14th Annual Meeting
Dutch Society for
Stem Cell Research

October 20th, 2023

University Medical Center Utrecht

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Program 14th Annual Meeting of the Dutch Society for Stem Cell Research

October 20th 2023, Roze Collegezaal, Heidelberglaan 100, AZU/UMC Utrecht

9:00	**Registration opens**
9.30-09:50	Coffee/tea
09:50-10:00	Welcome (Paul Coffer)

Session 1
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10.15-10.30	Jolien Jacobs: GO with the WNT: synchronising the co-development of blastocyst tissues
10.30-10.45	Mehmet Yildiz: The effect of retinoic acid during early embryonic development in a mouse gastruloid model
10.45-11.15	**Keynote lecture –** Peter Rugg-Gunn, Babraham institute, Cambridge, UK, Establishing the epigenome in human development and pluripotency
11.15-11.45	Coffee/tea

Session 2
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11.45-12.00	Albert Blanch-Asensio: A rapid and efficient platform for targeted large gene insertions in human induced pluripotent stem cells
12.00-12.15	Rebecca Snabel: A single-cell genomics roadmap of in vitro multi-lineage cardiac differentiation
12.15-12.30	Yolanda Chang: In vitro maturation of human primordial germ cell-like cells (hPGCLCS) in the female human somatic niche
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Session 3
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13.30-13.45	Han Verhagen: Genome editing of hematopoietic stem and progenitor cells to cure hemoglobinopathies.
13.45-14.00	Konradin Müskens: Clonal hematopoiesis is common in long-term survivors of pediatric stem cell transplantation, including umbilical cord blood transplantation.

14.00-14.15 Loukia Yiangou: Human iPSC–derived 2D and 3D cardiac models for investigating hypertrophic cardiomyopathy mechanisms.

14.15-14.45 **Keynote lecture –** Tiago C. Luis, Haematopoietic Stem Cells Laboratory, Imperial College London, UK: Bone marrow niche regulation of hematopoietic stem cells response to stress.

14.45-15.15 Coffee/tea

Session 4
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15.15-15.30 Winnie van den Boogaard: Disease modelling and RNA therapy in human inner ear organoids – Usher syndrome type 2

15.30-15.45 Pere Català: Volumetric bioprinting of an endocrine pancreas, a platform for drug screening

15.45-16.00 Dulce Lima Cunha: Restoration of functional PAX6 in aniridia patient iPSC-derived ocular tissue models using repurposed nonsense suppression drugs

16.00-16.30 **Keynote lecture –** Rob Coppes, Department of Radiation Oncology, University Medical Center Groningen, Netherlands:
Towards and beyond autologous Submandibular Gland Organoids to Ameliorate Xerostomia After Radiotherapy

16.30-16.45 Award ceremony (Ans van Pelt)

16.45-17.00 Introduction of new board members (Paul Coffer)

17.00-18.00 Drinks

Keynote speakers

Peter Rugg-Gunn

Babraham Institute, Cambridge, UK

Establishing the epigenome in human development and pluripotency

Peter Rugg-Gunn is a Senior Group Leader at the Babraham Institute in Cambridge. He is also an Affiliate Principal Investigator at the Cambridge Stem Cell Institute and at the Centre for Trophoblast Research. He is the scientific lead for the Wellcome-funded Human Developmental Biology Initiative (https://hdbi.org).

Peter's research interests centre on understanding how gene activity is controlled during human development and how this affects cell fate decisions in embryos and also in stem cells grown in the laboratory. His team have recently identified epigenetic mechanisms that influence the first lineage decision occurring in the developing human embryo, and also uncovered chromatin modifying pathways that are required for human pluripotent stem cells to transition between different states. Their current research focus is on investigating the establishment of epigenetic programmes during the second week of human embryo development.
Peter is also Head of Public Engagement at the Babraham Institute. He has recently completed a national public dialogue project that explored society's views on human developmental biology research including the '14-day' rule and stem cell-based embryo models.

Tiago C. Luis

Haematopoietic Stem Cells Laboratory, Imperial College London, UK

Bone marrow niche regulation of hematopoietic stem cells response to stress.

Tiago Luis research focuses on the hematopoietic system and more specifically on the mechanisms regulating hematopoietic stem cells (HSC) function and the extracellular signals these cells receive from their niches.
During his PhD in the laboratory of Prof Frank Staal at Erasmus Medical Centre (Rotterdam, The Netherlands) he studied the role of the Wnt signaling pathway in hematopoiesis and demonstrated a stage/lineage-specific and dosage-dependent effect of canonical Wnt signaling throughout hematopoiesis. After his PhD, Tiago joined the laboratory of Prof. Sten Eirik Jacobsen at the University of Oxford (UK) as an EMBO postdoctoral fellow, to investigate the cellular pathways of lineage commitment from HSCs. He characterized the first hematopoietic progenitors that migrate and colonize the fetal thymus, where they encounter a specific microenvironment eliciting further differentiation through the T-lymphocyte lineage. In 2014 he was awarded a Kay Kendall Leukaemia Fund Fellowship to investigate the bone marrow niche of platelet-biased HSCs. In 2018 he was awarded a Sir Henry Dale Fellowship from the Wellcome Trust and the Royal Society to start his laboratory at Imperial College London to investigate the molecular mechanisms by which the bone marrow niche regulates HSCs, in homeostasis, regeneration and in the development of hematological malignancies..

Rob Coppes
Department of Radiation Oncology, University Medical Center Groningen, The Netherlands

Towards and beyond autologous submandibular gland organoids to ameliorate xerostomia after radiotherapy.

Rob Coppes obtained his PhD in Molecular Pharmacology at the University of Groningen in 1993. Attracted by the possibility of using a pharmacological approach to attenuate radiation-induced normal tissue damage upon cancer treatment, he became a post-doctoral fellowship at the Department of Radiobiology at the University of Groningen under the supervision of Profs A.W.T. Konings, A. Vissink and later Prof H.H. Kampinga.
In 2000 he moved to the Department of Radiation Oncology as a clinical radiation biologist. He started his own lab at the Department of Cell Biology to focus on the effect of irradiation on normal tissues such as lung, heart and salivary glands. He became a full professor of Radiotherapy with a focus on the radiation biology of normal tissues in 2012. His lab developed in vivo and in vitro organoid models for purification and characterization of stem/progenitor cells from mice, rats and human salivary and thyroid gland. His research on salivary gland organoids led to the development of a protocol for adult stem cell therapy for radiation-induced hyposalivation and consequential xerostomia, which is now tested in the clinic in a Phase I/II trial.
Currently, his group is investigating the radiation-induced changes in the stem cell niche of the salivary gland and the difference between proton and photon radiation on the DNA damage-related immune response, using organoids as a model system. In 2015 he received the Bacq-and-Alexander Award from the European Radiation Research Society for an outstanding European researcher to recognize achievements in radiation research. In 2022 he received the Societal Impact Award from the W.J. Kolff Institute for Biomedical Engineering and Materials Science.

Abstracts - Invited speakers

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Comparative Transcriptomics of Mouse Gastruloids and Embryos using total RNA sequencing (VASA-seq)

Noëlle Dommann[1], Mehmet Yildiz[1], Clara Martinez[1], Pascale Dijkers[1], Niels Geijsen[1], Anna Alemany[1]

[1] Department of Anatomy and Embryology, Leiden University Medical Center (LUMC), Leiden, the Netherlands

Gastruloids are aggregates of embryonic stem cells that display an embryo-like organization and allow to closely recapitulate the first steps of mammalian development in vitro. They are an ideal model system for the early post implantation development as they can be grown in large quantities and display key features of axial organization, germ-layer specification and somitogenesis. However, the presence and function of non-coding transcript biotypes such as short or long non-coding RNA remains poorly characterized in gastruloids. To investigate this with single-cell resolution, here we use single cell total RNA-sequencing (VASA-seq) which provides full gene body coverage and a wider spectrum of RNA biotypes as compared to standard single cell RNA sequencing. In this study, we compared the total transcriptomic profiles of the mouse gastruloids with our previously generated dataset looking at mouse embryo cells stages (E6.5-E9.5). Our detailed analysis revealed subtle transcriptomic differences and similarities between various embryonic cell types that were not previously known between gastruloids and mouse embryos. In addition, VASA-seq revealed expression profiles of long non-coding RNA specific to germ layers. Finally, VASA-seq allows the investigation of differential histone usage, revealing up-regulation of H2bu2 in ectoderm during the S-phase of the cell cycle. In conclusion, our study provides a comprehensive characterization of the total transcriptomic landscape in gastruloids and their comparison to mouse embryos, which reveals novel insights into the expression profile and the role of non-coding RNA biotypes during early embryogenesis.

GO with the WNT: synchronising the co-development of blastocyst tissues

Jolien Jacobs[1,2*], Viktoria Holzmann[3,4*], Emiel van Genderen[1], Esther Baart[2], Nicolas Rivron[3#], Derk ten Berge[1#]

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* equal contribution # equal contribution

During embryonic development, multiple tissues organize themselves into increasingly complex structures. In mammals, formation of the body axis during gastrulation is critically dependent on the close association of the primed pluripotent epiblast with the visceral endoderm, an extraembryonic tissue. These two lineages first arise in the pre-implantation embryo as naïve pluripotent epiblast and primitive endoderm (PrE), respectively. We recently showed that downregulation of WNT signals in the pre-implantation embryo induces epiblast morphogenesis as well as its differentiation towards primed pluripotency. However, the mechanisms that coordinate differentiation and morphogenesis of the PrE and synchronise its development with that of the epiblast are poorly understood. Here we show that WNT signals synchronize development of the epiblast and primitive endoderm by maintaining the pre-implantation state of both tissues. Using embryos as well as a variety of 2D and 3D stem cell-based embryo models, we show that WNT signals prevent differentiation of the primitive endoderm towards visceral endoderm. Furthermore, we found that WNT signals prevent deposition of a basal lamina by the PrE, a prerequisite for epiblast morphogenesis. Consequently, downregulation of WNT signals prior to implantation indicates 'GO' to both the epiblast and PrE, triggering their coordinated differentiation and morphogenesis. Our findings illustrate how WNT signals orchestrate the formation of multi-lineage structures.

The effect of retinoic acid during early embryonic development in a mouse gastruloid model

Mehmet Yildiz[1,2], Pascale F. Dijkers[1,2], Niels Geijsen[1,2]

[1]Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands
[2]The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden node, The Netherlands

Gastruloids are 3D stem cell models of gastrulation, a process where the germ layers and the body axes are formed. The anterior-posterior axis is formed by two opposing signalling gradients: FGF-WNT and retinoic acid. The role of retinoic acid is not clear. In this study, we aimed to understand the effects of retinoic acid in a mouse gastruloid model and treated gastruloids with different doses of retinol, a precursor of retinoic acid. Here, we demonstrate that retinol has a dose-dependent effect on axial elongation. While gastruloids reached their maximum length with 300nM retinol, they were shorter with lower and higher doses. The expression of a critical modulator of posterior elongation, Fgf8, was highest with the maximum elongation. We next examined whether retinoic acid has an effect on body plan formation by examining expression of posterior trunk marker Hoxb9 and anterior trunk marker Hoxb1 by whole mount in situ hybridization (WISH). Unlike gastuloids treated with higher retinol doses, gastruloids treated with 300nM or less displayed Hox patterns as observed in vivo. Finally, we examined whether retinoic acid can influence germ layer formation by analyzing reporter lilnes. With retinol treatment, ectoderm markers SOX1/2 were upregulated. However, mesoderm marker T was downregulated. Expression of endoderm marker SOX17 was highest at 300nM retinol and decreased with lower and higher doses. These data showed that retinoic acid has effects on cell fate decisions in a dose-dependent manner.
Altogether, we demonstrate that retinoic acid modulates axial elongation, body plan formation as well as cell fate decision.

A rapid and efficient platform for targeted large gene insertions in human induced pluripotent stem cells

Albert Blanch-Asensio[1,2], Catarina Grandela[1], Babet van der Vaart[1,2,3], Nikola Popovic[1], Nini Schotman[1], Mariana Vinagre[1,2], Christian Freund[1,2,3], Niels Geijsen[1,2,3], Christine Mummery[1,2], Richard Davis[1,2]

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[2] The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden University Medical Center, 2300RC Leiden, the Netherlands
[3] LUMC hiPSC Hotel, Leiden University Medical Center, 2300RC Leiden, The Netherlands

In mammalian cells, such as human induced pluripotent stem cells (hiPSCs), incorporating large DNA payloads (>10 kb) or replacing genomic segments remains challenging. Here, we have merged the strengths of different classes of site-specific recombinases and combined these with CRISPR-Cas9-mediated homologous recombination to develop a platform termed STRAIGHT-IN (Serine and Tyrosine Recombinase Assisted Integration of Genes for High-Throughput INvestigation) for site-specific integration of large DNA payloads or replacement of genomic fragments of at least 50 kb. Only a single copy of the payload is integrated with full control of its location and no cargo size limitation detected to date (>170 kb). The procedure also permits the excision of nearly all the auxiliary DNA sequences which can lead to post-integrative silencing. We have demonstrated its applicability by I) characterizing and validating a multi-parameter reporter hiPSC line to assess the excitation-contraction coupling cascade in hiPSC-derived cardiomyocytes, II) establishing an inducible system for the fine-tunable and temporal expression of genes for various purposes including disease modelling, gene editing, lineage tracing and forward programming, and III) simultaneously generating >10 hiPSC lines containing disease variants in *KCNH2* that can recapitulate the arrhythmogenic phenotypes observed in patients. Furthermore, we have developed upgraded STRAIGHT-IN versions that allow genetically modified hiPSCs to be generated with 100% efficiency within two to three weeks (v2), as well as integrating two DNA payloads orthogonally in both alleles of either *CLYBL* or *AAVS1*, two genomic safe harbour loci, also with high efficiency (v3).

A single-cell genomics roadmap of *in vitro* multi-lineage cardiac differentiation

Rebecca R. Snabel, Carla Cofiño Fabrès, Marijke Baltissen, Verena Schwach, Robert Passier, Gert Jan C. Veenstra

Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University, Nijmegen, The Netherlands

The ability to culture many cell types from human pluripotent stem cells offers great potential in mimicking organogenesis. The earliest steps of human heart development are inaccessible *in vivo*, especially since the first cardiac progenitors already arise when the embryo just starts to form its three germ layers during gastrulation. Numerous cardiac culture models have been established over the years. These culture systems increased in complexity (e.g. from two- to three-dimensional cultures) leading to advanced maturity of the cardiomyocytes, as well as simultaneous development of other cardiac cell types.

We present a compendium of single-cell transcriptomic and chromatin accessibility datasets from multiple cardiomyocyte culture models, in which the cells were directed to both atrial and ventricular lineages. This roadmap enabled us to characterize the *in vivo* resembling cardiac progenitor states in our culture systems, as well as the gene regulatory network involved in specific lineage choices. Currently, we are performing follow-up experiments to validate these transcriptional regulator hits by modulating their expression during cardiac differentiation. The gene-regulatory capabilities of transcription factors makes them interesting driver gene candidates with potential key roles in cardiac lineage decisions. A better understanding of the molecular mechanisms involved in these processes of cardiac lineage commitment and plasticity, will help to further advance the culture systems and increase its applicability as a developmental model and in the study of congenital cardiac diseases.

In vitro maturation of human primordial germ cell-like cells (hpgclcs) in the female human somatic niche

Yolanda W. Chang, Celine M. Roelse, Sanne Hillenius, Talia van der Helm, Arend W. Overeem and Susana M. Chuva De Sousa Lopes

Leiden University Medical Centre, Leiden, The Netherlands

The female reproductive reserve is finite due to the limited number of oocytes that are generated only during embryonic development. Hence recapitulation of human oogenesis *in vitro* using induced pluripotent stem cells (hiPSCs) could be a useful tool to improve female fertility. Currently hiPSCs can be differentiated into primordial germ cell-like cells (hPGCLCs), but the maturation of hPGCLCs into oocytes has not been achieved. Different from the mouse, human fetal germ cells develop asynchronously, with some of the germ cells entering meiosis I and forming primordial follicles, while some remain at the PGC stage at any given time. Due to limited access to human material and lack of in vitro models, it is not well understood what biochemical cues trigger fetal germ cells to mature. Here we demonstrated that by reaggregating single cell suspension of human fetal gonads and encapsulating them in 3D hydrogel, we are able to preserve the somatic microenvironment necessary for fetal germ cells to proliferate and mature. After 10 days in culture, the number of DDX4+ fetal germ cells increased up to 5 fold and more than 25% of those entered meiosis I, indicated by the presence of SYCP3 and pH2AX(Ser139). Using this platform, we reaggregated hPGCLCs generated in 2D differentiation protocol (Overeem et al., 2023) with human fetal ovarian cells and observed upregulation of fetal germ cell marker DDX4 and evidence of germ cell epigenetic reprogramming. Our study demonstrated the potential of the somatic niche to support in vitro gametogenesis using hiPSCs.

Overeem AW, Chang YW, Moustakas I, et al. Efficient and scalable generation of primordial germ cells in 2D culture using basement membrane extract overlay. Cell Rep Methods. 2023;3(6):100488. Published 2023 May 23. doi:10.1016/j.crmeth.2023.100488

Genome editing of hematopoietic stem and progenitor cells to cure hemoglobinopathies.

Han JMP Verhagen[1], Julien Karich[1], Eelke T Brandsma[1], Steven Heshusius[1], Gerard van Mierlo[1], Martijn Veldthuis[1], Bart J. Biemond[2], Erfan Nur[2], Derk Amsen[1], Carlijn Voermans[1] and Emile van den Akker[1].

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Background: Sickle cell disease (SCD) and β-thalassemia are prevalent monogenic disorders caused by mutations in beta-hemoglobin (*HBB*), leading to chronic hemolytic anemia and organ damage. Allogenic stem cell transplantations offer a curative option but are hindered by donor availability and post transplantation complications. Gene editing of hematopoietic stem and progenitor cells (HSPCs) is a promising alternative. Our goal is to develop protocols to genome engineer HSPCs and prepare them for autologous stem cell transplantations, to form healthy red blood cells. Re-expressing gamma-hemoglobin (HBG) in 25% of the cells can compensate for mutated HBB and will be sufficient for patients to become symptom free.

Methods and results: We identified erythroid-specific intronic enhancers in *BCL11A* ($^{+}$55kb, $^{+}$58kb, and $^{+}$62kb), controlling *HBG* expression. Using CRISPR/Cas9 ribonucleoprotein (RNP) nucleofection, we identified multiple gRNAs to efficiently disrupt the 55kb+ and 58kb+-enhancer regions. Our optimized RNP protocol achieved over 85% survival of healthy HSPCs with 80% knockout of the BCL11A enhancer, leading to up to 35% HBG re-expression in differentiated red blood cells.

Our approach successfully reversed SCD formation *in vitro* and demonstrated unaffected outgrowth of diverse human hematopoietic cell types *in vivo*.

Conclusion: Our data supports the clinical implementation of genome edited HSPCs for curing hemoglobinopathies. We aim to make this therapy more accessible by reducing production costs, which is beneficial for patients in the Netherlands and other European countries with limited access to treatments. Our approach has the potential to significantly improve patients' quality of life by offering curative single-dose therapy.

Clonal hematopoiesis is common in long-term survivors of pediatric stem cell transplantation, including umbilical cord blood transplantation

K.F. Müskens, MD[1], M. van Bergen, PhD[2], A. de Graaf, PhD[2], N. Wieringa, MD[1], B. te Pas[1], A.P.J. de Pagter, MD PhD[3], J.E. Bense, MD PhD[3], A.C. Lankester, MD PhD[3], L.C.M. Kremer, MD PhD[1], J.H. Jansen, PhD[2], G.A. Huls, MD PhD[4], S. Nierkens, PhD[1], C.A. Lindemans, MD PhD[1], M.E. Belderbos MD PhD[1]

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Background: Clonal hematopoiesis (CH) is the selective expansion of hematopoietic stem and progenitor cell (HSPC) clones with driver mutations. CH is an age-related condition that predisposes to hematologic malignancy and all-cause mortality. Hematopoietic cell transplantation (HCT) recipients are at increased risk of CH, but the mechanisms driving the emergence and expansion of CH clones remain unknown. Here, we investigate the prevalence and risk factors of CH in long-term survivors of HCT at pediatric age.
Methods: CH was assessed by targeted, error-corrected sequencing of mutations in 27 cancer driver genes in whole blood. Clinical characteristics were compared using the Wilcoxon signed-ranked test or Chi-squared test.
Results: CH was present in 13.3% of survivors (n=16), much higher than the reported prevalence in the general population. Mutated genes were *DNMT3A* (n=15), *TET2* (n=4) and *MPL* (n=1), with three individuals having more than one driver mutation. The median variant allele frequency of the CH clones was 2.2%, but allele frequencies up to 30% were found. The age of the HSCs was significantly higher in individuals with CH compared to those without (38.6 versus 26.3 years, $p < 0.01$). Surprisingly, we detected CH in two recipients of umbilical cord blood grafts, at an interval of 11 and 15 years after HCT.
Conclusion: Pediatric HCT recipients are at increased risk of CH compared to the general ageing population and related to the age of transplanted HSCs. Ongoing research is aimed at investigating how factors such as stem cell source and post-transplant complications affect clonal expansion after HCT.

Human iPSC–derived 2D and 3D cardiac models for investigating hypertrophic cardiomyopathy mechanisms

Loukia Yiangou[1], Eline Groen[1], Jeroen Stein[1], Maury Wiendels[1], Berend van Meer[1], Christine L. Mummery[1, 2], Richard P. Davis[1, 2]

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[2]Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW

Hypertrophic cardiomyopathy (HCM) is a life-threatening disease caused by mutations in sarcomeric genes of the heart, manifesting with a range of disease phenotypes including left ventricular hypertrophy, myofibrillar disarray, myocardial hypercontractility and fibrosis. Among the most commonly mutated genes is *MYBPC3*, encoding the cardiac myosin binding protein C. The disease is characterised by reduced penetrance and variable disease manifestation. Some patients will develop severe disease while others carrying the same mutation will have no symptoms, confounding optimal patient treatment. Moreover, there are no HCM-specific medications with many patients not responsive to general cardiac medications.

Here, we developed models of HCM using hiPSCs derived from patients carrying the *MYBPC3*-2373insG founder mutation and subsequently generated a corrected isogenic control. Characterisation of 2D hiPSC-cardiomyocytes (hiPSC-CMs) showed that HCM hiPSC-CMs have defective calcium handling properties. Moreover, HCM 2D-aligned hiPSC-CMs show lower contraction amplitude and sarcomere disorganization compared to corrected cells. Furthermore, we generated 3D engineered heart tissues and observed lower contractile force, in line with our 2D findings. Lastly, we generated 3D cardiac microtissues (MTs) and cardioids as alternative HCM models. We observed that mutant MTs are smaller, have a hypocontractile phenotype and altered calcium transients. Similarly, mutant cardioids show contractile and calcium handling abnormalities. The hypocontractile phenotype seems to be genotype-specific, as a hypercontractile phenotype is generally expected in HCM. Overall, both the 2D and 3D hiPSC-derived models of HCM reveal similar contractile, calcium handling and structural abnormalities, providing relevant tools to further explore HCM disease mechanisms and identify novel therapeutics.

Disease modelling and RNA therapy in human inner ear organoids – Usher syndrome type 2

Esther Fousert[1,2], Winnie M.C. van den Boogaard[1,2], Wouter H. van der Valk[1,2], Amy W.A. Lucassen[1,2], John C.M.J. de Groot[1], Peter Paul G. van Benthem[1], Hannie Kremer[3,4],Erik de Vrieze[3], Erwin van Wijk[3], Heiko Locher[1,2]

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Usher syndrome type 2 (USH2) is the most common cause of inherited deaf-blindness, primarily caused by mutations in the USH2A gene. These mutations lead to retinitis pigmentosa and congenital hearing loss. To study the effect of USH2A mutation in vitro, we used inner ear organoids generated from human induced pluripotent stem cells (iPSCs). Because inner ear organoids mimic aspects of the human inner ear's structure and function they can be used to model genetic inner ear disorders in vitro.

In our study, we differentiated inner ear organoids from a USH2A-mutated patient and employed RNA therapy to counter the disease phenotype. These patient-derived organoids matured for 75-110 days, producing vestibular and cochlear hair cells. Control organoids were derived from healthy donor iPSCs. USH2A encodes the usherin protein which is expressed in the ankle links. Mutations in USH2A destabilize stereocilia bundles in hair cells. We assessed the stereocilia bundles' phenotype in both disease-specific and control hair cells using immunohistochemistry (IHC) and scanning electron microscopy (SEM).

Subsequently, we attempted to rescue the disease phenotype in USH2A-organoids using RNA therapeutics, specifically antisense oligonucleotides (AONs). Organoids were sectioned into 200µm slices with a vibratome to expose enclosed otic vesicles. AONs (1-15 µM) were added to the culture medium for an additional 15 days. We evaluated the therapeutic effect using IHC, PCR, and SEM. Concluding, this work provides a proof-of-concept for the use of human inner ear organoids to model inner ear diseases and treatment.

Volumetric bioprinting of an endocrine pancreas, a platform for drug screening

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Alpha and beta cells present in the islets of Langerhans provide a fine tuning of circulating glucose levels. A progressive loss of beta cells results in diabetes mellitus, which affects over 500 million people worldwide. The current treatment for diabetes mellitus consists on the delivery of exogeneous insulin but does not fully recapitulate the innate normoglycemia. There is the need to develop novel therapies to address the limitations of the current treatment. Bioprinting technologies, biomaterial engineering, and (stem) cell modelling provide a novel technological arsenal to pave the ground towards the design of bioinspired systems that recapitulate full organs. This study combines the generation of iPS-derived islets of Langerhans with volumetric bioprinting to engineer an advance in vitro model of the endocrine human pancreas for drug testing.

Differentiation of iPS to pancreatic islets of Langerhans was performed with a seven-stage protocol[1] . When reaching stage 7, iPS-derived islets were collected and pancreatic constructs were generated via layerless and shear stress-free, light-based Volumetric B(VBP)[2,3]. Concentrations ranging from 2%-7% (w/v%) of optically tuned bioresins, namely gelatin methacrylate and alginate methacrylate, were used for bioprinting. Bioresins were further functionalized with different combinations of collagen IV, laminin 511, and laminin 211, extracellular matrix components characteristic of the native human pancreas. Insulin release on the bioprinted constructs was assessed with a static glucose stimulated insulin secretion assay.

Volumetric bioprinting allowed to produce mm^3 constructs in 30 s. This ultra-fast techniques posed no mechanical or chemical stress on iPS-derived islets, which are highly viable for 21 days after printing. Stiffer, higher polymer content gelatin hydrogels presented the highest islet viability. Islet metabolic activity remains constant over time. Volumetric bioprinted iPS-derived islets showed mature, insulin, glucagon and somatostatin, single-hormone producing cells expression after 21 days. Bioprinted islets are functional and possess a glucose-responsive insulin secretion profile, and the system is compatible with supplementation with anti-diabetic drugs. Overall, this technology opens up to new possibilities on developing a novel platform for the *in vitro* testing of islet functionality.

[1]Balboa, D. et al. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells, Nature Biotechnology, 2022, 40: 1042-1055

[2]Bernal, P. et al. Volumetric bioprinting of complex living-tissue constructs within seconds. Advanced Materials, 2019, 31 (42): 1904209

[3]Bernal, P. et al. Volumetric bioprinting of organoids and optically tuned hydrogels to build liver-like metabolic biofactories, Advanced Materials, 2022, 34 (15): 211005

Restoration of functional PAX6 in aniridia patient iPSC-derived ocular tissue models using repurposed nonsense suppression drugs

Dulce Lima Cunha,[1,2] Hajrah Sarkar,[2,3] Jonathan Eintracht,[2] Philippa Harding,[2] Jo Huiqing Zhou,[1,4] and Mariya Moosajee[2,3,5]

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Congenital aniridia is a rare, pan-ocular disease causing severe sight loss, with only symptomatic intervention offered to patients. Approximately 40% of aniridia patients present with heterozygous nonsense variants in *PAX6*, resulting in haploinsufficiency. Translational readthrough-inducing drugs (TRIDs) have the ability to weaken the recognition of in-frame premature termination codons (PTCs), permitting full-length protein to be translated. We established induced pluripotent stem cell (iPSC)-derived 3D optic cups and 2D limbal epithelial stem cell (LESC) models from two aniridia patients with prevalent *PAX6* nonsense mutations. Both *in vitro* models show reduced PAX6 protein levels, mimicking the disease. The repurposed TRIDs amlexanox and 2,6-diaminopurine (DAP) and the positive control compounds ataluren and G418 were tested for their efficiency. Amlexanox was identified as the most promising TRID, increasing full-length PAX6 levels in both models and rescuing the disease phenotype through normalization of VSX2 and cell proliferation in the optic cups and reduction of ABCG2 protein and *SOX10* expression in LESCs. This study highlights the significance of patient iPSC-derived cells as a new model system for aniridia and proposes amlexanox as a new putative treatment for nonsense-mediated aniridia.

Abstracts – Submitted abstracts

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Revolutionizing Renal Research: Microfluidic Kidney Tubuloids-on-a-Chip for Improved Drug Translation

Dorota Kurek, Manuel Caro Torregrosa, Linda Gijzen, Marije van Beek, Julia Grasegger, Henriëtte Lanz

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Current 2D in vitro models of renal epithelium lack the key features of the in vivo setting, such as tubular structure and perfusion, resulting in low translatability to human situations and failure in clinical translation. Microfluidic techniques are becoming increasingly recognized as a valuable tool for adding physiologically relevant cues to traditional cell cultures. These cues include long-term gradient stability and continuous perfusion. Microfluidic technology also allows for the patterning of cell layers as stratified co-cultures that are free from artificial membranes, enabling the capture of complex tissue architectures found in vivo. Here, we introduce the OrganoPlate microfluidic platform, which can accommodate up to 64 independent microfluidic chips in a microtiter plate format, allowing the growth of 64 independent kidney tubuloid-derived barrier tissues in the form of perfused tubules. These renal tubules can be formed in just four days of culture in the device showing rapid and reproducible cell polarization, tight junction formation and proper expression of renal markers. When integrated into an OrganoPlate system, kidney tubuloids form leak-tight, perfusable tubes with stable Trans Epithelial Electrical Resistance (TEER), and are suitable for high-throughput screening of compound effects through assessment of barrier integrity by use of OrganoTEER and by real-time imaging of transport. OrganoPlate grown kidney tubes treated with Pgp inhibitor Verapamil show significant reduction of Rhodamine123 transport through kidney tubule barrier which confirms show stable activity of Pgp transporter and usability of the model in studying renal drug clearance. Our results demonstrate the suitability of our in vitro microfluidic kidney tubuloid-on-a-chip model in mimicking key physiological aspects of the kidney and offer new ways for studying organ physiology and renal disease mechanisms and drug toxicity.

Tubular organoid-derived gut-on-a-chip modelsuitable for drug development.

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Adult stem cell derived organoids are the most physiologically relevant cell source used in in vitro research, as they are composed of multiple differentiated cell types and resemble biology of an organ they were established from. In the standard culture protocols, organoids are grown as polarized cystic structures embedded in an ECM with limited apical access. Microfluidic techniques are increasingly recognized as an important toolbox to add physiologically relevant cues to traditional cell culture. There is a need to develop better models that capture the 3D morphology, heterogeneity, and boundary aspects of tissues while having potential to be implemented in high throughput screening pipelines. We established a human gut-on-a-chip model composed of intestinal organoids derived epithelial cells patterned inside of the microfluidic channel of an OrganoPlate®. Such tubular epithelial model of the intestinal tract shows rapid cell polarization, tight junction formation, functional transporters and expression of major intestinal markers. Reproducible barrier formation has been shown with the measurement of transepithelial electrical resistance (TEER) of tubules generated from different organoid lines' donors. Developed barriers proved to be sensitive to the pro-inflammatory triggers. The model is suitable for high-throughput screening of compound effects through real time imaging and rapid barrier integrity assessment. Complexity can be added to the miniaturized gut tubules model by incorporation of the other cell types (i.e. endothelial) into the system to mimic in vivo immunological responses. These next generation gut-on-a-chip models allow mimicking disease phenotypes such as inflammatory bowel disease (IBD) and support screening for potential drug targets. By combining adult human stem cell derived intestinal organoids with the microfluidic technology we provide a powerful platform to study physiology and disease mechanisms in patient specific gut models.

Single-cell analysis reveals a differential response of salivary gland stem/progenitor cells to photon versus proton irradiation

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An increasing number of head and neck cancer patients are being treated with proton therapy. Besides its physical advantages over conventional photon-based radiotherapy, little is known about the biological response to protons on healthy salivary gland tissue. To investigate and compare the potential differences of these radiation types, we used a murine salivary gland organoid (SGO) model and performed single-cell RNA sequencing to identify transcriptomic changes at the single cell level.
Analysis of 7 and 11 days old SGOs showed for the first time the presence of a population of salivary gland (SG) stem/progenitor cells defined by *Cd24a* and *Sox9*; acinar precursor cells defined by *Cd44, Itgb1* and *Trp63*; SG luminal duct cells defined by *Krt8/18* and differentiating cells defined by *Aqp5, Acta2* and *Krt19.*
Interestingly, photon and proton irradiation (IR) led to a similar reduction of SG stem/progenitor cell populations and an enrichment of the more differentiated type of cells. Nevertheless, proton-irradiated samples maintained a higher self-renewal capacity, measured as secondary organoid forming efficiency (OFE). Cell-cell interaction analysis showed a higher upregulation of Notch pathway after proton irradiation, which has been shown to be important for stem cell maintenance and proliferation following irradiation. Indeed, inhibition of Notch pathway reduced the OFE of irradiated organoids while its activation improved the OFE of photon-IR organoids. In conclusion, our study identifies new potential SG stem/progenitor cell populations and suggests the involvement of Notch pathway in the maintenance of these populations following proton irradiation.

This work was supported by the Dutch Cancer Society KWF Grant nr: 12092

iPSC disease models to study and treat Diamond-Blackfan Anemia Syndrome (DBAS)

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Introduction: Disease mechanisms driving anemia in DBAS are not completely elucidated, and research is hampered by the scarce availability of patient samples and disease models. Recently, we have developed an induced pluripotent stem cell (iPSC) culture to RBC differentiation platform, providing a powerful tool to study erythroid development. The TRACER-consortium aims to generate novel DBAS disease models, to genetically correct DBAS-iPSCs to produce cultured RBCs for transfusion, or corrected HSCs for autologous stem cell transplantation (Fig.1A). Currently, DBAS treatment is limited to glucocorticoids, blood transfusions, or allogeneic SCT, associated with toxicity and risk.

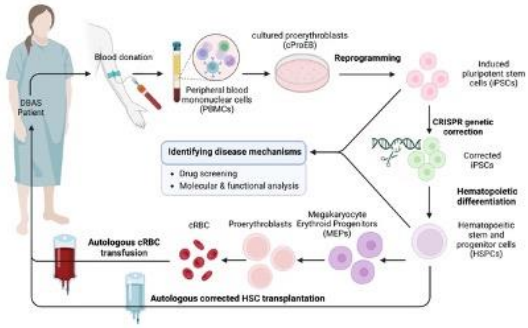
Methods: Patients were selected from the Dutch Registry (DBAN; Fig.1B) and PBMC-derived cultured proerythroblasts (proEB) were reprogrammed using the non-integrative Cytotune-Sendai-IPS2.0-kit. The DBAS-iPSC pluripotency marker expression and differentiation potential is demonstrated. DBAS-iPSC derived embryoid bodies (EBs) give rise to 'hematopoietic organoids' (HO) that produce hematopoietic production cells (HPCs) which are characterized by flow cytometry, and further expanded to produce proEBs and enucleated cRBCs.

Results: ProEBs of three DBAS patients were reprogrammed and these DBAS-iPSCs form EBs, HO, and HPCs, similar to healthy control iPSCs. However, the DBAS-HPCs display a slight bias to the myeloid lineage, and a reduced proEB expansion potential. Nonetheless, this platform allows the sustainable production of HPCs for further mechanistic studies.

Conclusion: In DBAS there is a clinical need for novel therapeutic strategies to treat severe anemia and reduce organ toxicity. DBAS-iPSC lines provide a sustainable source of DBAS models, in which erythropoiesis, novel therapeutics, including gene therapy, can be studied for clinical applications.

Figure 1.

A.



B.

Patient	Molecular defect	Current treatment	glucorticoid response	*In vitro* proerythroblast culture	Input reprogramming
#P1	Unknown*	Transfusions	Partial	Possible	Cultured Proerythroblasts
#P2	Unknown*	Transfusions	Partial	Possible	Cultured Proerythroblasts
#P4	Unknown*	Transfusions	Unresponsive	Unable, poor	PBMC
#P5	*RPS26*	None	Good	Good	Cultured Proerythroblasts
#P6	*RPS24*	GC low dose	Good	T.B.D.	PBMC
#P8	*RPS26*	None	Good	Very Good	NA
#control1 (parent of P1 & P2)	Unknown	NA		Possible / Good	NA
#control 2 (parent of P1&P2)	Unknown	NA		Possible / Good	NA

* diagnosis confirmed based on preRNA analysis (Northern Blot, Gleizes Lab Toulouse)

Blood organoids from induced pluripotent stem cells: generating hematopoietic stem cells

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To overcome the unmet need for an increased source for hematopoietic stem cell (HSC) transplantations, novel approaches generating *de novo* HSCs need to be developed. Ideally, HSCs would be obtained from autologous human induced pluripotent stem cells (hiPSCs) to overcome this therapeutic bottleneck, but this has not been achieved yet. In an effort to bridge this gap, we aim to recapitulate the *de novo* generation of HSCs using an hiPSC-derived differentiation model. This model, with minimal cytokine input, relies on spontaneously inducing embryoid-body (EB)-like structures, which develop into hematopoietic organoids that produce progenitor cells. Given the fact that these progenitors can be terminally differentiated into enucleated red blood cells, we would like to explore whether this model could also be exploited to obtain HSCs.

First, we immunophenotypically characterized the different cell populations that arise within these hematopoietic organoids by different membrane markers. Data indicates the sequential shift of endothelial towards hematopoietic cells, similar to events happening in the aorta-gonad-mesonephros region during embryogenesis. Additionally, we observe an HSC-like population occurring along this transition. Moreover, we have performed transcriptomic characterization of these hematopoietic organoids by Single-cell RNA sequencing, allowing in-depth detection of different populations within these organoids. Preliminary data identify distinct populations, including a hematopoietic cluster, which confirms our flow cytometry data.

Further tweaking of the model and functional assays (e.g. CFU/CAFC assays, transplantation assays) should point out whether this suspected HSC population actually consists of putative functional HSCs. This knowledge could eventually be used to broaden opportunities for clinical applications.

Mutant GFAP disrupts lineage commitment of neural organoids in a mechanosensitive manner

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As a type-3 intermediate filament protein involved in cell motility and structure, glial fibrillary acidic protein (GFAP) is primarily expressed by astrocytes in the central nervous system. Mutations in GFAP cause Alexander Disease (AxD), a rare, but fatal neurological disorder. Since AxD is classified as an astrocytopathy, where GFAP-expressing astrocytes are drivers of white and grey matter deterioration, most research in animal models has focused on astrocytes. However, GFAP can also be expressed by neural stem cells in the developing fetal brain, which allows for speculation that mutant GFAP can disrupt neurodevelopment. A recent paper by Arellano et al. (2021) suggests that the onset of GFAP expression in humans starts at the onset of corticogenesis, whereas in rodents it starts when corticogenesis has been completed. As such, any potential neural stem cell pathology due to mutant GFAP can only be investigated in a human model of brain development. In order to investigate the effects of mutant GFAP on human brain development, we generated neural organoids from AxD patient-derived iPSCs carrying heterozygous mutations in GFAP. We found that GFAP is expressed by iPSCs and that mutant GFAP disrupts early neural lineage commitment of embryoid bodies in a mechanosensitive manner. We show that this neuroectodermal acquisition defect can be manipulated and rescued through interventions in TGFβ, BMP and Wnt signaling.

A perfusable ureteric bud-on-chip model

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Background: We aim to develop perfusable human ureteric bud (UB) and collecting duct (CD) on chip models for drug testing, disease modeling, and, ultimately, integration within biomanufactured kidney tissues. The main motivation for our work is two-fold. First, existing CD models are limited by the availability of appropriate human cell lines or lack of relevant biophysical cues, such as flow. Second, the ability to integrate an open-ended CD within biomanufactured kidney tissues would enable urine drainage.

Methods: We adapted a recently developed UB differentiation protocol from human pluripotent stem cells. Next, we evaluated several possible candidates for the extracellular matrix that surrounds the open lumen and is seeded with these cells on chip. Finally, we generate a confluent UB monolayer and subject this to controlled flow on chip.

Results: We successful scaled existing UB organoid protocols from a 96-well to a microwell / transwell culture format. Similar growth potential, morphology, and gene expression were observed between these approaches. Cells obtained from dissociated organoids form a stable confluent UB monolayer when seeded on an engineered matrix composed of Geltrex and Collagen I. The resulting UB-on-chip was perfused and maintained UB-like marker expression and morphology over several days.

Conclusion: Looking ahead, we plan to investigate flow effects on UB-to-CD maturation and function with the goal of creating a human CD model that can be used directly or embedded within biomanufactured kidney tissues.

Heart and kidney organoids maintain organ-specific function in a microfluidic system

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Heart and kidney communicate with one another in an interdependent relationship and they influence each other's behavior reciprocally, as pathological changes in one organ can damage the other. Although independent human in vitro models for heart and kidney exist, they do not capture their dynamic crosstalk. We have developed a microfluidic system which can be used to study heart and kidney interaction in vitro. Cardiac microtissues (cMTs) and kidney organoids (kOs) derived from human induced pluripotent stem cells (hiPSCs) were generated and loaded into two separated communicating chambers of a perfusion chip. Static culture conditions were compared with dynamic culture under unidirectional flow. Tissue viability was maintained for minimally 72 hours under both conditions, as indicated by the presence of sarcomeric structure coupled with beating activity in cMTs and the presence of nephron structures and albumin uptake in kOs. We concluded that this system enables the study of human cardiac and kidney organoid interaction in vitro while controlling parameters like fluidic flow speed and direction. Together, this "cardiorenal-unit" provides a new in vitro model to study the cardiorenal axis and it may be further developed to investigate diseases involving both two organs and their potential treatments.

Growth factors independently control pluripotent state transitions and morphogenesis

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Mouse embryogenesis encompasses a pluripotent phase starting at the blastocyst stage and ending with lineage commitment at gastrulation. During this phase the epiblast acquires apicobasal polarity and forms a rosette that subsequently undergoes lumenogenesis to create a monolayer epithelium. Concomitantly, pluripotency proceeds from naïve to primed pluripotency via an intermediate rosette pluripotent state. Both polarization and differentiation to rosette pluripotency are induced by the downregulation of WNT signals upon implantation. Transition to rosette pluripotency then enables the cells to respond to MEK signals which induces both lumenogenesis as well as transition to primed pluripotency.

How WNT and MEK signals coordinate differentiation and morphogenesis of the epiblast remains unclear. Here, we show how control over pluripotency progression pivots from WNT to MEK signals in the rosette and identify how these same signals drive morphogenesis independently from pluripotent state transitions.

The downregulation of WNT factor WinTF1 enables expression of rosette-specific transcription factor OTX2. OTX2 subsequently allows the rosette to respond to MEK signals and differentiate to primed pluripotency. Remarkably, OTX2KO cells were able to undergo lumenogenesis, despite not being able to differentiate to primed pluripotency. We find that MEK-induced chloride channels drive lumenogenesis independent of differentiation to primed pluripotency. Similarly, we find that WNT blocks polarization through inactivation of the Rap1 pathway and that this also happens independent of differentiation to rosette pluripotency.

Our results demonstrate how growth factors can coordinate cell state transitions with morphogenesis through independent mechanisms and identify remarkable safeguarding mechanisms that ensure coordinated differentiation and epiblast morphogenesis.

Genomic correction of the FGFR2-C342Y Crouzon mutation using prime-editing and identification of distinctive downstream signaling profiles in Craniosynostosis patient-derived hiPSC lines

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We generated human induced Pluripotent Stem Cell (hiPSC) lines from three Craniosynostosis patients with distinct mutations in FGFR2 resulting in Crouzon- ($FGFR2^{+/C342Y}$; $FGFR2^{+/E565G}$) and Apert- ($FGFR2^{+/S252W}$) syndrome. We aim to utilize these patient hiPSCs as *in vitro* disease models to profile the potential changes in FGFR2 signaling in Craniosynostosis pathophysiology. Additionally, genomic correction of causative mutations should restore patient cells to clinically healthy clones.
Unstimulated FGFR2 signaling is profiled by exposing patient hiPSCs to bFGF-free conditions for 24 hours. Protein expression and phosphorylation of FGFR2 and two direct FGFR2-docking proteins PLCγ and FRS2α are assessed by Western Blot. The Crouzon-C342Y patient hiPSCs were targeted by CRISPR/Cas9 prime-editing for genomic correction. Both mixed population as well as individual clones are screened for successful editing.
Crouzon-C342Y patient samples show increased FGFR Tyr653/654 compared to the controls despite the absence of bFGF for 24 hours. We observe a noticeable increase of PLCγ- and FRS2α -phosphorylation in the Apert-S252W and the Crouzon-E565G samples compared to controls. Additionally, we notice a significant downregulation of FRS2α protein in all patients compared to the control samples. Applying the prime-editing constructs for FGFR2 correction to Crouzon-C342Y hiPSCs resulted in successful edits in the mixed population and genotyping suggested of 3 out of 13 clones are successfully repaired.
The data suggest that ligand-dependent FGFR2-mutant hiPSCs may distinctively activate downstream signaling in the absence of bFGF and FGFR2 activation. Additionally, genomic correction in Crouzon-C342Y patient-derived hiPSC appears feasible, and should be utilized for the Apert-S252W and Crouzon-E565G lines as well.

Single-cell proteogenomic analysis of hematopoietic stem cell regeneration in pediatric transplant recipients

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Hematopoietic cell transplantation (HCT) is a life-saving therapy for various diseases, including leukemia. Its success relies on the engraftment of donor hematopoietic stem and progenitor cells (HSPCs), which regenerate all differentiated blood cell types. Despite its application in >40.000 patients annually, the mechanisms by which human HSPCs regenerate blood in a foreign host, and the impact of transplantation on the HSPC population, remain unclear. Using CITE-seq (cellular indexing of transcriptomes and epitopes by sequencing), we compared the characteristics of HSPCs, immune and niche cells, before and at multiple time points after transplantation in pediatric patients. We created a detailed map of 41.294 bone marrow cells from four pediatric HCT donors. We identified various distinct HSPC subsets, their cell states and differentiation trajectories towards erythroid, myeloid and lymphoid cells. Ligand-receptor analysis revealed multiple significant ($p<=0.05$), interactions with HSCs as the receiving partner. Most signals were sent from mesenchymal stromal cells, including known signals (e.g., collagen-CD44, CXCL12-CXCR4), as well as signals that have not been reported before in the human hematopoietic system. Ongoing experiments are directed at comparing these pre-transplant data to samples obtained after HCT, during early and late regenerative phases. Preliminary results will be available at the DSSCR meeting. Collectively, these studies will provide unprecedented insight into the regenerative and adaptable nature of human HSPCs. Understanding the signaling pathways by which transplanted HPSCs interact with the recipient's niche could lead to new strategies to predict or improve HCT outcome.

Base editing as a therapeutic approach for LMNA Q493X cardiomyopathy

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Dilated cardiomyopathy (DCM) is a heart disease characterized by left ventricular dilation and consecutively, contractile dysfunction. It is one of the most common causes of heart failure with an estimated prevalence of approximately 1:250–400. In about 35% of patients, genetic mutations can be identified that usually involve genes responsible for cytoskeletal, sarcomere and nuclear envelope proteins. One of these genes is LMNA, it encodes for Lamin A and Lamin C which have roles in maintaining nuclear structural integrity, regulating gene expression, mechanosensing and mechanotransduction. LMNA mutations cause up to 10% of dilated cardiomyopathies and have a complete penetrance by the age of 60 years. The exact mechanisms by which mutations in LMNA cause heart disease are insufficiently understood, and there are no current therapies that can cure LMNA-associated cardiomyopathy. The LMNA Q493X mutation, is a pathogenic LMNA mutation which has been identified in multiple families with hereditary DCM. Our research aims to obtain a better understanding of the disease mechanism and to develop base editing approaches to correct the pathogenic LMNA Q493X mutation. To achieve this, we will utilize human induced pluripotent stem cell-derived cardiomyocytes, engineered heart tissue, cardioid models and a mouse model.

Assessing the safety of pluripotent stem cell therapies: tumour formation risk

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The field of regenerative medicine is advancing towards the clinic at an unprecedented pace owing to immense efforts focused on developing human pluripotent stem cell (hPSCs)-based therapies as a potential cure for a large number of diseases. Nevertheless, successful implementation of hPSC-based therapies is hampered by multiple safety concerns, including the risk of tumour formation. Despite ongoing improvements in hPSC directed differentiation protocols, the presence of residual undifferentiated cells or other off-target cell populations within the final cell product is a valid concern. Moreover, hPSCs are known to acquire recurrent (epi)genetic aberrations during reprogramming (in the case of induced hPSCs, i.e. hiPSCs) and long-term culture which are strongly reminiscent of those commonly observed in cancer. Therefore, a complete understanding of the causes and consequences of unwanted cell populations that arise during differentiation as well as of the recurrent (epi)genetic aberrations in hPSCs is vital to ensure the safety of hPSC-based therapies. This line of research can greatly benefit from research on germ cell tumours (GCTs), which originate from the deregulation of the hPSCs that arise during early embryonic development, including embryonic stem cells and primordial germ cells and therefore represent a malignant caricature of pluripotency. This project aims to further interrogate the similarities observed between cancer and hPSCs (and their derivatives), with a specific focus on GCTs. Therefore, this project aims to contribute to the development of techniques that assess, prevent or eliminate potentially malignant cells obtained during hPSC directed differentiation, bringing safe hPSC-based therapies closer to the clinic.

Cell Therapy Scale-up: Bioprocess Development for the Production of Hematopoietic Cells

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Abstract

Blood transfusion is currently the most common cell therapy applied worldwide to treat various medical purposes [1]. One of the purposes is the treatment of severe anemias, as these diseases can only be treated with blood transfusions and stem cell transplantations. An attractive therapy that could overcome the risks involved with donor-derived transfusion products is in vitroproduced red blood cells (RBCs) or the corresponding stem cells, namely hematopoietic stem cells (HSCs) [2,3]. In vitro RBCs can be produced from induced pluripotent stem cells (iPSCs), which are pluripotent cells created by reprogramming human somatic cells. This reprogramming technique started a new era in regenerative medicine due to their self-renewing properties and multilineage differentiation potential to produce patient-specific progenitor or functional effector cells [4, 5]. However, hematopoietic cells and RBCs from iPSC are mostly produced via multiple differentiation steps using static adherent protocols which hinders scalability towards clinically relevant volumes. A dynamic shake flask cultivation was developed to produce hematopoietic stem/progenitor cells (HSPCs), which can further be differentiated into functional RBCs. The dynamic cultivation led to a ~16,000 fold increase in cell number compared to static cultivation. This project aims to transfer the developed protocol to a suspended stirred-tank bioreactor to produce HSPCs. Employing bioreactors will improve reproducibility and facilitate upscaling towards the desired volumes, as mini-transfusions (1011 RBC, required in phase I trial) could be generated with 3 to 4 three-litre bioreactors. The main challenges to be optimized are specific nutrient and oxygen requirements for each differentiation step, as well as shear stress effects.

[1] WHO. (2022). Blood transfusion. https://www.who.int/news-room/facts-in-pictures/detail/blood-transfusion
[2] Hansen, M., et al. (2019). Human-induced pluripotent stem cell-derived blood products: state of the art and future directions. FEBS Letters, 593(23), 3288–3303. https://doi.org/10.1002/1873-3468.13599
[3] Wilkinson, A. C., et al. (2019). Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. Nature, 571(7763), 117–121. https://doi.org/10.1038/s41586-019-1244-x
[4] Martins Fernandes Paes, B. C., et al. (2017). Ten years of iPSC: clinical potential and advances in vitro hematopoietic differentiation. Cell Biology and Toxicology, 33(3), 233–250. https://doi.org/10.1007/s10565-016-9377-2
[5] Li, Y., et al. (2017). Engineering-derived approaches for iPSC preparation, expansion, differentiation and applications. Biofabrication, 9(3), 32001. https://doi.org/10.1088/1758-5090/aa7e9

The secretome of pulmonary microvascular endothelial cells supports alveolar epithelial growth

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The intricate role of the pulmonary microvascular endothelium in orchestrating alveolar epithelial repair remains enigmatic. This study explores the potential involvement of the secretome from human pulmonary microvascular endothelial cells (HPMECs), consisting of extracellular vesicles (EVs) and soluble factors (SFs), in alveolar regeneration.
HPMEC-derived EVs and SFs were purified using ultrafiltration and size exclusion chromatography. Murine lung organoids were generated by co-culturing alveolar epithelial progenitors with lung fibroblasts. The impact of the HPMEC-secretome was assessed by co-culturing HPMECs underneath the organoid insert. Additionally, organoids were exposed to HPMEC-derived EVs and SFs. Proteomic analysis unveiled the protein content of EVs and SFs, while RNA sequencing (RNA-seq) revealed transcriptomic changes in primary ECs from cigarette smoke (CS)-exposed mice.
Presence of untreated HPMECs significantly increased alveolar organoid count, while organoid size decreased. Treatment with HPMEC-derived EVs or SFs enhanced organoid count, without affecting size. Proteomics analysis on the HPMEC-secretome highlighted proteins related to VEGFA-VEGFR2, NRF2, WNT, and BMP signalling. Remarkably, HPMEC pre-treatment with cigarette smoke extract diminished the supportive function of the secretome, as was observed by a reduced alveolar organoid count. RNA-seq analysis showed decreased expression of BMP1, BMP4, and BMP6 in ECs from CS-exposed mice. Recombinant BMP6 treatment promoted alveolar organoid formation.
HPMECs support alveolar organoid growth through the secretion of EVs and SFs. Secreted factors, particularly BMPs, are involved in this supportive function. These data suggest an important role of HPMECs and its secretome as orchestrators in lung repair.

Development of an in vitro model of sensory neurons differentiated from human pluripotent stem cells for the study of neuroplasticity.

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Introduction: One of the pathological features observed in asthma is neuroplasticity of sensory neurons. In asthma specifically, this manifests as increased neuron density, increased neurite length and a lowered firing threshold, resulting in hyperresponsiveness to (a-)specific stimuli. To study this phenomenon of neuroplasticity in lung tissue, we developed an in vitro human model of sensory neurons.

Material and method: For differentiation of H9WA09 human pluripotent stem cells (hPSCs) into sensory neurons, hPSCs were first differentiated into neural vagal crest cells (vNCCs). Subsequently formed vNNCs spheroids were then differentiated into pre-mature sensory neurons using STEMdiff™ differentiation and maturation kits (Stemcell TechnologiesTM) in 6 days, followed by a minimum of 10 days of maturation. Thereafter the differentiation was validated.

Results and discussion: Using immunofluorescent staining we found successful generation of β3-tubulin+ neurons, with a subset of them being NAV1.7+/TRPV1+ sensory neurons. Successful differentiation of these sensory neurons was also accomplished in an axon-guidance NeuroChip model. Similarly, flow cytometry showed an average yield of 27% ±19% β3-tubulin+/TRPV1+ and 34% ±11% NAV1.7+sensory neurons. MEA analysis revealed spontaneous firing of the hPSC-derived sensory neurons. Ca2+ measurements indicated activation of sensory neurons upon exposure to capsaicin (10nM-10μM) through the TRPV1--channel.

Conclusion: We successfully generated sensory neurons from hPSCs as supported by data from immunofluorescence, flow cytometry, MEA and Ca2+ measurements. Furthermore, sensory neurons were successfully cultured in a NeuroChip, facilitating the study of neuro-effector interactions in cocultures, by separation of the neurites and cell bodies. This was further confirmed by initial co-culture experiments of hPSC-derived sensory neurons with mast cells.

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Employing the epigenetic memory and instructive stimuli to stimulate iPS-NLC differentiation

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Employing notochordal-like cells (NLCs) derived from human induced pluripotent stem cells (hiPSCs) is a promising strategy for rejuvenating the degenerated intervertebral disc (IVD). Therefore, this study aimed to build on the tissue-specific epigenetic memory of hiPSCs derived from IVD-progenitor cells (TIE2$^+$-cells) to improve hiPSC differentiation towards mature, healthy matrix-producing NLCs. For this, hiPSCs were generated from TIE2$^+$-cells (*n*=3) and, as a comparison, donor-matched peripheral blood mononuclear (PBM)-derived iPSCs were used. Firstly, hiPSCs were differentiated into mesendodermal progenitors by Wnt-pathway activation. Thereafter, cells were further driven towards the NC-lineage by transfection with *NOTO*-mRNA and matured by switching to a 3D-pellet culture in discogenic medium supplemented with 10ng/mL TGF-β_1 or 3mg/mL decellularized notochordal cell-derived matrix (dNCM). Both TIE2$^+$- and PBM-cell derived hiPSC showed successful differentiation towards mesendodermal progenitor cells following Wnt-activation, indicated by a decreased gene expression of pluripotency markers and upregulation of Wnt-target genes and mesendodermal markers compared to mTeSR1 controls. This was confirmed by immuno-stains for FOXA2 and TBXT. After transfection, we confirmed a significant increase in *NOTO* mRNA levels in all donor lines. 3D-pellets of all donor lines showed glycosaminoglycan (GAG)- and collagen type II-rich areas after dNCM- but not TGF-β_1-treatment. This was confirmed with the DMMB-assay, showing a significantly increased GAG content in the 3D-pellets treated with dNCM compared to TGF-β_1. Next to that, TIE2$^+$-cell derived iPSC pellets contained a higher GAG content compared to the PBM-cell derived hiPSC pellets. In conclusion, using tissue-specific TIE2$^+$-cell derived hiPSCs combined with dNCM-treatment may allow for an improved iPS-NLC differentiation capacity.

Ototoxic effects of cisplatin and gentamicin in human inner ear organoids and human adult vestibular organs

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Hearing loss and balance disorders can result from inner ear damage after treatment with antitumor drugs or antibiotics. Current animal and *in vitro* models do not completely represent the human inner ear, therefore, safety testing and identification of pathophysiology remain challenging. Recently, inner ear organoids (IEO) derived from human pluripotent stem cells (hiPSCs) have been described [1, 2]. These organoids contain cochlear or vestibular hair cells [2, 3], which are morphologically and physiologically similar to their counterparts in the normal inner ear.

Using IEOs and human adult vestibular organs, we aim to elucidate the pathophysiological mechanisms underlying the ototoxic effects of the antitumor drug cisplatin and the antibiotic gentamicin.

To this end, IEOs were generated from hiPSCs in three-dimensional culture and, next, 200 µm thick vibratome sections were obtained at day 75 to access the hair-cell-containing inner ear vesicles within the organoids. The ototoxic compounds were applied for 24 hours; cisplatin doses ranged from 0-100 µM and gentamicin doses from 0-1000 µM. Immunofluorescence was used to evaluate stereocilia bundle presence, apoptosis marker expression and neuronal integrity in the treated and untreated samples. These findings were compared with human vestibular organs obtained during translabyrinthine vestibular schwannoma surgery and subjected to identical treatments.

Preliminary results indicate increased apoptosis within the inner ear vesicle epithelium of the vibratomized IEOs and loss of neuronal integrity after gentamicin treatment. Ongoing work focusses on the time course of these changes and the ototoxic effects of both compounds on the human adult vestibular organs and the human IEOs.

1. *Van der Valk, W.H., et al., A single-cell level comparison of human inner ear organoids and the human cochlea and vestibular organs. Cell Reports, 2023.*
2. *Koehler, K.R., et al., Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. Nat Biotechnol, 2017. 35: p. 583-589.*
3. *Moore, S.T., et al., Generating high-fidelity cochlear organoids from human pluripotent stem cells. Preprint in Cell Stem Cell, 2022.*

Cytogenetic and phenotypic profiling of peri-implantation human embryo development Maldonado Guzman, M.R.[1,2], Chavli, E.A.[1] , Van Opstal, A.R.M.[3] , ten Berge, D.[2] , Baart, E.B.[1,4]

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Early human embryo development partially tolerates an abnormal chromosome content. After in vitro fertilization (IVF), up to 80% of pre-implantation embryos show a heterogeneous karyotype with a mix of diploid and aneuploid cells, referred to as chromosomal mosaicism. Transfer of mosaic embryos after IVF is associated with lower implantation rates and miscarriage, but can also lead to healthy live birth. This study aims to investigate the developmental potential of mosaic embryos and the impact of aneuploid cells on early development. We performed a Trophectoderm biopsy on embryonic day 5 (E5), followed by culture of the remaining embryo until E8 with immunofluorescence analysis of lineage markers and karyotype analysis using an NGS-approach. By comparing the chromosomal constitution between E5 and E8 we observed that only half of the embryos with a mosaic or abnormal E5 biopsy result could be confirmed at E8. Remarkably, within the mosaic group a majority of embryos showed a diploid composition on E8. Furthermore, our results showed that E5 mosaic embryos with a diploid composition on E8 have a similar diameter to embryos with diploid composition in both stages. In contrast, abnormal constituted embryos showed an Epiblast and Hypoblast cell reduction up to 80% compared to diploid embryos. Spatial distribution of ICM derived-cell lineages was also affected as embryos with delayed development showed peripheral location of the Epiblast and Hypoblast markers. Development of all embryos was monitored by time lapse imaging, allowing clinically relevant embryo grading with chromosomal constitution and morphology evaluation during the unexplored peri-implantation stages.

A data-driven approach towards mature iPSC-derived keratinocytes

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Human induced pluripotent stem cells (iPSC) bear great potential to study chronic skin diseases, since they can be derived from patients or can be genome-edited. So far, the generation of human organotypic skin modelsfrom iPSC-derived keratinocytes (iKC) is hampered by limited iKC proliferation, and heterogeneous and immature phenotypes. We aimed to overcome these pitfalls by a data-driven optimization strategy, in addition to previous hypothesis-driven approaches. We observed that CELLnTEC (CnT)-30 medium greatly increased the iKC culture homogeneity as compared to standardly used keratinocyte serum free medium (KSFM). Transcriptomic analysis revealed that iKC generated in CnT-30 have significantly higher basal keratinocyte marker expression (e.g., 100-fold higher keratin 5) and reduced expression of fibroblast markers (e.g., 10-fold lower vimentin), thereby more closely resembling the transcriptome of primary keratinocytes. Pathway analysis of differentially expressed genes indicated potential cascades to target with small molecule compounds to improve iKC generation, including inhibition of PI3K-Akt and JAK-STAT signaling. Unfortunately, PI3K and JAK inhibition did not improve iKC maturation based on morphology and K5/K14 expression. Furthermore, iKC expansion remains challenging, which is crucial for organotypic skin model development. Importantly, the addition of ROCK inhibitor to improve iKC proliferation appeared crucial, yet highly dependent on the exposure time periods during differentiation. To further improve differentiation, we plan to perform single cell RNA sequencing to quantify cell culture heterogeneity and to discover cell surface markers that allow for enrichment of the mature iKC population for further investigations.

Steering epithelial and mesenchymal cell type composition in an iPSC-derived Intestine-Chip

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Introduction: Intestinal model systems often lack a physiological cell type diversity as either proliferating or differentiating cells are induced. Our aim was to replicate a growth factor gradient in an Intestine-Chip, hereby maintaining proliferating stem cells and inducing the diverse stages of differentiating epithelial and mesenchymal subtypes.
Methods: Human intestinal epithelial and mesenchymal cells were generated from three control induced pluripotent stem cell (iPSC) lines and introduced in an Emulate Chip-S1 system. The cells were exposed to expansion medium (EM) mimicking the crypt condition, differentiation medium (DM) mimicking the villus condition or a combination (EM-DM) by introducing EM to the lower and DM to the upper compartment of the system. Intestinal epithelial and mesenchymal populations were assessed via immunofluorescent staining, flow cytometry and single-cell RNA sequencing.
Results: Both EM-DM and DM conditions induced mature epithelial subtypes (e.g. goblet, enteroendocrine, Paneth cells, enterocytes) when compared to EM. However, EM-DM better preserved proliferating transit-amplifying cells, progenitor stages of mature epithelial subtypes and tissue morphology than DM. The mesenchymal population was reduced in EM-DM and DM when compared to EM and shifted from a proliferating to a fibroblast-like phenotype. Comparing the Intestine-Chip dataset with reference data of the human intestine indicated resemblance to the small intestine in composition and function.
Conclusion: We present a thorough characterization of the intestinal epithelial and mesenchymal populations in an iPSC-derived Intestine-Chip. Applying a growth factor gradient yielded a physiologically relevant intestinal epithelial composition, capturing the entire differentiation trajectory from stem cells to progenitor stages and mature cells.

Understanding the role of TP53 in the malignant behavior of human pluripotent stem cells

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The malignant potential of human pluripotent stem cells (PSC) is not fully understood and its evaluation currently relies solely on the assessment of the cells' behavior *in vivo* upon their engraftment into mouse models (teratoma assay). *In vitro* however, long-term culture of PSCs can lead to (epi)genetic drift, potentially activating processes that resemble malignant transformation as an adaptive mechanism to the culture conditions. Here we investigated the relevance of *TP53*, a key gene regulating cell cycle control and (epi)genetic stability and found mutated in various PSC lines. With this goal, *TP53* knockout cell lines were generated through CRISPR-Cas9 technology of representatives of an embryonic (H9) and an induced (Lu07) PSC line. We demonstrate that despite the loss of *TP53* expression and related depletion of *P21* expression (mRNA & protein), the knockout lines did not show an increased expression of the pluripotency and malignancy-associated miRNA-371 and miRNA-373. Similarly, we did not observe changes in the expression levels of pluripotency markers both through qPCR (*OCT4*, *SOX2*) and flow cytometric analysis (*OCT4, SSEA3, TRA-1-60, NANOG*). Additionally, *TP53* knockout cell lines displayed proliferation rates similar to those of their isogenic wild-type counterparts, despite showing a greater colony formation capacity. We also explored the effect of the depletion of *TP53* to cisplatin exposure, a DNA-damaging agent commonly and successfully used in clinics for the treatment of malignant germ cell tumors, showing partial similarities to PSC-derived (*in vivo*) tumors. We observed that removal of *TP53* led to increase of cisplatin resistance of PSC lines, similar to clinical observations in malignant germ cell tumors. Overall, this study shows that *TP53* mutations in PSCs *in vitro* can lead to a greater resilience of the cells, while not necessarily triggering their oncogenic transformation, supporting the potential value of studying PSCs in the context of pathogenesis and clinical handling of malignant germ cell tumors and vice versa.

Extracellular matrix is a critical factor in the divergence of the germline and the amniotic lineages.

Arend W. Overeem, Yolanda W. Chang, Susana M. Chuva De Sousa Lopes

The human germline is established around week 2-3 of embryonic development, with the emergence of primordial germ cells (PGCs). The origin of human PGCs is still unclear: PGCs may be derived from either the posterior epiblast, the amnion or both. Specification of PGCs depends on bone morphogenic proteins (BMPs), which also induce the formation of the amnion during the same period of development. What determines the separation of the germline from other BMP-induced lineages remains unresolved. We report that the extracellular matrix (ECM) is important in hPGCLC versus amniotic fate determination. By adding diluted basement membrane extract (BMEx overlay) with BMP4, hPGCLCs form with up to 70% efficiency in conventional 2D culture. Single-cell transcriptomics revealed that hPGCLCs differentiated by BMEx-overlay are highly similar to PGCs from a Carnegie stage 7 human embryo. Interestingly, without BMEx overlay, only TFAP2A+/GATA3+ amniotic ectoderm-like cells were formed, but not hPGCLCs. We observed that BMEx overlay allows the feeder-free maintenance of isolated hPGCLCs and that hPGCLCs cultured without BMEx do not survive, lose germ cell marker expression, or revert to a SOX17-/POU5F1+ pluripotent state. Current hPGCLC differentiation methods have variable efficiencies and are difficult to scale up. Our results not only revealed an important role for ECM in hPGCLC specification and survival, but we also present a robust and scalable method of generating hPGCLCs which will benefit the field of *in vitro* gametogenesis in humans.

Mpox infection and drug treatment modelled in human skin organoids

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Mpox virus (MPXV) initially infects human skin to cause lesions. Currently, robust models that recapitulate skin infection by MPXV are lacking. Here, we demonstrate that human induced pluripotent stem cell-derived skin organoids are susceptible to MPXV infection and support infectious virus production. Keratinocytes, the predominant cell type of the skin epithelium, effectively support MPXV infection. Using transmission electron microscopy, we visualised the four stages of intracellular virus particle assembly: crescent formation, immature virions, mature virions and wrapped virions. Transcriptional analysis showed that MPXV infection rewires the host transcriptome with triggers abundant expression of viral transcripts. Early treatment with the antiviral drug tecovirimat inhibits MPXV infection, preventing infectious virus production and altered host transcriptional responses. Delayed treatment with tecovirimat also inhibits infectious MPXV particle production, albeit to a lesser extent. This study establishes human skin organoids as an experimental model for studying MPXV infection, mapping virus-host interactions and testing therapeutics.

Generation of 7 human induced pluripotent stem cell lines with CRISPR/Cas9-mediated deletions in the *DMD* gene

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Duchenne muscular dystrophy (DMD) is an X-linked progressive neuromuscular disorder caused by mutations in the *DMD* gene which result in the absence of dystrophin. In healthy muscle only the full-length dystrophin isoform Dp427m is expressed, while the brain expresses the full-length isoforms Dp427c and Dp427p and the shorter isoforms Dp140 and Dp71/40. Depending on the position of the mutation within the *DMD* gene, one or multiple isoforms are lacking in the brain, which is associated with cognitive and behavioral problems. Knowledge on the role of the isoforms and the consequences of a lack thereof is limited.

We utilized the CRISPR/Cas9 genome editing technology to generate isogenic human iPSC lines lacking one or multiple dystrophin isoforms from two healthy male control hiPSC lines to improve our current understanding. The expression of single isoforms (either Dp427c, Dp427p, Dp140 or Dp71) was abolished by targeting their unique promoter. The expression of multiple isoforms was abolished by the deletion of specific exons. Exon 11, 52 or 66 was deleted to respectively disrupt expression of either Dp427, Dp427+Dp140 or all isoforms. After gene editing, clones were picked and expanded. Mutated clones were selected by Sanger sequencing. hiPSCs and subsequently neuronal progenitor cells (NPCs) will be characterized by karyotyping, expression of pluripotency markers and differentiation into cells of the three germ layers.

These isogenic DMD hiPSC lines will be a valuable resource for the characterization of the role of dystrophin isoforms in brain cell development by studying the morphological, molecular and functional consequences of their absence.

Understanding dilated cardiomyopathy in epidermolysis bullosa patients carrying KLHL24 mutation using patient-derived induced pluripotent stem cells

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The unique aspect of epidermolysis bullosa patients with mutation in KLHL24 gene is their life threatening heart deterioration. The encoded protein, KLHL24, has been suggested to regulate protein turnover as part of the ubiquitin-proteasome system. The mutated version of KLHL24 was shown to be more stable, implying that excessive degradation of targeted substrates underlies disease development, however, the pathological mechanisms remain poorly defined. To better understand the function of KLHL24 in heart, we generated human induced pluripotent stem cells (hiPSCs) from two patients with cardiac condition and corrected the mutation to obtain two pairs of isogenic mutated (KLHL24mut) and corrected lines. We performed mass spectrometry on monolayer cultures of hiPSC-derived cardiomyocytes (CMs), which enabled us to gain insight into the substrates of KLHL24. Several intermediate filament proteins were found to be reduced in KLHL24mut CMs, most notably desmin, reduction of which we confirmed in the heart explants of patients. Since patient heart explants were fibrotic, we investigated whether KLHL24 affects intermediate filaments also in cardiac fibroblasts (CFs), as this could promote the development of the heart disease in these patients. We observed KLHL24-dependent reduction in levels of vimentin in CFs differentiated from hiPSCs as 3D cardioids, which we validated in patient heart explants. Our study shows that mutation in KLHL24 leads to reduced levels of several proteins in multiple cell types, demonstrating the need for holistic approach when designing diagnosis, treatment and research models tackling this EB type.

A robust and standardized method to isolate and expand mesenchymal stromal cells from human umbilical cord

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Background aims: Human umbilical cord–derived mesenchymal stromal cells (hUC-MSCs) are increasingly used in research and therapy. To obtain hUC-MSCs, a diversity of isolation and expansion methods are applied. Here, we report on a robust and standardized method for hUC-MSC isolation and expansion.

Methods: Using 90 hUC donors, we compared and optimized critical variables during each phase of the multi-step procedure involving UC collection, processing, MSC isolation, expansion, and characterization. Furthermore, we assessed the effect of donor-to-donor variability regarding UC morphology and donor attributes on hUC-MSC characteristics.

Results: We demonstrated robustness of our method across 90 UC donors at each step of the procedure. With our method, UCs can be collected up to 6 h after birth, and UC-processing can be initiated up to 48 h after collection without impacting on hUC-MSC characteristics. The removal of blood vessels before explant cultures improved hUC-MSC purity. Expansion in Minimum essential medium α supplemented with human platelet lysate increased reproducibility of the expansion rate and MSC characteristics as compared with Dulbecco's Modified Eagle's Medium supplemented with fetal bovine serum. The isolated hUC-MSCs showed a purity of ∼98.9%, a viability of >97%, and a high proliferative capacity. Trilineage differentiation capacity of hUC-MSCs was reduced as compared with bone marrow-derived MSCs. Functional assays indicated that the hUC-MSCs were able to inhibit T-cell proliferation demonstrating their immune-modulatory capacity.

Conclusion: We present a robust and standardized method to isolate and expand hUC-MSCs, minimizing technical variability and thereby lay a foundation to advance reliability and comparability of results obtained from different donors and different studies.

Generation of a novel human ACAN-mScarlet induced pluripotent stem cell reporter line and its differentiation into matrix-producing cells

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Differentiating induced pluripotent stem cells (iPSCs) toward matrix-producing cell types has become increasingly important in regenerative medicine. Yet, there is an unmet need for iPS reporter cell lines to visualise and track matrix genes and to provide an attractive option to trace transplanted cells. Aggrecan (*ACAN*) is an integral part of the extracellular matrix and is the most abundant aggregating proteoglycan in cartilage and the nucleus pulposus of the intervertebral disc, being continually synthesized and secreted by chondrocytes and nucleus pulposus cells. Aggrecan is also present in cardiac jelly, developing heart valves, and blood vessels during cardiovascular development and is also found in brain[1]. Here we use CRISPR/Cas9-based interruption to establish a human iPSC line expressing the red fluorescent protein mScarlet knock-in at the last exon of the ACAN locus[2,3]. Stable mScarlet expression was confirmed upon activation of the ACAN promoter using CRISPR-mediated trans-activation of the ACAN locus in the iPSC-derived reporter line. The reporter iPSC line was characterized for the expression of normal stem cell markers and shows trilineage differentiation potential. We further differentiated the reporter line into the notochordal and chondrogenic cell lineages and confirm endogenous mScarlet expression upon activation of the ACAN locus. The generation of the *ACAN* reporter line will be beneficial for end point matrix production determination and visualization and would advance iPSC differentiation strategies.

[1]Koch et al, 2020, Journal of Histochemistry & Cytochemistry, doi: org/10.1369/0022155420952902
[2]Santos et al, 2016, Curr Protoc Stem Cell Bio, doi: 10.1002/cpsc.15
[3]Maguire et al. 2019, Curr Protoc Stem Cell Biol, doi: 10.1002/cpsc.64

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Transfusion-ready red blood cell generation from human induced pluripotent stem cells

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There is constant need of blood products that are generally rely on blood donations, although the world-wide demand do not meet availability. *In vitro* red blood cell (RBC) production would substantially supplement this need, and would provide further benefits such as thoroughly screened products, possibility of genetic manipulation and therapeutic loading. IPSC is a promising cell source to derive transfusable RBCs and blood products due to their immortality, donor independency, availability in GMP-grade and as universal source (e.g. HLA-matched, blood group matched). However till to date the field lacks a sufficient iPSC differentiation protocol, that is capable of reaching required erythroid yield suffice transfusion, due to developmental immaturity, inefficient enucleation (5-25%) and traditionally applied small-scale, static culture conditions. RBC-transfusion products generally contain large cell quantity (10^11-12 cells/unit) and to be able to propagate that, feasible iPSC differentiation with high enucleation and scalable, suspension culturing allowing bioreactor applications is required.

Here we describe a 3 phase iPSC to RBC differentiation platform and their translation process from static/adherent to dynamic/suspension culture condition, allowing scalability and eventual bioreactor application. Our system gives rise to ~16000 fold increase in cell number, with a constant 50-60% enucleation, thus a mini-transfusion (10^11 RBC, required in phase I trial) according to our calculation could be generated from 6x10^6 iPSC in bioreactors. The iRBC derived here, passed the necessary functional assays, including but not limited to morphological analyses, hemoglobin content, blood group phenotype, deformability, oxygen dissociation. The stability of iRBC is currently tested in mouse transfusion experiments using humanized MISTRG mice. Translation to bioreactors, including novel bioreactor design, culturing parameters, feeding regimen, in house GMP-grade media development is momentarily ongoing.

In conclusion, an efficient 3 phase iPSC-RBC differentiation and their translation to dynamic culturing described here for the first time, provides a bridge from small-scale static culturing to large-scale bioreaction RBC production aiding clinical transfusion application

Generation of human ILC3 from allogeneic and autologous CD34$^+$ hematopoietic progenitors for adoptive transfer

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Type 3 innate lymphoid cells (ILC3) are important in tissue homeostasis. In the gut, ILC3 repair damaged epithelium and suppress inflammation. In allogeneic hematopoietic cell transplantation (HCT), ILC3 protect against acute graft-versus-host disease (GvHD), most likely by restoring tissue damage and preventing inflammation. We hypothesized that supplementing HCT grafts with IL-22-producing ILC3 may prevent acute GvHD. Here, we explored whether IL-22-producing ILC3 can be generated from CD34$^+$ hematopoietic stem and progenitor cells (HSPC) obtained from different sources. We were able to generate IL-22-producing NKp44$^+$RORγt$^+$ *bona fide* ILC3 from CD34$^+$ HSPC derived from cord blood (CB), healthy adult granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood (MPB), bone marrow and fetal liver in a stromal cell-free culture system. The addition of the EZH1/2 inhibitor UNC1999 and recombinant human IL-15 further promoted the generation of ILC3 irrespective of the source of CD34$^+$ HSPC. Importantly, the HSPC-ILC culture protocol described here only requires a feasible input of approximately 5x10^4 CD34$^+$ CB-HSPC or 9x10^4 CD34$^+$ G-CSF MPB-HSPC to obtain sufficient ILC3 (approximately ~10^7) for adoptive transfer of ILC in a 70kg human transplant recipient. Our next step is to investigate whether CD34$^+$ HSPC derived from G-CSF mobilized adult peripheral blood and cord blood can give rise to the different ILC subsets in the blood and tissues of humanized (MISTRG) mice. Finally, we plan to determine the effects of adoptively transferring cultured IL-22-producing ILC3 on the overall ILC reconstitution in the same humanized mouse model.

Patient-specific iPSC 3D vessel-on-chip model of CADASIL disease reveals vascular smooth muscle cell phenotypic changes

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CADASIL is a hereditary small vessel disease caused by *NOTCH3* mutations leading to an accumulation and deposition of NOTCH3 protein around vascular smooth muscle cells (VSMCs). The major pathogenic feature of CADASIL is the progressive degeneration of VSMCs of small and middle-sized cerebral arteries leading to vascular dysfunction. Here, we established three CADASIL and isogenic gene-corrected iPSC lines from two CADASIL patients. By using a set of standard 2D assays, we observed comparable levels of NOTCH3 and reduced contractile proteins such as SM22 and αSMA in CADASIL iPSC-VSMCs. As endothelial cell (EC) expression of NOTCH3 ligands, such as Jagged-1, is required for activation of NOTCH3 in VSMCs, we recapitulated EC-VSMC interactions by using an iPSC-based engineered 3D vessel-on-chip (VoC) model. In this system, CADASIL iPSC-VSMCs showed elongated morphology and higher proliferative behavior while microvascular structure was comparable. In contrast to 2D assays, immunofluorescent analysis revealed an increment of NOTCH3 protein in CADASIL iPSC-VSMCs and high levels of contractile proteins SM22 and αSMA. Importantly, NOTCH3 downstream targets PDGFRβ and HEY1 were also increased in CADASIL iPSC-VSMCs. The aforementioned results were validated by incorporating CADASIL patient-derived primary brain VSMCs in the VoC model. Functional analysis in 3D identified altered intracellular Ca^{2+} kinetics upon vasoactive stimulation in CADASIL iPSC-VSMCs. Inhibition of NOTCH3 cleavage and activation with the γ-secretase inhibitor DAPT rescued CADASIL iPSC-VSMCs phenotypical and functional differences. Collectively, our findings suggest that NOTCH3 increment in CADASIL VSMCs result in profound phenotypical and functional changes and these results may guide treatment development in CADASIL.

A multiscale pipeline for 3D image analysis of intact cerebral organoids

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To unravel pathomechanisms for a novel Amyloid Precursor Protein (APP) variant, we reprogrammed patient fibroblasts into induced-pluripotent stem cells (iPSCs). We will employ these iPSCs to generate cerebral organoids and perform a 3D structural analysis using the recently published SCOUT pipeline. SCOUT allows for the multiscale characterization of intact organoids, extracting features capturing molecular, cellular, spatial, cytoarchitectural, and organoid-wide properties from 3D fluorescence imaging datasets. Currently, we have optimized the SCOUT pipeline using cerebral organoids generated from healthy individuals. We prepared cerebral organoids using the standard Lancaster protocol, followed by optical clearing and whole-organoid staining with various markers including *SOX2, TBR1, PAX6, and CTIP2.* 3D fluorescent microscopy enabled three-channel imaging of intact organoids at single-cell resolution. We optimized the detection and segmentation of single cells allowing us to measure absolute frequencies of different cell populations and to characterize cell organization. We will apply this pipeline to study the effects of the novel APP variant on cerebral organoid development.

EP300 facilitates human trophoblast stem cell differentiation

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During early human placenta development, cytotrophoblasts differentiate into extravillous trophoblast and syncytiotrophoblast. Defective trophoblast differentiation may result in severe pregnancy complications, including early-onset fetal growth restriction and pre-eclampsia. The incidence of these complications is increased in pregnancies of fetuses affected by Rubinstein-Taybi syndrome, a developmental disorder predominantly caused by heterozygous CREBBP or EP300 mutations. Although the acetyltransferases CREBBP and EP300 are paralogs with many overlapping functions, the increased incidence of pregnancy complications is specific for EP300 mutations. We hypothesized that these complications have their origin in early placenta development and that particularly EP300 plays an important role in that process. We therefore investigated the role of EP300 and CREBBP in trophoblast differentiation, using human trophoblast stem cells (TSCs) and trophoblast organoids. We found that knockdown of EP300, and not CREBBP, blocked differentiation of TSCs into both the syncytiotrophoblast and extravillous trophoblast lineages. In fact, it resulted in proliferation of TSC-like cells under differentiation-inducing conditions. By transcriptome sequencing we found that several ligands for the EGF receptor were upregulated upon EP300 knockdown. This upregulation could partially explain the inhibitory effect of EP300 knockdown on differentiation, as blocking the EGF receptor rescued trophoblast differentiation to a large extent. These findings suggest that EP300 facilitates trophoblast differentiation by interfering with at least EGFR signaling, whereas its paralog CREBBP is not as important in this process. This points towards a crucial role for EP300 in early human placenta development, and may explain the increased incidence of complications seen in pregnancies of EP300-mutated fetuses.

Modelling and understanding human embryo development beyond the implantation stage

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Human reproduction is considered relatively inefficient as only about 30% of successful IVF-cycles results in a live birth. Chromosomal abnormalities in embryos are known to contribute to implantation failure and (early) pregnancy loss. Interestingly, up to 90% of pre-implantation embryos may contain chromosomally abnormal cells. Most of these embryos are mosaic: they consist of both normal and abnormal cells. Part of the mosaic embryos arrest, while a large proportion can compensate for the abnormal cells and continue development. This compensation is found to occur during and after implantation. However, after implantation, not much is known on how the human embryo proceeds development: not only due to inaccessibility, also a limited number of embryos are available for in vitro research and these are further subject to many ethical constraints.

Recently, a stem-cell based embryo model, the blastoid, has been developed that can recapitulate the human blastocyst stage embryo shortly before implantation. We aim to use blastoids as a tool to identify and study cellular interactions as well as the molecular pathways that direct lineage segregation, differentiation, and morphogenesis. Furthermore, we aim to mimic the mosaic embryo using chromosomally abnormal stem cells to study their impact on early human embryo development and the ability of the embryo to cope with such abnormalities.

Advanced (3D) culture techniques, (live) immunofluorescence imaging as well as single cell RNA sequencing and low-input proteomics will be used to establish an embryo model not only recapitulating the pre-, but also the peri-, and post-implantation stage.

The exploration of cell-cell communication during somitogenesis

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The development of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas as a gene editing system has led to an explosion of new CRISPR-Cas-based technologies. Where CRISPR-Cas13 systems differ from other Class II CRISPR systems, is that they exclusively target and cleave RNA. Another peculiarity of Cas13 is that in prokaryotic cells and in vitro, Cas13 cleaves not only target RNA, but also non-target RNA when it is activated by a specific target RNA. For eukaryotic cells, contradicting results have been shown as CRISPR-Cas13 initially seemed to exclusively cleave the target RNA. However, after exploring the collateral activity of six Cas13 systems, we found that LbuCas13a also exhibits strong collateral RNA cleavage activity in human cells. LbuCas13a displayed unbiased, collateral cleavage whilst targeting both exogenous and endogenous transcripts. In response to collateral RNA cleavage, cells upregulate stress and innate immune response genes and depending on target transcript levels, RNA degradation resulted in apoptotic cell death. To understand how cells go into apoptosis after collateral cleavage by LbuCas13a, we will perform a full genome CRISPR knockout screen. If a gene is involved in activating apoptosis, its knockout will be enriched and if a gene prevents apoptosis, its knockout will be depleted. As we also demonstrated that LbuCas13a can serve as a cell selection tool, killing cells in a target RNA specific manner, we aim to create an optimized LbuCas13a expression system. By using this expression system on gastruloids and reporter cell lines, embryonic development and cancer progression can be studied.

1. Jorik F. Bot, Zhihan Zhao, Darnell Kammeron, Peng Shang, Niels Geijsen
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Long-term donor chimerism in survivors of hematopoietic cell transplantation at pediatric age – first results.

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Background: The effect of hematopoietic cell transplantation (HCT) relies on donor hematopoietic cells regenerating the recipient's blood system. If donor chimerism is lost over time, this may impact the risk of relapse, and second malignancies originating from recipient cells previously exposed to myeloablative therapy. Yet, the incidence, risk factors and consequences of mixed donor chimerism in long-term HCT survivors remain incompletely known.
Aim: To determine the prevalence and risk factors of mixed chimerism in long-term survivors of HCT at pediatric age.
Methods: The results are part of an ongoing study aiming to determine the integrity of hematopoiesis in long-term HCT survivors at pediatric age. Donor chimerism was assessed using a targeted panel of 202 single-nucleotide polymorphisms (SNPs). Data analysis was performed using the AlloSeq HCT software (Alloseq HCT, CareDx, California, USA). Mixed chimerism was defined as $\leq$99.78% donor.
Results: Thus far, 136 survivors are enrolled in the study, at a median of 11.7 years (range 8.0-15.9) after HCT. The median age of the survivors was 20.5 years (range 15.9-26.6). 79% were transplanted for malignant indications. Analysis of the first 76 HCT survivors showed mixed chimerism in 32%, with a median of 99.25% donor signal (range 88.16-99.77%). Analyses of clinical determinants associated with long-term chimerism are ongoing and will be presented during the symposium.
Conclusion: This project will provide insight into the competitive fitness of patient versus donor HSCs in HCT recipients, and on the prevalence and consequences of mixed chimerism after HCT for malignant and non-malignant diseases.

Activation of the cAMP pathway induces structural and functional maturation in hiPSC-derived cardiomyocytes

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The immaturity of cardiomyocytes differentiated from human induced pluripotent stem cells (hiPSCCMs) limits their application to cardiovascular research. Our group has previously shown that maturation of hiPSC-CMs was improved in three-dimensional cardiac microtissues containing hiPSCderived cardiac fibroblasts (hiPSC-CFs) and cardiac endothelial cells (hiPSC-ECs). Gene expression anlaysis showed that the cAMP pathway was significantly upregulated in hiPSC-CMs within the microtissues. cAMP in the heart is responsible for regulating the contraction rate and force along with multiple other functions. Here, we investigated whether cAMP pathway activation can induce hiPSCCMs maturation. We used the cell permeable analogue of cAMP, dibutyryl cAMP (dbcAMP), to increase the intracellular cAMP in two-dimensional cultured hiPSC-CMs. We measured sarcomere organisation and show that it is significantly improved after dbcAMP treatment for 72 hours. Additionally, RNAsequencing revealed a clear separation between dbcAMP treated cells and control. Gene ontology (GO) analysis confirmed that phosphodiesterases, which are enzymes that regulate the intracellular cAMP, were significantly upregulated post-treatment. Ion channel activity and transporter GO terms were also significantly modified, which hints towards an alteration of the electrical properties. This matches with the altered action potential profile of hiPSC-CMs after 72 hours of dbcAMP treatment, measured by patch-clamp. Here, the resting membrane potential was more hyperpolarized, while upstroke velocity and action potential amplitude were increased. These results show functional consequences after increasing the intracellular cAMP, suggesting that the cAMP pathway contributes maturation of hiPSC-CMs.

Primary ciliary dyskinesia patient-specific model to study and treat ciliary dysfunction

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Respiratory primary ciliary dyskinesia (PCD) is a rare genetic ciliopathy characterized by anomalous mucociliary clearance caused by immotile or abnormal motion of the cilia lining the respiratory epithelium and/or by a severe reduction in the number of respiratory cilia. Restoration of ciliary function has not been possible as no causative treatment for PCD is available, resulting in a lifelong recurrent respiratory tract infection. Currently, there is still a lack of a proper in vitro model that (partially) recapitulates the in vivo situation of PCD patients. While these models resemble the different morphological and biochemical features of the original tissue and might be beneficial in the understanding and treatment of ciliary dysfunction in these patients. In the present study, we developed a novel PCD patient-specific model using human nasal airway epithelial cells and used ciliary beating frequency (CBF), rotation of 3D-apical out organoids, and bead movement as a functional readout for the ciliary activity. We observed patient-specific differences in CBF and rotation of 3D-apical out airway organoids, which were in agreement with the patients genetic mutation. Furthermore, we showed that 15 µm-sized polystyrene microbeads added on healthy epithelia moved in the same direction, consistent with coordinated beating of the underlying cilia, whereas beads added on PCD epithelia were immobile, suggesting an impairment of the mucociliary clearance function. Our findings suggest that our stem cell-based PCD model allows to study ciliary dysfunction and can be used for the identification, development, and high throughput drug screenings for the treatment of PCD.

An efficient transgene-free integrated model of the gastrulating mouse embryo

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In the last few years, an increasing number of stem cell-derived in vitro models of mammalian embryogenesis have been developed. The formation of integrated gastrulation-like synthetic mouse embryos (called ETX) typically relies on the self-assembly of embryonic stem cells (ESCs), trophoblast stem cells (TSCs), and extraembryonic endoderm cells (XEn) that are reprogrammed through ectopic overexpression of transcription factors. However, the requirement for transgenes restricts flexibility in the choice of cell lines, while using primary XEn cells limits the potential in supporting gastrulation-like morphogenesis. Here, we developed a reliable and efficient integrated gastrulation-like ETX embryo of the mouse by using solely chemical induction. Transient exposure of three-dimensional (3D) cultures of naïve ESCs to a combination of signaling factors gave rise to the co-formation of epiblast (Epi) and XEn. Successive co-culture with TSCs within agarose hydrogel microwells allowed for the generation of chemically induced integrated ETX embryos (chemETX embryos) that recapitulate morphogenesis of the post-implantation embryo including gastrulation, symmetry breaking, somitogenesis, and neural plate formation. In comparison to ETX embryos that are created through genetic induction (genETX embryos), chemETX embryos were produced with greater efficiencies. Overall, chemETX embryos provide a robust and accessible tool for studying early embryogenesis *in vitro* using non-genetically modified cells.

LSD1/KDM1A and GFI1B repress endothelial fate and induce hematopoietic fate in iPSC-derived hemogenic endothelium

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Differentiation of induced pluripotent stem cells (iPSCs) to hematopoietic lineages carries substantial potential to generate therapeutic and medicinal products. During embryogenesis, hemogenic endothelium (HE) gives rise to hematopoietic stem and progenitor cells (HPSCs) through a process called endothelial-to-hematopoietic transition (EHT). Understanding this process is crucial for establishing protocols to generate bone marrow repopulating HSCs, a currently unmet challenge. The transcription factor GFI1B and its cofactor, the chromatin demethylase LSD1 (KDM1A), are essential to produce functional HSPCs during EHT in mice. To study the role of GFI1B and LSD1 in this process, we used iPSC lines derived from patients with a bleeding disorder that express a dominant negative dysfunctional GFI1BQ287*, and we treated wild type iPSCs with an irreversible LSD1 inhibitor, GSK-LSD1.

Neither the use of GFI1BQ287* patient lines nor the inhibition of LSD1 changed the formation of CD144+CD309+ progenitor cells with hemogenic endothelial capacity during iPSC differentiation. However, the hematopoietic committed population as well as the yield of hematopoietic progenitors was severely reduced using GFI1BQ287* iPSC lines and completely absent upon inhibiting LSD1. To unravel the molecular mechanisms implicated in the affected hematopoietic commitment via HE under these conditions, we conducted single cell RNA sequencing (scRNAseq) on the heterogeneous CD144+/CD31+ HE population harvested from iPSCs differentiated in the absence or presence of GSKLSD1. scRNAseq revealed a dynamic expression of genes associated with EHT in vivo, and a complete absence of cells with a hematopoietic gene expression profile in GSK-LSD1 treated conditions.

Additionally, we identified an uncommitted HE population that lacked GFI1B expression yet was expressing its cofactors, including LSD1. When GFI1B was overexpressed in the uncommitted HE sorted via CD34 magnetic beads, a downregulation of endothelial programs and an upregulation of hematopoietic programs were observed. In summary, our findings underscore the essential role of the LSD1/GFI1B axis in driving hematopoiesis from iPSCs.