for stress reduction in primary and PSC-derived neural cells**

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Existing cell recovery supplements have shown improved survival in Pluripotent Stem Cells (PSCs); however, there are limited solutions for improving survival in neural cell applications. In this study, we aimed to address this issue by evaluating the utility of CultureCEPTTM Supplement, a new small molecule cell survival supplement based on published data (1), in improving survival and recovery of neural cells. To demonstrate the versatility of CultureCEPTTM Supplement, testing included a range of neural cell types (primary neurons, PSC-derived neurons, and glial cells) and applications (recovery from cryopreservation, dissociation, and differentiation).

Poor cell survival is a substantial barrier for many neurobiology applications due to the complexity and fragility of neural cells. To investigate the utility of CultureCEPTTM Supplement, specific neural cell models and applications were identified that are known to result in poor cell survival. Cryopreserved primary mouse neurons (cortical and hippocampal) are particularly sensitive to cell stress as demonstrated by poor post-thaw survival and recovery. Differentiation of primary rat NSC's to Oligodendrocytes (OL) is a stress inducing process and often leads to poor yield of OL. PSC-derived NGN2 induction leads to significant cell stress and poor survival of cells going into neuronal maturation as well as poor recovery from cryopreservation. Finally, when working with neuronal cells it is important to assess functionality and whether reagents will impact neuronal activity. Multi-Electrode arrays (MEA) were employed to assess spontaneous neuronal activity in cells treated with CultureCEPTTM Supplement compared to untreated cells.

Results indicate CultureCEPTTM Supplement effectively mitigated stress and improved survival of neural cells in several distinct applications. CultureCEPTTM Supplement provides a solution for poor in-vitro neural cell survival and may show utility in a wide-range of applications and workflows.

Chen, Y. et al.: Nat. Methods, 18(5), 528 (2021). A versatile polypharmacology platform promotes cytoprotection and viability of human pluripotent and differentiated cells

Abstract No. P097**Modeling PURA syndrome in neural progenitor cells and cerebral organoids using PURA KO and patient iPSCs**

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Spontaneous heterozygous mutations in the PURA gene cause the neurodevelopmental disorder PURA syndrome. Among the over 600 reported patients, symptoms such as neurodevelopmental delay, intellectual disability, hypotonia and epilepsy are observed. Besides its nucleic acid binding ability, PURA's molecular role in cells is poorly understood. To model PURA syndrome in vitro, homozygous knock-out (KO) iPS cell lines and reprogrammed patient iPS cell lines harboring the most recurrent PURA mutation (Phe233del) were generated as well as their respective isogenic controls. The iPSCs were differentiated into neural progenitor cells (NPCs) and cerebral organoids to study the impact of PURA's absence and the effect of Phe233del mutation during neurodevelopment. While morphological and size analysis of NPCs and organoids showed no difference between KO, patient cells, and their isogenic control, multi-omic approaches revealed significant cellular differentiation changes in early development upon loss of PURA.

Abstract No. P098**Advancing and modulating human heart-forming organoids (HFOs) for drug testing**

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Although their numbers were drastically decreased since the 1970s, animal models still play a major role in life sciences and drug development, despite having numerous drawbacks such as high costs and low predictive capabilities for the human organism. Therefore, organoids derived from human pluripotent stem cells (hPSCs) play a key role in the replacement of animal models in disease modelling and drug screening. The heart-forming organoid (HFO) system is a hPSC-derived pre-vascularized and patterned multi-tissue organoid model that closely resembles early heart and foregut development. To establish HFOs as a platform for drug screenings, we exposed them to FDA-approved antiangiogenic chemotherapeutic compounds. Their effect on the vascular network within the HFO was evaluated by flow cytometry (FC) and whole-mount immunofluorescence staining (WMS). Confocal images were analyzed using AngioTool, which allowed a quantitative analysis and characterisation of the vascular network. Treating HFOs with Axitinib (Ax) and Cabozantinib (Cabo) in a clinically relevant concentration range resulted in a strong antiangiogenic effect. These preliminary results highlight the HFOs' potential as a robust drug screening platform to test antiangiogenic compounds while simultaneously investigate possible off-target effects on cardiac and foregut tissues. To simplify and accelerate downstream analysis, we have started to generate a double reporter hPSC line for the early cardiac marker NKX2.5 and the endothelial marker CD31. To increase our model's complexity, first steps for the design of a vascularising chip have been made. The chip design will connect the HFO with an external and perfusable endothelial network. This will allow oxygen, nutrients and drugs to be introduced into the organoid in a physiological manner.

Drakhlis L, Biswanath S, Farr CM, et al. Human heart-forming organoids recapitulate early heart and foregut development. Nat Biotechnol. 2021;39(6):737-746. doi:10.1038/s41587-021-00815-9

Abstract No. P099**Elucidating SYNGAP1 isoform functions in human neurodevelopment using cerebral organoids**

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SYNGAP1, encoding Ras/Rap GTPase-activating protein, is a critical gene involved in synaptic signaling and neurodevelopment. Mutations in SYNGAP1 are associated with intellectual disability and autism spectrum disorder (ASD). However, the specific functions of its multiple isoforms, generated by alternative splicing and transcription start sites, remain poorly understood. Current methods for studying SYNGAP1 functions rely on animal models, which do not fully recapitulate human neurodevelopment. This project aims to elucidate the roles of different SYNGAP1 isoforms using human cerebral organoids as an alternative to animal models. Cerebral organoids, derived from pluripotent stem cells, offer a promising 3R approach by mimicking the human brain architecture and functionality. We will employ cutting-edge techniques including single-cell RNA sequencing, CRISPR-based gene perturbation, and BaseScope in situ hybridization to comprehensively profile SYNGAP1 isoform expression, manipulate their levels, and visualize their spatiotemporal distribution across different developmental timepoints: 15, 30, 60 days, roughly equivalent to 4, 10, 18 post-conceptional weeks of human development in vivo. By developing this innovative 3D model system, we aim to uncover isoform-specific roles of SYNGAP1 in human brain development. Our approach will not only advance mechanistic understanding of SYNGAP1 biology, but also exemplify how cerebral organoids can serve as a powerful alternative to animal use. This project showcases the potential of human cerebral organoids to replace animal experiments in studying neurodevelopmental disorders, aligning with the 3R principles (Replacement, Reduction, and Refinement).

10.1016/j.ijdevneu.2019.08.003.

10.7554/eLife.56273

Abstract No. P100**Using a bioreactor to improve retinoblastoma model with stem cell-derived retinal organoids***Junling Liu*, Maren Kaufmann, and Laura Steenpass*

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Retinoblastoma is a rare retinal cancer mainly affecting young children. It is caused by biallelic mutation of the retinoblastoma gene (RB1). Until now, no effective animal model faithfully recapitulates key features of human retinoblastoma. In vitro, RB1ko retinal organoids differentiated from human stem cells displayed main characteristics of retinoblastoma in patients, such as proliferation of cone photoreceptors and aberrant retinal differentiation. Disintegration at late stages of differentiation was detected in pure RB1ko and mixed RB1ko and RB1wt retinal organoids, but not in wt organoids, indicating cell-cell interactions between the two cell lines. Furthermore, genes associated with extracellular matrix organization were enriched in RB1ko retinal organoids compared to wt retinal organoids.

The project aims to investigate the tumorigenic potential of RB1ko retinal cells in iPSCs-derived retinal organoids. To improve the differentiation protocol, retinal organoid differentiation was performed in a CERO 3D Incubator & Bioreactor using H9 hESCs until day 65 of differentiation. Retinal organoids cultured in the CERO system were larger and more homogeneous compared to control organoids. The neuroretina of CERO-cultured organoids showed higher density of photoreceptor precursors, immature photoreceptors and retinal ganglion cells compared to controls. Further analysis is needed to assess later stages of differentiation and reproducibility.

In 2D culture, RB1ko and RB1wt iPSCs displayed similar proliferation rates. To investigate intercellular interactions between RB1ko and RB1wt cells, a doxycycline-inducible GFP RB1ko iPSC line was generated and mixed with RB1wt iPSCs at various ratios. Flow cytometry analysis revealed no abnormal proliferation or growth suppression of either cell line in all setups.

To determine if and how RB1 mutant cells interact or affect RB1wt cells in differentiated retinal cell types, retinal organoid cultures derived from mixed RB1ko and RB1wt iPSCs in the CERO system will be performed, followed by in-depth morphological and molecular analysis.

Abstract No. P101**UV-micropatterning for high-throughput generation of embryoid bodies**

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Embryoid bodies (EBs) are three-dimensional aggregates of pluripotent stem cells. Due to their self-organized differentiation, they are of growing interest for drug screening and regenerative medicine. However, current methods for EB formation are difficult to implement for standardized, large-scale production in automated cell culture. We recently demonstrated that geometrically confined iPSC colonies spontaneously detach after 5-7 days of culture. The confinement was within circular coated areas of vitronectin generated by micro-contact printing. This resulted in 3D aggregates that correspond to EBs of controlled size without enzymatic or mechanical treatment (Elsafi Mabrouk et al., *Biomaterials* 2022). In this study, we have further improved this approach by using ultraviolet (UV)-micropatterning for the generation of homogeneous EBs. The micropatterning process involves generating a passivated surface, which is then selectively exposed to UV light to locally degrade cell-repellent molecules. The irradiated regions of the surface are then coated with vitronectin to enable cell adhesion and growth. Similarly to micro-contact printing, the iPSCs detached from these UV-microstructured substrates as cellular aggregates and are capable of self-organization into the three lineages of endoderm, mesoderm, and ectoderm. Our improved method provides a more homogeneous generation of large numbers of EBs. We are currently investigating the effects of long-term storage, different protein coatings, and size distribution of the material structures on self-detachment of the cellular aggregates. Our UV-micropatterning may facilitate a simple and cost-effective approach for high-throughput generation in cell culture automation. Furthermore, it provides a standardized tool to investigate self-organization and differentiation potential for quality control of iPSC lines, such as PluripotencyScreen.

Abstract No. P102**REAL human skin: realistic, ethical, automated and layered**

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Skin organoids derived from induced human pluripotent stem cells hold immense promise as in vitro models for studying skin biology and disease. These organoids mimic the complexity of native skin, including hair follicles and sebaceous glands, providing a more physiologically relevant environment for research. Despite these benefits, current differentiation methods face significant challenges, such as inside-out orientation and lack of vasculature or immuncells.

To overcome these challenges, we have automated the skin organoid differentiation process as well as the cultivation in a bioreactor. Our results showed successful differentiation, producing all requisite cell types (Keratinocytes, Melanocytes, Fibroblasts, Adipocytes) and structures (hair follicle, sebaceous glands, endogenous collagen ECM) with reduced hands-on time.

A key aspect of our research is the establishment of a static air-liquid interface (ALI) culture for inside-out oriented skin organoids. By culturing the organoids on porous nanofibers to mimic the in vivo environment a skin barrier. Culturing for 28 days at the ALI resulted in the correct orientation and maturation of the epidermis, indicated by the expression of undifferentiated keratinocytes (CK5) and differentiated keratinocytes (CK1). As well as key skin barrier markers such as loricrin and ZO-1. The presens of hairfollicles was characterized by Ki67, CK17 and E-Cad.

These advancements in automation, bioreactor cultivation, and ALI culture with specialised nanofibers significantly enhance the functionality and reproducibility of in vitro skin models. This progress holds great promise for applications in disease modeling, drug testing, and the development of new therapeutic strategies for skin-related diseases.

Abstract No. P103**MRAS KO in iPSC-derived BVOs inhibits sprouting mediated by UNC5b**

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Coronary artery disease (CAD) is the third leading cause of mortality worldwide accounting for over 17 Mio deaths per year¹. So far, more than 300 genetic regions linked to CAD have been discovered through genome-wide association studies². Neovascularization, which involves the formation of new blood vessels, is crucial in the progression and vulnerability of atherosclerotic plaques. One of the identified genetic regions includes the MRAS gene which has been linked to inflammation and cell proliferation, but also with angiogenesis/neovascularization. Our research aims to investigate the role of MRAS in angiogenesis using blood vessel organoids (BVOs).

In our study, we utilized our 3D angiogenesis model of blood vessel organoids (BVOs), which was initially introduced in 2019 by Wimmer and colleagues³. We generated BVOs from induced pluripotent stem cells (iPSCs) with MRAS knockout (KO) as well as their corresponding parental lines. Subsequently, we conducted a comprehensive analysis of the MRAS-KO iPSC line at the genetic, RNA, and protein levels, and utilized them for the angiogenesis assay. In brief, we differentiated the iPSCs into spheric aggregates containing vascular progenitors, embedded them in a hydrogel on day 6, and monitored sprouting and morphology for 8 days. We then examined gene expression of thirteen tip cell and angiogenesis markers in the vessel networks immediately after sprouting and in mature BVOs on day 25 for both the MRAS KO and parental lines. Our findings indicate that MRAS KO led to decreased sprout growth in our iPSC-derived BVO angiogenesis model compared to the parental lines and also influenced the morphology of the BVOs. Furthermore, initial results from gene expression analysis suggest a potential involvement of UNC5B in this process.

1 Brown, J.C., Gerhardt, T.E., and Kwon, E. (2024). Risk Factors for Coronary Artery Disease. In StatPearls (StatPearls Publishing).

2 Erdmann, J., Kessler, T., Munoz Venegas, L., and Schunkert, H. (2018). A decade of genome-wide association studies for coronary artery disease: the challenges ahead. *Cardiovasc. Res.* 114, 1241–1257. <https://doi.org/10.1093/cvr/cvy084>.

3 Wimmer, R.A., et al. Penninger, J.M. (2019). Human blood vessel organoids as a model of diabetic vasculopathy. *Nature* 565, 505–510. <https://www.nature.com/articles/s41586-018-0858-8>

Abstract No. P104**MO:RE lab platform: a new automated device to standardize organoid culture**

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Human cortical organoids derived from induced pluripotent stem cells (iPSCs) represent an important in vitro system for disease modeling and drug screening (1). The applicability of organoids for high-throughput assays, however, requires automated liquid handling workflows that enable a robust and homogeneous organoid production and downstream workflows. We therefore developed the automated Mo:re lab platform to standardize organoid culture. In this study, we demonstrate the use of Mo:re's lab platform to support the production of cortical organoids in screening-compatible 96-well plates. iPSCs were differentiated to cortical organoids following the STEMdiff Dorsal Forebrain Organoid Differentiation Kit (StemCell Technologies) with minor modifications (2). At the organoid maintenance stage, half medium changes were automated by the Mo:re lab platform and bright-field images were collected to evaluate the organoid area and roundness. Moreover, at day 50, the cellular viability was evaluated using the CellTiter-Glo 3D Kit (Promega). As a control, the medium of another organoid plate was changed manually. The Mo:re lab platform performed the medium exchanges of cortical organoids without aspirating or disrupting the 3D structures. Moreover, the area and roundness of the cortical organoids were maintained during all medium exchanges demonstrating the accuracy and precision of the Mo:re pipetting technology. The organoids cultured with the automated device presented a higher cell viability than organoids cultured manually. Mo:re lab platform enables the automation of medium exchanges of iPSCs-derived cortical organoids maintaining the 3D morphology. Therefore, this automated device represents a key tool for organoid standardization in high-throughput plate formats.

1. VANDANA, J. Jeya, et al. Human pluripotent-stem-cell-derived organoids for drug discovery and evaluation. *Cell Stem Cell*, 2023, vol. 30, no 5, p. 571-591.

2. <https://www.stemcell.com/products/stemdiff-dorsal-forebrain-organoid-differentiation-kit.html>

Abstract No. P105**Single-cell transcriptomics of hiPSC-derived endothelial cells reveals neovascularization dynamics in 3D microenvironments**

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The development of vascular structures is essential in *in vitro* tissue engineering to ensure effective nutrient supply and enhance the success of organ transplants. This study investigated the neovascularization potential of endothelial cells (ECs) derived from human induced pluripotent stem cells (hiPSCs) within a 3D microenvironment. Utilizing a comprehensive single-cell transcriptomic approach, we conducted time-resolved analyses to track the differentiation of ECs and co-evolving mural cells within different 3D cell cultures. This analysis provided crucial insights into these cell types' development, stability, and plasticity. Upon transfer to a 3D hydrogel matrix from a 3D suspension culture, the hiPSC-derived ECs demonstrated neovascularization, characterized by distinct cellular states, including migration, coalescence, and tubulogenesis. Furthermore, the maturation process revealed the development of two distinct pericyte subtypes, which exhibited specific interactions with ECs, playing a central role in vessel stabilization and maturation.

Through ligand-receptor interaction studies, we identified key angiogenic signals that regulate these processes. In particular, several *in silico*-predicted ligands were experimentally validated for their ability to attract ECs within a microfluidic chip, featuring their potential to guide vascular development. Combining single-cell transcriptomics and functional assays has allowed us to draw an *in vitro* roadmap of vascularization through culture conditions. This has implications for the advancement of tissue engineering and regenerative medicine. Our findings enhance the understanding of EC and pericyte dynamics within engineered tissues and provide a valuable platform for developing targeted therapeutic strategies to promote vascularization in clinical applications.

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Abstract No. P106/T25**Optimization of cardiomyocyte cryopreservation and quality control aspects for potency and residual iPSC contaminations**

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Screening of Cord blood units (CBUs) for most frequent HLA haplotypes allows the selection of corresponding HLA homozygous (HLA^h) CBUs. After isolation of CD34⁺ hematopoietic stem cells (HSC) from these HLA^h CBUs, cells are reprogrammed towards iPSC.

Within the HEAL Project (101056712), the HLA^h iPSC were differentiated into cardiomyocyte-aggregates (CMAs) via 3D-suspension culture. For possible applications of the CMAs in a clinical setting, certain quality-assessments /-controls (QA/QC) need to be established to ensure the final safety of the product. Cryopreservation is a good option for the optimal clinical process to allow time for QA/QC assays.

CMA differentiation was achieved by WNT pathway modulation with an optimized protocol (Terheyden-Keighley et al. 2024, accepted in SCTM). The CMAs were analyzed for their phenotype and residual cell contaminations applying flow cytometry and qPCR. Detection of potency/function was conducted by video-analysis. CMs were cryopreserved either as aggregates or as single cells. In case of single cells, re-aggregation after thaw was performed for a maximum of 5 days.

Cardiomyocytes with >85% cTnT⁺ were used for downstream analysis. Spontaneous contraction, as an analysis for the function, was monitored as early as differentiation-day 7, resembling immature cardiomyocytes, with low beating frequencies (<20 bmp). Cryopreservation resulted in an overall recovery of <60% CMAs after 5d of culture and recuperation of spontaneous contraction (~10 bmp). Cryopreservation of single cell cardiomyocytes was associated with a greater loss of cells (overall recovery <40%). For Screening of residual iPSC contaminations, qPCR could detect contaminations of <0.1% iPSC with specific markers, e.g. miR-302.

Cryopreservation is a useful step of processing, however both the freezing of aggregate and single cell cardiomyocytes need further optimization. Although <0.1% iPSC contamination can be detected by qPCR, *in vivo* animal studies are a mandatory requirement in the development of a save product (Tamura et al. 2024 SCTM).

Terheyden-Keighley et al. GMP-compliant IPS cell lines show widespread plasticity in a new set of differentiation workflows for cell replacement and cancer immunotherapy, accepted in Stem Cells Translational Medicine, 2024

Tamura et al. Study on the Extrapolability of Current Tumorigenicity Test With Mice by Comparing the Syngeneic or Allogeneic Mouse Transplantation Model, Stem Cells Translational Medicine, 2024, <https://doi.org/10.1093/stcltm/szae019>

Abstract No. P107/T13**Tracking bone marrow regeneration after CAR-T cell therapy in myeloma at spatial single-cell resolution**

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Anti-BCMA CAR-T cell therapy is a widely used treatment strategy of relapsed/refractory multiple myeloma. Despite its notable efficacy and substantial overall response rate, this treatment is frequently accompanied by severe undesirable side effects. In particular, the pathophysiology of impaired bone marrow (BM) regeneration post therapy, which is critical for patient recovery, is not understood. This study investigates BM regeneration after anti-BCMA CAR-T cell therapy in multiple myeloma patients, focusing on hematopoietic stem cells (HSCs) and their potential niche communications. We analyzed BM biopsies from 16 patients, integrating single-cell RNA-sequencing and single cell resolution spatial transcriptomic technologies. This approach allowed spatial mapping of >20 cell types in the post-CAR-T BM, and revealed significant inter-patient variability in cell states and BM architecture. Importantly, elevation of mesenchymal cell inflammation is prevalent in patients experiencing prolonged cytopenia, a severe side effect of CAR-T therapy, characterized by a lower-than-normal density of erythroid, platelet or neutrophil blood count. Ligand-receptor interaction analysis and spatial niche information suggested that these mesenchymal cells act as signaling hubs, relaying inflammatory signals to myeloid cells, thereby maintaining an inflammatory state. In vitro culture systems showed that this persistent inflammation impaired functional outputs of hematopoietic stem and progenitor cells (HSPCs), potentially causing prolonged cytopenia. Gene set enrichment analysis indicated a senescence signature in HSCs from cytopenic patients, suggesting that HSPC dysfunction might be linked to their senescence state. In conclusion, our study showed that the inflammatory microenvironment in BM may induce HSPC senescence, contributing to the prolonged cytopenia observed in some patients post-CAR-T therapy. This research provides insights into the mechanisms of BM regeneration and the challenges associated with cytopenia in multiple myeloma treatment.

Abstract No. P108**Time-efficient large-scale manufacturing of pluripotent stem cell-derived cardiomyocytes**

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Cardiovascular diseases are the foremost cause of death worldwide. Postnatal mammals lack the regenerative capacity to replace cardiomyocytes that are lost in cardiac diseases, resulting in reduced contractility of the heart. A promising strategy to restore heart function is the transplantation of pluripotent stem cell-derived cardiomyocytes (PSC-CMs).

Here, we describe the generation of large numbers of PSC-CMs in a closed system. Initially, we screened a library of more than 100 human cytokines on PSC-CMs to identify potential inducers of cell cycle activity. We identified several cytokines that increased Ki-67 expression and/or the number of nuclei in PSC-CM populations derived from multiple PSC clones. Interestingly, none of these cytokines were able to induce cardiomyocyte division on their own. However, when combining one of the cytokines with a Wingless/int1 pathway modulator, we observed a significant increase in PSC-CM proliferation that exceeded the effect of Wingless/int1 modulation alone. Subsequently, we applied the combination in the closed cell manufacturing system and could achieve substantial expansion of PSC-CMs.

We show that exogenous activation of multiple signaling pathways can induce cardiomyocyte proliferation, an approach that could be harnessed to accelerate the production of PSC-CMs e.g. for transplantation or drug screening purposes.

Abstract No. P109**A novel marker to enrich human pluripotent stem cell-derived pancreatic β cells***Yilin Di^{1, 2,*}, Chenglei Tian¹, and Henrik Semb¹*¹Helmholtz Zentrum München, Institute of Translational Stem Cell Research²Technical University of Munich, Munich, School of Medicine and Health

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Type 1 diabetes (T1D) is an autoimmune disease in which β cells are selectively destroyed, leading to severe insulin deficiency requiring daily insulin injections for survival. Due to the inefficiency of insulin injection and the shortage of donor islets, human pluripotent stem cells (hPSCs) have emerged as a promising and unlimited source of functional β -cells for improved therapy for more T1D patients. Although there have been many attempts to improve the differentiation efficiency of generating glucose-responsive stem cell-derived β cells (SC- β cells), the challenge of producing a highly purified β cell population remains, primarily due to contamination by off-target and non-functional polyhormonal cells, which can potentially compromise therapeutic efficacy and safety. In this study, we developed a robust cell sorting-based purification strategy to enhance β cell purity while eliminating other types of by-products and off-target cells. Based on our previous findings, we identified a novel SC- β cell-enriched surface marker that can selectively isolate β cells in the endocrine population. Integrating this surface marker with the pan-endocrine cell surface marker CD49a (ITGA1) achieved a substantial 2.7-fold increase in β cell enrichment (from 28.0% to 75.5%) and a significant 4.3-fold reduction in the proportion of α and polyhormonal cells (from 13.8% to 3.2%). Consistently, quantitative PCR and immunocytochemistry analyzes also confirmed this enrichment. Moreover, the enriched SC- β clusters exhibited enhanced insulin secretion in response to glucose challenge compared to the un-enriched controls, underscoring their functional superiority. This strategy markedly improves β cell purity, thereby advancing the safety and therapeutic potential of SC- β cells for T1D cell therapy.

Abstract No. P110**Human dental pulp stem cells attenuate streptozotocin-induced parotid gland injury in rats**

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Background

Diabetes mellitus causes deterioration in the body, including serious damage of the oral cavity related to salivary gland dysfunction, characterized by hyposalivation and xerostomia. Human dental pulp stem cells (hDPSCs) represent a promising therapy source, due to the easy, minimally invasive surgical access to these cells and their high proliferative capacity. It was previously reported that the trophic support mediated by these cells can rescue the functional and structural alterations of damaged salivary glands. However, potential differentiation and paracrine effects of hDPSCs in diabetic-induced parotid gland damage have not been investigated. Our study aimed to investigate the therapeutic effects of intravenous transplantation of hDPSCs on parotid gland injury in a rat model of streptozotocin (STZ)-induced type 1 diabetes.

Methods

Thirty Sprague–Dawley male rats were randomly categorised into three groups: control, diabetic (STZ), and transplanted (STZ + hDPSCs). The hDPSCs or the vehicles were injected into the rats' tail veins, 7 days after STZ injection. Fasting blood glucose levels were monitored weekly. A glucose tolerance test was performed, and the parotid gland weight, salivary flow rate, oxidative stress indices, parotid gland histology, and caspase-3, vascular endothelial growth factor, proliferating cell nuclear antigen, neuronal nitric oxide synthase, endothelial nitric oxide synthase, and tetrahydrobiopterin biosynthetic enzyme expression levels in parotid tissues were assessed 28 days post-transplantation.

Results

Transplanted cells decreased blood glucose, improved parotid gland weight and salivary flow rate, and reduced oxidative stress. The cells migrated to the STZ-injured parotid gland and differentiated into acinar, ductal, and myoepithelial cells. hDPSCs downregulated the expression of caspase-3 and upregulated the expression of vascular endothelial growth factor and proliferating cell nuclear antigen, likely exerting pro-angiogenic and anti-apoptotic effects and promoting endogenous regeneration. In addition, the transplanted cells enhanced the parotid nitric oxide-tetrahydrobiopterin pathway.

Conclusions

hDPSCs may have potential in the treatment of diabetes-induced parotid gland injury.

Abstract No. P111**Versatile high-speed microfluidic cell sorting of human pluripotent stem cells and their derivatives**

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iPSCs with their potential to differentiate into numerous different cell types hold great potential for cellular therapy. Innovative therapeutic approaches often entail the application of clearly defined cells types or subsets that need to be isolated and enriched from heterogenous cell populations. Conventional flow sorters were predominantly designed for research applications. Open systems and harsh sorting conditions therefore often prevent the translation of protocols into the clinic. We hereby show how a gentle microchip-based sorting technology can enable enrichment of PSCs and their derivatives in the safety of a sterile cartridge.

Human fibroblasts were reprogrammed via repeated mRNA transfections and resulted in a mixture of successfully generated iPSCs and non- or partly reprogrammed undefined cells. This heterogenous cell culture was stained with Tra-1-60-PE and sorted immediately after reprogramming. The iPSCs could be successfully enriched from purities of 4-41% to 85-99 %. Furthermore, transgenic iPSC constitutively expressing GFP to a differing degree could be effectively enriched from 13-51% to a population of 72-98%. To obtain monoclonal transgenic iPSCs the sorted fraction was applied to a single-cell dispenser and seeded in 96-well plates. The cells showed high plating efficiency after the combination of sorting and subsequent single-cell dispensation while maintaining typical iPSC morphology. Next, various iPSC-derived cell types were enriched after differentiation. Retinal pigmented epithelial cells could be enriched from a purity of 51-69% to 93-98% and showed a strongly pigmented phenotype after sorting. In other approaches cardiomyocytes were enriched from 56% to 96% and CD144-positive endothelial cells could be enriched from 48-49% to 90-96%. In all applications a high viability of over 85% was maintained after sorting.

In summary, this gentle, sterile sorting technology allows for efficient, reliable enrichment of PSCs and their derivatives and therefore facilitates working with clearly defined cell populations in a wide variety of PSC applications.

Abstract No. P112**Three dimensional and multiplexed characterization of hPSC spheroids generated with different three dimensional cultivation conditions**

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Three-dimensional cultivation as new manufacturing strategy of human pluripotent stem cells (hPSCs) has gained more importance over the last few years to serve the needs for hPSCs in the field of autologous and allogenic regenerative medicine applications, as for example Parkinson's disease or age-related macular degeneration (AMD), as well as for drug screening or disease modeling approaches.

3D cultivation of hPSCs spheroids is still challenging with respect to homogeneity, reproducibility of cell compositions and quality and holds various difficulties regarding the access to and analysis of all cells within the culture. Furthermore, cultivation systems could vary significantly regarding shear force, type of movement and control of the culture conditions. We already established a workflow of combined standard analytical methods (FlowCytometry, media analysis and microscopy) and spatial biology techniques to characterize pluripotent hPSC spheroids. The multiplexing Imaging Platform MACSima™ enables automated staining, acquisition and analysis of hundreds of markers to reveal the protein expression within the hPSC spheroids, while the UltraMicroscope Blaze™ facilitates access to the three-dimensional structure within the spheroids by light sheet microscopy.

We investigated in the development of hPSC spheroids at different timepoints and under different conditions of a 3D cultivation in a manual laboratory scale or a fully controlled stirred tank bioreactor system. Our spatial biology techniques revealed pluripotency and enables a deep insight into the three-dimensional architecture and organization of the spheroids.

Abstract No. P113**Sendai viral vectors for efficient prime editing in in vitro and in vivo applications**

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Prime editing, a variation of CRISPR/Cas, is a particularly promising system for the precise manipulation of the human genome. In contrast to other methods, prime editing allows for all twelve base-to-base conversions and small insertions and deletions and is not limited to dividing cells. However, application of this system in human cells is limited by its method of delivery. Though conventional transfection approaches that are used in vitro yield sufficient correction efficiencies, such delivery methods are not efficient enough or may not even be possible for therapeutic in vivo application. Viral vectors are advantageous in vivo because they hijack the host machinery for robust expression of virus-encoded genes. More specifically, the Sendai virus (SeV) is of particular interest due to its safety, as it is an RNA virus with a purely cytoplasmic lifecycle and no DNA intermediate. Thus, it avoids unwanted integration into the host genome, which is a potential concern for the widely used adeno-associated and lentiviruses. We have therefore established the production of a replication-deficient SeV carrying transgenes. As a proof-of-principle for prime editing, we have assembled a viral vector encoding the SeV genome alongside the prime editing tools for an eGFP>BFP switch by two base conversions. Following production from the vector, this virus can be applied for editing in respiratory and vascular cells derived from induced pluripotent stem cells (iPSCs) capable of the eGFP>BFP switch, as well as in an eGFP rat model. Ultimately, this SeV-based prime editing would be used for the correction of disease mutations in numerous lung diseases both in vitro and in vivo. In parallel, we have achieved highly efficient editing in iPSCs using modified RNA encoding prime editing tools for the eGFP>BFP switch and are now packaging these components for lipid nanoparticle-based delivery in vitro and in vivo.

Abstract No. P114**Polyamines sustain epithelial regeneration in aged intestines by modulating protein homeostasis**

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Aging hampers the regenerative potential of intestinal epithelium across species including humans, yet the underlying causes remain elusive. Here, using proteomic and metabolomic profiling of intestinal tissues together with functional assays, we characterized the temporal dynamics of regeneration following injury induced by 5-fluorouracil, a commonly used chemotherapeutic agent. Comparison of regeneration dynamics in mice of different ages revealed the emergence of a proteostasis stress signature and increased levels of polyamines following injury exclusively in old epithelia. Mechanistically, we show that delayed regeneration is an intrinsic feature of aged epithelial cells that display reduced protein synthesis and accumulation of ubiquitylated proteins. Notably, dietary restriction followed by re-feeding prior to injury increases polyamine pathway activation, enhances protein synthesis, and restores the regenerative capacity of aged intestines. Our findings highlight promising epithelial targets for interventions aimed at tackling the decline in tissue repair mechanisms associated with aging.

Abstract No. P115/T38**Humanized osteoclasts: an interspecies chimeric immune cell***Gülce Perçin^{1,*}, and Claudia Waskow^{2,3}*¹Leibniz Aging Institute/FLI, Jena²Leibniz Institute on Aging, Immunology of Aging, Jena³Friedrich-Schiller University Jena, Faculty of Biological Sciences

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Osteoclasts are multinucleated immune cells that are critical for bone remodelling to ensure space for adult hematopoiesis. Initially, because of their dependence of Csf1r signalling, osteoclasts were suggested to be of macrophage/monocyte origin that are constantly generated from Hematopoietic Stem Cells (HSCs), laying the foundation of the current clinical treatment for osteopetrosis. However, mouse models that specifically inhibited Csf1r signalling in embryonic or adult hematopoiesis revealed that osteoclasts have an embryonic origin. Adult Csf1r depletion revealed that embryo-derived osteoclasts are maintained in adults by fusing with HSC-derived monocytes, forming a syncytium with genetic material from two ontogenically distinct immune cells. Taking it a step further, we are interested in understanding the mechanism of osteoclast maintenance in xenograft models. In comparison to other commercially available models, NSGW41 was chosen because of the increased and stable human hematopoietic cell engraftment over time with increased myeloid cell output. Thus, we discovered mouse osteoclasts with one or more human nuclei in humanized NSGW41 bones, indicating an interspecies chimeric immune cell. We isolated distinct blood monocytes from cord blood or adults to find the human donor cell that can fuse with pre-existing osteoclasts. We discovered that osteoclastogenesis in the blood monocyte compartment was limited to CD14⁺ inflammatory blood monocytes. As a result of this *in vitro* finding, CD14⁺ blood monocytes potentially be the target cells that can fuse with pre-existing mouse osteoclasts in xenograft models to maintain osteoclasts. By adoptive transfer experiments to the humanizable NSGW41oc/oc model, we hope to demonstrate the osteoclast potential of human CD14⁺ blood monocytes *in vivo* in the near future.

Abstract No. P116**Large-scale expansion of 3D pluripotent stem cell spheroids in a new xeno-free suspension culture medium**

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Scalable and efficient PSC expansion systems are an important need for pluripotent stem cell (PSC)-derived allogeneic cell therapies. Clinical applications can be supported by three-dimensional (3D) suspension culture, which enables the large-scale production of high-quality PSCs. However, suspension culture adoption is hindered by a few key barriers to execution. In particular, clinical suspension culture is limited by the lack of a regulatory compliant suspension culture medium. To address this, we have recently developed Gibco™ Cell Therapy Systems (CTS) StemScale™ PSC Suspension Medium, which enables single cells to easily self-aggregate into 3D spheroids. CTS StemScale Medium has a xeno-free formulation and is related to our research-use only (RUO) StemScale Medium, with the two versions of culture medium exhibiting similar performances. Both induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) are capable of growing in CTS StemScale Medium, with cell line-dependent growth in the range of 5X – 10X expansion per passage. This enables PSCs grown in CTS StemScale Medium to easily scale up in multiple types of vessels, from small-scale (<100 mL) to large-scale (>1L) culture systems. Up to 5 billion cells can be harvested from spheroids grown in a 3L bioreactor system. Due to the large quantities of PSCs harvested from these bioreactors, we have cryopreserved these cells at high densities to facilitate ease of use in future large-scale studies or subsequent downstream applications that require high quantities of cells. Ultimately, cells grown in CTS StemScale Medium have the flexibility to: differentiate as 3D spheroids, dissociate into single cells and be utilized in downstream applications, or be cryopreserved as single cells for future use.

Abstract No. P117**Vascularization of stem cell-derived β -cells for diabetes research**

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Diabetes is a complex disease characterized by β -cell destruction, impaired insulin secretion, and hyperglycemia. For the treatment of diabetes, new therapies like pancreatic islet transplantation are emerging¹, offering up to ten years of glycemic control. However, challenges such as donor scarcity, immunosuppression, and impaired graft vascularization, which is crucial for graft survival and function, remain. In this regard, human induced pluripotent stem cells (hiPSCs) offer a promising alternative to generate an unlimited source of stem cell-derived islet-like clusters (SC-ILCs). However, these SC-ILCs are immature and lack vascularization¹. To this end, understanding the interactions in the islet microenvironment, including endothelial cells (ECs), is crucial as it has been shown that islet ECs can improve β -cell development, maturation, and insulin secretion².

Here, we present a hiPSC-derived *in vitro* model for SC-ILC vascularization. We use stem cell-derived ECs (SC-ECs) and stromal cells to mimic the islet microenvironment and gain insights into cell-cell interactions and maturation pathways. Using single-cell RNA sequencing and cell-cell communication analysis, we identify specific interactions between SC-ECs and endocrine cells that represent potential targets for SC-ILC maturation. Transcriptomic analysis reveals a maturation of SC- α cells and a toning of SC-ECs towards an islet-EC profile. The vascularization of SC-ILC leads to increased insulin secretion from SC- β cells and positively influences glucagon secretion from SC- α cells. This emphasizes that the cell-cell interactions in the islet niche, especially with ECs, enhance endocrine cell functionality and that deciphering interaction pathways can help to improve SC-ILC maturation *in vitro*. Overall, the vascularization model can enhance our understanding of the interaction between ECs and endocrine cells, thus contributing to vascularization strategies for cell replacement therapies. In the future, our model could be used to generate more physiologically relevant *in vitro* models for diabetes research and help to reduce islet transplantation studies within animals.

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Abstract No. P118/T40**Generation of designer stem cell-derived islets (SC-islets) to improve safety and efficacy of SC-based islet replacement therapy**

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Stem cell (SC)-derived pancreatic islets are an alternative source of islets for Type 1 Diabetes replacement therapy. Despite their great potential as an unlimited cell source, SC-islets still have limitations due to current protocol efficiency to generate large numbers of mature endocrine cells. Infact, unspecified non-endocrine cell types generated from this method could give rise to cysts and unwanted outgrowths, posing a potential safety risk for patients. Additionally, differentiation protocols towards endocrine pancreatic lineages have been focused on SC-beta-cells and overlooking other islet cell types, despite their importance. In particular SC-alpha-cells, for their important role in regulating SC-beta-cells functionality. For these reasons, with this project, we aim to generate a superior SC-islets with improved purity, functionality, and safety.

To generate more functional SC-islets with defined composition and depletion of unwanted cell types, we have established sorting strategies for enrichment of SC-beta-cells and SC-alpha-cells. We generated an ARX-CFP x C-Peptide-mCherry reporter iPSC cell line and utilized the reporter expression to enrich for SC-beta-cells (C-Peptide⁺) and SC-alpha-cells (ARX⁺), thus allowing the generation of SC-islets with a defined ratio of SC-beta-cells and SC-alpha-cells. Here we showed that enriched SC-beta-islets and SC-beta/alpha-islets showed an improved insulin secretion upon glucose challenge compared to the non-enriched SC-islets.

With this result, we confirmed our initial hypothesis that the presence of SC-alpha-cells are crucial for SC-beta-cells function. Next, we aim to do a comprehensive transcriptomic analysis of our SC-islet product before and after in vivo transplantation. This would provide a novel insight on SC-derived product efficacy and safety upon transplantation and pave the way for future clinical trials.

Abstract No. P119**PluripotencyScreen – improving the quality control of pluripotent stem cells and their derivatives**

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Cells from our tissues can be reprogrammed into induced pluripotent stem cells (iPSCs) by overexpressing specific transcription factors. Such reprogrammed iPSCs have the ability to differentiate into the cell types of from our body. Thus, iPSCs are defined by their potential to give rise to cells of all three embryonic germ layers: mesoderm, endoderm, and ectoderm. As a quality control for this ability, gene expression patterns and immunofluorescence images are commonly used. However, there remains an need to further improve and optimize the tracking of early cell fate decisions and to improve quality control of iPSCs.

DNA methylation (DNAm) has been shown to be involved in cellular differentiation and manifests throughout development. Hence, it can be used to classify cell types and to estimate the composition of tissues. To better understand epigenetic changes in early differentiation events, we investigated DNAm changes during endodermal, mesodermal, and ectodermal differentiation of iPSCs in monolayer culture, as well as in 3D culture as embryoid bodies. Based on these epigenetic changes, we were able to derive a pluripotency score and differentiation scores for the individual germ layers, each consisting of three specific genomic regions (CpG sites). These epigenetic markers can support the quality control of pluripotent stem cell preparations and possibly also estimate their propensity to differentiate into specific germ layers (Schmidt et al., Stem Cell Reports, 2023).

While the selected sites have been established on different cell lines and differentiation conditions, the next step is to further validate this assay on more cell lines, more maintenance medium, and more differentiation protocols to make them universally applicable. To this end, we received funding by the Bundesministerium für Bildung und Forschung (BMBF) to further develop this application into a service or kit that facilitates quality control of iPSC for other scientists.

Schmidt, M., Zeevaert, K., Elsaifi Mabrouk, M.H., Goetzke, R., and Wagner, W. (2023). Epigenetic biomarkers to track differentiation of pluripotent stem cells. Stem Cell Reports 18, 145-158.

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Abstract No. P120**Advanced matrix-free human pluripotent stem cell manufacturing by seed train approach and intermediate cryopreservation**

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Human pluripotent stem cell (hPSCs) derivatives have great potential for advanced drug screening, in vitro disease modelling and regenerative therapies. However, the envisioned routine application of these cells will require robust and economically viable production processes, compatible with industry and regulatory standards.

Instrumented stirred tank bioreactors (STBR) are routinely applied for mammalian cell lines cultivation in the biopharmaceutical industry. This platform has also been adapted to the matrix-free suspension culture of hPSCs and recently enabled advanced high density bioprocessing of hPSCs by metabolic control and in silico modelling by our group.

To further close the gap between the research state and industry-compliance, we here demonstrate a seed train approach to ensure straightforward process upscaling. Chemical, STBR-controlled dissociation of the matrix-free cell-only hPSC aggregates was established; this crucially supported efficient single cell recovery and process control at each upscaling step.

In contrast to prior suspension culture processes, which relied on conventional monolayer culture (2D) for process inoculation, our advanced strategy enabled the uninterrupted maintenance of exponential hPSC cell growth for 8 passages (4 days per passage; 32 days in total) in serial suspension culture. Furthermore, we show the applicability for intermediate high-density cryopreservation of suspension-derived hPSCs followed by the direct re-inoculation of 3D suspension culture in STBRs, thereby entirely excluding the requirement of 2D cultivation. Gene expression profiling reveals novel insights into molecular mechanisms associated with continuous hPSCs 3D culture compared to conventional 2D controls; importantly, hPSCs' karyotype stability and differentiation potential was fully maintained after long-term suspension culture.

By completely omitting the need for conventional 2D matrix-dependant cultivation of hPSC, the novel culture strategy fosters process automation and facilitates the development of GMP-compliant closed system manufacturing, paving the way for hPS cells expansion and differentiation at clinically relevant conditions and quantities.

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Abstract No. P121**Differentiation of iPSCs to functional iNK cells in suspension and applications in cancer research**

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Pluripotent stem cells (PSCs) are a renewable cell source that could be used to generate cell therapies to treat many diseases including cancer. Numerous methods have been established to generate different cell types however significant challenges remain to ease the transition from discovery to commercial manufacturing. Recently we developed CTSTM StemScale™ PSC suspension culture medium, designed for CGT manufacturing that enables the large-scale culture of PSCs. Natural killer (NK) cells are innate, cytotoxic lymphoid immune cells that can kill malignant cells without HLA matching and are a major focus for allogeneic therapy development. NK cell therapy clinical trials indicate that ~5x10⁶ - 1x10⁸ NK cells per dose may be required for effective treatments. Yet requirements such as donor sourcing and successful expansion hamper the ability to efficiently generate large quantities of functional NK cells. Here, we describe a method to produce PSC-derived NK (iNK) cells, from CTS-StemScale suspension cultures enabling the generation of highly enriched, functional iNKs in a scalable culture format. PSCs grown in suspension as spheroids were induced using growth factor cocktails to differentiate into CD34+ hematopoietic progenitor cells and subsequently to CD56+ iNK cells without the use of feeder cells. Further culture of iNK cells in CTSTM NK-Xpander™ led to significant enrichment of CD56+CD3- and CD56+CD16+ phenotypes. The cytolytic potential of these iNKs was further demonstrated by their ability to kill K562 cancer cells as well as patient-derived 3D colon tumoroids. In summary, the use of CTS StemScale highlights the potential for feeder-free PSC suspension cultures to be differentiated into cytolytic iNKs at scale.

P122 – P126 Somatic stem cells & development

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Sara Zocher

Abstract No. P122**Reconstructing the development of liver architecture at single-cell resolution***Gosia Golda^{1, 2, *}, Lisa Marschhäuser², and Dominic Grün²*¹Max Planck Institute of Immunobiology and Epigenetics, Freiburg²Würzburg Institute of Systems Immunology

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The liver is the largest visceral organ responsible for essential metabolic processes and has a unique regenerative capacity after damage. Understanding liver development can shed light on new therapeutic possibilities for improving liver regeneration. However, our understanding of the differentiation and maturation pathways of liver cell types remains incomplete. One important aspect of liver development is the establishment of zonation, where hepatocytes organize into distinct functional domains. Although studies have provided insights into adult metabolic zonation, the mechanisms driving early liver zonation during development remain largely unexplored. Here, we addressed this limitation by investigating the developmental trajectories of liver stem and progenitor cells at single-cell resolution. Analyzing approximately 50,000 single-cell transcriptomes from mouse embryos at stages E8.5 to E15.5, we discovered substantial heterogeneity among developing liver epithelial cells with varying cell fate potential reflected by differential organoid forming capacity. In particular, we identified a population of hepatoblasts present from E10.5 onwards, exhibiting an early zonation-associated gene signature, indicating that liver zonation initiation occurs earlier than previously thought.

In summary, our study provides a comprehensive single-cell resolution roadmap of liver development, shedding light on the organization of primitive hepatic structures and the establishment of fetal liver zonation. Our findings challenge prevailing notions that liver zonation primarily occurs postnatally and shed light on the early stages of zonation establishment during embryonic development. These insights not only enhance our understanding of liver organogenesis but also provide valuable insights for future interventions targeting liver regeneration and disease.

Abstract No. P123**Spatial transcriptomics of developing pancreas: a long journey for spatiotemporal mapping of morphogenesis and differentiation**

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Single-cell RNA sequencing has been a prevailing method to identify and discover cell types in an organ and has been generated a vast amount of data across tissues and species. However, some caveats, such as loss of positional arrangements of cells and under- and/or over-representation of certain cell types, are inevitable due to cell dissociation.

Spatial transcriptomics is an emerging, cutting-edge technology enabling not only transcriptomics but also spatial mapping of cells in an organ. Recent advancement of this method has resulted in data visualization in single-cell resolution.

Pancreas is an organ consisting of exocrine and endocrine cells, which arise from pancreatic progenitor cells during development. Architectural organization of branched pancreas with terminal acini connected to ductal network and nearby islets of Langerhans coincides with cell differentiation in embryos. Although snapshots of different cell type emergence in pancreas have been shown by multiple techniques and animal models, spatial transcriptomics in high resolution has not yet been tested. Here, we use Visium HD (10X Genomics) with formalin-fixed paraffin-embedded sections of embryonic pancreata (E12.5, E14.5, E16.5, E18.5) for spatiotemporal mapping of morphogenesis and differentiation in development. We are in the process of cell type analysis and mapping after clustering of transcriptomics data based on cell segmentation in hematoxylin-and-eosin-stained images. Our preliminary data confirm lineage progression of bi-potent progenitors, early acinar progenitors, early and late islets, and surrounding mesenchyme. One of technical difficulties is to prepare samples with a good RNA quality, especially later stages of pancreas at E16.5 and E18.5, due to the prevalent expression of RNases in acinar cells and slow penetration of fixative. We are seeking enhanced sample preparation procedures for the late stages of pancreas samples. When samples are ready at different stages, spatial transcriptomics will be an excellent benchmark for unbiased spatiotemporal development of the organ.

Abstract No. P124/T20**Epithelial-stromal communication via BMP signaling is a critical regulator of site specificity in the mouse oral mucosa**

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The mouth or oral cavity consists of the tongue, cheeks, gums, and palate. Without these specialized tissues, eating, tasting, and speaking would not be possible. As a multifunctional organ, the oral cavity has a high degree of structural heterogeneity. Each oral site is unique and contains distinct niches for oral epithelial stem cells. Despite their importance, our understanding of oral stem cell niches remains limited. Here, we use single-cell and spatial transcriptomics, and organoid technology to define the molecular mechanisms governing oral site-specific stemness. Using a Ki67 knock-in mouse model, we found that proliferation in the epithelial basal layer is site-specific, with the dorsal tongue being the most proliferative tissue and the buccal mucosa being the least proliferative. We then generated a comprehensive single-cell atlas of major oral sites in the mouse. Our atlas comprises 100,000 cells from all major cell lineages. Sub-clustering of epithelial cells confirmed site-specific molecular signatures of stemness and differentiation. Ligand-receptor analysis revealed robust communication between epithelial basal cells and stromal cells. Subsequent sub-clustering of stromal cells revealed that oral fibroblasts have site-specific gene expression profiles, including expression of bone morphogenetic protein (BMP) signaling molecules. The BMP antagonist Noggin was robustly expressed in all oral sites except the buccal mucosa. BMP ligands likewise showed oral-site-specific expression. For instance, BMP5 transcripts were enriched in tongue fibroblasts, whereas BMP7 robustly expressed by palatal fibroblasts. To functionally assess BMP signaling, we established site-specific epithelial organoid cultures. Organoid-forming efficiency assays showed that tongue and palate organoids were sensitive to BMP inhibition, whereas buccal mucosa organoids grew independently of BMP modulation. Our comprehensive cell atlas of the mouse oral mucosa revealed a critical role of epithelial-stromal communication via BMP signaling for site-specific stemness. It represents a rich resource for dissecting other key modulators of site specificity in the mouse oral mucosa.

Abstract No. P125**The power of BMP: inducing a quiescent-like state in distinct human neural stem cells**

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Quiescence is a reversible state characterised by profound metabolic, transcriptional and signaling changes. In adult tissues, quiescence decelerates stem cell exhaustion and contributes to homeostasis and regeneration. During development, neural stem cells (NSCs) acquire quiescence in distinct niches in the mouse brain to ensure lifelong generation of new neurons. Adult neurogenesis has also been reported in humans, but the origin of new neurons remains unknown. This is due to a combination of poor accessibility of human brain tissue and the lack of bona fide NSC markers. Therefore, we tested the competence of human NSCs (hNSCs) to acquire and maintain quiescence using an in vitro approach.

Mouse NSCs from embryonic and adult origin acquire a quiescence-like state in vitro upon addition of BMP4 for 72h. We exposed hNSCs of different origins to BMP4 and assessed different quiescence hallmarks like decreased proliferation, upregulation of quiescence-associated markers (GFAP, ID4) and the ability to re-enter the cell cycle (re-activation). We confirmed that only radial glia-like (and not neuroepithelial-like) NSCs drop proliferation and upregulate GFAP in response to BMP4. Furthermore, correct patterning to specific brain areas might be required for re-activation. We observed that a subset of genes strongly associated with mouse NSC quiescence, such as ID4, are not upregulated in hNSCs in response to BMP4. Preliminary bulk and scRNAseq data showed differences in the transcriptional response to BMP between mouse and human NSCs. We are currently using transcriptomic approaches to identify markers of hNSC BMP-induced quiescence and generate a transcriptional signature to explore the potential presence of quiescent NSCs in published human datasets. This will help us determine whether hNSCs acquire a quiescence signature during development and whether this allows them to be maintained throughout life, a requirement for them to be the origin of new neurons in the adult human brain.

Abstract No. P126/T19**Lifelong persistence of nuclear RNAs in the rodent brain**

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Genomic DNA that resides in the nuclei of mammalian neurons can be as old as the organism itself. Also some nuclear proteins are known to be long-lived and contribute to life-long epigenetic regulation. However, the life span of nuclear RNAs, which are critical for proper chromatin architecture and transcription regulation, has not been determined in adult tissues. In this work, we identified and characterized nuclear RNAs that do not turn over for at least 2 years in the mouse brain. These long-lived RNAs were stably retained in nuclei in a neural cell type-specific manner, with postnatally generated granule cells and quiescent neural stem cells in the hippocampus showing high capacity for the RNA long-term retention. Using next generation sequencing, we revealed the molecular identity of long-retained RNAs in quiescent neural stem cells and identified many non-coding RNAs, predominantly repetitive RNAs such as major satellite RNAs, as particularly stable RNAs. Knock-down and overexpression of major satellite RNAs confirmed their long-term retention in quiescent neural stem cells. Perturbation experiments further demonstrated that major satellite RNAs are required for heterochromatin maintenance and for the reactivation of quiescent neural stem cells into proliferation, indicating their functional significance for the maintenance of neural stem cells. These results suggest that the life span of neural cells may depend not only on the longevity of DNA for the storage of genetic information and on long-lived proteins for epigenetic regulation, but also on the extreme stability of RNA for the functional organization of chromatin.

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National Office Gene & Cell-Based Therapies GCT Germany

Who we are

The National Office Gene & Cell-Based Therapies | GCT Germany was established as part of the National Strategy for Gene and Cell Therapies. We see ourselves as a nationwide contact point and platform for national and international actors, stakeholders from the fields of science, industry, politics, society, authorities, foundations and patient organizations.

Our vision

We are unveiling the vast potential that gene and cell therapies offer for patient care, the healthcare economy and Germany's pharmaceutical landscape. By uniting local expertise and excellence in this highly innovative and forward-thinking field we are facilitating a new era of collaboration. Ensuring that patients can seamlessly and swiftly access gene and cell therapies in the future. Together we are creating a pioneering and patient-centered healthcare.

What we do & what we offer

- 1 Platform & Contact Point
- 2 National GCT Atlas
- 3 Patient & Stakeholder Engagement
- 4 Networking & Cooperation
- 5 Education & Training, e.g. GeneNovate
- 6 Science Communication
- 7 Regulatory Support Unit

What is GeneNovate about?

- 1 national entrepreneurship program GCT
- 2 For early career scientists & physicians
- 3 Collaboration across Germany
- 4 Coaching by mentors
- 5 6 modules focusing on a.o.:

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