GSCN working group: Pluripotency and re-programing

Dear GCSN Committee,

in the first place I would like to thank again for awarding me with this travel grand and giving me thereby the possibility to join the big ISSCR community at the meeting in Stockholm in 2015.
The advent of stem cell research can be seen from different sites, like emphasised in my application I wanted to view on the field not only from the scientific community but also from the industry side. The following points draw my attention as they reflect either the transition from the bench to bedside or how ideas from the lab converged into a product.
The talk from Shinya Jamanaka impressed me with his idea to reprogram and to build up a hiPSC bank, from Japanese persons focusing on selected MHC loci. Thereby he aligns closer to the working principle of donor organ banks, which search for proper matches prior to transplantation. Once established, such bank could cover the entire population of Japan and serve as a starting source for cellular material. Hereby a comparably small number of several hundred correctly selected patients are enough to achieve this task.

Concerning reprogramming activities also further contributions from the industry side continue to boost the field. Applications that allow mass production or parallelization of patient reprogramming continuously increase. Methods and devices emerge, which allow for transfecting in parallel up to 96 patients individually. Thereby GMP compatible media and protocols for expansion and reprogramming will contribute to ease transition of such cellular material into the clinic.

Despite the availability of simplified and GMP compatible media, which is aimed to harmonize and standardize the expansion of PSC the awareness increases to the amount and quality of information and data available in the scientific community.

The ISSCR 2015 in the great city of Stockholm was sure a hotspot to engage and network with people and groups beyond your own country.

Sincerely

Andreas Elanzew

Report about the annual meeting of the ISSCR - Working group 'Pluripotency and reprogramming'

by Henning Kempf

From June 24th to 27th the annual meeting of the international society for stem cell research (ISSCR) took place in the Exhibition and Convention Center in Stockholm. The meeting gathered more than 3500 stem cell scientist from more than 60 nations. During the opening plenary session president Rudolf Jaenisch highlighted the success of the societies' peer-reviewed journal 'Stem Cell Reports' since its launch 2 years ago and emphasized its role as an important platform for stem cell researchers to publish their research. Furthermore, he pointed out the new ethical guidelines of the ISSCR addressing the ethical concerns of gene-editing in human embryos. The ISSCR clearly states that such experiment resulting in germline modification should not be conducted and be banned.

Later that afternoon, Nobel Laureate Shinya Yamanaka presented the progress of his team in iPS cell applications. Next to presenting a method called 'miRNA switch' enabling purification of differentiated progenies by application of a specific miRNA labeling technique and the progress in a preclinical study of application iPSC-derived dopaminergic neurons, Yamanaka focused on the problem of possible immune rejection of transplanted iPS derivatives. To solve this issue, his group is searching for HLA haplotype matched persons as donors to derive iPS cell lines. A single HLA haplotype matched iPS cell line serves as transplantable cell source for a broader range of recipients (about 10% of the Japanese population) with reduced risk of immune rejection. Yamanakas group has successfully generated a first HLA haplotype matched cell line that is planned to be included in the first human iPS-based clinical trial. This trial is conducted in Japan by the group of Masayo Takahashi, who also reported the current state of the trial at the ISSCR. To date, the initial safety trial is ongoing with an autologous-derived iPS cell sheet of differentiated retinal pigment epithelial cells that was transplanted into the eye of a patient suffering from age-related macular degeneration. In the future, it is planned to include Yamanakas HLA haplotype matched lines to reduce overall costs (production of the first autologous sheet was about 1 million US$) and thereby enabling a broader applicability of the treatment.

During the conference, the ongoing debate of the differences between mouse and human iPS cells and the way of how to generate and maintain human iPS cells in a pluripotent ground state was addressed. Austin Smith from the Cambridge Stem Cell Institute presented his recent progress in maintaining the human iPS cells in the naïve, mouse-like pluripotency state. The conditions used by his group included the addition of two small molecules, the atypical PKC inhibitor Gö6983 and low concentrated GSK3 inhibitor CHIR99021 that is also applied to maintain naïve murine pluripotent cells. The interest in naïve human PSCs became apparent in the talk by Cynthia Bamdad representing Minerva Biotechnologies that is commercializing the findings on culturing naïve human cells using a single factor, NME-P binding to the receptor MUC1* on human pluripotent stem cells. Although stated to be independent of certain signaling pathways (TGFβ and LIF/bFGF, respectively), both studies still need to be confirmed in terms of quality, stability and reproducibility of the putative naïve cells.

Overall, this year's ISSCR meeting pointed out the increasing in-depth understanding of the pluripotency state and reprograming process. These findings form the profound basis of the continuous progress towards the cells' application in the clinics that the field is driving to since the breakthrough finding of reprogramming somatic cells to iPSC by Shinya Yamanaka.

ISSCR2015 meeting report

Kee-Pyo Kim
Max Planck Institute in molecular biomedicine.

1. Flight

24th of June: Münster – München – Stockholm
28th of June: Stockholm – München – Münster

2. Accommodation

24th of June to 28TH of June: Hetell Älvsjö

3. Poster

I presented my poster on 25th of June from 6 pm to 8 pm. For this 2 hours, many students and principal investigators visited my poster and discussed my project. I got many feedbacks from them. Specially, Paul Tesar (Case western Reserve university), Guoping Fan (UCLA), Steven Goldman (University of Rochester), Frank Edenhofer (University of Bonn) and Hideyuki Okano (Keio University) visited my poster, asked several questions and provided valuable comments.

4. Meeting

I met friends and formal professors who worked together with me in different labs, Ji-Su KIM (Korea National Primate Center), Sun-Wook Kim (Korea National Primate Center), Dong-Wook Han (Konkuk University), Ralf Jauch (Guangzhou Institute of Biomedicine and Health), Jung Keun Hyun (Dankook University), Gunnar Hargus (Columbia University), Yong-Mahn Han (KAIST), Janghwan Kim (KRIBB), Yee Sook Cho (KRIBB), In-Hyun Park (Yale University), Paul Burridge (Northwestern University). I had dinners with them and discussed potential collaborative projects.

5. Listening talks

I enjoyed listening all the talks. Among those talks, I surmise three talks below. I liked the story what Konrad Hochedlinger presented on the 26 th of June. To identify molecular barriers, his group initially performed loss-of-function screening with RNAi during reprogramming of mouse fibroblasts into induced pluripotent stem cells (iPSCs). Interestingly, they found that subunits (Chafa, Chaf1b, Rbbp4) of the chromatin assembly factor-I (CAF-1) complex, which were previously known to be involved in heterochromatin maintenance, DNA repair, asymmetric cell division, notch signaling and regulation of cell identity, emerged as the most prominent hits from screens. The suppression of either CAF-1 or their subunits indeed increased reprogramming efficiencies and shortened reprogramming kinetics. To understand molecular mechanisms underlying this, they performed Chip-seq and found that the inactivation of CAF-1 increased the chromatin accessibility. As consequence of this, Sox2 can easily bind to its targets and activate its downstream genes. Finally, they found that the suppression of CAF-1 facilitated direct conversion of pro-B cells to machrophages as well as fibroblasts to neurons.

Another talk I was very excited is from Austin Smith. His talk was related to the generation of naïve pluripotent stem cells (PSCs) in humans. He found overexpression of NANOG and KLF2 together with 2i/LIF/Gö6983 was sufficient to convert primed PSCs into Naïve PSCs. The derived naïve PSCs were independent of transgenes and co-expressed TFCP2L1, NANOG and KLF4. The naïve PSCs showed mitochondrial activation and metabolic realignment. Using this condition, they also derived naïve ES cells from human blastocyst stage embryos. RNA-seq analysis indicated that they were closely clustered with naïve PSCs as well as naïve

cells generated from Jaenisch lab, but distinct from primed PSCs. He also found genome-wide demetylation in naïve ES cells that was correlated with the loss of DNMT3B expression. He mentioned at the end of his talk that there were still many things needed to be improved; 1) improving homogeneity, 2) robustness of *in vitro* culture, 3) not yet ground state, 4) replacing feeders with defined substrates, and 4) defining the proximity of naïve cell identity to *in vivo* epiblasts.

Thomas Graf talked about his recent finding regarding direct reprogramming of pro-B cells to iPS cells. He found that transient expression of C/EBPa (18 hr) and constitutive overexpression of Oct4, Sox2, Klf4 and c-Myc allowed reprogramming of pro-B cells to iPS cells with 100% efficiency. This high efficiency was mainly due to the fact that the transient expression of C/EBPa activated GMP in pro-B cells. Using Chip-seq, RNA-seq and 4C-technolgy, he found that genes that were known to bind to Oct4 and Nanog enhancers were dramatically activated within a few days. Furthermore, the genes (EBf1 Rag2 Rag1) related to the B cell state were rapidly silenced at the early phase of reprogramming. He also found that transient expression of C/EBPa increased chromatin accessibility that allowed exogenous factors, such as Oct4, Sox2, Klf4 and c-Myc, could easily bind to their targets. Finally, he found a rapid accumulation of 5hC at CpG sites during reprogramming that was correlated with an increased expression of Tet2.

5. Attending the GSCN Meet-up Hub

To me, it was great to attend the GSCN meet-up Hub. I met several scientists who were working in Germany. We discussed future directions of the filed of pluripotency and reprogramming as well as potential collaborative works

6. Acknowledgement

I sincerely thank committee members of GSCN who provide me such a nice travel grant. Furthermore, I would like to thank Dr. Daniel Besser who organizes all the events and travel awards.

GSCN working group: Somatic stem cells and development

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Report for GSCN about ISSCR Conference, Stockholm June 2015

On the meeting like this you have a chance to see and listen to great scientists and learn about their research not only from printed out articles. It was very inspiring to listen to such outstanding scientists as Elly Tanaka, Erika Sasaki, Joanna Wysocka**,** Jürgen Knoblich who are doing groundbreaking research in tissue engineering and stem cell filed. Or to attend talk given by Nobel Prize winner – Shinya Yamanaka.ISSCR conference was also a great way to interact with others and making new friends and meeting old ones. Poster session – which is a way to present your project in a graphical, condensed form – was also very fruitful. I could share with my latest results, I've gotten some input to my work and I could also see what other scientists are working with.And of course beside all of this – conference is a great opportunity to go to social events after conference hours and enjoy the city.

Particularly, I was enjoying the talk of Prof. Götz who is focusing on identificationof mechanisms regulating lineage progression from undifferentiated neural stem cells to committed neuronal or glial precursors. However, the most inspiring me talk was given by Prof. Yamanaka and his remarks about an efficient method for purifying cell populations based on endogenous miRNA activity. For differentiation and sorting Yamanaka's team have developed a method called miRNA switch. The technique is mainly aimed at those cell types for which we do not have good cell surface markers for FACS sorting. Basically expression of two fluorescent proteins indicates transfected cells, which upon differentiation to the desired cell type will lose expression of one of the fluorescent indicators under the control of a cell type specific miRNA. These single positive cells can then be sorted or selected with chemical resistance. Simple and elegant though may require significantly larger numbers of cells, dependent on transfection efficiency.While Shinya may be the big name, his humility and genuine desire to make a change in the lives of patients is a great inspiration.

Dr. Dorota Kaniowska

GSCN working group: Somatic stem cells and development

ISSCR 2015 Report: Peggy Matz, Düsseldorf

The ISSCR is the biggest conference I have ever been. There you get an impression what is going on in the field all over the world. For me the ISSCR serves as source of inspiration. This time so many ideas came to my mind. Nowhere else you can get such a wide-ranging review of the own field of interest and beyond. Moreover you get a unique opportunity to talk to the big people in your area. It is possible to talk with anyone on your research and to share issues, problems and experiences. Additionally, it is worth to talk to the companies in the exhibition hall about a problem you have or ask for things you need for your research. Sometimes they have a solution and sometimes you find another way to solve the problem. The other way around the companies get impressions of what researchers looking for and need. Both can profit from each other. It happened so much in the field. More and more will be learned about adult stem cells and their differentiation potential. Identification of regulatory networks in stem cells and immature progenitors help to understand the mechanisms behind their development and differentiation. Today it is possible to generate little functional organs in a dish, e.g. little brain or intestine. They are generated to analyze the developmental processes of these organs and their characteristics. Furthermore, these organs can be used e.g. to analyze the processes during the healing procedure after injury. Additionally, drug screenings and toxicology screens in clinical trials can be done by using adult stem cells and developed organs or cell types. Furthermore, it is worth to gain an insight into other areas. It is helpful for your specific topic when it comes to problem solving or methods. In addition, you get to know people with whom you can collaborate or share their experiences. Finally, I believe that everyone have to be once been on an ISSCR annual meeting. This experience everyone should do once!

GSCN working group: Somatic stem cells and development

Medizinische Hochschule Hannover

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06.09.2015

Organizer:

German Stem Cell Network (GSCN)

c/o Max Delbrück Center

Robert-Rössle-Str. 10

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ISSCR 2015 Bericht

Ich habe im Oktober 2014 meine Doktorarbeit an mesenchymalen Stamm- oder Stromazellen (MSCs) und ihrer klonalen Entwicklung bei der Expansion begonnen. Das ISSCR 2015 in Stockholm war somit der erste Kongress, den ich besuchte. So hatte ich schon früh in meiner Laufbahn die Gelegenheit in einer konzentrierten Form die Arbeiten von anderen kennenzulernen. Auf der einen Seite stellten Firmen ihre Produkte in sogenannten *Innovation Showcases* oder an ihren Ständen vor. Darunter war die SmartFlare Technologie von Merck Millipore, die es einem ermöglicht spezifische RNA in lebenden Zellen zu detektieren und die Zellen durch Fluorochrome reversibel anzufärben. So können die Zellen ohne Fixierung und auf Basis von mRNA auch anhand von intrazellulären Proteinen sortiert und weiter kultiviert werden. Auf der anderen Seite stellten andere Wissenschaftler ihre Arbeiten in Vorträgen und auf Postern vor. Die Themen MSCs und klonale Entwicklung spielten eine untergeordnete Rolle. Der Fokuspunkt 2015 lag bei induzierten pluripotenten Stammzellen (iPS), ihrer Charakterisierung und ihrer Anwendung. Insbesondere stach der Vortrag von Frau Dr. Takahashi heraus, die die erste klinische Studie mit iPS-abstammenden Zellen in Japan leitet. Dazu werden iPS Zellen zu Pigmentepithelzellen des Auges differenziert und durch Implantation versucht die Makuladegeneration im Auge zu behandeln. Diese Studie könnte den Weg für weitere Studien auch außerhalb von Japan und bei anderen Einsatzgebieten der iPS Zellen ebnen. Einen persönlicheren Austausch fand an den Postern oder bei dem Angebot *Meet the Experts Luncheon* für Trainee-Mitglieder statt. Für die Posterpräsentationen erstellte ich meinen eigenen Beitrag und erhielt die Gelegenheit auch meine Arbeit vorzustellen und zu diskutieren, weil ich leider für keinen Vortrag ausgewählt wurde. Ein weiterer Vorteil war der ausführlichere Austausch als bei den Vorträgen, bei denen maximal 5 Minuten Diskussion gestattet waren. Bei dem *Meet the Experts Luncheon* saß ich mit Herrn Dr. Paul Frenette, der einige Publikationen zu MSCs im Zusammenhang mit der hämatopoetischen Stammzellnische veröffentlichte, und weiteren Trainee-Mitglieder, die hauptsächlich an iPS Zellen und hämatopoetischen Zellen interessiert waren. Nichtsdestotrotz war auch diese Runde für mich interessant, was für das ganze Meeting gilt. Ich habe nicht viel über meine Zielzellen gelernt, aber die Problemlösungen und Ansatzwege der anderen Wissenschaftler und die neuen Technologien der Industrie haben mir sehr geholfen auch Fragestellungen anzugehen, die vorher nicht zu beantworten waren, neue Fragen zu stellen und bestehende Probleme zu lösen.

GSCN working group: Basic, translational, and applied hematopoiesis

ISSCR 2015 – Mania Ackermann – Travel Award Report

This year's ISSCR meeting took place in Stockholm, Sweden and was attended by 3800 scientists from all over the world. More than 2000 abstracts of high scientific quality in the field of basic research as well as preclinical and clinical progress were submitted and 100 of those were selected for oral presentations. The scientific program was excellent and covered various areas of stem cells research including basic and translational studies applying multipotent and pluripotent stem cells. One special highlight was the presentation of preliminary data from the first in-human clinical trial using autologous iPSC-derived tissues. Here, Masayo Takahashi reported the first transplantation of iPSC-derived retinal pigmented epithelium to an age-related macular degeneration patient in September 2014, after extensive safety analysis of the cell product. So far, no adverse effects are observed.

Also new and interesting findings in the field of "basic, translational and applied hematopoiesis" were presented in lots of oral and poster presentations. Especially, attention was drawn to embryonic hematopoietic development. This might reflect the problems in the generation of long-term engraftable true hematopoietic stem/progenitor cells (HSPCs) from (human) PSCs. Here a better understanding of the early hematopoietic specification processes and signaling networks involved in the establishment of a definitive hematopoietic program might help in the generation of improved differentiation protocols. In this line, Hanna Mikkola from the University of California reported the important role of the transcription factor (TF) Scl in the early hemogenic vs. cardiac specification of mesodermal cells. Although Scl does not represent a pioneering factor during this process, it can control gene expression and cell fate by recruitment of Polymerase2 to primed enhancers of hematopoietic genes and activation of a hematopoietic program. Besides the action of Scl during early hematopoietic specification, Peter Kim from G. Daleys Group in Boston Children's Hospital highlighted the importance of interferon (INF) signaling for the maturation of AGM HSCs associated to JAK/STAT signaling. He demonstrated that the treatment of AGM HSCs with INFα induces JAK/STAT signaling and significantly increases the long-term hematopoietic engraftment potential as well as the competitiveness of these cells in transplantation experiments. Moreover, he showed that the AT-rich interactive domain 3a protein (Arid3a) most likely seems to be the upstream regulator of this inflammatory signaling pathway involved in hematopoietic development. Several talks and posters also emphasized the importance of Notch signaling during definitive HSPC development, which plays a critical role at several stages of hematopoietic development ranging from the somite stage to the endothelial to hematopoietic transition (David Traver from the University of California). Another highly interesting talk was given by Adrea Ditadi from G. Kellers Group who demonstrated that hemogenic endothelium (HE) and arterial and venous vascular endothelium represent different lineages and can be distinguished by surface expression of CD34, CD73 and CD184. Only HE shows a CD34+/CD73-/CD184- phenotype and under defined conditions can undergo endothelial to hematopoietic transition.

Also a number of interesting methodologies/technologies to support stem cell research were presented. Especially, several talks introduced novel algorithms for the analysis of RNA-Seq data in order to better identify and trace different differentiation stages in heterogeneous cell populations. Whereas conventional analysis of single-cell RNA-Seq data has a low temporal resolution and relies on the day of differentiation a specific probe was harvested, a new algorithm (Monocle) introduced by C. Trapnell from the University of Washington, drastically increases the temporal resolution of time series in gene expression studies. The ordering of cells in "pseudotime" allows for a more stringent identification of key regulators in differentiation processes, as he exemplarily analyzed for

the direct conversion of fibroblasts in muscle cells by overexpression of the TF MyoD. However, this algorithm might also be applied for the detailed analysis of signaling networks involved in hematopoietic differentiation or trans-programming processes.

In terms of adult hematopoiesis, Heather Ann O'Leary from the Indiana University School of Medicine investigated the effects of extra physiological oxygen shock stress (EPHOSS) on murine and human HSPC. When applying real hypoxic conditions throughout the whole isolation process, the number and competitiveness of LT-HSCs from murine bone marrow or human cord blood could be increased. Importantly for a practical application, the same effect could be observed after addition of Cyclosporin A to the culture reagents. Another focus session was dedicated to the stem cell niche and the importance of understanding how the microenvironment of the niche can influence the behavior of stem cells. This might be of special importance in the case of leukemia when healthy and malignant cells potentially compete for space and signals. Targeting the leukemic niche might help to improve anti-cancer therapy in relapsing patients as indicated by Christina Lo Celso from Imperial College London. The Ernest McCulloch Memorial Lecture this year was given by Hiromitsu Nakauchi who also highlighted the importance of the stem cell niche in normal hematopoietic development, but also in leukemic settings. Besides several known cellular and extracellular components of the niche, he emphasized the important role of specific amino-acids. For example, he demonstrated that Valin and Cystein are critical for the efficient growth of HSC cultures.
In order to identify novel regulators of murine HSCs engraftment Shannon Mc Kinney-Freeman and colleagues from the St Judes Children's research Hospital performed a functional screening of several candidate genes selected by database search. In competitive repopulation assays following a knock down of specific transcription factors by sh-RNAs they could identify 18 regulators of HSPCs repopulation. Interestingly, some of the genes important for efficient repopulation are involved in vesicular trafficking, receptor turnover, and secretion of extracellular matrix proteins. These findings suggest that HSCs must actively participate in the establishment of the niche to allow for efficient engraftment.
It might be of interest to the society that a new special lecture was initiated by the "Tobias Foundation". The "ISSCR Tobias Award Lecture" will recognize original and promising basic hematology research, as well as direct translational or clinical research related to cell therapy in hematological disorders from around the world. It is accompanied by an award of $15000 USD.

To conclude my report, I would like to take the opportunity to say "THANK YOU" to the GSCN for giving me the chance to attend this great meeting. Besides getting an update on various areas of the fast developing field of stem cell research, I also had the opportunity for networking and meeting different experts in the field. Here, a clear highlight was to meet Dr. Takahashi at the "meet the expert lunch" and discuss the first clinical trial utilizing iPSCs-derived cell products. Moreover, during the poster presentation, I had the chance to present and discuss my data with a lot of scientists of the field. The numerous interesting talks and posters gave an important impact on my project.

GSCN working group: Basic, translational, and applied hematopoiesis

Report ISSCR 2015 – André Görgens, Essen

The visit to the 2015 annual meeting of the International Society for Stem Cell Research (ISSCR) in Sweden this June was really a great experience for me, not only because it was held in the beautiful city of Stockholm and the plethora of high-quality talks and posters, but also because of the inspiring and motivating atmosphere. I met many interesting researchers from all over the world, and experienced in many discussions that everyone was very open and positive.

The most impressive aspect, compared to previous stem cell meetings, was that translation of basic research to clinical applications, the long-term goal for many stem cell research projects nowadays, has come in reach for many projects. Several presenters mentioned increasing success and feasibility to translate findings based on basic research to the clinic. Many talks included data from first in human studies and clinical trials based on both human primary and induced pluripotent stem cell (iPSC) derived cells like central nervous system stem cells, mesenchymal stem cells, beta cells, and hematopoietic stem cells. For example, Shahin Rafii (Weill Cornell Medical College, New York, USA) demonstrated how his team reprogrammed mid-gestation amniotic cells into vascular endothelial cells using a mix of inhibitors and without the use of transcription factors. He showed in mice and non-human primates that following intra-venous transplantation, these vascular endothelial cells home to regenerating tissues and promote their regeneration. Currently, his lab aims to translate this approach to the clinics to treat various diseases, and he plans to establish public banks as a repository for tissue-specific niche cells for organ regeneration. Other highlights included several talks demonstrating major technical advances for stem cell in vivo imaging: Owen Tamplin from Leonard Zon's lab (Boston Children's Hospital, USA), demonstrated how a new technique called lightsheet live imaging can be used to visualize and study how hematopoietic stem cells (HSCs) colonize their niches in developing zebrafish embryos. Similarly, Cristina Lo Celso (Imperial College London, UK) presented how she and her team used intravital time-lapse confocal microscopy of mouse calvarium bone marrow to study HSC-niche interactions.

The scientific program was flanked with numerous additional offers like meet the expert luncheons, opportunities to talk to journal editors and industry representatives, great social events and very successful meet-up hubs, e.g. organized by the Karolinska Institutet (Stockholm) or the GSCN. In summary, this meeting was a big success, and I am thankful for the support by the GSCN to attend it.

GSCN working group: Basic, translational, and applied hematopoiesis

ISSCR ANNUAL MEETING 2015

Stockholmmässan, Stockholm, Sweden, June 24-27 2015

Conference Report / Highlights

with Focus on Basic, translational and applied hematopoiesis

Daniel Klimmeck

Division of Stem Cells and Cancer (A010; Prof. Andreas Trumpp) and HI-STEM gGmbH (Heidelberg Institute for Stem Cell Technology and Experimental Medicine); German Cancer Research Center (DKFZ), Heidelberg.

The Stem Cell Niche *-- new concepts:*

*** In his *Ernest McCulloch Memorial Lecture* entitled `Stem Cell Niche - from cells to organs and beyond´, **Hirumitsu Nakauchi** (University of Tokyo, Stanford, CA) presented unpublished work on the dependency of human and mouse **HSCs** on **amino acids**. Building on findings on protein deficiency-based anemias by Kornberg back in the 1940s (Kornberg et al., 1946), Dr. Nakauchi and colleagues cultured HSCs in different single amino-acid depleted media and found that HSCs are strictly dependent on valine and cysteine, while loss of all other amino acids was tolerated. In more detail, Val(-) diet lead to strongly reduced frequency specifically of HSCs and mimicked the effect of myeloablative treatment to empty bone marrow niches. Although the exact mechanism in the niche context is yet to be resolved, these findings hold the potential for alternative conditioning regimens in transplantation assays. The second part of the lecture was dedicated to the concept of the **organ niche**, a micro-environment for organ development. Compensation of e.g. pancreas development in Pdx1-/- or kidney development in Sall1-/- mice by injection of pluripotent stem cells (PSC) into the blastocyst (Kobayashi et al., 2010; Usui et al., 2012) is feasible, even across species barriers. Interestingly, the host organ niche still instructs and regulates organ size. While current efforts aim to generate human organs in large organ-deficient animals such as pigs, which then could serve as bioreactors, technical hurdles as well as ethical concerns exist with regards to this approach. Here, novel stragegies to overcome these concerns were presented e.g. targeted organ generation using Mixl1-inducible PSCs (Kobayashi et al., 2015; Rashid et al., 2014).

*** **Heather O´Leary** (Boxmeyer laboratory; Indiana University School of Medicine, IN) reported on her recently published finding, that brief exposure to ambient oxygen strongly decreases recovery of long-term repopulating HSCs and increases progenitor cells, a phenomenon termed *extraphysiologic oxygen shock/stress* (**EPHOSS**) (Mantel, O´Leary et al., 2015). The authors conclude, that after HSC extraction and processing in norm-oxic environment, true numbers of HSCs in the hypoxic bone marrow and cord blood are routinely underestimated. While ROS production and induction of the mitochondrial permeability transition pore (MPTP) via cyclophilin D and p53 can be linked as mediators of EPHOSS, the MPTP inhibitor cyclosporin A (CSA) protects mouse and human HSCs at extraction, resulting in increased recovery of transplantable HSCs. Minimizing the impact of the EPHOSS mechanism during cell extraction and processing by e.g. pharmacological interference may be applicable to a variety of stem cells and clinically advantageous for transplantation.

GSCN working group: Basic, translational, and applied hematopoiesis

*** **Christina Schreck** (Oostendorp laboratory, Klinikum rechts der Isar, TU Munich) presented a study on **stromal Wnt5a** and its role in the **regulation of HSCs**. Wnt5a was earlier described to affect *in vitro* maintenance (Buckley et al., 2011) and aging of HSCs (Florian et al., 2013). In the context of osteoblastic differentiation HSCs are subject to intrinsic and extrinsic regulatory cues. Studying HSCs derived from $Wnt5a^{+/-}$ mice in serial transplantation experiments, Wnt5a deficiency had little effect on engraftment and self-renewal capacities. However, Wnt5a levels are high in stromal cells, and the reverse transplant of WT bone marrow cells into a $Wnt5a^{+/-}$ deficient niche showed severe self-renewal failure in secondary recipients. To mechanistically address, how the Wnt5a-deficient micro-environment modulates HSC engraftment, RNA-seq whole transcriptome analysis of recipient $Wnt5a^{+/-}$ HSPCs was performed. This analysis highlighted altered non-canonical planar cell polarity (PCP) Wnt signalling, cytoskeleton re-arrangement as well as cell cycle regulation. In line, while CXCL12 induces actin re-arrangement/ polarization in WT HSCs, their migratory phenotype got entirely lost in $Wnt5a^{+/-}$ HSCs. In summary, perturbated PCP/Actin signaling seems to be a key player in defective engraftment capabilities of $Wnt5a^{+/-}$ HSCs.

*** **Il-Hoan Oh** (Catholic University of Korea) presented **micro-environmental remodeling** as a **prognostic marker for acute myeloid leukemia** (AML) (Kim et al., 2015). The impact of the bone marrow niche on progression of leukemia is controversely discussed. This study screened age-matched AML patients w/o previous treatment and found transcriptional reprogramming of niche MSCs by leukemic cells. Molecular changes included cell-cycle, Notch-jagged signalling, as well as cell-cell interaction (CXCL12-CXCR4 axis). In the MN-1 mouse AML model (Kwon et al., 2010), the human phenotype of deteriorated BM microenvironment is recapitulated, e.g. loss of CAR cells. In summary, the clonal dominence of leukemia over normal HSCs might at least in part originate from the leukemic micro-environment. Because niche remodelling was found to be distinct for different AML patients throughout treatment history, the stromal pattern could be a useful predictive parameter.

The Stem Cell Niche *-- new techniques:*

*** **Cristina Lo Celso** (Imperial College London) presented an update on her work illustrating **plastic interactions of HSCs and LSCs** with the **bone marrow niche** by use of **intravital time-lapse imaging of the calvarial bone**. Addressing the impact of stress on HSC - niche interactions, animals were infected with the parasite *Trichinella spiralis* (Rashidi et al., 2014). In contrast to steady-state HSCs, which were relatively stable over time, stressed HSCs dynamically migrated and got in contact with larger bone marrow niches. In order to reduce analysis bias, an automated image analysis and integration algorithm was developed (Khorshed et al., 2015). Next, the authors used this approach to address the existence of a leukemic niche in T-cell acute lymphoblastic leukemia (T-ALL; Hawkins et al., unpublished). In T-ALL, no particular LSC phenotype is defined. Using Col2.3-GFP mice, Nestin-GFP mice and 3D-scanning of the calvaria, transplanted and engrafted T-ALL leukemic cells showed a random distribution, arguing that BM seeding and colonisation is a stochastic, niche-independent process. In contrast, the behavior of engrafted leukemic cells over time was highly heterogeneous. In mice tracked before or after therapy, recipient leukemic cells were left and fastly moving. The authors conclude that rather than targeting specific niches, one needs to target the fast-changing character of interactions between LSCs and their micro-environment. Notably,

Nestin-GFP cells are preserved and might be useful to target the micro-environment within restricted areas. Similarly, this technique could be applied to investigate wether other leukemias are characterized by distinct LSC - micro-environment interactions.

*** **Owen Tamplin** (Zon laboratory; Boston Children´s Hospital) summarized his recent approach for in-depth visualization of **hematopoietic stem cell niche dynamics** in zebrafish using **light-sheet imaging microscopy** (Tamplin et al., 2015). To investigate colonization of the kidney marrow niche *in vivo* might give broader insights into how homing and engraftment are organized in the vertebrate in general and can be facilitated e.g. after stem cell transplantation. Establishing a Runx:mCherry transgenic reporter line to track individual HSC nuclei, and using snake bungarotoxin-based to immobilize the embryo for long-term live imaging, light-sheet microscopy (Huisken and Stainier, 2009) was performed in 250µm-thick tissue. Notably, after the first wave of colonization, the size of the HSC population remained stable (appr. 50 cells). The established high-resolution technique has the potential to track varying HSC pool size in response to environmental cues (e.g. Lycorine treatment) and identify other cells the HSC cluster associates with (e.g. perivascular endothelial cell hemospheres (Wang et al., 2013)).

*** **Melih Acar** (Morrison laboratory; UT Southwestern Medical Center, Dallas, TX) reported on a **3D confocal imaging** approach to investigate **spatial localization of quiescent HSCs residing in the murine bone marrow niche**. This work comprises 1) the finding of a-catulin (a-CAT) as a new endogenous unique HSC marker, 2) developing a technique to do 4-color imaging using 2-photon microscopy, and 3) developing biosoftware tools to integrate and analyze these data. a-catulin is highly enriched in HSCs, and a-CAT / cKit double-positive HSCs were found to be mostly quiescent. a-CAT KO mice do not show any HSC phenotype, thus a-CAT seems to be a highly specific, but solely descriptive marker for HSCs. Deep imaging of reporter mice expressing GFP-a-catulin after clearing of the bone showed that most HSCs were not adjacent to the bone. Instead, HSCs reside at the vasculature, as shown by co-staining of sinusoidal vessels. 3D-visualization of HSCs over the entire BM length was achieved and showed that HSCs are largely depleted around the bone surface. In contrast, at the vasculature HSCs are peri-vascular with the majority residing at sinusoids (80%; dividing or non-dividing) and only a minority (12-16%) at arterioles. Transition zone- vessels at the bone surface were devoid of KI67 negative quiescent HSCs, in line with overall HSC depletion from the bone surface. In addition, allmost all HSCs were found to be adjacent to both Cxcl12 high and LeptR high perivascular stromal cells (10um distance).

Fate Decisions in Hematopoietic Development

*** **Hanna Mikkola** (UCLA) presented her work on embryonic cell fate decisions at mesoderm diversification in the hemogenic endothelium (HE) and **Scl as a driver of the hematopoietic to cardiac fate switch** (VanHandel et al., 2012; Org et al., 2015). While Scl does not act as a pioneer factor, it seems to exploit an enhancer landscape already primed in mesoderm. Scl binding to cardiac genes was found to be mostly transient, at the same time hematopoietic gene binding was stably sustained. ATAC-seq was used to study accessibility of chromatin at the transition from mesoderm to hemogenic endothelium. Scl instructs openness of chromatin in hematopoietic enhancers, and Pol-II binding to enhancers like Gfi1b and Eto2, while priming of cardiac genes is lost. In addition, Scl may regulate enhancer RNA (eRNA) expression. While the same motifs can be activated or repressed, GATA co-factors,

which can simultaneously occupy same enhancers, are only required for hematopoietic activation. In summary, Scl suppresses cardiac genes by blocking their activation by cardiac TFs. Enhancer decommissioning at dually accessible enhancers which serve as switch platforms, might be a concept applicable to a wider range of of mutually exclusive cell fate decisions during development. In the second part of her talk, Dr. Mikkola reported on a strategy to identify molecular barriers which block the emergence of definitive HSCs. Characterization of multi-potent hESC-HSPCs with human fetal HSC immuno-phenotype generated in a 2-step differentiation protocol, revealed that HOXA programs could not be activated, and argued for incomplete maturation. These findings suggest HOXA cluster genes as major roadblocks in the regulation of self-renewal, an aspect which is now further investigated using Hox reporter lines.

*** **Trista North** (Harvard Medical School, Boston) presented her study on **regulation of HSCs by the serotonergic nervous system**. Performing a screen on modulators of HSPC formation in the zebrafish embryo, unexpectedly neuromodulators were found to in addition act as regulators of HSPCs. Administration of e.g. serotonin (5-hydroxytryptamine, 5-HT) increased the formation of embryonic HSPCs in the aorta-gonad-mesonephros (AGM). While 5-HT can be synthesized by tryptophan hydroxylase I (TPHI) and TPH2 activity, inhibition of these enzymes by morpholinos decreased HSPC frequency, which could be rescued by exogeneous 5-HT. Interestingly, the 5-HT mediated effect was independent of the sympathetic nervous system, as shown by sympathetic nerve lesions. Instead, it was mediated by the hypothalamic-pituitary-adrenal (HPA) axis, as shown by increased gene expression and cortisol levels. As glucocorticoid receptors (GR) are expressed on HSPCs and niche cells, and GR-KO largely reduced HSPC production, these data suggest, that 5-HT acts via the HPA (teleost HPI) system and peripheral coritisol levels.

*** **Peter Geon Kim** (Daley laboratory, Boston Children´s Hospital) presented work on **promotion of AGM HSC maturation by IFN signalling**. Starting from the ontogeny of mouse embryonic HSCs at day E11.5, the authors addressed the question, how AGM HSCs develop into definitive adult-like HSCs? Recent reports revealed involvement of inflammatory signalling in the specification of the hematopoietic lineage at the AGM (Espín-Palazó et al., 2014; Sawamipak et al., 2014). While IFNg has been demonstrated to confer HSC emergence (Li et al., 2014), the role of IFNa is much less clear. Here, the authors found JAK-STAT/IFNa signaling to be increased in FL-HSCs and adult HSCs as compared to AGM HSCs and show that IFNa increases the competitiveness of AGM HSCs *ex vivo*. AT-rich interactive domain 3a (Arid3a), which has been shown to be critical for FL-HSCs, is identifed as an upstream regulator of IFNa/STAT signalling, since Arid3a KO embryos fail to develop functional AGM HSCs, but this effect can be rescued by IFNa treatment. In summary, parallel inflammatory pathways converge on STAT1 to regulate the emergence of AGM HSCs.

Regulatory Circuits of Stem Cell States

*** **Victoria Moignard** (Göttgens laboratory, MRC, Cambridge, UK) presented her work on **single-cell analysis of transcription factor networks active in the specification of HSC cell fate** between embryonic days E7.0 to E8.5 (Moignard et al., 2015). Extending on earlier characterization of TF networks operating in hematopoietic stem progenitors (Moignard et al., 2013), the authors investigated blood emergence in the primitive streak of Runx1-ires-GFP reporter

mice (Lorsbach et al., 2004) by single-cell qPCR (46 TF genes; 4,000 cells; 100 Fluidigm C1 runs). Integrating these data using a diffusion maps approach (Haghverdi et al., 2015), developmental trajectories were captured. Clustering revealed a bifurcation event between blood and endothelial cell fate, confirmed by established differential markers like Runx1, Gata1 and Cdh5. Boolean modelling allowed to address hierarchies by computing a network model from blood development which identified core hubs of most interlinked modules of 20 TFs, which are suggested to organize transcriptionally stable states of blood vs. endothelial cell fate differentiation.

*** **Daniel Klimmeck** (Trumpp laboratory, DKFZ, Heidelberg) presented his recently published work on **identification of regulatory networks in HSCs and multipotent progenitors via integrated proteome, transcriptome, and DNA methylome analysis** (Cabezas-Wallscheid, Klimmeck, et al., 2014). To explore essential HSC features, the authors performed integrated OMICs analyses of five FACS-sorted mouse HSC and MPP populations (MPP1-4), as defined earlier (Wilson et al., 2008). From the characterization of more than 6,000 proteins, 27,000 transcripts, and 15,000 differentially methylated regions (DMRs), coordinated changes associated with early differentiation steps were identified. The data reveal the differential expression landscape of 500 TFs and 700 lncRNAs and highlight specific expression clusters operating in HSCs, including Wnt and Lin28-Hmga signaling, the imprinted-gene-network and Hox genes. A dynamic pattern of transcript isoform regulation was apparent, suggesting a critical regulatory role during HSC commitment. To address differentiation lineage potential of MPP2-4, the OMICs data were linked with functional reconstitution experiments, which highlighted multi-lineage potential of MPP2 cells, but limited and lineage-biased capacities of MPP3 and MPP4 progenitors. Together, this study represents a robust global resource to further dissect critical molecular transitions in early adult hematopoiesis.

*** **Salemiz Sandoval** (Crook laboratory, UCLA) presented her work on **regulation of human bone marrow HSCs by opposing microRNAs let7c and miR125b**. Starting from an microRNA array profiling the authors found the let7c/miR99a/miR125b cluster to be highly enriched in HSCs, in line with earlier work (O´Connell et al., 2010). Notably, expression of this cluster is developmentally regulated, i.e. the cluster is not present in FL-HSCs, but expressed in adult HSCs, and this molecular switch is conserved in humans. To test for functional relevance in HSC regulation, each let7c and miR125b individually as well as the intact cluster were overexpressed in human HSCs and functionally tested in different *in vitro* and immunophenotypic assays. *In vitro* differentiation protocols showed that miR125b GOF increased proliferation (LT-CIC; CFSE) and maintenance of CD34+ cells, in accordance with earlier studies showing expansion of LT-HSCs at overexpression of miR-125b (Ooi et al., 2010). In contrast, let7c GOF resulted in decreased number and frequency of CD34+ cells, while the intact cluster had an intermediate phenotype. These results were confirmed by *in vivo* serial transplantation assays of transformed cells and suggest that let7c functions to balance and counteract miR125b proliferative effects on HSCs.

*** **Shannon McKinney-Freeman** (St. Jude Children's Research Hospital, Memphis, TN) presented a **screen for novel regulators which promote murine HSC engraftment** (Holmfeldt et al., 2013; Holmfeldt and Ganuza, submitted). Interrogating earlier global expression profiles (Mc-Kinney-Freeman et al., 2012) as well as a number of public databases (StemSite, ImmGen Consortium etc.) the authors collected a compendium of 50 candidate genes, which were subjected to shRNA screening on inhibitors. HSCs were transformed, injected 24 hours after *ex vivo* knockdown and HSC transplantation (HSCT) capabilities were evaluated 16w post transplant. Using this approach, 19 hits were detected and re-screened with a

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different set of shRNAs, summing up to a total of 1,300 transplanted mice. Among the 16 confirmed genes of diverse cellular roles, which contribute positively to HSCT, two members of the GASP (GPCRs via GASP domain) family of proteins were found: loss of both Gprasp2 and Armcx1 promotes HSPC repopulating potential. Other hits include Fstl1, an inhibitor of TGFb-signalling, Foxa3 and Nfix, some of which might exert roles specific to a post-transplant stress scenario.

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POSTERS (selected):

>> W-1083: **David G. Kent** (MRC Stem Cell Institute, University of Cambridge, UK) **Molecular signatures of heterogeneous stem cell populations are resolved by linking single-cell functional assays to single-cell gene expression.** Combining single cell functional assays with flow cytometric index sorting and single-cell gene expression assays the authors investigate gene expression programs of self-renewing murine HSCs as defined by four commonly used HSC isolation strategies (Wilson, Kent et al., 2015). The molecular map derived from 90,000 qRT-PCR reactions was subjected to weighting algorithm clustering, which reavealed that HSCs lacking durable self-renewal were transcriptionally primed for proliferation and differentiation.

>> T-1090: **Michael Rieger** (LOEWE Center for Cell and Gene Therapy, Goethe University, Frankfurt/M.). **STAT5 regulated microRNA-193B controls hematopoietic stem and progenitor cell expansion by modulating cytokine receptor signaling.** Characterization of a miR-193b knockout-out mouse model revealed a regulatory circuit that prevents excessive HSC self-renewal by up-regulation of miR-193b upon self-renewal promoting Thrombopoietin-MPL-STAT5 signaling. miR-193b LOF led to selective accumulation of functional LT-HSCs and increased levels of STAT5 and AKT signaling. In contrast, miR-193b overexpression decreased LT-HSCs and restricted cytokine signaling via targeting of the tyrosine kinase c-KIT.

>> T-1092: **Kevin Rouault-Pierre** (Cancer Research UK, London). **RARS (Refractory Anemia) clonal evolution supports the Hypothesis of the existence of MDSstem cells.** To test wether recent reports of the existence of rare multipotent Myelodysplastic syndrome (MDS) stem cells (MDS-SCs) in 5q- MDS patients can be generalized and HSCs could also be the initiating cells in other MDS subgroups, the authors studied Refractory anemia with ring sideroblasts (RARS) patients, with mutations of the pre-mRNA splicing gene SF3B1, and demonstrate that SF3B1 mutations arise from HSCs and are the initiating mutations in these patients. Xenotransplantation assays prove evolution of multiple, genetically diverse subclones of mutant SF3B1 in mice indicating a branching multi-clonal as well as ancestral evolutionary paradigm, which is mirrored in the patient.

>> T-1098: **Özge Vargel** (Lancrin laboratory, EMBL). **Exploring the emergence of first blood cells by using single cell gene expression analysis.** The authors utilized single-cell qRT-PCR to investigate gene expression signatures and cell heterogeneity at mouse embryonic endothelial to hematopoietic transition (EHT) in yolk sac (YS) and aorta-gonad-mesonephros (AGM). 96 genes were analyzed in hemogenic endothelial (HE) cells, pre-hematopoietic stem progenitor (Pre-HSP) cells, progenitor cell (HPC) or hematopoietic stem cell (HSC) at E9.0 to E11.0 stages. The results indicate that pre-HSPs in AGM and YS are transcriptionally similar, pointing to different microenvironmental cues potentially instructing different hematopoietic programs in AGM and YS, respectively. Notably, the analysis also revealed a novel small population with both endothelial and mature erythroid profile.

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REFERENCES:

- Buckley SM, et al., 2011. PMID: 20933051
- Cabezas-Wallscheid N, et al., 2014. PMID: 25158935
- Espín-Palazón R, et al., 2014. PMID: 25416946
- Florian MC, et al., 2013. PMID: 24141946
- Haghverdi L, et al., 2015. PMID: 26002886
- Holmfeldt P, et al., 2013. PMID: 24041575
- Huisken J, et al., 2009. PMID: 19465594
- Khorshed RA, et al., 2015. PMID: 26120058
- Kim JA, et al., 2015. PMID: 25791383
- Kobayashi T, et al., 2010. PMID: 20813264
- Kobayashi T, et al., 2015. PMID: 25192056
- Kornberg A, et al., 1946. PMID: 21028152
- Kwon KR, et al., 2010. PMID:20024912
- Li Y, et al., 2014. PMID: 25395663
- Lorsbach RB, et al., 2004. PMID:14630789
- Mantel CR, et al., 2015. PMID: 26073944
- McKinney-Freeman SL, et al., 2012. PMID: 23122293
- Moignard V, et al., 2013. PMID: 23524953
- Moignard V, et al., 2015. PMID: 25664528
- O'Connell RM, et al., 2010. PMID: 20660734
- Ooi AG, et al., 2010. PMID: 21118986
- Org T, et al., 2015. PMID: 25564442
- Rashid T, et al., 2014. PMID: 25280216
- Rashidi NM, et al., 2014. PMID: 24850759
- Sawamiphak S et al., 2014. PMID: 25490269
- Tamplin et al., 2015. PMID: 25594182
- Usui J, et al., 2012. PMID: 22507837
- Van Handel B, et al., 2012. PMID: 22863011
- Wang L, et al., 2013. PMID: 23188081
- Wilson A, et al., 2008. PMID: 19062086
- Wilson NK, et al., 2015. PMID: 26004780
- Yamazaki S, et al., 2011. PMID: 22118468

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ISSCR 2015 Report: Maria Stecklum

At first some words about the conference and my experience in general. It was my first time at such a big conference and it is fascinating how many people are working on such so different topics of stem cell research. The talks (of the key note speaker) were impressive. The conference gave me an unique chance to hear talks about the the latest trends of iPSCs from their inventor and Nobel Prize winner Shinya Yamanaka on the one day as well as the unpublished data from Austin Smith about embryonic stem cells on the other day.

The percentage of posters, talks and the number of projects showed the shift from working with hESC to iPSC, which display a great tool to work with patient- and disease-specific pluripotent stem cell lines. Yamanakas work explained how we have to use iPSC lines in the future and built-up an iPSC stock to treat different and a multitude of patients with the same iPSC line (derived transplants). The same HLA background decreases the immune response and the risk of transplant rejection.

Especially the plenar session „Immunology and Stem Cells" and different talks of "Hematopoiesis" were interesting for me and my current work and gave me some new inputs and ideas for the future.

The work of O'Leary for example used a simple idea to improve the percentage of long-term HSCs. HSC studies normally are performed in ambient air, but HSC reside in hypoxic niches. Decreasing the percentage of oxygen improves the quality and long time survival of HSC. This results suggest to improve the bone marrow transplantation by changing the harvesting and transplantation process into an oxygen reduced environment.

Furthermore the topic "immunotherapy" finds its way into the stem cell field. There are different ideas to use iPSC to generate functional T cells, but also HSC are used to investigate the function of immune cells in vitro and in vivo. Zuniga-Pflucker described for example how to improve the percentage of T cells in humanized mice by the pre-differentiation of HSC into pre-T-cells and their co-transplantation with HSC into immunodeficient mice. This idea I will use also to enhance our humanized mouse model.

At least, during my poster session I got a positive feedback about my recent work and there were some new contacts, which maybe result in a cooperation in the future. On the one hand our humanized mouse model is maybe also interesting to investigate or to test organ transplantation and the rejection process in vivo. And on

the other hand is a group in Canada, which develops an humanized thymus in vivo and this is maybe also interesting to improve our humanized mouse model.
In summary, stem cells are still a great tool with unexpected properties which will be used in so different fields of research (drug discovery, generation of humanized models in animals, understanding the development process) and the meeting reunite all this in only 4 days.

Juliane Strietz
University Hospital Freiburg
Germany

Report on the ISSCR Annual Meeting 2015 in Stockholm

The Annual Meeting of the International Society for Stem Cell Research (ISSCR) is one of the largest stem cell meetings, attracting researchers of all different areas of stem cell science. I am very grateful for the GSCN travel grant which allowed me to attend this renowned conference in 2015.

It was one of the first large conferences I experienced during my PhD studies and I was amazed by the variety of stem cell topics presented during the talks.

During one of the plenary sessions I had the possibility to hear a talk given by the Nobel prize winner Dr. Shinya Yamanaka who together with colleagues discovered how to reprogram mature cells into stem cells (iPS cells). He vividly showed what is possible today in medicine by using iPS cells. This was further demonstrated during the talks of Dr. Douglas A. Melton and Dr. Allan Robins who showed how functional beta cells can be differentiated from human iPS cells and how this technology can be combined with a transplantable encapsulating device to cure diabetes. Coming from the field of cancer stem cell research where stem cells are usually the "bad guys", for me it was really interesting to see how to use "good" stem cells to cure a disease.

During my poster session I had the possibility to talk to other scientists from different backgrounds about my own project. I realized that depending on the focus of one's work everybody has different perspectives on how to interpret and assess the results and I got valuable input and new ideas for my work. Furthermore while talking to other presenters about their posters I got a comprehensive overview of the topics other research groups are working on.

My visits to the Exhibition Hall gave me insights into the newest technologies and state-of-the-art gadgets that could improve my work in the lab and would bring great benefits. I learned about different approaches how to handle problems I came across during my own work and I got new ideas on how to solve them.

Nonetheless the scientific exchange was only one part of the interaction with other researchers. During the Meet the Experts Lunch Event I had the privilege to meet Dr. Connie J. Eaves who is not only known for her work in the hematopoietic stem cell field but also to be a very dedicated researcher. The conversation with her gave me insights on how to organize a good work-life balance and how important it is to be persistent in science. Additionally I had the chance to meet the GSCN stem cell community during the well-organized WunderBar Event and the GSCN Meet-up Hub.

Taken together the ISSCR Annual Meeting was a great opportunity for me to gain new experience on both the scientific and the personal level.

Stella Stepputtis
PhD Student
University Hospital Freiburg, Germany
DKTK Partnersite Freiburg

Report on my attendance at the ISSCR 2015

I am very grateful to the GSCN for giving me the opportunity to attend the ISSCR meeting in Stockholm this year (2015). It was fascinating to see the extent of such a large conference with scientists of the stem cell field gathered from all over the world. The sheer amount of knowledge present during the conference was apparent in the enthusiastic talks which were given, as well as the lively discussions which followed. Especially during the plenary sessions, I was able to hear well-renowned scientists speak, among them e.g. Hans Clevers, who plays an important role in my field of research. Although a little bit outside of my field, I was extremely fascinated by the talks given by Dr. Melton and Dr. Robins about their progress in generating insulin-producing cells and an implantation device, respectively, which could prove an excessive improvement for diabetics in the future.

I was also allowed to present my work in form of a poster. As I had hoped, I could gather many valuable insights from the discussion of my research with other scientists during the poster session. It was great to connect to other researchers from around the globe and I was able to deepen the scientific exchange during the relaxed atmosphere of the WunderBar event which was organized by the GSCN.

Furthermore, I was lucky to catch a seat at Dr. Connie Eaves' table at the 'Meet the Experts luncheon'. To be able to eat lunch in a comfortable atmosphere, with someone you have such a high regard for, was a great experience. She talked openly about her opinion on work/life balance, the future of science and career opportunities which helped me form my own opinion.

To sum up, it was a great conference which widened my horizon in scientific but also in social aspects and last but not least it was great to see the beautiful city of Stockholm.

Stem cells in regenerative therapies – ISSCR 2015 Report: Antje Appelt-Menzel

Science is overcoming disease by waving the way for clinical and preclinical applications in stem cell research. What the ISSCR conference in Stockholm was really able to provide is to put the main focus on applications in regenerative therapies. The use of stem cells enables the restoration of normal tissue functions by engineering, replacing, and regenerating diverse cell types; tissues as well as whole organs. At the moment, therapies with stem cells are evaluated in clinical trials or have been already approved for therapeutical issues. As an illustration, it was mentioned that neural stem cells can be transplanted into the central nervous system. Further, it was shown that human beta cells can functionally regenerate the insulin production in the pancreas to overcome lifestyle diseases such as diabetes. An important advantage of stem cells in context of regenerative therapies is the independency from postnatal tissue biopsies, including associated variations and limitations during *in vitro* culture. In addition, using stem cells it is possible to get the huge amount of cells, which is necessary to engineer tissues and organs. Upscaling processes, for example by use of stirred-tank reactors, make this possible and let different disciplines come together. Further possible fields of applications which are provided by the use of stem cells are retinal repair through transplantation of photoreceptor cells, treatment of spinal cord injury, and regeneration of cardiac tissue. In addition, stem cells can be used to support the bodies self-healing capacities.

In conclusion, what I found most stimulating and impressing was to see that stem cell science is transforming clinical practice and can indeed pose a future attractive opportunity to create organs for transplantation. This is even more important in times of retrogressive willingness to donate an organ. In addition, problems like immunological rejection after transplantation can be overcome and the life of patients will be improved.

Short Report -- ISSCR meeting 2015 – Martin Pfeiffer

Having had the opportunity to join the annual meeting of the International Society for Stem Cell Research in Stockholm truly led to an astounding experience. Not only could I meet people from already existing and past collaborations, but also finally got to talk to researchers whose works were only known to me from the literature. It is truly inspiring to hear the stories behind papers and how certain projects evolved.

Many interesting talks could be heard throughout the meeting. Rusty Gage talked about modeling human ageing in the dish in the realm of neuronal disorders and introduced novel age related genes in the brain like RanBP17, LAMA3 and PCBH10. Also Jürgen Knoblich introduced new research on ectdodermal organoids and how they might be put to use to study brain development and disease. A true highlight was also Shinya Yamanaka's lecture in which he portrayed the current projects and visions that drive the scientists in his laboratory. It was amazing to see that he is doing basic research while, at the same time, striving to drive the iPS technology further to become potentially useful to the clinics. A truly eye-catching presentation was given by Hans Clevers and again it became clear that organoids seem to be the next big thing in stem cell research which may allow to not only see processes that otherwise only become visible in patient biopsies, but to also allow to model and test treatment options in a more natural three dimensional environment.

Many other talks followed over the next days which ranged from large overviews over seemingly decades of research up to very precise and specific talks from less senior but very high profile scientists. It was interesting to me to not early hear about the scientific results themselves, but also which methods had been applied to achieve the discoveries. The CRISPR technology rapidly has transformed the field and now allows for genetic modifications in unprecedentedly short timeframes. Also, the application of high-throughput methods based on sequencing and mass spectrometry have seemingly become standard techniques. While I was familiar with most of the methods, there were still quite some specialized methods I had not known before like, HiC-Seq, ChiRP-Seq or CHART-Seq, the use of miRNA switches to purify differentiating cells or locked nucleic acids. It is always great to know about all the possibilities that exist and my presence at the ISSCR meeting greatly extended my knowledge.

Apart from the talks true highlights of the meeting have been the poster sessions with thousands of projects being on display. While it took some time during the pre-meeting preparation to pick the ones most relevant to my research, these sessions have been more than helpful. I could dismiss some parts of planned projects simply because other people already had done these experiments and at the same time new ideas sparked while talking to the poster presenters working in my own and other fields of research.

Another interesting encounter was the 'meet the experts' lunch with Natasha Buschati, an editor at Nature Cell Biology, who could give an inside view on the inner workings of science journals and review processes. This knowledge, hopefully, will make successful publishing easier in the future.

Aside from the pure science, it was great to join the 'Wunderbar event", in which researcher from the GSCN met for a casual evening. Rarely could I talk to peer scientists in such a relaxed environment, which is always a great inspiration and left me with new ideas and high enthusiasm.

In Summary, having attended the meeting was a great success for me. I gained insights into the current state of stem cell research beyond my own field, I could learn about new methods that will be helpful in the future, I could refine my own projects based on input from senior researchers and information from the poster sessions, and I could enlarge my scientific network which will be very helpful for putative future collaborations.

I want to thank the GSCN for having given me this great opportunity.

Martin J. Pfeiffer

Report for the GSCN travel award to the ISSCR - Laura Stappert

Dear GSCN committee,

First of all, I would like to express my thanks for awarding me with this travel grant and giving me the opportunity to participate in the 2015 ISSCR meeting. Besides listening to the interesting talks and engaging in scientific discussions, I also enjoyed the social program and the sight seeing hours in beautiful summery Stockholm.

During my daily work in the lab, I am engaged in a project aiming at the generation of dopaminergic neurons for cell replacement therapies in Parkinson's Disease (NeuroStemCellRepair). Thus, I was very interested in learning about the new advances and ideas in the field of "Stem cells in regenerative therapies". I was very impressed about **Shinya Yamanaka's talk**, who presented the idea of generating a bank of HLA-matched hiPS cells. In detail, he is planning to derive hiPS cells from donors homozygous for the most common HLA haplotype in Japan, which would be sufficient to provide immune matched cells to a large portion of the Japanese population. This would evade the problems linked with allogenic transplantation and also represents a cost advantage to each time generating patient-specific hiPS cells for autologous transplantations. Since I am also working with microRNAs, I was very interested in hearing about Yamanaka's new approach to use so-called microRNA switches for the purification of the desired terminally differentiated cells, which still represents one of the main challenges in the field. Finally he also gave an update of the recent advances made by Jun Takahashi moving towards pre-clinical studies for Parkinson's Disease transplantation of dopaminergic neurons.

During the meet the expert's lunch, I had the chance to talk to the fascinating **Masayo Takahashi**. She gave us an overview on how she came to use hiPS cells as a source for retinal pigment epithelial cells, which she has already transplanted into a patient suffering from age-related macular degeneration. Moreover, she also talked in general about the role of women in science and how she managed to pursue her career. Finally, she ended with the philosophical advice for us " to look for the blue ocean, where we can do our research in peace, instead of joining the fight in the red ocean".

In general, I was impressed with the excellent quality of the junior speaker's presentation and the high quality of the poster presentations. For me as a young scientist, it is always very rewarding to have the chance to discuss my data with the peers in the field. Although the ISSCR is such a big meeting, the atmosphere is still very open-minded and provides lot of opportunity to engage with the PIs either during the poster presentation time or during the social events. I also had many fruitful discussions with other young scientist and I am still in contact with some of them for exchanging protocols.

All in all, the meeting was a great success for me and I want to thank the GSCN again for founding me for this conference.

Sincerely,

Laura Stappert

ISSCR 2015 Annual Meeting – Short Report

Manuela Völkner, German Center for Neurodegenerative Diseases (DZNE), Dresden

The ISSCR is the largest stem cell and regenerative medicine community worldwide and brings together leading research in the field. Supported by a travel award from the GSCN working group “Stem cells in regenerative medicine”, I attended the ISSCR 2015 Annual Meeting in Stockholm.

One of the highlights of the conference was the workshop on clinical translation, which was organized the day before the conference. In this workshop I gained a lot of knowledge on legal issues and the process of clinical translation in general. Even more importantly, I benefited from other researchers experiences by receiving valuable advice on upscaling and GMP compliance of PSC differentiation, as well as possible solution to problems we are facing in this regard.

In the field of stem cell in regenerative therapies many novel highlights were presented at the conference. For example in the field of heart research and cardiomyocyte cell replacement much work has been done toward large scale clinical grade hPSC expansion and differentiation. Thus, experience gained in this field will now be invaluable to also develop such production protocols for other cell types of interest. Further, a number of very interesting new advances in more basic research were presented. For example Agnete Kirkeby presented a novel system to achieve linear gradients using microfluidic systems and its application to improve neural tube patterning from hPSC in vitro. Such systems may not only allow improved in vitro disease modeling using complex tissue, but may also give more precise control over differentiating defined cell types of interest in high purity. Kevin Eggan presented a novel technique for electro-physiological measurements of a large number of neurons simultaneously utilizing a novel channelrhodopsin. This technique will facilitate the characterization of stem cell derived neurons, potentially even other cell types, by allowing to look at much more cells than previously possible and thus enabling more precise characterization of e.g. cell to cell heterogeneity.
Another recent development in the field is several groups efforts on the use of more defined media and matrices for cell expansion and differentiation, as well as surface markers for cell enrichment. In this regard, e.g. Jürgen Knoblich showed data on the generation of more reproducible cerebral organoids with improved morphology using pre-fabricated scaffolds as growth substrate and Robin Ali presented data on improved PSC-derived photoreceptor precursor enrichment using surface markers and MACS.
Further, Masayo Takahashi presented first data from the ongoing first clinical trial on iPSC-derived retinal pigment epithelium cell replacement therapy. She presented details on safety characterization of the graft, the clinical procedure and patient selection criteria. In the first treated patient with 6 month follow up after treatment with RPE derived from autologous iPSC, they observed no rejection reaction or tumor formation. The graft survived during the six month follow up and visual acuity was stabilized in the patient, suggesting the procedure may be safe.

More personally, I benefited mostly from the poster sessions and all the advice I received there. In the field of retina research many groups presented novel data on improved differentiation of retinal cells from PSCs during the poster session. Thus, I gained a lot of advice on improving our differentiation protocols for human PSC. Further, in the field several groups are now trying to improve the generation of cone

photoreceptors from PSCs. Our group has already been able to significantly increase the numbers of cone precursors generated from mESC, which I presented at the poster session. I received advice on how to better characterize the cells we generated and how the protocol may possibly be improved even further.

In summary, at the ISSCR 2015 Annual Meeting much novel data in the field of stem cells in regenerative therapies was presented. Especially discussion at the poster sessions and the workshop on clinical translation was highly inspiring and will be very helpful for our future research.

GSCN working group: Stem cells in disease modelling and drug development

Short Report ISSCR – Raul Bukowiecki

I would like to thank the GSCN for providing the opportunity to join this fantastic meeting. I would like to highlight several points and talks that were of particular interest to me. First of all it was impressive how far clinical applications of iPSCs and other stem cells reach already to date!
Shinya Yamanaka, Doug Melton, and Masayo Takahashi stand out there for me since all stringently proved quality and applicability of iPSC. All answered crucial questions about transgene delivery and silencing as well as quality control in stem cells but additionally patient-follow-up showing improvement and no pathological development of the iPSC derivatives. The possibility of producing organs over the species barrier (human heart in pig etc.) as presented by Hiromitso Nakauchi was very intriguing. Methodically, Sara Howden presented a possibly ver powerful approach of combining episomal vector-based reprogramming with CRISPR/CAS-based gene targeting in a single step. Thereby, you can select for correctly targeted iPSC clones while establishing the line already. This approach could fasten the generation of isogenic lines for disease modeling exponentially and the episomal-based reprogramming is still a cheap and effective method for iPSC generation.

To name single posters would be excessive. In general, disease modeling took a huge step forward. Especially, in the neurodegeneration field differentiation into several lineages of affected tissue was improved on all fronts (microglia, astroglia, and neuronal subtypes). Metabolism, epigenetics and transcription was assessed for various diseases including RETT, SCA-3, Huntington's, Parkinson's, and Alzheimer's disease.

The interaction during the poster sessions gave me invaluable input for my projects and also provided several new ideas for cooperation with people visiting my poster. Thus, again, I'm very grateful that I had the chance to take part in Stockholm 2015.

International Society Stem Cell Research 2015 Annual Meeting – GSCN Report

I have attended the ISSCR meeting in Stockholm (23rd to 27th June 2015) with a Travel Grant kindly awarded by the German Stem Cell Network. In this meeting I have presented a poster entitled: "Human induced Pluripotent Stem Cell- based keratinocytes for patient specific skin models of congenital keratinization disorders".

It was for me a new experience to attend the ISSCR meeting, and a most rewarding one. As a PhD student working in disease modeling using iPS cells, I was very impressed by the amount of applications and new developments in this field. Even though the meeting seemed more inclined to neural stem cells than to other areas, with ESCs and iPSCs already being used for studying and developing new therapies for diseases like Parkinson, Spinal Cord Injuries as well as neuropsychiatric diseases, it was interesting to acknowledge the great achievements in this field and to have the opportunity to learn from the greatest minds in stem cell research.

It was also of particular interest to me the sessions where Transdifferentiation and Reprogramming mechanisms were presented as well as the Plenary Sessions on "Therapies with Stem Cells" and "Making Tissues and Organs".

Concerning my specific area of focus, modeling skin diseases using iPSCs, there were very few presentations related to it; however, I still managed to meet and have very interesting discussions and feedback from delegates working in similar projects, which I am sure will be very fruitful for my project.

On a more personal note, I acknowledge once more the German Stem Cell Network for allowing me to attend ISSCR with this Travel Grant and for the great opportunity to meet and share my work with great scientists. I also enjoyed the events organized by GSCN, especially the "WunderBar Evening", and I look forward to meeting the GSCN members again already this September for the GSCN Annual Conference and hopefully next year for the ISSCR16 as well.

Kind regards,

Dulce Cunha

Topic: **"Stem cells in disease modeling and drug development"**

I would like to start my report thanking the Society for allowing my participation at the ISSCR meeting with the award support. It has been a great experience that definitely gave an impact in my research. The meeting not only allowed me to discover new insights in my topic but also broaden the spectrum into other fields.

The conference counted 3800 participants with 2000 selected abstracts and 100 talks and aimed not only to give updates on the research topics but also deliver new guidelines for valuable research. Very important issues have been discussed from the scientific and ethical point of view in the perspective development of the stem cell field and its clinical application.

This meeting gave me the chance to hear talks from "VIP" of my research area such as Dr. Yamanaka, who is currently working on transferring induced pluripotent stem cells (iPSC) research into the clinic by creating HLA homozygous iPSC stocks from healthy donors that would constitute a cell bank for allogenic transplantation scenarios. Moreover, I got the opportunity to meet ground breaking scientists such as Dr.Takahashi, responsible for the first clinical trial using iPSC-derived retinal pigment epithelium (RPE) for the treatment of age related macular degeneration. During the last day she gave the first update on the first transplanted patient showing safety and stabilization of sight loss upon administration. These results, as preliminary as they are, still open a new field with potential perspectives for other diseases.

Concerning the topic of "Stem cells in disease modeling and drug development" many aspects were presented with applications in almost every subject. Many methods have been introduced and highly significant discovery have been presented that may innovate the available treatment options for different diseases.
To mention just one example of a novel highlight in the field, in the talk "Human glial progenitors cell-based treatment and modeling of neurological disease", Dr. Goldman introduced a novel strategy to induce chimerism of human cells in the murine brain. In detail, oligodendrocyte progenitors cells derived from fetus or human iPSC were transplanted in the brains of shiverer mice (models for schizophrenia). These progenitors, upon transplantation, infiltrated the brain and over time substituted the murine glial cells and astrocytes with human once. This model will allow to better study alteration in patients affected by neurological pathologies such as schizophrenia by evaluating behavioral changes and potentially provide an in vivo system to test therapeutic options.

Particularly, iPSCs have been shown to hold a great potential in the area of disease modeling and drug testing as highlighted in many talks and posters during this meeting. From neurological to metabolic diseases the range of application of these cells is increasingly expanding and with the development of screening assays for small molecules, drugs and miRNA novel therapeutic options are being discovered. Just to mention one

example, in the talk from Dr. Nissan 11 compounds were identified using a high throughput screening on iPSCs derived from Hutchinson Gilford Progeria patients that upon differentiation recapitulate the disease phenotype. The identified compounds showed improvement in the differentiation process of diseased cells therefore potentially representing treatment options.

Another tool with increasing impact for the field is the generation of specific organoids. These can be generated by tridimensional culture of different tissue/iPSC derived stem cells and form structures that resemble the organ they originated from. Formation of such organoids allows to study the development and biology of the corresponding organs and also better understand specific diseases. Dr. Carla Kim nicely introduced this concept carrying the example of how gut organoids can be used to study the biology of cystic fibrosis and to test drugs. In her talk she also introduced the concept of using liver buds to study diseases such as alpha1 anti-trypsin deficiency. Furthermore, organoids can also be generated from cancer biobanks to study, for example, patient-specific colon cancer phenotypes and develop the perfect drug treatment for each single case.

I had the possibility to gain insight in many other topics not directly related to my work and to meet scientists from all over the world with expertise in different areas of interest. In addition, I could discover the city of Stockholm. Therefore I take the chance once more to thank you for giving me this opportunity.

Stem cells in disease modeling and drug development

ISSCR 2015 Report: Lydia Wagner

I am very thankful to have received the travel award of the German Stem Cell Network (GSCN). It was a great opportunity to attend the annual meeting of the ISSCR in Stockholm, for various reasons. The impressive line-up of speakers was unique compared to other conferences and poster presentations from various fields of stem cell research made the meeting an interesting and inspiring event for a young researcher. Additionally, meeting and interacting with other researchers from similar but also distant areas really helped me in getting new ideas and developing my future research.

My personal interest focuses on iPSC-based disease modeling and translational applications of stem cell-derived cell types for neurodegenerative research and drug discovery. These topics were dealt with very extensively at this meeting, and international high profile speakers gave insight into their actual and unpublished research and progress. Several presentations stood out exceptionally from the meeting lineup, such as Rusty Gage's talk about modeling human aging. Aging is one of the major risk factors for many diseases, including neurodegenerative disorders. Although iPSC are ideal for modeling age-related diseases, reprogramming erases the age-related gene expression profile. To overcome this problem iNeurons were derived from patient fibroblasts to compare their expression profile with patient fibroblasts, and post-mortem brain tissue from patients to look for overlapping gene expression. Interestingly, the overlapping gene found in this analysis is part of the nuclear export-import-machinery, highly correlated with aging, and might give us insight into the mechanisms how aging contributes to developing age-associated diseases. Because of interesting and inspiring presentations, discussions and interactions, I can do nothing less than recommend the ISSCR annual conference to every young scientist!

Brief report on Computational Stem Cell Biology at the ISSCR 2015 in Stockholm

Thomas Zerjatke (Institute for Medical Informatics and Biometry, Faculty of Medicine Carl Gustav Carus, TU Dresden)

There was no particular session on mathematical modelling or bioinformatics at the ISSCR. Nonetheless several speakers reported on the application of novel computational methods, some of which I want to highlight here.

Session **Single cell biology:**

Victoria Moignard from the University of Cambridge presented an approach using single-cell gene expression measurements to decode the regulatory network for blood development. The presented work used the method of diffusion maps to reduce the dimensionality of the gene expression data in order to explore paths of differentiation. Contrary to other methods for dimensionality reduction like PCA the proposed approach is capable to give a pseudotemporal resolution of the differentiation process into several branches. The approach was recently published by the group of Fabian Theis (*Haghverdi et al., Bioinformatics 2015,* doi: 10.1093/bioinformatics/btv325), its application can be found in *Moignard et al., Nat. Biotechnol. 2015 (*doi:10.1038/nbt.3154*)*.

Pseudotemporal analysis was also the topic of the next speaker, Cole Trapnell from Harvard University. He presented an open-source software package called *Monocle* that increased the temporal resolution of time series gene expression data. This is done by treating the measurement of each individual cell as a separate time point and placing it according to its progress in the differentiation progress on a pseudotime axis. Moreover, the tool can be used to align several pseudotime series to each other to make them comparable. The approach was applied to myogenic differentiation and reprogramming and was published as *Trapnell et al., Nat. Biotechnol, 2014* (doi:10.1038/nbt.2859).

Session **Stem cell niches**:

Several speakers presented results of single-cell tracking approaches and the application of image analysis methods: Cristina Lo Celso from Imperial College London talked about the localisation of murine haematopoietic stem cells (HSCs) within their niche in the bone marrow. The work is based on 3D in vivo imaging using customised automated image analysis methods to segment stem cells and bone marrow components in combination with a machine learning approach to distinguish viable cells from image noise caused e.g. by death cells. The methodology they used was recently published as *Khorshed et al., Stem Cell Reports, 2015* (doi:10.1016/j.stemcr.2015.05.017).

Live single cell tracking techniques were also used by Melic Acar from the Morrison Lab at the UT Southwestern Medical Center. A special labelling technique with a unique stem cell marker (alpha-catulin) made it possible to image HSCs deep in the bone and determine their localisation relative to other bone components and blood vessels.

Owen Tamplin of Boston Children's Hospital used in vivo tracking of HSCs in the Zebrafish embryo. Using lightsheet microscopy (SPIM) made it for the first time possible to image the process of HSC homing during embryogenesis in the Zebrafish kidney marrow (that is the niche analogue to mammalian bone marrow) for several hours. The used image analysis techniques have been published recently in Tamplin et al., Cell, 2015 (doi:10.1016/j.cell.2014.12.032).

Besides this, attending the ISSCR in Stockholm was a unique possibility for me to get a broad overview of all the novel developments in stem cell research.