Programme / Abstracts
XXI Wilsede Meeting

Modern Trends in Human Leukemia & Cancer

June 18-21, 2016 – Wilsede, Germany

We cordially thank for supporting the Wilsede Meeting

Partners

Welcome to the XXI Wilsede Meeting on
»Modern Trends in Human Leukemia and Cancer«

This year the village of Wilsede hosts the XXI. »Modern Trends in Human Leukemia and Cancer« meeting. Traditionally, this meeting presents the most recent and exciting developments in the field of leukemia and cancer biology – from basic science to new clinical concepts. We are proud and honored that again many leading specialists in the fields of stem cell and cancer biology as well as immune- and gene therapy accepted our invitation to come here.

This year, stem cell biology is a major theme of the meeting. Several sessions will concentrate on the role of stem cells in homeostasis and disease as well as the interaction of stem cells with their stroma. Another focus of the meeting will be the field of myeloproliferative neoplasia, a disease that can be regarded as a paradigm of a stem-cell driven malignancy. Furthermore, the role of »canceromics« in our understanding of malignant diseases will be addressed. Finally, following the Wilsede tradition two sessions will be dedicated to novel therapies, highlighting the impressive progress of targeted and immuno-gene therapies. We highly appreciate the willingness of all speakers and chairs to share their knowledge and results with the next generation of scientists and researchers at this unique platform of scientific exchange.

We would like to thank the Mildred Scheel Foundation of the Deutsche Krebshilfe e.V. (German Cancer Aid) for their generous support. The decision of the Deutsche Krebshilfe to »adopt« the Wilsede meeting as its second »key meeting« has been vital to keep up the tradition of the Wilsede meetings. The DKH takes over not only the »usual costs«, but in particular it finances many stipends for young scientists making the Wilsede meeting an almost unique platform for both »young« and »experienced« scientists to interact. We are also grateful to the Deutsche Forschungsgemeinschaft, another traditional supporter of the Wilsede meetings, for continuous funding.

The »Magic« of Wilsede lies just not in the outstanding science and the tranquil location, but also in the people that have spent much time and energy in keeping the spirit of the 1973 meeting alive. At particular, Prof Rolf Neth, who was the initiator and driving force of the Wilsede meetings, but also Prof Axel Zander and Dr Carol Stocking who kept the tradition alive through the 1990s and 2000s, deserve special thanks.

For those of you who do not know Wilsede, we are sure that you will also come to appreciate this tiny village in the Lüneburg Heath, which provides the ideal setting for scientific exchange and stimulating discussions, for which the Wilsede meetings are known. Please push aside your other obligations for a few days, shut off your mobile devices and join us during the lectures, poster sessions, walks in the heath, and at the social events in the evenings with delicious local food, drinks, and music to discuss scientific concepts and new ideas.

Let's keep up with the Wilsede tradition!

Boris Fehse Nicolaus Kröger

The Local Organizing Committee

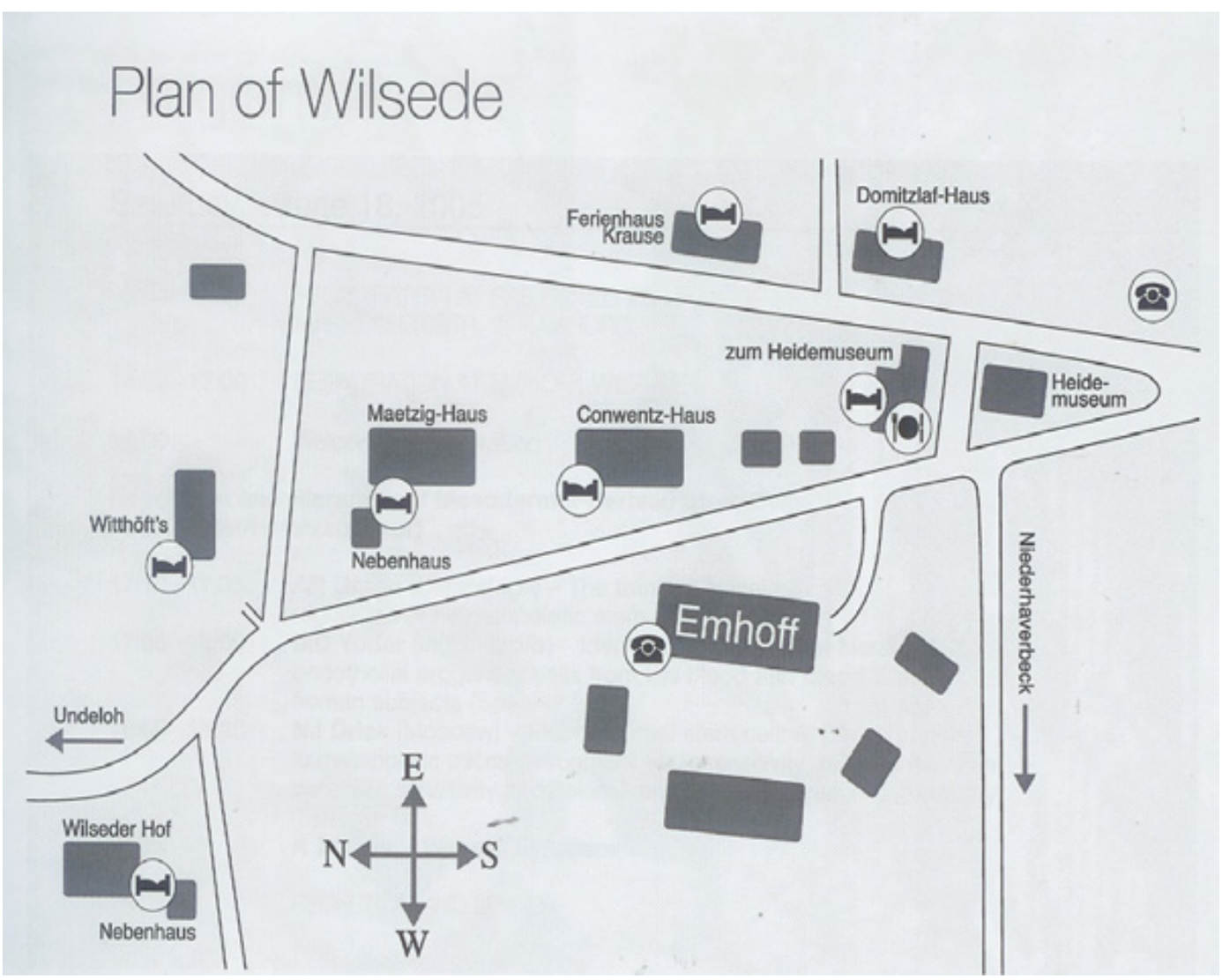
Facts

Wilsede

... is located in the heart of the Lüneburger Heide (Heath), one of the most magnificent and oldest national parks in Europe. The conservation society »Naturschutzpark Verein« (NVP) was founded in 1909 to preserve this unique moorland between Hamburg and Hannover, including the small village of Wilsede, whose existence can be traced back to 1287. Located within the nature reserve, where neither cars or other motorized vehicles are allowed, Wilsede can only be reached by foot, horse carriage, or bicycle. It has been the site of the »Wilsede Meetings: Modem Trends in Human Leukemia« since 1973, gathering Haematologists and Oncologists from around the word.

A history lesson: »De Emhoff«

... was built in 1609 by family Emmann, who lived there for more than 300 years. In 1960 the meanwhile dilapidated building wastobe pulled down, but the NVP, together with the Lower Saxony Authority for Preservation of Historical Monuments, succeeded to place the building under the Preservation Act. About one third of the building was reconstructed under the direction of the architect Prof. Maetzig using the original techniques, e.g. grooved planks instead of nails. The former hallway, cowsheds and stables were rebuilt into a large meeting room and the »Dönz«, the former living room, is today the kitchen. The shape of the lamps is to remind the visitor of the old pitch torches. The architectural style of the Emhoff is said to be the oldest of the moorland region and it is especially typical for the southern part of this region. Nowadays buildings of this kind have almost completely disappeared due to disrepair and deterioration, making Emhoff especially precious.



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On-site Registration · Emhoff/Wilsede

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Meeting point

University Medical Center Hamburg-Eppendorf (UKE)
Campus Lehre | Building N55
Martinistrasse 52 | 20251 Hamburg
(via main entrance or back entrance at Süderfeldstrasse)

From 08.30

Registration and pick up of conference material

Bus transfer at

10.00, **12.00** and **14.00** to the National Park (entrance Undeloh) For transport from Undeloh to Wilsede, horse carriages will be departing or please feel free to get a bike or enjoy a nice walk.

Horse Carriage (Schedule)

June 18, 2016 (Arrival)

11.30	from Undeloh	to Wilsede
13.30	from Undeloh	to Wilsede
15.30	from Undeloh	to Wilsede
22.30	from Wilsede	to Undeloh

June 19 & 20, 2016

08.00	from Undeloh	to Wilsede
22.30	from Wilsede	to Undeloh

June 21, 2016 (Departure)

08.00	from Undeloh	to Wilsede
12.00	from Wilsede	to Undeloh
13.00	from Wilsede	to Undeloh

Undeloh: parking lot near »Undeloher Hof«

Registration Hamburg/Wilsede

June 18, 2016 Campus Lehre, Building N55 University Medical Center Hamburg-Eppendorf (UKE)	08.30 to 14.00
June 18, 2016	14.00 to 17.00
June 19-20, 2016	08.00 to 18.00
June 21, 2016	08.00 to 12.00

Bicycles (»rent-a-bike«)

A deposit of 10,- Euros is required and will be refunded after return of the bike.

Undeloh: »Hotel Heiderose«
Wilsede: »Wilseder Hof«

Departure (June 21, 2016)

Bus transfer from parking lot in Undeloh to Hamburg airport and/or central station:

Departures 12.30 and 13.30

Attire of the meeting: Casual

General Information

Wilsede

Zum Heidemuseum | 20646 Wilsede
Phone: +49 4175-217
Contact: Klaus Parpart

- Conwentz-Haus (Guesthouse)
- Domitzlaff-Haus (Guesthouse)
- Maetzig-Haus/Nebenhaus (Guesthouse)

Wilseder Hof | 29646 Wilsede
Phone: +49 4175-311
Contact: Stefan Wischhof

Witthöfts Gästehaus | 20646 Bispingen
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Contact: Dr. Uta Büttinghaus

Undeloh

Ferienhof Heins | 21274 Undeloh
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Contact: Inge Brunkhorst

- Dependance Brunnencafé
- Dependance Uhlchen

Breakfast

June 19-21, 2016
Guests accommodated in
Wilsede: at Gasthof "Zum Heidemuseum" (starting at 7.00)
exeption:
Guests accommodated in
Wilseder Hof at Wilseder Hof
Guests accommodated in
Undeloh: at their hotel or guesthouse
exeption:
Guests accommodated in
Ferienhaus vorm Westerhoop:
at "Hotel Heiderose"

Welcome Reception and Dinner

Saturday, June 18, 2016 - 19.30
»Hotel Wilseder Hof«

Lunch

Sunday, June 19, 2016 - 12.45-14.00
Monday, June 20, 2016 - 12.30-13.30
Tuesday, June 21, 2016 - packed Lunch
»Gasthof "Zum Heidemuseum"«

Barbecue/Farewell Dinner

Sunday, June 19 and
Monday, June 20, 2016 - 19.00
»Emhoff«

General Information

Poster Session (including presenting authors)

The opportunity to present research or clinical results will be provided in two poster sessions (please see schedule). Posters will be grouped by sections. All poster presenters are called to present their contribution in a 4-minute poster fast-forward presentation. Please arrive to your session on time. Your Poster-Number was announced by E-Mail and the poster wall is marked with your number.

Poster Session I
Sunday, June 19, 2016 | 17:00-19:00

Leukemogenesis (III),
OMICs (Genomics, Transcriptomics, Proteomics, Epigenomics…) (IV)
Targeted therapies (V),
New treatments (Cell, Gene, Immune therapy) & agents (VI)

#
S-III-01 · S-III-02 · S-III-03 · S-III-04 · S-III-05 · S-III-06 · S-III-07 S-III-08 · S-III-09 · S-III-10 · S-III-11 · S-III-12 · S-III-13 · S-III-14 · S-III-15
#
S-IV-16 · S-IV-17 · S-IV-18 · S-IV-19 · S-IV-20 · S-IV-21
#
S-V-22 · S-V-23 · S-V-24 · S-V-25 · S-V-26 · S-V-27
#
S-VI-28

Poster Session II
Monday, June 20, 2016 · 13:30-15:30

Tumour heterogeneity & metastasis (I),
Stem cells in homeostasis and disease (II),
New treatments (Cell, Gene, Immune therapy) & approaches (VII)

#
M-I-01 · M-I-02 · M-I-03 · M-I-04 · M-I-05 · M-I-06 · M-I-07 · M-I-08 · M-I-09 · M-I-10
#
M-II-11 · M-II-12 · M-II-13 · M-II-14 · M-II-15 · M-II-16 · M-II-17 · M-II-18 · M-II-19 · M-II-20 · M-II-21 · M-II-22 · M-II-23
#
M-VII-24 · M-VII-25 · M-VII-26 · M-VII-27 · M-VII-28 · M-VII-29 · M-VII-30

M = Monday · S = Sunday Roman numerals = Topic

Location

Conwentz-Haus, Maetzig-Haus

Poster Size

Portrait formatted posters in DIN A0 size (118.9 cm x 84.1 cm) are recommended. The size of your printed poster should not exceed 145 cm in height and 95 cm in width.

Poster Hanging

On the evening or morning before the session, but may be set up 1 hour at latest, before the start of the session.

„Sunday-Poster-Session-presenter":
We would highly appreciate the dismantling of your Poster directly after the Session. Please keep in mind that your Poster-wall is shared with the presenters of the Monday-Poster-Session. Thank you for your consideration.

Saturday | June 18, 2016

08.30 – 14.00	**Registration** at UKE N55 (Hamburg) and in Wilsede	
16.30 – 16.40	**Welcome address (Emhoff)** Boris Fehse & Nicolaus Kröger, Hamburg - DE	

Session I: Stem cells & stroma
Chairs: Tsvee Lapidot, Rehovot/IL; Simon Mendez-Ferrer, Cambridge/UK

16.40-17.05	**Remodelling of Mesenchymal stroma cells by Acute Myeloid Leukemia (AML) Cells** Dominique Bonnet, London - UK	Speaker 01
17.05-17.30	**Microenvironmental HSC regulation in space and time** Simon Mendez-Ferrer, Cambridge - UK	Speaker 02
17.30-17.55	**Molecular and Functional Heterogeneity of Dormant HSCs** Nina Cabezas-Wallscheid, Heidelberg - DE	Speaker 03
18.00-19.00	**Special Lecture** **Metabolic regulation of blood and bone forming stem cells by the endothelial barrier: the role of ROS and nitric oxide** Tsvee Lapidot, Rehovot - IL	Speaker 04
19.30	**Welcome Reception and Dinner** Wilseder Hof	

Sunday | June 19, 2016

Session II: Myeloproliferative neoplasia I Chairs: Tony Green, Cambridge - UK; Carol Stocking, Hamburg - DE		
09.00-09.25	**Structural and Molecular Bases of Pathologic Signaling by JAK2 and Tpo Receptor Mutants in Myeloproliferative Neoplasms: Perspectives for Therapy** Stefan Constantinescu, Brussels - BE	Speaker 05
09.25-09.50	**Stem cell origin and disease initiation of myeloproliferative neoplasms** Radek Skoda, Basel - CH	Speaker 06
09.50-10.15	**Genetic basis of Ph- myeloproliferative neoplasms** Robert Kralovics, Vienna - AT	Speaker 07
10.15-10.30	**The metabolite R-2-hydroxyglutarate (R-2HG), but not S-2HG, collaborates with HoxA9 to induce monocytic leukemia in vivo** (selected abstract) Anuhar Chaturvedi, Hannover - DE	Speaker 08
10.30-11.00	**Coffee break**	

Session III: Myeloproliferative neoplasia II Chairs: Robert Kralovics, Vienna - AT; Radek Skoda, Basel - CH		
11.00-11.25	**CALR mutants induce a myeloproliferative disorder through activation of the thrombopoietin receptor MPL and JAK2** William Vainchenker, Villejuif - FR	Speaker 09
11.25-11.50	**The myeloproliferative neoplasms, JAK/STAT signalling and stem cell subversion** Tony Green, Cambridge - UK	Speaker 10
11.50-12.15	**Acute myeloid leukemia: roadblock to differentiation** Michael Heuser, Hannover - DE	Speaker 11
12.15-12.30	**HSC genetic heterogeneity determines clonal dynamics in PMF** (short talk) Ioanna Triviai, Hamburg - DE	Speaker 12
12.30-12.45	**Attenuation of PKC Activity Augments Hematopoietic Stem and Progenitor Cells Poll Size and Fitness** (short talk) Tata Nageswar Rao, Basel - CH	Speaker 13
12.45-14.00	**Lunch (Heidemuseum) & Poster viewing**	

Sunday | June 19, 2016

Session IV: Canceromics Chairs: Bertie Goettgens, Cambridge - UK; Phil Koeffler, Los Angeles - US		
14.00-14.25	**Acute Leukemia: Genomic Abnormalities, Even Before the Disease Develops** Phil Koeffler, Singapore - Singapore	Speaker 14
14.25-14.50	**Meis transcription factors in normal and leukemic hematopoiesis** Keith Humphries, Vancouver - CA	Speaker 15
14.50-15.15	**MicroRNA-155 and microRNA-708 are opposing modulators of Hoxa9 activity in acute myeloid leukemia** Florian Kuchenbauer, Ulm - DE	Speaker 16
15.15-15.30	**Integrated Analysis of the Human Hematopoietic Non-Coding RNA Landscape Reveals Lnc-RNA Stem Cell Signature in AML** (selected abstract) Jan-Henning Klusmann, Hannover - DE	Speaker 17
15.30-16.00	**Coffee break**	

Intermezzo: Immunotherapy I Chair: Archana Thakur, Charlottesville - US		
16.00-16.25	**Off-the-shelf engineered cord blood NK cells for eradication of leukemia** Katy Rezvani, Houston - US	Speaker 18
16:25-16:40	**Identification of Immune Checkpoint Ligands PVR and PVRL2 as Novel Therapeutic Targets in Acute Myeloid Leukemia** (selected abstract) Hauke Stamm, Hamburg - DE	Speaker 19
17.00-19.00	**POSTER SESSION I** **(Conwentz-Haus, Maetzig-Haus)** Chairs: Michael Heuser, Hannover - DE Florian Kuchenbauer, Ulm - DE Leukemogenesis (III), OMICs (Genomics, Transcriptomics, Proteomics, Epigenomics…) (IV), Targeted therapies (V), New treatments (Cell, Gene, Immune therapy) & agents (VI)	
19.00	**Barbecue and Live Music ‚Les amis'** »Emhoff«	

Monday | June 20, 2016

Session V: Stem cells in homeostasis and disease I Chairs: Ulrich G. Steidl, New York - US; David Williams, Boston - US		
09.00-09.25	**Molecular analysis of individual primitive human hematopoietic cells before and after their stimulation** Connie Eaves, Vancouver - CA	Speaker 20
09.25-09.50	**Regulatory Network Control of Blood Cell Development** Bertie Goettgens, Cambridge - UK	Speaker 21
09.50-10.15	**Model-based prediction of long-term molecular response in TKI treated CML** Ingo Roeder, Dresden - DE	Speaker 22
10.15-10.30	**Analysis of the subclonal architecture of adult B-ALL reveals highly plastic surface marker expression contradicting a common stem cell phenotype** (selected abstract) Bartosch Wojcik, Frankfurt - DE	Speaker 23
10.30-11.00	**Coffee break**	

Session VI: Stem cells in homeostasis and disease II Chairs: Connie Eaves, Vancouver - CA; Keith Humphries, Vancouver - CA		
11.00-11.25	**The role of RhoGTPases in normal and malignant hematopoiesis** David Williams, Boston - US	Speaker 24
11.25-11.50	**Transcriptional Dysregulation in Precancerous Stem Cells and their Progression to MDS and AML** Ulrich G. Steidl, New York - US	Speaker 25
11.50-12.15	**Stem cell gene therapy - what cells should we target?** Hans-Peter Kiem, Seattle - US	Speaker 26
12.15-12.30	**Genetic and functional diversity between single stem cell clones of a patient's ALL sample growing in mice** (selected abstract) Irmela Jeremias, Munich - DE	Speaker 27
12.30-13.30	**Lunch (Heidemuseum) & Poster viewing**	
13.30-15.30	**POSTER SESSION II** **(Conwentz-Haus, Maetzig-Haus)** Chairs: Nicolaus Kröger, Hamburg - DE Sonja Loges, Hamburg - DE Tumour heterogeneity & metastasis (I), Stem cells in homeostasis and disease (II), New treatments (Cell, Gene, Immune therapy) & approaches (VII)	
15.30-15.45	**Vorstellung der Förderprogramme der Deutschen Krebshilfe** Matthias Serwe, Bonn - DE	

Monday | June 20, 2016

Session VII: New targets, novel therapies Chairs: Olaf Heidenreich, Newcastle upon Tyne - UK; Richard Lock, Sydney - AU		
15.45-16.10	**New Agent Testing in Paediatric Leukaemia** Richard Lock, Sydney - AU	Speaker 28
16.10-16.25	**A novel selective inhibitor of mutant IDH1 promotes differentiation in vivo and prolongs survival in a mouse model of leukemia** (selected abstract) Ramya Goparaju, Hannover - DE	Speaker 29
16.25-16.55	**Coffee break**	
16.55-17.20	**Axl-targeting - a novel treatment approach for AML?** Sonja Loges, Hamburg - DE	Speaker 30
17.20-17.45	**Integrative Genomics Identifies the Molecular Basis of Resistance to Azacitidine Therapy in Myelodysplastic Syndromes** John Pimanda, Sydney - AU	Speaker 31
17.45-18.10	**t(8;21) AML is susceptible to G1 CDK inhibition** Olaf Heidenreich, Newcastle upon Tyne - UK	Speaker 32
19.00	**Farewell Dinner** »Emhoff«	

Programme

Tuesday | June 21, 2016

Tuesday | June 21, 2016

Session VIII: Immunotherapy I Chairs: Tuna Mutis, Amsterdam - NL; Paul Veys, London - UK		
09.00-09.25	**First-in-man use of gene-edited universal (U)CAR19 T cells to induce remission of refractory relapsed acute lymphoblastic leukaemia** Paul Veys, London - UK	Speaker 33
09.25-09.50	**Adoptive T-cell therapy** Gerald Willimsky, Berlin - DE	Speaker 34
09.50-10.05	**Receptor-transgenic T cells for the Immunotherapy of Multiple Myeloma** (short talk) Sara Yousef, Hamburg - DE & Utah - US	Speaker 35
10.10-10.40	**Coffee break**	
10.40-11.05	**Immunotherapy of Multiple Myeloma by targeting CD38: antibodies, CART cells and beyond** Tuna Mutis, Amsterdam - NL	Speaker 36
11.05-11.30	**Enhanced Transfer of Specific Anti-Cancer Immunity by Infusions of Bispecific Antibody Armed T cells Given Prior to Stem Cell Transplant** Archana Thakur, Charlottesville - US	Speaker 37
11.30-11.45	**Prognostically favorable B-cell immune responses after allogeneic stem cell transplant define a unique targetable epitope fingerprint of multiple myeloma** (selected abstract) Anna Oberle, Hamburg - DE	Speaker 38
11.45	**Concluding remarks**	
from 12.00	**Departure (packed Lunch)**	

We thank you for your active participation and wish you a pleasant and safe trip home. Hope to see you again for the Wilsede Meeting in June 2018!

Speaker Abstracts

Stem cells & stroma

Speaker 1

Remodelling of Mesenchymal stroma cells by Acute Myeioid Leukemia (AML) cells

Dominique Bonnet

The Francis Crick Institute HSC laboratory, 44 Lincoln's Inn Fields, London, WC2A 3LY, United Kingdoms

Acute myeloid leukemia (AML) is a hematologic malignancy, arising within the bone marrow, which is characterized by the uncontrolled proliferation of leukemic blasts, often in association with a disruption of normal hematopoiesis. Like their normal counterparts, AML cells depend upon both cell-intrinsic and -extrinsic regulatory signals generated by their surrounding microenvironment, for their survival and proliferation. One cell-intrinsic hallmark of malignant cells is an altered metabolic profile, due to the increased energetic demands required for their deregulated proliferation and survival. This aerobic glycolysis has most commonly been described in solid tumors, but several investigations suggest that some leukemias also utilize the Warburg effect. It has been demonstrated that enhanced glycolysis was associated with poor overall survival in patients with AML and a decreased sensitivity to chemotherapy *ex vivo* (Chen et al., 2014). Studies have also shown that direct targeting of metabolism, either glycolysis or mitochondrial respiration, within AML cells is effective at impairing leukemogenesis (Lagadinou et al., 2013; Wang et al., 2014). However, despite the increasing evidence that describe the cell autonomous metabolic reprogramming of leukemic cells, our understanding of how this affects the surrounding microenvironment still remains limited.

It has long been established that leukemia cells depend upon interactions with cells of the bone marrow (BM) niche. More recently, studies have implicated a link between altered metabolism of cancer and its niche. Further characterization of the key mechanisms controlling the metabolic communication between leukemia cells and their supporting niche warrants further investigation.

We will present data showing that primary AML patient samples in contact with mesenchymal stroma cells (MSC) produce increased amounts of lactate and identified monocarboxylate transporter 1 (MCT1) and MCT4 as the prominent pathway for lactate transport by MSC and AML cells, respectively. Targeting of MCT1 in MSC and MCT4 in AML abrogated the remodeling of MSC by AML, profoundly inhibited leukemogenesis and restored normal hematopoiesis. Collectively, our results describe a mechanism of communication that enables AML cells to remodel their surrounding niche through the secretion of metabolic by-products in order to create a permissive microenvironment for the promotion of leukemia.

References

Chen, W.L., Wang, J.H., Zhao, A.H., Xu, X., Wang, Y.H., Chen, T.L., Li, J.M., Mi, J.Q., Zhu, Y.M., Liu, Y.F., *et al.* (2014). A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. Blood *124*, 1645-1654.

Lagadinou, E.D., Sach, A., Callahan, K., Rossi, R.M., Neering, S.J., Minhajuddin, M., Ashton, J.M., Pei, S., Grose, V., O'Dwyer, K.M., *et al.* (2013). BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell stem cell *12*, 329-341.

Wang, Y.H., Israelsen, W.J., Lee, D., Yu, V.W., Jeanson, N.T., Clish, C.B., Cantley, L.C., Vander Heiden, M.G., and Scadden, D.T. (2014). Cell-state-specific metabolic dependency in hematopoiesis and leukemogenesis. Cell *158*, 1309-1323.

Speaker 2

Microenvironmental HSC regulation in space and time

Simón Méndez-Ferrer

Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute Department of Haematology. University of Cambridge and National Health Service Blood and Transplant, Long Road, Cambridge, CB2 0PT, United Kingdoms

Haematopoietic stem cells (HSCs) traffic between bone marrow and circulation, what allows for life-saving clinical transplantation procedures. Our previous work has shown that HSC numbers in blood follow circadian oscillations that are regulated by the central pacemaker in the brain. The central nervous system indirectly regulates HSC traffic through peripheral innervation of the HSC environment ('niche'). Among niche cells, we have found a prominent role for mesenchymal stem cells (MSCs) identified by the expression of the intermediate filament protein nestin. Like HSCs, MSCs are heterogeneous cell populations. In the perinatal bone marrow, HSC-niche forming MSCs might be different from those that form the skeleton and some of them might be neural crest-derived, like peripheral neurons and supporting glial cells. Thus, tight regulation of the bone marrow stem-cell niche in vertebrates might build upon developmental relationships of several niche components. We have found recently that cholinergic signals regulate HSC maintenance, proliferation and migration in different niches. We will present unpublished evidence of how both branches of the autonomic nervous system cooperate to regulate HSC maintenance and function in space and time. In certain blood disorders named myeloproliferative neoplasms, mutated HSCs overcome the normal niche control by damaging this regulatory network. Protecting the HSC niche might represent a novel complementary therapeutic strategy for these disorders. Patients with myeloproliferative neoplasms have a higher risk of developing acute leukaemia. However, cells driving acute myelogenous leukaemia might be less sensitive to the normal control by the microenvironment and, instead, these cells might transform the bone marrow niches to support their own survival. We will discuss potential microenvironmental contributions to normal HSC regulation and to different myeloproliferative disorders.

Speaker 3

Molecular and Functional Heterogeneity of Dormant HSCs

Nina Cabezas-Wallscheid[1,2], Buettner Florian[3], Klimmeck Daniel[1,2], Sommerkamp Pia[1], Thalheimer Frederic B.[4], Pastor-Flores Daniel[5], Roma Leticia P.[5], Renders Simon[1], Scognamiglio Roberta[1], Zeisberger Petra[1], Przybylla Adriana[1], Collier Paul[6], Pavlinik Dinko[6], Schroeder Timm[7], Benes Vladimir[6], Dick Tobias P.[5], Rieger Michael A.[4], Stegle Oliver[3], Trumpp Andreas[1,2]

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[2]HI-STEM, Heidelberg, Germany ;

[3]EBI, Cambridge, United Kingdoms;

[4]Goethe University Frankfurt LOEWE Center for Cell and Gene Therapy and Department of Medicine, Frankfurt, Germany ;

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Rare dormant hematopoietic stem cells (dHSCs) harboring the highest long-term reconstitution capacity define the top of the hematopoietic system. The molecular identity of dHSCs and immediate progeny, as well as the mechanism regulating maintenance and the transition out of dormancy remain unknown. We now show by single-cell RNA-seq analysis that the transition from dormancy towards cell cycle entry is achieved by a continuous and coordinated up-regulation of all major biosynthetic processes rather than a switch on/off mechanism. We generate a novel transgenic reporter mouse that specifically labels dHSCs avoiding label retention assays and we decipher the key pathways that are important for keeping dormancy.

Special Lecture

Speaker 4

Metabolic regulation of blood and bone forming stem cells by the endothelial barrier: the role of ROS and nitric oxide

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Most long-term repopulating hematopoietic stem cells (LT-HSC) are retained in their bone marrow (BM) niches in a quiescent non-motile, low metabolic state, via adhesive interactions. BM retained $EPCR^+$ LT-HSC endowed with the highest repopulation potential, are protected from chemotherapy induced cell death via EPCR/PAR1 signaling, to prevent hematology failure which is lethal . $EPCR^+/PAR1^+$ LT-HSC BM retention is mediated by specialized less permeable arteries and arterioles adjacent to bone which functionally express thrombomodulin and secrete the EPCR ligand aPC. Activation of PAR1 by aPC/EPCR inhibits nitric oxide (NO) production, increasing VLA4 affinity, adhesion and retention. Nitric oxide limitation also increases the levels of bone forming progenitors. In contrast thrombin activates different PAR1 signaling, inducing NO generation, TACE mediated EPCR shedding and enhanced CXCL12/CXCR4 mediated mobilization. Cell surface CXCL12 expression by BM stromal cells is essential for LT-HSC BM retention and is dependent on connexin (Cx) 43 gap junction communication. BM retained LT-HSC have low levels of reactive oxygen species (ROS) which prevent their migration and development. Chemotherapy insult increases stem cell ROS levels which in turn are transferred to BM stromal cells in a Cx43 dependent, suggesting mitochondria transfer from blood to bone forming stem cells. This leads to opposite ROS and metabolic states between the two populations and suggests energy sharing and either new bone or blood production on a one at a time basis. The blood bone marrow endothelial barrier prevents circulating mature red blood cells and platelets entry to the bone marrow. BM sinusoids are more permeable and fenestrated, transfer signals from blood which elevate ROS levels. A functional consequence of high blood vessel permeability is that exposure to blood plasma increases BM HSPC ROS levels, augmenting their migration and differentiation potential while compromising their long term repopulation and survival capacity. BM sinusoids are also the exclusive site for immature and mature leukocyte trafficking from and to the bone marrow. Less permeable arteries and arterioles adjacent to bone, form ROS^{low} peri arterial cites which maintain both blood and bone forming stem cells, in a ROS^{low} metabolic state. In conclusion, the dynamic BM stem cell niches regulate both blood production and new bone formation via metabolic generation of ROS and nitric oxide by distinct barrier blood vessels.

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Myeloproliferative neoplasia I

Speaker 5

Structural and Molecular Bases of Pathologic Signaling by JAK2 and Tpo Receptor Mutants in Myeloproliferative Neoplasms: Perspectives for Therapy

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BCR-ABL-negative myeloproliferative neoplasms (MPN) are associated with phenotypic driver mutants, namely JAK2 V617F, thrombopoietin receptor (TpoR, c-MPL) W515 mutants and exon 9 calreticulin mutants. Constitutive activation of JAK2/STAT5/STAT1/STAT2 and JAK2/MAP-kinase and JAK2/PI-3'-kinase pathways constitute hallmark features of these pathologies, leading to absence of physiologic cytokine-control on the survival, proliferation and differentiation of erythroid, megakaryocytic and granulocytic progenitors. Persistent STAT5 activation is required for MPN pathology.

A major challenge has been the structural understanding of how mutations induce JAK2 and TpoR activation. Here we show that such oncogenic conformations differ substantially from the transient cytokine-activated Tpo receptor and JAK2. We also show that chromatin effects of persistent STAT5 activation differ from cytokine-induced activation.

Using structure-guided modeling, mutagenesis and structural approaches we identified the mechanisms by which JAK2 kinase domain is activated by the prevalent pseudokinase (JH2) V617F mutation. A community of residues was identified starting from the JH2 helix αC F595, SH2-JH2 linker and JH1 kinase domain that mediate V617F-induced activation. This circuit could be broken by altering the charge of residues along the solvent-exposed face of the JH2 αC. Mutations in JH2 such as E596A/R, did not alter the JH2 V617F fold, as shown by our crystal structure of JH2 V617F E596A. Instead, they prevent kinase domain activation via modulation of the C-terminal residues of the SH2-JH2 linker. Furthermore, using mutagenesis, in vivo bone marrow transplantation and biophysical approaches we identified a novel required conformational change in TpoR cytosolic juxtamembrane domain that is associated with W515 mutations and with the S505N transmembrane mutation. This conformational change leads to close apposition of JH1 JAK2 kinase domains and their activation. Overall, ou results suggest novel strategies for selective inhibition of mutated JAK2 and TpoR.

Finally, we show that persistent STAT5 activation in MPNs leads to pathologic recruitment of p53 as well as p53 mutants to non-canonical STAT5-target promoters that contributes to progression of MPNs to secondary acute leukemia. We also suggest that STAT5 is critical for MPN progression, further emphasizing that an early block in pathologic JAK-STAT signaling might required to stop progression to secondary acute leukemia.

Speaker 6

Stem cell origin and disease initiation of myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN) are clonal disorders of the hematopoietic stem cell. Somatic gain of function mutations in the genes for JAK2, CALR or MPL can be found in about 90% of patients with MPN and are considered to be the disease driving mutations. Indeed, expressing these driver mutations in mouse models result in MPN phenotypes closely recapitulating the human disease. While CALR, MPL and JAK2 exon 12 mutations have a very defined phenotype, the phenotypic expression of JAK20V617F mutations can range from isolated thrombocytosis, leukocytosis to a tri-lineage disease resembling polycythemia vera. The genotype-phenotype correlation of JAK2 mutations will be discussed. In addition, mutations in epigenetic regulators, transcription factors and signaling components can co-exist with driver mutations. They modify the course of the disease and can contribute to disease initiation and/or progression. Using limiting dilution transplantations in mice, we have shown that the JAK2-V617F mutation alone can initiate clonal MPN starting from single hematopoietic stem cells (HSCs). Mice expressing JAK2-V617F in combination with a loss of function mutation in Ezh2 displayed more severe disease with rapid progression to myelofibrosis due to hyperactive megakaryopoiesis. Furthermore, single cell disease initiation was more efficient with the double mutant HSCs and MPN could be also initiated from short-term HSCs that normally do not have the capacity for long-term repopulation. Analysis of RNA expression in HSCs and megakaryocytic erythroid progenitor cells (MEPs) identified several upregulated genes that are candidates to mediate the acceleration of disease. Functional tests are under way to define the potential contribution

Speaker 7

Genetic basis of Ph- myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN) are characterized by clonal overproduction of terminally differentiated myeloid cells, increased risk of thrombosis, bleeding and leukemic transformation. Both acquired and constitutional genetic alterations contribute to the MPN pathogenesis. An overview of genomic data generated over the past decade using SNP microarray analysis and exome sequencing will be provided. The genetic defects associated with MPN are classified into the following categories: 1) germline genetic predispositions, 2) disease initiating mutations (JAK2, MPL, CALR) and 3) mutations driving disease progression (TP53, CBL, ASLX1). The germline genetic predispositions include a variety of factors with weak and strong effect that predispose carriers to acquisition of somatic mutations that initiate MPN. These include the GGCC JAK2 haplotype, polymorphisms in the TERT gene, and the recently identified RBBP6 mutations. All these factors also contribute to familial clustering of MPN. The disease initiating mutations are targeting the JAK/STAT signaling pathway. Three genes (JAK2, CALR, MPL) are mutated in a mutually exclusive manner in more than 95% of MPN cases. JAK2 mutations are present most frequently in all three MPN subtypes while the less frequent CALR and MPL mutations are present only in primary myelofibrosis and essential thrombocythemia and have not been seen in polycythemia vera. JAK2, CALR, and MPL induce overlapping phenotypes but also influence the clinical course specifically. A fraction of MPN patients that are negative for the common JAK2, CALR, and MPL mutations often carry unusual JAK2 and MPL mutations. The last group of mutations associated with disease progression is the most diverse. Both somatic point mutations and chromosomal aberrations are identified that strongly influence leukemic transformation and in each patient both types of defects are often detected in complex mono- or bi-clonal hierarchies. At the leukemic stage each patient seems to be a unique transformation event and patient stratification at this stage of the disease will be challenging.

Speaker 8

The metabolite R-2-hydroxyglutarate (R-2HG), but not S-2HG, collaborates with HoxA9 to induce monocytic leukemia in vivo (selected abstract)

Anuhar Chaturvedi[1], Michelle Maria Araujo Cruz[1], Nidhi Jyotsana[1], Amit Sharma[1], Ramya Goparaju[1], Adrian Schwarzer[2], Kerstin Görlich[1], Renate Schottmann[1], Eduard A. Struys[3], Erwin E. Jansen[3], Christian Rohde[4], Carsten Müller-Tidow[4], Robert Geffers[5], Gudrun Göhring[6], Arnold Ganser[1], Felicitas Thol[1], Michael Heuser[1]

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Canonical mutations in IDH1 and IDH2 produce high levels of the R-enantiomer of 2-hydroxyglutarate (R-2HG), which is a competitive inhibitor of αKG-dependent enzymes and a putative oncometabolite. Mutant IDH1 collaborates with HoxA9 to induce monocytic leukemia in vivo. Almost all patients with *IDH1/2* mutations express high levels of intracellular R-2HG, while an increase of the S-enantiomer of 2HG has never been described. To investigate whether oncometabolites R-2HG and/or S-2HG, have a causative function in leukemogenesis or instead are rather only biomarkers for oncogenic IDH. To this end, we daily administered R-2HG and S-2HG at a dose of 1mg/mouse in vivo in three independent mouse models (HoxA9, MLL-AF9 and an AML patient derived xenotransplantation model) containing only wild-type IDH1. We show that R-2HG, but not S-2HG or αKG, is an oncometabolite in vivo, induces hyperleukocytosis and accelerates the onset of murine and human leukemia. A question of current investigation is whether R-2HG alone is responsible for the transforming effects of mutant IDH or if the mutant protein contributes additional oncogenic functions. Interestingly, both cohorts, HoxA9+IDH1mut and HoxA9 treated with R-2HG, developed leukemia, albeit with different kinetics. In gene expression data R-2HG treated cells clustered with IDH1 mut cells, while IDH1 wt cells clustered with HoxA9 control cells. The differentially methylated regions (DMRs) between R-2HG treated cells and IDH1 wt cells were well represented in IDH1 mut cells. However, DMRs between IDH1 mut and IDH1 wt cells were not well represented in R-2HG treated cells and these cells clustered with IDH1 wt and not IDH1 mut cells. The higher number of hyper- and hypomethylated regions in IDH1 mut cells compared to R-2HG treated cells, supports an additional function of the mutant protein on transcriptional regulation and DNA methylation beyond the function of the metabolite R-2HG and can explain the earlier disease onset of IDH1 mut cells compared to R-2HG treated cells. We show that circulating R-2HG acts in a paracrine fashion and can drive the expansion of many different leukemic and preleukemic clones that may express wildtype IDH1, and therefore can be a driver of clonal evolution and diversity. We therefore propose R-2HG independent oncogenic functions of mutant IDH1 that may need to be targeted in addition to R-2HG production to exploit the full therapeutic potential of IDH1 inhibition.

Myeloproliferative neoplasia II

Speaker 9

CALR mutants induce a myeloproliferative disorder through activation of the thrombopoietin receptor MPL and JAK2

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BCR-ABL-negative myeloproliferative neoplasms (MPN) are classified into polycythemia vera, essential thrombocythemia (ET), and primary myelofibrosis (PMF). MPNs are driven by mutations in genes such as *JAK2* and *MPL*, triggering abnormal activation of the cytokine receptor/JAK2 pathway. The recent discovery of calreticulin (*CALR)* mutations has closed a gap in our knowledge of MPN driver mutations. However, CALR is not a signaling molecule, but an ER chaperone. All discovered mutations are located in exon 9 and induce a frameshift leading to a CALR with a new common C-terminus devoid of the ER retention KDEL sequence. *CALR*del52 and *CALR*ins5 are the 2 predominant mutations. Using cell lines, mouse models and patient cells, we demonstrated that the different CALR mutants activate MPL and consequently JAK2, as well as the different downstream signaling pathways, but more particularly the STATs. Mutant CALR bind to MPL through an interaction between its lectin binding domain, and the extracellular N-linked sugars of MPL. However, MPL activation absolutely requires the new CALR C-terminus domain. There is evidence that MPL associated with mutant CALR traffics to the cell surface in an immature N-glycosylated form. CALR mutants are not able to activate other cytokine receptors, except G-CSF-R. However this activation is weak and does not allow the growth of factor-dependent cell lines without cytokines. In mouse transplantation assays, CALR mutants give rise to an ET-like disorder, progressing frequently to myelofibrosis for the CALRdel52 mutant. In this model, MPL is also indispensable for the development of a disease. In humans, the presence of CALR mutants is only found in ET and PMF, two diseases essentially involving the megakaryocyte (MK) lineage. The *CALR*-mutated MPNs are characterized by a clonal dominance occurring at the level of hematopoietic stem cells and a cytokine-independent growth of MK progenitor, which is dependent of MPL and JAK2. Therefore, CALR mutations define a new subgroup of MPNs restricted to ET and PMF, which is explained by a restricted activation of MPL, whereas JAK2V617F activates other cytokine receptors. It remains to be determined whether the differences in the phenotype between *CALR*- and *JAK2*V617F- mutated MPNs are only explained by the types of activated cytokine receptors or whether other mechanisms are involved.

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Speaker 10

The myeloproliferative neoplasms, JAK/STAT signalling and stem cell subversion

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The human myeloproliferative neoplasms (MPNs) are a spectrum of clonal haematological malignancies which arise in the haematopoietic stem cell compartment. These disorders are experimentally tractable, permit clonal analysis and provide a window on the earliest stages of tumorigenesis. A single somatic gain-of-function mutation in JAK2 is present in most MPN patients. This observation emphasised the significance of the JAK signalling pathway which plays a key role in stem cell biology and has now been implicated in many human malignancies. This talk will focus on the molecular and cellular consequences of normal and mutant JAK2 signalling. Highlights of general relevance for stem cell and cancer biology include unexpected insights into chromatin biology, clonal evolution and haematopoietic stem cell function. We have recently demonstrated for the first time in any cancer, that mutation order influences stem/progenitor cell behaviour, clonal evolution, clinical presentation and response to therapy (Ortmann et al NEJM 2015). In addition, we have made several observations which establish new paradigms for cytokine signalling. These include the demonstration that nuclear JAK2 functions as a histone kinase and regulates transcription (Dawson et al Nature 2009; Griffiths et al Nat Cell Biol 2011), and our recent identification of a genome-wide role for tyrosine-unphosphorylated STATs (Park et al EMBOJ 2016).

Speaker 11

Acute myeloid leukemia: roadblock to differentiation

Michael Heuser

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AML patients are at high risk of fatal infections as they are highly immunocompromised already at diagnosis, yet they require an aggressive, immunocompromising treatment to be cured. A conceptual solution to this problem would be instant differentiation of leukemic blasts to mature, functional immune cells that can quickly restore immunosurveillance in AML patients. Such an immune reconstituting treatment has been achieved in APL patients with a chemotherapy-free drug regimen. However, in all other AML patients cure is only achieved so far by myelotoxic chemotherapy. Thus, to modulate the myeloid differentiation block towards differentiation remains a key goal in AML research. We have characterized the MN1 oncogene as a key component of blocked myeloid differentiation. MN1 is a strong antagonist of retinoic acid signaling in mouse and human hematopoiesis and coordinates with MEIS1 to repress the immune response signature in leukemic cells. The function of MN1 in leukemogenesis and clinical consequences will be presented.

Speaker 12

HSC genetic heterogeneity determines clonal dynamics in PMF (short talk)

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PMF is a myeloproliferative neoplasm of stem cell origin, characterized by sequential distinctive waves of aberrant myeloid differentiation.The continuum of clonal dynamics dominating the chronic phase disease, as exhibited in the variability of divergent myeloid progeny in PMF progression, reflects the molecular heterogeneity of malignant HSCs that sustain fluctuations in clone propagation.In our previous work, we described a CD133+ stem cell population circulating in PMF patient peripheral blood, which exhibits multi-lineage differentiation capacity *in vivo* and *in vitro* and induces the PMF phenotype in the first xenotransplantation model of PMF. Single cell analysis of CD133+ patient-derived HSCs revealed multi-clonal lineage restricted differentiation potential of the stem cell pool in PMF. CD133+ HSCs from 100 PMF patients were molecularly characterized by whole exon sequencing. Sorted HSC cells were functionally analyzed at a single cell level for variable myeloid colony formation. 2230 colonies were phenotypically characterized and isolated. Analysis of the PMF HSC clonogenic potential indicates that the presence of mutations in the epigenetic regulator EZH2 correlates with granulo/monocytic differentiation but limited erythroid colony formation potential (0-0,05%), as determined in three different patient samples (2 JAK2-V617F+, 1 CALR-fs*+). Transplantation of these patient samples gave the highest engraftment in our mouse model and in one case, $EZH2^{mu}JAK2^{wt}$ leukemic transformation.CD133+ HSC-derived single colony analysis indicated 8 different genotypic clones of HSC, which exhibit variable granulo/monocytic differentiation capacity *in vitro*. PCR analysis of colonies for JAK2-V617F and Sanger sequencing for EZH2-D265H and EZH2-Y733C mutations indicates that the presence of JAK2-V617F in hetero- or homozygosity can occur in the EZH2-D265H background without influencing the granulo/monocytic commitment of these mutated HSCs. Our results indicate that mutations in epigenetic regulators precede the expanding mutations of the chronic phase PMF (JAK2, CALR). Thus they confer the genomic instability connected with subsequent emergence of following mutations and shape the genomic landscape supporting the expansion of pre-leukemic clones.

Speaker 13

Attenuation of PKC Activity Augments Hematopoietic Stem and Progenitor Cells Poll Size and Fitness (short talk)

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Hematopoietic stem cells (HSCs) are the only adult stem cells that can sustain lifelong blood cell production through their self-renewal and multi lineage blood differentiation capacity. A finely tuned balance of self-renewal, differentiation, cell proliferation and survival governs the pool size and fitness of HSCs. However, the signaling pathways that preside over fine-tuning of these HSC key properties are incompletely understood. Improved understanding of the molecular machinery that determines HSC function will aid the development of innovative strategies to expand HSCs ex vivo or to prevent their involvement in hematopoietic cancers. Here, we report that protein kinase C (PKC) is a critical regulator of adult HSC pool size and function that couples proliferative and survival activities in HSCs. Using both germline and hematopoietic specific inducible mouse models deficient for functional PKC, and in vitro approaches we demonstrate that PKC restricts HSC number and function in the steady-state and during hematopoietic stress conditions in a hematopoietic cell-intrinsic manner. Intriguingly, despite accelerated proliferation, PKC-deficient HSCs do not exhaust in serial transplant assays or induce leukemia. Finally, exploring the mechanism of HSC expansion in PKC-deficient mice, at the molecular level, we uncovered that PKC coordinately governs multiple regulators within signaling pathways implicated in HSC homeostasis. Our results implicate a pivotal role of PKC in the regulation of HSC proliferation and apoptosis and identify PKC as a critical rheostat controlling HSC expansion. Thus, reversible targeting of PKC may represent an attractive strategy to stimulate ex vivo expansion of HSCs and enhance hematopoietic recovery following HSPC transplantation. Such an approach could increase the likelihood of successful engraftment following HSC transplantation therapies.

Canceromics

Speaker 14

Acute Leukemia: Genomic Abnormalities, Even Before the Disease Develops

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Preleukemia: confusion concerning nomenclature. Myelodysplastic syndrome (MDS) was called **preleukemia** in the 1950s; in fact, about 30% of MDS patients develop acute myelogenous leukemia (AML). Genomic changes differentiate most MDS cases from AML. A recent discovery: Some AML patients who have been placed into a complete morphologic remission can have a clone of cells carrying mutations associated with the original leukemia (IDH2, DNMT3A, U2AF1, TET2). These are also called **preleukemic clones**. This, of course, is different from MDS. Compared with those with no preleukemic clone at remission, these individuals may have a shorter event-free survival suggesting that they should be monitored carefully, and more aggressive therapy should be considered. Another confounding discovery is that "normal" individuals as they age, develop hematopoietic clones marked by mutations (especially DNMT3A). At the ages of 60, 70 and 90 years, about 5%, 10%, and 20% of individuals, respectively have an aberrant hematopoietic clone; and they have a modest increased chance of developing leukemia (≈4% develop leukemia). These abnormal clones have occasionally been called **preleukemic**, but they also markedly differ from MDS. Relationship between developing cancer in a lifetime is related to the number of stem cell divisions within that tissue. A frequently asked query by a patient is why did I develop MDS and/or AML? We have very few early hematopoietic stem cells (HSC); and they rarely divide. The random mutation rate is about 1 mutation per 10^8 cell divisions. By mere bad luck, a mutation occurs in a dividing HSC that gives it a growth advantage. The clone accumulates additional driver mutations resulting in leukemia over time. Exposure to mutagens (e.g. benezine/radiation) or having a familial predisposition (e.g. germline mutations of GATA2, RUNX1, C/EBPα) can enhance the rate of cell divisions and DNA mutations in the HSC which can increase their risk of developing MDS and AML.

Speaker 15

Meis transcription factors in normal and leukemic hematopoiesis

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Meis1 is a homeodomain transcription factor and known Hox cofactor extensively studied for its roles in both normal and leukemic hematopoiesis. Meis1 is a strong leukemogenic collaborating gene with many Hox transcription factors and NUP98-Hox fusion genes as well as being a critical downstream effector of MLL-fusion gene leukemias. Our recent studies of a conditional knockout model of Meis1 (Miller et al, PLOS ONE, 2016) add to the now extensive evidence that Meis1 is also an essential transcription factor for normal hematopoiesis. In recent work we have turned our attention to Meis1 regulation and (Xiang et al, Leukemia, 2014) and its possible protein partners. As one approach we have used CRISPR-Cas9 gene editing to introduce a GFP reporter and an HA tag into the MEIS1 gene of several human leukemic cell lines as well as in a mouse Embryonic Stem cell line. From the latter we have also derived a GFP-HA-Meis1 mouse line and then tagged murine leukemic cell lines. These models now provide novel approaches to identify and characterize Meis1 enhancer regions and to identify novel interacting proteins. Meis1 also attracted our attention from studies of Meningioma 1 (MN1), a transcriptional cofactor whose overexpression is a poor prognostic marker in normal karyotype acute myeloid leukemia. MN1 is also a potent and sufficient oncogene in murine leukemia and its leukemogenic function is strongly dependent on the MEIS/AbdB-like HOX Protein Complex (Heuser et al Cancer Cell 2011). To identify additional genes critical to MN1 leukemogenesis, we generated a shortlist of genes differentially expressed between leukemic and non-leukemic states. Amongst the top ranked genes, Meis2 was of particular interest as it has not previously been implicated in MN1 induced leukemia and is expressed at much lower levels than Meis1 in normal hematopoietic cells. While, knockdown of Meis1 by shRNAs in MN1 leukemic cell lines had little to no detectable effect in vitro, knockdown of Meis2 significantly impaired cell growth, increased differentiation and increased apoptosis. Meis2 knockdown in transplanted MN1 cells increased the median latency of disease and impaired overall engraftment. These new finding reveal Meis2 as a novel player in MN1 induced leukemia and stimulate further studies to understand the basis for the profound upregulation of Meis2 and its roles in additional leukemic settings.

Speaker 16

MicroRNA-155 and microRNA-708 are opposing modulators of Hoxa9 activity in acute myeloid leukemia

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In order to identify microRNAs (miRNA) relevant in acute myeloid leukemia (AML), we profiled global miRNA expression in a murine AML progression model. We found miR-155 and miR-708 upregulated in leukemic Hoxa9/Meis1 cells (H9M) compared to the preleukemic Hoxa9/ctrl cells. Subsequent analysis of AML cases showed significantly elevated levels of miR-155 and miR-708, indicating potential oncogenic roles for these miRNAs.We further investigated, *in vivo*, the role of miR-155 by ectopic overexpression with Hoxa9 (H9) in murine bone marrow (mbm) cells (H9/miR-155). H9/miR-155 transplanted cells caused a significantly accelerated onset of AML, but still a less aggressive course of disease compared to H9M. In order to assess if miR-155 is dispensable for the onset of AML, we transformed miR-155-/- and miR-155+/+ mbm with H9M. No difference in onset of AML was observed. The absence of miR-155 impaired homing of H9M cells but did not impact their proliferation rate, which eventually compensated in this AML model.

We then hypothesized that the combination of miR-155 and miR-708 could further replace the oncogenic potential of Meis1. Therefore, mbm cells were transduced with H9/miR-155/miR-708 or H9/miR-708 and functionally analyzed. To our surprise, miR-708 abrogated the leukemogenic effect of H9, alone or in combination with H9/miR-155 *in vivo*, with little or no engraftment. Transcriptome analysis revealed that miR-155 and miR-708 have opposite effects on H9-induced transcription.To further understand why miR-708, a potent tumor suppressor miRNA, is upregulated in the highly aggressive H9M cells, we explored the role of miR-708 in leukemia initiating cells (LIC). H9M cell subpopulations enriched for LIC based on c-kit, Mac-1 and Gr-1 expression were transplanted. The c-kit$^+$Gr-1$^-$Mac-1$^-$ cells caused a significantly shorter survival compared to the other subpopulations. This was mirrored by lower expression of miR-708 in the LIC-enriched subpopulation compared to bulk, whereas there was no difference in miR-155 expression. Similar findings were made in human AML samples sorted into LIC-enriched subpopulations. Together, these results highlight the role of miR-708 as an orchestrator of the leukemic hierarchy through its tumor suppressor activity.In conclusion, we demonstrate for the first time a functional role for miR-155 in homing of AML cells. In addition, we propose a novel concept where miR-708, a tumor suppressor miRNA, stratifies the leukemic hierarchy.

Speaker 17

Integrated Analysis of the Human Hematopoietic Non-Coding RNA Landscape Reveals LncRNA Stem Cell Signature in AML (selected abstract)

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Long non-coding RNAs (lncRNAs) and miRNAs have emerged as crucial regulators of gene expression, epigenetics and cell fate decisions. Here we present an integrated quantitative and functional analysis of the miRNA-, lncRNA- and mRNA-transcriptome of purified human hematopoietic stem cells (HSCs) and their differentiated progenies, including granulocytes, monocytes, T-, NK-, B-cells, megakaryocytes and erythroid cells, which we correlated with the ncRNA expression profile of 46 pediatric AML samples. For each blood cell population more than 40,000 lncRNAs, 25,000 mRNAs and 900 miRNAs on 146 arrays were quantified.T-distributed stochastic neighbor embedding (t-SNE) on lncRNA and miRNA genes robustly structured the dataset into groups of samples that matched the input populations, demonstrating their unique ncRNA expression profiles. Self-organizing maps (SOMs) revealed clusters of lncRNAs and mRNAs that were coordinately expressed in HSCs and during lineage commitment. To demonstrate their functionality, we knocked down LINC00173 from the granulocytic core signature using two independent shRNA constructs, which resulted in diminished granulocytic *in vitro* differentiation, myeloid colony-formation and nuclear lobulation. Accordingly, CRISPR-mediated transcriptional repression of nuclear localized *LINC00173* using dCas9-KRAB and three sgRNAs per locus reduced proliferation of myeloid NB4 cells.We extended our findings to malignant hematopoiesis. t-SNE of 46 pediatric AML samples mapped most samples to a space between HSCs and differentiated cells together with the myeloid progenitors. A subset of AML-samples mapped closely to healthy HSCs, including most of the megakaryoblastic and MLL-rearranged AMLs. We identified a stem-cell associated lncRNA signature that was absent in healthy differentiated progenies, but upregulated in AML samples. A mesoscale CRISPRi screening in AML cell lines suggested the importance of the lncRNA stem cell core signature for the maintenance of leukemic growth.

Thus, our study provides a comprehensive resource for the exploration of the mRNA-, lncRNA- and miRNA-transcriptome across the normal and malignant human hematopoietis. The definition of a core lncRNA stem cell signature in normal HSCs and AML blasts will guide our way towards an improved understanding of self-renewal and the underlying transcriptional program, which is hijacked during malignant transformation.

Intermezzo: Immunotherapy I

Speaker 18

Off-the-shelf engineered cord blood NK cells for eradication of leukemia

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Natural killer (NK) cells play critical roles in host defense against cancer. Our group is exploring a number of avenues to enhance NK cell function against leukemia. These include novel strategies to expand off-the-shelf cord blood (CB) derived NK cells based on their co-culture with genetically-modified leukemic cells that express membrane-bound cytokines and co-stimulatory molecules. The methods ensure reliable expansion and activation of human CB NK cells and have been implemented in a GMP-grade large-scale setting to support ongoing clinical trials of CB-NK adoptive therapy.

To redirect NK cell specificity and enhance their in vivo persistence, we have successfully transduced expanded CB NK cells with chimeric receptors directed against CD19 (a molecule expressed on lymphoid malignant cells) linked to CD28 and CD3z, and IL15 to enhance their in vivo persistence and survival following adoptive transfer. CAR.CD19.IL15 CB-NK cells exhibited enhanced anti-leukemic activity and in vivo persistence in a nod-SCID gamma null (NSG) mouse model of B lymphoid malignancy. Based on these data, we are planning a clinical trial of 'off-the-shelf' CAR.CD19.IL15 CB-NK cells in patients with relapsed/refractory lymphoid malignancies.

Speaker 19

Identification of Immune Checkpoint Ligands PVR and PVRL2 as Novel Therapeutic Targets in Acute Myeloid Leukemia (selected abstract)

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T-cell activity is regulated by immune checkpoints to maintain the sensitive balance of co-stimulatory and inhibitory immune signals. Therapeutic blockade of checkpoint molecules on tumor or T cells, such as CTLA-4 or PD-L1, has shown clinical success in several tumor entities. In this investigation we show that AML cell lines and patients highly express the novel immune checkpoint ligands PVR (CD155) and PVRL2 (CD112), whose interaction with their receptors TIGIT, CD96 or CD112R confers a negative immune signal and dampens an ongoing immune response. Using two independent AML patient cohorts (n=139 and n=290, respectively), we could demonstrate that high PVR and PVRL2 expression correlates with a poor clinical outcome. The antibody blockade of PVR, PVRL2 or the combination of both antibodies significantly enhanced the cytotoxic activity of immune effector cells against the AML cell lines MV4-11, TF-1, Molm-13 and Kasumi-1 in *in vitro* cytotoxicity assays. Bispecific T-cell engager (BiTE®) antibody constructs have been shown to be an effective therapeutic approach for the treatment of hematologic malignancies, but studies show that the cytotoxic capacity of BiTE® antibodies is influenced by checkpoint molecule interactions. We could show that the anti-leukemic effects of the CD33/CD3 BiTE® antibody construct AMG 330 could be enhanced upon combination with PVR and PVRL2 blocking antibodies, using the above mentioned AML cell lines in cytotoxicity assays. In order to prove that the enhanced AML cell lysis was due to the immune checkpoint molecule blockade rather than being antibody dependent cellular cytotoxicity, several control experiments were performed. Blocking of CD117 on Kasumi-1 cells with an anti-CD117 antibody did not have any effect on cell killing either in the absence or presence of AMG 330. Besides, saturation of Fcγ-receptors on effector cells prior to the assay did not affect the increased cell killing of MV4-11 cells in either single or combined blocking of PVR and PVRL2 with or without additional AMG 330.

Furthermore, CRISPR/Cas9 mediated double knockout of PVR and PVRL2 in MV4-11 cells recapitulated the antibody effects and prolonged the survival of NSG mice reconstituted with human T cells compared to MV4-11 wildtype cells. Altogether, our results show for the first time that the PVR/PVRL2 axis is of major importance in AML and blocking of this immune checkpoint ligands on AML blasts represents a promising target for the treatment of AML.

References

Jasmin Wellbrock and Walter Fiedler contributed equally to the work.

Stem cells in homeostasis and disease I

Speaker 20

Molecular analysis of individual primitive human hematopoietic cells before and after their stimulation

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Hematopoietic differentiation is generally conceptualized as a series of irreversible, stepwise division-associated molecular changes that occur in response to external stimuli. These stimuli are envisaged to initiate an intrinsically determined and co-ordinated program in which proliferative capacity and lineage competence are synchronously lost and terminal differentiation is ultimately activated. Simultaneous analysis of 40 proteins in hundreds of thousands of individual human CD3-CD11b-CD19-CD34+ cord blood cells with parallel examination of their activation, and clonal growth and differentiation in vitro and in vivo reveals a more complex picture. This includes extensive molecular heterogeneity in progenitor subsets currently defined as distinct phenotypes as well as the detection of new ones. The latter include phenotypes that distinguish hematopoietic stem cells (HSCs) with 30 week regenerative activity in primary vs primary plus secondary hosts. Analysis of their activation shows different mechanisms controling HSC survival, mitogenesis and self-renewal. These findings uncover a new molecular landscape of primitive human hematopoietic cells that display less coordinated control than previously envisaged.

Speaker 21

Regulatory Network Control of Blood Cell Development

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The Göttgens group uses a combination of experimental and computational approaches to study how transcription factor networks control the function of blood stem cells and how mutations that perturb such networks cause leukaemia. This integrated approach has resulted in the discovery of new combinatorial interactions between key blood stem cell regulators, as well as experimentally validated computational models for blood stem cells. I will present results from our current research, which focuses on single cell genomics of early blood development, the development of computer models to chart the transcriptional landscape of blood stem and progenitor cell differentiation, and investigations on how leukaemogenic transcription factor mutations cause dysregulation of wider transcriptional networks.

Speaker 22

Model-based prediction of long-term molecular response in TKI-treated CML

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Systems biology, i.e. the application of mathematical models to quantitatively describe complex biological systems, is still a nascent discipline. However, even in the context of translational and clinical applications, the use of computational methods is becoming more and more accepted. In my presentation I will discuss potentials and limitations of mathematical modeling and simulation studies to broaden our understanding of treatment dynamics in chronic myeloid leukemia (CML). Specifically, I will present a simulation strategy that allows to predict patient-specific, individual long-term BCR-ABL kinetics. The simulation study is based on the analysis of the 5-year follow-up data from about 500 CML patients, who were treated within the DASISION trail [1,2], which compares first-line Imatinib and Dasatinib treatment. Our results demonstrate specific differences in the treatment response between the two drugs. Furthermore, we show that the proposed simulation strategy can be used to reliably predict individual BCR-ABL kinetics under both, Imatinib and Dasatinib treatment. Most importantly, the application of the mathematical model allows to predict the BCR-ABL kinetics in the population of hematopoietic stem cells in the bone marrow, which is extremely difficult to be monitored in clinical practice, but which is most predictive for potential relapses after treatment cessation.

References

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Speaker 23

Analysis of the subclonal architecture of adult B-ALL reveals highly plastic surface marker expression contradicting a common stem cell phenotype (selected abstract)

Bartosch Wojcik[1,2], Fabian Lang[1], Thomas Oellerich[1], Timm Schroeder[3], J. H. Frederik Falkenburg[4], Monika Brüggemann[5], Oliver G. Ottmann[6], Michael A. Rieger[1,2]

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The existence of a leukemic hierarchy and a stable phenotype of leukemia initiating (stem) cells (LICs) in acute lymphoblastic leukemia (ALL) remain elusive, in contrast to acute myeloid leukemia. In ALL, the heterogeneous subclonal composition, the process of clonal evolution and a variety of different ALL subphenotypes are a hallmark of the disease. But the investigation of the genealogy and molecular composition of functionally defined subclones, that may be unrelated to their surface marker phenotype, require stable maintenance of cellular identity in culture systems. Since cell lines do not represent the above mentioned heterogeneity of the disease, culture of primary ALL patient material is essential to solve questions regarding plasticity of marker expression, cancer stem cell hierarchy or clonal evolution. As culturing primary ALL cells remain very difficult, analyses of hierarchy and identification of a common LIC phenotype are impeded.

We established a patient-derived ALL culture system with a stable karyotype for 6 months to isolate distinct clonal subpopulations from one patient. In these patient-derived ALL cultures, we identified single cell clones, showing both stable and plastic surface marker profiles. The ability to revert to a phenotype that contradicts the differentiation hierarchy is common in all subclones, as more than 90% of all single clones isolated from every subpopulation are capable to reestablished cultures with all other markers. This phenomenon also appears at the single cell level, where ALL cells showed a dynamic change of surface markers within one cell generation in continuous time-lapse-microscopy-based single cell tracking. However, subclones isolated with the same immunophenotype showed a huge difference in their leukemogenic potential upon transplantation in NSG mice: some subclones are highly leukemogenic, others have low or no leukemogenic potential in vivo. Quantitative proteomics of these subclones revealed distinct differentially-regulated pathways which might open new therapeutic windows for treatment of highly aggressive LICs in ALL, especially regarding metabolism and glycolysis. In here we were able to identify a distinct aggressiveness pattern which is linked to glycolysis. Further, new surface markers and molecular mechanisms which are directly correlated to the aggressive phenotype will help to prospectively identify and specifically target LICs to improve therapy and minimize therapy related side effects.

Stem cells in homeostasis and disease II

Speaker 24

The role of RhoGTPases in normal and malignant hematopoiesis

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In previous studies we have demonstrated an essential role of Rac GTPases, one of their upstream activators, the guanine exchange factor (GEF) Vav1, and a major downstream effector, p21 activated kinase (Pak), in hematopoietic stem cell (HSC) homing and engraftment. In HSCs, activation of the Rac node via chemokines, integrins and growth factor receptors regulates cell shape and migration via F-actin assembly and proliferation/survival via kinase pathway activation (reviewed in Troeger and Williams, Experimental Cell Research, 2013). We and others have also demonstrated abnormal activation of Rac in several leukemias and validated Rac as a relevant therapeutic target in BCR-ABL induced chronic myelogenous leukemia (Thomas et al. Cancer Cell, 2007), MLL-rearranged (Mizukawa et al., Blood, 2011) and RAS-mutated leukemias (unpublished). Vav and Pak are multi-domain proteins that subserve multiple Rac-independent cellular functions in addition to their specific role as regulators and effectors of Rac. In current studies we are defining the specific role of Vav and Pak proteins in HSC homing and engraftment. Preliminary studies suggest that Vav1 has a specific role in HSC responses to pro-inflammatory cytokines induced by radiation utilized in transplant conditioning regimens and Vav1 deletion is associated with defective HSC engraftment specifically following conditioning. Pak2 kinase activity and its interaction with the protein β-Pix, another GEF, regulate HSC directional migration, actin-remodeling, homing and engraftment (Reddy et al. Blood, 2016). Unexpectedly we have found that Pak2 regulates homing of HSC to the bone marrow via CDC42 activation. To explore Rac as a potential therapeutic target in leukemias, we have also developed small molecule inhibitors designed based on the tool compound, NSC23776 (Gao et al. PNAS, 2004). We have generated compounds with ~20-fold lower IC:50 in leukemia cell lines and are currently testing these compounds *in vivo* in *RAS*-mutated and *MLL*-rearranged leukemia cell lines and patient-derived xenografts. These data demonstrate the essential role of Rac GTPases in normal HSC biology, its relevance in leukemia and its potential as a therapeutic target in a subset of leukemias that are currently difficult to effectively treat.

Speaker 25

Transcriptional Dysregulation in Precancerous Stem Cells and their Progression to MDS and AML

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Relapse continues to be the most common cause of death in MDS and AML and overall cure rates remain low. Recent evidence has shown that the accumulation of stepwise genetic and epigenetic changes in HSC lead to the formation of pre-cancerous/pre-leukemic stem cells (pre-LSC) that play a pivotal role not only in disease origination but also in relapse. While the existence and essentiality of such pre-cancerous cell states has been demonstrated in mice and humans, still very little is known about the molecular mechanisms driving pre-LSC formation and progression. We have recently performed molecular studies of pre-leukemic cell states in mouse genetic models as well as primary cells from patients, and discovered new transcription factors and regulatory mechanisms in pre-LSC in MDS and AML. Specifically, we discovered critical roles for several non-clustered homeobox transcription factors in pre-LSC, including HLX, and identified PAK1 as a functionally critical and pharmacologically targetable downstream effector. We also found that enhancer haplodeficiency causing minimal reduction of key transcription factors is sufficient to induce pre-LSC formation and subsequent progression to MDS and AML. We developed a novel mouse model that co-models the genomic context found in aging human individuals and patients with MDS/AML and crossed Msh2–/– mice with animals carrying a heterozygous deletion of an enhancer of the PU.1 gene. Strikingly, the resultant minimal (~30%) reduction of PU.1 levels led to the emergence of an aggressive transplantable AML. Overt leukemia was preceded by a preleukemic phase hallmarked by an expanded pre-LSC population with a myeloid bias and reduced quiescence. Longitudinal monitoring revealed progression to MDS, followed by AML. Progression to AML was mediated by additional inhibition of a PU.1 co-transcription factor, Irf8. Comparative cross-species analysis revealed a high degree of molecular resemblance with human MDS and AML. These results demonstrate that minimal reduction of a key lineage-specific transcription factor that commonly occurs in human disease is sufficient to initiate leukemia development, and improve our molecular understanding of the formation and progression of pre-LSC in MDS and AML. Our model provides a new tool for further mechanistic study of pre-LSC and their progression to overt disease, and for the development and testing of pharmacological approaches to therapeutically interfere with these processes.

Speaker 26

Stem cell gene therapy - what cells should we target?

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We have pursued hematopoietic stem cell (HSC) gene therapy for a number of diseases including Fanconi anemia, HIV and cancer. Like most other investigators we have been isolating and targeting CD34+ cells for retroviral mediated gene therapy and for gene editing. We have seen very promising results for patients with glioblastoma. Temozolomide (TMZ) is one of the most potent chemotherapy agents for the treatment of glioblastoma. Unfortunately, more than half of glioblastoma tumors are TMZ resistant due to overexpression of methylguanine methyltransferase (MGMT). Co-administration of O6-benzylguanine (O6BG) can restore TMZ sensitivity, but causes off-target myelosuppression. We conducted a prospective clinical trial to test whether gene therapy to confer O6BG resistance in HSCs would improve chemotherapy tolerance and outcome. We used the MGMTP140K gene therapy strategy also in our preclinical nonhuman primate (NHP) model to select and track hematopoietic repopulating clones. We tracked unique clones in 8 pigtail macaques for up to 10 years following myeloablative transplantation with autologous, lentivirus (LV) gene-modified CD34+ cells. Before in vivo selection with O6BG and BCNU, we observed a cell dose-dependent, successive pattern of hematopoietic reconstitution by integration site analysis (ISA) analysis, with short-term clones declining within 100 days after transplantation. Long-term clones however were observed as early as 1 month after transplant. Thus we were interested whether there might be a cell population that contributes to early and long-term repopulation. We have studied this question in the NHP model and have identified evolutionarily conserved hematopoietic stem and progenitor cell (HSPC) phenotypes and transcriptional profiles between human and NHP HSPCs. Transplantation studies in NHPs confirmed the identification of a unique cell population / phenotype that provides for highly efficient early repopulation with sustained engraftment for now more than 6 months. Importantly this engraftment was possible with only about 500,000 cells / kg. Thus the identification and characterization for this HSC phenotype will allow further studies into the basic HSC biology and the development of novel HSC therapies, the testing of novel expansion strategies and most importantly, the development of gene targeting and editing approaches to correct HSC and lineage-specific diseases.

Speaker 27

Genetic and functional diversity between single stem cell clones of a patient's ALL sample growing in mice (selected abstract)

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Acute lymphoblastic leukemia (ALL) consists of genetically heterogeneous cell subpopulations, but little is known about how genetic differences lead to functional differences between the clones. Aggressive subpopulations determine the prognosis of patients and require eradication by treatment. We aimed at characterizing functional and genetic characteristics of single stem cell clones in order to identify new treatment options for adverse subclones.

Primary tumor cells from a 5-year old girl at first relapse ALL with chromosomal hyperdiploidy involving a trisomic X chromosome were transplanted into severely immune-compromised mice and lentivirally modified to express the fluorochromes red, blue and green at different amounts and combinations (RGB marking, Weber et al., Nat. Protoc. 2012). Eight single stem cell clones were generated by limiting dilution transplantation and their uniqueness verified by ligation-mediated (LM) PCR. Several single cell clones were mixed in a single mouse for in vivo assays and distinguished from each other by flow cytometry due to their unique color staining.

We found that clones differed substantially in growth rate and that faster and slower growing clones co-existed in the sample. Slow growth was associated with marked resistance against chemotherapy treatment of mice. The slowest subclone was most resistant to in vivo treatment with Glucocorticoids. The slowly proliferating, Glucocorticoid-resistant clone had lost the additional X chromosome, which was present in all other clones and the bulk.

Taken together, our studies allow identifying and characterizing single cell clones in order to develop efficient novel treatment approaches to eliminate aggressive stem cell clones in ALL.

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New targets, novel therapies

Speaker 28

New agent testing in paediatric leukaemia

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While the overall cure rate for paediatric acute lymphoblastic leukaemia (ALL) is approximately 90%, patients with high-risk subtypes remain relatively intractable to treatment. The objective of the Pediatric Preclinical Testing Program (PPTP, NCI USA funded 2005-2015) and the Pediatric Preclinical Testing Consortium (PPTC, NCI funded 2015-2020) is to provide reliable preclinical testing data for paediatric drug candidates that can be used to inform new agent prioritisation decisions for children with malignancy. The PPTC consists of 5 Research Programs with collectively almost 400 patient-derived xenografts (PDXs), which encompass the major paediatric cancer histotypes. The paediatric ALL PDXs (~100) were established by direct transplantation of patient biopsies into immune-deficient mice, with PDX sub-panels representing the heterogeneity of paediatric ALL and specific high-risk subtypes. Novel agents to be tested are selected on the basis of: (1) strong preclinical or clinical evidence of efficacy against adult cancer(s); (2) rationale for efficacy against paediatric cancer(s); and, (3) a tangible pathway for clinical development in the paediatric cancer setting. *In vivo* testing is conducted in a blinded fashion against panels (n=8-10) of PDXs, with responses evaluated by event-free survival (T/C) measurements and stringent objective response criteria modelled after the clinical setting.

Over 70 new agents/combinations have been evaluated against the PPTP paediatric ALL PDXs, including small molecules, antibodies, and antibody-drug conjugates. Less than 25% of the new agents tested elicited objective responses in $\geq$ 50% of PDXs, despite several drug classes being represented by multiple agents. For example, 6 agents representing the PI3K/AKT/mTOR pathway were completely ineffective. The antimitotic agent eribulin, the kinesin spindle protein inhibitor ispinesib, the Aurora Kinase A inhibitor alisertib (MLN8237), the hypoxia-activated pre-prodrug PR-104, and the SMAC mimetic birinapant were highly effective. The most consistently active drug class was MDM2 inhibitors, with MK-8242 and RG7112 inducing objective responses in 6/7 and 7/8 ALL PDXs, respectively. In conclusion, *in vivo* efficacy testing of novel drugs against paediatric ALL PDXs has identified several candidates for clinical evaluation. The p53/MDM2 axis appears to be of particular interest as a target for the development of novel treatment strategies in paediatric ALL.

Speaker 29

A novel selective inhibitor of mutant IDH1 promotes differentiation in vivo and prolongs survival in a mouse model of leukemia (selected abstract)

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Mutations in the metabolic enzymes isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) are frequently found in several cancers including acute myeloid leukemia (AML). Mutant IDH produces R-2-hydroxyglutarate (R2HG), which induces histone- and DNA-hypermethylation through inhibition of epigenetic regulators, thus linking metabolism to tumorigenesis.We recently established an in vivo mouse model and investigated the function of mutant IDH1. By computational drug screening, we identified an inhibitor of mutant IDH1 (HMS-101), which had a significantly lower IC50 in mouse bone marrow cells transduced with IDH1mut compared to IDH1wt (1 μM vs. 12 μM, respectively, P<.001). In order to study the specificity of HMS-101 towards the IDH1mut protein, we performed an activity assay with pure protein. HMS-101 significantly decreased the activity of IDH1mut, while activity of IDH1wt was not affected even at higher concentrations (IC50 0.8 mM). We further evaluated the effect of HMS-101 in the IDH1 mouse model. The R/S-2HG ratio in serum was significantly reduced in HMS-101 treated mice after 8 weeks of treatment compared to control treated mice. HMS-101 or PBS treated mice had similar levels of transduced leukemic cells in peripheral blood at 2 and 6 weeks after transplantation. However, from week 6 to week 15 leukemic cells in peripheral blood decreased in HMS-101 treated mice. While the control cohort developed severe leukocytosis, anemia and thrombocytopenia, mice treated with HMS-101 still had normal WBC, RBC and platelet counts at 15 weeks after transplantation. Moreover, the HMS-101 treated mice had significantly more differentiated Gr1+CD11b+ cells in peripheral blood than control mice at 6 weeks and 15 weeks after transplantation and at death. Importantly, HMS-101 treated mice survived significantly longer with a median latency of 87 days (range 80-118), whereas PBS-treated mice died with a median latency of 66 days (range 64-69) after transplantation (P<.001). This data demonstrates that HMS-101 specifically inhibits R2HG-production of mutant IDH1 in vivo, inhibits proliferation, induces differentiation in leukemic cells, and thus prolongs survival of IDH1mutant leukemic mice. Therefore, HMS-101 - a novel inhibitor of mutant IDH1 - shows promising activity in vivo and warrants further development towards clinical use in IDH1 mutated patients.

Speaker 30

Axl-targeting - a novel treatment approach for AML?

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Acute myeloid leukemia (AML) represents a clonal disease of hematopoietic progenitors characterized by acquired heterogenous genetic changes that alter normal mechanisms of proliferation, self-renewal and differentiation. Although 40-45% of patients younger than 65 years can be cured with current therapies, only 10% of older patients reach long-term survival. As only very few novel AML drugs were approved in the last two decades there is an urgent need to identify novel targets and therapeutic strategies to treat underserved AML patients. We identified Axl, a member of the Tyro3, Axl, Mer receptor (TAMR) tyrosine kinase family, as independent prognostic marker and therapeutic target in AML. AML cells induce expression and secretion of the Axl ligand growth arrest-specific gene 6 (Gas6) by bone marrow-derived stromal cells (BMDSCs). Gas6 in turn mediates proliferation, survival and chemoresistance of Axl-expressing AML cells. This Gas6-Axl paracrine axis between AML cells and BMDSCs establishes a chemoprotective tumor cell niche, which can be abbrogated by Axl-targeting approaches. Therefore, Axl-inhibition alone or in combination with chemotherapy might represent a novel therapeutic avenue for AML. We are currently this approach by applying the small molecule Axl inhibitor BGB324 to AML patients in the Phase 1a/b trial BGBC003.

Speaker 31

Integrative genomics identifies the molecular basis of resistance to azacitidine therapy in myelodysplastic syndromes

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Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukaemia (CMML) are haematological disorders that develop in haematopoietic stem or progenitor cells (HSPCs) and are characterised by ineffective haematopoiesis. 5'-Azacytidine (AZA), a DNA demethylating agent, is the primary drug for high-risk MDS and CMML. However, only half of treated patients will ever respond to AZA and the molecular basis for poor response is currently unknown. Additionally, AZA response is rarely sustained and a substantial fraction of responders eventually relapse. We aimed to: 1.) understand the molecular basis for poor response to AZA, and 2.) characterise the in vivo effect of AZA therapy on dysplastic cells in responders, as a first step towards understanding eventual relapse. We enrolled high-risk MDS and CMML patients on a compassionate access program. Bone marrow was collected at different points and we isolated high-purity CD34+ HSPCs. We performed RNA-seq to query the transcriptomes and deduced the clonal evolution in the bone marrow in response to AZA therapy by whole exome-sequencing and single-cell genotyping. We hypothesised that primary AZA resistance would be driven by pre-existing molecular differences between responders and non-responders. Analysis of the pre-treatment RNA-seq data revealed differential gene expression between responders and non-responders. Pathway analyses of these genes indicated that cell cycle was relatively up-regulated in responders compared to non-responders. We validated these gene expression differences in independent patient cohorts. We then adapted a flow cytometry based assay, amenable to a clinical diagnostic setting to directly detect the increased quiescence of haematopoietic progenitors in unsorted bone marrows of non-responders. Finally, to reverse the quiescence, we developed a stromal co-culture drug testing platform and discovered that inhibiting integrin-linked signalling combinatorially with AZA improved the functionality of non-responder dysplastic cells. To trace the fate of dysplastic cells, we performed whole exome sequencing of all patients and deduced the clonal architecture in each individual. We have discovered that although AZA alters the sub-clonal contribution to different lineages, founder clones are not eliminated and continue to drive hematopoiesis even in complete responders. Lastly, we have also discovered that AZA response is associated with induction of inflammation-associated pathways *in vivo*.

Speaker 32

t(8;21) AML is susceptible to G1 CDK inhibition

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The translocation t(8;21) is the most frequent chromosomal rearrangement in AML and generates the RUNX1/ETO fusion protein. Integration of ChIP-seq and RNA-seq revealed a transcriptional programme that interferes with myeloid differentiation and that depends on a dynamic equilibrium between RUNX1/ETO and RUNX1. Functional interrogation of RUNX1/ETO target genes by in vitro and in vivo RNAi screens identified CCND2 as crucial mediator of RUNX1/ETO-driven leukaemic propagation. Knockdown of CCND2 phenocopied the antiproliferative effect of RUNX1/ETO inhibition suggesting a dependence of RUNX1/ETO-positive leukaemia on CDK activity in the G1 phase of the cell cycle. Pharmacologic inhibition of G1 CDK complexes impaired leukaemic proliferation and colony formation in tissue culture and increased median survival of mice transplanted with RUNX1/ETO-expressing leukaemic cells. These combined findings suggest inhibition of G1 cell cycle progression as a new non-genotoxic option for the treatment of t(8;21) AML.

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Immunotherapy I

Speaker 33

First-in-man use of gene-edited universal (U)CAR19 T cells to induce remission of refractory relapsed acute lymphoblastic leukaemia

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Hematological malignancies are attractive targets for engineered T cell therapies, but autologous approaches have limited availability or may not be feasible in specific circumstances. Applications using "off-the-shelf"' therapies comprising non-HLA matched T cells must address barriers of host mediated rejection and GvHD. We have used transcription activator-like effector nucleases (TALEN)s to simultaneously disrupt expression of endogenous alpha/beta T cell receptor (TCR) expression and CD52 (target antigen for Alemtuzumab) in T cells transduced to express a chimeric antigen receptor against CD19 (CAR19). These cells are designed to survive in vivo lymphodepletion of $CD52^+$ immune cells by Alemtuzumab, and to target $CD19^+$ B cell leukaemia without TCR mediated alloreactivity. We manufactured a bank of universal CAR19 T cells (UCART19) from volunteer donor mononuclear cells under GMP conditions in collaboration with Cellectis SA. Cells were first activated and transduced with a self-inactivating lentiviral vector encoding a 4g7 CAR19 (CD19scFv-41BB-CD3ζeta) linked to RQR8, an abbreviated selection / suicide gene encoding both CD34 and CD20 epitopes. Next TALEN mRNA was delivered by electroporation for multiplex targeting of both the TCR alpha constant chain and CD52 gene locus. Residual TCR expressing cells were depleted using CliniMacs αβ TCR depletion, yielding a T cell product with <1% TCR expression, 85% CAR19 expression, and 64% $CD52^-$ T cells. Here we describe first-in-man use of UCART19 cells in two infants with refractory infant ALL ($CD19^+$) who experienced bone marrow relapse 3/9 months after myeloablative SCT. Their disease progressed despite treatment with Blinatumomab. Following lymphodepletion using Fludarabine Cyclophosphamide and Alemtuzumab, each received a single dose (4×10^6/kg) of UCART19 T cells without significant toxicity at a time when bone marrow exhibited persisting disease. FACS analysis demonstrated expansion of well-demarcated UCART19 cells in the circulation from day 14, which persisted till conditioning for 2nd SCT (same donor) 12 weeks later, designed to remove UCART cells and restore immunity/counts. The UCART expansion remained $CAR^+CD3^-CD52^-$ in one patient with additional expansion of CAR^+CD3^+ cells in the other patient, associated with skin GvHD and neutropenia. Molecular remission was achieved from day 28 in both patients and has continued at follow-up of 4 &10 months. Both patients are clinically well at home.

Speaker 34

Adoptive T cell therapie

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Adoptively transferred T cells have been shown to reject large established tumors. In such models, T cell recognize the tumor antigen als foreign. The task is to generate human T cell receptors (TCR) that recognize human tumor-associated (self) antigens as foreign and use these TCRs for gene therapy. We use mice with a humanized TCR repertoire is isolate therapeutic TCRs.

Speaker 35

Receptor-transgenic T cells for the Immunotherapy of Multiple Myeloma (short talk)

Sara Yousef[1,2], *Tim Luetkens*[1], *Nikolaus Kröger*[2], *Djordje Atanackovic*[1], *Boris Fehse*[2]

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In recent years the therapeutic use of genetically modified T cells has proven highly efficacious in clinical trials of different hematological malignancies. However, this approach is still hampered by (i) the limited availability of functional T cell receptors (TCRs) targeting tumor-specific autoantigens and (ii) the lack of specific surface antigens for chimeric antigen receptors (CARs).

To address the former we introduce here a high-throughput approach for the generation of tumor-reactive TCRs in order to reprogram T cells for the immunotherapy of multiple myeloma (MM). To this end we use peptide-loaded or genetically modified autologous dendritic cells to stimulate T cells with cancer testis autoantigens. We were able to induce specific T cells against SLLP1, MAGE-C2, and SSX-2, which are highly expressed in the majority of MM patients. After identification of tumor-specific T cell cultures and immunogenic epitopes by interferon-γ elispot, TCR genes can be retrieved from single cells using a novel cloning technique which enables us to obtain full TCR α/β pairs. The receptor genes are then cloned into a lentiviral self-inactivating vector lentiviral vector. Prior to transduction of primary T cells with the new receptor genes the endogenous TCR is knocked out using TALENs to avoid the creation of chimeric TCRs which may result in autoimmune reactivity and to enhance surface expression of the transgenic receptor. Receptor-negative T cells are then used for reprogramming with our lentiviral vectors.

To address the lack of specific target antigens recognized by CAR T cells we have also developed a rapid approch for the development of CAR binding domains against novel antigens and cell-based screening assays for identification of CARs with superior function. First we are using a fully human antibody phage display library for selection of binders to a given antigen. These binders are then immediately reformatted into CAR screening constructs and expressed in human cells to assess surface expression and antigen binding. Activating constructs are then identified using a T cell line expressing a fluorescent reporter upon antigen-specific T cell activation. Using this platform we have generated several cytotoxic CAR constructs targeting different antigens expressed on MM cells.

Our approaches allow the rapid parallel development of TCRs and CARs with different specificities and, in the case of TCRs, a wide range of HLA restrictions with the goal to develop clinical products for the treatment of MM and other cancers.

Speaker 36

Immunotherapy of Multiple Myeloma by targeting CD38: antibodies, CART cells and beyond.

Tuna Mutis

VUmc Medical Center Dept. of Hematology, Amsterdam, Netherlands

Multiple myeloma (MM), the malignant disorder of antibody producing clonal plasma cells is the second most common hematologic neoplasia worldwide. Despite four decades of drug innovation and combination therapies, drug resistance remains the main cause of therapy failure. Targeted immunotherapy with the human anti-CD38 antibody Daratumumab is recently emerged as a promising strategy for the treatment of multidrug resistant patients with multiple myeloma (MM). In recently completed clinical trials Daratumumab achieved highly promising results with little or no toxicity. Encouraged by this, we explored the feasibility of development of a CD38CART cell therapy. Using high-affinity human CD38 antibodies, we generated second generation CD38CARs, and have shown that T cells transduced with CD38CARs, optimally proliferate and effectively lyse MM cells and AML cells in a CD38-dependent fashion. Since these CD38CART cells appeared to lyse also the CD38+ fractions of normal hematopoietic cells, we generated ±100 new antibodies with 100-1000x lower affinity to CD38 and hereby succeeded in generating affinity-optimized CD38CART cells that can clearly discriminate MM cells from normal hematopoietic cells. CD38CART cells with optimized affinity may therefore be effective therapeutics for MM patients. In addition, we addressed the marked heterogeneity in Daratumumab response of patients in order to improve its efficacy. We demonstrated that the CD38 expression level of MM cells is an important parameter for Daratumumab sensitivity, and the Daratumumab response can be significantly improved by up regulation of CD38 with all-trans retinoic acid (ATRA). We also addressed the impact of MM microenvironment on the efficacy of T cell- and antibody-based immunotherapeutic approaches. We discovered that the stromal cells of bone marrow microenvironment induce a cell-cell adhesion mediated immune resistance mechanism, hereby protecting MM cells from cytotoxic T cell and from Daratumumab-dependent NK cell mediated ADDC. The possible mechanisms and the potential therapeutic modulation of this generally ignored immune escape mechanism will be highlighted and discussed.

Speaker 37

Enhanced Transfer of Specific Anti-Cancer Immunity by Infusions of Bispecific Antibody Armed T cells Given Prior to Stem Cell Transplant

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The non-MHC restricted cytotoxicity mediated by anti-CD3 activated T cells (ATC) can be redirected by arming with bispecific antibody to target tumor associated antigens (TAAs). Preclinical studies show that anti-CD3 x anti-CD20 (CD20Bi) or anti-CD3 x anti-HER2 (HER2Bi) **B**ispecific antibody **A**rmed activated **T** cells (BATs) not only exhibit cytotoxicity at the appropriate targets but also proliferate, and secrete cytokines/chemokines upon tumor engagement. In a phase I study, we targeted CD20+ clonogenic "stem cells" by giving two infusions of CD20 BATs to patients with multiple myeloma to determine whether pretargeting of CD20+ cells could induce anti-myeloma immunity that could be transferred in the stem cell inoculum. Immune responses to RPMI 8226 (non-CD20+ target), Daudi (CD20+ target), and K562 (NK target) could be detected after SCT and these responses could be boosted by a single armed ATC infusion. In another phase I study in women with stage IV breast cancer, 8 infusions of HER2 BATs in combination with low dose IL-2 and GM-CSF induced endogenous cytotoxic immune responses. Those immune responses could be transferred by infusions of ATC produced from a second leukapheresis to obtain immune T cells prior to SCT. Fresh peripheral blood mononuclear cells (PBMC) exhibited cytotoxic responses to breast cancer as early as 2 weeks after SCT that persisted more than 1 year. These data suggest that anti-cancer immunity could be established prior to SCT and transferred into a myeloablated host, and boosted after SCT. These data have implications for vaccine strategies in both liquid and solid tumors.

Speaker 38

Prognostically favoral B-cell immune responses after allogeneic stem cell transplant define a unique targetable epitope fingerprint of multiple myeloma (selected abstract)

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Multiple myeloma (MM) is a common hematological malignancy suitable for immunotherapeutic interventions and immune-based treatments are currently in development. Many of these rely on the identification of highly disease-specific, strongly and stably expressed antigens.

Here we profiled the myeloma B-cell immunome both to explore its predictive role in the context of autologous and allogeneic hematopoietic stem cell transplantation (HSCT) and to identify novel immunotherapeutic targets. We established a highly myeloma-specific epitope fingerprint by dissecting the B-cell responses of 18 patients in clinical remission with oligoclonal antibodies to the epitope level using random peptide phage display, reverse immunization and next-generation sequencing assisted antibody phage display.We found that myeloma patients after allogeneic HSCT more efficiently allowed production of myeloma-specific antibodies compared to autologous HSCT and show largely overlapping epitope recognition profiles that are highly disease-specific and correlate with superior clinical response to treatment.Next, we performed myeloma cell surface screenings of phage-displayed patient transplant immune repertoires. While some of the screenings yielded clear-cut surface binders, the majority of screenings did not, suggesting that many of the targeted antigens may in fact not be accessible to the B-cell immune system in untreated myeloma cells. This fitted well with the identification of heat-shock proteins as a class of antigens that showed overall the broadest reactivity with myeloma patient sera after allogeneic HSCT and that may be significantly translocated to the cell surface upon treatment as a result of immunogenic cell death.

Our data reveal a disease-specific epitope signature of multiple myeloma that is predictive for response to treatment. Mining of transplant immunomes for strong myeloma surface binders may open up avenues for myeloma immunotherapy.

M = Monday
S = Sunday
Roman numerals = Topic

Leukemogenesis

Poster S-III-01

Human acute myeloid leukemia cell lines for a study of acquired resistance to Ara-C

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The nucleoside analog cytarabine (ara-C) is commonly used for the treatment of acute myeloid leukemia (AML). However, the treatment is frequently associated with the development of resistance. To study mechanisms involved in acquired Ara-C resistance we adapted a panel of ten human AML cell lines to growth in medium in the presence of Ara-C resulting in highly Ara-C resistant sublines which were compared with parental (Ara-C sensitive) cell lines. Expression of proteins involved in Ara-C uptake and conversion of Ara-C in its active metabolite Ara-C triphosphate including human equilibrative nucleoside transporter 1, deoxycytidine kinase, cytidine deaminase, deoxycytidilate deaminase, 5'-nucleotidase, cytosine triphosphate synthase and ribonucleotide reductase were measured by western blot or flow cytometry. Intracellular Ara-C triphosphate levels were measured by liquid chromatography tandem mass spectrometry. Ara-C resistant sublines exerted multiple changes in expression of proteins involved in metabolism of Ara-C which correlated with decreased Ara-CTP levels in resistant cells. The resistant AML cells showed cross-resistance to several other nucleoside analogs including fludarabine, clofarabine, cladribine, nelarabine, gemcitabine and decitabine but not to nucleoside analogs azacytidine and ribavirin and to topoisomerase II inhibitors daunorubicin and etoposide. The resistance could be at least in part reverted by genetic and pharmacological modulation of expression and/or activity of proteins involved in Ara-C metabolism. In conclusion the results demonstrated high complexity of mechanisms involved in Ara-C resistance of AML cells. Moreover, our panel of well characterized Ara-C resistant cell lines provides a model for studies of resistance mechanisms and testing strategies for treatment of resistant AML cells.

Poster S-III-02

Macrophages/megakaryocytes crosstalk in regulation of bone marrow microenvironment in myelofibrosis development

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Myelofibrosis with myeloid methaplasia together with polycythemia vera and essential thrombocytemia is classified as Ph(-) chronic myeloproliferative disorder (CMPD). The key role in pathogenesis of CMPD belongs to a somatic mutation (V617F) of the JAK2 gene. In CMPD megakaryocytes and macrophages are the major regulators of fibrosis development. In this study we investigated the influence of platelet factors on the cellular characteristics of the macrophages with oncogenic mutation JAK2 V617F. THP-1 cell line was modified by lentiviral vector. Two cell lines were established: mutant oncogene JAK2 V617F-expressing cells and wild type (WT) JAK2-overexpressing cells. Cell lines were differentiated into macrophages by culturing with 80 nM PMA in RPMI supplemented with 10% fetal bovine serum (FBS) or 5%, 10% and 15% platelet lysate (PL). Expression levels of transforming growth factor-β1 (TGF-β1), basic fibroblast growth factor (bFGF), pentraxin-3, galectin-3, matrix metalloproteinases (MMP) 2, 9, 12, 13 and tissue inhibitors of MMP (TIMP) were assessed by specific RT-qPCR. MMP-2 and -9 activity was analyzed by gelatin zymography. In our model elevated levels of expression of TIMPs and MMPs were observed. Gelatin zymography also revealed increased amount of pro-enzymes MMP-2 and -9 and their active forms in media conditioned by JAK2 WT-overexpressing and JAK2 V617F-expressing macrophages compared to control cells. Mainly we observed upregulation of pro-forms rather than active forms of MMP-2 and -9. It can be explained by TIMP-mediated inhibition of MMPs activation as a result of elevated expression levels of TIMPs. Expression levels of TGFβ1 and bFGF were significantly increased in JAK2 V617F expressing macrophages compared to non-transgenic cells only in the presence of 15% PL. We conclude that megakaryocytes in CMPD could stimulate macrophages to produce pro-fibrotic factors through increased platelets production. Interestingly, the increased level of TIMPs in the presence of FBS or low concentration of PL was observed only in JAK2-overexpressing macrophages, not in mutant cells. From our data we could suggest that platelet factors are essential for JAK2 V617F-expressing macrophages to promote bone marrow fibrosis.

Poster S-III-03

SHIP1 suppresses proliferation of leukemic cells *in vivo*

Maike Täger[1], *Jasmin Wellbrock*[2], *Emily Latuske*[2], *Patrick Ehm*[3], *Marcus Nalaskowski*[1], *Matthias Schaks*[1], *Walter Fiedler*[2], *Carol Stocking*[4], *Manfred Jücker*[1]

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The PI3K/AKT signaling pathway plays a critical role in regulating several cellular processes including cell growth, survival and differentiation. Constitutive activation of this pathway is found in approximately 50-70% of AML patients. The SH2-containing inositol 5-phosphatase 1 (SHIP1) is a negative regulator of PI3K/AKT signaling in hematopoietic cells and is assumed to play a functional role as tumor suppressor in the pathogenesis of AML. Striking evidence came from studies on knockout mice where the ablation of SHIP1 leads to a myeloproliferative syndrome and concomitant deletion of SHIP1 and the tumor suppressor PTEN leads to the development of lethal B cell lymphomas. The impact of SHIP1 on proliferation in leukemic cells has been studied so far in different *in vitro* models. In this study we investigated the role of SHIP1 as a tumor suppressor in an *in vivo* xenograft transplantation model. The AML cell line UKE-1 was transduced with the lentiviral vector LeGO expressing either SHIP1/EGFP or EGFP alone. For leukemia induction transduced UKE-1 cells were transplanted intravenously into NSG mice (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ). Mice transplanted with cells overexpressing SHIP1 showed a significant extended lifespan in comparison to the vector control cohort in three independent experiments. Thus, the reproducible prolongation of the lifespan indicates that SHIP1 can decelerate the proliferation of human myeloid leukemia cells in our mouse model *in vivo*.Further evidence for SHIP1 as a tumor suppressor in leukemia is the occurrence of missense mutations detected in 3% of AML patients. The patient derived loss-of-function mutation SHIP1-Y643H is located in the 5-phosphatase domain of SHIP1 leading to a nearly complete loss of the specific enzymatic activity. Using our mouse model, we observed that SHIP1-Y643H mice showed a significant reversion of the SHIP1 induced prolongation of the survival time.Taken together, these results demonstrate that SHIP1 acts as a tumor suppressor in our *in vivo* mouse model. In contrast to this the AML patient derived SHIP1-Y643H mutation was not able to reduce proliferation of the leukemic cells. These findings are in line with a long-standing hypothesis that the disruption of genes acting as tumor suppressor is a critical step in leukemogenesis. In addition our *in vivo* model provides an excellent tool to study additional leukemia-associated SHIP1 missense mutations to gain insight in the maintenance and progress of AML.

Poster S-III-04

Requirement and Function of MOZ in normal Haematopoiesis and Leukaemia

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The gene encoding the Histone acetyl transferase (HAT) MOZ (Monocytic leukaemia Zinc finger) is altered in a subtype of acute myeloblastic leukaemia with a dismal prognosis. Previous studies have indicated that this protein, or its enzymatic activity, is indispensable for the development of embryos, in particular the blood system, their absence leading to embryonic lethality. This early defect has largely impeded further examination of the relevance of this protein in adult mice. To circumvent this problem, we have developed a mouse strain where *MOZ* can be deleted in the haematopoietic system of adult mice. With this powerful tool, we aim to determine the function and requirement for MOZ, or its enzymatic activity, in the normal adult blood system. Our results indicate that the mice reach adulthood and are healthy, although the deletion of *MOZ* is highly efficient in the different haematopoietic organs. Analysis of the different haematopoietic compartments in these mice revealed a major defect in B lymphopoiesis. In the immature compartments of the bone marrow, we observed a profound loss of phenotypic HSCs, associated with a decrease in CMP (Common Myeloid Progenitor), MEP (Megakaryocyte-Erythroid Progenitor) and an increase in GMP (Granulocyte-Monocyte Progenitor) populations. Preliminary data also suggests a role of MOZ loss in the development of T-cell leukaemia. Moreover, we want to gain new insights in the understanding of the leukaemogenesis mechanism driven by MOZ fusion proteins. These chimeric proteins retain the HAT domain of MOZ and the identified fusion partners are also associated directly or indirectly with HAT activities. It has been suggested that these "super HAT" proteins aberrantly acetylate targets of MOZ, leading to the deregulation of expression of key genes associated with the development of leukaemia. We decided to investigate this theory in a genome wide manner, by performing ChIP-seq of MOZ-TIF2 and Histone acetylation in a model of MOZ-TIF2 leukaemia. We identified the direct targets of the MOZ-TIF2 fusion protein. Some of these genes are known leukemic oncogenes such as *Cebpa*, *Lmo2*, *Myc* and *c-Myb*. They display an increased expression in the MOZ-TIF2 leukemic cells compared to the ckit^{+} cells, however, we couldn't detect an increase in Histone acetylation (H3K9/14Ac) at these targets or globally. We are currently investigating the involvement of other histone modifications in the MOZ-TIF2 leukemogenesis process.

Poster S-III-05

Activation of the Hematopoietic-Specific Lyn-Substrate 1 (HCLS1) Protein by NAMPT/SIRT1-Triggered Deacetylation in Chronic Myeloid Leukemia (CML)

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G-CSFR-triggered phosphorylation and activation of the HCLS1 protein is important for the granulocytic differentiation of hematopoietic stem cells (HSCs) (Skokowa J. et al., Nat Med 2012). G-CSF also activates protein deacetylation via Nicotinamide phosphoribosyltransferase (NAMPT)/NAD+/sirtuin 1 (SIRT1) pathway (Skokowa J. et al., Nat Med 2009). SIRT1 is NAD+-dependent protein deacetylase playing an important role in the regulation of gene transcription. Interestingly, having strong pro-differentiation effects on myeloid progenitors, both HCLS1 and NAMPT are markedly hyper-activated in myeloid leukemias, inducing proliferation and survival of undifferentiated blasts. We investigated whether HCLS1 could be activated by NAMPT/NAD+/SIRT1 pathway via deacetylation in HSCs. Indeed, we found that HCLS1 is deacetylated in HSCs by NAMPT/SIRT1 and that G-CSF treatment of these cells induced deacetylation and phosphorylation of HCLS1 via NAMPT/SIRT1. Moreover, we found that deacetylation of HCLS1 is important for myeloid differentiation and proliferation of HSCs. HCLS1 protein was hyperactivated by deacetylation in blasts of chronic myeloid leukemia (CML) patients, as compared to HSCs of healthy individuals. This was in line with elevated levels of NAMPT. We further investigated, whether inhibition of NAMPT-triggered deacetylation of HCLS1 has any effect on the survivial and differentiation of CML blasts in vitro. We found that treatment of CML blasts with the specific NAMPT inhibitor FK866 resulted in increased acetylation of HCLS1. Treatment of CML blasts with FK866 resulted in marked inhibition of proliferation and elevated cell death as well as in strong suppression of their CFC. This inhibitory effect was further amplified by combination of FK866 with Imatinib. Taken together, we demonstrated that NAMPT and HCLS1 have dose-dependent effects on hematopoietic cells and that NAMPT activates HCLS1 functions by SIRT1-triggered deacetylation. Inhibition of NAMPT in CML blasts markedly reduced their survival, proliferation and differentiation. In conclusion, we suggest a possible therapeutic application of NAMPT inhibition in the treatment of CML patients via deactivation of HCLS1 function by acetylation. This therapy could be applied in combination with Imatinib, or in Imatinib-resistant CML patients.

Poster S-III-06

Co-acquisition of RUNX1 and CSF3R Mutations Transforms Hematopoietic Progenitor Cells in More Primitive Cells with Stem Cell Features and High Clonogenic Capacity

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Severe congenital neutropenia (CN) is a bone marrow failure syndrome with a high risk of evolving into leukemia or myelodysplastic syndrome (MDS). Recently we demonstrated a very high frequency of cooperating RUNX1 and CSF3R mutations in CN patients who developed leukemia or MDS. In the majority of CN patients, CSF3R mutations were acquired prior to RUNX1 mutations. CSF3R mutations alone are unable to induce leukemia in CN patients or in mice expressing a transgenic d715 G-CSFR. To characterize the expression signature of hematopoietic cells of CN/AML patients carrying CSF3R mutations prior to and after acquisition of RUNX1 mutations, we analyzed expression profiles of CD34+and CD33+ hematopoietic cells of CN patient who developed AML. This patient acquired CSF3R mutation (p.Q718X) five years and RUNX1 mutation (p.R139G) 16 months prior to leukemia. We compared expression profiles of CD34+ and CD33+ cells harboring CSF3R mutation only, or both CSF3R and RUNX1 mutations. Co-acquisition of RUNX1 and CSF3R mutations led to marked reduction of the expression of hematopoietic growth factors such as IL6 and NAMPT, as well as components of neutrophil granules. At the same time expression levels of pro-proliferative downstream effectors of G-CSF such as SMAD1 and CCNA1 were dramatically elevated. Moreover, genes overexpressed in early hematopoietic stem/progenitor cells (HSPCs), such as DNTT, BAALC, CD109, HPGDS, MLLT11 and FLT3, were strongly upregulated in CN/AML blasts harboring both RUNX1 and CSF3R mutations, as compared to more mature progenitors. Next, we transduced lineage negative (lin-) bone marrow HSPCs of WT or transgenic d715 G-CSFR mice with lentiviral expression constructs containing either WT or mutated forms of RUNX1 cDNA. We used RUNX1 p.R139G and p.R174X mutants. We found that transduction of HSPCs from transgenic d715 G-CSFR mice with RUNX1 mutant constructs resulted in a markedly elevated clonogenic capacity in replating experiments, as compared to cells transduced with WT RUNX1 or control vector. Moreover, granulocytic differentiation of lin- cells from d715 G-CSFR mice transduced with RUNX1 p.R139G mutant was severely diminished, in comparison to cells transduced with WT RUNX1. Taken together, co-acquisition of RUNX1 and CSF3R mutations shifted the hematopoietic differentiation program towards more primitive hematopoietic progenitors with elevated proliferative capacity and reduced myeloid differentiation, which ultimately lead to AML.

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Poster S-III-07

Acquired CSF3R Mutations in Cyclic Neutropenia.

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We recently reported a 17-year-old female with cyclic neutropenia (CyN) harboring ELANE mutations presenting with cycling hematopoiesis involving neutrophils, platelets and reticulocytes who developed AML (FAB M2) (presented at ASH 2015, Blood 126 (23) abstract 885). At the time of AML diagnosis, all CSF3R-expressing BM leukemic blasts were positive for the p.Gln741X mutation. We tested the patient's BM MNCs, obtained at the time of leukemia development, for RUNX1 mutations and found that the RUNX1 mutation p.Asp171Asn was present at an allele frequency of 10%. So far, CSF3R mutations have been reported in congenital neutropenia patients (CN) only but never in patients with CyN.Next, we performed deep sequencing of CSF3R in 22 additional CyN patients. Out of these 19 patients we identified two additional patients harboring acquired CSF3R mutations. One CyN patient aged 15.4 years and her sister inherited the ELANE mutation from their father. Time-course analysis of the acquisition of CSF3R mutations in this patient showed that 2.6% of the CSF3R alleles in BM MNCs were obtained at the age of 13 years possessing the p.Gln749X mutation. After additional 1.5 and 2.4 years, respectively, the mutant allele frequency increased to 9% and 8%. A third CyN patient, a 7 year old girl, harboring spontaneous ELANE mutations and revealing typical cycling of neutrophils, platelets, and monocytes was treated with G-CSF at a dose of 4.5 μg/kg/day and recently acquired CSF3R mutation (p.Gln739X) in approx. 30% of the CSF3R allele. The latter two CyN patients have not yet developed AML or myelodysplastic syndrome (MDS).Taken together, these findings suggest that CyN patients with typical CyN-associated ELANE mutations may also acquire CSF3R mutations and are therefore at risk for leukemic development. Long-term data of the Severe Chronic Neutropenia International Registry (SCNIR) have shown that the risk of acquisition of CSF3R mutations and of myeloid transformation is low but not absent in patients with CyN compared to patients with CN. This new knowledge is important for prognostic counseling and long-term management of ELANE-CyN patients.

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Poster S-III-08

LSD1 inhibition induces differentiation and reduces the frequency of leukemia-initiating cells in a murine model of AML

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The histone demethylating enzyme LSD1 represents a promising epigenetic target in the treatment of Acute Myeloid Leukemia (AML) and inhibition of LSD1 has been shown to facilitate a response of AML cells to all-*trans* retinoic acid (ATRA). In this project, we have now modeled the effect of pharmacological inhibitors of LSD1 in a murine leukemia model. Hematopoietic progenitor cells isolated from murine bone marrow were transformed by retroviral overexpression of a combination of Hoxa9 and Meis1 turning them into cytokine-dependent progenitor cultures that elicit AML upon transplantation into mice. We have now used these cells to study the effect of different pharmacological inhibitors of LSD1. In detail, we treated the Hoxa9/Meis1-transformed cells with three irreversible (tranylcypromine, AW69, AW84) and three different reversible LSD1 inhibitors (MS120 (=Namoline), MS142, SP2509) alone or in combination with ATRA. When analyzing the morphology of Hoxa9/Meis1 cells after treatment with AW69, AW84 or high-doses of tranylcypromine we observed signs of granulocytic differentiation. Flow cytometric analysis showed an increase in Mac1 expression and a reduction in the expression of c-Kit from 53% to 17% after treatment with AW69. Colony formation of Hoxa9/Meis1-transformed myeloid progenitors treated with AW69 for 96 hours was reduced by 70% compared to control cells. Treatment with AW69 and AW84 also enhanced the response of leukemia cells to ATRA by enhancing the anti-proliferative effect and by reducing colony formation by 99% in Hoxa9/Meis1 cells in the combination. The reversible LSD1 inhibitors exhibited only minor effects on differentiation even when used at effective concentrations. In order to assess the effect of LSD1 inhibition on leukemia-initiating cells (LICs) we performed a limiting dilution transplantion assay. In this assay AW69 significantly reduced the frequency of LIC in our Hoxa9/Meis1 model from 1/40 (95 % CI 1/10.7 to 1/175) to 1/3200 (95 % CI 1/835.5 to 1/12101) ($p < 0.001$). We conclude that pharmacological inhibition of LSD1 by tranylcypromine and its derivatives induces differentiation and drastically reduces LIC frequency in a model of Hoxa9/Meis1-driven AML. This model will allow us to select LSD1 inhibitors based on their functional effects and will facilitate molecular studies investigating the role of LSD1 as a target in the treatment of AML.

Poster S-III-09

Hoxa9 and Meis1 cooperatively induce addiction to Syk signaling by suppression of miR-146a in acute myeloid leukemia

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Acute myeloid leukemia (AML) is driven by cell populations with stem cell-like characteristics, so called leukemia stem cells (LSC). The transcription factor Meis1 is one of the critical regulators of LSC and is capable to rapidly induce AML in murine models in the context of Hox gene overexpression. Despite sophisticated studies identifying Hox- and Meis1-regulated genes the knowledge about their impact on intracellular signaling pathways and its functional consequences is still limited. Since Hox and Meis1 gene overexpression is often found in high risk AML and as both factors are currently considered as undruggable, we aimed to elucidate their role in regulating intracellular signaling and to investigate, if cells transformed by Hoxa9 and Meis1 are addicted to certain signaling processes. To characterize the effect of Meis1 in the context of Hox gene overexpression on protein expression and intracellular signaling, we have performed a comprehensive (phospho)proteomic analysis and correlated it with transcriptome sequencing data. Our analysis revealed that Meis1 upregulates expression of spleen tyrosine kinase (Syk) without affecting its mRNA expression level. This was confirmed in patient-derived AML cells. By global analysis of microRNA expression and subsequent functional analyses we could identify the downregulation of miR-146a, which turned out to be PU.1-dependent, as a mediator for this post-transcriptional upregulation of Syk in Hoxa9/Meis1 overexpressing cells. To further investigate, if an activation of Syk signaling can mimic Meis1 in inducing leukemia in our murine AML transplantation model, we overexpressed Syk in Hoxa9-transformed myeloid progenitors and found that this resulted in an acceleration in leukemia development comparable to the acceleration observed upon combined Hoxa9/Meis1 overexpression. We also found that Syk overexpression resulted in an increased expression of Meis1 and an induction of Meis1-dependent gene expression signatures. Notably Hoxa9/Meis1-transformed cells also exhibited a remarkable sensitivity to Syk inhibition and Syk knockdown *in vitro* and *in vivo*, while Hoxa9-transformed cells did not. In summary, we identified a previously unknown signaling loop between Meis1 overexpression and Syk signaling involving miR-146a as a regulator of Syk expression. Hence, we believe that Syk inhibition by small molecules might be a potential therapeutic option for AML particularly in the context of Hox/Meis1-overexpression.

Poster S-III-10

An autonomous *CEBPA* enhancer specific for myeloid-lineage priming and neutrophilic differentiation

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Neutrophilic differentiation in the bone marrow (BM) is coordinated by C/EBPa, a leucine zipper transcription factor (TF) that is also critical for terminal cell differentiation in different non-hematopoietic tissues. We hypothesize that *CEBPA* levels in the bone marrow are modulated by myeloid-specific enhancer(s). Thus, we aim to identify potential *CEBPA* enhancer(s) and study their role in hematopoiesis. ChIP-seq$^{H3K27ac(active)}$ revealed 14 potential enhancers within the *CEBPA* locus when investigated in myeloid^{CEBPA+} and non-hematopoietic^{CEBPA+} human tissues (n=15). Two of these enhancers located at +35kb and +42kb were exclusively active in mature neutrophils, whilst only the +42kb enhancer was active and bound by TFs in CD34+ hematopoietic stem cells (HSCs), indicating a role of this enhancer in immature and mature myeloid differentiation stages. Using 4C-seq, the enhancer engages with the *CEBPA* promoter at higher frequencies in myeloid^{CEBPA+} cell lines compared to non-hematopoietic^{CEBPA+} cell lines. Deletion of the+42kb enhancer by CRISPR/Cas9 in the myeloid^{CEBPA+} cell line THP-1 showed 3-5fold reduction of *CEBPA* mRNA levels, suggesting transcriptional control of the enhancer on *CEBPA*. In mice, the +42Kb enhancer is located within a conserved sequence at +37Kb of *Cebpa*. We deleted the +37Kb enhancer in one-cell stage zygotes using CRISPR/Cas9. Flow-cytometric analysis of peripheral blood and BM at 8-10 weeks of age, showed 10-20fold decrease in neutrophil absolute counts in homozygous mice (+37kb$^{-/-}$) compared to age matched controls. In line with the block of neutrophil development, common-myeloid-progenitors (CMPs) were increased by 20 fold, whereas granulocyte/ monocyte progenitors (GMPs) were decreased by 8 fold in +37Kb$^{-/-}$ mice, which is reflected by a significant reduction of *Cebpa* levels in CMPs and CMPs by RNA-seq. However, no changes in *Cebpa* levels were found in the lung and liver, indicating tissue specificity of this enhancer. Moreover, loss of HSCs was observed in +37Kb$^{-/-}$ mice and accompanied by an increase in the multipotent progenitors (MPPs). The +37Kb$^{-/-}$ MPPs were able to serially replate *in vitro* under growth factors, suggesting that the absence of the enhancer has transforming potential. We conclude that the +37Kb enhancer is tissue specific and plays a central role in hematopoiesis to regulate *Cebpa* levels. Absence of the enhancer disturbs neutrophilic differentiation and predispose the bone marrow to leukemic transformation.

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Poster S-III-11

Loss of p62/SQMSTM1 in malignant transformation impairs mitophagy and delays myeloid leukemia development

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The lysosomal degradation pathway of autophagy has dual roles in cancer, acting as a tumor suppressor during tumor development and as a survival mechanism for established tumors. Autophagy is induced by starvation, hypoxia and upon anticancer treatment. Recently, autophagy has been shown to be fundamental for maintaining hematopoietic stem cell (HSC) integrity and preventing malignant transformation. Besides the bulk degradation process, autophagy also serves as an intracellular quality control by selective removal of protein aggregates, damaged or redundant organelles, which is referred to as selective autophagy. This process is mediated by substrate ubiquitination and requires autophagy receptors such as p62, which specifically bridge the ubiquitinated cargos into the membrane of the autophagosome. Here we investigate the contribution of p62 in the development of acute myeloid leukemia (AML) by using a genetic model of p62 knock-out (p62-/-) mice and acute knock-down of p62 (shRNA). Our data demonstrate that loss of p62 impairs the colony forming ability *in vitro* and delays leukemia onset *in vivo*. Mechanistic studies demonstrate that loss of p62 prevents the effective clearance of damaged mitochondria by autophagy (mitophagy), thereby causing the accumulation of mitochondrial reactive oxygen species (ROS) and defects in basal and maximal mitochondrial respiration. Our results suggest a prominent role of p62 in leukemia development and open new opportunities for drug development to target selective autophagy in the treatment of AML.

Poster S-III-12

Investigation of T cell Subsets and Germinal Center Reaction and their Role in Lymphomagenesis in a murine Model of *Rhoh* Deficiency

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Deficiency of RhoH has recently been linked to T cell immune deficiency and an association with the genetic condition *Epidermodysplasia Verruciformis* (EV) in humans. However, in patients with EV, despite a severe lack in naïve T cells, T cell counts were near normal in two patients with chronic viral infections. They demonstrated an increase in memory T cells that exhibit an exhausted phenotype. To decipher the impact of RhoH deletion and the role of antigenic stimuli on changes in specific T cell populations we crossed *Rhoh*$^{-/-}$ mice with the murine *Em-TCL1*^{Tg} model of CLL. In these animals, leukemia progression is significantly delayed in spite of the T cell immunodeficiency of the *Rhoh*$^{-/-}$ phenotype. We observed defective *in vivo* development of CXCR5^{+} follicular T helper (Tfh) cells in young *Rhoh*$^{-/-}$ mice, resulting in defective germinal center (GC) formation and immunoglobulin switching. We detected a severe defect in CXCR5^{+} Tfh cells in the spleens (*WT* vs *Rhoh*$^{-/-}$ mice: 10.09% +/-1.23 vs. 1.71% +/-0.45; mean+/- SEM; p=0.01) and peripheral blood (*WT* vs *Rhoh*$^{-/-}$ mice: 4.69% +/-0.51 vs 0.36% +/-0.13) of young *Rhoh*$^{-/-}$ mice. While IgM levels were only mildly reduced in *Rhoh*$^{-/-}$ mice, these animals exhibited significantly reduced IgG1 serum levels 21 days after TNP-KLH treatment (*WT* vs. *Rhoh*$^{-/-}$ mice: IgG1 3706951 ng/ml +/-871537 vs 122176 ng/ml +/-14006; mean+/- SEM; p=0.01), indicating a defect in immunoglobulin class switching. Moreover, while both naïve CD4^{+} and CD8^{+} T cells were decreased, effector memory and activated effector memory T cells were significantly increased in *Rhoh*$^{-/-}$ mice prior to development of significant leukemia burden. Finally, adoptive transfer experiments suggest that delayed disease onset in *Rhoh*$^{-/-}$;*Em-TCL1*^{Tg} mice is primarily leukemia cell autonomous and independent of a coexisting T cell defect. WT recipients of *WT* CLL cells demonstrated a significantly accelerated disease progression after 6 weeks compared to those injected with *Rhoh*$^{-/-}$ CLL cells (*WT* recipients: 238.1 +/-65.8 K/µl K/µl *WT* CLL cells vs. 2.3 +/-1.4 K/µl *Rhoh*$^{-/-}$ CLL cells) and accordingly also demonstrated improved survival (survival probability *WT* recipients of *WT* CLL cells: 0.33+/-0.16 vs. *Rhoh*$^{-/-}$ CLL cells: 0.78+/-0.14; p<0.05). These data suggest that the exhausted T cell phenotype may be directly related to RhoH deficiency rather than secondary to chronic antigenic stimulation. Furthermore, RhoH may represent an attractive tool for future targeted therapies in CLL.

Poster S-III-13

Mathematical Model for Oncogenesis Control in Mature T-Cell Populations

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It is known for roughly two decades that T-cell clones (uniquely defined through their antigen-specific T-cell receptor) maintain homeostasis in the periphery almost independent from new thymic output through competition for self-peptides presented on MHC molecules (on the surface of antigen presenting cells) (Rocha & Boehmer 1991, Kirberg et al. 1997). Beyond existing mathematical models, we add leukemic clone variants to the repertoir of T cells and analyse the system with respect to competitive exclusion of the oncogenic variants (Diebner et al. 2016). An analysis known from the studies of evolutionary stability allows for the derivation of a fitness function that relates systems parameters with clonal diversity in order to gain conditions under which the leukemic invaders are suppressed. The model well captures the experimental observation that transgenic clones are outcompeted under a polyclonal condition whereas monoclonality leads to tumour outgrowth (Newrzela et al. 2012). The conditional function allows to investigate the system with respect to other dynamical behaviours as, for example, co-existence of both healthy as well as leukemic clone variants. Since quality and quantity of available sp-MHC complexes appear to vary over the lymphatic system (lymph node dependent niche hierarchies), our model may stimulated further experiments in this direction as, for example, local and temporal niche variations and their impact on clonal diversity.

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Poster S-III-14

Defining the roles of AKT isoforms in hematopoietic cell transformation *in vitro* and *in vivo*

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The phosphoinositide 3-kinase (PI3K) signaling cascade plays a pivotal role in multiple cellular processes and is commonly dysregulated in human cancers including leukemia, lymphoma and myeloma. The serine/threonine kinase AKT is the major effector kinase downstream of activated PI3K. Three isoforms (AKT1, 2 and 3) sharing a high degree of homology in sequence and structure have been identified. In this study, we determined the relevance of AKT activation in leukemic cells and defined the specific roles of AKT isoforms in hematopoietic cell transformation and leukemogenic growth. To evaluate the relevance of AKT activation in leukemogenesis, we established a bone marrow transplantation model by reconstituting BALB/c mice with HSPCs expressing an activated mutant of the catalytic subunit of class IA PI3K (PIK3CA H1047R). We demonstrate development of hematologic neoplasia in multiple lineages in conjunction with constitutive PI3K-dependent activation of AKT. Proliferation, colony formation and survival of leukemic cells isolated from moribund mice were sufficiently blocked by treatment with either PI3K or AKT inhibitors. In contrast, mTOR inhibition was less effective in targeting the leukemic cells. In agreement with these results, hematopoietic progenitor cells (Ba/F3) expressing oncogenic PI3K mutants (E542K or H1047R) were highly sensitive to AKT, but less sensitive to mTOR inhibition. Knockdown (KD) of AKT1 or AKT2 specifically and significantly reduced the potential of activated PI3K to confer factor-independency to various progenitor cell lines (Ba/F3, FDCP-1 and 32D) by at least 1 log whereas silencing of AKT3 only slightly affected transformation in all three cell models. Consistently, down-regulation of AKT1 and AKT2, but not AKT3 inhibited the proliferation of Ba/F3-H1047R cells, and interfered with cell cycle progression and leukemogenic growth after transplantation into BALB/c mice resulting in a prolonged survival of recipient mice. Furthermore, targeting two AKT isoforms by double KD revealed non-redundant functions of AKT1 and AKT2 or AKT3 in both cell transformation *in vitro* and leukemogenic growth *in vivo*, whereas the combined KD of AKT2 and AKT3 demonstrated overlapping functions of these isoforms. Our data show that AKT isoforms are critically involved in PI3K-mediated mitogenic and anti-apoptotic signaling in leukemic cells and have differential roles in hematopoietic cell transformation and leukemogenic growth.

Poster S-III-15

CRISPR-Cas9 induced MLL-ENL translocations in human hematopoietic progenitor cells are leukemogenic *in vivo*

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Reciprocal chromosomal translocations are the causative genetic aberration in almost 60% of the pediatric acute myeloid leukemia cases. Amongst these, rearrangements of the MLL1/KMT2A gene are most frequent. Whether bona fide endogenous MLL-rearrangements -with loss of one wildtype allele of each fusion partner and potential expression of two fusion genes- are sufficient to transform human HSPCs remains unknown. With the aim to elucidate the transformative nature of endogenous MLL-rearrangements in primary human HSPCs, we developed and advanced an all-in-one lentiviral CRISPR-Cas9 system with two sgRNA expression cassettes (LentiCRISPR-CT2.0). Utilizing established reporter-based sgRNA testing, we selected highly efficient sgRNAs targeting MLL1 and ENL intronic sequences (cleavage rates >80%) to generate the t(11;19)/MLL-ENL translocation. Based on these results, we tested generation of chromosomal rearrangements in hematopoietic cell lines. MLL-ENL expression and genomic breakpoints were robustly detectable in the transduced bulk population (K562 cells). To determine the impact of endogenous MLL-ENL on HSPCs, we transduced cord blood derived CD34+ HSPCs. In three independent experiments using methylcellulose-based colony-forming assays, MLL-ENL expression was detectable. MLL-ENL containing cells had an extended –but not unlimited- replating capacity. With these results, we next aimed to interrogate the transforming capacity of endogenous MLL-rearrangements *in vivo*. CD34+ HSPCs, freshly transduced with the LentiCRISPR-CT2.0, were transplanted into immunodeficient mice. MLL-ENL expression was detectable in 6/8 mice. Within an observation time of up to 30 weeks 2/8 mice developed malignant, transplantable monocytosis as determined by flow cytometry and histopathology. Malignant cells showed robust expression of MLL-ENL, ENL-MLL and MLL-rearrangement as determined by FISH. Furthermore, secondary transplantation of mice with detectable MLL-ENL rearrangement but absence of disease resulted in leukemic transformation (B-ALL) in one case. In aggregate, our study uncovers the oncogenic potency and limitations of endogenous MLL translocations in human HSPCs *in vitro* and *in vivo* and highlights the power of the CRISPR-Cas9 system to generate precise cancer models, which will allow us to test the efficacy of targeted therapies, and to investigate the mechanisms of drug resistance.

Poster S-IV-16

Plasma and saliva expression of miR-21 in colorectal cancer

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miR-21 expression was quantified by real-time qPCR in peripheral blood and saliva samples obtained from patients diagnosed with colorectal cancer (CRC) of varying degrees of malignancy and healthy volunteers. All patients had adenocarcinoma located in distal colon at different stages. Significant differences were detected between the control group and the general group of CRC patients (plasma - $P = 0.005$, saliva - $P < 0.001$). miR-21 expression was also significantly different in the separate groups of patients with CRC disease stages II-IV compared to the control group. No correlation of miR-21 expression was found with gender and age. Also no significant individual correlation and linear regression of miR-21 expression in plasma and saliva were detected. The diagnostic sensitivity and specificity of expression miR-21 were estimated in 55% and 90% in plasma, 97% and 93% in saliva, respectively. Both salivary and plasmatic miR-21 can be proper biomarkers for CRC screening, but salivary miR-21 expression test looks more preferably due to its higher sensitivity, specificity and technical simplicity

Poster S-IV-17

mircroRNA-21 expression profiling in the monitoring of cerebral's gliomas progression

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The early verifying of cerebral's gliomas progression is an actual problem. It was found that the level of mircroRNA-21 expression is a highly specific in a tumor's evolutional process. That the aim of the study was the assessment with level of mircroRNA-21 expression in blood and saliva of patients with cerebral glioma progression and patients without it which were verified by PET using [11C] methionine. It was found that in majority of cases, the negative dynamics of cerebral neoplastic lesions coincided with an increase in metabolic changes in the residual glioma and highly correlated with the level of mircroRNA-21 expression. Development of cerebral's gliomas is accompanied by a convincing increase in the expression of mircroRNA-21, reflecting the epigenetic manifestation of genetic dysfunctions. Method of determining the level of expression microRNA-21 highly specific for the diagnosis of cerebral's gliomas in comparison with other pathological (including cancer) processes central nervous system ($p < 0.001$). The expression level of mircroRNA-21 in patients with cerebral's gliomas is highly correlated with the precision method as PET study with 11 C methionine ($r = + 0,96$). The method of determining the availability of the dynamics of microRNA-21 in the monitoring of patients with cerebral's gliomas allows to substantiate indications for PET studies. This allows us to consider a highly significant the level of mircroRNA-21 expression as a screening technique in monitoring the evolution of the tumor process and the effectiveness of the treatment.

Poster S-IV-18

An integrated approach to AML diagnostics using Next Generation Sequencing

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Acute Myeloid Leukemia (AML) is one of the best characterized malignancies on the molecular level, but this extensive knowledge is only slowly translating into novel therapeutic concepts. Over the past 30 years, virtually all younger patients have undergone the same induction chemotherapy, and additional disease-specific characteristics have been considered only for choosing an appropriate consolidation regimen. Traditionally, the karyotype of the malignant blasts determined by classical cytogenetics has been the most important parameter for risk stratification. However, chromosomal aberrations indicating a favorable or, respectively, poor prognosis such as t(8;21), t(15;17), certain monosomies and complex karyotypes, are found only in approximately 50% of AML patients. On the other hand, the outcome in cytogenetically normal AML is essentially determined by the presence / absence of mutations in certain genes such as *NPM1*, *FLT3* or *CEBPA*. Therefore, the karyotype is not sufficient to guide treatment decisions in AML and extensive mutational analysis of leukemia-associated genes is required for proper risk assessment in at least half of the patients. Here we present an integrated approach to AML diagnostics that incorporates analysis of both structural variants and mutations in a single *Next Generation Sequencing* (NGS) run. We applied our method on leukemia cell lines and peripheral blood leukocytes from healthy donors and were able to reliably distinguish between normal and complex karyotypes based on copy number variation analysis of low coverage whole genome sequencing data. Translocations and mutations in > 40 AML-relevant genes were identified with higher sensitivity and deeper coverage using an amplicon-based enrichment strategy for targeted RNA- and DNA-resequencing rather than in-solution hybrid capture of genomic DNA. Although our protocol requires preparation of three sequencing libraries from one sample, the entire procedure can be completed within one week. Therefore, NGS not only appears a suitable substitute for difficult and time-consuming cytogenetics, but also allows for more comprehensive translocation- and mutation-screening than current (q)PCR methods. We plan to further validate our NGS-approach to AML diagnostics in clinical samples to pave the way for a more differentiated management of AML patients in the near future.

Poster S-IV-19

Mesenchymal stroma protection of leukemia involves epigenetic and metabolic processes

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Bone marrow mesenchymal stroma cells (BM-MSC) protect leukemia cells from drug-induced cytotoxicity. A greater understanding of the mechanisms involved in the interaction of stroma with leukemia cells may identify the 'protective' factors involved in drug resistance. The main challenge to successful treatment is the genetic and epigenetic heterogeneity of leukemia cells. Accumulating evidence also implicates the microenvironment as the optimal setting for malignant leukemia cells to thrive and undoubtedly affect gene expression. In this regard, BM-MSC interaction with leukemia cells may cooperatively affect epigenetic and genetic adaptation of leukemia cells for cell survival. Thus, we hypothesize that leukemia-stroma interactions promote drug resistance through epigenetic modulation. We used an epigenetic drug library of 80 compounds modulating epigenetic protein families including histone deacetylases (HDAC), histone methyl transferases, bromodomain, DNA demethylases, and sirtuins to monitor KG1a cells in coculture with HS5 stroma. We found that that anti-leukemic efficacy of HDAC inhibitors is dramatically reduced by stroma protection indicating histone acetylation may also play a role in stroma-induced drug resistance. These findings persisted in primary AML blasts (n=25) cocultured on stroma treated with 15 epigenetic modifying drugs. Treated AML blasts clustered into three distinct response patterns which may give insight into the efficacy of epigenetic modifiers in AML blasts. Furthermore, to further probe for leukemia-stroma pro-survival pathways through crosstalk cellular signaling, quantitative proteomic methods were applied to trace phosphorylated and dephosphorylated peptides upon KG1a-Hs5 stroma interaction. In addition, a HDAC inhibitor was used to further enhance dynamic phosphorylation changes in response to stroma protection. Significantly enriched pathways from 544 detected peptides included proteoglycans, VEGF signaling, focal adhesion, adherens junction, insulin, MAPK and cytoskeleton. Also, enriched cellular process included key chromatin and metabolic enzymes. Increased phosphorylation of metabolic targets from within the stroma compartment may provide the metabolic support for cell survival of leukemia cells. Furthermore, we predict that targeting metabolic pathways in the stroma may override stroma-induced drug resistance of leukemia cells.

Poster S-IV-20

Genomic and transcriptomic landscape of t(4;11)(q21q24)/MLL-AF4+ Infant pro-B acute lymphoblastic leukemia.

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B-cell Acute Lymphoblastic Leukemia (B-ALL) is the most common pediatric cancer, representing the 25% of all malignant tumors in childhood. A particular subtype of B-ALL characterized by the genomic translocation t(4;11)(q21q24) which fuses the *MLL* with *AF4* is frequently found in newborns/infants and is associated with dismal prognosis [Pui *et al*., 2011]. In spite of its short latency and high aggressiveness, it has been shown both *in vitro* and *in vivo* that *MLL-AF4* does not seem sufficient to initiate leukemogenesis, suggesting that secondary cooperating events may be necessary for leukemogenesis [SanJuan-Pla *et al*., 2015]. Nevertheless, whole-genome sequencing studies revealed a silent landscape of somatic mutation in MLL-rearranged ALL [Dobbins *et al*., 2013, Andersson *et al*., 2015]; in fact, infant MLL-rearranged B-ALL has one of the lowest frequencies of somatic mutations of any sequenced cancer. Here, we have performed a high-coverage (110X) exome sequencing and RNAseq in a cohort of 25 diagnostic samples and 7 matched relapsed from *MLL-AF4*+ B-ALL infants. Our results show a slight higher mutation load than previously described, revealing the presence of some somatic mutations in minor tumor subclones. In agreement with Andersson et al., recurrent somatic mutations were exclusively found in genes of PI3K-RAS signaling pathway. In addition, RNAseq studies have revealed new genes/pathways differentially expressed in this leukemia which could play an important role in the development of the disease. Taken together, our results confirm an extremely low mutation rate in infant MLL-AF4+ B-ALL, and suggest that the target cell of origin and epigenetic portraits may be crucial in the pathogenesis of this dismal leukemia.

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Poster S-IV-21

Regulation and interactions of the oncogenic transcription factor MEIS1

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MEIS1 is a critical regulator of normal hematopoiesis and its overexpression is implicated in a wide range of leukemias. While there has been progress in identifying some of the target genes and pathways controlled by MEIS1, we know little about what regulates MEIS1 expression and what proteins interact with MEIS1 to mediate its function as a transcription factor. Our overall objective is to identify transcriptional regulators as well as interacting partners of MEIS1 with the long term goal of identifying new ways to alter MEIS1 expression and function for therapy. Here we describe recent progress focused on delineating MEIS1 cis-regulatory elements. In a previous study (Xiang et al., Leukemia, 2014, 28(2):433-6) we showed that in addition to the MEIS1 promoter, multiple candidate cis-regulatory elements reside within the 140 kb MEIS1 genomic locus and are potential key components contributing to increased MEIS1 expression seen in leukemia. Using CRISPR-Cas9 gene editing, we have now introduced a GFP reporter and an HA tag into the MEIS1 gene of several human leukemic cell lines as well as in a mouse Embryonic Stem cell line. From the latter we have also derived a GFP-HA-MEIS1 mouse line and then tagged murine leukemic cell lines. As a first application of these new tools, we have exploited GFP-HA-MEIS1 U937 lines that expresses relatively high levels of MEIS1 and used CRISPR/Cas9 editing to introduce mutations around 22 sites (many of them within the candidate enhancer regions) within the MEIS1 genomic locus. Mutation around two regions, one located ~2kb upstream (E1) and one ~10 kb downstream (E2) of the transcription start site of MEIS1, result in marked decrease of MEIS1 expression as indicated by lower GFP (by FACS) and HA-MEIS1 (by western blot) expression. Deep sequencing of the targeted E2 region has further identified a "minimal deleted region" associated with a significant decrease in MEIS1 expression and a candidate binding site for TFAP2alpha. These initial data provide important new functional evidence of the regulatory role of the E1/E2 enhancer regions for *Meis1* expression and identify a candidate new upstream regulator of MEIS1. Further application of these new GFP-HA-MEIS1 models to resolve MEIS1 regulatory regions and to identify novel interacting proteins are now in progress.

Poster S-V-22

Targeting CRLF2-rearranged Ph-like acute lymphoblastic leukaemia using global phosphotyrosine profiling

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Cases of high-risk acute lymphoblastic leukaemia (ALL) frequently harbour activating mutations in protein kinases, leading to dysregulated cellular signalling pathways[1]. Approximately 50% of Philadelphia-like (Ph-like) ALL cases overexpress cytokine receptor-like factor 2 (CRLF2) due to gene rearrangements[2]. CRLF2 signalling via interaction with its ligand thymic stromal lymphopoietin (TSLP) is critical for the development, proliferation and survival of normal lymphocytes[3]. While clinically approved agents targeting CRLF2 do not currently exist, tyrosine kinase inhibitors (TKIs) can be used to target kinases downstream of CRLF2. This study aimed to identify targetable tyrosine kinases deregulated downstream of CRLF2 that are critical for the survival of Ph-like ALL cells. To investigate the activation of tyrosine kinases regulated by TSLP, a phosphotyrosine profiling technique coupled with stable isotope labelling of amino acids in cell culture (SILAC) was used. The Ph-like ALL cell lines MHH-CALL-4 and MUTZ-5 were cultured in SILAC (Heavy) media, stimulated with TSLP (30 min) and lysed, before equal amounts of protein were mixed with protein extracts from their respective control cells cultured in normal (Light) media. Samples were subsequently immunoaffinity enriched for tyrosine-phosphorylated peptides and analysed by mass spectrometry. As a result, the level of TSLP stimulation was quantified for each paired tyrosine kinase using the Light:Heavy ratio. Increased tyrosine phosphorylation was detected in several well-known TSLP-activated tyrosine kinases and substrates, including JAK1, JAK2, STAT5, and ERK1/2. Interestingly, TSLP stimulation also increased tyrosine phosphorylation of the insulin receptor (INSR) and fibroblast growth factor receptor 1 (FGFR1). Based on these findings, fixed-ratio cytotoxicity assays were carried out to investigate the possible synergistic effects of combining specific pathway inhibitors in Ph-like ALL cells. In both MHH-CALL-4 and MUTZ-5, dual inhibition of INSR and FGFR1 using the TKIs BMS-754807 and ponatinib respectively was strongly synergistic, which was replicated in patient-derived Ph-like ALL xenografts cultured *ex vivo*. In conclusion, this study has demonstrated the feasibility of rationally designing combination drug treatments using unbiased phosphotyrosine profiling of TSLP-stimulated Ph-like cell lines. The dual inhibition of INSR and FGFR1 represents a novel strategy to target CRLF2-rearranged Ph-like ALL.

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Poster S-V-23

Upregulation of RUNX1 by radiation and chemotherapy induces apoptosis and augments the response to cytotoxic therapies

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The transcription factor RUNX1 (AML1) has a well-documented role in blood cell differentiation and hematopoietic stem cell (HSC) renewal. Its function is frequently impaired in AML and MDS and this has been clearly linked to the development of the disease. Recent clinical studies have also revealed a strong association between RUNX1 mutations and chemotherapy resistance/poor prognosis in AML, ALL and MDS. Consistently, RUNX1-deficient HSCs were protected against radiation and cytarabine-induced cell death, supporting a model in which the complete loss of RUNX1 function promotes the survival of therapy-resistant leukemic stem cells. However, little is known about the mechanisms regulating the apoptotic activity of RUNX1 and the relevance of it in more differentiated blood cells. To elucidate these questions we examined the response of C57BL/6 mice to whole-body gamma irradiation. Significantly, we saw a strong upregulation of RUNX1 in the thymus and (to a lesser extent) in spleen and bone marrow that correlated with induction of apoptosis. Interestingly, radiation induced upregulation of RUNX1 in the thymus can be attributed exclusively to post-transcriptional mechanisms, although elevated RUNX1 mRNA was observed in other cell types. These findings identify RUNX1 as a novel DNA damage responsive protein. To test whether elevated RUNX1 levels directly promote apoptosis and/or affect the response to cytotoxic therapies, we overexpressed different RUNX1 constructs in BA/F3 cells and monitored cell growth, death and response to cytotoxic drugs. Our experiments revealed the following novel findings: (1) ectopic overexpression of either AML1b or AML1c isoforms inhibit cell proliferation and induce apoptosis, (2) elevated RUNX1 levels augment the apoptotic response in response to radiation and cytarabine, (3) transcriptionally impaired RUNX1 mutants lack the anti-proliferative and pro-apoptotic activity of wild-type protein. However, when overexpressed in the presence of wild-type RUNX1, these mutants do not alter the response to cytarabine or radiation suggesting that biallelic inactivation of RUNX1 is necessary for mutant RUNX1-related therapy resistance. In summary, we identified RUNX1 as a stress-responsive pro-apoptotic factor whose activity is linked to its abundance. Our findings support a model in which upregulation of RUNX1 contributes to the elimination of cancer cells by cytotoxic therapies, a process that is impaired by loss-of-function mutations.

Poster S-V-24

BGB324 inhibits BCR-ABL TKI-resistant chronic myeloid leukemia

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Resistance and CML stem cell persistence preclude cure for the majority of patients treated with TKI therapies. We demonstrated that the RTK Axl is expressed by TKI-sensitive and –resistant CML cells, and that inhibiting Axl by BGB324 (BerGenBio) represents a therapeutic target in AML. We hypothesised that Axl represents a tractable therapeutic target even in the most resistant forms of CML. Upon treatment with imatinib KCL-22 and K562 cells showed upregulation of Axl at the protein level indicating that Axl might be involved in resistance towards TKIs in CML. Axl levels were higher in MNCs of TKI-resistant patients compared to –sensitive patients after 6 months of treatment. Upon combined treatment of KCL-22 and K562 cells with BGB324 and imatinib we detected an additive effect of growth inhibition. We also found that Axl induces phosphorylation of Stat5 by BCR-ABL independent pathways because we detected an additive effect of inhibition of Stat5 phosphorylation when combining imatinib and BGB324. We investigated Axl activation in TKI insensitive BCR-ABL$^+$ cell lines and a novel Ponatinib-resistant cell line KCL-22 PonR. BCR-ABL is unmutated in these cells. We found that Axl phosphorylation was higher in the TKI-resistant cell lines BaF3/T315I, KCL-22 T315I and KCL-22 PonR when compared to the parental cell lines. Treatment with BGB324 inhibited cell viability by inducing apoptosis and reducing proliferation in these cell lines. Furthermore, BGB324 blocked growth of colonies and induced apoptosis of T315I-mutated and pan-TKI-resistant (including ponatinib) primary cells.The finding that BGB324 inhibits TKI-resistant CML was corroborated with KCL-22 T315I mutated and KCL-22 PonR xenograft models, resulting in a significant tumor growth reduction upon treatment with 25 mg/kg BGB324 compared to placebo leading to a 34% and 58% reduction. Cell proliferation was quantified by pHH3 analysis indicating a significant reduction. Concomitantly, a significant decrease of Axl, Erk and Stat5 phosphorylation was observed after treatment with BGB324. We also investigated the therapeutic effect of BGB324 in a systemic model, by transplantation of KCL-22 PonR into sublethally irradiated NSG mice. Treatment with 25 mg/kg BGB324 resulted in significant prolongation of overall survival (median OS 36 days vs 43 days). Our data highlight the advantage to be gained from inhibition of Axl even in the most resistant CML cells, and support the need for human clinical trials.

Poster S-V-25

Targeting Rac-GTPases Reverses Stroma-Induced Resistance to Notch and mTOR-Inhibition in Acute T-cell leukemia

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mTOR and NOTCH signalling pathways have been investigated as promising therapeutic targets in T-ALL, since their aberrant activity has been linked to therapeutic resistance and high frequencies of leukemia-initiating cells. However, treatment of acute leukemia patients with the mTOR inhibitor Rapamycin and treatment of T-ALL mouse models with Notch inhibitors has been disappointing. To investigate the impact of mTOR and Notch inhibition in a genetically complex T-ALL, we developed a murine T-ALL model driven by tyrosine kinase signaling, loss of Pten, Cux1-haploinsufficiency and constitutive Notch signaling. In vitro, T-ALL blasts were highly sensitive to inhibition of AKT, mTOR and Notch signaling. After transplantation of the leukemias into secondary recipients Rapamycin treatment prolonged survival. Eventually, all Rapamycin treated animals succumbed to T-ALL despite continuous drug administration. In vitro, Rapamycin-resistant blasts became susceptible to Rapamycin again, proving a context-dependent resistance rather than outgrowth of intrinsically resistant clones. Transcriptome profiling pointed towards upregulation of Rac-associated cell-cell interactions in vivo. Indeed, stroma cell support recapitulated the in vivo effect and induced robust resistance to mTOR and Notch inhibition in T-ALL blasts. PAK-pull down assays revealed an increased Rac1 activation in the tumor cells when in contact with stroma cells. To determine whether Rac activation plays a role in stroma-induced resistance, we used Clostridium difficile serotype F strain 1470 produced toxin B isoform (TcdBF) to inactivate Rac(1, 2, 3) in a dose-dependent manner in the T-ALL blasts, but not in the stroma cells. Strikingly, in the TcdBF-pretreated T-ALL, the stroma-induced resistance effect was abrogated and clusters of apoptotic cells were clearly visible (>2 fold reduction of the input, $p=0.002$). In contrast, the carrier-treated T-ALL exhibited resistance to the inhibitors on stroma (>10 fold expansion of the input, $p<0.0003$). Altogether, we identify the Rac-GTPases as a nexus of stroma-induced drug resistance and show that inhibition of Rac and mTOR is synthetically lethal to T-ALL blasts that are in contact with stromal cells, thus paving the way to augment the effectiveness of small molecule inhibitors in acute leukemia.

Poster S-V-26

Discontinuing MEK inhibitors in tumor cells with an acquired resistance increases migration and invasion.

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Background: Development of small molecular inhibitors against BRAF and MEK has been a breakthrough in the treatment of malignant melanoma. However, the long-term effect is foiled in virtually all patients by the emergence of resistant tumor cell populations. Therefore, mechanisms resulting in the acquired resistance against BRAF and MEK inhibitors have gained much attention and several strategies have been proposed to overcome tumor resistance, including interval treatment or withdrawal of these compounds after disease progression.

Methods: Using a panel of cell lines with an acquired resistance against MEK inhibitors, we have evaluated the sensitivity of these cells against compounds targeting AKT/mTOR signaling, as well as novel ERK1/2 inhibitors. Furthermore, the effects of withdrawal of MEK inhibitor on migration in resistant cell lines was analyzed.

Results: We demonstrate that withdrawal of BRAF or MEK inhibitors in tumor cells with an acquired resistance results in reactivation of ERK1/2 signaling and upregulation of EMT-inducing transcription factors, leading to a highly migratory and invasive phenotype of cancer cells. Furthermore, we show that migration in these cells is independent from AKT/mTOR signaling. However, combined targeting of AKT/mTOR using MK-2206 and AZD8055 efficiently inhibits proliferation in all resistant tumor cell lines analyzed.

Conclusions: We propose that combined targeting of MEK/AKT/mTOR or treatment with a novel ERK1/2 inhibitor downstream of BRAF/MEK suppresses proliferation as well as migration and invasion in resistant tumor cells. We provide a rationale against the discontinuation of BRAF or MEK inhibitors in patients with an acquired resistance, and provide a rationale for combined targeting of AKT/mTOR and MEK/ERK1/2, or direct targeting of ERK1/2 as an effective treatment strategy.

Poster S-V-27

A mathematical model approach to study the immunological effects in CML patients during and after TKI treatment

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Molecular monitoring of BCR-ABL levels in the peripheral blood of patients with CML under continuous tyrosine kinase inhibitor therapy revealed that the majority respond well to the treatment and achieve sustained molecular remission. This encouraged the initiation of treatment cessation trails, in which approximately 50% of the patients relapsed within the first 12 months after therapy stop. The rare observation of relapses later then 12 months after cessation indicates that the residual leukemic disease is either completely eradicated in the non-relapsing patients or that other factors such as immunological effects constrain leukemia regrowth. The latter hypothesis is supported by observations of closely monitored patients, with very low but detectable BCR-ABL burden, which do not relapse even without treatment resumption. Previously, we developed a mathematical model to functionally describe CML pathogenesis and treatment as a competition phenomenon between healthy and leukemic stem cells. By fitting our model to time course data of BCR-ABL levels under TKI-treatment from the peripheral blood, we can estimate residual disease levels among the stem cell population and derive estimates on leukemia reoccurrence after therapy cessation. Now, we explicitly integrate a population of immune cells within our model to account for the particular role of immunological effects and to critically discuss the role of anti-cancer immune competence in the treatment-free maintenance of CML patients. In brief, a high abundance of CML cells represses the immunological population while a therapy induced decline in tumor load releases this limitation and supports anti-cancer immune competence, which in case of treatment cessation can lead to a sustainable control of the leukemic cell population. We study the influence of certain model parameters, like the level of immune stimulation and the effectiveness of immune cell induced leukemic control, to identify suitable factors and feedback mechanisms that account for the observed heterogeneity between relapsing and non-relapsing patients. We further aim to use this approach to speculate about accessible clinical parameters that can potentially support clinical decision making for therapy cessation trails. Our model represents a further step towards deriving a more holistic view on leukemia occurrence and treatment in the context of multiple, interacting tissues and cell types.

Poster S-VI-28

The Helix-Loop-Helix Protein ID2 Governs NK Cell Homeostasis by Tuning TheSensitivity to Interleukin-15

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The recent success of checkpoint blockade has highlighted the potential of immunotherapy approaches for cancer treatment. While the majority of approved immunotherapy drugs target T cell subsets, it is appreciated that other components of the immune system have important roles in tumor immune-surveillance as well and thus represent promising additional targets for immunotherapy. Natural killer (NK) cells are the body's first line of defense against infected or transformed cells as they kill target cells in an antigen-independent manner. An epidemiological 11-year follow-up study in patients demonstrated that high cytotoxic activity in peripheral-blood lymphocytes is associated with reduced cancer risk (Imai et al, 2000). These data indicate that therapies augmenting the number of NK cells in patients are likely to result in improved clinical outcome. It is therefore important improve our understanding of the regulation of NK cell homeostasis. The inhibitor of DNA binding 2 (Id2) is essential for natural killer (NK) cell development with its canonical role being to antagonize E-protein function and alternate lineage fate. Here we have identified a key role for Id2 in regulating interleukin-15 (IL-15) receptor signaling and homeostasis of NK cells by repressing multiple E-protein target genes including Socs3. Id2 deletion in mature NK cells was incompatible with their homeostasis due to impaired IL-15 receptor signaling and metabolic function and this could be rescued by strong IL-15 receptor stimulation or genetic ablation of Socs3. During NK cell maturation, we observed an inverse correlation between E-protein target genes and Id2. These results shift the current paradigm on the role of ID2, indicating that it is required not only to antagonize E-proteins during NK cell commitment, but constantly required to titrate E-protein activity to regulate NK cell fitness and responsiveness to IL-15.

Poster M-I-01

Reactivating Latent Endo- and Exoviruses in Resistant Tumor cells. Discovering the Tip of the Iceberg.

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The fact that malignant transformation is a multi-stage event based on sequential biological accidents is easily consistent with the understanding that many tumors arise from single or serial viral infections. Until now, ten exoviruses have been linked to the evolution of cancer, with protective vaccine and immunization programs existing for only two of these: the human papilloma virus and the hepatitis B virus. The aim of the present work was to screen the viral background in different tumor cells, both wildtype and chemotherapy resistant. Using a viral expression subtraction approach in highly chemotherapy-resistant vs. treatment-naïve parental tumor cells, we discovered multiple viral gene transcripts upregulated in the resistant entities. A set of both endo- and exoviruses was found significantly overexpressed in well-established cell lines, primary tumor cells cultures, and afterwards confirmed in tumors. We particularly observed a set of viral entities in atypical tumor cells isolated from resistant entities. This viral reactivation background seems to be pivotal in the cellular biology of refractory tumors. Thus, our findings may provide novel insights into the viral background in tumor cells, indicating a virus-cell interaction and therefore the possible contribution of some viruses to carcinogenesis.

Poster M-I-02

Role of sphingosine-1-phosphate receptor 4 in tumor-associated inflammation

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The inflammatory tumor microenvironment is a critical element for tumor development and progression, as well as a parameter predicting the response to cancer treatment (1). An increased abundance of suppressive immune cells (TAMs, Tregs) appears thereby as a limiting factor for a successful treatment due to the inhibition of tumoricidal lymphocytes such as cytotoxic T cells (2). The bioactive lipid sphingosine-1-phosphate (S1P), which signals mainly through its five G-protein-coupled receptors (S1PR1-5) (3), is an important regulator of carcinogenesis by promoting tumor growth, angiogenesis and metastasis (4). We propose S1PR4, which is exclusively expressed by immune cells, as a negative regulator of anti-tumor immunity. Using a transgenic mouse model of spontaneous invasive ductal mammary carcinoma (PyMT mice) we show that S1PR4 deficiency leads to a significant decrease in lung metastasis and a delay of tumor growth. Furthermore, tumors of $S1PR4^{-/-}$ mice show a decrease in the amount of pro-tumoral regulatory T cells (Tregs) and Th17 cells. This might be attributed to a reduced production of the Treg and Th17 cell-attracting chemokine CCL22 by tumor-associated macrophages (TAMs), which are also reduced in $S1PR4^{-/-}$ tumors. Due to the less immunosuppressive tumor microenvironment, accompanied by an increased abundance of cytotoxic CD8+ T cells in tumors, $S1PR4^{-/-}$ mice also exhibit a significantly improved response to chemotherapy characterized by the absence of relapse. Chemotherapy-treated $S1PR4^{-/-}$ tumors showed increased expression of tumoricidal factors such as TNFα and TRAIL. Our current experiments investigate the role of TRAIL and CD8+ T cells in the improved response to chemotherapy using neutralizing antibodies in PyMT cell-grafted $S1PR4^{+/+}$ and $S1PR4^{-/-}$ mice. Taken together, our data suggest a major role for S1PR4 in restricting anti-tumor immunity, especially following chemotherapy.

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Poster M-I-03

Regulation of the anti-apoptotic enzyme PON2 by Wnt/β-catenin-signalling and its role in tumorigenesis and leukemias

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Dysregulated Wnt-signaling is involved in various cancer sites. Overexpression of Wnt proteins and defective GSK-3β were found to be associated with pre-B-cell leukemia and chronic myeloid leukemia (CML), while in acute myeloid leukemia (AML) overexpression of β-cat is associated with a poor prognosis. The intracellular human enzyme paraoxonase 2 (PON2) is known to have antiapoptotic properties, among others in leukemia. PON2 is a ubiquitously expressed enzyme localized to mitochondria and the endoplasmic reticulum where it fulfills vital functions in the control of ROS generation. We revealed that PON2 diminished mitochondrial superoxide generation causing reduced cytochrome C release, which prevented cell death and protected various human tumor cell lines against chemotherapeutics. We could demonstrate that PON2 was upregulated in many malignancies, including leukemias. Its overexpression raised cancer cell resistance against chemotherapeutics, whereas PON2 deficiency enhanced the susceptibility, for instance seen with Imatinib-treatment of CML-like K562 cells. However, the distinct regulating pathways are poorly understood. Given its overexpression in leukemias and the in vivo and in vitro findings, we here addressed endogenous regulatory pathways controlling PON2 expression. To reveal underlying mechanisms we used several methodologically different approaches, which identified some promising candidates. We focused on Wnt/β-catenin as most promising pathway, since it appears in every approach. These studies revealed that inhibition of GSK-3β results in up-regulation of PON2 expression in K562 cells and that the expression is regulated by LEF-1 and TCF4. Additionally ex vivo analysis of oral squamous cell cancer (OSCC) patients revealed a correlation between PON2 and β-catenin expression in tumor tissue. Higher PON2 expression in OSCC is associated with relapse independently of treatment, which emphasize the clinical impact of the regulation of PON2 through Wnt/GSK3β/β-catenin. Collectively, our study systematically addressed endogenous PON2 regulation and how this links to tumorigenesis, demonstrating a significant role for the Wnt–β-catenin–Lef1/TCF axis. This appears similarly relevant for both CML and OSCC and may also apply to other forms of malignant cell transformation involving increased β-catenin activity. Based on these findings, targeting PON2 in cancer cells through Wnt/β-catenin may contribute to restored death signaling in tumor cells.

Poster M-I-04

Hybrid cells derived from human breast stem-like cells and human breast cancer cells exhibit stem cell properties and an EMT phenotype

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It is well recognized that cell fusion plays a pivotal role in several physiological events, such as fertilization and wound healing, as well as pathophysiological processes including cancer. Here it is assumed that hybrid cells, derived from fusion events between tumor cells and normal cells, like macrophages or stem-like cells, will foster tumor progression due to possessing novel properties, such as an increased metastatogenic capacity. However, in order to metastasize and in accordance to the cancer stem cell hypothesis hybrid cells should possess stem cell properties enabling them to successfully seed secondary lesions at distant organ site. We thus investigated M13HS-2 and -8 hybrid cell clones, which originated from spontaneous cell fusion events between human HS578T-Hyg breast cancer cells and human M13SV1-EGFP-Neo breast epithelial cells exhibiting stem cell properties, for the expression of mammary breast (cancer) cells related stemness-factors as well as EMT genes. cDNA-microarrays and Western Blot analysis revealed that M13HS-2 and -8 hybrid cell clones and M13SV1-EGFP-Neo cells were positive for SOX9 and SLUG, which have been defined as mammary stem cell factors and which could reprogram mammary cancer cells towards a cancer stem cell like phenotype. On the contrary, HS578T-Hyg breast cancer cells were solely positive for SLUG. Co-expression of SOX9 and SLUG was further correlated to an increased fraction of $ALDH^{bright}$ cells, an increased mammosphere formation capacity and an increased colony formation capacity of M13HS-2 and -8 cells in comparison to their parental cells suggesting that M13HS hybrid cell clones might exhibit cancer stem cell properties. Additionally, M13HS-2 and -8 hybrid cells do exhibit an EMT phenotype (being positive for N-CADHERIN, VIMENTIN, TWIST, ZEB1 and ZEB2), do express both a lung and bone metastasis gene signature and do respond to the chemokine CCL21, which has been associated with lymph node metastasis. In summary, cell fusion between human breast epithelial cells exhibiting stem cell properties and human breast cancer cells could give rise to hybrid cells possessing cancer stem cell properties concomitant with a metastatic phenotype.

Poster M-I-05

Effect of TLR 4 and 9-mediated inflammatory response in hybrid clones derived from human breast cancer and human breast epithelial cells

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Cell fusion is a well-known biological phenomenon and plays a fundamental role in several physiological and pathological events including cancer. The fusion of tumor cells between themselves or with other cells like macrophages results in hybrid cells, which could exhibit new properties such a higher metastatic potential or drug resistance. Moreover, tumor progression might also be triggered by Toll like receptors (TLRs), which are critical mediators of the innate immune system. Indeed, TLR stimulation in a variety of tumor cell lines leads to increased survival and proliferation in vitro. Accordingly, TLR-4 expression has been associated with tumor growth, whereas TLR-9 seems to be involved in metastasis formation. Western Blot analysis revealed expression of TLR-4 and -9 as well as components of the specific signal transduction cascades in human MDA-MB-435-Hyg breast cancer cells, M13SV1-EGFP-Neo breast stem-like cells and M13MDA435-1 and -3 hybrid clones. However, treatment of cells with LPS was correlated with nuclear translocation of NF-kB only in M13MDA435-1 and -3 hybrid clones suggesting a differential kinetics of TLR signaling. In accordance with that, up-regulation of TRAIL, IL-6, IFN-beta and TNF-alpha was solely observed in LPS treated M13MDA435 hybrid cells, but not parental cells. In addition, altered phosphorylation levels of AKT und MAPK were only observed after CpG application in hybrids. The migratory activity was examined by using 3D collagen matrix migration assay. In hybrid and parental cells a CpG and LPS dependent decrease in migration activity was observed. On the contrary, cell proliferation studies showed a significant inhibition of proliferation rate by LPS, but not CpG, in both hybrid clones due to induction of apoptosis. In conclusion, the obtained data show that TLR signaling pathways are differently regulated in breast cancer cells and hybrid cells, which could provide important information about the mechanism of metastasis and proliferation rate as well as tumor aggressiveness in breast cancer and how these results could be positively to use as a new therapy approaches for novel anticancer strategies.

Poster M-I-06

Mast cell-derived granzyme b mediates resistance against anti-angiogenic therapy

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Targeted therapies have revolutionized the treatment of cancer. However, efficacy of anti-angiogenic therapies is limited due to significant resistance. Based on the correlation of mast cell (MC) density with tumor growth and angiogenesis we put forward the hypothesis that MC might be implicated in anti-angiogenic therapy resistance. C57BL/6J, NSG or MC-deficient $Kit^{W\text{-}sh}$ (Wsh) mice were subcutaneously injected with $5x10^5$ (Panc02 and EL4) or $1x10^6$ (TD2) cells +/- bone marrow derived MC. Tumors were treated with 20 mg/kg anti-VEGFR2 antibody (DC101) or 25 mg/kg cromoglicic acid (Cromo). BrdU was injected 12 h before sacrifice. We show that MC alter the proliferative and organizational state of endothelial cells (EC). MC dose-dependently induced EC-proliferation (HUVEC + MC: 158 ± 12 %; *p<0.05) and tube formation (HUVEC + MC: 290 ± 12 %; *p<0.05). In MC-deficient mice, tumor growth was reduced by 36 % (*p<0.05) and the efficacy of AAT was increased (WT + DC101: 1420 ± 134 mg; Wsh + DC101: 599 ± 107 mg; *p<0.05). Histomorphometric analyses unraveled that MC-deficiency decreased the numbers of mature pericyte-covered vessels by 80 % (*p<0.05) rendering them more prone for therapy. Indeed, an additive anti-angiogenic effect of MC-deficiency and AAT was observed resulting in reduced microvessel density (MVD) and tumor cell proliferation. This "angiosensitizing" effect could be abrogated by adoptive transfer of MC. In WT mice, AAT only initially reduced the proliferation of tumor vessels by 60 % (*p<0.05), a process that got reverted after long-term treatment. This pro-angiogenic rescue phenotype did not occur in MC-deficient mice. By blocking MC degranulation with Cromo we could increase the efficacy of AAT (DC101: 703 ± 48 mg; Cromo + DC101: 386 ± 92 mg; *p<0.05), leading to reduced vessel proliferation, MVD and tumor cell proliferation. Microarray analysis of tumor-resident MC unraveled increased expression levels of ECM-degrading *granzyme b* (*gzmb*) in response to therapy. We showed that MC-specific knock down of *gzmb* rendered tumors more susceptible for AAT and lowered the levels of alternative, pro-angiogenic mediators beside the VEGF-VEGFR2-axis in the tumor microenvironment. Our results indicate that tumor-resident MC interfere with AAT. We provide evidence that MC-derived *gzmb* liberates ECM-bound pro-angiogenic factors besides the targeted VEGF-VEGFR2 axis by fine-tuning vessel maturation and proliferation, which ultimately induces therapy resistance.

Poster M-I-07

Major heterogeneity in homing ability between different samples from patients with acute leukemias

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Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are hematologic malignancy associated with poor outcome. Acute leukemias are bone marrow diseases and leukemia proliferation as well as treatment response is influenced by the interaction between the leukemia cell and bone marrow. A better understanding of the interaction between tumor cell and bone marrow might enable novel treatment options to treat acute leukemias. Here we studied how PDX AML and ALL cells home to the bone marrow in the patient-derived xenograft (PDX) mouse model. We amplified primary tumor cells from several patients with AML or ALL cells in NSG mice and used genetic engineering to express transgenes in resulting patient-derived xenograft (PDX) cells. For enrichment of PDX cells from mouse bone marrow, murine cells were depleted using MACS followed by a FACS enrichment step targeting the mCherry transgene. A maximum of 4% of the injected PDX cells could be re-isolated from bone marrow 3 days after injection. The different samples showed highly different cell recruitment to bone marrow differing by more than 2 orders of magnitude with a minimum efficiency of only 0,01%. These data indicate that the interaction between the specific leukemic sample and murine bone marrow depends on specific characteristics of the individual samples. Homing efficiency depended on the cell number injected and was higher upon injecting high cell numbers. Across all samples, PDX ALL samples showed highly similar homing behavior than PDX AML samples and heterogeneity was higher within each disease type than between both disease types. Our data argue against the existence of a fix number of niche places present in the bone marrow of each NSG mouse, which are able to house PDX cells. Our data strengthen the assumption that the specific characteristics of the individual leukemia sample determines the ability of the sample to home to bone marrow.

Poster M-I-08

Digital PCR panel for sensitive hematopoietic chimerism quantification after allogeneic stem cell transplanation

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Monitoring of hematopoietic chimerism is a routine method to evaluate engraftment of donor cells after allogeneic stem cell transplantation. Various techniques have been used for chimerism quantification with PCR-based STR amplification (STR) being the most popular one. Whereas STR has high power of discrimination, its detection limit is restrained to ≥1%. Consequently, for the early detection of engraftment failure, secondary rejection or relapses, more sensitive techniques are needed. In this regard, digital PCR represents a promising alternative method, which combines excellent sensitivity (routinely ≤0.1%) with exact quantification and high reproducibility over a very wide measurement range (Stahl et al 2015). In addition, dPCR is easy to perform and requires only small DNA amounts (50-100 ng) for routine chimerism monitoring. Here we aimed at introducing a whole panel of digital-PCR based assays for routine chimerism quantification. Therefore, we investigated the suitability of 53 deletion/insertion polymorphisms (DIPs) for duplex analysis in combination with a reference gene and a Y-chromosome specific locus. 29 DIPs with high power of discrimination and good performance in duplex PCR were identified, optimized and technically validated. To verify applicability of the dPCR panel for diagnostics, we performed comparative analyses of artificial serial dilutions as well as patient samples in parallel with STR and qPCR. Finally, we developed a screening plate for initial genotyping with DIP-specific digital duplex assays for convenient use in routine diagnostics. In conclusion, we have established a complete, easy-to-use dPCR system for highly sensitive and precise measurement of hematopoietic chimerism. We propose that our system will be highly useful for follow-up diagnostics after allogeneic transplantation.

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Poster M-I-09

Clonal evolution in primary and relapsed follicular lymphoma: Insights from a mathematical model analysis

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Introduction: Germinal centers (GCs) are sites within secondary lymphoid organs that provide signals for B cells to become high affinity antigen-detecting cells. Somatic hypermutation (SHM) and clonal selection play a pivotal role in this optimization process. As a result, mutations in immunoglobulin (IG) genes are accumulated. Malignant transformation of GCs to follicular lymphoma (FL) is associated with genetic lesions. The disease can be controlled but not cured, with a median survival of approximately 10 years. Furthermore, most patients suffer from a relapse, in some cases additionally associated with transformation to more aggressive diffuse large B-cell lymphoma. **Objective**: In order to gain insight into processes underlying clonal evolution and lymphoma relapse, we applied a mathematical modeling approach. We particularly focused on the explanation of qualitatively different types of lymphoma evolution, as observed in FL patients, such as divergent, sequential and no evolution. **Materials and Methods:** We developed an agent-based model of physiological GC reaction to study the dynamics of GC expansion and B cell affinity maturation. We also applied our dynamical model to the situation of FL. We compared our simulation results to phylogenetic trees estimated from clinical measurements (sequences of IGHV rearrangements) of primary and relapse tumor in FL patients [1]. **Results:** Based on parameter changes in a single cell, representing the malignant transformation event, the model is capable of reproducing typical features of FL emergence and development. Specifically, different types of evolution can be explained. We identified distinct model scenarios for the "no evolution" case that predict either total absence of SHM in FL evolution or the existence of extra-follicular niche-like environments in which primary FL cells lie dormant for extended periods of time. For sequential and divergent evolution, the model predicts the SHM machinery to work at a significantly reduced rate during the period until relapse as compared to normal GCs. **Conclusion:** Our model is capable of reproducing both physiological GC reaction and transformation to FL. Different scenarios to explain lymphoma evolution need to be validated experimentally. Our findings also have potential clinical implications on e.g. immune therapeutic approaches.

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Poster M-I-10

Downregulation of AKT3 Increases Migration and Metastasis in Triple Negative Breast Cancer Cells by Upregulating S100A4

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Treatment of breast cancer patients with distant metastases represents one of the biggest challenges in today's gynecological oncology. Therefore, a better understanding of mechanisms promoting the development of metastases is of paramount importance. The serine/threonine kinase AKT was shown to drive cancer progression and metastasis. However, there is emerging data that single AKT isoforms (i.e. AKT1, AKT2 and AKT3) have different or even opposing functions in the regulation of cancer cell migration *in vitro*, giving rise to the hypothesis that inhibition of distinct AKT isoforms might have undesirable effects on cancer dissemination *in vivo*. The triple negative breast cancer cell line MDA-MB-231 was used to investigate the functional roles of AKT in migration and metastasis. AKT single and double knockdown cells were generated using isoform specific shRNAs. Migration was analyzed using live cell imaging, chemotaxis and transwell assays. The metastatic potential of AKT isoform knockdown cells was evaluated in a subcutaneous xenograft mouse model *in vivo*. We could show that depletion of AKT3, but not AKT1 or AKT2, resulted in increased migration *in vitro*. This effect was even more prominent in AKT2,3 double knockdown cells. Furthermore, combined downregulation of AKT2 and AKT3, as well as AKT1 and AKT3 significantly increased metastases formation *in vivo*. Screening for promigratory proteins revealed that downregulation of AKT3 increases the expression of S100A4 protein. In accordance, depletion of S100A4 by siRNA approach reverses the increased migration induced by knockdown of AKT3. In conclusion, our results show that knockdown of AKT3 can increase the metastatic potential of triple negative breast cancer cells and provides a rationale for the development of AKT isoform specific inhibitors.

Poster M-II-11

Extracting the key regulators of leukemia stem cell self-renewal using an advanced competitive in-vivo gain-of-function screen

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A growing body of evidence indicates that leukemia stem cells (LSC) can survive chemotherapy and initiate disease relapse in acute myeloid leukemia (AML). For development of novel targeted therapies it is therefore crucial to understand the mechanisms underlying LSC survival and self-renewal, stem cell properties shared with normal hematopoietic stem cells (HSC). To identify the key regulators of LSC/HSC self-renewal we assessed the potential of 64 candidate genes to enhance self-renewal in an advanced competitive *in vivo* screen. Candidate genes were selected based either on high expression in functionally validated sorted populations of LSC vs. non-LSC or densely interconnected genes derived from protein interaction analysis of LSC genes. In two parallel screens we transduced HSC (CB $Lin^-CD34^+CD38^-$) and progenitor (CB $Lin^-CD34^+CD38^+$) enriched fractions with 64 lentiviral vectors and assembled 16 pools, each consisting of 8 individual gene-transduced populations, that were transplanted into NSG (for 20w) or NSG-SGM3 (for 4.5w) mice, respectively. We implemented a small-scale unbiased barcoding approach to facilitate in depth analysis of an individual vector's contribution within a competitive gene pool by digital droplet PCR (ddPCR) and established a highly predictive titration protocol to obtain initially balanced cell pools. The transplanted pools (mean input %BFP^+: 13% and 28%) resulted in up to 69% BFP^+ human $CD45^+$ cells in the bone marrow and individual gene abundance reached up to 97% of BFP^+ cells. We observed multiple diverse patterns of competition and robustness across cell subsets, individual mice, pools and both screens. We developed a scoring algorithm that considers individual gene abundance, competition and robustness across pools and both screens. The 9 highest scoring genes were selected for individual evaluation *in vivo*. Of note, in the progenitor screen *HOXA4*, a known positive regulator of murine repopulating cells, received the highest score. In a first *in vivo* validation assay transducing $CD34^+CD38^+$ cells *HOXA4* outperformed all other candidates analyzed highlighting the power of our screening approach to find stemness regulators. Thus, we successfully developed an advanced competitive *in vivo* gain of function screen and thereby extracted potential regulators of self-renewal**.** Detailed functional studies on the candidate genes under investigation will uncover new therapeutic targets, setting the stage for eradication of LSC in AML.

Poster M-II-12

The majority of hematopoietic stem cells is dispensable for steady-state and stress hematopoiesis

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Hematopoietic stem cells (HSCs) are the most undifferentiated cells at the top of the hematopoietic hierarchy. The regulation of HSC pool size and its contribution to hematopoiesis are incompletely understood. We depleted long-term repopulating (LT)-HSCs in adult mice *in situ* and found that this population recovered from initially very low levels (< 1%) to below 10% but not more, of normal numbers. In spite of this persistent and massive reduction of LT-HSCs, however, steady-state hematopoiesis was unaffected and residual HSCs remained quiescent. Hematopoietic stress, although reported to recruit quiescent HSCs into cycle, was well tolerated by HSC-depleted mice and did not induce expansion of the small LT-HSC compartment. Hence, a contracted HSC compartment cannot recover *in situ* to its original size and steady-state blood cell generation can be achieved from less than 10% of normal LT-HSC number without increased contribution of these cells. Only upon 5-Fluorouracil treatment, hematopoiesis of HSC-depleted bone marrow collapsed demonstrating that LT-HSCs are crucial for re-initiation of hematopoiesis after efficient eradication of cycling progenitor cells.

Poster M-II-13

Modulation of hematopoiesis by the anti-oxidative enzyme Paraoxonase-2

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The enzyme Paraoxonase-2 (PON2) is one of three members of the paraoxonase family. Among other functions, PON2 grants robust anti-oxidative and anti-apoptotic benefit to tumor cells due to enhanced evasion from cell death and resistance to chemotherapeutics. The enzyme locates to the endoplasmic reticulum and mitochondria where it fulfills vital functions in the control of ROS generation. This is in line with the association of PON2 levels with response to front-line therapies in pediatric ALL and imatinib resistance in CML patients. These findings and the known impact of redox signaling on quiescence, apoptosis, differentiation and self-renewal of hematopoietic stem cells (HSCs) led us to investigate the role of PON2 in the hematopoietic system. First analysis showed severe alterations of the hematopoietic stem cell compartment, i.e. a significant increase in the LSK fraction as well as at the level of the LT-, ST-HSCs and MPPs and altered apoptotic rates especially in old $PON2^{-/-}$ mice. In line with the anti-oxidative function of the Enzyme there are enhanced ROS Levels in all fractions of the LSK population, what becomes most obvious at the level of MPPs which exhibit the highest PON2 expression rate within the LSK fraction in wild-type mice. Transplantation of old $PON2^{-/-}$ BMCs into young recipient mice resulted in decreased number of LT-, ST-HSCs and MPPs, that prompted us to more in-depth analysis. Reciprocal bone marrow transplantation assays revealed that PON2 functions through intrinsic cell signalling rather than the niche. Recently, competitive bone marrow transplantation assays, that display the functionality of HSC, exposed significant differences in multi-lineage reconstitution. To shed some light on, we treated $PON2^{-/-}$ mice with NAC (N-acetyl-L-cysteine) to uncover whether the observed effects are ROS-dependent. We detected that antioxidant-treatment lowers the effects caused by PON2 deficiency and thereby increased ROS level. Among other effects, the percentage of apoptotic LT- and ST-HSCs was significantly decreased in comparison to untreated $PON2^{-/-}$ mice. Collectively, these studies propose PON2 as crucial redox control enzyme in hematopoietic stem cells and potential target in anti-leukemia therapies.

Poster M-II-14

A double-edged sword: distinct role of the phosphatases STS1/STS2 in normal and malignant hematopoiesis

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The class III receptor tyrosine kinases (RTKs) FLT3 and c-KIT play a central role in hematopoietic stem and progenitor cell maintenance and proliferation. After binding to their natural ligands, these RTKs dimerize, become autophosphorylated and activate an intracellular signaling cascade. Negative RTK regulation by dephosphorylation, ubiquitination and degradation are equally important to prevent uncontrolled kinase activity and downstream signaling in normal hematopoiesis. Activating mutations in FLT3 (and more rarely in c-KIT) are found in more than 30% of acute myeloid leukemia (AML) patients and we investigated the role of negative regulators in normal and malignant hematopoiesis. Recently, we identified STS1/STS2 as the phosphatases responsible for the dephosphorylation of FLT3 and c-KIT (Zhang et al., Stem Cell Reports, 2015). STS1/STS2-deficient mice show a profound expansion of multipotent progenitor and lymphoid primed multipotent progenitor cells with elevated colony-forming capacity. Although long-term HSCs are not increased in numbers, lack of STS1 and STS2 significantly promotes long-term repopulation activity, demonstrating a pivotal role of STS1/STS2 in regulating HSC fitness. Mechanistic analyses identified STS1 (and to a lesser degree STS2) as a direct phosphatase of FLT3 *in vivo*. Loss of STS1/STS2 induces hyperphosphorylation of FLT3, enhances AKT signaling, and confers a strong proliferative advantage. These data demonstrate that STS1 and STS2 serve as negative regulators for wild-type FLT3 kinase activity and normal hematopoiesis. Surprisingly, loss of STS1/STS2 decreased the transforming potential of the oncogenic FLT3-ITD mutant. Specifically, although STS1 slightly dephosphorylates FLT3-ITD, the overexpression of STS1 enhances FLT3-ITD induced cytokine independent cell growth *in vitro* in a phosphatase-dependent manner. Bone marrow transplantation results demonstrate a collaboration of STS1 or STS2 with FLT3-ITD with increased engraftment *in vivo*. Mechanistic analyses indicate that differential phosphorylation and ubiquitination of other STS1 substrates may be involved. These data suggest two distinct roles of STS1/STS2 in the context of normal and malignant hematopoiesis. Importantly, these data may have therapeutic implication as the phosphatase inhibition of STS1/STS2 may both improve hematopoietic recovery of normal hematopoiesis and decrease the leukemic potential of FLT3-ITD-expressing blasts.

Poster M-II-15

Predicting CML residual disease in bone marrow by mathematical modelling

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Background: Cessation of tyrosine kinase inhibitor (TKI) therapy has developed into a feasible treatment option for patients with chronic myelogenous leukemia that present with sustained deep molecular remission over extended time periods. However, the relapse of about 50 % of the stopped patients indicates that residual CML stem cells remained. We have previously demonstrated that functional mathematical models are a valuable tool to describe the abundance of CML cell in the peripheral blood under TKI treatment while at the same time estimating the number of residual leukemic stem cells. As of now, we could not determine the uncertainty of these estimates.

Methods: We utilize a screen of 270,000 model simulations for different parameter values of our CML model to overcome the limitations in uncertainty approximation. For each individual set of patient data we use a likelihood ratio to determine a set of the best fitting model simulations.

Results: Based on the identified set of optimal simulations we can (i) determine an uncertainty of the model fit to the patient's peripheral blood data and (ii) approximate a distribution of residual leukemic cells for each time point during treatment. We use a standard deviation measure to describe and visualize this uncertainty. Based on the distribution of residual leukemic cells for an intended time point of therapy cessation we can derive an estimate for the patient's particular risk of relapse.

Conclusions: We have established a method to quantify the uncertainty of our model predictions with respect to predicting residual leukemic stem cell numbers in CML patients under long-term TKI therapy. The method is an important step in the development of tools to assist clinical decision making.

Poster M-II-16

Estimation of mesenchymal stem cells frequency in the bone marrow by combining genetic marking, next generation sequencing, and in vivo hematopoietic microenvironment transfer

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Mesenchymal stem cells (MSCs) have become attractive tools for cell therapy, but their actual numbers in the bone marrow (BM) remain obscure. MSCs can be identified functionally by their ability to transfer and maintain hematopoietic microenvironment (HME) in vivo. We reasoned that HME transfer in vivo combined with stable marking of individual MSCs could provide the possibility to assess MSC numbers. We applied a method of ectopic hematopoiesis foci formation combined with genetic barcoding and next-generation sequencing. Each long-term BM culture (LTBMC) was established from one BM plug of C57Bl/6xCBA F1 female mice (n=20). LTBMCs were transduced by a barcoded lentiviral library two weeks after BM explantation to the flasks. Two weeks later LTBMCs' adherent cell layers were implanted under the renal capsules of syngeneic mice (n=20). Six weeks later the developed ectopic foci were partially retransplanted (n=12). Some of the retransplanted foci (n=6) were further retransplanted for the second time after additional 6 weeks. DNA was isolated from LTBMCs, initial, secondary, and tertiary ectopic foci. Barcode sequences were determined on the Illumina platform. The inner cell mass of each initial ectopic focus and the corresponding bone shell contained 155±24 and 181±14 barcodes, respectively. Secondary ectopic foci contained 166±13 barcodes within the internal cell mass and 96±9 barcodes in the ossicles. The inner cell mass of one tertiary focus contained 87 barcodes. Together, this data indicate that roughly equal numbers of MSCs took part in differentiation into bone and maintenance of internal microenvironment. It also suggests that only about half of MSCs capable of transferring HME have a high proliferative potential sufficient for repetitive reconstitution of the BM stroma. Where possible total barcode numbers (internal cell mass + surrounding bone shell) were calculated for individual foci. Primary foci (n=7) contained 338±34 and secondary foci (n=4) 265±16 barcodes, further indicating that part of HME transferring cells has limited proliferative potential. These numbers were used to estimate frequencies of barcoded MSCs within the hematopoietic microenvironment ($\geq 3.3 \pm 1.2 \times 10^{-5}$). After correction for the transduction rate (app 25%) and partial transplantation of LTMBC, the actual frequency of MSCs could be approximated to 3 per 10^4 BM cells. In conclusion, this work provides evidence for the definite number of serially transplantable MSC in mouse BM.

Poster M-II-17

Functional activity of hematopoietic progenitor cells and CD34-positive cells in chronic myeloid leukemia patients with different response to imatinib therapy

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The problem of finding predictive test systems for quick assessment of resistance to TKI (tyrosine kinase inhibitors) remains open. Therefore, it appeared reasonable to compare the results of bone marrow (BM) cells cultivation with the number of CD34+ cells in patients with chronic myeloid leukemia (CML). It is believed that the reason of leukemic clone resistance to treatment with TKI during CML is mutations in the genome of early BM progenitors which are CD34+. Such cells, regardless of treatment, acquire ability to proliferation and differentiation, which leads to their re-expansion. We studied BM samples of 39 CML patients during the chronic phase of CML treated with imatinib for at least 12 months. Their average age was 54.0 ± 7.41 years. According to the criteria for assessing effectiveness of TKI therapy of The European Leukemia Net, patients were divided into 3 groups: with optimal (n = 15), suboptimal response (n = 10) and failure (n = 14) of the imatinib therapy. To determine functional activity of hematopoietic cells, we applied *in vitro* 13-day cultivating of BM mononuclear cells in semisolid agar with a use of RPMI-1640 medium, fetal calf serum and antibiotics. Clusters included no more than 40 cells; colonies consisted of more than 40 up to several hundred cells. Proliferative potential was determined as the ratio of colonies to clusters numbers. Analysis of cytometry results showed that with the acquisition of imatinib resistance the number of CD34+ cells increases. The number of these cells in BM and peripheral blood of the patients with different response to therapy was significantly different. Correlation analysis was conducted to identify correspondence between the number of CD34+ cells in BM and peripheral blood of patients treated with imatinib. These results indicate direct correlation between the number of colonies and clusters in culture and the number of CD34+ cells in patients' BM ($r = 0.83$ and $r = 0.58$, $p < 0.05$). Thus, the number of CD34+ cells in BM of patients increases with the acquisition of imatinib resistance. Correlation between the number of CD34+ cells and cell aggregates in culture *in vitro*, which may be used for predicting the future course of CML, shows prognostic value of determining CD34+ cells in patients' BM; parallel increase of their number in the peripheral blood will allow developing express methods for the detection of individual patient's response to imatinib therapy.

Poster M-II-18

Assessment of the phenotypic and functional characteristics of hematopoietic progenitor cells in patients with chronic myeloid leukemia

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The mechanism of chronic myeloid leukemia (CML) progression was postulated basing on in vitro modeling even before it was detected in clinical practice. It is known that after CML therapy with tyrosine kinase inhibitors (TKI) relapse is observed in 59%. Such high relapses level occurs due to persistence of quiescent CD34+ hematopoietic stem cells (HSCs). In this study we analyzed phenotypic and functional characteristics of HSCs and progenitors with respect of correlation with CML progression in each individual patient. Totally 51 bone marrow (BM) samples were analyzed from patients with CML in chronic phase treated with TKI. BM samples of 13 patients were analyzed before they started TKI treatment. Expression of surface markers CD34, CD95, Bcl-2, Pgp-170, and Ki67 side by side with CFU assay was investigated. BM mononuclear cells were stained with monoclonal antibodies. Cytometry study was performed using flow cytometer FACScan. Cells were cultivated for 14 days in RPMI-1640 with Bactoagar and GM-CSF. Correlation analysis performed individually for patients independently of response to TKI therapy demonstrated negative correlation ($\rho = -0.7648$) between the number of Ki67+ and CD34+ cells. Negative correlation was also estimated for co-distribution of Ki67+ and CD34+CD95– cells ($\rho = -0.8556$), but positive correlation was found for Ki67+ and CD34–CD95+ cells ($\rho = 0.7363$). No correlation was obtained between Ki67+ and CD34+CD95+ cells in patients' BM ($\rho = 0.0424$). CFU assay indicated significant difference in clonogenic potential between groups of patients with different response to therapy ($p < 0.05$). As to colony to cluster ratio our results showed correlation ($\rho = 0.6783$) between index and number of cells with Philadelphia chromosome. To have a possibility of identification of cells composing aggregates obtained in CFU assay, positive correlation was also found between numbers of colonies, formed during cultivation, and level of CD34+ cells ($\rho = 0.4658$), between number of clusters and CD33+ cells ($\rho = 0.7612$). Strong negative correlation was indicated between CD34+ and Ki67+ cells, meaning that with increase of CD34+ cells number, higher proportion of them are Ki67– and can be indicated as quiescent. Even more, those cells were CD95–. In our study we also defined positive correlation between mitotic index and percentage of Pgp+ cells. This finding also supports assumption regarding involvement of both BCR-ABL dependent and independent mechanisms in CML progression.

Poster M-II-19

HOXB4 acts as a fate determinant in hemangioblasts by promoting hematopoietic specification

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Generation of hematopoietic stem cells (HSCs) from pluripotent stem cells, in vitro, holds great promise for future gene and cell therapy. To date, the generation of HSCs remains challenging and has primarily been achieved in mouse cells by ectopic expression of the homeotic selector protein HOXB4. The exact cellular stage at which HOXB4 enhances hematopoietic development, in vitro, is not yet known. However, its identification is a prerequisite for unambiguously identifying the molecular circuits controlling HSC development. To identify this cell population and the mode of action of HOXB4, we retrovirally expressed HOXB4 in differentiating mouse embryonic stem cells (ESCs). Through the use of Runx1(-/-) ESCs containing a Doxycycline-inducible Runx1 coding sequence, we uncovered that HOXB4 promotes the formation of the hemogenic endothelium from which hematopoietic stem and progenitor cells arise. Furthermore, we show that HOXB4 does so by priming bipotent hemangioblasts towards the hematopoietic fate. Thus, HOXB4 acts as a hematopoietic cell fate determinant in hemangioblasts.

Poster M-II-20

Fetal microchimerism is associated with lower risk of aGVHD after haplo BMT.

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Introduction: Haploidentical transplantation of hematopoietic stem cells (haplo-HSCT) is a feasible therapeutic approach in patients lacking an HLA-identical donor. However, high risk of graft-versus-host disease (GVHD) is among major complications in haplo-BMT. Currently, there exist some data concerning associations between maternal microchimerism levels (detection of maternal cells in recipient's blood) and increased risk for GVHD development. Meanwhile, the effects of microchimerism upon GVHD probability are still controversial. The aim of our study was to adapt a technique for quantitative evaluation of fetal (recipient-cell) and mathernal (donor-cell) microchimerism and investigation of its effects upon actual risk of common complications after HSCT.

Methods: Determination of microchimerism was carried out by real-time alle-specific polymerase chain reaction (AS RQ-PCR). The analytic panel included 20 InDels markers located at different chromosomes. Evaluation of PCR sensitivity was carried out using DNA mixtures from several established cell lines (HL60, K562, 293T, MOLT3, A549) at serial dilutions. The samples from 20 donor-recipient pairs were analyzed. The patients' age ranged from 2 to 27 years.

Results: The sensitivity thresholds for the InDel-based test panel ranged from 10^- 4 to 10^-5. We have found out that detection of fetal microchimerism in the donor organism is associated with lower risk, or lower degree of acute GVHD ($p=0.01$). Moreover, we observed a trend to higher timeframe for transplant engraftment, and lower probability of full donor chimerism achievement, if the donor exhibited fetal microchimerism ($p=0.12$). The patients in our setting transplanted from donors with detectable fetal microchimerism had a tendency to a higher overall survival ($p=0.14$). Hovewer, we did not reveal any significant association between microchimerism levels (fetal and maternal), and higher probability of chronic GVHD development, as well as any differences in other posttransplant complications for recipients with maternal microchimerism.

Conclusions: Evaluation of fetal microchimerism may be considered a useful and informative approach to selection of potential donors, or a method of GVHD prediction in haploidentical HSCT.

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Poster M-II-21

Monitoring of Calreticulin mutations and hematopoietic chimerism by digital PCR after allogeneic stem cell transplantation in patients with myelofibrosis.

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Myelofibrosis (MF) is a myeloproliferative disorder often associated with poor prognosis. Allogeneic stem cell transplantation (aSCT) remains the only potentially curative treatment for MF. Mutations in JAK2, MPL, and Calreticulin (CALR) have been described as initiating events in its pathogenesis. Molecular monitoring for minimal residual disease (MRD) after aSCT has been suggested as a vital tool to guide possible adoptive immunotherapy. CALR mutations occur in 30% of all MF patients and 80% of JAK2- and MPL-negative patients. Two types were identified: type 1 represents a 52-bp deletion producing the protein change p.L367fs*46 and type 2 results from a 5-bp insertion (p.K385fs*47). To monitor MRD we here used digital-PCR techniques recently described for CALR type-1 (Mansier et al) and type-2 (Badbaran et al) mutations. MRD data was compared with chimerism data, also obtained by digital PCR. Out of 143 patients with MF, 92 were JAK2V617F positive, 4 MPL positive and 35 CALR positive (21 harbored type-1 and 8 the type-2 mutation). Samples for follow-up analyses were available for eight patients with type-1 and seven with type-2 mutation. Dynamics of CALR mutation levels after aSCT were in excellent agreement with clinical disease status. For CALR type 1, three patients reached MRD negativity within two months and remained in stable complete molecular remission (CMR) since. In the other five patients, dPCR revealed increasing levels of CALRt1-positive cells indicative of MRD. In four of them, ceasing immune suppression was sufficient to establish CMR and full chimerism. In contrast, the fifth patient required therapeutic intervention (HSC boost) due to a high increase (>30%) in CALRt1-positive cells, but went into CMR thereafter. Similar data was found for CALR type 2. Three patients became MRD-negative after 3 and two after 6 months. All remained in CMR since. The two other patients required intervention due to increasing numbers of CALRt2-positive cells in conjunction with decreasing chimerism. While one patient achieved CMR after ceasing immune suppression, the other eventually relapsed, but was successfully treated with a second aSCT and is now in CMR. In conclusion, both type-1 and type-2 CALR mutations represent sensitive MRD markers for follow-up diagnostics after aSCT. Digital PCR is an excellent method for precise monitoring of CALR mutations and chimerism.

Poster M-II-22

Knockdown of the leukemia stem cell marker GPR56 impairs normal and leukemic engraftment capacity

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Knowledge about the differences and similarities between normal and malignant hematopoietic stem and progenitor cells (HSPCs) is crucial for the development of more efficacious anti-leukemic therapeutic approaches. While the novel leukemia stem cell marker G-protein coupled receptor 56 (GPR56) has been attributed a role in homing in mice its functional role in the human hematopoietic system and leukemia has not been characterized. Here we knocked down GPR56 via lentiviral shRNA vectors in cord blood (CB) CD34+ and AML cells to determine its impact on in vitro and in vivo functional characteristics of these cells. The fraction of GFP+ CD34+ and AML cells infected with two different shRNA vectors against GPR56 was rapidly lost in vitro, whereas GFP+ cells infected with shLuciferase control vector were stably maintained over time. Furthermore, colony formation capacity of CD34+ cord blood cells, in particular the formation of the more immature GEMM and BFU-E colonies, was severely impaired upon knock-down (KD) of GPR56. Moreover, the repopulating potential of CB CD34+ and AML cells was significantly impaired in cells with near complete KD of GPR56 upon transplantation in NSG mice. Moreover, the lympho-myeloid-lineage-ratio in NSG mice engrafted with CB CD34+ cells was shifted to the advantage of the myeloid lineage when harvesting human cells from mouse bone marrow 20 weeks post transplantation of GPR56 KD HSCs compared to controls. In line with these observations we found enrichment for GO terms connected to the regulation of hematopoiesis, apoptosis, and survival when analyzing RNA-Seq data obtained from CB CD34+ cells upon GPR56 KD vs controls. In summary, our findings indicate that GPR56 might play an important role in the maintenance of the multipotent undifferentiated state of human HSPCs and GPR56 positive AML.

Poster M-II-23

Models to study Thpo/Mpl signaling in normal and pathological hematopoiesis

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The hematopoietic cytokine Thrombopoietin (THPO) regulates hematopoietic stem cell (HSC) self-renewal, maintenance and megakaryocyte (MK) differentiation via binding its receptor Mpl. Dimerization of Mpl after Thpo binding activates the Janus kinase 2 (JAK2) and signaling via the three main pathways Stat3/5, PI3K/Akt and MapK/Erk. Loss of function mutations in patients and mice cause thrombocytopenia and aplastic anemia. In contrast, activating mutations in MPL induce myeloproliferative disorders (MPN). In an attempt to correct Mpl-deficiency in the Mpl-/- mouse model we showed that Mpl expression has to be well controlled and directed to the physiological target cells, namely HSC and MK. To achieve this we developed a lentiviral vector platform for controlled expression in HSC and MK by the use of lineage-specific promoters and studied the effects of Mpl expression after transplantation of transduced bone marrow cells in various models. Ectopic Mpl expression by viral promoters on non-target cells acted in a dominant-negative fashion by receptor competition for Thpo binding, a phenotype that could be mimicked by overexpression of an intracellular truncated and therefore signaling deficient Mpl in wildtype mice. In addition, high Mpl expression in HSC and MK of Mpl-/- mice caused aberrant megakaryopoiesis and marrow fibrosis without correcting the thrombocytopenia probably due to abnormal Mpl signaling in MK. To dissect Mpl signaling we generated Mpl mutants by exchange of tyrosines to phenylalanine at positions 582, 616 and 621, three important phosphorylation sites of Mpl that are also recognized by negative regulators such as SYK and SHP-1. Mutation of Y616 reduced Mpl-induced phosphorylation of Erk, Stat5 and Akt similar to Mpl mutants with truncations of the C-terminal 53 or 69 amino acids, while mutations of Y582 and Y621 increased signaling of all three main pathways by >50%. In agreement with that, MplY616F did not correct MK differentiation of Mpl-/- BM cells in vitro, while lentiviral expression of MplY582F and MplY621F enabled MK differentiation with almost the same efficiency than wildtype BM cells in vitro. MplY582F and MplY621F were still dependent on Thpo induction, in contrast to activating mutations in Mpl, such as W515 or S505. Retroviral overexpression of MplW515L in mice by strong promoters induced MPN within three weeks. In an attempt to develop a murine model that resembles the human situation more closely we expressed MplW515L by the glycoprotein Iba promoter in BALB/c mice. Mice developed progressive MPN without succumbing to the disease symptoms which allowed the study of the long term consequences of constitutive active Mpl overexpression, such as effects on the stem and progenitor cell pool.

Poster M-VII-24

Associations of recipient's bone marrow stromal cells functional characteristics with post-transplant hematopoiesis reconstitution kinetics.

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Introduction. Deficient graft functioning observed in some cases necessitates development of functional tests for the stromal cells, in order to provide clinical indications for co-transplanting of hematopoietic and bone marrow stromal cells (BMSC), and evaluable introduction of alternative therapeutic approaches. The aim of this study was to investigate the role of bone marrow stromal cells in the course of donor marrow cells engraftment and their significance in post-transplant complications. Materials and methods. The study included clinical observation of the post-transplant course in ten patients with acute myeloid leukemia (AML) and 7 healthy donors. Bone marrow nucleated cells were selectively harvested prior to BMT and followed by monolayer culture. Upon growth of fibroblast-like cell colonies (CFU-F), their hematopoiesis-supporting activity was determined, as well as their differentiating ability along adipogenic and osteogenic pathways. We have also analyzed the relative expression of Selectin and CXCR4 genes in these cells. Results. When comparing functional characteristics of BMSC from healthy donors and AML patients, an increased hemostimulatory activity of the latter was noted, as reflected by an increase in large and small CFU-GM numbers ($p < 0.02$). In addition, an increase in differentiation along adipogenic and osteogenic pathways was observed in AML patients ($p = 0.03$). Moreover, the number of CFU-Fs, capable for adipogenic differentiation was inversely correlated with platelet recovery time ($p = 0.05$). In contrast, higher numbers of osteogenic colonies in culture were associated with an increased time to leukocyte lineage recovery ($p = 0.05$). When analyzing gene expression in BMSC population, a decreased expression of the CXCR4 gene responsible for the homing effect, with age of the patient's ($p = 0.05$) was noted. The Selectin gene expression in BMSC was higher by AML patients as compared to healthy donors. Conclusion. Stromal cells derived from the patients' bone marrow exhibit higher proliferative activity and marked expression of molecules mediating HSC homing, as compared with a group of healthy donors. BMSC from AML patients taken before bone marrow transplantation are characterized by a more pronounced capacity to osteogenic and adipogenic differentiation than those from healthy donors. Increased adipogenic differentiation ability of the BMSCs is associated with a more rapid recovery of hematopoiesis.

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Poster M-VII-25

mRNA transfection of hematopoietic stem cells with a TALE nuclease provides efficient knockout of HIV co-receptor CCR5 in HSC

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Aim: To assess efficiency of CCR5-Uco-TALEN mediated CCR5 gene knockout in hematopoietic stem cells (HSC).

Introduction: The case of successful allo-HSCT in an HIV-infected patient ("Berlin patient") from a CCR5Δ32 -homozygous donor triggered development of novel approaches in HIV treatment, particularly HSCT-based cellular therapy with site-specific genome editing targeting the CCR5 gene. HSCs are among the most promising targets for gene modifications, since they comprise a multipotent progenitor population of stem cells giving rise to all kinds of blood cells. Thus, new methods of HSCs genome editing with using designer nucleases may have potential applications in gene therapy of HIV.

Materials and methods: In-vitro transcription of mRNA for CCR5-Uco-TALEN and eGFP was performed with T7-mScript Standard mRNA Production System on the DNA-plasmids template. CD34+ cells were isolated by MACS and resuspended at $1x10^6$ cells/ml in StemSpan Medium supplemented with 1% Sodium Pyruvate, 2% L-Glutamine and cytokines: IL-6, SCF, TPO and FLT3-ligand. HSC were prestimulated for 24 h at 37°C. mRNA electroporation was performed using 25 μg of mRNA for each TALEN arm and 2,5 μg for eGFP. Thereafter, cells were incubated at 32°C for 24 h, and transfection rates were estimated by flow cytometry (FC) based on eGFP expression. CCR5 gene editing efficiency was assessed by droplet digital PCR (ddPCR). Colony-forming ability of transfected hematopoietic progenitors was evaluated using MethoCult medium.

Results: To adapt the mRNA-electroporation protocol for HSC we determined optimal electroporation conditions for eGFP mRNA transfection on UKE-1 cells: 300V, 10ms, 1 pulse. As determined for eGFP by FC analysis, mRNA transfection facilitated efficient (up to 90%) transient gene expression in HSC. In HSC transfected with mRNA for CCR5-Uco-TALENs gene editing was achieved in up to 38% of CCR5 alleles as determined by ddPCR results. We did not observe any impact of the gene-editing procedure on the ability of manipulated HSC to form colonies in methylcellulose.

Conclusions: Here we show that CCR5-Uco-TALEN, successfully used in T lymphocytes, does also facilitate efficient CCR5-gene editing in hematopoietic stem cells without affecting their CFU potential. Also, we confirm that mRNA electroporation represents an efficient means for transient expression of designer nucleases.

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Poster M-VII-26

Multiple genes within the proximity of a retroviral insertion site may enhance the competitive advantage of hematopoietic cells

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Retroviral insertional mutagenesis (RIM) is a known risk factor in gene therapy trials, but also a powerful tool for identifying novel oncogenes and cooperative pathways. However, focusing only on the gene closest to the insertion site may lead to underestimation of RIM effects, as deregulation of distal and/or multiple genes by a single retroviral insertion site (RIS) was reported in multiple studies. We hypothesized that multiple genes within a defined region of a vector insertion site could influence the outcome of RIM. In a proof-of-principle study, we examined the ±150 kb region of a common RIS, the Bcl-xL locus, and found eight protein-coding genes. A conventional transplantation setting would require high numbers of experimental mice to study the effects of these eight genes in hematopoietic stem cells (HSCs). Therefore, we developed a multiple-competitors' competitive repopulation (MCCR) assay to simultaneously screen the effects of multiple genes on HSCs engraftment in vivo. In short, HSCs transduced with individual, but distinct genes were pooled prior to transplantation. The composition of each HSCs population in recipient animals was later determined by transgene-specific primers using quantitative PCR. The MCCR assay revealed two potential competitive fitness-enhancing genes within the Bcl-xL locus – Bcl-xL and Tpx2. Bcl-xL is an anti-apoptotic member of the Bcl2 family. Overexpression of Bcl2 and Bcl-xL is known to enhance cell survival and increase HSCs number and repopulating potential. Tpx2 has important roles in mitosis and spindle assembly. Tpx2 is expressed in a wide variety of tumors and is a known collaborating partner of the proto-oncogene Aurora A kinase. Interestingly, the Id1 gene, which was previously shown to be important for HSCs maintenance, was outcompeted in our MCCR assay. Confirmatory HSCs transplantations with Id1- and Bcl-xL-transduced HSCs demonstrated that Id1-overexpressing HSCs failed to sustain long-term hematopoiesis. In contrast, Bcl-xL overexpression sustained long-term hematopoiesis and counteracted the negative influence of Id1 when co-expressed. This work highlights the importance of studying multiple genes located within the proximity of an RIS to uncover all important players in RIM events. The MCCR assay developed in this study provides a platform for screening multiple genes in HSCs transplantation studies and supports the 3R principle by reducing the numbers of required recipient animals.

Poster M-VII-27

Simultaneous genetic barcoding of the hematopoietic system with different lentiviral vector constructs to evaluate genotoxicity

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Introduction: Based on their ability to stably integrate in their host cells' genome, retroviral vectors have successfully been used for gene marking as well as gene therapy, mainly in the hematopoietic system. However, γ-retroviral vectors have been shown to influence the fate of hematopoietic stem cells (HSC) by insertional mutagenesis, whereas lentiviral vectors have a less genotoxic insertion profile. In order to establish the optimal vector for neutral cell marking to study the reconstitution of the blood in mice after stem cell transplantation, we made use of different lentiviral vectors equipped with genetic barcodes (BC). The use of our multicolor barcode system enables us to study all vector constructs in parallel.

Methods: We constructed lentiviral marking vectors with diverse promotors driving different fluorescent proteins (FP) and one promotor-deprived construct. In each vector, one of our BC32-sequences flanked by sequences for Illumina-Adaptors was introduced. Lentiviral vectors were generated and used to transduce lineage-negative bone marrow cells from male CD45.1 donors. Prior to transplantation into lethally irradiated female CD45.2 recipients, cells independently transduced were mixed to compose one graft with up to three different constructs. Mice were observed for 8-11 months. Monthly blood samples (PB), as well as samples of PB and the hematopoietic organs from the final analysis, were analyzed by flow cytometry (FC) and next generation sequencing (NGS) for barcode identification.

Results: The complexity of each plasmid library was up to $4x10^6$ different BCs. Transduction rate was kept low to avoid multiple vector insertions per cell. FC analysis revealed stable engraftment (>90% chimerism) and decreasing levels of FP expression over time, whereas as preliminary NGS data from the first cohort revealed stable, polyclonal, marking with an average of 930 BC's per sample (average 220 - 493 BC's per construct). However, in most samples 20 or less BC's contributed >75% of read counts. Most barcodes, which were dominant at the final analysis, were already detected in early PB samples. Analysis of more animals is currently ongoing.

Conclusion: Our multicolor barcode system is a suitable tool to follow-up clonal dynamics of different vector constructs. The similar structure of the different "colored" barcodes allow for simultaneous analyses and comparison of different vector constructs in one sample.

Poster M-VII-28

Integrative Analysis of LincRNA Expression in 922 Acute Myeloid Leukemia Patients Reveals Multiple Prognostic Gene Signatures

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Background: Acute myeloid leukemia (AML) is a heterogeneous myeloid neoplasm that develops in hematopoietic stem and progenitor cells (HSPCs). The karyotype and recurrent lesions in *NPM1*, *FLT3-ITD* and *CEBPA* are associated with patient remission, relapse and survival and have been integrated into AML prognostic indices such as MRC and ELN. However, the utility of these guidelines for clinical decision making has only been validated in the ~40% of patients younger than 60 years. The human genome encodes a relatively small number of coding and a larger number of non-coding transcripts. The association between the coding genome and the biology, pathogenesis and clincal parameters in AML have been extensively studied. However, the contributions from the large pool of non-coding RNAs to these events is largly unknown.

Aims: Long intergenic non-coding RNAs (lincRNA) are emerging as key regulators of molecular processes including stem cell activity and function, but their role in AML is poorly understood. We aimed to:

1. Comprehensively describe and evaluate the expression of lincRNAs in large and clinically annotated AML cohorts, AML cell lines and in normal HSPC subsets.

2. Develop and validate lincRNA signatures as robust predictors of patient survival across multiple large patient cohorts, requiring the measurement of a small numbers of genes.

Methods: We analysed expression profiles of lincRNAs measured by three technologies in 922 patients from three independent AML patient cohorts (USA: The Cancer Genome Atlas, NL: HOVON, GER: CALGB) and sourced a fourth independent AML dataset in Australia to experimentally validate our findings. We also analysed expression profiles in normal hematopoietic cells (BLUEPRINT) and AML cell lines (Cancer Cell Line Encyclopedia).

Results: We developed and validated a novel approach to quantify the expression of 1664 lincRNAs in 736 AML patients from 3 patient cohorts representing the largest resource of lincRNA expression profiles available in AML (Figure 1a). Interrogation of this database (i) reconfirmed previously known regulatory relationships and uncovered lincRNAs in close proximity to key haematopoietic genes that showed strong expression correlations across AML datasets. We identified that distinct lincRNAs expression profiles are (ii) associated with recognized cytogenetic and mutational subgroups of AML (Fig 1b) and (iii) are expressed in a cell type specific manner (Fig 1c). We also (iv) identified 48 lincRNAs that were significantly correlated with patient outcomes and (v) expression signatures, composed of 2-4 lincRNAs, that have prognostic value in addtion to current clinical risk algorithms based on karyotype and mutations (vi) that these signatures can be measured by RT-PCR and used as a standalone tool or in

Poster M-VII-29

Optimization of barcode construct: the longer, the better

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Introduction: Genetic barcodes (BC) are a promising tool for tracking cell clones not only in vitro but also in vivo. The uniform barcode length allows for a more evenly amplification and, furthermore, for a quantitative assessment of clonal contributions in a marked cell population. The quantification of clonal contribution depends entirely on counting barcode-containing reads after next-generation sequencing (NGS). To our knowledge, data about detailed and systematic analyses of this procedure is very limited. We used our recently published BC16 system (consisting of 16 random nucleotides) to analyse recurrent amplification bias and possible error-sources. To overcome observed limitations of the BC16 system, we doubled its length and improved our BC design.

Methods: Barcoded and sanger-sequenced 293T cell clones were evenly mixed to establish so-called miniBulks, containing 4 or 5 different clones for a systematic and repeated analysis. We successively reduced the number of PCR steps to analyse the influence of the PCR and used additionally restriction digest and artificially generated 1kb fragments as templates to study the impact of genomic accessibility of the BC sequences. Furthermore, we prolonged our BC16 construct to 32 nucleotides (BC32) and additionally equipped the vector backbone with truncated Illumina-Adaptor sequences to facilitate amplification and reduce PCR steps.

Results: The shorter BC16 construct resulted in heavily uneven BC abundances after the standard protocol for PCR and NGS. Surprisingly, two of the five BC16 barcodes, due to a double integration are carried by one particular cell-clone, also resulted in a final uneven read-count distribution. This was not improved by a reduction of PCR cycles or enhanced genomic accessibility. In contrast to these results, our newly developed BC32 construct showed much more balanced read-count abundances for the same experimental settings. Furthermore, we conducted spike-in experiments, which also resulted in a balanced read-count distribution for all BC32 barcodes even down to a dilution of 0.01%.

Conclusion: Our new BC32 construct shows superior performance in terms of BC quantification after amplification from genomic DNA. Especially the balanced recovery of the BCs only present in 0.01% of a cell population is of particular interest. Finally, we conclude that validation experiments for BC quantification are ultimately required before BCs are used in a particular experimental setting.

Poster M-VII-30

Anticancer marine natural compounds

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In the last decades, marine organisms have served as a source of new potent anticancer drugs [1]. We investigated anticancer activity of three novel marine natural compounds *in vitro* and *in vivo* models of urogenital tumors. Monanchocidin A [2] - an alkaloid from sponge *Monanchora pulchra* – was found to be active against cisplatin-resistant germ cell tumor (GCT) cells, drug-resistant prostate and bladder cancer. In contrast, non-malignant cells were less sensitive. Monanchocidin A was highly synergistic with cisplatin in GCT cells. Its mechanism of action includes autophagy and lysosomal membrane permeabilization, while apoptosis is of minor importance. Antimigratory activity was predicted by proteomics-based approach and validated in functional assays. Rhizochalinin [3] – a sphingolipid-like compound from sponge *Rhizocahlina incrustata* – was evaluated in castration-resistant prostate cancer (CRPC) models. Rhizochalinin significantly reduced cell viability at low micromolar concentrations showing most pronounced effects in AR-V7 positive (enzalutamide and abiraterone resistant) cells. Caspase-dependent apoptosis, inhibition of pro-survival autophagy, downregulation of AR-signaling as well as inhibition of voltage-gated potassium channels were identified as mechanisms of action. Remarkably, Rhizochalinin re-sensitized AR-V7 positive cells to enzalutamide and increased efficacy of taxanes. *In vivo*, rhizochalinin significantly reduced growth of androgen-independent tumors with an increased fraction of tumor cells showing apoptosis, while no severe side effects were observed. Frondoside A (FrA) [4] – a triterpene glycoside isolated from sea cucumber *Cucumaria frondosa* – was tested in CRPC cell lines *in vitro* and *in vivo*. FrA induced cell cycle arrest and caspase-dependent or -independent apoptosis. Global proteome analysis revealed regulation of keratin 81, CrkII, IL-1β and cathepsin B. Inhibition of pro-survival autophagy was observed following FrA exposure. *In vivo*, FrA reduced tumor growth of PC-3 and DU145 cells with a notable decrease of lung metastasis, as well as circulating tumor cells in the peripheral blood. In conclusion, we were able to identify three interesting marine substances with unique mechanisms of action for the treatment of urogenital malignancies. In order to promote their clinical development chemical synthesis from commercially available reagents as well as the "hit to lead" drug development step, are currently performed.

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