16th Annual Meeting
Dutch Society for
Stem Cell Research

October 17th, 2025

University Medical Center Utrecht

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Our Mission: To promote the quality of stem cell research and to disseminate knowledge in the Netherlands

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Program 16th Annual Meeting
of the Dutch Society for Stem Cell Research

October 17th 2025, Roze Collegezaal / Pink Lecture Hall, Heidelberglaan 100, AZU/UMC Utrecht

9:00	**Registration opens**
9.30-09:50	Coffee/tea
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	Session 1 Chair: Hendrik Marks
10.00-10.15	Viviana Meraviglia (Post doc) *Characterization of cardiac models from an isogenic allelic series of LMNA-mutated hiPSC lines generated using the novel and highly-efficient targeting platform, STRAIGHT-IN*
10.15-10.30	Anna Bertocci (PhD) *iPSC Population Dynamics In The "Village-In-a-Dish" Model*
10.30-10.45	Casper de Visser (PhD) *Dissecting the sources of variation in neuronally differentiated iPSC lines through multi-omics analysis*
10.45-11.15	**Keynote lecture – Laura Pellegrini** Center for Developmental Neurobiology, King's College, UK ***Building and breaking the blood-CSF barrier:*** ***Human choroid organoids reveal injury-repair dynamics***
11.15-11.45	Coffee/tea
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12.00-12.15	Charlotte Brice (PhD) *Ultrafast Volumetric Bioprinting Enables Precise Morphological Control of Perfusable Stem Cell-Derived Microphysiological Endocrine Pancreatic Units*

12.15-12.30 Laura van Dijk (PhD)
Modeling Respiratory Virus Infections in Nasal Organoids

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From stem cells to somites: revealing genetic and environmental factors of human embryogenesis

14.15-14.30 Mathangi Lakshmipathi (PhD)
Time to change: how the foetal and prepubertal testicular somatic niche prepares for sperm formation

14.30-14.45 Ridvan Cetin (PhD)
Distinct Roles of Atf3, Zfp711, and Bcl6b in Early Embryonic Hematopoietic and Endothelial Lineage Specification

14.45-15.15 **Keynote lecture – Cedric Blanpain**
Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB), Belgium
Stem cell plasticity during tumor initiation

15.15-15.45 Coffee/tea

Session 4 Chair: Emile van den Akker

15.45-16.00 Virgínia Andrade (Post Doc)
Spatiotemporal proteomics reveals dynamic antagonistic gradients shaping signalling waves

16.00-16.15 Hiromune Eto (post Doc)
Frequency encoding regulates cell type composition in the small intestine

16.15-16.30 Nick Bovee (PhD)
A PRC2 roadmap: Proximity proteomics of PRC2 uncovers its regulatory interactome from naïve human pluripotency to early differentiation

16.30-17.00 **Keynote lecture – Ruben van Boxtel**
Princess Máxima Center for pediatric oncology, Utrecht
Tracing stem cell fates with single-cell genomics

17.00-17.05 Award ceremony: Ans van Pelt

17.05-18.00 Closing and Drinks: Koen Braat

Keynote speakers

Keynote lecture - Laura Pellegrini

Center for Developmental Neurobiology, King's College, UK

Building and breaking the blood-CSF barrier:
Human choroid organoids reveal injury-repair dynamics

Keynote lecture - Cedric Blanpain

Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB), Belgium

Stem cell plasticity during tumor initiation

Keynote lecture - Ruben van Boxtel

Princess Máxima Center for pediatric oncology, Utrecht

Tracing stem cell fates with single-cell genomics

Laura Pellegrini

Laura Pellegrini is a Group Leader at the Centre for Developmental Neurobiology (CDN), King's College London. She established her lab in October 2023 after receiving the Wellcome Trust Career Development Award. Her research investigates human brain development using cerebral and choroid plexus organoids.

Previously, Laura conducted her postdoctoral research at the MRC Laboratory of Molecular Biology in Cambridge, in the lab of Dr. Madeline Lancaster. There, she developed an in vitro model of the choroid plexus to study cerebrospinal fluid (CSF) secretion. This breakthrough, published in Science (Pellegrini et al., 2020a), earned her the NC3Rs International Award (2021) and was later applied to investigate SARS-CoV-2 infection in the brain (Pellegrini et al., 2020b).

Her current research explores the development of the choroid plexus in humans, the secretion of disease-related biomarkers into CSF, and the mechanisms underlying barrier repair following brain injury.

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Keynote lecture - Laura Pellegrini
Center for Developmental Neurobiology, King's College, UK

Abstract **Building and Breaking the Blood–CSF Barrier:**
Human choroid plexus organoids reveal injury-repair dynamics

The choroid plexus (ChP) is a vital tissue located in the brain ventricular system. This tissue displays a number of important functions such as forming a protective epithelial barrier and secreting the cerebrospinal fluid (CSF). To explore the development and function of the human ChP, we recently established a protocol to generate human ChP organoids using a combination of signalling molecules that are physiologically present during the stages of development of this tissue. These organoids recapitulate fundamental functions of ChP such as CSF secretion and formation of a tight epithelial barrier selectively permeable to small molecules. To characterise the development of ChP cell populations over time, we have performed a longitudinal scRNA sequencing analysis of the organoids. We found that, similarly to ChP tissue in vivo, organoids stop proliferating in vitro and develop features of mature tissue, comparable to adult human ChP. Next, we used this model to investigate the epithelial response to mechanical injury and we discovered that ChP epithelial cells secretes cytokines and chemokines in response to injury and undergo a transitional state involving tissue remodelling leading to repair. Finally, we are using this model as a platform to study mechanisms of peripheral regulation in the ChP in response to environmental stimuli, CNS-targeting drugs and inflammatory agents. In conclusion, ChP organoids have been proven useful in multiple applications and represent a powerful tool to study not only developmental diseases but also tissue repair response.

Cedric Blanpain

Cedric Blanpain graduated as a Medical Doctor (1995), received his PhD in Medical Sciences (2001) and was board certified in internal medicine (2002) from the Université Libre de Bruxelles (ULB), Belgium. He performed a postdoctoral training in the laboratory of Elaine Fuchs, at the Rockefeller University from 2002 to 2006. Cédric Blanpain started his laboratory in October 2006 as "chercheur qualifié" of the Belgian Fonds National de la Recherche Scientifique (FNRS).

Cedric Blanpain received several prestigious and highly competitive awards including EMBO Young investigator award, ERC starting, ERC consolidator and ERC advanced grants, the outstanding young investigator award of the ISSCR 2012, the Liliane Bettencourt award for life sciences 2012, the Joseph Maisin Award for basic biomedical Science 2015, the Francqui prize 2020, the European Association for Cancer Research's Mike Price gold medal 2022, the momentum award of the ISSCR 2023 and the Fondation ARC Léopold Griffuel Award for basic research 2024 . He has been elected member of the EMBO, the Belgian Royal Academy of Medicine, the Academia Europaea, the French Academy of Science, and the American Academy of Arts & Sciences.

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Keynote lecture - Cedric Blanpain
Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB), Belgium

Abstract **Stem cell plasticity during tumor initiation**

Glandular epithelia, such as the mammary gland and the prostate, develop from multipotent stem cells (SCs), which are replaced in adult life by different types of lineage-restricted basal and luminal unipotent SCs. Upon oncogenic hits, the lineage restricted SCs can re-activate multipotent features reminiscent of the embryonic progenitors. However, the molecular mechanisms regulating cell plasticity and oncogene-induced reprograming during mammary gland and prostate tumorigenesis are poorly understood. I will present new studies combining lineage tracing, clonal analysis, single cell RNA sequencing, chromatin profiling as well as in vitro and in vivo functional experiments investigate the cellular and molecular mechanisms regulating cell fate and plasticity during the early stage of mammary gland and prostate tumor initiation. Understanding how the mechanisms regulating SC fate and plasticity are corrupted during tumor initiation will have important implications for cancer prevention and therapy.

Ruben van Boxtel

Ruben van Boxtel is Group Leader at the Princess Máxima Center for Pediatric Oncology and Professor of Stem Cell Genomics at the Faculty of Veterinary Medicine, Utrecht University. His research focuses on uncovering the origins of childhood cancers and the long-term effects of cancer therapy by studying the genomes of human stem cells at nucleotide resolution. His group develops and applies single-cell genomic approaches, retrospective lineage tracing, and mutational signature analyses to understand why children develop leukemia and lymphoma, and how cancer treatments impact the hematopoietic system.

Among his key discoveries are the identification of carcinogenic mutational signatures caused by genotoxic gut bacteria and antiviral drugs, as well as insights into how chemotherapy induces clonal evolution and accelerates stem cell aging in childhood cancer survivors. These findings provide fundamental understanding of cancer origins and therapy-related toxicities, with direct relevance for diagnosis, prevention, and the development of safer treatments.

Van Boxtel has been recognized with several prestigious awards, including an ERC Consolidator Grant (2019) and the NYSCF Robertson Stem Cell Investigator Award (2022).

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Keynote lecture - Ruben van Boxtel
Princess Máxima Center for pediatric oncology, Utrecht

Abstract **Tracing stem cell fates with single-cell genomics**

Understanding how cancer arises is key to developing better prevention and treatment strategies. Our research focuses on uncovering the origins of cancer by analyzing DNA at the single-cell level. We aim to answer two fundamental questions: (1) why do children develop cancer despite minimal age-related DNA damage, and (2) what processes drive the DNA mutations that are required for carcinogenesis?

To address these questions, we apply single-cell whole-genome sequencing to patient samples and use mutational patterns to reconstruct the earliest stages of cancer. This approach allows us to trace back its origins and identify critical events that lead to malignancy. In parallel, we use genome editing technologies to introduce cancer-driving mutations into in vitro stem cell models or expose them to potential carcinogens, modeling the mechanisms that shape cancer development.

By combining retrospective lineage tracing with experimental validation, our work provides unique insights into the preventable causes of cancer and the molecular mechanisms that enable tumors to arise and evolve.

Abstracts - Invited speakers

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Characterization of cardiac models from an isogenic allelic series of LMNA-mutated hiPSC lines generated using the novel and highly-efficient targeting platform, STRAIGHT-IN

Viviana Meraviglia, Albert Blanch-Asensio, Richard Davis, Christine L. Mummery, Milena Bellin

Bellin/Mummery group (Anatomy and Embryology department), LUMC

Cardiolaminopathy is a form of dilated cardiomyopathy (DCM) associated with conduction defects caused by mutations in LMNA, encoding Lamin A/C protein. Human induced pluripotent stem cells (hiPSCs) are valuable tools for in vitro disease modelling, and genetically modified hiPSCs carrying LMNA variants would be particularly useful for this purpose.
Here, the STRAIGHT-IN platform (Serine and Tyrosine Recombinase Assisted Integration of Genes for High-Throughput INvestigation) was applied, combining CRISPR/Cas9-mediated homologous recombination with different classes of site-specific recombinases to efficiently and precisely integrate genomic fragments. We used STRAIGHT-IN to simultaneously generate a library of 12 genetically matched hiPSC lines carrying multiple heterozygous mutations in LMNA gene.
For further characterization, we selected two LMNA-mutated lines (H222P and Q493X) alongside with WT isogenic control. LMNA-mutated lines and WT isogenic control were successfully differentiated into four different cardiac cell types: cardiomyocytes, epicardial cells, cardiac fibroblasts and endothelial cells. Both LMNA mutated lines revealed abnormal nuclear morphology and increased DNA damage in all the four different cardiac cell types in 2D mono-culture compared to WT isogenic control.
Three-dimensional (3D) cardiac microtissues (MTs) including LMNA mutated cardiomyocytes have been successfully generated and preliminary functional characterization on 3D MTs has been carried out; this showed abnormal electrical properties and higher propensity to arrhythmia in spheroids containing LMNA mutated cardiomyocytes compared to WT.
STRAIGHT-IN allows simultaneous generation of a panel of 12 isogenic hiPSC lines carrying selected LMNA mutations rapidly, efficiently and cost-effectively, thus facilitating the production and characterization of specific mutated hiPSC lines for disease modelling.

iPSC Population Dynamics In The “Village-In-a-Dish" Model

Anna Bertocci[1], Li You[2], Sara Costa[3], Pierangela Chiafele[2], Mehrnaz Ghazvini[4], Joana de Pinho Gonçalves[3], Miao-Ping Chien[3], Joyce van Meurs[1,5], Roberto Narcisi[1]

[1]Department of Orthopedics & Sports Medicine, Erasmus MC; [2]Department of Molecular Genetics, Erasmus MC; [3]Department Computer Science (VMB), TU-Delft; [4]iPSC core facility, Erasmus MC, [5]Department of Internal medicine, Erasmus MC

The “village-in-a-dish” (ViaD) model provides a new platform for in vitro population genetics and uncovers how genetic variation influences gene expression. Unlike standard culture, induced pluripotent stem cell (iPSC) lines from genetically diverse donors are co-cultured in the same dish. This approach increases throughput, reduces variability, and lowers costs, making it valuable for population-scale studies and modeling complex diseases. Despite its promise, little is known about how donor–donor interactions might confound interpretation of genetic effects on gene expression. To address this, we investigated the impact of co-culturing iPSC lines from different donors from multiple perspectives. First, we examined qualitative composition of iPSC colonies within a ViaD setting. Using cytoplasmic dyes and confocal microscopy, we found clusters could consist of cells from one or multiple donors, and time-lapse imaging revealed cluster composition arises largely by chance. Second, we used the Functional Single Cell Selection pipeline (FUN-seq) to compare transcriptional profiles of donors in mono- versus multi-donor clusters, showing composition did not significantly influence transcriptomic profile. Third, we observed the ViaD setting does not affect donor-specific growth rates. Overall, our data support robustness of the village model.

Dissecting the sources of variation in neuronally differentiated iPSC lines through multi-omics analysis

Casper de Visser[1], Lisa Rahm[3], Elly Lewerissa[4,5], Rachel Mijdam[2], Cenna Doornbos[1], Junda Huang[1], Luke O'Gorman[1], Firdaws Badmus[1], Hans van Bokhoven[3], Nael Nadif Kasri[4,5], Dirk Lefeber[2,3], Peter A.C. 't Hoen[1], Alain van Gool[3], Purva Kulkarni[3]

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Induced pluripotent stem cells (iPSCs) are powerful in vitro models for studying human disease mechanisms across various cell types, within a patient's own genetic context. However, many studies report unexplained variation in such models. This is particularly pressing in omics studies, where sources of variation may obscure disease-specific signatures. Our study aims to uncover the main sources of variation in iPSC models to support the development of reliable disease models.
We analyzed multiple omics layers (lipidomics, metabolomics, proteomics, transcriptomics, and epigenomics) from iPSC-based models of neurological disease, including multiple patients per disease, multiple clones per patient, and multiple differentiation experiments per clone (biological replicates). We focused on Ngn2-transduced iPSCs from patients diagnosed with Myotonic Dystrophy Type 1 (DM1), chromodomain-DNA-helicase-binding protein 2 (CHD2) related disorder, and N-acetyl-d-neuraminic acid synthase congenital disorders of glycosylation (NANS-CDG), at day 7 of neuronal differentiation.
We observed large differences in neuronal marker expression across cell lines, even among clones from the same donor, while biological replicates showed minimal variation. Omics correlations revealed clone-level variability comparable to inter-patient differences. Multi-Omics Factor Analysis (MOFA) revealed the main sources of variation shared among omics layers. The second-largest latent factor was associated with neuronal differentiation, indicating that differentiation is a major source of variation in iPSC-based studies. We propose novel biomarkers across omics layers to monitor differentiation status.
Despite variability, robust molecular disease signatures were identified using nested linear mixed models. Our findings emphasize the importance of including multiple donors and clones, rather than repeating differentiation experiments, to ensure statistically valid conclusions.

KLHL24 Mutation Drives Intermediate Filament Degradation, Mitochondrial Dysfunction and Fibrosis in heart failure patients

Veronika Ramovs1,2, H. Sophia Chen1,3, Athina Patra1, Rayman Tjokrodirijo4, Peter van Veelen4, Aat A Mulder5, Roman I Koning5, Lauran Stöger6, Catalina Hubner7, Rodrigo Ibañez-Arenas8,9, Bernardo Morales Catalan10, Pilar Morandé7, Rosario dell'Oro7, Cristian Poblete11, Andrés Schuster9, María Joao Yubero7,12, Francis Palisson7,13, Cristina Has14, Monique Jongbloed1,3, Ignacia Fuentes7,15,16, Christine L Mummery1,2, Karine Raymond1,2,17, *

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A striking aspect of epidermolysis bullosa patients with a mutation in KLHL24 (KLHL24mut) is their life-threatening deterioration of heart function. KLHL24 is part of the ubiquitin-proteasome system and acts as a substrate-specific adaptor protein for E3 ubiquitin ligase. KLHL24mut represents a gain-of-function mutation, with associated cardiac and skin pathologies arising from the excessive degradation of its target proteins. Although reduced desmin levels in cardiomyocytes (CMs) have already been documented, the involvement of additional mechanisms in KLHL24mut-driven heart pathology remains unexplored. To better understand the pathophysiology of KLHL24 mut-driven heart failure, we integrated proteomic analyses of heart tissue of two KLHL24mut patients with human induced pluripotent stem cell (hiPSC) models carrying KLHL24mut. Mass spectrometry analysis of CMs derived from patient hiPSCs mirrored the proteomic profile of their left ventricle tissue. KLHL24mut resulted in a reduction of several intermediate filament (IF), mitochondrial and muscle fiber proteins as well as the emergence of an early fibrotic signature. By utilizing various hiPSC-derived cardiac models, we uncovered that KLHL24mut mediates the excessive degradation not only of desmin, but also of synemin and vimentin. In cardiac tissue, the effects of KLHL24mut extend beyond CMs or IF proteins, affecting cardiac fibroblasts and a wide array of proteins. This leads to impaired PKA signalling, disrupted mitochondrial function and localization, alterations in autophagy and sarcomere structure, and a pronounced fibrotic signature. Importantly, the close similarity between hiPSC-derived CMs and patient cardiac explants validates hiPSC-derived CMs as a relevant model for future mechanistic and therapeutic studies.

Ultrafast Volumetric Bioprinting Enables Precise Morphological Control of Perfusable Stem Cell-Derived Microphysiological Endocrine Pancreatic Units

Charlotte Brice1,2,3, Pere Català1,2, Davide Ribezzi1,2, Gabriel Größbacher1,2, Frank Riemers1,3, Mattie van Rijen1,2, Francesca Stillitano1,4,5, Massimiliano Caiazzo6,7, Bart Westendorp1,8, Riccardo Levato1,2,3

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8. Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

Introduction. Functional islets from induced pluripotent stem cells (iPSCs) hold great promise as a renewable cell source for pancreatic tissue engineering. Despite advances in differentiation protocols, iPSC-derived islets often remain immature and lack the complex microenvironmental cues necessary for full functionality. This limits the development of robust in vitro platforms, impeding deeper insights into islet biology and function. In this study, we present an engineered pancreatic construct that integrates iPSC-derived islets with light-based volumetric bioprinting (VBP) to engineer advanced endocrine units.

Methods. iPSC-derived pancreatic islets were generated using a seven-stage differentiation protocol (Balboa+, 2022). At stage 7, islets were suspended in gelatine methacryloyl and bioprinted using shear stress-free, light-based VBP. Constructs were analysed by immunostaining, single-cell transcriptomics, and glucose-stimulated insulin secretion (GSIS) assays under static and dynamic conditions (+/- GLP-1 analogues or streptozotocin).

Results. VBP enabled rapid fabrication of centimetre-scale constructs (t<30 s), preserving iPSC-islet viability and metabolic activity for at least 21 days. These constructs contained mature, single-hormone producing β-, α-, and δ-cells, confirmed by staining and single-cell transcriptomics. Mathematically-defined gyroids (strut size:750□m, infill:40%) containing a homogenous distribution of iPSC-islets were successfully perfused for up to 21 days and further functionally validated using dynamic GSIS assays. Additionally, these perfusable constructs supported anti-diabetic drug screening as shown by their responsiveness to GLP-1 analogues and streptozotocin.

Conclusion. VBP allows to successfully pattern iPSC-derived islets into geometrically-defined morphologies while retaining their functionality. This technology paves the way for new possibilities on developing novel tissue engineered platforms for disease modelling and therapeutic development.

Modeling Respiratory Virus Infections in Nasal Organoids

Laura L.A. van Dijk1,#, Mark Power1,#, Pau Ribó-Molina1, Jinghsu Zhang1, Debby Schipper1, Ilona I. Tosheva1, Tim I. Breugem1, Stefan van Nieuwkoop1, Corine H. Geurts-vanKessel1, Ralph Stadhouders2, Bernadette van den Hoogen1, Sander Herfst1, Bart L. Haagmans1, Rory D. de Vries1,*, Melanie Rissman1,*

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Viral respiratory tract infections are a leading cause of morbidity and mortality worldwide. Young infants, older adults, and immunocompromised individuals are disproportionally affected; however, in many cases, the factors underlying severe disease are unknown. Here, we established a pipeline for the rapid isolation of nasal organoids from a healthcare worker cohort and employed this to investigate donor-specific differences in susceptibility to respiratory viruses.

Nasal epithelial stem cells from four healthy donors were isolated, expanded, and differentiated at an air-liquid interface (ALI). Basic characteristics such as phenotype and virus receptor distribution were studied. Donor phenotypes varied, from flat to ridged pseudostratified structures, and receptor frequency/distribution for respiratory viruses was variable. ALI cultures were then infected with eight clinically relevant viruses: SARS-CoV, SARS-CoV-2, MERS-CoV, HCoV-OC43, IAV, HRSV, HPIV3, and HMPV. We assessed viral replication kinetics, cytopathic effects, and innate immune responses for up to 96 hours. Long-term cultures were monitored for viral persistence over one month. Susceptibility and permissiveness varied across donors and viruses, often linked to receptor distribution, epithelial differentiation, and cytokine production. Notably, all viruses persisted over a month, showing epithelial cells alone support prolonged infection.

These findings re-emphasize that nasal organoids are an important tool to model respiratory virus infections. Even though organoids from all donors were susceptible to infections with respiratory viruses, intrinsic donor-to-donor variability in nasal epithelial responses to respiratory viruses were detected which underscore the importance of host factors in shaping infection outcomes.

From stem cells to somites: revealing genetic and environmental factors of human embryogenesis

Maria Teresa Alejo-Vinogradova, Lieke A. Lamers, Michiel Vermeulen, Suzan Stelloo

Department of Experimental Urology, RadboudUMC

Stem cell-based human embryo models offer an ethically tractable platform for studying early human development. This study employs somitoids, three-dimensional structures that mimic human somitogenesis, to investigate how transcriptional programs and environmental conditions influence axial patterning and segmentation. We show that the choice of pre-differentiation culture medium impacts the developmental potential of induced pluripotent stem cells (iPSCs), with StemFit medium outperforming mTeSR Plus in generating morphologically robust somite-like structures. Transcriptomic profiling reveals increased expression of somitogenesis-related genes under StemFit conditions, consistent with the segmented phenotype. P300-based proximity labeling, targeting active enhancers, identifies a largely overlapping set of chromatin-associated regulators across conditions, while also uncovering medium-specific transcriptional regulators. Knockout of three candidate genes, BPTF, RBPJ, and CITED2, reveal their essential roles in somite formation. Together, these findings highlight the influence of environmental cues and enhancer-associated networks in early human development and demonstrate somitoids as a scalable system for functional genomics.

Time to change: how the foetal and prepubertal testicular somatic niche prepares for sperm formation

Iris Sanou[1,2,3]†, Mathangi Lakshmipathi[1,2]†, Lisette Schönhage[1,2], Saskia K. M. van Daalen[1,2], Cindy M. de Winter- Korver[1,2], Andreas Meißner[1,2,4], Geert Hamer[1,2], Dirk G. de Rooij[5], Rod T. Mitchell[3,6]*, Callista L. Mulder[1,2]*

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Abstract:
Co-development of male gonadal Sertoli and germ cells is fundamental to male reproductive potential. Due to limited tissue availability and lack of suitable research models, their maturation and proliferation dynamics in foetal and early postnatal testis remain poorly understood, limiting progress in reproductive regenerative medicine.
In this study, we systematically mapped the normal developmental trajectories of the human testis. We utilized quantifiable multiplex immunofluorescence on a unique set of foetal (PCW 7-21) and prepubertal (1.8-13 years) human testicular tissues to assess cell maturation and proliferation states. We identified OCT3/4+ germ cells as the main proliferating germ cell type during foetal development, while MAGE-A4+ spermatogonia divide slowly after the loss of the pluripotency factor both *in utero* and early childhood. This suggests foetal life as a critical window for establishing a sufficient germ cell pool for spermatogenesis. In contrast, immature AMH+ Sertoli cells exhibit proliferation marker Ki-67 at low levels, with a peak in early childhood. A gradual transition from Sertoli cells exhibiting immaturity marker AMH to mature AR expression was observed between 6 and 8 years, coinciding with lumen formation in the tubules and cessation of Sertoli cell proliferation, occurring at an earlier developmental stage than expected. Notably, this data suggest that Sertoli cell maturation precedes the initiation of spermatogenesis by several years, thereby establishing a permissive environment well before puberty.
Our data provide insights into the sequence of maturation events in normal testis development, offering guidance for researchers in reproductive biology and the development of fertility and regenerative treatments.

Distinct Roles of *Atf3*, *Zfp711*, and *Bcl6b* in Early Embryonic Hematopoietic and Endothelial Lineage Specification

Ridvan Cetin[1], Giulia Picco[1], Jente van Staalduinen[1], Eric Bindels[2], Remco Hoogenboezem[2], Gregory van Beek[2], Mathijs A Sanders[2], Yaren Fidan[1], Ahmet Korkmaz[3], Joost Gribnau[1,4], Jeffrey van Haren[1], Danny Huylebroeck[1], Eskeatnaf Mulugeta[1], Frank Grosveld[1,±]

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Hematopoiesis occurs in three consecutive overlapping waves in mammals, regulated by transcription factors. We investigated the role of three relatively poorly studied transcription factors in early embryonic hematopoietic development at single-cell resolution: *Atf3*, *Zfp711* and *Bcl6b*. These transcription factors are upregulated early in development when hematopoietic and endothelial lineages separate from cardiac and other mesodermal lineages. We combined multiplexed single-cell RNA sequencing and flow cytometric analysis with knockouts in *in vitro* differentiating mouse embryonic stem cells to dissect the function of these transcription factors in lineage specification. Δ*Atf3* cells showed increased mesodermal differentiation but decreased endothelial cells and erythro-myeloid progenitors, accompanied by aberrant interferon signaling. Mechanistically, loss of *Atf3* disrupted key hematopoietic regulators (*Runx1*, *Egr1*, *Jun*, *Fos*, *Mafb*, *Batf3*) required for erythro-myeloid progenitors' formation. Δ*Zfp711* cells exhibited increased blood progenitors and erythroid cells but decreased endothelial cells, with a striking shift from *Hoxa*+ mesoderm (allantois, limb-mesoderm) to *Hoxb*+ mesoderm (mesenchyme, epicardium). Notably, *Zfp711* binds the *Atf3* promoter, suggesting a hierarchical regulation. In contrast, Δ*Bcl6b* had no observable effects on early hematopoiesis despite specific expression in hemato-endothelial progenitors.

KEYWORDS: *Atf3*, *Bcl6b*, *Zfp711*, Hematoendothelial Development, Erythro-Myeloid Progenitors, Endothelial-to-Hematopoietic Transition.

Spatiotemporal proteomics reveals dynamic antagonistic gradients shaping signalling waves

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Embryonic development is driven by dynamic protein networks, yet how these dynamics shape morphogenesis is not fully understood. Somitogenesis, the rhythmic segmentation of vertebrate embryos, is governed by signalling gradients and oscillations in the presomitic mesoderm (PSM), but the corresponding protein dynamics remains largely unknown. We developed an integrated proteomics and microfluidics approach to resolve spatiotemporal protein expression in the developing mouse tail embryos. We established a microfluidic system to synchronize oscillations in mouse embryo tail explants grown in 3D, and combined it with mass-spectrometry and RNA sequencing. We found oscillatory proteins and differentially expressed genes along the anteroposterior axis. We revealed an antagonistic, dynamic ligand–receptor expression pattern in R-Spondin/LGR signalling explaining how Wntoscillation amplitude increases despite decreasing ligand levels in anterior PSM. Dynamic ligand expression was validated in mouse gastruloids and perturbation of ligand dynamics reduced oscillation amplitude and impaired somite formation. Our study revealed a previously unrecognized regulatory strategy in which dynamic antagonistic gradients fine-tune signalling strength, providing new mechanistic insight into how protein dynamics control tissue patterning. Our dataset can serve as foundation for mechanistic investigations of mammalian somitogenesis. This approach combining microfluidics-based synchronization of signalling dynamics in multicellular systems with omics analyses can be used to study dynamics in other contexts such as in tissue homeostasis and regeneration.

Frequency encoding regulates cell type composition in the small intestine

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Notch signalling is one of the most important signalling pathways that regulates multiple cell fate decisions in the intestine. Notch plays a pivotal role in maintaining the stem cell niche, and it is also is essential for differentiating these cells into absorptive or secretory lineages. During homeostasis, Notch signalling coordinates multiple decisions to preserve intestinal tissue organization. However, the precise mechanisms by which Notch maintains this balance is far from understood.

In this study, we generated a transgenic mouse line expressing a fluorescent reporter for Notch pathway activity, from which we derived intestinal organoids for live-cell imaging. Single-cell tracking revealed, for the first time, that Notch signalling exhibits oscillatory behaviour in certain epithelial cell types. To investigate the functionality of these oscillations, we developed a microfluidics system to dynamically modulate the oscillation frequency, and observed their effects on tissue maintenance. With this, we demonstrated that specific frequencies of Notch signalling oscillations regulate differentiation of certain specialized cells in the small intestine. This research reveals a new mechanism of how Notch regulates cell fate decisions in the intestine. It also establishes a new in vitro platform technology to control of signalling processes in multicellular system.

A PRC2 roadmap: Proximity proteomics of PRC2 uncovers its regulatory interactome from naïve human pluripotency to early differentiation

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Polycomb Repressive Complex 2 (PRC2), which catalyzes H3K27me3 through its EZH2 subunit, is one of the best known regulators of lineage-specific gene silencing during early embryogenesis. While PRC2's chromatin-based functions are well characterized, how its activity is regulated and coordinated in the early embryo remains incompletely understood. To address this, we engineered human pluripotent stem cells (hPSCs) with a miniTurboID knock-in at the EZH2 locus, enabling proximity labeling and mass spectrometric identification of proteins within the PRC2 microenvironment. In primed pluripotency, this approach revealed a rich network of interactors, including canonical PRC2 subunits, PRC1 components, transcription factors, and chromatin regulators connected to mRNA processing and developmental pathways such as WNT and TGF-β signalling. To investigate lineage-specific regulation, we profiled the PRC2 proxeome in cardiomyocytes (mesoderm) and neural progenitor cells (ectoderm). We further chemical resetted the hPSCs to naïve pluripotency, modeling the pre-implantation epiblast, and characterized the PRC2 interactome in this ground-state context. Together, these analyses provide a comprehensive roadmap of cell state-specific PRC2 interactions, highlighting dynamic regulatory networks that shape PRC2 function during early embryogenesis and lineage commitment.

Abstracts – Other submitted abstracts

Content – Other submitted abstracts

Piecing Cornelia de Lange syndrome together: one mosaic brain organoid at a time

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Cornelia de Lange syndrome, a developmental disorder characterized by cognitive impairment, typical facial features, and growth delay, is in >70% of cases associated with mutations in the cohesin-loading factor NIPBL. Around 20% of NIPBL-CdLS cases are mosaic, meaning the variant is found only in a fraction of cells. Similar to non-mosaic CdLS, mosaic patients span the full phenotypic spectrum from mildly affected to severe, hinting at potential non-cell autonomous effects. We model mosaic CdLS in iPSC-derived mosaic cortical organoids composed of control and NIPBL variant cells, fluorescently labelled to visually distinguish between genotypes. Our results show that NIPBL variant organoids are smaller and have fewer progenitor zones, potentially caused by a premature shift of mitotic spindle orientation in NIPBL variant cells. This recapitulates CdLS patient symptoms, such as microcephaly. Mosaic organoids show an intermediate phenotype, where NIPBL variant cells are outcompeted over time. Furthermore, we set up sparsely labelled mosaic organoids, where only few cells are labelled, to image and track migration and morphology of single cells, as well as interaction dynamics, in live tissue. In conclusion, we generated mosaic brain organoids that recapitulate patient phenotypes and set up promising systems to further explore the non-cell autonomous effects of NIPBL variant cells in brain organoids with confocal microscopy and single-cell RNA sequencing.

Cell Tropism, Replication Dynamics, and effect on neural network activity to Seasonal and Pandemic Influenza A Virus Infection in a hiPSC-Derived Neural Co-Culture Model

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Influenza A virus infection is associated with a wide variety of neurological complications, which vary from severe encephalitis to mild complications like impaired cognitive functioning. There is substantial evidence that seasonal and pandemic influenza A viruses can enter the central nervous system (CNS) through cranial nerves, but once inside the CNS, the cell tropism, replication efficiency and functional consequences are largely unknown. Therefore, the aim of our study was to investigate the interaction of seasonal and pandemic influenza A viruses with cells of the CNS using a human induced pluripotent stem cell (hiPSC)-derived neural model, consisting of neurons and astrocytes. Analyses included electrophysiology to asses neural functioning using a multi-electrode array (MEA) system. All viruses were able to infect neurons in the co-culture model, although this infection did not result in efficient replication and release of progeny virus. Additionally, infection did not result in visible cell death or apoptosis. However, functional analyses revealed that pandemic influenza A virus infection resulted in electrophysiological changes, including a reduction in firing rate, burst frequency and network burst frequency in the first days after inoculation. Furthermore, a partial reduction of neural excitability in inoculated cultures was observed after employing long-term potentiation protocols. This study shows that seasonal and pandemic influenza A viruses can disrupt neural homeostasis, without efficient virus replication or the induction of cell death. However, these functional changes in neural activity can contribute to cognitive problems during influenza A virus infections in the acute and potentially post-acute phase of the infection.

Tubuloids as a human disease model to study drivers of chronic kidney disease progression

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Chronic kidney disease (CKD) is a progressive disease affecting approximately 880 million people worldwide. The progressive nature of the disease can lead to kidney failure, requiring dialysis or transplantation, which significantly decreases quality of life. Therefore, there is an unmet need to better understand the factors driving CKD progression as well as biomarkers to predict CKD progression and guide its treatment. It has been hypothesized that factors in urine can directly contribute to tubular injury. However, models that recapitulate human tubules to investigate this were lacking until recently. Tubuloids, which represent an in vitro 3D model of adult tubular epithelial cells, have emerged as a promising tool for studying disease processes in the kidney tubule. In this study, a polarized tubuloid epithelium on Transwell™ filters with an accessible apical and basolateral side has been developed to test the effects of potential disease-inducing factors in urine. To create tubuloids, primary epithelial cells were isolated from healthy kidney biopsies and embedded in a basement membrane matrix that supports three-dimensional (3D) growth. The generated tubuloid cultures were dissociated into single-cell suspensions, seeded onto Transwell™ filters, and expanded into a confluent two-dimensional (2D) monolayer. These monolayers were then exposed to artificial urine (AU), allowing full control over urine composition during experimentation. Differentiated 2D cultures expressed segment specific transporters for the proximal tubule (SGLT2), thick ascending limb (NKCC2), distal convoluted tubule (NCC), and collecting duct (alpha-ENaC). Transepithelial electrical resistance (TEER) measurements confirmed the integrity of the monolayer. Exposure to AU led to almost 2 fold increased TEER, indicating improved barrier function. Spiking AU with elevated oxalate and calcium concentrations induced calcium oxalate (CaOx) microcrystal formation on the monolayer, mimicking a well-established kidney injury pathway in tubular cells. In conclusion, this model can serve as a novel tool to study urine-derived injury pathways in kidney tubules and may help identify new biomarkers for CKD progression.

Cell Nanoinjections in Retinal Organoids: a Novel Platform for Retinal Ganglion Cell Therapy

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Background:
Glaucoma is the second leading cause of blindness worldwide, characterized by retinal ganglion cell (RGC) and optic nerve degeneration. The current treatment options can only slow down disease progression. Therefore, the development of regenerative medicine therapies is essential to reverse neurological damage and restore vision in the patients. Retinal organoids offer a suitable platform for novel therapy development as they recapitulate retinal development, cell lamination and functionality. Here, we present a novel technique to transplant stem cell-derived RGCs into retinal organoids, via micromanipulator-controlled organoid nanoinjections.
Methods:
WA01 human embryonic stem cells were differentiated towards retinal organoids. A micromanipulator setup with an injector arm was used to perform nanoinjections into retinal organoids at a targeted site with a standardized tip diameter, providing controlled volume and injection rate. The technique was validated using fluorophore-tagged microspheres to trace the injections inside the organoids. Next, two genetically modified tagged lines were generated – one for transient mCherry expression, and one for Brn3b-specific tdTomato-Thy1.2 expression in retinal ganglion cells – and used as source of donor RGCs. Retinal organoids generated with these 2 lines were dissociated and Thy1+ or BRN3b+ RGCs were isolated using MACS or FACS. Finally, the sorted RGCs were injected into the WA01 retinal organoids. Viability and integration of the transplanted cells was investigated.
Results:
100% of the fluorescent bead injections were successful and targeted the desired area in the organoid lamination. The morphology of the organoid was impacted after injection, but recovered within 2 weeks compared to sham controls. The differentiated retinal organoids presented neural retina lamination and functionality. RGCs were successfully isolated from stem cell-derived retinal organoids based on Thy1 or BRN3b expression. The isolated RGCs were successfully transplanted into WA01 retinal organoids, as confirmed by mCherry expression in live imaging 2 weeks post injection. The integration and functionality of the transplanted RGCs will be further investigated by RNA-Seq, IHC and MEA electrophysiology.
Conclusions:
Retinal organoids are an effective platform for RGC replacement therapy development, as they can be used both as a source of donor stem cell-derived RGCs but also as a host/patient model. Retinal organoid nanoinjections using a micromanipulator can be used as a highly controlled novel tool for targeted and precise transplantation of new cells into organoids. The present work is a proof of concept of successful transplantation of RGCs into human retinal organoids. In the future, we hope to use this novel technique as an in vitro alternative for retinal cell replacement therapy development.

Using Stem-Cell-Derived Bovine Organoids to Predict Food Safety

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Stem-cell-derived organoids offer powerful new opportunities to model organ function in vitro and to address questions relevant to both animal and human health. While human and rodent organoids are well established, large-animal models are still scarce, despite their relevance for veterinary medicine and translational research.
We have established bovine organoids from intestine, liver, and mammary gland—three key tissues that determine whether compounds from veterinary medicines, feed, or dietary supplements reach edible products such as beef and milk. Derived from primary bovine stem/progenitor cells, these organoids can be used to model functional features of their tissue of origin and can be kept long-term in culture. Together, they form the first bovine organoid models for assessing metabolism and transport of dairy cows.
Functional assays in these organoids enable measurement of compound uptake, metabolism, and secretion. The resulting data are incorporated into a physiologically based kinetic (PBK) model that predicts the distribution of compounds throughout the animal and into food products. In this way, bovine organoids provide the biological foundation for predictive modelling of chemical residues in milk and meat.
This combined organoid–modelling approach shows how stem-cell-based systems can replace or reduce animal testing in pharmacological studies. By enabling controlled investigation of bovine-specific mechanisms, these models provide a cost-effective and ethical alternative to traditional animal experiments. Ultimately, this research contributes to animal welfare, supports the development of safer veterinary medicines and feeding strategies, and safeguards human health through improved food safety.

Retinoic acid and FGF signaling interact to control elongation and lineage specification in a mouse gastruloid model

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Axial elongation and cell fate decisions during early embryonic development are regulated by signaling gradients, such as Retinoic Acid (RA) and Fibroblast Growth Factor (FGF). To assess the role of RA in vitro, studies have relied on its direct addition to the medium, potentially resulting in teratogenic effects. Here we examined its role on axial elongation, anteroposterior development and FGF-dependent signaling in gastruloids, an in vitro model system for early embryogenesis. Rather than ectopically applying RA we used its precursor, retinol, in a retinoid-free medium which prompts the cells to produce RA endogenously.

Our results show that the spatiotemporal RA and FGF gradients can be manipulated with different doses of retinol, influencing gastruloid elongation and lineage specification. In gastruloids cultured in high doses of retinol, RA was successfully metabolized, prompting the appearance of ectoderm and primordial germ cell-like cell (PGCs) differentiation. In low doses we observed early mesoderm specification and increased FGF signaling. We further used our approach to validate that RA signaling inhibits FGF in gastruloids. Thus, retinol can modulate cellular composition in gastruloids through interaction with FGF signaling. Our novel model will allow us to investigate fate commitment of ectoderm and mesoderm progenitors in vitro.

CRISPR engineering in kidney tubuloids to model ciliopathies

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Almost all kidney epithelial cells are continuously sensing their surroundings with an antenna-like projection called the primary cilium. This cilium is a hub containing many growth factor receptors like those involved in hedgehog and non-canonical Wnt signaling. Cilium function is dependent on many different proteins, amongst which are the 20 nephronophthisis-associated genes. When these proteins are defective they lead to tubular atrophy, cysts, fibrosis and eventually renal failure with no treatment options available as of yet. Some preclinical studies have attempted intervene in the pathways associated with nephronophthisis, but have not been able to successfully treat the disease. This could well be caused by the complexity of multiple different signal pathways being disturbed simultaneously in nephronophthisis. Adult kidney organoids (tubuloids) are a model system that allows us to study ciliary signaling in primary epithelial kidney cells. While tubuloids can be made from diseased patients' tissue or urine, the rarity of the patients makes systematically researching disease variants hard. Therefore, we set out to CRISPR engineer nephronophthisis mutations into tubuloids in order to study the signaling involved in this disease and to find common, treatable, mechanisms among the variants. Until now however, CRISPR technology has not been applicable to tubuloids due to efficiency and viability challenges. Here, through employing novel and optimized techniques, we were able to create knockouts of a selection of cilium- and nephronophthisis-associated genes. We show the initial characterization and future experimental plans for these lines.

Quality Control and non-clinical testing of iPSC- and ESC-derived medicinal products: a systematic review

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Cell-based therapies derived from induced pluripotent (iPSCs) and embryonic stem cells (ESCs) are increasingly used in clinical trials for various diseases. To bring these therapies to patients, non-clinical studies and robust Quality Control (QC) strategies are necessary to demonstrate safety and efficacy of the medicinal products. However, due to the novelty of the technology, specific safety concerns, and diversity of products, the evaluation of these therapies is not fully defined in regulatory guidelines. This raises uncertainty among developers and regulators on the optimal means to assess the products for clinical translation.

We conducted a systematic review focusing on QC and non-clinical in vivo studies of iPSC/ESC-derived products developed for clinical translation. Over 2400 records from 2022-2024 were retrieved, from which 50 reports containing relevant information were included. Product quality testing were categorized by identity, purity, content, safety, potency, and stability. A custom-made ranking system allowed to distinguish which tests were already employed as QC assays for clinical release; were proposed as QC tests for future implementation; or were used for characterization during product development. In addition, insights into the non-clinical study design revealed common practices for selecting animal models, cohort size, and product dosing.

This comprehensive overview of the latest trends in assessing iPSC/ESC-derived products serves as a resource for informing on suitable methods for QC testing and efficient non-clinical study design. It can aid in selecting an informative and appropriate testing strategy, ultimately contributing to the harmonization of the pre-clinical development and improving product quality and patient safety.

Generation of a dual reporter female hPSC line to monitor X chromosome inactivation

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In females, one of the two X chromosomes is inactivated early in development to balance X-linked gene dosage with males. In female human pluripotent stem cells (hPSCs), however, X chromosome inactivation (XCI) is unstable. This instability leads to XCI erosion and aberrant expression of X-linked genes, defects that persist after differentiation. As a result, female hPSCs are often excluded from studies, limiting our ability to model female-specific mechanisms in health and disease.
To address this problem, we engineered a dual reporter female hPSC line. A nanoluciferase–tdTomato cassette was inserted downstream of the MECP2 gene on the inactive X chromosome, while a Green Lantern fluorescent reporter was targeted to the active allele. This design enables sensitive, real-time monitoring of XCI dynamics across pluripotent states and during differentiation.
Using this system, we tested three published naïve conversion protocols, assessing naïve marker expression by FACS and quantifying XIST, the master regulator of XCI. Following naïve induction, cells were subjected to capacitation back to the primed state to evaluate whether proper XCI could be re-established.
In addition, the reporter line contains an inducible NGN2 cassette, allowing rapid and homogeneous differentiation into cortical neurons. This combination provides a unique platform to investigate the impact of XCI on neuronal differentiation and to model X-linked neurodevelopmental disorders in a female context.
Our aim is to establish a standardized culture pipeline that restores and maintains proper XCI in female hPSCs, enabling their broader use as robust and physiologically relevant models of human development and disease.

Unravelling the role of the miRNA 371-373 cluster in human germ cell tumors and human pluripotent stem cells

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Background: The microRNA cluster 371-373 (miR371-3) is known to be essential in human pluripotent stem cells (hPSCs) for maintaining pluripotency (1-4). Similarly, malignant germ cell tumors (GCTs), which arise from early pluripotent embryonic (germ) cells, exhibit high levels of this cluster, including the pluripotent embryonal carcinoma subtype. MiR371-3 is a highly specific and sensitive biomarker for GCTs, and is close to clinical implementation, however, its functional role remains incompletely understood (5-10).
Methods: We performed sequential CRISPR-Cas9 mediated knockouts of miR371-3 in two GCT cell lines (2102Ep and NCCIT, both embryonal carcinoma) and the hPSC cell line H9. MicroRNA and mRNA profiling, Western blot analysis, FACS, drug sensitivity assays, proliferation assays, SNP microarrays, and in vivo xenografting experiments were performed.
Results: We successfully knocked out miR371-3 in the GCT cell lines. Compared to their wild type counterparts, no consistent changes were observed. Moreover, despite a high degree of cell death, we also successfully knocked out miR371-372 in the H9 cell line, whereas miR373 persistently remained wildtype in the surviving clones.
Conclusion: The GCT cell lines tolerated loss of all members of miR371-3 and no consistent changes in in vitro or in vivo behavior were observed. As the H9 line did not tolerate loss of miR373, this highlights a potential key difference between untransformed (hPSC) and transformed (malignant GCT) pluripotent cell lines. We therefore preliminarily conclude that miR371-3 does not function as essential oncogenes (anymore) in malignant GCT (cell lines), and that its elevated levels may be remnants of their pluripotent cell origin.

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Radiation-induced interferon-I response impairs thyroid organoid function

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Abstract

Radiotherapy is a standard cancer treatment, but radiation exposure to surrounding healthy tissues may lead to adverse side effects that compromise patient quality of life. In patients with head and neck cancer treated with radiotherapy, thyroid damage is a frequent complication, resulting in hypothyroidism or secondary thyroid malignancies. Although clinically recognized, the molecular mechanisms underlying these side effects remain mostly unexplored. This study aims to characterize the radiation-induced molecular alterations in thyroid organoids.
Bulk RNA sequencing was performed to investigate transcriptomic changes in tissue-derived thyroid organoids following gamma-irradiation. Observed changes were further validated and explored using qPCRs, western blotting, immunofluorescence, caspase 3/7 activity and organoid forming efficiency.
Our findings identify interferon-β (INF-β) signaling as a key mediator of radiation-induced inflammation in the thyroid. Additionally, the intrinsic apoptotic pathway was found to be the predominant mechanism of radiation-induced thyroid cell death. Notably, while INF-β exhibited a protective effect against apoptosis, it concurrently reduced thyroid stem progenitor cell potential.
These results highlight the dual role of INF-β signaling in modulating thyroid cell fate after irradiation, potentially promoting survival upon injury at the expense of self-renewal.

Developing a hPSC-derived teratoma in vitro model for hPSC malignancy assessment: current possibilities and limitations

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Human pluripotent stem cells (hPSCs) have a myriad of potential clinical applications due to their capacity to self-renew and differentiate towards any of the embryonic germ layers. However, hPSC-derived cell products have shown to have the risk of tumor formation when used in vivo. To circumvent this issue, the safety of their use needs to be addressed, which includes the evaluation of the intrinsic hPSC malignant potential in vitro. Due to our limited knowledge on the underlying mechanisms causing this malignant potential, this assessment has traditionally been performed focusing on the histological traits of malignancy by xenografting the cells into immunodeficient mice (teratoma assay), for later evaluating the tissues found in the resulting tumor. Although informative, this assay faces scrutiny due to the lack of standardization of the procedure, the long waiting times and high related costs as well as the limited predicted value and questionable ethics behind animal testing. Here, we aimed at developing a reliable, standardized and fully animal-free in vitro model able to recapitulate the teratoma assay for addressing the histologic features of hPSC malignancy in an efficient manner. We generated embryoid bodies (EBs) as basic models of unbiased cell differentiation from validated malignant and safe stem cells, and exposed them to xeno-free and xeno-derived culture conditions. These were then compared on the basis of their capacity to generate structures displaying tissues with the same histopathological traits as those observed upon in vivo xenografting. Immature, fully differentiated, and mixed tissues were obtained in EBs from malignant stem cells, safe hPSCs, and iPSCs with impaired differentiation, respectively, cultured in both xeno-derived and -free conditions, although with low efficiency. Our results suggest that it is possible to develop an in vitro system able to recapitulate (immature) teratoma formation using xeno-free differentiation conditions, although efficient tissue formation can be improved potentially by better mimicking the mouse injection site microenvironment through co-culture and hydrogel embedding conditions.

In vitro **expansion and differentiation of Lgr5 positive supporting cells from normal hearing adult mice**

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Background: Over 400 million people globally experience hearing loss, often due to irreversible damage to cochlear hair cells. Unlike mammals, non-mammalian species can naturally regenerate their hair cells. Studies in neonatal mice show Lgr5+ supporting cells can generate new hair cells, and our research aims to translate this potential to adults. We investigated the regenerative capacity of adult mouse-derived Lgr5+ supporting cells by studying cochlear organoid proliferation and differentiation.
Methods: Mature adult (P40 – P60) Lgr5GFP transgenic mice were used. Harvested cochleae were dissociated, digested, filtered, and single cells were cultured in 3D Matrigel drops to generate organoids. We developed optimised media, tailored for adult tissue, building upon protocols from published data. The organoids were cultured for 10 days in expansion conditions and 10 days in differentiation conditions before being harvested for immunofluorescence microscopy.
Results: Organoids were successfully cultured from Lgr5GFP-positive supporting cells of the cochlea of normal-hearing adult mice. Our optimised media supported organoid expansion to larger sizes than previously established protocols. Published media showed limited differentiation to Myosin7A+ cells indicative of cochlear hair cells. Importantly, our newly optimised media resulted in structured organoids with organised MYO7A+ cells located at the rim, suggesting de novo produced hair cell-like cells are found after differentiation.
Conclusions: Optimisation of the culture media led to healthy expansion and robust, structured regeneration of hair cell-like cells. To our knowledge, this has not yet been described in adult derived tissue.

Harnessing Nasal Organoids for Functional Immune Profiling: An Autologous Model for Measuring Virus-Specific Immunity

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Virus-specific immune responses are mainly studied in immortalized cell systems, using artificial antigens like recombinant proteins or peptide pools as proxies for viral infection. Autologous model systems that examine humoral and cellular immune responses in primary cells remain limited but could be an important future direction for better understanding host responses targeting respiratory viruses. Since human nasal organoids provide a physiologically relevant platform for these viruses, we established a donor-matched system that combines single-donor-derived nasal organoids, virus isolates, and immune effectors to assess both virus-specific antibody and T cell responses.

Sera were assessed for the presence of VN antibodies targeting severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), human respiratory syncytial virus (HRSV), or human parainfluenza virus type 3 (HPIV3), isolated from the respective donors. Virus neutralization (VN) assays were performed on differentiated nasal organoids cultured at air–liquid interface (ALI) in a 96-well format. Neutralization was measured using sera collected before and after XBB.1.5 vaccination (for SARS-CoV-2), or before and after natural infection (for HRSV or HPIV3). To assess cellular immunity, virus-specific T cell lines and clones reactive to SARS-CoV-2, HRSV, or HPIV3 were generated. Submerged, apical-out nasal organoids were co-cultured with autologous T cells to assess activation and cytotoxic capacity upon viral infection. These proof-of-concept assays demonstrated that virus-specific antibody and T cell responses can be reproduced in fully autologous nasal organoid systems.

Overall, this work established nasal organoid platforms for comprehensive evaluation of antibody- and T cell–mediated immunity, offering a translational approach to understanding human antiviral responses.

Understanding Yolk Sac Tumors and Yolk Sac Elements in Pluripotent Stem Cell–Derived Tumors: Implications for Malignancy of Pluripotent Stem Cells

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Concern about the safety of pluripotent stem cell-based therapies, predominantly related to potential malignant transformation, hinders clinical translation of these therapies. The teratoma assay—where stem cells are xenografted into immunocompromised mice—remains the (gold) standard for risk assessment. Histologically, a pluripotent stem cell line is considered malignant when embryonal carcinoma-like cells are present in the xenograft. Yolk sac elements might also be present, but their significance for the safety of stem cell products is unclear. Yolk sac elements that are derived from pluripotent stem cells resemble, from a pathology point of view, yolk sac tumor, a clinically malignant human germ cell tumor. This study aims to develop tools for identifying and characterizing human yolk sac tumors as well as yolk sac elements in teratoma (xenograft-derived or in vitro generated). We generated a single-cell RNA expression profile from a 20-year-old paraffin-embedded sample. Preliminary data indicate that RNA of sufficient quality can be obtained and that biologically relevant sub-clustering of single-cell data can be performed. We observed the presence of a (cancer) stem cell cluster inside the tumor cluster, indicating a potentially clinically relevant intratumoral heterogeneity. We are now expanding our panel to include nine more samples from our archive, spanning both pediatric and adult yolk sac tumors. The final goal is to understand yolk sac tumor better, find new transcriptional or molecular markers, and identify malignancy-related molecular pathways of value for risk stratification of pluripotent stem cell products and clinical yolk sac tumors.

Towards Transplantable Liver Constructs: Organoid-Based Bioprinting and Automated Cell Expansion

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Organ transplantation is the only curative treatment available for end-stage liver disease patients. However, the availability of donor organs remains a critical challenge. Approximately 20% of patients on the liver transplant waitlist either die or become too ill to be transplanted, highlighting the urgent need for solutions to the donor shortage. The aim of our project is to produce dense, functional, bioprinted liver constructs with a vascular bed that can be scaled up and integrated into a standardized and automated process, providing a potential alternative to organ donors. The project is currently in its early stage. Adult liver stem cells will be isolated from 6 healthy donors and expanded as organoids. The organoids, together with supporting mesenchymal and endothelial progenitor cells, will be used to form multicellular spheroids to mimic in vivo liver tissue. To restore 10-20% of the hepatocyte liver mass of an adult person, an estimated 10-20 billion cells are required. To reach clinically adequate cell numbers for bioprinting and transplantation, we are currently developing GMP-conform protocols for large scale expansion of organoids and supporting cells in controlled bioreactors. In addition, we are optimizing xenogeneic-free culture medium to meet the criteria for human cell-based therapies. Key parameters such as cell viability, functionality, and scalability will be evaluated as part of this process.

Leveraging bioreactor technology and automation, this project aims to establish a reproducible and scalable process for generating clinically relevant numbers of cells for the production of transplantable liver constructs. Future steps of the project include bioprinting of the spheroids on a bioengineered vascular bed, construct maturation and vascularization, and transplantation of the constructs in a large animal model for validation. Our approach could offer an alternative solution to organ shortages and improve access to life-saving treatments for end-stage liver disease patients.

Revealing the impact of Gravity on Skeletal Muscle physiology

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During prolonged manned spaceflight the human body is inherently exposed to the lower gravitational force, or microgravity. One of the most impacted systems in the human body is the musculoskeletal system, where both bones and skeletal muscle are significantly reduced in mass and strength. The skeletal muscle system reveals a significant atrophy of the tissue in response to reduced gravity, but the molecular mechanism behind this atrophy remains unknown. In this study, we propose to explore the impact of the gravity spectrum on several skeletal muscle in vitro models. A combination of transcriptomics, qPCR and immunocytochemistries approaches will be used to obtain a better understanding of the molecular and cellular events induced in hyper or microgravity conditions. We will validate our in vitro model by comparing our observations with those made in studies describing muscle atrophy during spaceflight. Additionally, by employing the Reduced Gravity Paradigm / Gravity Continuum we also use simulated hypergravity to extrapolate the effects observed, providing a fundamental understanding of how the system behaves in different gravity conditions. Our study will provide new knowledge on the role and mechanism by which gravity controls skeletal muscle mass and potentially identify key players in this process that could be candidates for pharmaceutical intervention.

Whole genome CRISPR screening reveals RO60 as a protective factor against Cas13 induced stress

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Cas13 is a single-effector CRISPR system within the class 2 type VI CRISPR family. Unlike its class 2 siblings, Cas9 and Cas12, Cas13 exhibits RNase activity that is activated by RNA-guided RNA targeting. Such induced RNase activity not only degrades the target RNA, but also non-target RNAs (collateral RNA degradation). The Cas13 orthologue, LbuCas13a, manifests collateral RNA degradation whilst targeting both exogenous and endogenous transcripts in human cells. In response to the collateral RNA degradation, cells upregulate the expression of stress and innate immune response genes. By targeting a highly expressed transcript, the Cas13-induced collateral RNA degradation could result in apoptotic cell death1. To understand the mechanism underlying the collateral RNA degradation-induced apoptosis by LbuCas13a targeting, we performed a full genome CRISPR knockout screen where KO cells were challenged by LbuCas13a protein together with either a targeting or a non-targeting guide RNA (gRNA). We found that RO60 was one of the top depleted genes in our screening. Knocking out RO60 makes the cells more sensitive to Cas13-induced collateral RNA degradation. As an RNA binding protein, RO60 is involved in the quality control of non-coding RNAs.
Two different responses to stress were observed when cells were exposed to the Cas13-induced collateral RNA degradation, indicating two possible roles for RO60. On the one hand, RO60 regulates stress response by protecting ribosome RNAs when the cells are exposed to stimuli independent of Cas13. On the other hand, VASA-sequencing shows RO60 regulates a specific stress response via YRNAs, and apoptotic genes are upregulated in RO60 WT cells opposed to RO60 KO cells when targeted by Cas13. Further RO60 rescue and overexpression experiments, as well as YRNA KOs and their overexpression in cells will provide us more insights into the mechanics of RO60-YRNA coordination in response to the Cas13-induced collateral RNA degradation.

1. 1. Jorik F. Bot, Zhihan Zhao, Darnell Kammeron, Peng Shang, Niels Geijsen
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The long-term effects of hyperglycemia on human iPSC-derived retinal pigment epithelial cells and retinal organoid

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Abstract

Purpose: Diabetic retinopathy is a common complication of diabetes that damages retina, leading to visual impairment. Retinal pigment epithelial (RPE) cells are crucial for maintaining retinal function. Retinal organoid (RO) is a 3D structure tissue which is composed of major retinal cell types. This study aims to evaluate the long-term effects of hyperglycemia on human iPSC-derived RPE cells and RO as an *in vitro* model for future therapeutic strategies.

Methods: Two human iPSC lines from healthy donors were differentiated into RPE cells and ROs. To mimic DR conditions, iPSC-RPEs were exposed to high glucose (HG; 30 mM, 40 mM) and to HG combined with inflammatory factors (TNF-α, IL-6) every other day for 2 or 4 weeks. ROs at day 150 were similarly treated with 40 mM HG plus inflammatory factors. Gene expression of inflammatory, oxidative stress, and angiogenesis markers was analyzed by qPCR. Cone density in ROs was assessed by immunofluorescence.

Results: *IL6*, *IL1B*, and *NFKB1* were significantly upregulated after 2- and 4-week HG exposure, with greater increases when combined with inflammatory factors. *SOD1* expression was elevated under all HG conditions, while *SOD1*, *SOD2*, and *HIF1A* were further enhanced by combined treatments. Notably, cone density was reduced in ROs after 4 weeks of 40 mM HG exposure.

Conclusion: Exposing to 40 mM high glucose and inflammatory factors jointly for long term could induce upregulation of inflammatory cytokines, oxidative stress markers, angiogenesis factors in human iPSC-RPE and cones loss in RO, which could serve as a pre-clinical model for studying diabetic retinopathy.

Ligand-based differentiation of induced pluripotent stem cells into granulosa-like cells

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Introduction: Polycystic ovary syndrome (PCOS) is a common reproductive and metabolic disorder in women, yet its etiology remains unclear. Anti-Müllerian hormone (AMH) is produced by granulosa cells (GCs) of preantral and small antral follicles, and acts as a gatekeeper in follicular differentiation. In PCOS, AMH expression is increased and its window of expression is prolonged. However, the mechanisms regulating AMH expression, in healthy and PCOS conditions, remains unknown. This study aims to investigate the transcriptional regulation of AMH using human induced pluripotent stem cells (iPSCs).
Methods: To differentiate iPSC into granulosa-like cells, several ligand-induced differentiation protocols were analyzed in combination with two types of base medium. Assessment for correct lineage commitment and GC markers was performed by gene expression analysis.
Results: Our preliminary results indicated all protocol combinations suppress expression of pluripotency markers. However, protocol-dependent differences in expression of early and late stage GC markers was observed. In human embryonic stem cell medium, expression of the early GC marker FOXL2 increased 3-10 fold and expression of the late follicular stage marker CYP19A1 increased ~100 fold. Despite a 3-5 fold increase in AMH expression, expression remained low. In contrast, a strong induction in AMHR2 expression (200-300 fold) was observed, but only when iPSCs were differentiated in RPMI medium.
Conclusion: We show that iPSCs can be differentiated into granulosa-like cells. Differences in GC marker expression between protocols may reflect differentiation into GCs of distinct follicular stages. Studies are ongoing to induce AMH expression, and to determine AMHR2 responsiveness in these cells.

Revealing novel contributors to arrhythmogenic cardiomyopathy phenotypes through hiPSC-derived 2D and 3D cardiac models

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Arrhythmogenic cardiomyopathy (ACM) is a rare inherited cardiac disorder, characterized by fibro-fatty replacement of ventricular myocardium, leading to contractile dysfunction, arrhythmias, and sudden cardiac death. While the genetic basis of ACM is well established – primarily involving mutations in desmosomal genes, most frequently plakophilin-2 (PKP2) – the pathogenic mechanisms remain unclear, and no treatments currently target ACM tissue remodeling.
Human induced pluripotent stem cell (hiPSC)-derived cardiac models provide a powerful platform to investigate disease mechanisms and discover novel therapeutic strategies. Here, we present a fully human, multicellular model to dissect ACM pathophysiology and investigate novel cellular contributors to the disease.
Patient-derived hiPSCs carrying a heterozygous PKP2 c.2013delC mutation and their isogenic corrected control were successfully differentiated into cardiomyocytes (CMs), endothelial cells (ECs), epicardial cells (EPIs), and cardiac fibroblasts (CFs), each confirmed by lineage-specific marker expression. To dissect the multicellular contribution to ACM, we investigated the currently understudied epicardial cells and we found that ACM EPIs showed altered desmosomal proteins expression and signs of mesenchymal transition.
To capture the multicellular complexity of the human heart, three-dimensional cardiac microtissues (MTs) were assembled by combining hiPSC-derived CMs, CFs, and ECs. ACM MTs revealed structural and functional abnormalities, which are currently under deeper investigation through omics-based approaches.
In summary, our work highlights the utility of hiPSC-derived multicellular cardiac models to study ACM in a physiologically relevant manner. By uncovering cell-specific phenotypes and emphasizing the role of non-myocytes in disease progression, our approach offers a next-generation platform for precision disease modeling and preclinical drug testing.