Programme / Abstracts
XXII Wilsede Meeting

Modern Trends in Human Leukemia & Cancer

June 30 – July 03, 2018 – Wilsede, Germany

We cordially thank for supporting the Wilsede Meeting

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

We are very happy to keep up the tradition of more than 30 years of Wilsede meetings focusing on recent developments in the field of leukemia and cancer biology. Tumor heterogeneity as well as the concepts of normal and malignant stem cells will be central themes of this year's meeting. Myeloproliferative neoplasias reflect many general aspects of malignant transformation and progression, but also stem cell biology. Therefore, MPNs represent another major topic of the XXII. Wilsede meeting. Traditionally, translation of basic findings into targeted therapies is a recurrent subject at Wilsede meetings. This year the meeting will particularly highlight the recent progress in immunotherapy – from checkpoint inhibitors to gene-modified lymphocytes. The impressive progress made in these different areas will be presented by experts in their respective fields, but also junior scientists that are embracing new technologies to bring new light into the open questions and problems. We thank all speakers and chairs for donating time and sharing results that allows this unique platform of scientific exchange.

Meetings do not happen without financing. Thus, we would like to gratefully acknowledge the continued support of Mildred Scheel Foundation of the Deutsche Krebshilfe e.V. (German Cancer Aid). The commitment of the Deutsche Krebshilfe to be our main sponsor and to finance stipends for young scientists has been vital to keep up the tradition of providing in Wilsede a platform for both "young" and "experienced" scientists to interact. We are also grateful to the Deutsche Forschungsgemeinschaft, another traditional supporter of the Wilsede meetings.

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. In particular, the never-ending energy of Prof Rolf Neth, who was the initiator and driving force of the Wilsede meetings, as well as Prof Axel Zander, who carried the baton from the 1990ies to the 2000s, deserve special thanks. In the first decade of this century, Dr Carol Stocking was the key person to keep the tradition alive.

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 responsibilities for a few days, and join us during the lectures, poster sessions, walks in the heath, and evenings with food, drink, and music to discuss scientific concepts and new ideas.

Welcome to Wilsede!

Boris Fehse Nicolaus Kröger

The Local Organizing Committee

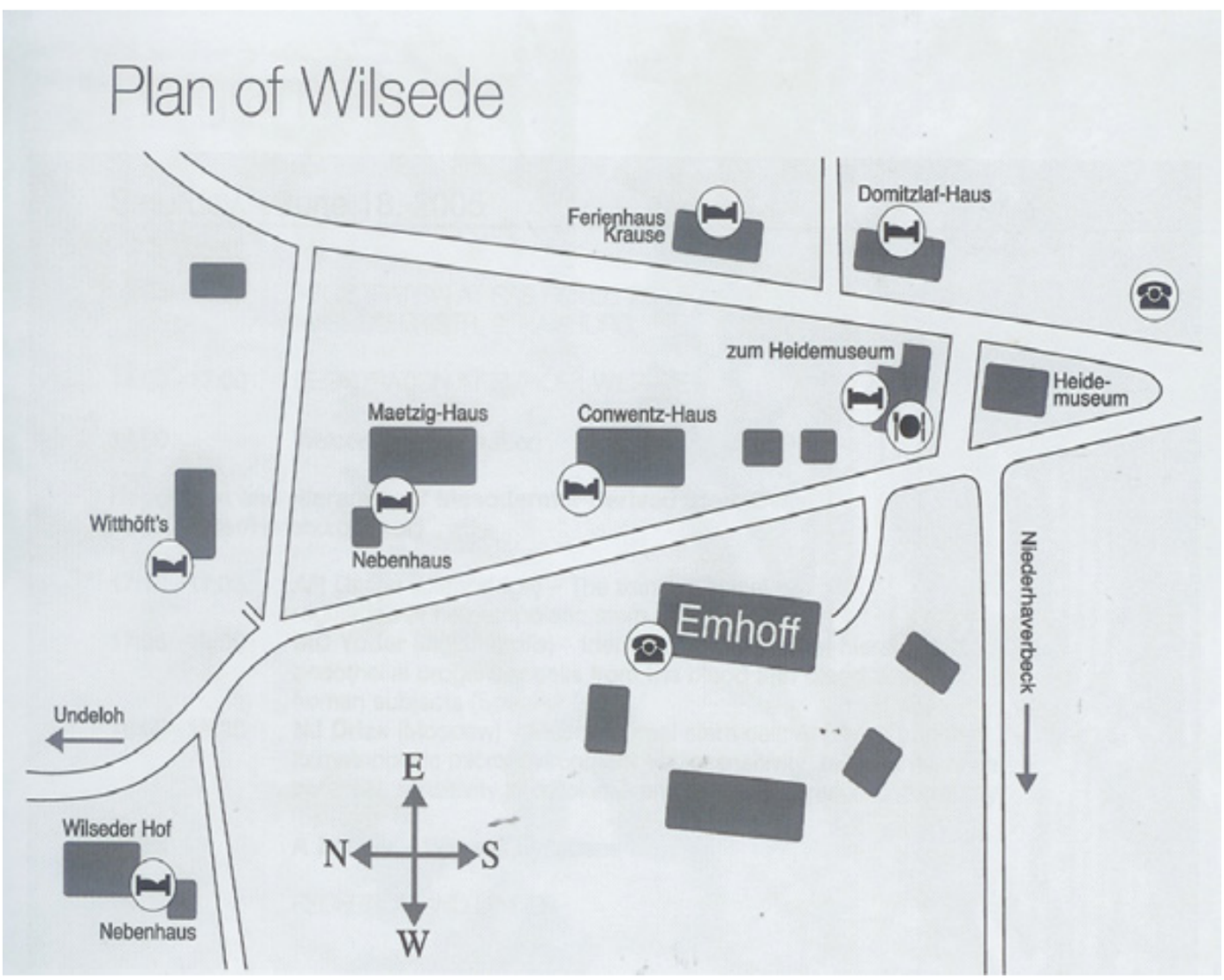
Facts

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 nor motorized vehicles are allowed, Wilsede can only be reached by foot, horse carriages, or bicycle. It has been the site of the »Wilsede Meetings: Modern Trends in Human Leukemia« since 1973, gathering Hematologists 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 was to be 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 doubly precious.



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Organization and Support

Scientific and Organizing Committee

Dr. rer. nat. Maike Buchner
Technische Universitaet Muenchen
Institute for Clinical Chemistry and Pathobiochemistry

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University Medical Center Hamburg-Eppendorf
Dept. of Stem Cell Transplantation
Research Dept. Cell and Gene Therapy

Dr. med. Michael Hudecek
University Medical Center Wuerzburg

Prof. Dr. med. Nicolaus Kröger
University Medical Center Hamburg-Eppendorf
Center of Oncology | Dept. of Stem Cell Transplantation

Dr. med. Oliver Weigert
LMU Munich

Main-Sponsor

Deutsche Krebshilfe e.V.

Co-Sponsors

Deutsche Forschungsgemeinschaft (DFG)

University Medical Center Hamburg-Eppendorf (UKE)

Meeting Office | Contact

CSi Hamburg GmbH
Conferences | Symposia | Incentives
Goernestraße 30
20249 Hamburg, Germany
Phone: +49 40 30770300

On-site Registration · Emhoff/Wilsede

Contact

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On-site Registration

June 30, 2018 Campus Lehre, Building N55 University Medical Center Hamburg-Eppendorf (UKE)	09.00 to 14.00
June 30, 2018	14.00 to 17.00
July 01-02, 2018	08.00 to 18.00
July 03, 2018	08.00 to 12.00

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 09.00
Registration and pick up of your meeting documents

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 (Timetable)

June 30, 2018 (Arrival)

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

July 01 & 02, 2018

08.00	from Undeloh	to Wilsede
22.30	to Wilsede	from Undeloh

July 03, 2018 (Departure)

08.00	from Undeloh	to Wilsede
12.00	from Undeloh	to Wilsede

Undeloh: parking lot near »Undeloher Hof«

Bicycles (»rent-a-bike«)

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

Departure (July 03, 2018)

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

Departures 13.00, 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
Phone: +49 4175-545,
Contact: Dr. Uta Büttinghaus

Undeloh

Ferienhof Heins | 21274 Undeloh
Phone: +49 4189-541
Contact: Constance Heins

Hotel Heiderose | 21274 Undeloh
Phone: +49 4189-311
Contact: Ullrich Wischhof

Landhaus Heideschmiede | 21274 Undeloh
Phone: +49 4189-81310
Contact: Martina Wischhof

Undeloher Hof | 21274 Undeloh
Phone: +49 4189-81 89 10
Contact: Inge Brunkhorst

Breakfast

July 01-03, 2018
Guests accommodated in
Wilsede: at Gasthof "Zum Heidemuseum" (starting at 7.00)
Undeloh: at their hotel or guesthouse

Welcome Reception and Dinner

Saturday, June 30, 2018, 19.30
Hotel Wilseder Hof

Lunch

Gasthof "Zum Heidemuseum"

Sunday, July 01, 2018	12.30-14.00
Monday, July 02, 2018	12.45-13.30
Tuesday, July 03, 2018	packed Lunch

Barbecue/Farewell Dinner

»Emhoff«

Sunday, July 01 and Monday, July 02, 2018	19.00

General Information

Poster Session (with 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 1-minute poster fast-forward presentation. Please arrive punctually to your session.
Your Poster-Number was announced by E-Mail and the poster wall is marked with your number.

Poster Session I
Sunday, July 01, 2018 | 17:00-19:00

Tumor heterogeneity & metastasis (I),
Stem cells in homeostasis and disease (II),
Leukemogenesis (III)

#
S-I-01 · S-I-02 · S-I-03 · S-I-04 · S-I-05
#
S-II-01 · S-II-02 · S-II-03 · S-II-04 · S-II-05 · S-II-06
S-II-07 · S-II-08 · S-II-09 · S-II-10 · S-II-11 · S-II-12
S-II-13
#
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-09 · S-III-10 · S-III-11
S-III-12 · S-III-13 · S-III-14 · S-III-15 · S-III-16 · S-III-17
S-III-18 · S-III-19

Poster Session II
Monday, July 02, 2018 · 13:30-15:30

OMICs & Targeted therapies (IV),
Gene and Immunotherapy (V)

#
M-IV-01 · M-IV-02 · M-IV-03 · M-IV-04 · M-IV-05 · M-IV-06
M-IV-07 · M-IV-08 · M-IV-09 · M-IV-10 · M-IV-11 · M-IV-12
M-IV-13 · M-IV-14 · M-IV-15 · M-IV-16 · M-IV-17 · M-IV-18
M-IV-19 · M-IV-20 · M-IV-21 · M-IV-22 · M-IV-23
#
M-V-01 · M-V-02 · M-V-03 · M-V-04 · M-V-05 · M-V-06
M-V-07 · M-V-08

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 latest, before the start of the session.

Saturday | June 30, 2018

09.00 - 14.00	**Registration** at UKE (Hamburg) and in Wilsede	
16.30 - 16.40	**Welcome address** Boris Fehse & Nicolaus Kröger, Hamburg - DE	

Session I: Molecular Leukemia / Lymphoma
Chairs: Boris Fehse; Nicolaus Kröger, Hamburg - DE

16.40 - 17.05	**Epitranscriptomic regulation of stemness in AML** Carsten Müller-Tidow, Heidelberg - DE	Speaker 01
17.05 - 17.30	**Patients' ALL and AML stem cells reveal reversible drug resistance and dormancy when growing in mice** Irmela Jeremias, Munich - DE	Speaker 02
17.30 - 17.55	**Discovery of a new Burkitt lymphoma survival signal by the CRISPR/Cas9 method** Michael Reth, Freiburg - DE	Speaker 03
18.00-19.00	**Special Lecture** **Genetically modified pigs for Biomedicine** Angelika Schnieke, Munich - DE	Speaker 04
19.30	**Welcome Reception and Dinner** Wilseder Hof	

Sunday | July 01, 2018

Session II: Stem cells in homeostasis and disease Chairs: Tony Green, Cambridge - UK; Markus Manz, Zurich - CH		
09.00 - 09.25	**Long-term single-cell quantification: New tools for old questions** Timm Schroeder, Zurich - CH	Speaker 05
09.25 - 09.50	**Autoimmunity checkpoints in B cell malignancies** Markus Müschen, San Francisco - USA	Speaker 06
09.50 - 10.15	**Cross-talk between Leukemic stem cells and the bone marrow microenvironment** Dominique Bonnet, London - UK	Speaker 07
10.15 - 10.30	**In vivo hematopoietic stem cell expansion by the thrombopoietin-target Epcr in Mpl-deficient mice** (selected abstract short talk) Saskia Kohlscheen, Langen - DE	Speaker 08
10.30 - 11.00	**Coffee break**	

Session III: Immunotherapy I Chairs: Michael Hudecek, Würzburg - DE; Andrea Schietinger, New York - USA		
11.00 - 11.25	**Virus specific T cells - Broadening applicability** Catherine Bollard, Washington DC - USA	Speaker 09
11.25 - 11.50	**Targeting microenvironment biology in B cell lymphomas** Christian Steidl, Vancouver - CAN	Speaker 10
11.50 - 12.15	**CART cell engineering and clinical development** Mario Assenmacher, Bergisch-Gladbach - DE	Speaker 11
12.15 - 12.30	**Super-resolution microscopy dSTORM reveals CD19dim expression on a subset of myeloma cells that can be targeted with CD19-CAR T cells** (selected abstract short talk) Thomas Nerreter, Würzburg - DE	Speaker 12
12.30 - 14.00	**Lunch (Heidemuseum) & Poster viewing**	

Sunday | July 01, 2018

Session IV: Epigenetics Chairs: H. Phillip Koeffler, Los Angeles - USA; Daniel G. Tenen, Boston - USA		
14.00 - 14.25	**Experiments of Myeloid Differentiation** H. Phillip Koeffler, Los Angeles - USA	Speaker 13
14.25 - 14.50	**A novel class of noncoding RNAs (SPEARS) recruit acetylated histone H2A.Z for gene activation** Daniel G. Tenen, Boston - USA	Speaker 14
14.50 - 15.15	**Committed hemopoietic progenitors, not stem cells, are the predominant responders to Hox gene transduction** Norman Iscove, Toronto - CAN	Speaker 15
15.15 - 15.30	**Targeting LSD1 in Hoxa9/Meis1-driven AML Induces Differentiation and Interferes with GFI1-mediated Repression** (selected abstract short talk) Tobias Berg, Frankfurt - DE	Speaker 16
15.30 - 15.45	**Prolyl hydroxylase 3 (Phd3) is essential for mutant IDH1-mediated leukemogenesis in vivo** (selected abstract short talk) Anuhar Chaturvedi, Hannover - DE	Speaker 17
15.45 - 16.15	**Coffee break**	
16.15 - 17.00	**Special Lecture** Chair: H. Phillip Koeffler Los Angeles - USA **Myeloproliferative Neoplasms - from Pathogenesis to Personalized Predictions** Tony Green, Cambridge - UK	Speaker 18
17.00 - 19.00	**POSTER SESSION I** Chairs: Dr. Maike Buchner - DE Prof. Dr Manfred Jücker - DE Prof. Dr. Carsten Müller-Tidow - DE Tumor heterogeneity & metastasis; Stem cells in homeostasis and disease; Leukemogenesis	
19.00	**Barbecue** »Emhoff«	

Monday | July 02, 2018

Session V: CANCEROMICS & new targets Chairs: Ulrich G. Steidl, New York - USA; Oliver Weigert, Munich - DE		
09.00 - 09.25	**When cell differentiation goes side-ways: Subtype-specific regulatory network rewiring in acute myeloid leukemia** Peter Cockerill, Birmingham - GB	Speaker 19
09.25 - 09.50	**DUSP1/6 inhibition as a novel therapeutic approach in chronic lymphocytic leukemia** Maike Buchner, Munich - DE	Speaker 20
09.50 - 10.15	**The role of RhoGTPases in normal and malignant hematopoiesis** Oliver Weigert, Munich - DE	Speaker 21
10.15 - 10.30	**Combined Targeting of Oncogenic JAK2 Signaling and Metabolic Dependencies of Mutant Clones Elicits Synergistic Therapeutic Efficacy in Myeloproliferative Neoplasms** (selected abstract short talk) Tata Nageswara Rao, Basel - CH	Speaker 22
10.30 - 11.00	**Coffee break**	

Session VI: Cancer stem cells Chairs: Maike Buchner, Munich - DE; Peter Cockerill, Birmingham - UK		
11.00 - 11.25	**Actionable Genetic Signatures in Large B-cell Lymphoma** Björn Chapuy, Göttingen - DE	Speaker 23
11.25 - 11.50	**Transcriptional Dysregulation in Precancerous Stem Cells and their Progression to MDS and AML** Ulrich G. Steidl, New York - US	Speaker 24
11.50 - 12.15	**Clonal dynamics in head and neck squamous cell carcinoma: seeking the recurrence-initiating cells by cellular barcoding** Genrich Tolstonog, Lausanne - CH	Speaker 25
12.15 - 12.30	**A stemness screen reveals the endogenous p21-activated kinase inhibitor C3ORF54/INKA1 as a regulator of stem cell latency that functions through global epigenetic changes in normal human hematopoietic and leukemia stem cells** (selected abstract short talk) Kerstin B. Kaufmann, Toronto - CAN	Speaker 26
12.30 - 12.45	**Grant program of the German Cancer Aid** Matthias Serwe, Bonn - DE	
12:45 - 13:30	**Lunch (Heidemuseum) & Poster viewing**	

Monday | July 02, 2018

13.30 - 15.30	**POSTER SESSION II** Chairs: Dr. Michael Hudecek - DE Dr. Oliver Weigert - DE Prof. Dr. Irmela Jeremias - DE OMICs & Targeted therapies; Gene and Immunotherapy	
15.30 - 16.00	**Coffee break**	

Session VII: Stem cells and their niche I Chairs: Carsten Müller-Tidow, Heidelberg - DE; Timm Schroeder, Zurich - CH		
16.00 - 16.25	**Dissecting cellular and molecular dynamics in the bone marrow niche in myelofibrosis** Rebekka Schneider-Kramann, Rotterdam - NL	Speaker 27
16:25 - 16:50	**The evolving view of the hematopoietic stem cell niche (preliminary title).** Simon Mendez-Ferrer, Cambridge - UK	Speaker 28
Session VIII: Genome editing – Research & translation Chairs: Boris Fehse, Hamburg - DE; Wolfgang Uckert, Berlin - DE		
16:50 - 17:15	**Antiviral HSC gene-therapy using genome editing** Joachim Hauber, Hamburg - DE	Speaker 29
17:15 - 17:40	**Enhancing CAR T cell activity by linking IL-12 expression to the endogenous PDCD1 promoter** Alexander Astrakhan, Seattle - USA	Speaker 30
17:40 - 17:55	**Transient retroviral MS2-CRISPR/Cas9 all-in-one particles for efficient targeted gene knockout** **(selected abstract short talk)** Melanie Galla, Hannover - DE	Speaker 31
19.00	**Farewell Barbecue** »Emhoff«	

Tuesday | July 03, 2018

Session IX: Stem cells and their niche II Chairs: Simon Mendez-Ferrer, Cambridge - UK; Markus Manz, Zurich - CH		
09.00 - 09.25	**Inflammageing of HSCs** Markus Manz, Zurich - CH	Speaker 32
09.25 - 09.50	**ASXL1/EZH2 mutations promote clonal expansion of neoplastic HSC and impair erythropoiesis in PMF** Ioanna Triviai, Hamburg	Speaker 33
09.50 - 10.05	**Functional dominance of CHIP-mutated hematopoietic stem cells in patients undergoing autologous stem cell transplantations** (selected abstract short talk) Michael A. Rieger, Frankfurt - DE	Speaker 34
10.05 - 10.30	**Coffee break**	

Session X: Immunotherapy II Chairs: Christian Steidl, Vancouver - USA; Wolfgang Uckert, Berlin - DE		
10.30 - 10.55	**Pre-clinical T cell receptor gene therapy of cancer** Wolfgang Uckert, Berlin - DE	Speaker 35
10.55 - 11.20	**Adoptive T-cell therapy** Michael Hudecek, Würzburg - DE	Speaker 36
11.20 - 11.45	**Molecular programs defining tumor-specific T cell dysfunction** Andrea Schietinger, New York - USA	Speaker 37
11.45 - 12.00	**A Reporter Cell Platform for High-Throughput Screening of Chimeric Antigen Receptor (CAR) Libraries** (selected abstract short talk) Julian Rydzek, Würzburg - DE	Speaker 38
12.00	**Concluding remarks**	
from 12.05	**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 2020!

Speaker Abstracts

Molecular Leukemia / Lymphoma

Speaker 1

Epitranscriptomic regulation of stemness in AML

Carsten Müller-Tidow

Department of Medicine, Hematology, Oncology and Rheumatology, University of Heidelberg, Germany

Leukemic stem cells (LSC) possess unique capabilities to induce leukemia relapse despite intensive chemotherapy and even allogenic stem cell transplantation. LSC differ from bulk leukemia cells by distinct RNA and protein expression patterns due to altered epigenetic and epitranscriptomic regulation. Epitranscriptomics analyzes the mechanisms and the outcome of post-transcriptional RNA modifications. Small non-coding nucleolar RNAs (snoRNAs) are generated from spliced intronic sequences and are required for pseudouridylation and 2´-O-methylation of rRNA. Recently, we discovered that snoRNAs are essential for AML1-ETO induced self renewal and leukemogenesis (Zhou et al., Nat Cell Biol 2017). C/D box snoRNAs act as guides for an enzymatic complex that induce 2'-O-methylation of rRNA. Our data show that snoRNAs are prominently expressed in LSC enriched primary AML specimens. Fibrillarin, the methyltransferase enzyme that catalyzes the 2'-O-methylation is also highly expressed in LSC. Loss of Fibrillarin activity decreases protein synthesis and inhibits proliferation of leukemia cells. In focused CRISPR/CAS9 knockout screens we identified several of snoRNAs that are crucial for leukemia cell proliferation in vitro and in vivo. Knockout of a single snoRNA (SNORD42A) inhibited protein synthesis and cell growth of leukemia cells. In a bioinformatics screen we found more than 20 so far unknown C/D box snoRNAs. At least three of these snoRNAs were required for leukemia cell proliferation in vitro. These data highlight that snoRNAs play important functions in fine tuning ribosome functions in AML. The direct targeting of these mechanisms by leukemogenic oncogenes suggest an important role in leukemogenesis and highlight the possibility to utilize snoRNAs as potential therapy targets for LSC.

Speaker 2

Patients' ALL and AML stem cells reveal reversible drug resistance and dormancy when growing in mice

Irmela Jeremias

Helmholtz Zentrum München, Apoptosis in Hematopoietic Stem Cells - MUNICH, Germany

Drug resistant tumor stem cells represent a major threat for tumor patients as they induce relapse with dismal prognosis. Here we aimed at unraveling basic mechanisms determining drug resistance and dormancy in tumor stem cells. We studied acute leukemia (AL), both acute lykphoblastic as well as acute myeloid leukemia and used genetic engineering in the individualized xenograft mouse model. Recombinant surface markers enabled an unbiased enriching of minute numbers of PDX cells from mice; proliferation-sensitive dies enabled monitoring PDX growth in vivo. Using in vivo imaging, we established a, clinic-related model to mimic the challenging disease stage of minimal residual disease (MRD) in mice. These preclinical approaches allow to characterize patients' dormant stem cells in detail including functional in vivo assays. We identified a distinct, rare subpopulation of PDX AL stem cells that displayed long term dormancy in vivo and resistance against drug treatment in vivo. Long-term dormant stem cells thus combined the three challenging characteristics of relapse-inducing cells dormancy, drug resistance and stemness with re-growth upon withdrawal of treatment pressure. Single cell RNA sequencing revealed that dormant cells resembled cells at minimal residual disease and primary high-risk AL cells and dormant sub-fractions in patients' leukemia samples. Of high clinical relevance, both dormancy and drug resistance revealed transient characteristics in PDX AL cells. When PDX long-term dormant AL cells were distracted from their in vivo environment, they started proliferating similarly as their previously highly proliferative counterparts. When in vivo drug resistant PDX AL cells were retrieved from murine bone marrow, they showed similar drug sensitivity in vitro as their sensitive counterparts. As adverse stem cell characteristics are reversible, dissolving AL cells from their environment might sensitize them towards treatment. Novel therapies might target the interaction between AL cells and their niche to prevent AL relapse.

References

Ebinger S et al., Jeremias I, *Characterization of rare, dormant and therapy resistant cells in acute lymphoblastic leukemia,* Cancer Cell. 2016 Dec 12;30(6):849-862. doi: 10.1016/j.ccell.2016.11.002.

Speaker 3

Discovery of a new Burkitt lymphoma survival signal by the CRISPR/Cas9 method

Michael Reth

BIOSS Centre For Biological Signaling Studies and Department of Molecular Immunology, Biology III, Faculty of BiologyUniversity of Freiburg and MPI of Immunobiology and Epigenetics, Freiburg, Germany - Freiburg, Germany

With help of the CRISPR/Cas9 method it is now possible to rapidly generate defective (KO) genes, not only in the mouse linage, but also in human B cell lines. We have chosen the Burkitt Lymphoma B cell line Ramos for a detailed genetic engineering and gene function analysis. In particular, we have KO the genes encoding the four components, HC, LC, Iga (CD79a) and Igb (CD79b) of the B cell antigen receptor (BCR). This allowed us to express wt or mutated form of the human BCR and to test their signaling function.
Two identical HC form together with two identical LC the tetrameric membrane bound immunoglobulin (mIg) molecule carrying the antigen binding sites whereas Iga and Igb form a heterodimer that via their immunoreceptor tyrosine-based activation motif (ITAM) functions as signaling subunit of the BCR. The BCR requires the assembly of the mIg molecule together with the Iga/Igb heterodimer for its transport on the cell surface. With our BCR gene KO approach, we found that in the absent of mIg the Igb, but not the Iga subunit is able to come alone on the B cell surface as an Igb:Igb homodimer. With a Fab-based proximity ligation assay (Fab-PLA) we showed that on the surface the Igb:Igb homodimer is localized in close proximity to the BCR-coreceptor CD19. Interestingly, by a competitive growth assay we found that Ramos cells require the expression of both, Igb and CD19, for their fitness and competitive growth in culture. We propose, that, by emitting a continuous ITAM/PI-3 kinase signal the Igb:Igb/CD19 module is part of a new tonic signaling receptor that promotes the fitness and survival of Burkitt Lymphoma as well as that of normal B cells with reduced or defective BCR expression. In summary, we demonstrate that the CRISPR/Cas9 technique in combination with nanoscale studies by Fab-PLA can not only result in new insight in the organization of the BCR and the B cell surface, but also in the discovery of new functional receptor modules on normal B or B tumor cells.
This study was supported by the German Cancer Foundation grant 111026 and by the Deutsche Forschungsgemeinschaft through SFB746-P07, TRR130-P02 and EXC 294 as well as by the advanced ERC-grant 32297

Special Lecture

Speaker 4

Genetically modified pigs for Biomedicine

Angelika Schnieke

Technische Universität München, Livestock Biotechnology - München, Germany

Mice have come to dominate basic research in mammals because they are convenient, cheap to house and have long been amenable to precise genetic modification. They have provided powerful insights into the molecular basis of many human diseases and enabled proof-of-principle studies for potential biomedical applications. However, mice differ significantly from humans in size, lifespan, physiology, anatomy and diet, limiting their usefulness for some studies. Pigs are increasingly recognised as a valuable adjunct to pre-clinical research, and their value is considerably increased by the engineering of precise genetic modifications that replicate lesions responsible for human disease conditions. The production of genetically modified pigs has been technically challenging, but can now be significantly streamlined by using gene editing enzymes.
In our group combinations of genetic engineering technologies are being employed to follow two main aims: altering the porcine genome to enable xeno-organ transplantation into humans and to provide a series of genetically-defined pigs that model serious and common human cancers. The latter will allow new diagnostic and therapeutic strategies to be investigated at human scale, and longitudinal studies under conditions that mimic the human patient.
To model colorectal cancer we have generated gene-targeted cloned pigs carrying a nonsense mutation in the adenomatous polyposis coli tumour suppressor gene (APC^{1311}), orthologous to a mutation responsible for the inherited predisposition, familial adenomatous polyposis (FAP). Histological and molecular analyses showed that the porcine model recapitulates all major features of early stage FAP. Somatic mutations of the major tumour suppressor gene *TP53* are present in most human cancers. We have therefore created gene-targeted pigs carrying a latent $TP53^{R167H}$ mutant allele orthologous to human mutant $TP53^{R175H}$ that can be activated in chosen tissues by Cre recombination. Mutations in the proto-oncogene KRAS are indicative of poor prognosis in several cancers, and mutant KRAS is a driver of pancreatic cancer. Pigs have also been generated carrying a Cre-inducible $KRAS^{G12D}$ mutation that can be activated locally to mimic pancreatic and other cancers. In order to monitor Cre-activity we have placed a dual reporter construct in the porcine *ROSA26* locus

Stem cells in homeostasis and disease

Speaker 5

Long-term single-cell quantification: New tools for old questions

Timm Schröder

ETH Zurich, Department of Biosystems Science and Engineering - Basel, Switzerland

Despite intensive research, surprisingly many long-standing questions in stem cell research remain disputed. One major reason is the fact that we usually analyze only populations of cells - rather than individual cells – and at very few time points of an experiment – rather than continuously. We therefore develop imaging systems and software to image, segment and track cells long-term, and to quantify e.g. divisional history, position, interaction, and protein expression or activity of all individual cells over many generations. Dedicated software, machine learning and computational modeling enable data acquisition, curation and analysis. Custom-made microfluidics devices improve cell observation, dynamic manipulation and molecular analysis. The resulting continuous single-cell data is used for analyzing the dynamics, interplay and functions of signaling pathway and transcription factor networks in controlling hematopoietic, pluripotent, skeletal and neural stem cell fate decisions.

Speaker 6

Autoimmunity checkpoints in B cell malignancies

Zhengshan Chen[1], Teresa Sadras[1], Chan Lai N.[1], Lowell Clifford A.[2], Eric Meffre[3], Hassan Jumaa[1], Markus Müschen[1,2]

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Background: Reflecting its central role in normal B-cell development, the B-cell receptor (BCR) and its downstream signaling pathway is the target of oncogenic transformation in the majority of B-cell leukemia and lymphomas. Oncogenic BCR-mimics promote survival and proliferation even in the absence of a functional BCR. Oncogenic mimics include viral oncoproteins (e.g. LMP2A and K1), activating mutations in the Iga and Igb signaling chains or oncogenic tyrosine kinases that intersect with the BCR-signaling pathway (e.g. *BCR-ABL1*).
Rationale: Oncogenic activation of BCR-signaling represents the functional equivalent of *positive selection*. Unlike other types of cancer, B-cell malignancies are unique in that they are also subject to *negative selection* to eliminate B-cells that express autoreactive antibodies. As safeguard against autoimmune diseases, B-cell development *evolved autoimmunity checkpoints (AIC) to eliminate autoreactive clones*.
Results: We have recently discovered that targeted hyperactivation of SYK, PI3K and ERK in B-cell malignancies represents the functional equivalent of an autoimmunity checkpoint (AIC) for elimination of autoreactive B-cells. Despite malignant transformation, B cell- leukemia and lymphoma cells are fully sensitive to negative selection and AIC-activation. AIC-activation is achievable by pharmacological hyperactivation of BCR-signaling above a maximum threshold. Targeted AIC-activation was not affected by known mechanisms of drug-resistance and may be particularly useful in patients with refractory disease.
Conclusion: Normal B-cells are positively selected for BCR signaling of *intermediate* strength (moderate activation of SYK, PI3K and ERK). In the absence of a functional BCR, activity of SYK, PI3K and ERK fall *below a minimum* threshold, resulting in death by neglect. Hyperactivation *above maximum* thresholds (e.g. autoreactive BCR) triggers negative selection and cell death via AIC-activation. Targeted therapy of cancer typically focuses on agents that *suppress* oncogenic signaling *below a minimum threshold*. Our results support a novel strategy to overcome drug-resistance in B-cell malignancies based on targeted AIC-activation for removal of autoreactive cells.

Speaker 7

Genetic basis of Ph- myeloproliferative neoplasms

Dominique Bonnet

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Acute myeloid leukaemia (AML) has long been considered a haematopoietic-cell autonomous disorder in which disease initiation and progression is driven by haematopoietic cell intrinsic genetic events. Recent experimental findings in diverse model systems have challenged this view, implicating different stromal cells of the bone marrow in disease pathogenesis. Thus, it is now accepted that leukaemic haematopoiesis can turn the BM niche into a "leukaemic niche" which promotes leukemic stem cell (LSC) function and impairs the maintenance of normal HSC. However, much remains to be understood about how different leukaemic cells impacts the BM microenvironment and, in turn, how changes in the activity of specific BM niche cells contribute to AML pathogenesis. This talk will showcase specifically the interactions between AML and the vascular niche and how by restoring a normal vascular niche we could impede leukemia development (Passaro *et al.*, 2017). We will also discuss the development of a humanized 3D scaffold closely mimicking the human BM niche, providing us with a valuable and versatile tool to translate our observations to the human niche (Abarrategi *et al*, 2017).

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Speaker 8

In vivo Hematopoietic Stem Cell Expansion by the Thrombopoietin-target Epcr in Mpl-deficient Mice

Saskia Kohlscheen, Marcel G. E. Rommel, Franziska Schenk, Katharina Cullmann, Ute Modlich

Paul-Ehrlich Insitute Research Group for Gene Modification in Stem Cells and the LOEWE Centre for Cell and Gene Therapy Frankfurt - Langen, Germany

Genetic modification of (autologous) hematopoietic stem cells (HSCs) is an attractive therapeutic option for patients with monogenic disorders who lack a suitable HSC donor. The important self-renewal potential of HSCs to maintain lifelong blood cell production is cytokine controlled. The Thrombopoietin (Thpo) receptor Mpl is selectively expressed on HSCs and during megakaryocyte differentiation. Thpo/Mpl-signaling is important in maintaining HSC quiescence during steady-state as well as expanding HSC under stress conditions. MPL-deficiency in men results in thrombocytopenia and aplastic anemia, an inherited disease called congenital amegakaryocytic thrombocytopenia (CAMT), however, activating mutations of MPL lead to the development of myeloproliferative disorders. Thus, the identification and characterization of Thpo-target genes would be a benefit in HSC transplantations.

In former studies we investigated the transcriptome of Mpl-regenerated Mpl-deficient (MplKO) HSC and wildtype HSC inhibited for Mpl-signaling, in order to identify Thpo/Mpl-target genes. Five candidates were evaluated by transplantation of lentiviral transduced hematopoietic cells in the Mpl-deficient mouse model for their ability to rescue the Mpl-deficient phenotype. The overexpression of Epcr (endothelial protein C receptor) expanded the number of phenotypic MplKO long-term (LT)-HSC by 11-fold compared to the negative control (neg ctrl) and were functional, as they engrafted and expanded (2.6-fold) in secondary recipient mice. Epcr is a known HSC marker and was recently associated with HSC BM retention and recruitment. We correlated HSC expansion by Epcr to the upregulation (3-fold) of the anti-apoptotic gene Bcl-xL and to an altered cell cycle profile. Epcr overexpressing MplKO HSC established the quiescent HSC population faster (4 weeks) after transplantation (Tx) compared to the neg ctrl and cycled actively (60-80% G1) and with this expanded LT-HSC at >16 weeks post Tx, arguing for favored HSC self-renewal rather than exhaustion. Notably, Epcr overexpressing MplKO HSC also efficiently homed to the BM at 4 weeks post Tx in contrast to the neg ctrl. In vitro Thpo clearly supported the surface expression of Epcr on C57BL/6 primary HSC. Our results put Epcr downstream of Thpo and identify Epcr as major surface receptor involved in HSC engraftment and self-renewal after transplantation.

Immunotherapy I

Speaker 9

Acute Leukemia: Genomic Abnormalities, Even Before the Disease Develops

Catherine Bollard

Children's Research Institute and The George Washington University, Center for Cancer and Immunology Research - Washington, United States

Despite recent advances in the field of allogeneic hematopoietic cell transplantation (HCT), viral infections are still a major complication during the period of immune suppression that follows the procedure. Adoptive transfer of donor-derived virus specific cytotoxic T cells (VSTs) is a strategy to rapidly restore virus-specific immunity to prevent or treat viral diseases after HCT. Early proof of principle studies demonstrated that the administration of donor-derived T cells specific for CMV or EBV could effectively restore virus-specific immunity and control viral infections. Subsequent studies using different expansion or direct selection techniques have show that donor-derived virus-specific T cells confer protection in vivo after adoptive transfer in 70-90% of recipients. Since a major cause of failure is lack of immunity to the infecting virus in a naïve donor, more recent studies have infused closely matched third-party virus specific T cells and reported response rates of 60-70%. Current efforts have focused on broadening the applicability of this approach by: (i) extending the number of viral antigens being targeted, (ii) simplifying manufacture, (iii) exploring strategies for recipients of virus naïve donor grafts and (iv) developing and optimizing „off the shelf" approaches which will be discussed in this session.

Speaker 10

Meis transcription factors in normal and leukemic hematopoiesis

Christian Steidl

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Lymphoid cancers represent a heterogeneous group of neoplasms composed of malignant lymphoid cells with variable infiltration by non-neoplastic, mostly immune cells (tumor microenvironment). The tumor microenvironment is increasingly recognized to play a pivotal role in the pathogenesis of many lymphoma subtypes. However, the clinical potential of an improved understanding of related biology remains largely untapped. Past discovery and functional studies by our group and others have pointed to the pathogenic importance of acquired immune privilege and altered cellular crosstalk between cells in the tumor microenvironment driven by somatic gene alterations. The genomic changes discussed in this talk can be broadly categorized according to the effect that they exert on the tumor microenvironment: 1) Loss or down-regulation of (surface) molecules leading to decreased immunogenicity of tumor cells (e.g. loss of MHC I/II expression); 2) Increased expression of surface molecules suppressing immune cell function (e.g. structural genomic changes of *PDL1, PDL2*); 3) Recruitment or induction of a regulatory cellular milieu (e.g. mutations in JAK-STAT and NFkB signaling pathways). The discovery of gene mutations underlying immune privilege, properties of the altered molecules, downstream functional consequences and clinical rationales for therapeutic intervention will be presented in the context of specific lymphoma subtypes. Moreover, it will be discussed how precise description of genomic and molecular alterations underlying immune privilege might accelerate effective targeting of microenvironment-related biology in the clinical setting.

Speaker 11

CART cell engineering and clinical development

Mario Assenmacher

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The clinical success of gene-engineered T cells expressing a chimeric antigen receptor (CAR) warrants solutions for remaining limitations in the design, clinical application and the manufacturing of CAR T cells.
Single CD19 CAR-based immunotherapy for B cell malignancies is accompanied by disease relapse due to antigen-escape variants of the tumor cells. Targeting multiple antigens is an attractive strategy to address this. To this end we have developed tandem CARs, where CD19 and CD20 antigen binding domains were linked in different configurations in a single CAR construct. CD20-CD19 tandem CARs were expressed on primary human T cells by lentiviral transduction and compared to single antigen targeting CARs in vitro and in vivo. Tandem CAR T cells efficiently killed leukemic cell lines both in vitro and in vivo. In co-culture in vitro experiments a down-modulation of CD19 expression was seen on leukemic cells at low effector to target ratios with both single- and tandem- CAR T cells. Thus CD20-CD19 tandem CARs may provide a promising approach to reduce the chance of escape and relapse by antigen-loss variants in certain B cell malignancies.
The clinical success of CAR T cells also creates a challenging demand for simple and cost-efficient manufacturing, which can be easily upscaled for large number of patients. Conventional protocols for cell processing, but also analytics are complex and labor intensive, and therefore are expensive and difficult to scale up and/or out. An automated GMP–compliant process has therefore been developed for the generation of gene-engineered T cells in a closed, single-use tubing set on the CliniMACS Prodigy™. The robustness and reproducibility of the manufacturing of therapeutic doses of highly viable CAR T cells was verified for starting material from healthy donors, lymphoma or melanoma patients. Anti-tumor reactivity of the produced CAR T cells was shown in vitro as well as in vivo. Also the in-process and quality control has been integrated, largely by highly automated flow cytometric assays. With these elements we have established a blueprint process for efficient CAR T cell manufacturing in our ballroom cell factory in Bergisch Gladbach. This will serve as a basis and template for multiple academic manufacturing sites, i.e. decentralized CART cell manufacturing in a multi-center CD19 CAR trial in ALL and B-NHL.

Speaker 12

Super-resolution microscopy *d*STORM reveals CD19^{dim} expression on a subset of myeloma cells that can be targeted with CD19-CAR T cells

Thomas Nerreter[1], *Sebastian Letschert*[2], *Sören Doose*[2], *Sophia Danhof*[1], *Hermann Einsele*[1], *Michael Hudecek*[1]

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Background: Since the inception of chimeric antigen receptor (CAR) development, especially since the transfer of CAR T cell therapy to the clinic, there has been controversy over the amount of target molecules required to activate T cells via the CAR. Here, we evaluate the use of (CAR)-engineered T-cells targeting CD19 (CD19CART) in multiple myeloma, a clonal proliferation of plasma cells. A recent study (Garfall et al, NEJM 2015) reported complete remission in a patient that had received CD19CART even though only 0.05% of myeloma cells expressed CD19 as judged by flow cytometry (FC), the routine detection method. The mechanism for this response has remained unclear and sparked debate over low level CD19 expression on myeloma cells that may not be detectable by FC but trigger elimination by CD19CART.
Methods: We generated expression profiles of CD19 on myeloma cells from n=14 patients by single-molecule sensitive super-resolution microscopy (dSTORM - direct stochastic optical reconstruction microscopy) and FC. In parallel, we treated myeloma cells with CD19CART and control T cells in vitro.
Results: In 10/14 patients, we detected CD19 on a fraction of myeloma cells (range: 10.3%-80%) by dSTORM, while FC detected CD19 only in two out of these 10 patients on a smaller cell fraction (range: 4.9%-30.4%). Four patients were classified as CD19-negative by dSTORM. The majority of myeloma cells expressed CD19 at very low levels, far below the FC detection limit. Treatment with CD19CART led to specific elimination of CD19dim myeloma cells, even when CD19 was undetectable by FC. The threshold for CD19CART recognition was below 100 CD19 molecules per myeloma cell.
Conclusions: In a prevailing subset of patients, CD19 is expressed on a large fraction of myeloma cells at a very low density, only detectable by super-resolution dSTORM microscopy. These patients might be candidates for therapy with CD19 CART cells. Our data rationalize anti-myeloma responses that have been reported after CD19CART therapy. dSTORM analysis allowed defining the threshold of antigen expression for T cell activation via a CD19 CAR, which was found to be less than 100 molecules per cell and may be in a similar range for other target antigens.

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Epigenetics

Speaker 13

Experiments of Myeloid Differentiation

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C/EBP family of transcription factors is important for normal hematopoiesis. Six members in this family exist. C/EBPε is important for terminal granulocytic differentiation. The granulocytes of C/EBPε knockout mice differentiate only to intermediate granulocytes and are lacking specific and many tertiary granule proteins. These mice have impaired chemotaxis and phagocytose. Mutations in C/EBPε have been detected in individuals with neutrophil-specific granule deficiency; their phenotype is reminiscent of the C/EBPε knockout mice. We have performed Chip-Seq for C/EBPε as well as RNA-seq using C/EBPε knockout versus wildtype bone marrow cells as well as intermediate granulocytes. We also examined other epigenetic marks during hematopoietic differentiation. We have identified miRs and long non-coding RNAs that are specifically transcribed by C/EBPε. I will describe these in the meeting. Furthermore, we noted that 6kb upstream of the C/EBPε gene was a binding site for C/EBPε. In vitro, we used a DEAD-CAS9 having a KRAB sequence and 5 guide RNAs and inhibited binding of C/EBPε to this C/EBPε binding site. This inhibited the ability of these cells to express C/EBPε and inhibited their ability to differentiate to granulocytes and transcribe secondary granule genes. In addition, in vivo using CRISPR technology, we deleted this C/EBPε binding site in embryos and noted that the expression of C/EBPε and its target genes were lost in the developed mice. In further studies, we are analyzing the hematopoiesis of mice that have C/EBPβ "knocked-in" into the C/EBPα locus. These the mice do not make C/EBPα but do make C/EBPβ at a time when C/EBPα would normally be expressed. These data will also be presented at the meeting.

Speaker 14

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

Daniel Tenen

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An intact CEBPA pathway is required for myeloid differentiation, and this key transcription factor pathway is mutated, downregulated, or inactivated in many forms of Acute Myeloid Leukemia (AML). A number of therapeutics utilized in AML actually serve to restore CEBPA expression and/or function, including all trans retinoic acid (ATRA) in Acute Promyelocytic Leukemia; FLT3 tyrosine kinase inhibitors in FLT3-ITD positive AMLs; CDC2/CDK1 inhibitors; Histone Deacetylase (HDAC) inhibitors; CDDO; DOT1L inhibitors; and Imatinib in CML myeloid blast crisis. We and others have recently developed a number of novel therapeutics resulting in activation of CEBPA expression and/or function, including small molecules, long noncoding RNAs, and short activating RNAs. These result in more specific activation of CEBPA and subsequent differentiation of AML cells. In addition, similar approaches can be utilized to induce demethylation and activation of expression of other tumor suppressors, including p15 and p16, with potential applicability in both leukemias and solid tumors, such as liver cancer and lung cancer.

Speaker 15

Committed hemopoietic progenitors, not stem cells, are the predominant responders to Hox gene transduction

Harvey Lim[1], Salima Janmohamed[2], Patricia Benveniste[2], Robert Herrington[1], Carol Stocking[3], Jana Krosl[4], Guy Sauvageau[4], Norman Iscove[5]

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As hemopoietic stem cells differentiate, their proliferative lifespan shortens by poorly understood and understudied mechanisms. Homeobox cluster (Hox) genes are candidates for involvement because they enhance self-renewal when transduced into hemopoietic cells, but unambiguous determination of their role by gene deletion has been elusive because of their functional redundancy. We enforced HOXB4 expression in purified murine hemopoietic precursor stages, and compared responses of early stages that expressed the endogenous genes with later stages that did not. Contrary to the prevalent view that transduced Hox genes enhance the self-renewal of hemopoietic stem cells, we found little response of stem cells or their multipotent progeny when we repeated earlier published infection protocols. Instead, working at the level of individual clones, we found immortalization, extensive self-renewal and reconstituting potential in apparently committed erythroid and myeloid progenitors at stages where the endogenous genes were shutting down. Further, sustained in vivo reconstitution of myeloid, erythroid and lymphoid lineages by transduced precursor cells was abrogated upon cre-mediated excision of the HOXB4 transgene, starkly illustrating the dependence of committed precusor cells on the transgene for sustained self-renewal. The results show that sustained expression of a Hox gene product in committed progenitor cells is both sufficient and required by these cells for sustained self-renewal. The findings are compatible with the notion that natural shutdown of the endogenous Hox genes is a principal determinant of the shortened clonal lifespans of committed progenitor cells. They also lay the groundwork for an experimental system that provides high efficiency, single-hit immortalization of normally transient clonogenic cells via insertion of a single proviral HOXB4 copy. The system is currently being exploited to test the role of immortalization in initiation and propogation of spontaneously arising leukemias.

References

Committed hemopoietic progenitors, not stem cells, are the principal responders to Hox gene transduction. H Lim, S Janmohamed, P Benveniste, R Herrington, M Barbara, C Frelin, D Hyam, CJ Paige, J-C Zuñiga-Pflücker, C Stocking, J Krosl, G Sauvageau, and NN Iscove. BioRxiv/2017/174490

Speaker 16

Targeting LSD1 in Hoxa9/Meis1-driven AML Induces Differentiation and Interferes with GFI1-mediated Repression

Jessica Barth[1,2,3], Anna-Maria Scheder[1], Denis Dalic[1], Khalil Abou-El-Ardat[1,2,3], Sebastian Mohr[1], Johannes Schulz-Fincke[4,5,3], Martin Schmitt[4], Milica Tosic[6], Eric Metzger[6], Cyrus Khandanpour[7], Gesine Bug[1], Sebastian Wagner[1,2,3],Michael Lübbert[8,5,3], Manfred Jung[4,5,3], Hubert Serve[1,2,3], Roland Schüle[6,9], Tobias Berg[1,2,3]

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Lysine specific demethylase 1 (LSD1) has emerged as a promising epigenetic target in the treatment of acute myeloid leukemia (AML). Inhibition of LSD1 has been shown to induce differentiation and facilitate the responsiveness of AML cells to all-trans retinoic acid. We used two murine AML models based on retroviral overexpression of Hoxa9/Meis1 (H9M) or MN1 to study the effect of Lsd1 knockout (KO) and inhibition in AML.
The conditional KO of *Lsd1* resulted in differentiation with both granulocytic and monocytic features in H9M-induced AML cells and enhanced their responsiveness towards ATRA treatment, which was not seen in MN1-driven AML. *Lsd1* KO also extended the survival of mice with H9M-driven AML.
We used RNA sequencing to investigate the observed differentiation effect on the molecular level. *Lsd1* KO led mostly to the upregulation of genes, and only very few genes were found downregulated. The computational prediction tool ISMARA helped us to identify transcription factors mediating these expression changes. The important regulators of myeloid differentiation Spi1 (PU.1), Cebpb and Gfi1/Gfi1b were among the top 10 most strongly enriched motifs in both H9M and MN1 cells, and the important monocytic transcription factor Irf8 was found exclusively in H9M cells. Transcript levels of *Gfi1b* and *Irf8* increased upon *Lsd1* KO which was also seen upon KO of *Gfi1*. GFI1B and IRF8 were also upregulated in the majority of human AML samples upon treatment with an LSD1 inhibitor making them potential biomarkers.
We also compared the effectiveness of different irreversible and reversible LSD1 inhibitors in our two AML mouse models. We could show that only irreversible inhibitors were capable of inducing a differentiation response in AML cells. We therefore employed a conditional knock-in model of inactive, mutant LSD1 to study the effect of only interfering with LSD1 enzymatic activity without affecting its interactions with different protein complexes. While this was sufficient to initiate differentiation, it did not result in a survival benefit *in vivo*. We therefore believe that interfering with both enzymatic and scaffolding functions of LSD1 is required to efficiently treat AML.
These findings may be relevant for the treatment of AML patients with LSD1 inhibitors. We will further investigate the identified biomarkers in the context of a translational program accompanying the ongoing German multicenter phase I/II trial (TRANSATRA).

Speaker 17

Prolyl hydroxylase 3 (Phd3) is essential for mutant IDH1 mediated leukemogenesis in vivo

Anuhar Chaturvedi[1], Michelle Maria Araujo Cruz[1], Ramya Goparaju[1], Razif Gabdoulline[1], Renate Schottmann[1], Kerstin Görlich[1], Peter Carmeliet[2], Arnold Ganser[1], Michael Heuser[1]

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Understanding Isocitrate Dehydrogenase 1 mutant (IDH1mut) biology has led to the development of specific IDH inhibitors. Around one third of IDH1mut AML patients respond to IDH1 inhibitors with median survival of 8 months, which strongly argues to characterize downstream targets to improve treatment response. IDH1mut produces R-2-hydroxyglutarate (R-2HG), which activates PHD1 and PHD2 but have negligible effects on PHD3. To investigate whether PHDs have an R-2HG independent role in IDH1mut pathogenesis, we investigated if PHD3 is required for IDH1-induced transformation. Bone marrow cells from Phd^{wt} and $Phd3^{ko}$ mice were immortalised with HoxA9, and IDH1wild-type (IDH1wt) and IDH1mut respectively, were constitutively expressed. In in-vitro functional assays, loss of Phd3 impaired proliferation, apoptosis and clonogenic capacity of HoxA9 IDH1mut but not HoxA9 IDH1wt cells. Likewise, in mouse transplantation assays, loss of Phd3 eliminated HoxA9 IDH1mut induced leukemia. However, Phd3 was dispensable to the engraftment and proliferation of HoxA9 IDH1wt cells. Additionally, the IDH1-independent model of MN1-induced leukemia remained unaltered in the absence of Phd3, indicating the specificity of the role in mutant IDH1-induced transformation. To study the functional relevance of PHD3 inhibition in patients, IDH1mut cells from AML patients were transduced with shRNA against *PHD3* and were transplanted in NSG mice. Interestingly, inhibiting PHD3 depleted human AML cells in a mutant IDH1 patient-derived xenograft model. To identify molecular pathways that might explain in vitro and in vivo phenotypes gene expression profiling was performed. Immune and stress-response pathways as well as metabolism-related genes were most prominently dysregulated in $Phd3^{ko}$ IDH1-mutant cells. Analysis of dysregulated transcription factors by gene set enrichment analysis revealed a depletion of key oncogenic transcription factors such as Myc, as well as antioxidants in $Phd3^{ko}$ IDH1mut cells, which was supported by the observed increase in ROS in $Phd3^{ko}$ IDH1mut cells compared to $Phd3^{ko}$ IDH1wt cells. Thus, metabolic perturbations and cellular stress ensuing from the loss of normal IDH1 function in mutant IDH1 cells might render these cells more dependent on non-oncogenes that are dispensable in other cell types. As PHD enzymes can be inhibited pharmacologically our findings suggest combinatorial treatment of PHD and IDH1 inhibitors to eradicate leukemic stem cells in IDH1 mutant AML.

References

AC and MMAC contributed equally

Special Lecture

Speaker 18

Myeloproliferative Neoplasms - from Pathogenesis to Personalized Predictions

Tony Green

University of Cambridge Department of Haematology - Cambridge, United Kingdom

The human myeloproliferative neoplasms (MPNs) are haematological malignancies which arise in blood stem cells. These disorders are experimentally tractable and provide a window on the earliest stages of tumour formation in a way that is not possible in other cancers. Most MPN patients have a somatic mutation that upregulates the JAK/STAT signalling pathway. This pathway plays a key role in regulating the behaviour of multiple stem cell types, and its dysregulation is seen in many human malignancies.

The Green lab continues to explore the molecular and cellular basis for the MPNs, and to translate this understanding into improved management of patients with leukaemia and other blood cancers. These studies have had rapid clinical impact with new approaches to both diagnosis and therapy embedded in international guidelines. In addition we have been able to provide unexpected insights of general relevance to both cancer biology and cytokine signalling. Highlights of broad biological relevance include: the identification of somatic CALR mutations thus revealing an unexpected link between endoplasmic reticulum biology and malignancy; the demonstration (for the first time in any cancer) that mutation order alters stem/progenitor cell behaviour, clinical presentation and response to therapy; the demonstration that nuclear JAK2 directly regulates transcription by functioning as a histone kinase ; and the identification of an unexpected nuclear role for tyrosine-unphosphorylated STATs in regulating lineage-affiliated transcriptional programmes. Most recently, with our collaborators, we have shown that comprehensive genomic characterization identifies distinct genetic subgroups and refines disease classification. Integration of genomic data with clinical parameters enables personalized predictions of patient outcome and will support management of MPN patients.

CANCEROMICS & new targets

Speaker 19

When cell differentiation goes side-ways: Subtype-specific regulatory network rewiring in acute myeloid leukemia

Salam Assi, Maria Rosaria Imperato, Daniel Coleman, Anetta Ptasinska, Anna Pickin, Sandeep Potluri, Peter Cockerill, Constanze Bonifer

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Acute myeloid leukemia is a heterogeneous disease which can be subdivided into different categories defined by disease-causing mutations in transcription factors, epigenetic regulators and signalling molecules. How different mutant regulators establish AML-specific transcriptional networks is unclear. Here we addressed these questions by collecting global data from purified leukemic blasts from AML patients with defined TF and signaling molecule mutations. We focussed on a carefully selected cohort of patients with TF mutations in genes encoding the core-binding factor complex (*RUNX1*/*CBFB*) and the *CEBPA* locus. In addition, we collected data from patients with the FLT3-ITD mutation, with or without NPM1 mutation, and with NPM1 mutations alone. We examined the complete mutation status, collected transcriptome (RNA-Seq) and cistrome (DNase-Seq) data. In addition, using digital DNaseI footprinting, we identified occupied transcription factor binding sites. Our data show that different types of mutations in AML cells result in differential transcription factor expression, cooperation and occupancy, leading to differentially programmed chromatin and gene expression. Moreover, we show that lineage-inappropriate transcription factors are co-opted into aberrant interactions. Finally, for two major classes of AML we performed promoter capture HiC to identify AML type-specific promoter-enhancer interactions to assign cis-regulatory elements to their correct genes. Integrated analysis of these data and experimental validation demonstrates that each mutant regulator establishes a specific transcriptional and signalling network unrelated to normal cells which is required to maintain AML growth.

Speaker 20

DUSP1/6 inhibition as a novel therapeutic approach in chronic lymphocytic leukemia

Veronika Ecker[1], Aida Varela Moreira[2], Martina Braun[1], Bernhard Küster[3], Marcel Fens[2], Jürgen Ruland[1], Maike Buchner[1]

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Chronic lymphocytic leukemia (CLL) is one of the most prevalent B cell malignancies in Western countries and infectious complications are a major cause of morbidity and mortality. Extensive research has led to significant improvement in understanding its pathogenesis, which resulted in the development of novel and promising treatment options. However, none of these approaches is curative and the evolving relapses are challenging to treat, with most drugs aggravating the immunosuppression. Here, we test an approach to simultaneously target the malignant cells and revert the CLL-induced immunosuppression. We found that negative regulators of the MAPK signaling pathway, namely the phosphatases DUSP1 and DUSP6, are readily expressed in CLL. By treatment with BCI, a small molecule inhibitor of DUSP1/6, we induce hyperactivation of the MAPK signaling pathway and rapid cell death in CLL but not other lymphoma or normal B cells. This has been tested on primary CLL samples as well as the Tcl1-driven CLL mouse model. Global phosphatome analysis revealed significant alterations in the BCR signaling pathway upon BCI treatment in primary CLL cells, including hyperphosphorylation of ERK1/2 and rapid induction of a DNA damage response. This was confirmed by immunoblot in murine and human CLL, and does not occur in but in BCI resistant cell lines. We observed that BCI-induced cell death is highly immunogenic with dose dependent release of the immunogenic cell death biomarkers HMGB1 *in vitro*. Furthermore, treatment of primary CLL peripheral blood mononuclear cells with BCI resulted in selective enrichment for cytotoxic T cells. Further validation of BCI effects *in vivo* was impeded by the short biological half-lives in the circulation. Hence, we tested different drug delivery approaches and their suitability for CLL treatment in mice. Interestingly, we found that similar to solid tumors, certain nanoparticles specifically target CLL proliferation sites. Therefore, these represent a potent tool for BCI delivery, which we are currently evaluating. Taken together, we propose that inhibition of DUSP1/6 can simultaneously induce direct, immunogenic cell death in the malignant CLL clone(s) and lower the threshold for activation of other immune cells. Thereby DUSP1/6 inhibition may selectively reduce the amount of malignant cells and reverse the dysfunctional immune system observed in CLL to support effective antitumor immune responses.

Speaker 21

Translational Genetics in Follicular Lymphoma: Towards Risk- and Biology-Adapted Treatment Strategies

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Follicular lymphoma (FL) is a clinically and molecularly highly heterogeneous disease. Most patients achieve long-lasting remissions and have excellent overall survival (OS) with current treatment. However, approximately 20% of patients have early progression-of-disease and short OS. At present, therapies are not guided by individual risk or disease biology. Reliable tools for patient stratification are urgently needed to avoid overtreatment of low-risk patients and to prioritize alternative approaches in high-risk patients. A rapidly expanding repertoire of promising therapeutic options is available for clinical evaluation; however, the numbers of patients with FL and the resources to conduct adequately powered trials are limited.
Combining clinical factors into various FL-specific indices is widely performed to assess individual patient risk, but none are currently used for treatment stratification, partly because they –inherently– cannot inform about the underlying biology of the disease. Recent studies have shown that somatic gene mutations can serve as prognostic and/or predictive biomarkers, in particular when integrated into composite risk models. Gene mutations, however, are only one component of complex biological networks. Novel omics technologies are increasingly applied in unbiased manners to FL samples, but it will be challenging to capture the full complement of biologically- and clinically-relevant factors. Among the most promising developments are recently improved technologies for robust and reliable gene expression profiling from challenging biopsy specimens, to comprehensively capture the "net transcriptional phenotype" which result from molecular alterations within the malignant cells and interactions with the distinct microenvironment. Before these findings can be translated into routine clinical practice, however, several challenges loom. Ultimately, a new era of collaboration and harmonization is required if we hope to transition from empiric selection of therapeutics to risk-based, biology-guided treatment for patients with FL.

Speaker 22

Combined Targeting of Oncogenic JAK2 Signaling and Metabolic Dependencies of Mutant Clones Elicits Synergistic Therapeutic Efficacy in Myeloproliferative Neoplasms (selected abstract short talk)

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Background: Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem and progenitor cells (HSPCs) characterized by overproduction of erythroid and myeloid lineages. The mechanisms downstream of driver mutations such as JAK2-V617F on MPN initiation and propagation are incompletely understood, and clinically utilized JAK2 inhibitors have limited ability to reduce MPN burden. Mouse models expressing mutant JAK2 exhibit MPN phenotypes and display early mortality. We noticed that these mice also display markedly decreased body weight and adipose tissue. Therefore, we studied the metabolic basis of MPN pathogenesis in these mice and primary samples from MPN patients. Elucidating the unique metabolic demands of MPN cells could be exploited as therapeutic targets in MPN.
Results: We found that activation of mutant JAK2 induces metabolic alterations including adipose atrophy, and resistance to high-fat diet (HFD) induced obesity in mice. Intriguingly, HFD treatment significantly ameliorated early lethality in MPN mice, which was not due to reduction in elevated platelet and erythrocyte numbers. In addition, mice under normal dietary conditions were severely hypoglycemic and showed increased glucose tolerance despite normal insulin levels, and this was correlated to extensive erythrocytosis. Integrated transcriptomics and metabolomics analysis together with metabolic functional assays of MPN propagating HSPCs revealed heightened utilization of nutrients and subversion of metabolic pathway derivatives to biosynthetic processes attributable for expansion of MPN propagating HSPCs and severity of MPN. Some of these metabolic changes were also detected in primary samples from MPN patients highlighting the clinical relevance of our findings. Combined pharmacological targeting of metabolic dependencies of mutant cells and JAK2 activity resulted in marked reduction in splenomegaly and MPN burden.
Conclusions: Our data show that metabolic rewiring in mutant clones is fundamental for MPN initiation and propagation. Activation of mutant JAK2 in the hematopoietic system induced bystander metabolic reprogramming in non-hematopoietic tissues leading to energy crisis, which may contribute to early lethality of MPN mice. Importantly, our study identified therapeutically viable metabolic targets of MPN, and provided the rationale for a “two-pronged” approach of co-targeting distinct metabolic features and oncogenic JAK2 activity in MPN.

Cancer stem cells

Speaker 23

Actionable Genetic Signatures in Large B-cell Lymphoma

Björn Chapuy

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Diffuse large B-cell lymphoma (DLBCL), the most common lymphoid malignancy in adults, is a clinically and genetically heterogeneous disease that is further classified into transcriptionally defined activated B-cell (ABC) and germinal center B-cell (GCB) subtypes. Primary central nervous system lymphomas (PCNSLs) and primary testicular lymphomas (PTLs) are extranodal large B-cell lymphomas (LBCL) with inferior responses to current empiric treatment regimens. To identify actionable genetic features of PCNSL and PTL, we characterized their recurrent somatic mutations, chromosomal rearrangements, copy number alterations and associated driver genes and compared these comprehensive genetic features to those of diffuse LBCL and primary mediastinal large B-cell lymphoma (PMBL). These studies identify unique combinations of genetic alterations in discrete LBCL subtypes and subtype-selective bases for targeted therapy. PCNSLs and PTLs utilize multiple genetic mechanisms to deregulate key targets and pathways and exhibit near-uniform oncogenic Toll-like receptor signaling due to MYD88 mutation and/or NFKBIZ amplification, frequent concurrent B-cell receptor pathway activation and deregulation of BCL6 by multiple mechanisms. PCNSLs and PTLs also have frequent 9p24.1/PD-L1/PD-L2 amplifications and additional translocations of these loci, structural bases of immune evasion that are shared with PMBL and provide a framework for analyzing genetic bases of PD-1 ligand overexpression that is broadly applicable to other tumor types. The comprehensive genetic signatures of PCNSL and PTL reveal actionable alterations including oncogenic TLR signaling, concurrent BCR activation and PD-1 mediated immune evasion and structural bases for genomic instability. By comparing the genetic signatures of PTL and PCNSL to those of systemic DLBCL and PMBL, we also define unique combinations of structural alterations in these discrete LBCL subtypes.
In addition, Dr. Chapuy will describe a comprehensive genetic analysis of 304 primary DLBCLs that identifies low-frequency alterations, captures recurrent mutations, somatic copy number alterations (SCNAs) and structural variants (SVs) and defines coordinate signatures in patients with available outcome data. We integrated these genetic drivers using consensus clustering and identified 5 robust DLBCL subsets, including a previously unrecognized group of low-risk ABC-DLBCLs of extrafollicular/marginal zone origin; 2 distinct subsets of GCB-DLBCLs with different outcomes and targetable alterations; and an ABC/GCB-independent group with biallelic inactivation of TP53, CDKN2A loss and associated genomic instability. The genetic features of the newly characterized subsets, their mutational signatures and the temporal ordering of identified alterations provide new insights into DLBCL pathogenesis. The coordinate genetic signatures also predict outcome independent of the clinical International Prognostic Index and suggest new combination treatment strategies. More broadly, these studies provide a roadmap for an actionable DLBCL classification.

Speaker 24

Targeting the Stem Cell Origins of MDS and AML

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Relapse and malignant progression continues to be the most common cause of death in myelodysplastic syndromes (MDS) acute myeloid leukemia (AML) and many other cancers. Recent evidence has shown that the accumulation of stepwise genetic and epigenetic changes in tissue-specific stem cells 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 myelodysplastic syndromes (MDS) and AML. Our models and data sets at the stem cell level provide novel tools for mechanistic study of pre-LSC and their progression to overt MDS and AML, and for the development and testing of pharmacological approaches to therapeutically interfere with these processes. In summary, recent studies have started to shed light on pre-cancerous stem cell states as the earliest origin of various malignancies including MDS and AML, as well as molecular mechanisms driving their formation and progression. These advances provide a basis for the specific therapeutic targeting of pre-cancerous stem cells for the causative treatment and potential prevention of MDS and AML and other cancers developing from pre-cancerous conditions.

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Speaker 25

Clonal dynamics in head and neck squamous cell carcinoma: seeking the recurrence-initiating cells by cellular barcoding.

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Local recurrences of head and neck squamous cell carcinoma (HNSCC) are generally suspected to originate from residual cancer cells left behind during the initial surgery. It is suggested that recurrent HNSCC tumors originate from cancer stem cells that acquired an invasive phenotype, disseminated beyond the border of the resected primary tumor, survived after therapy, and resumed growth. To test this concept at a single-cell resolution, we developed a surgical mouse model using HNSCC cells tagged with multiple fluorescent proteins and a highly complex library of DNA barcodes. Using this approach, we provide the first clonal analysis of matched HNSCC primary and recurrent tumors and highlight the distinct phenotypic and genetic nature of clones prevailing in postsurgical local recurrences. The massive decrease of clonal diversity together with the substitution of the most abundant clones in relapsed tumors emerged as hallmarks of local recurrence. Although scarce in primary tumors, the recurrence-initiating lineages locally disseminated and underwent further clonal selection after tumor resection. Given the epithelial-mesenchymal transition (EMT) seen in clones enriched in recurrent tumors, pharmacological targeting of EMT-related pathways is attractive to achieve better local control for invasively growing HNSCC tumors. Overall, we provide evidence that clonal mechanisms drive the growth of recurrent HNSCC, and demonstrate that our approach is well suited to explore the biology of recurrence-initiating cells.

Speaker 26

A stemness screen reveals the endogenous p21-activated kinase inhibitor C3ORF54/INKA1 as a regulator of stem cell latency that functions through global epigenetic changes in normal human hematopoietic and leukemia stem cells

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The controversy generated from recent murine studies as to whether hematopoietic stem cells(HSC) contribute to steady-state hematopoiesis emphasizes how limited our knowledge is of mechanisms governing HSC activation and latency, a problem even more acute for human HSC and leukemia stem cells(LSC). Since LSC can survive chemotherapy and initiate relapse in acute myeloid leukemia(AML), they are clinically relevant and a greater understanding of the mechanisms underlying their long-term survival and self-renewal is also imperative.
We investigated 64 differentially expressed genes as potential HSC and LSC self-renewal genes using a competitive *in vivo* gain of function screen. Strikingly, individual overexpression(OE) of 5 high scoring candidates revealed delayed repopulation kinetics of human HSC/progenitor cells(HSPC) in xenografts. For INKA1 we found that OE increased primitive CD34+ cells at 20 w and secondary transplantation revealed a 5 fold increase in HSC frequency. Hence, we have linked the protracted stem cell latency induced by INKA1 OE to HSC self-renewal.
INKA1 has been described as an inhibitor of p21(Cdc42/Rac)-activated kinase 4 (PAK4), however, no role for PAK4 is described in hematopoiesis. Its regulator Cdc42 is implicated in aging of murine HSPC by affecting H4K16 acetylation(H4K16ac) levels and polarity and has recently been described to regulate AML cell polarity and division symmetry. Along this line, our preliminary data comparing hCB and adult BM HSC shows changes in H4K16ac levels upon aging. H4K16ac immunostaining of HSPC subsets following xenotransplantation indicates that INKA1-OE differentially affected epigenetics of these subsets linking H4K16ac to the regulation of stem cell latency.
Moreover, transcriptional upregulation of INKA1 in LSC vs non-LSC fractions and in subsets of paired diagnosis-relapse samples from AML patients implicates INKA1 as a regulator of LSC self-renewal and persistence. Indeed, INKA1-OE in a primary AML derived culture system with a phenotypic and functional hierarchy revealed a strong latency phenotype; *In vitro* and *in vivo* we observed label retention along with a steady increase of %CD34+ cells, differentiation block, reduced growth rate, G_0 accumulation and global reduction of H4K16ac.
Taken together, our data implicates INKA1 as a gate-keeper of stem cell latency in normal human hematopoiesis and leukemia and studying the pathways involved may uncover new therapeutic targets for LSC eradication in AML.

Stem cells and their niche I

Speaker 27

Dissecting cellular and molecular dynamics in the bone marrow niche in myelofibrosis

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Bone marrow (BM) fibrosis is associated with a variety of malignant hematopoietic disorders and is a central pathological feature of primary myelofibrosis (PMF). The specific mechanisms that cause BM fibrosis in hematological disorders are not understood, in particular as the cells driving fibrosis have remained obscure.
We just demonstrated using genetic fate tracing in two murine models of PMF and patient samples that 1) Gli1$^+$ mesenchymal stromal cells (MSC) are are fibrosis-driving cells in PMF, 2) that their frequency correlates with fibrosis severity in patients, and 3) that their ablation ameliorates BM fibrosis (Schneider et al. Cell Stem Cell 2017). Our data in patient samples and in murine models thus indicate that targeting Gli/Hedgehog (Hh)-signaling is an attractive strategy for the treatment of PMF.
We now sought to determine the underlying mechanisms for the fibrotic transformation of sort-purified Gli1+ cells in bone marrow fibrosis and homeostasis using RNA sequencing. Differential gene expression analysis demonstrated that megakaryocyte-associated genes were significantly enriched in the fibrosis-driving cells, in particular the chemokine Cxcl4, also called platelet-factor 4 (PF4). Using in vitro co-culture assays and genetic knock-down experiments, we now demonstrate that Cxcl4 plays a central role in the activation of Gli1$^+$ stromal cells and their myofibroblastic differentiation. We asked if Hh/Gli inhibition blocks the cellular interaction between dysplastic megakaryocytes and Gli1+ fibrosis-driving cells. We tested the Gli antagonist 61 (GANT61) in in vitro assays and also in murine models with bone marrow fibrosis in vivo and demonstrate that Gli inhibition 1) significantly decreases the expression of CXCL4 in fibrosis-driving cells, 2) inhibits the fibrotic transformation of Gli1+ cells, 3) induces apoptosis in the MPN clone, and 4) normalizes the megakaryocyte ploidy in dysplastic megakaryocytes.
Our data demonstrate that CXCL4 plays a central role in the fibrotic transformation of Gli1+ cells in PMF and is a potential therapeutic target.

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Speaker 28

HSC niches in ageing and MPN

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Haematopoietic stem cells (HSCs) residing in the bone marrow (BM) accumulate during aging but are functionally impaired. However, the role of HSC-intrinsic and -extrinsic aging mechanisms remains debated. Premature aging in Hutchinson-Gilford progeria syndrome (HGPS) recapitulates physiological aging features, but whether these arise from altered stem and/or niche cells is unknown. We will present evidence showing that the murine BM microenvironment promotes stem/progenitor cell myeloid bias in normal aging and HGPS. During physiological aging, HSC supporting niches decrease near bone (endosteal BM) but expand in the BM further from bone. Increased sympathetic noradrenergic innervation in BM microenvironment promotes β2-adrenergic-receptor(AR)-interleukin-6-dependent myeloid bias. Reduction of endosteal niches decreases β3-AR-nitric-oxide-NO-dependent inhibition of cytokines driving myeloid expansion. However, chronic treatment with β3-AR-agonist does not rejuvenate overall haematopoiesis, but decreases exacerbated megakaryopoiesis and improves myelofibrosis in mice and humans with myeloproliferative neoplasms (MPNs). Therefore, niche aging promotes stem/progenitor myeloid bias and might represent a therapeutic target in age-related pathological disorders.
MPNs can be considered preleukemic disorders because MPN patients have higher risk of developing acute myeloid leukaemia (AML). However, for reasons not fully clear, the transformation rates are very different across distinct MPN subsets (myelofibrosis > polycythemia vera (PV) > essential thrombocythemia (ET), which is generally more benign), despite the fact that mutated HSCs may carry the same oncogenic driver mutation (e.g., JAK2-V617F). We hypothesized that the niches where LSCs reside might affect disease evolution in MPN.
BM niches, which can be found either closer (endosteal) or further away (non-endosteal) from the bone, contain different neurovascular beds controlling distinct HSC functions. We used intravital imaging to visualize JAK2-V617F-mutated HSCs able to initiate PV or ET, or control WT HSCs, in real time in their natural microenvironment (skull BM). Three days after transplantation, hematopoietic stem and progenitor cells (HSPCs) from ET mice located significantly closer to the bone surface, while PV HSPCs proliferated preferentially in the BM area further away from bone. Surprisingly, despite their location close to the bone surface, ET HSPCs migrated twice as much as control/PV cells and with doubled speed. High motility of ET HSPCs correlated with their increased adhesive capacity *in vitro*, compared with PV cells. Increased adhesion correlated with higher expression of integrin-beta3 in different ET mouse models.
Lodging of ET HSPCs close to the bone is followed by expansion of the endosteal niche in ET mice, characterised by increased bone formation and expansion of osteoblasts and osteoclasts. Likewise, the central sinusoidal vasculature of ET mice, but not PV mice, is partially replaced by bone-forming $CD31^{hi}EMCN^{hi}$ and integrin $\beta1^{+}$ blood vessels. Increased endothelial Notch signalling and high expression of VEGFC and PDGFb in the ET BM might trigger vascular remodelling in ET mice.
In summary, we demonstrate that HSCs carrying the same mutation (JAK2-V617F) exhibit different microenvironmental requirements in murine models of ET and PV. The results also suggest that HSPCs carrying the same mutation can cause differential niche remodelling in two diseases associated with different progression.

Genome editing - Research & translation

Speaker 29

Antiviral HSC gene-therapy using genome editing

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Chronic viral infections are often incurable because current antiviral strategies do not target chromosomally integrated or non-replicating episomal viral genomes. The rapid development of technologies for genome editing in combination with gene-therapy may possibly soon allow for therapeutic targeting of viral genomes and, hence, for development of curative strategies for persistent viral infection. Thus, this presentation focusses on the experimental strategies to treat persistent viral infection by genome modification. Pros and cons of current genome editing tools, including engineered site-specific recombinases and the CRISPR/Cas9 system, are discussed. Moreover, the study outline of a first-in-human HSC gene therapy trial to remove chromosomally integrated HIV-1 genomes from infected patients is presented.

Speaker 30

Enhancing CAR T cell activity by linking IL-12 expression to the endogenous *PDCD1* promoter.

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Chimeric antigen receptor (CAR) T cells have shown great promise in treating certain late stage hematological malignancies. While very encouraging, current CAR T cell therapies have not shown the same level of success in targeting solid tumors and alternative approaches are required to achieve clinical efficacy in solid tumor patients. We describe a combinatorial approach to boost CAR T cell activity by combining T cell checkpoint deletion with immunomodulatory transgene insertion, resulting in more potent CAR T cells for solid tumor applications.
While inflammatory cytokines can stimulate vigorous antitumor responses, the clinical application of cytokine therapy has been limited by systemic toxicity, particularly for strong effector cytokines such as IL-12. Linking IL-12 expression to T cell activation may reduce unwanted toxicity while enhancing CAR T cell functionality. The expression of immune checkpoint gene programed death 1 (*PDCD1*) is tightly linked to T cell activation and inactivation of the PD-1 signaling pathway has been shown to enhance CAR T cell functionality. Here, we used genome editing/homology directed repair (HDR) to place an *IL-12* transgene under the control of the *PDCD1* promoter, linking IL-12 production with CAR T cell activation as well as eliminating PD-1 expression. CAR expression was combined with targeted transgene expression by treating lentivirally transduced T cells with a *PDCD1*-specific megaTAL and an adeno associated virus-6 (AAV6) containing an *IL-12* transgene flanked by *PDCD1* homology regions. We observed highly efficient HDR, with activation-dependent induction of IL-12 expression. Minimal IL-12 production was detected under resting conditions, whereas PMA/Ionomycin or co-culture with tumor cell lines resulted in higher IL-12 secretion. Expression of IL-12 under the *PDCD1* promoter enhanced CAR T cell cytokine production and cytotoxicity, especially under conditions of repeated antigen exposure.
In summary, we describe a novel genome editing strategy to enhance CAR T cell functionality. Using HDR, we were able to engineer CAR T cells to simultaneously disrupt the *PDCD1* gene and place a potentially therapeutic transgene under inducible transcriptional control. The IL-12/CAR T cells exhibited activation-dependent IL-12 production and enhanced cytokine and cytotoxicity responses against tumor cells *in vitro*. HDR may represent a promising approach to enhance CAR T cell functionality in solid tumor applications.

Speaker 31

Transient retroviral MS2-CRISPR/Cas9 all-in-one particles for efficient targeted gene knockout

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The recently discovered CRISPR/Cas9 system is widely used in basic research and is an interesting tool for the treatment of genetic diseases. However, we and others showed that stable overexpression of DNA modifying enzymes leads to cytotoxicity and cells may suffer from unwanted genomic off-target events. Here, we developed transient retrovirus-based CRISPR/Cas9 all-in-one particles that co-deliver the Streptococcus pyogenes Cas9 (SpCas9) and the single guide RNA into target cells. Initially, we redirected the gammaretroviral (GV) packaging mechanism to generate GV MS2 bacteriophage chimera (GV.MS2) and achieved up to 70% knockout of a reporter gene in murine and human cell lines. GV.MS2-CRISPR/Cas9 all-in-one particles were dependent on Gag.MS2 structural proteins as well as the VSVg envelope protein and required co-transfection of both SpCas9 and sgRNA expression plasmids during particle production. Remarkably, knockout of the TP53 gene in primary human foreskin fibroblasts by GV.MS2-CRISPR/Cas9 led to a growth advantage of TP53 knockout cells versus control cells under competitive culture conditions. To analyze whether the SpCas9 enzyme is cytotoxic per se, we stably overexpressed SpCas9 in murine NIH3T3 fibroblasts at high MOIs (>10) and observed a substantial G0/G1 cell cycle arrest associated with reduced cell growth and metabolic activity. Strikingly, these adverse side effects were absent in cultures that were treated with transient GV.MS2.SpCas9, suggesting that the observed SpCas9-mediated cytotoxicity is dose-dependent. Next, we generated alpharetrovirus-based MS2-CRISPR/Cas9 (AV.MS2-CRISPR/Cas9) all-in-one particles. Compared to GV-based CRISPR/Cas9 all-in-one particles, AV.MS2-CRISPR/Cas9 elicited similar knockout rates of our reporter gene when using up to 20-fold less supernatant. Subsequent immunoblot analysis of both chimeric particles revealed ~23-fold higher Gag.MS2 protein levels in AV.MS2-CRISPR/Cas9 supernatants, which may explain their better performance. Since chemokine receptors CXCR4 and CCR7 are promising targets for novel CRISPR/Cas9-based therapies for HIV-1infections and/or chronic inflammatory diseases, we next tested our novel particles as potential therapeutic agents. We transduced human Jurkat cells and efficiently knocked out the endogenous CXCR4 receptor to render the cells resistant against infection with human CXCR4-tropic HIV-1-based vector particles. As further proof-of-concept, we successfully knocked out the CCR7 receptor in primary murine T cells and achieved up to 18% CCR7 negative cells. In conclusion, we introduced transient retrovirus-based CRISPR/Cas9 all-in-one particles from two different retroviral genera for efficient and non-cytotoxic, targeted gene knockout.

Stem cell and their niche II

Speaker 32

Inflammageing of HSCs

Markus Manz

Speaker 33

ASXL1/EZH2 mutations promote clonal expansion of neoplastic HSC and impair erythropoiesis in PMF

Ioanna Triviai

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Primary Myelofibrosis (PMF) is hematopoietic stem cell (HSC) disease, characterized by aberrant differentiation of all myeloid lineages and profound disruption of the bone marrow niche. PMF samples carry several mutations, but their cell origin and hierarchy in regulating the different waves of clonal and aberrant myeloproliferation from the prime HSC compartment is poorly understood. Genotyping of >2000 colonies from CD133+ HSC and progenitors from PMF patients confirmed the complex genetic heterogeneity within the neoplastic population. Notably, mutations in chromatin regulators *ASXL1* and/or *EZH2* were identified as the first genetic lesions, preceding both *JAK2-V617F* and *CALR* mutations, and are thus drivers of clonal myelopoiesis in a PMF subset. HSC from PMF patients with double *ASXL1*/*EZH2* mutations exhibited significantly higher engraftment in immunodeficient mice than those from patients without histone modifier mutations. *EZH2* mutations correlate with aberrant erythropoiesis in PMF patients, exemplified by impaired maturation and cell cycle arrest of erythroid progenitors. These data underscore the importance of post-transcriptional modifiers of histones in neoplastic stem cells, whose clonal growth sustains aberrant myelopoiesis and expansion of pre-leukemic clones in PMF

Speaker 34

Functional dominance of CHIP-mutated hematopoietic stem cells in patients undergoing autologous stem cell transplantations

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Hematopoiesis is maintained by a polyclonal pool of hematopoietic stem cells (HSCs). Single somatic mutations in recurrent genes cause the emergence of expanded clones in elderly persons, a phenomenon called clonal hematopoiesis of indeterminate potential (CHIP). While the multilineage differentiation of these clones suggests an involvement of HSCs in CHIP, direct evidence of the fitness of CHIP-mutated human HSCs in blood reconstitution is lacking. Interestingly, previous studies showed a constant clone size for decades under steady-state conditions. Myeloablative treatments and stem cell transplantation put enforced stress on HSCs to reconstitute the blood system, and the fitness of HSCs is challenged.

To assess whether human CHIP-mutated HSCs outcompete their wildtype counterparts in stress hematopoiesis, we took advantage of a well-characterized cohort of currently 74 patients undergoing high-dose chemotherapy conditioning and autologous stem cell transplantation (autoPBSCT) for the treatment of solid tumors or lymphoid diseases in our center.

Using deep next generation sequencing of 56 myeloid cancer genes, we found that 18 patients (24%) are affected by CHIP (variant allele frequency >2%) in their blood at a time 6-102 months after autoPBSCT, with a mean variant allele burden of 4.5%. DNMT3A was affected most often, followed by TET2, PPM1D, ASXL1, TP53, KRAS, and KDM6A. Most patients carry only one mutation while few have 2-3 mutations. To explore whether the mutations had been present before the high-dose chemotherapy, and whether the CHIP-mutated HSCs expanded after autoPBSCT, we sequenced the frozen samples of the transplanted grafts. Importantly, the mutations can be detected in the majority of patients already in the transplanted cells, suggesting that they are not induced by high dose chemotherapy; however, the allele burden in the graft is significantly lower, often below 0.5%, than in the blood months to years after autoPBSCT suggesting a selective advantage of mutated HSCs outcompeting normal HSCs upon stress hematopoiesis.

Immunotherapy II

Speaker 35

Pre-clinical T cell receptor gene therapy of cancer

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TCR gene therapy to fight cancer requires the successful interplay of individual components: The therapeutic TCR gene, the selected target antigen, the gene transfer vector, and the subset of T cells to be modified.
Efficient strategies to isolate potent TCRs for gene therapy are still limited. We developed a TCR isolation platform for the detection of antigen-specific TCRs. T cells of healthy donors were stimulated with antigen-expressing dendritic cells to enrich antigen-specific T cells *in vitro*. Screening of antigen-specific T cell responses was performed using a novel MHC I cell library consisting of single MHC-expressing antigen-presenting cells covering all six cognate MHC class I alleles of the T cell donor. T cells responding to a specific antigen-MHC I combination were enriched and TCR gene sequences were determined. Dominant TCR alpha and beta chains were combined in a g-retroviral vector and expressed in T cells. The approach was successfully applied to isolate virus- and cancer antigen-specific TCRs, and in parall, allowed the identification of new antigenic epitopes as targets for TCR gene therapy.
Antigens encoded by cancer-specific somatic mutations (neo-antigens) are potentially the best targets for TCR gene therapy. However, using neo-antigens as targets requieres the fast availability of TCR-engineered T cells. Thus, commonly used lenti- and g-retroviral vectors need too much time to fulfill this requirement. On the other hand, non-viral vector systems, such as transposons, do not reach the efficiency of viral transduction and are accompanied with toxic side effects for T cells. Therefore, we optimized the Sleeping Beauty transposon system. We used transposon minicircle vectors to reduce the size of the DNA plasmid, replaced the SB100X transposase plasmid by transposase-encoding RNA and introduced miRNAs to silence the expression of endogenous TCRs. We achieved stable TCR expression in more than 50% of human T cells. TCR-engineered T cells were highly functional in *in vitro* and *in vivo* pre-clinical cancer models.
We generated immune-deficient, human MHC I-transgenic mice and a syngeneic tumor cell line that can be modified to express human cancer antigens of interest. In this model, all cellular components are of murine origin, while all components of immunological interactions are of human origin. The model allows a precise prediction of the therapeutic efficacy of TCRs and cancer antigens, which are selected for TCR gene therapy.

Speaker 36

Novel targets and technologies for CAR-T cells

Michael Hudecek

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Adoptive immunotherapy with tumor-reactive T cells expressing a chimeric antigen receptor (CAR) has continued its breathtakingly rapid development. CARs are designer molecules comprised of several components: an extracellular antigen-binding domain, usually the variable light and heavy chains of a monoclonal antibody (scFv); a spacer and transmembrane region to anchor the receptor on the T-cell surface; and an intracellular signaling module, most commonly CD3 zeta and one or more costimulatory domains that mediate T-cell activation after antigen binding.
The most advanced clinical development (incl. two products that obtained clinical approval in the U.S.) is the use of CARs specific for the B-lineage marker CD19 in patients with chemotherapy- and radiotherapy-refractory B-cell ALL, NHL and CLL. Side effects of CD19 CAR-T-cell therapy include cytokine release syndrome, neurotoxicity and depletion of normal B-cells. With longer follow-up, resistance mechanisms to CD19 CAR-T cell therapy have become apparent, often due to the loss of CD19 antigen expression, particularly in ALL. CD19-low/negative leukemia cells may still express CD20, CD22 and/or CD123 that are being pursued as rescue antigens. Therefore, combinatorial targeting of CD19 with either CD20, CD22 or CD123 is being explored, either through bi-specific CAR constructs with two scFvs *in cis* or through co-expression of two CAR constructs in the same T cell.
There is an ongoing quest to identify and validate novel CAR target antigens in hematologic malignancies and solid tumors. We have demonstrated that the ROR1 tumor antigen can be exploited as a CAR target antigen in lymphoma, lung and breast cancer. In recent work, we have also demonstrated that CAR-T cells targeting SLAMF7 in multiple myeloma and FLT3 in acute myeloid leukemia confer potent reactivity in pre-clinical models. In addition to highly expressed target antigens that are readily detectable in routine histopathology, there are antigens that are expressed at levels below the detection threshold of flow cytometry and immunohistochemistry, but can still be recognized by CAR-T cells. Therefore, we have recently implemented super-resolution microscopy into our work flow of identifying novel antigens for CAR-T cells. These advances also mandate the development of novel safety technologies that enable physicians to maintain control over the infused CAR-T cell product and to steer their function in vivo. We will review these recent advances in antigen discovery and CAR-T technologies that are emerging from our research laboratory and in the field.

Speaker 37

Molecular Programs Defining Tumor-Specific T cell Dysfunction

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Tumor-specific T cells in solid tumors are dysfunctional, allowing tumors to progress. The epigenetic regulation of T cell dysfunction and therapeutic reprogrammability (e.g. to immune checkpoint blockade) is not well-understood. We recently found that T cells in tumors differentiated through two discrete chromatin states: a plastic dysfunctional state from which T cells could be rescued, and a fixed dysfunctional state resistant to reprogramming. We identified novel surface markers associated with each chromatin state that demarcated reprogrammable from non-reprogrammable PD1^{hi} dysfunctional T cells within heterogeneous T cell populations in tumors. *In vivo* pharmacologic modulation of transcription factors associated with each chromatin remodeling step improved therapeutic reprogrammability of dysfunctional T cells. Our study has important implications for cancer immunotherapy by defining key transcription factors and epigenetic programs underlying T cell dysfunction and surface markers that predict therapeutic reprogrammability.

Speaker 38

A Reporter Cell Platform for High-Throughput Screening of Chimeric Antigen Receptor (CAR) Libraries

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Background: Chimeric antigen receptor-modified T-cells (CAR-T) are under intense investigation in cancer immunotherapy. Several parameters in CAR design including binding domain affinity and extracellular spacer length affect CAR-T function. At present, the approach to identify CAR constructs with optimal function is empiric testing in primary T-cells, which is time consuming and error prone due to variations in receptor expression and T-cell subset composition. Here, we established a CAR screening platform, which enables the rapid identification of CAR variants with unique properties from large libraries in high-throughput fashion.
Methods: The platform is based on reporter cells that were derived from the immortalized Jurkat T-cell line and modified with NF-κB- and NFAT-inducible CFP and GFP reporter genes. CARs were stably integrated in reporter cells, which were then activated with stimulator cells expressing the target antigen to analyze reporter gene-activation by flow cytometry.
Results: First, we studied the importance of the transcription factors NF-κB and NFAT in CAR activation. Stimulation of ROR1-CAR T-cells revealed an accumulation of both proteins in the nucleus. Likewise, ROR1-CAR expressing reporter cells demonstrated NF-κB- and NFAT-induced reporter activation 24 hours upon stimulation with $ROR1^+$ target cells. Based on the induced CFP and GFP expression, reporter cells also readily identified the optimal CAR construct from a mini-library of n=3 ROR1-CARs that differed in spacer length. Next, we challenged our reporter platform to a CAR library screen with ~$2x10^5$ receptor variants derived from the R11 ROR1-CAR and containing four randomly altered amino acids in the scFv CDR3 region. After enrichment of CAR positive reporter cells and ROR1-dependent stimulation, we performed single cell sorting of CFP^+ GFP^+ reporter cells, which established 20 clones with strong reporter signal. CDR3 sequencing revealed 3 unique CAR variants that are currently functionally analyzed in primary T-cells in comparison to wild-type ROR1-CAR.
Conclusions: We established a versatile CAR screening platform with NF-κB- and NFAT-inducible reporter genes. Our data show that this platform can be utilized to identify CAR constructs from CAR libraries that are otherwise not amenable to evaluation in primary T-cells. We are currently adapting this platform for screening campaigns on naive antibody libraries to identify CAR constructs that recognize novel tumor antigens.

Poster Abstracts

M=Monday
S=Sunday
Roman numerals = Topic

Tumor heterogeneity & metastasis

Poster S-I-01

Characterization of the Functional Heterogeneity of a Patient's Acute Myeloid Leukemia Cells in a Xenograft Mouse Model

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Cancers, such as acute myeloid leukemia (AML), are heterogeneous diseases consisting of cells with different genetic and epigenetic alterations as well as different functional phenotypes. If a subpopulation of treatment resistant cells exists, relapse might occur after chemotherapy. Understanding genetic and functional heterogeneity of tumors will help to better prevent relapse. A model system allowing functional studies on leukemia cells in vivo is the individualized xenograft mouse model, where patients´ primary tumor cells are transplanted into severely immunocompromised mice. These so called patient derived xenografted (PDX) AML cells can be serially retransplanted, can be genetically manipulated using lentiviruses, and maintain the characteristics of the transplanted tumor cells. Therefore, this model represents a valuable tool for the study of cancer heterogeneity and/or clonal evolution. We have established a PDX AML sample pair, the first and second relapse of the same patient, which was genetically characterized by next generation sequencing and functionally characterized using the in vivo mouse model. Both first and second relapse consist of a main clone and several minor subclones, indicating the presence of clonal heterogeneity. An additional mutation was gained in the second relapse in the patient, indicating genetic evolution in men. Drug response towards systemic treatment with Cytarabine in mice in vivo was different between both relapses with increased treatment resistance in relapse 2, despite increased growth speed of relapse 2. Taken together and by combining genetic, epigenetic and functional characterization of patients´ tumor cells growing in mice, our in vivo model represents a suitable tool for the study of cancer heterogeneity and evolution. In the future, this model could help to identify mechanisms of therapy resistance and disease progression, and to define putative targets for novel therapeutic approaches.

Poster S-I-02

Clonal evolution is much more common in myelodysplastic syndromes than previously described

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Introduction: Clonal evolution (CE) is known to be associated with poor survival in myelodysplastic syndromes (MDS) and almost 30% of patients have been identified by conventional cytogenetics (CC) to acquire cytogenetic aberrations (CA) during the course of the disease [1-4]. To determine the true incidence of CE in MDS we considered obvious CA, cryptic CA (that cannot be detected by CC), and molecular mutations (MM, e.g. point mutations, indels) in our study thus allowing a more comprehensive view on genomic dynamics in MDS.
Methods: We retrospectively reviewed 108 pts with proven MDS for which CC, but also the molecular karyotype from SNP-array analysis (SNP-A) were available. Cryptic CA (cryptic copy number (CN) variations and CN neutral losses of heterozygosity (CN-LOH)) were detected by fluorescence in situ hybridization and SNP-A analyses. Results from NGS/Sanger panel-sequencing were included for 73/108 pts. Genetic follow-up from bone marrow and/or CD34+ peripheral blood cells was available for 61 pts (31 treated with disease modifying therapies).
Results: Obvious and cryptic CA were detected in 65/108 (60%) and MM in 49/73 (67%) pts at diagnosis or during the disease. In detail, 12 pts presented with cryptic CN variations and 24 pts with CN-LOHs, from which 17 showed a MM or a cryptic deletion in the region of the CN-LOH. The formation of a "double hit" aberration from a hetero- to a homozygous MM due to a CN-LOH was observed in consecutive samples of 3 pts.
CE involving any obvious CA, cryptic CA or MM was detected in 35/61 (57%) pts (median observation 42 mos). The first CE event was detected 0-176 mos after diagnosis (median 13 mos), including 8 pts showing patterns of antecedent CE at diagnosis (2x sub-clones; 6x homozygous MM in CN-LOHs). Median survival from first CE event was significantly shorter than median survival from first diagnosis for pts without CE (25 vs. 78 mos, P=0.007). Subsequent CE events were observed in 7/27 (26%) pts with detection of CE after diagnosis and in 7/8 (88%) pts with patterns of CE at diagnosis.
Summary: Considering obvious CA, cryptic CA, and MM, resulted in a much higher incidence of CE in our cohort than observed ever before in MDS. Particularly, the development of homozygous MM due to cytogenetic LOH seems to be an important step towards progression. The high incidence of CE is important for therapy management of MDS patients, especially if the targets in future targeted therapies are involved in CE.

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Poster S-I-03

Secretion of catalase drives a novel mechanism of resistance to oxidative stress in acute myeloid leukaemia

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Reactive oxygen species (ROS) are a group of inorganic molecules derived from diatomic oxygen (O_2). In biological systems, a key step in ROS formation is the univalent reduction of O_2 to produce superoxide radicals ($O_2^{\bullet-}$), which are an important precursor to many biologically important ROS, especially hydrogen peroxide (H_2O_2). H_2O_2 is a relatively long-lived and mildly reactive molecule which crosses biological membranes and can act as a second messenger.[1, 2] In normal and leukaemic contexts, $O_2^{\bullet-}$ and H_2O_2 are generated within the mitochondria as a by-product of oxidative phosphorylation,[3] and also by NADPH oxidase (NOX) proteins whose sole biological function is the generation of $O_2^{\bullet-}$.[4] ROS production is normally counterbalanced by an extensive repertoire of antioxidant defences. However, in the context of leukemia,[5] myelodysplasia[6] (and cancer generally[7, 8]) chronic elevated ROS production and depletion of antioxidant defences can promote oxidative stress, a state characterised by oxidative damage to DNA, lipids and proteins; and dysregulated redox signalling.[9, 10] Indeed, leukaemia cells may exploit this pro-oxidative state, which is known to promote proliferation,[11, 12] inflammation,[13] and genomic instability through DNA damage.[5, 14] The kinase $p38^{MAPK}$ is a key sensor of environmental stress including oxidative stress.[15, 16] $p38^{MAPK}$ activation drives cell-cycle arrest and DNA repair and in cases of chronic activation, senescence or apoptosis.[17] $p38^{MAPK}$ is therefore regarded as a tumour-suppressor in oncogenic transformation models.[18, 19] Cancer cells are known to adapt to evade the consequences of oxidative stress by increasing glutathione pools[20, 21] or by silencing of $p38^{MAPK}$ signaling.[19] We previously showed that over 60% of AML patients exhibit elevated extracellular superoxide and H_2O_2 production, and significant impairment of several antioxidant systems.[11] Despite this, these cells unexpectedly maintained minimal $p38^{MAPK}$ activation, reminiscent of the adaptive responses of solid tumours to oxidative stress.[19] However, the mechanism underlying this adaption was unknown. Here, we report for the first time that catalase plays a key protective role against the effects of excessive H_2O_2 in the context of primary AML. We also report that AML blasts constitutively secrete catalase and that this secretion is regulated in response to redox stress accounting for most if not all of the oxidative resistance characteristic of AML.

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Poster S-I-04

MN1 gene expression as a prognostic marker in patients with acute myeloid leukemia after allogeneic transplantation.

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Background: The meningioma 1 (MN1) gene is located at chromosome band 22q12 and it's overexpression is associated with a poor prognosis in patients with acute myeloid leukemia (Baldus et al., 2007, Heuser et al., 2006).
The **aim** of our research was to check the clinical significance of aberrant MN1 expression on patients after HSC. In addition, we investigated the possibility of using the MN1 gene as a marker of minimal residual disease.
Methods: The material was obtained from 68 AML patients both before and after the alloHSCT. After the RNA isolation and reverse transcription reaction, gene expression was analyzed by using the MN1 Oncoprime system (Inogene). The expression results were normalized to the ABL1 gene. As a threshold level, two values were used - 500 and 1000 copies of the MN1 gene per 10,000 copies of the ABL1 gene.
Results: To assess the possibility of using the MN1 gene expression in order to estimate the minimal residual disease, we compared the expression data with the number of blast cells and could not detect the presence of correlation dependence. A similar picture was observed when compared the expression data of MN1 and chimeric transcripts as well as with a level of donor chimerism.In addition, in patients characterized by resistance to therapy prior to transplantation, we detected a significant increase of the MN1 expression level. Higher values were observed in patients with adverse cytogenetic risk ($p < 0.05$). Moreover, we obtained data that the detection of MN1 overexpression after HSC did not have a significant prognostic value, while in patients with overexpression of this gene more than 1000 copies of MN1 to HSC, a decrease in overall survival was noted. At the same time, as the achievement of an expression level of 500 copies, 1000 copies of the MN1 gene were associated with a greater probability of posttransplant relapses ($p = 0.02$ and $p = 0.07$, respectively). When assessing the effectiveness of the conditioning regime type, we did not reveal they significant differences in prognostic significance in patients with registered hyperexpression before HSCT ($p = 0.9$).
Conclusions: Our results indicate that MN1 is not a marker of therapy efficiency and can not be recommended for estimating the minimum residual disease. At the same time, the obtained results indicate the most aggressive characteristics of the MN1 positive tumor clone, which may cause the development of posttransplantat relapses.

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Poster S-I-05

Calcitonin receptor-like is a novel prognostic biomarker and a potential therapeutic target in acute myeloid leukemia

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Calcitonin receptor-like (*CALCRL*) is a G protein-coupled receptor that mediates the pleiotropic effects of adrenomedullin and calcitonin gene-related peptide, two structurally related neuropeptides involved in the regulation of blood pressure, angiogenesis, cell proliferation and apoptosis. It is currently emerging as a novel target for the treatment of migraine and has also been described as a therapeutic target in models of solid tumours. A role in acute myeloid leukemia (AML) has not yet been described. We analysed *CALCRL* transcript levels in pretreatment samples from 492 intensively treated AML patients from the AMLCG-1999 trial. Cox regression was performed to evaluate associations with survival. We validated our findings in four independent international cohorts of intensively treated AML patients: HOVON (n=400), TCGA (n=125), and Leucegene (n=263) for *CALCRL* mRNA, and UKM (n=190) for protein expression. When trichotomized at the 25th and the 75th quartile, increasing mRNA levels of *CALCRL* were associated with poorer overall survival (5-year OS, 43.1%, 26.2%, and 7.1% for low, intermediate and high *CALCRL* expression; p=0.0007), event-free survival (5-year EFS, 29.9%, 15.8%, and 4.7%; p<0.0001), and lower complete remission (CR) rates (71.5%, 53.7%, and 49.6%; p<0.0001) in the AMLCG-1999 discovery cohort. *CALCRL* expression levels remained independently associated with OS (p=0.024), EFS (p=0.0091) and CR rate (p=0.015) on multivariable regression. The prognostic impact was confirmed in all three mRNA (HOVON, TCGA, and Leucegene) and in the protein expression validation cohort (UKM). Interestingly, *CALCRL*^{high} cases were significantly enriched for gene sets found in previously established leukemic stem cell (LSC) signatures. In addition, *CALCRL* levels were positively linked to LSC frequencies in primary AML patient samples and to their engraftment capacity in immunocompromised mice. Finally, CRISPR-Cas9-mediated knockout of *CALCRL* resulted in significantly lower colony formation in three human AML cell lines. In summary, we identified *CALCRL* expression as a novel biomarker of stemness in AML, that predicts response to chemotherapy and survival beyond existing clinicopathologic and genetic risk factors. These findings, together with the favourable safety profiles of therapeutic antibodies interfering with CALCRL signalling in recently reported clinical trials, will stimulate future research on repositioning CALCRL targeting drugs to the context of AML.

Stem cells in homeostasis and disease

Poster S-II-01

Alterations in the bone marrow stromal microenvironment precursor cells in patients with Diffuse Large B-cell Lymphoma without bone marrow involvement

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In most patients with Diffuse Large B-cell Lymphoma bone marrow (BM) is not affected. Patients, depending on IPI, are treated either by R±CHOP or R±mNHL-BFM-90 courses. Changes in the BM stroma are observed 6-12 years after treatment. Alterations in stromal precursor cells MSCs and CFU-F are obscure.
The aim of the study was to investigate the changes in MSCs and CFU-F from the BM of patients at the onset of the disease and 6-12 years after treatment.
The study included 10 patients at the onset of the disease, 20 after R±mNHL-BFM-90 and 11 after R±CHOP. BM MSCs were isolated by standard method and the concentration of CFU-F was determined. The gene relative expression level (REL) in MSCs was determined by real-time PCR. As a control 31 MSC samples from the BM of healthy donors selected for each group of patients according to age were used.
In primary patients, the concentration of CFU-F in the BM is reduced by 30%. After R±CHOP, it is 2 times reduced compared with donors and 1.6 times with primary patients. After R±mNHL-BFM-90 it is 1.5 times higher than that of donors and 2 times higher than in patients after R±CHOP.
The total cell production of MSCs in primary patients is 2.3 times higher than that of donors. Many years after R±CHOP, it almost reaches the level of donors, after R±mNHL-BFM-90, it remains 1.7 times higher.
The *FGF2* REL in MSCs is reduced in primary patients and years after treatment. The REL of *FGFR1* is lower in patients compared to donors. The *FGFR2* REL is doubled in primary patients compared to donors and remains significantly increased for years. The *ICAM1* and *MMP2* REL involved in the adhesion of hematopoietic cells, are 2 times reduced. It indicates irreversible changes in the ability to maintain hematopoiesis. In primary patients the *SPP1* REL is increased 3 times in comparison with donors. It decreases after R±CHOP but it rises even more after R±mNHL-BFM-90. The *BGLAP* REL is reduced in primary patients and remains low for years. Patients bone tissue suffers and these changes are not restored. The REL of interleukin 6 (IL6) is reduced in primary patients by 3.5 times in comparison with donors. Years after chemotherapy, it remains reduced 2 times. These changes can be attributed to the HSC protective mechanisms used by the MSCs.
Stromal BM precursor cells respond to a lymphoid tumor even if there is no BM involvement. Analysis of all factors shows that many years after treatment, the characteristics of MSCs are only partially restored.

Poster S-II-02

Intraosseous administration of multipotent mesenchymal stromal cells to patients with graft failure after allogeneic bone marrow transplantation simultaneously with repeated transplantation of hematopoietic cells

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One of the reasons of graft failure (GF) in patients after allogeneic bone marrow transplantation (allo-BMT) could be the damage of hematopoietic microenvironment by chemotherapy and tumor cells. As a result, the bone marrow (BM) stroma loses its ability to support hematopoiesis. Multipotent mesenchymal stromal cells (MSCs) participate in the formation of niches for hematopoietic stem cells. The aim of the study was to investigate the possibility of restoring niches for hematopoietic cells by means of donor MSCs. In National Research Center for Hematology for each patient, MSCs from their own BM donor are stored individually. There were 8 patients with GF after allo-BMT. In all cases, an attempt was made to repeat allo-HSCT with simultaneous intraosseous (IO) introduction of MSC from the BM donor. MSCs were injected into the iliac crest. MSCs were thawed and suspended in 2 ml of 6% polyglucin. Cells (200-370 x 10^6) were introduced into the bone tissue in small portions of 100-200 µl through 2 punctures of skin and multiple punctures of periosteum. In patients after IO MSCs administration and repeated allo-HSCT from the same donor, restoration of donor hematopoiesis was noted. At 1-2, 4-5, and 9 months after IO injection of donor MSCs, BM punctures were performed. MSCs were isolated and cultured from 2-3 ml of BM from 2-4 independent punctures of iliac crest of each patient according to a standard protocol. DNA was isolated and chimerism was determined on the 1-3th passages from patients MSCs. It was shown that the MSCs introduced in a small amount remain localized at the site of administration and do not lose the ability to proliferate ex vivo. In fact, these cells survived 3 transfers: from the donor's organism to the culture, then again into the patient's body and repeatedly into the culture. The proportion of donor cells among patients MSCs was small (9.4 ± 3.3%). This may be due to the fact that the site of BM sampling did not coincide with the place of MSCs introduction or with the fact that a small amount of MSCs was injected. Probably MSCs performed a trophic function and participated in the restoration of niches for hematopoietic cells. The long-term existence of MSCs in the human body is shown for the first time. A large potency of MSCs to repeated transplantations was revealed. Donor MSCs improved the stromal microenvironment of patients and participated in the restoration of donor hematopoiesis.

Poster S-II-03

The dynamics of human multipotent mesenchymal stem cells' clonal composition

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Multipotent mesenchymal stromal cells (MSCs) could assist the regeneration of bone and cartilage tissues. To effectively use these cells, it is necessary to know MSCs' clonal composition and its dynamics. The aim of this study was to reveal the clonal composition of human MSCs and to follow its dynamics by marking the cells with a library of self-inactivating lentiviral vectors carrying genetic barcodes (BCs). The library included 671 BCs. MSCs were obtained from bone marrow of 6 healthy donors and for each donor 10^5 MSCs were infected at passage zero (P0) with the library. At each passage, 10^5 cells were transferred to the next passage, 120 cells were cloned 1 cell/well in 96-well plates, and DNA was extracted from the remaining cells. The latter was used to determine the average number of proviruses per diploid genome (VCN) by digital droplet PCR. The same DNA was used to reveal the repertoire and the relative abundance of each BC in the overall cell population by applying the Illumina-based sequencing. DNA was extracted from the single cell derived colonies, and BCs were determined in each colony by Sanger sequencing. The clonal composition of MSCs at each passage was analyzed by comparing the combinations of BCs in each colony. The analysis of BCs in cell clones showed that the MSCs population is polyclonal. When the cultures were passaged, their clonal composition changed substantially, while the proportion of cell clones containing BCs was stable in passages (it was 76 ± 6 % at P1). The VCN was in the range 7-35 at the P1 due to high MOI. VCN dropped to 4-6 at P2, and further to 2-5 at P3 and 1-3 at P4. It indicated that clones with multiple integration sites lost their proliferative potential (PP). Clones represented by large number of cells did not exceed 5-10% and were detected only at P1. The results of deep sequencing showed that the proportion of BCs having significant contribution decreased from 8% to 1%, and the proportion of BCs, which contribute imperceptibly, increased from 10% to 20% by P2. The maximum contribution of a BC to the total pool was 1-2%, which corresponded to 3000-6000 cells. The main population of MSCs consisted of cell clones that did not exceed 500 cells. So the MSCs population is represented by a lot of small clones. At each passage, only a portion of cells develops its PP and contributes to the culture. The majority of cells is dormant and realizes its PP in subsequent passages.

Poster S-II-04

Alterations in Multipotent Mesenchymal Stromal Cells from the Bone Marrow of Acute Myeloid Leukemia Patients at Diagnosis and During Treatment

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We analysed multipotent mesenchymal stromal cells (MMSCs) from the bone marrow of 33 acute myeloid leukemia (AML) patients at diagnosis (day 0), after first course of chemotherapy (day 37), and at days 100 and 180 after the diagnosis. All patients were treated according to AML 01.10 protocol. Cumulative cell production of MMSC cultures from AML patient at diagnosis did not differ from donors but it constantly increased during the treatment. Time needed to reach confluence after the initial bone marrow seeding (time to P0) was significantly increased at diagnosis and during the treatment in comparison with donors' MMSCs. At diagnosis of AML most of the studied genes were upregulated; during treatment some remained upregulated (e.g., *IL6*, *IL1B*, and *LIF*), others downregulated (e.g., *FGFR1*, *FGFR2*, and *ICAM1*) or normalized. A few genes were normal at diagnosis but decreased during treatment (*FGF2*, *VEGF*, *VCAM*, *SDF1*, *SOX9*, and *TGFB1*). *PDGFRB* expression was upregulated in MMSCs from patients who were not in remission at day 37.
Our data suggest that regulation of MMSC proliferation is a complex process and could not depend on FGF2, PDGF, or TGF pathways only. AML development strongly affects MMSCs in the BM resulting in the upregulation of the most genes studied. The upregulation of the expression of pro-inflammatory genes both at diagnosis and during the remission reflects constantly ongoing inflammation processes. AML 01.10 protocol downregulates the expression of the number of genes regulating proliferation, differentiation and niche formation.
The study presents unique data of MMSC status at diagnosis and during half-a-year of remission.

Poster S-II-05

A role of erythroid progenitor cells in pathogenesis of chronic myeloid leukemia

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Chronic myeloid leukemia, originated from leukemia stem cell, was the first oncological disease whose cause was clearly associated with the target molecule, tyrosine kinase BCR-ABL. This led to the discovery of targeted drugs – tyrosine kinase inhibitors (TICs), which provide selective action only on cells of the leukemic clone. The fact that the Philadelphia chromosome is detected not only in the granulocyte-macrophage, but also in the cells of the erythroid lineage of hematopoiesis is also confirmed by the existence of leukemia stem cell. It was known that in the long-term culture of the Dexter type CML granulocyte-macrophage progenitor cells are maintained longer than erythroid progenitor cells which are eliminated much faster (by 17-21 days). However, there is a large number of controversial data regarding the role of the erythroid progenitor cells in the pathogenesis of CML. Hematopoietic progenitor cells obtained from bone marrow of 32 patients with CML were studied in semisolid agar culture *in vitro* and *in vivo* (in diffusion chambers). Cultured cells were analyzed regarding their phenotypes and functions using flow cytometry, colony-forming unit (CFU) assay and long-term culture-initiating cells (LTC-IC) assay. It was founded that leukemic clone cells are characterized by the ability to proliferate and differentiate in the granulocyte-macrophage and erythroid directions, regardless of the presence of the granulocyte-macrophage factor and erythropoietin in the culture medium. With the application of the original model for determination of hematopoietic progenitor cells in gel diffusion chambers *in vivo*, the influence of soluble microenvironment factors on the erythroid progenitor cells in CML was detected. These factors inhibited proliferation of erythroid progenitor cells in *in vivo* culture. At the same time it was founded that in diffusion chambers, implanted into peritoneal cavity of irradiated animals, progenitor cells of the granulocyte-macrophage lineage in CML are insensitive to soluble microenvironmental factors from organism of healthy mouse. Consequently gel diffusion chambers cultivation *in vivo* might provide more adaptive microenvironment for leukemic stem/progenitor cell growth and might be an original tool for investigation of the behavior of leukemic hematopoietic stem cells/hematopoietic progenitor cells.

Poster S-II-06

Use of CCR5-DELTA 32, CCR2-64I, SDF1-3'A , IL6 -572 C/G polymorphic alleles as chimerism markers and possible prognostic factors for transplantation outcome.

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Background: Immunological tolerance after bone marrow transplantation (BMT) depends on complex interaction between graft and host cells and includes cytokine production and reception. Both host and graft cells may carry different polymorphic alleles in appropriate genes that may alter these processes. Possible impact of cytokine gene polymorphisms on the BMT outcome have not been thoroughly studied yet. **Aims:** To measure CCR5-delta 32, CCR2-64I, SDF1-3'A, IL6 -572 C/G allele frequencies for recipients and donors. To evaluate possible correlation of overall survival, reconstitution time and level of GVHD with appropriate polymorphisms in host and graft cells. **Methods:** Forty three patients 18 to 64 years old (median age 32), 13 males and 30 females who received allogeneic (14 bone marrow and 29 peripheral stem cells) hematopoietic transplantation for AML(26 cases), ALL(9), AA(3), MDS(2), CML(2) and MPN/CLL(1 case) in the National Research Center for Hematology were included in the study. DNA of donors and recipients were analyzed for CCR5-delta 32(rs333), CCR2-64I(rs1799864), SDF1-3'A(rs1801157), IL6 -572 C/G (rs1800796) polymorphisms by means of allele-specific PCR. SAS software was used to correlate genetic data with transplantation outcome. **Results:** In 27 patients (63%) unique recipient alleles and in 25 patients (58%) unique donor alleles were informative for chimerism calculation. For each polymorphism groups with no mutant alleles in both recipient and donor (1); equal number of mutant alleles (2); more mutant alleles in recipient (3); more mutant alleles in donor (4) were compared. All groups were equal in graft source, diagnosis, disease stage, gender and age. No correlations of most polymorphic alleles with overall survival, reconstitution time, frequency of a GVHD and infectious complications at day 30 were found. Hematopoesis reconstitution time was significantly longer ($p<0.0001$) and infectious complications were more frequent at day 30-120 ($p<0,0016$) when CCR2 mutated cells were delivered to wild type CCR2 recipient. **Conclusion:** Decreased reconstitution rate and increased levels of infectious complications could be seen in patients who received transplant from donors carrying mutant CCR2 alleles. Polymorphic alleles evaluated could be used for chimerism monitoring.

Poster S-II-07

JAK1-STAT3 signaling axis supports leukemic stem cell persistence in CML

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Tyrosine kinase inhibitors (TKI) block oncogenic Bcr-Abl signaling and greatly improved CML therapy, though the disease-initiating leukemic stem cells (LCSs) survive within the bone marrow (BM) niche independent of Bcr-Abl kinase activity[1]. The transcription factor STAT3 was shown to be highly activated in TKI resisting CML cells, supported by the BM microenvironment[2] and targeting STAT3 in combination with Bcr-Abl was potent to kill TKI-resistant LCSs[3]. Here, we investigated the mechanism of persisting STAT3 activation and applied specific JAK inhibitors to various CML cells. STAT3 mRNA expression was significantly increased in KCL-22 (1.9-fold, $p \leq 0.001$) and primary CML MNC (1.6-fold, $p \leq 0.05$) upon Bcr-Abl inhibition, exclusively in presence of mesenchymal stroma cell (MSC) derived conditioned medium (CM). Likewise, tyrosine phosphorylation of STAT3 was strongly increased in transgenic murine Bcr-Abl BM, KCL-22 and CML-MNC. Upon treatment with JAK1 (filgotinib/itacitinib) but not JAK2 (BMS-911543) specific inhibitors, persisting $pSTAT3^{Y705}$ was decreased to basal levels in transgenic murine Bcr-Abl BM, KCL-22 and CML MNC. A similar result was observed when applying an IL-6 blocking receptor fusion protein (RFP) suggesting this effect to be IL-6 mediated. Combined inhibition of Bcr-Abl and JAK1 strongly reduced the colony forming unit (CFU) capacity of transgenic murine Bcr-Abl BM (2.2-fold, $p \leq 0.001$) and primary human CML $CD34^+$ cells (6.9-fold, $p \leq 0.001$) compared to IM treatment alone. Tracking the proliferation of CML $CD34^+$ cells by CSFE staining and flow cytometry analysis revealed increased quiescence (3.2-fold, $p \leq 0.001$) and decreased proliferation (3.6-fold, $p \leq 0.001$) in Bcr-Abl- and JAK1-inhibited cells compared to single treatments. Interestingly, combined Bcr-Abl and JAK1 inhibition strongly induced apoptosis even in quiescent LSCs (2.4-fold, $p \leq 0.001$). In summary, STAT3 is strongly activated in Bcr-Abl leukemic cells upon oncogene inhibition in presence of BM microenvironment-derived CM. Here, we identified JAK1 as the STAT3-activating kinase, promoting CML LSC survival. Pharmacological JAK1 inhibition decreases persisting $pSTAT3^{Y705}$ levels, blocks proliferation and reduces CFU capacity. Importantly, apoptosis is strongly induced in quiescent LCSs upon combined Bcr-Abl and JAK1 inhibition. As a consequence, JAK1 emerges as a new potential therapeutic target for curative CML therapies.

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Poster S-II-08

Role of minimal residual disease in disease progression in children with acute lymphoblastic leukemia after allogeneic hematopoietic stem cell transplantation.

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Allogeneic hematopoietic stem cell transplantation (HSCT) is widely used treatment for acute lymphoblastic leukemia (ALL), but relapses are still the main cause of treatment failure. Clonal Ig/TCR rearrangements can be used as specific molecular markers for minimal residual disease (MRD) monitoring.

The aim of the study was evaluating the impact of PCR-based MRD on outcome after alloHSCT.

Patients and methods: The study included 45 children with ALL after the first alloHSCT. RQ-PCR with patient-specific primer was used for MRD measuring.

Results: Ig/TCR markers were identified for 37 (82.2%) of 45 patients. MRD monitoring was performed after 35 alloHSCT. MRD was negative in 21 (60%) patients, 2 (9.5%) of them relapsed (1 extramedullary relapse, 1 bone marrow relapse with the loss of the Ig/TCR target).

MRD was a positive in 14 (40%) patients and 7 (50%) of them had bone marrow relapse. In all 6 patients with a high MRD level $>10^{-3}$ we observed disease reoccurrence. In patients with negative MRD, with MRD $\leq 10^{-3}$ and $>10^{-3}$ 3-year overall survival (OS) was 83.6±8.8% vs. 57.1±18.7% vs. 0% (p=0.0083), event-free survival (EFS) was 66.6±11.4% vs. 43.8±18.8% vs. 0% (p=0.0012), CI of relapse was 10.7±7.4% vs. 14.6 ± 14.6% vs. 100% (p <0.0001).

MRD positivity combined with increasing mixed chimerism (MC) led to relapse in almost all cases. In patients with negative MRD and full donor chimerism (FDC), with positive MRD and FDC and with positive MRD and increasing MC 3-year OS was 94.4±5.4% vs. 44.4±22.2% vs. 20.0±17.9% (p=0.0029), EFS was 75.0±11.0% vs. 25.0±20.4% vs. 0% (p <0.0001), CI relapse was 11.9±8.2% vs. 41.7±29.5% vs. 80.0±23.9% (p=0.0008). MRD clearance was more often observed in patients with FDC having GvHD or after donor lymphocyte infusion.

The outcome of patients with negative MRD without aGvHD, with negative MRD without aGvHD, with positive MRD without aGvHD was significantly better than the outcome of patients in patients with positive MRD and no aGvHD. OS was 80.0±17.9% vs. 84.6±10.0% vs. 66.7±27.2% vs. 18.0±15.1% (p=0.026), EFS 60.1±21.9% vs. 67.3±13.6% vs. 66.7±27.2% vs. 0% (p=0.0004), CI relapse was 20.0±20.0% vs. 7.7±7.7% vs. 0% vs. 80.0±20.2% (p=0.008).

Conclusion. Positive MRD after HSCT is an unfavorable factor for OS, EFS and associated with ALL reoccurrence. We identified the high risk group of relapse for ALL patients after alloHSCT who are: 1. MRD positive with MC and no GvHD. 2. With MRD>10^{-3} regardless of the chimerism.

Poster S-II-09

WT1 and BAALC genes expression analysis in post-transplant relapse prediction.

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Background. Up to 50 percent of acute myeloid leukemia (AML) cases does not have any informative genetic markers of tumor cells. *WT1* and *BAALC* gene expression analysis might be one of the possible approaches in relapse development monitoring in patient with AML.
Aims. The evaluation of *WT1* and *BAALC* gene markers overexpression significance in assessment of post-transplant relapse risk and impact on the disease prognosis over a 2-year period after the allo-HSCT in patients with AML.
Methods. Our study includes bone marrow samples of 93 patients with AML (2 to 60 years old) (M0-6pts, M1-13pts, M2-19pts, M3-2pts, M4-29pts, M5-12pts, M7-1pts, unspecified – 10pts) who underwent allo-HSCT. The estimation of *WT1 and BAALC* relative gene expression levels and the level of chimeric transripts expression was performed by RQ-PCR with the normalization to ABL reference gene and were analyzing by using nonparametric criteria.
Results. We observed high correlational ratio between blasts cells and relative expression of investigated genes (Spearmen ratio - 0.489 and 0.433 respectively ($p<0.05$). High correlation level was observed also with chimeric transcripts. We established following cutoff levels for the investigated genes based on their analysis in patients without the relapse after allo-HSCT: WT1 – 250, and 60 for *BAALC*. The patients with *WT1* and *BAALC* overexpression had significantly lower OS and DFS ($p<0.001$). There was found that the monitoring of investigated expression markers simultaneously had higher summary specificity and sensitivity in the clinic-hematological relapse risk estimation ($S=0.881$), than the estimation of chimeric transcripts by QR-PCR ($S=0.865$) or expression levels of each expression marker separately ($S<0.863$). However in case of MRD monitoring the expression markers had lower values of specificity and sensitivity ($S<0.688$ for complex and separate monitoring) comparing to the fusion transcripts. This fact was confirmed also by positive fusion transcripts expression level (RUNX1/RUNX1T1, CBFB/MYH11 and PML/RARα), at the absence of *WT1*and *BAALC* markers overexpression.
Summary. Evaluation of universal gene marker expression panel is an attractive approach for assessing efficiency of therapy in patients with AML with normal karyotype. However, due to low specificity caused by basal expression the monitoring of tumor specific genetic markers, like chimeric transcripts or point mutations, is more preferable for MRD monitoring.

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Poster S-II-10

Mesenchymal stromal cell-derived extracellular vesicles provide long-term survival after radiotherapy without additional hematopoietic stem cell support

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Radiotherapy is widely applied for myelosuppressive therapy or myeloablative conditioning of patients with hematopoietic malignancies prior to hematopoietic stem cell (HSC) or bone marrow (BM) transplantation (Tx), often leading to high morbidity and mortality. Hence, reduction of adverse effects for a better outcome is pivotal. Based on efficacy of mesenchymal stromal cells (MSC) as therapeutics after irradiation *in vivo* we investigated the potency of MSC-derived extracellular vesicles (EV) as cell-free agent in this system.
EV were tested *in vitro* and *in vivo* for their protecting function on hematopoietic stem and progenitor cells (HPC) after radiotherapy. *In vitro*, mouse BM-derived mononuclear cells (MNC) were irradiated, incubated with or without EV and either tested directly in colony-forming assays (for HPC support) or after preincubation on stromal layers (for HSC protection). To investigate the effect *in vivo*, EV were transplanted in lethally irradiated mice and long-term survival was documented. Additionally, the fate and targeting of PKH26labeled EV was examined after Tx into lethally irradiated recipients. Two and 4h after Tx, BM was immunocytochemically analyzed via cytospins and image stream investigating the colocalization of labeled EV with HSC and HPC using the stem cell specific markers Sca1 and c-kit.
EV Tx into lethally irradiated mice resulted in long-term survival but no improvement in shortterm reconstitution. This result was in agreement with *in vitro* data showing EV protection of irradiated HSC but not HPC. After infusion into lethally irradiated mice, labeled EV traveled freely through the body reaching BM within 2h directly targeting reconstituting Sca-1^{+}and c-kit^{low} HSC matching the observed longterm survival of recipients.
Together, our data suggest EV as an effective treatment to combat radiation-induced hematopoietic failure improving patient survival. In addition, myelosuppression due to chemotherapy and toxic drug reaction might be compensated by EV as well. Based on data from proteomics and transcriptomics of mouse MSC-derived EV we show preliminary results which cargo of EV might be responsible for the protecting effect transferred to the hematopoietic system.

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Poster S-II-11

The Neogenin receptor represents a novel regulator of hematopoietic stem cell function during homeostasis and aging

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Hematopoietic stem cells (HSCs) hold the capacity to generate a series of multipotent progenitors that differentiate into lineage-committed progenitors and subsequently mature cells. Recently, we explored the molecular signatures employed by HSCs during differentiation by performing quantitative proteome, transcriptome (RNA-seq) and whole genome DNA methylation analyses on HSCs and multipotent progenitors and recently in dormant HSCs (Cabezas-Wallscheid et al., *Cell Stem Cell* 2014; Klimmeck et al., *Stem Cell Reports* 2014; Cabezas-Wallscheid et al., *Cell* 2017). These analyses revealed expression of the DCC-like cell surface receptor Neogenin (Neo) specifically in HSCs. Neo can act as a BMP co-receptor and as the receptor for neuronal guidance molecules such as Netrins. To investigate the role of Neo in HSC function, we analyzed mice carrying Neo-deficient HSCs during homeostasis and upon aging by performing long-term reconstitution assays. Strikingly, Neo-deficient HSCs showed a competitive repopulation advantage associated with reduced stem cell quiescence. RNA-seq analysis of young and aged Neo-deficient HSCs revealed reduced expression of dormancy related factors. In contrast, upon aging, Neo-deficient HSCs prematurely exhaust and show an increased myeloid differentiation bias. Furthermore, we will discuss the ligand in the bone marrow niche that maintains HSC quiescence through Neo. Taken together, our results introduce Neogenin as a novel receptor important for HSC quiescence, which may also be involved in the regulation of the hematopoietic aging process.

Poster S-II-12

Human hematopoietic stem cell engraftment in NSGW41 mice

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Xenotransplantation models enable the in-depth analysis of human hematopoietic stem cell (HSC) function *in vivo* [1,2]. However, human HSC engraftment and self-renewal are hampered in xenotransplantation settings restricting the study of mechanisms regulating human HSC function. Especially the study of interactions between human HSCs and niche cells is limited due to the requirement for irradiation preconditioning. By introducing a loss-of-function KIT receptor into NOD/SCID *Il2rg*$^{-/-}$ (NSG) mice we created a novel mouse strain, NOD/SCID *Il2rg*$^{-/-}$ *Kit*$^{W41/W41}$ (NSGW41), which combines an impaired endogenous HSC compartment with immunodeficiency [3]. This mouse strain efficiently supports stable long-term engraftment of human HSCs without the need for conditioning and shows highly improved multilineage engraftment, including the myeloid and megakaryocyte-erythroid lineages [4]. Moreover, by transplanting titrated numbers of human HSCs in primary and secondary recipients, we could show enhanced pick-up, maintenance and expansion of human HSCs *in vivo* compared to conventional KIT-proficient NSG mice. Thus, we show that human HSCs efficiently engraft in NSGW41 mice whereupon their self-renewal capacity und functionality are conserved in the recipient, making NSGW41 mice a useful tool to study human HSC function. We hypothesized that human KIT-proficient HSCs have a competitive advantage compared to endogenous HSCs with a defective KIT receptor and thus can stably engraftment in NSGW41 recipients. In fact, the endogenous HSC pool is significantly decreased in NSGW41 mice after engraftment of human HSPCs, supporting our hypothesis that endogenous HSCs are actively replaced from their niche. Remaining murine HSCs express wildtype levels of Kit receptor, suggesting competition between endogenous and human donor HSCs for the Kit-ligand stem cell factor (SCF). Surprisingly, analysis of the murine bone marrow niche revealed a significant expansion of CD51^{+} PDGFRα^{+} mesenchymal stromal cells (MSCs) after transplantation of human cells. MSCs are the main producers of SCF in the bone marrow. The increase in MSCs strictly correlated with the number of engrafted human HSCs, indicating that human donor HSCs modulate the murine niche. We suggest that MSCs provide a niche for human HSCs in mice and that human HSCs actively modify the murine bone marrow niche after xenotransplantation.

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Poster S-II-13

Dynamics of hematopoietic reconstitution in elderly recipients: old versus young grafts

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The surface molecule CD34 serves as a quality marker for human hematopoietic stem cell (HSC) grafts and is not only expressed on HSCs but also on a variety of progenitors. But the distinct role of these different subpopulations during hematopoietic reconstitution remains unknown. We applied our coloured genetic barcode system to analyse the influence and clonal contribution of four different subpopulations in a murine model. Fluorescence activated cell sorting (FACS) was used to sort common lymphoid, myeloid and multipotent progenitors (CLP, CMP, MPP) and stem cells (HSCs) from young (8 weeks, group 1) and aged (18 months, group 2) murine donors. After transduction with fluorescence protein (FP) expressing barcoded lentiviral vectors, populations were mixed and transplanted into lethally irradiated aged recipients. 1, 3, 8 and 16 weeks later, hematopoietic organs were analysed via FACS and genomic DNA was extracted for chimerism and barcode analyses. A maximum of 15% of cells in the peripheral blood expressed the encoded FPs, which were mostly derived from the MPPs and HSCs. CMP-derived cells were only detected 1 week after transplantation in the myeloid compartment. Cells derived from the CLPs were not detected at any time point. We analysed the barcode content of the differently marked cells after next-generation-sequencing. In accordance with the FACS-data, the majority of the clones during the 16 weeks of observation are derived from HSCs and MPPs. The CMP-derived clones were only contributing during the first weeks and the CLP-derived clones are barely detectable. The total number of clones is higher in the group, which received the "aged" graft, independently from the transduced cell population. The detected higher clonal diversity indicates the possible advantages of "aged" grafts in aged recipients. Our system allows clonal tracking of individual clones in four different subpopulations in parallel and analysing their contribution during reconstitution.

Leukemogenesis

Poster S-III-01

Dissecting an aberrant transcription factor: Convertibility of RUNX1/ETO fusion protein modules driving CD34+ human progenitor cell expansion

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Chromosomal translocations represent frequent events in human leukemia. In t(8;21)+ acute myeloid leukemia, the DNA-binding domain of RUNX1, which mediates specific target gene recognition, is fused to nearly the entire ETO protein, a nuclear repressor containing four conserved *nervy homology regions*, NHR1-4. Furthermore RUNX1/ETO interacts with ETO-homologous proteins via NHR2, thereby multiplying NHR domain contacts within the complex. As shown recently RUNX1/ETO retains oncogenic activity upon either deletion of the NHR3+4 N-CoR/SMRT interaction domain or substitution of the NHR2 tetramer domain. Thus, we aimed to clarify the specificities of the NHR domains.
A C-terminally NHR3+4 truncated RUNX1/ETO containing a heterologous, structurally highly related non-NHR2 tetramer interface translocated into the nucleus and bound to RUNX1 consensus motifs. However, it failed to interact with ETO-homologues, repress RUNX1 targets, and transform progenitors. Surprisingly, transforming capacity was fully restored by C-terminal fusion with ETO's NHR4 zinc-finger or the repressor domain 3 of N-CoR. With an inducible protein assembly system, we further demonstrated that repressor domain activity is critically required early in the establishment of progenitor cultures. Together we can show that NHR2 and NHR4 domains can be replaced by heterologous protein domains conferring tetramerization and repressor functions, thus showing that the NHR2 and NHR4 domain structures do not have specific functions concerning RUNX1/ETO activity for the establishment of human CD34+ cell expansion. It was archived to resemble the function of RUNX1/ETO through modular recomposition with protein domains from RUNX1, ETO, BCR and N-CoR without any NHR2 and NHR4 sequences.
As most transcriptional repressor proteins do not comprise tetramerization domains, our results provide a possible explanation as to the reason that RUNX1 is recurrently found translocated to ETO family members, which all contain tetramer together with transcriptional repressor moieties.

Poster S-III-02

***In vivo* CRISPR/Cas9 Screen to Identify Genes associated with high Leukemia Stem Cell (LSC) Frequency**

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Acute myeloid leukemia (AML) is a hematologic malignancy which is initiated by a small fraction of leukemia stem cells (LSCs). In previous work, we have applied RNA-Sequencing (RNA-Seq) on primary AML specimens and correlated gene expression data with LSC frequencies determined in xenograft assays. Eventually, 227 LSC associated candidates with significant difference in expression level between LSC^{high} and LSC^{low} AML were identified. The clustered regularly interspaced short palindromic repeats (CRISPR)/**C**RISPR-**a**ssociated **s**ystem (Cas) is a powerful technology to simultaneously and efficiently perform genome editing of multiple genes, but in vivo screening using human primary AML cells is technically challenging and limited by the rare number of LSCs in AML samples. Here we applied a two-step lentivirus CRISPR/Cas9 system to perform a loss and gain of function screen in a humanized NRGS mouse xenograft model to investigate essential leukemia drivers and suppressors. The CRISPR library was sequenced before and after parallel *in vivo* and *in vitro* selection and data were analyzed using the MAGeck software. Interestingly, we observed very little overlap between *in vitro* and *in vivo* screen results emphasizing the need for *in vivo* assays to study stem cell functions. We show here that CRISPR/Cas9 *in vivo* screening with humanized mouse models is feasible and powerful to identify novel LSC regulators in AML.

Poster S-III-03

Expression of retinoid acid receptor gamma (RARγ) is modulated by mIR-30a

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All-*trans*-retinoic acid (ATRA) plays critical regulatory roles in normal haematopoiesis and the pathogenesis of acute myeloid leukemia (AML).[1,2] Most notably, it has been curative when used in combination with arsenic trioxide in differentiation therapy of acute promyelocytic leukaemia (APL).[3,4] Although ATRA effectively inhibits growth and stimulates myelomonocytic differentiation of myeloid progenitors, it is equally potent in causing expansion of multipotent haematopoietic stem cells (HSC).[5,6] Results of studies utilizing mice that lack expression of a specific RAR and/or RAR subtype specific retinoids, as well as work addressing the molecular pathogenesis of acute promyelocytic leukaemia (APL), indicate that the influence of ATRA on differentiation and proliferation of HSC and myeloid progenitors is mediated via RARα and RARγ, respectively.[7,8] Using quantitative TaqMan PCR we have now shown that *RARG* mRNA is expressed in non-APL AML patients (over 80% of cases examined) and human cord blood derived stem cells but not in more mature myeloid progenitors or myelomonocytic cells. This change in the RARγ expression levels is paralleled by a reciprocal change in expression of *RARG* 3′-targeting miRNAs (mIR-24, mIR-30a, mIR-331), which we have identified and validated using multiple experimental strategies, including *RARG* 3′UTR-based reporter assays. RARγ protein and *RARG* mRNA are also expressed in cell lines derived from primary AML samples (MB61 and TEX)[9] but not in ATRA-responsive AML cell lines such as HL60. Using TEX cells as model of primary AML, we confirmed through lentiviral transduction by mIR-30α that this microRNA promotes differentiation and inhibits proliferation. Taken together, our results suggest that finely tuned and miRNA mediated down-regulation of RARγ expression in the myelomonocytic lineage switches ATRA responsiveness from RARγ-mediated pro-proliferation to RARα-mediated pro-differentiation. We therefore propose that the use of a RARα specific agonist, possibly in conjunction with a strategy that negatively targets RARγ (as with RARγ selective antagonist or siRNA), could be effective in retinoid-based differentiation therapy of non APL-AML.

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Poster S-III-04

Self-renewal pathways in DNMT3A, NPM1, and FLT3-ITD triple mutated Acute Myeloid Leukemia

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Normal karyotype (NK) AML patients most frequently harbour mutations in DNMT3A, NPM1 and FLT3-ITD. Concomitant occurrence of these three mutations is associated with very poor outcome, but only little is known about the pathology of this group hereafter called “triple AML”. We found that triple AML have high leukemic stem cell frequencies and can be immunophenotypically characterized by an aberrant GPR56 high/CD34 low profile. Strikingly, triple and double mutated subclones could be separated based on positive and negative GPR56 expression in a subset of these patients. Moreover, we identified a specific set of transcription factors significantly overexpressed in triple AML versus other NK-samples. Functional studies revealed a crucial role of these genes for in vivo engraftment of both normal and leukemic hematopoietic stem cells while being dispensable for short-term in vitro proliferation.

Poster S-III-05

A Systematic Evaluation of Prognostic Gene Expression Signatures in 1208 Acute Myeloid Leukemia Patients Reveals Novel Insights for Future Research and Clinical Application

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Prognostic gene expression signatures (pGES) have been proposed to assess risk and/or clarify therapeutic options for patients with acute myeloid leukaemia (AML). However, given uncertainties about the relationship of pGES to underlying biology, and difficulties generalising their utility across patient populations, none have been routinely adopted in clinical trial design or practise. To rectify this, we implemented and compared pGES proposed by Marcucci-7, Ng-17, Li-24, Eppert-LSCR-48, Metzeler-86, Eppert-HSCR-105 and Bullinger-133. A comparison of gene identities, leukaemia associated pathways, and expression in 7 stem/progenitor populations revealed no common associations. However, interrogation of the 7 pGES across 4 independent cohorts of intensively treated patients revealed that the majority were associated with overall survival (OS) in univariate and multivariate models. These included 6/7, 4/7, 5/7 and 3/7 pGES in univariate and 6/7, 4/7, 5/7 and 4/7 pGES in multivariate models when applied to the HOVON, TCGA, AMLCG-99 and AMLCG-08 cohorts, respectively. However, there was little agreement whether a particular patient was classified as favourable or adverse risk using the 7 pGES. We further investigated this lack of consensus in the largest patient cohort (HOVON). We found that 43/519 patients were classified to identical risk-groups by all 7 pGES (high consensus, CH), 189/519 and 143/519 patients were classified into the same risk-group by six and five pGES (intermediate consensus, CI-1 and CI-2). However, 144/512 patients were classified to opposing risk-groups by 3/4 pGES (low consensus, CL). An independent re-analysis of patients in CH and CL revealed that those with CH were dichotomised into favourable or unfavourable with significantly higher patient-consensus by twice as many pGES. Interestingly, patients with CH and CL had overlapping clinical characteristics but could be distinguished based a 17-gene classifier. Applied to two independent cohorts, we were able to classify 69 and 101 patients as CH, 70 and 33 patients as CL (AMLCG-2008 and TCGA, respectively). Strikingly, for patients in CH, 5/7 and 6/7 pGES and for patients with CL 1/7 pGES and 0/7 pGES were associated with patient-overall survival. In conclusion, for a subset of patients, most pGES were prognostic for OS despite any overt biological relationship of their constitutent genes. Importantly, these patients can be identified prospectively using a classifier of 17 genes.

Poster S-III-06

Genetic and Pharmacologic Targeting of LCP1 Impairs Eosinophilic Differentiation and Viability in Hypereosinophilia

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Hypereosinophilia (HE) is caused by a variety of disorders, ranging from parasite infections to autoimmune diseases and cancer. Only a small proportion of hypereosinophilia cases are clonal malignancies, and one of these, FIP1L1-PDGFRA (F/P) positive chronic eosinophilic leukemia (CEL), is sensitive to tyrosine kinase inhibitors, while most subtypes lack specific treatment. In this study, we investigated the role of LCP1, also termed L-plastin, in HE. LCP1 was dephosphorylated at Ser5, essential for LCP1 activity, after treatment with the PKCβ selective inhibitor enzastaurin in F/P-positive Eol-1 cells, and this was associated with reduced proliferation, metabolic activity, and colony formation as well as enhanced apoptosis and impaired migration. While enzastaurin did not alter F/P-induced STAT3, STAT5, and ERK1/2 phosphorylation, it inhibited $STAT1^{Tyr701}$ and AKT^{Ser473} but not AKT^{Thr308} phosphorylation, and shRNA knock-down experiments confirmed that this process was mediated by LCP1. Reduction of LCP1 protein or activity triggered mTORC2 activity loss, while PDK1 activity stayed essentially unchanged, explaining opposing phosphorylation pattern of the main regulatory tyrosines Ser473 and Thr308 of AKT. We confirmed that $SIN1^{T86}$ phosphorylation as a measure of mTORC2 activity was dependent on LCP1 protein, hypothesizing that LCP1 influences mTORC2 complex formation. In addition, both enzastaurin treatment and LCP1 knockdown led to impaired eosinophilic differentiation of HoxB8-immortalized murine bone marrow progenitor cells, and enzastaurin further reduced eosinophilic differentiation and survival of primary HE samples *in vitro*. In conclusion, our data show that HE involves active LCP1, which triggers mTORC2 activity, and that the PKCβ inhibitor enzastaurin may provide a novel treatment approach to hypereosinophilic disorders.

Poster S-III-07

Differencies in rearranged IGHV genes and stereotyped receptors in B-CLL and SMZL

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Immunoglobulin heavy chain V-gene (IGHV) mutational status is known to be a key factor for the long-term prognosis in B-cell chronic lymphocytic leukemia (B-CLL). In addition, the diversity of rearranged IGHV genes in B-CLL cells is very limited and significantly different from that of normal B cells. Recent data obtained suggest narrowing of the IGHV repertoire not only in B-CLL but also in SMZL. Our study included 683 patients with B-CLL and 45 with splenic marginal zone lymphoma (SMZL). Rearranged IGHV gene repertoire for two malignancies was compared. V-gene family usage was different for B-CLL and SMZL. In SMZL, half of all cases represent VH1 genes (51%), 27% - VH3 genes and 15% -VH4. In B-CLL cases 45% are VH3, 30% and 17.5% are VH1 and VH4 respectively. While typical distribution for normal B cells is VH3 - 57%, VH1 - 20%, VH4 - 18% [1]. Other VH genes usage was also rare for the cases investigated (1% -2%), similar to that for normal B cells. For SMZL patients from our sample with VH1 family genes all cases (with the exception of one) represent IGHV1-2 gene (49% of the total sample). In addition, there is a very high similarity of the nucleotide sequences of the CDR3 region for some of these cases. On the other hand, in B-CLL this gene is relatively rare and occurs in 5% of cases. Furthermore, in B-CLL this gene is preferably unmutated (84%), while in SMZL the frequencies of mutated and unmutated cases are similar. In B-CLL, the most common gene was IGHV1-69 (18%), it also participates in the formation of the most common stereotypic antigenic receptors (CLL # 3, 5, 6), but we didn't found this gene in SMZL patients. No stereotypic antigenic receptors described for B-CLL so far were found in our sample of SMZL patients. The narrowing of the IGHV gene repertoire in B-CLL and SMZL suggests antigen stimulation of B-cells could play an important role in the development of these diseases. At the same time differences in IGHV gene repertoire between these B-cell malignancies may indicate that different antigens may be involved. Unfortunately, our sample of patients with SMZL are much smaller than that for B-CLL. Further studies with extended samples of SMZL may be beneficial to reveal possible prognostic factors and probably CDR3 stereotype data for these diseases.

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Poster S-III-08

Estimation of hematopoietic progenitor cells in patients with chronic myeloid leukemia with resistance to tyrosine kinase inhibitors

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Chronic myeloid leukemia (CML) is a clonal oncological disease that is associated with the presence of the Philadelphia chromosome and the formation of the chimeric gene *bcr-abl*, the product of which is the active tyrosine kinase BCR-ABL, which plays a major role in the pathogenesis of CML. Data obtained in recent years, allow to assume the decisive role not only of hematopoietic stem cells (HSC), but also the progenitor cells of the bone marrow in both the onset and progression of the disease. However, information on the functional characteristics of bone marrow progenitor hematopoietic cells in different phases of the disease, in terms of therapeutic inhibition of tyrosine kinase BCR-ABL and resistance to imatinib is not sufficient today and the findings are contradictory. Based on the above, we considered it expedient to provide a morphofunctional analysis of hematopoietic progenitor cells removed from the bone marrow of 56 patients with CML in cell culture *in vitro*. The colony to cluster ratio (CCR) was calculated as number of colonies divided by the number of clusters. In this study we have analyzed changes in functional properties of hematopoietic progenitor cells, such as alterations in proliferation potential, ability to form cell aggregates during cultivation in CFU assay with further assessment of cytological and cytochemical composition of individually picked-up cell aggregates, concerning correlation with CML progression. The analysis of the results showed the ability of hematopoietic progenitor cells in CML to colony formation in a culture with semisolid agar and to maintain a long-term suspension culture without the addition of exogenous growth factors, depending on the response to the effect of the inhibitor of tyrosine kinase BCR-ABL. It was found that in samples of the bone marrow of patients who received busulfan before the use of imatinib, regardless of the number of months of its administration, was determined resistance to further imatinib therapy, high colony-forming activity and high proliferative potential. It is proved that indicators of proliferative potential and cellular composition of colonies formed during cultivation in a semisolid agar can serve not only to assess the state of the hematopoietic system at the time of the study, but also to be early predictors of the progression of the pathological process.

Poster S-III-09

The effect of ERK2 inhibitors on sensitivity of leukemia cells to KIT downregulation

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Cytokines, chemokines and growth factors are described as major regulators of several malignant diseases progression including AML. Also some of them were suggested to be responsible for the development of drug-resistant leukemia phenotypes. The majority of cytokine pathways are associated with ERK kinases. Early ERK2 kinase was suggested as a key regulator, responsible for malignant blood cells survival. In this study we used ERK2 small-molecular inhibitors to evaluate the sensitivity of leukemia cells to anti-cancer therapeutic agents treated with exogenous cytokines and growth factors. Here we demonstrated the increase in sensitivity of KIT-positive cells to anti-KIT small harpin RNA or imatinib when ERK2 inhibitors were added. Also we showed that leukemia cells treated with imatinib or after KIT downregulation become more responsive to cytokines and growth factors. Furthermore, we demonstrated how it may be modulated by ERK2 inhibitors. We suggest the strategy to reduce stress-response mechanisms existing in malignant myeloid cells by that they can avoid apoptosis and survive during the therapy.

Poster S-III-10

Activation of both pro-survival and pro-apoptotic signalling pathways in response to KIT downregulation in leukemia cells.

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Receptor tyrosine kinase KIT is found to be expressed in the high percentage of acute myeloid leukemia (AML) cases. Since that KIT is an attractive target for anti-leukemia treatment, but the input of non-mutant KIT up-regulation in AML still remains unclear. We used RNA interference (RNAi) technique to study the mechanisms responsible for survival of leukemia cells in response to KIT downregulation. We observed significant slowdown of cell growth when KIT is repressed. Also the alteration in expression of genes encoding cell cycle regulatory proteins such as cyclins and cyclin-associated kinases was demonstrated. Further we performed a genome-wide microarray-based screening of gene expression. We showed that KIT downregulation was followed by dramatic changes in signaling pathways activation profile. From the one side, most of them are responsible for cell death and suppression of cell growth. But from the other side, the huge number of activated pathways is associated with the kinases involved in cell survival, avoiding apoptosis and proliferation stimulation. Among them signaling pathways associated with ERK kinases were found to be up-regulated when KIT is suppressed and that could be defined as a compensatory mechanism. We suggest ERK2 as the key regulator, implying an existence of therapy resistant phenotype when anti-KIT drugs are used.

Poster S-III-11

Oxygen-specific vulnerabilities in acute myeloid leukemia

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Oxygen-specific vulnerabilities in acute myeloid leukemia
Acute myeloid leukemia (AML) is thought to be caused by multiple genetic and epigenetic alterations in hematopoietic stem and progenitor cells (HSPCs) that result in bone marrow infiltration by aggressive, differentiation-incompetent leukemic blasts. The biology of AML is dependent on environmental factors within the bone marrow. Besides the heavily studied cellular compartments, cytokines and other regulatory protein factors, the supply of nutrients and physicochemical conditions have recently received attention. For example, oxygen levels within the normal bone marrow vary from 1 – 6% oxygen, which is commonly (and misleadingly) termed as hypoxia. We are interested to study the effects of oxygen tension on the growth, survival and sensitivity to therapy of AML blasts.
In a first attempt to systematically analyze the effects of physiologically low oxygen levels on AML biology, we assayed the activity of key regulators of cell growth and proliferation under normal and hypoxic conditions in several AML cell lines with varying genetic abnormalities. Surprisingly, we saw a complex, but over several cell lines consistent regulation of mTOR-dependent pathways, depending on the length of time of the reduced oxygen conditions. In a second step, we performed SILAC-based quantitative mass spectrometry to identify oxygen-specific changes in protein expression. We saw an adaptation of the proliferation rate and dramatic metabolic changes as well as changes in major cellular signaling pathways under low oxygen tension, when compared to cultivation under atmospheric oxygen concentrations. The relevance of some of these changes is currently being validated by gene knock-down and knock-out methodology. In summary, we hypothesize that levels of hypoxia and oncogenic events cooperate to cause changes in the metabolic wiring in AML blasts. This may differentially impact on the biological behavior of malignant and benign HSPCs and also may modify molecular determinants of response to therapy.

Poster S-III-12

CRISPR/Cas9 mediated knockout of single snoRNAs and effects in leukemia cells

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Small nucleolar RNAs (snoRNAs) are small RNA molecules mostly known for their function as guide RNAs mediating 2'-O-methylation (box C/D snoRNAs) or pseudouridylation (box H/ACA snoRNAs) of other ribonucleic acids, primarily pre-ribosomal RNA.
Recently, our group has shown that box C/D snoRNA levels correlate closely with the leukemic stem cell frequency in AML patients. Further, in t(8;21)-AML the AML1-ETO fusion protein promoted snoRNA/RNP formation, thereby enhancing the self-renewal potential of leukemic stem cells and leukemogenesis. Therefore, snoRNA/RNP formation and function emerge as an important pathway in leukemogenesis and a promising leverage point for gaining a better understanding of the development and maintenance of acute myeloid leukemia.
Here, we study the impact of CRISPR/Cas9-mediated knockout of selected candidate box C/D and H/ACA snoRNAs on AML cells (Kasumi-1), including alterations in cell viability, colony formation capacity or chemotherapy resistance. Several single snoRNA knockouts decreased clonogenic potential of Kasumi-1 cells in CFU assays. Together with further analysis of the underlying mechanisms this will lead to a better understanding of the pathogenesis and might reveal novel therapeutic leverage points for the treatment of acute myeloid leukemia.

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Poster S-III-13

Transforming capacities of BCR-ABL in the context of AML: First come, first served?

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BCR-ABL$^+$ acute myeloid leukemia (AML) has recently been listed in the 2016 revised WHO classification of myeloid malignancies as a provisional entity. Its distinction from primary myeloid blast crisis of chronic myeloid leukemia (CML-BC) is often challenging and no definite criteria are yet established. Despite a well-established *BCR-ABL*-driven oncogene-addiction in both CML and acute lymphatic leukemia (ALL), the biological role of *BCR-ABL* remains unknown in AML. *BCR-ABL*$^+$ AML is rare, and collection of meaningful primary data sets is very difficult. Hence, we recently analysed all published cases with regard to their clinical and molecular features (1). In comparison, we analysed cases of primary myeloid CML blast crisis from several German University hospitals.
In AML, *BCR-ABL* predominantly occurs in the context of a complex/monosomal karyotype, CBF leukemia and "AML not otherwise specified (NOS)". Although the updated WHO classification excludes the co-incidence of *BCR-ABL* and CBF leukemia for the diagnosis of *BCR-ABL*$^+$ AML, this striking co-occurrence suggests the appearance of *BCR-ABL* in AML to be a secondary event. In contrast in CML, the late acquisition of typical AML aberrations like inv(16) upon transformation to blast crisis is well documented. This implicates that the sequence of acquisition determines the disease manifestation and this view is further supported by comparing cytogenetic features of *BCR-ABL*$^+$AML, primary and transformed CML blast crises. Whether this accounts also for *BCR-ABL*$^+$ with normal karyotype/NOS or whether *BCR-ABL* occurs as a primary event in this context remains to be clarified. In addition, *BCR-ABL* is rarely acquired during AML relapse or progression. We present cases in which *BCR-ABL* was acquired during relapse and successfully eradicated by 5-azacytidine or TKI treatment. This response was accompanied by disease progression and vivid clonal evolution, indicating that oncogene addiction on *BCR-ABL* played a minor role in these disease evolutions.
In conclusion, *BCR-ABL* seems to play a heterogenous role in AML but predominantly occurs as a late event during leukemogenesis.

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Poster S-III-14

Investigation of cell-contact dependent alterations in immune synapse formation between T cells and BCP-ALL cells

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Leukemia is the most common cancer in children with precursor B cell acute lymphoblastic leukemia (BCP-ALL) being the most prevalent immunophenotype.
Cancer-development is often associated with a state of immune attenuation and impaired antigen-recognition, contributing to the failure of immune surveillance. Recently defective immunological synapse formation between T cells and leukemia as well as normal B cells has been demonstrated in patients with chronic lymphocytic leukemia (CLL)[1]. Furthermore, it has been recognized that CLL cells induce T cell dysfunction in a cell-contact dependent manner. Both actin cytoskeleton changes and alterations in RhoGTPase regulation and activation have been reported in T cells of CLL patients[2]. Based on this knowledge we hypothesized that actin cytoskeleton reorganization, which is involved in immune synapse formation may also be altered in T cells of children with BCP-ALL. In order to investigate this hypothesis, we performed *in vitro* experiments to assess the dynamics of immune synapse formation between allogeneic T-cells from healthy donors and sAg activated precursor B-ALL cell lines (Nalm6 and REH) or activated, normal B-cells using immunofluorescence staining and confocal microscopy. Cell-interactions were selected at random from imaging, counted and scored for accumulation of F-actin at the immune synapse.
We found that CD8+ T cells from healthy donors show a significantly impaired ability to form an immunological synapse after direct short term contact with the cell line Nalm6 compared to normal B cells. Of note, this effect was less pronounced in the cell line REH. A decreased number of cell interactions was also seen between CD4+ T cells from healthy donors and the mentioned B-ALL cancer cell lines. However, this effect seemed to be time-dependent, as after slightly prolonged co-incubation of CD4+ T cells with the B-ALL cancer cell lines, synapse formation was improved.
Interestingly, the F-actin polymerization pattern at the immunological synapse appeared qualitatively different between allogeneic CD4+ T cells and normal B cells compared to allogeneic CD4+ T cells and B-ALL cancer cell lines. These preliminary data suggest that immune synapse formation may be quantitatively altered in a time-dependent manner in T cell/B-ALL interactions and result in modified actin cytoskeleton rearrangement. More detailed analysis also using primary BCP-ALL cells is warranted to further investigate the underlying mechanism.

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Poster S-III-15

Adult acute megakaryocytic leukemia. Rare association with cytopenias of undetermined signifiance and BCR-ABL transcripts

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Acute megakaryocytic leukemia is a rare form of acute myeloid leukemia (AML), associated with poor prognosis. In adult AML, it accounts for only 1% of the cases. The few published reports identified some particular features associated with M7-AML: frequent chromosomal abnormalities especially of chromosome 3, frequent previous hematologic disorders or myelodisplastic syndromes, poor prognosis. Herein, we describe the clinical case of a 47 years old male, diagnosed in our clinic with M7-AML, associated with antecedent hematologic disorder of undetermined signifiance and 2 BCR-ABL transcripts. MF, 47 years old male, presented at our clinic in September 2016 for asthenia, for the last month. The patient's medical history was consistent with intermittent low grade fever associated with bicytopenia (neutropenia and thrombocytopenia) for the last 3 years. Clinical exam there was slight palour, enlarged adenopathies of 2 cm, splenomegaly at 6 cm under the costal rib and oral candidiasis. The hematologic exam revealed leukocytosis at 120 x103/μl with 80% blasts, anemia (Hb 9 g/dl),, thrombocytopenia (45x103/μl), schistocytes. Biochemical results showed increased LDH at 1895 UI/l (230-460). A first bone marrow aspirate was a dry tap. A second bone marrow aspirate was performed, that showed an infiltrate of immature cells with megakaryocytic appearance, signs of dysplasia (micromegakaryocytes), reduced myeloid series. The bone marrow biopsy revealed 90% infiltration of tumoral monomorphic cells with round nuclei with nucleolei, increased nucleus/cytoplasma ratio. Immunohistochemically, the cells expressed CD 34, CD 117, CD 61 and Ki-67. The cells were negative for MPO and glycophorinA. The peripheral blood karyoptype was normal. Mutational analysis revealed 2 BCR-ABL transcripts: p210 and p190. FLT3 ITD and NPM mutations were negative. An induction chemotherapy was started consistent with idarubicine at 10 mg/m2/day for days 1, 3, 5 and cytarabine at 200 mg/m2/day continuous infusion for 7 days. Wa also added dasatinib 140 mg daily. There was no response to induction chemotherapy. Salvage therapy consisting in FLAG protocol was administered, again with no response. MEC protocol was then administered, but the patient succombed unfortunately. The case presented in the current report demonstrates the difficult diagnosis and management of M7 AML, in the context of antecedent hematologic disorder of undetermined signifiance and associated genetic abnormalities.

Poster S-III-16

DNMT3A-NPM1 mutated acute myeloid leukaemia shows sensitivity to PARP1 inhibitor combined with anthracyclines in an in-vitro model

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Acute myeloid leukaemia is a neoplasia in need of new treatment approaches. PARP inhibitors are a class of targeted therapeutics for cancer that disrupts dysfunctional DNA damage response in various neoplasia. MLL-AF9 mutated leukaemias are sensitive to combinations of PARP inhibitors and cytotoxic drugs. Moreover, DNMT3A and NPM1 mutations are linked to dysfunctions in DNA damage response. Therefore, we investigated if DNMT3A-NPM1 AML cell line are sensible to PARP inhibitors combined with anthracyclines. Our results show that DNMT3A-NPM1 mutated AML is as sensible to combinations of PARP inhibitors and anthracyclines as MLL-AF9 mutated leukaemias, in an *in vitro* setting.

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Poster S-III-17

Establishment of a novel human GM-CSF-dependent leukemia cell line (AML/HH1) – a versatile tool for the analysis of gene functions in leukemogenesis

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Hematopoietic cell lines have been widely used in the past to elicit relevant processes in the regulation of hematopoiesis and leukemogenesis. Indeed, different growth-factor dependent cell lines of murine origin, such as Ba/F3 or FDCP-1, have been useful for studying the transforming capacity of leukemia-associated mutations and testing effectiveness of new drugs in preclinical studies. In contrast, only few human cell lines have been available for those studies. Here we established a novel leukemia cell line (AML/HH1) from a 74-year-old woman with acute myeloid leukemia (AML) following chronic myelomonocytic leukemia (CMML). Primary patient's cells exhibited a G12S mutation in one allele of *NRAS*, whereas an additional G12D mutation was identified by NGS in the second allele in the cell line, indicating that homozygous activation might have contributed to the *in vitro* growth. Karyotypic and SNP analysis confirmed the authenticity of the novel cell line. Growth of AML/HH1 is strictly dependent on the addition of exogenous GM-CSF, as demonstrated by GM-CSF-dependent proliferation, survival and cloning capacity. AML/HH1 cells could be efficiently transduced with lentiviral vectors of various pseudotypes, allowing further genetic modifications. The usefulness of the novel cell line to study gene functions in leukemogenesis was tested by ectopic expression of receptor- (*FLT3*ITD, Δ*TRKA*) and non-receptor tyrosine kinases (*BCR*/*ABL*, *JAK2*V617F). Importantly, each oncogene led to a quantifiable induction of GM-CSF-independent growth in AML/HH1 cells even in unselected polyclonal setting. In a proof-of-principle experiment BCR/ABL-transformed AML/HH1 cells were treated with 1st- (imatinib) and 3rd-line (ponatinib) tyrosine kinase inhibitors resulting in dose-dependent inhibition of factor-independent proliferation and induction of apoptosis. Interestingly, an activating mutant (H1047R) of the catalytic subunit (p110α) of class IA PI3K was sufficient to drive AML/HH1 cells into factor independence. In contrast to vector-transduced cells, AML/HH1 cells with intrinsic PI3K pathway activation were tumorigenic in immune-deficient NSG mice, expanding into the bone marrow, liver and spleen of transplanted mice (median survival: 65 days pt). In conclusion, we have established a novel human leukemia cell line, AML/HH1, which may be a useful model for the functional analysis of leukemia-related genes in human cells.

Poster S-III-18

Identification of Meis2 as a crucial player in MN1-induced acute myeloid leukemia

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Meningioma 1 (MN1) is an independent prognostic marker for normal karyotype AML, with high expression linked to all-trans retinoic acid resistance and poor overall and relapse-free survival. Additionally, MN1 is a potent and sufficient oncogene in murine leukemia, able to block myeloid differentiation, promote leukemic stem cell self-renewal and transform cells at the common myeloid progenitor level – functions strongly dependent on the MEIS1/AbdB-like HOX protein complex (Heuser *et al.* 2011).
To identify key genes and pathways underlying leukemic activity, we functionally assessed MN1 cell phenotypic heterogeneity, revealing leukemic and non-leukemic subsets. Gene expression profiling of these subsets, combined with data from full-length MN1 and mutants with varying leukemogenic ability, identified candidate genes critical to leukemia. We transduced 2 independently-derived MN1 cell lines with lentiviral shRNAs against the upregulated genes and assayed for (1) *in vitro* proliferation, (2) self-renewal by CFU assay, (3) apoptosis, (4) differentiation and (5) *in vivo* leukemogenesis.
Meis2 was among the top-ranked genes not previously implicated in MN1-induced leukemia. Knockdown of Meis2 significantly impaired cell growth (17.2-fold fewer cells, $P<0.01$) and increased apoptosis (8.12%, $P<0.01$). Meis2 knockdown in transplanted MN1 cells increased median disease latency from 41 to 50.5 days ($P<0.01$) and impaired overall engraftment (7.1±4.5% vs. 32.4±18.5% at 4 weeks, $P<0.01$). Moreover, only 14.0±11.8% of engrafted Meis2-knockdown cells expressed the shRNA in moribund mouse bone marrow, compared to 86.3±15.3% in control cells, indicating strong negative selection against Meis2 knockdown. Intriguingly, analysis of the Leucegene RNASeq patient dataset reveals the MN1 target IRF8 as among the top genes negatively correlated with MEIS2 expression (r=-0.61). Moreover, MEIS2 is upregulated in patients expressing t(8;21) and downregulated in t(15;17) AML compared to other cytogenetic aberrations ($P<0.01$), suggesting that MEIS2 may be relevant in specific patient subsets.
Our findings reveal Meis2 as a novel player in MN1-induced leukemia with critical roles in proliferation, differentiation, and apoptosis. This work provides a platform to unravel the basis for the profound upregulation of Meis2 in MN1 leukemia and delineate potential functional differences between Meis2 and Meis1, while stimulating further study into the role of Meis2 in broader leukemic contexts.

Poster S-III-19

The role of thymic epithelial cells in T cell acute lymphoblastic leukemia

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Introduction: The thymus is a specialized hematopoietic organ, which is responsible for the generation of T cells. The central thymic cell type controlling T cell development are thymic epithelial cells (TECs). Based on their specific function and anatomic location TECs are separated into cortical and medullary subsets (cTECs and mTECs). cTECs express pivotal NOTCH-ligands such as DLL4 controlling T cell lineage commitment while mTECs play a central role in negative selection of developing T cells. Acquisition of *NOTCH1* gain-of-function mutations play a central role in acute T cell lymphoblastic leukemia (T-ALL) development. During T-ALL leukemogenesis aberrant expression of transcription factors such as SCL and LMO1 block T cell differentiation and increase self-renewal while *NOTCH1* mutations promote survival and proliferation. Since acquired *NOTCH1* mutations still require ligand-binding to exert augmented signaling we propose DLL4-expressing TECs playing a critical role during T-ALL leukemogenesis.
Materials and Methods: We used *Scl/Lmo1* double-transgenic mice to study TECs during T-ALL leukemogenesis by flow cytometry, microscopy and gene expression analysis. Furthermore, we studied the *in vitro* T-ALL pro-survival effect of the TEC cell lines ANV and TE-71.
Results: The *Scl/Lmo1* T-ALL preleukemic phase was characterized by the expansion of aberrant $CD8^+CD4^-TCR\beta^{intm}$ thymocytes. Strikingly, thymocytes of 6-week-old *Scl/Lmo1* mice displayed a more than 100-fold upregulation of the NOTCH1 target genes *Hes1* and *Dtx1* compared to wild-type controls. Fluorescence microscopy revealed a relative expansion of the $BP1^+$ cortical and reduction of the UEA^+ medullary thymic areas. Correspondingly, absolute numbers of cTECs and mature cTECs $MHCII^+$ expanded while mTEC and mature mTEC $MHCII^+$ numbers declined in *Scl/Lmo1* thymi. Gene expression profiling of sorted *Scl/Lmo1* TECs revealed upregulation of *CXCL12* and *IL18* which are known factors supporting T-ALL progression. Finally, we co-cultured T-lymphoblasts (from n=7 independent *Scl/Lmo1* T-ALLs) with the TEC cell lines ANV and TE-71. TEC cell lines promoted 72 hour *in vitro* survival in 4 out of 7 independent *Scl/Lmo1* T-ALLs studied.
Conclusion: During T-ALL leukemogenesis T-ALL promoting factors such as CXCL12 and IL18 are upregulated in TECs. Moreover, expansion of the T progenitor supporting cTEC compartment suggests a so far unrecognized role of this thymic stromal cell compartment in T-ALL leukemogenesis.

OMICs & Targeted therapies

Poster M-IV-01

Antagonism of retinoic acid receptor gamma (RARγ) blocks self renewal and induces cell death in prostate cancer.

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Prostate cancer (PC) tissue contains all-*trans* retinoic acid (ATRA) at a very low level (10^{-9} M), at least an order of magnitude lower than in adjacent normal healthy prostate cells or benign prostate hyperplasia.[1] When this is coupled with overexpression of CRABP1 and FABP5 and diminished expression CRABP2, that are frequently found in PC, this will have the effect of diverting available ATRA to oncogenic PPARβ/δ at the expense of retinoic acid receptors (RARs).[2,3] There are three isotypes of RARs (RARα, RARβ and RARγ) and recent studies have pointed to discrete physiological roles. For example, RARγ and RARα have opposing actions in self-renewal and differentiation, respectively, which are critical for proper hematopoietic stem cell maintenance.[4,5] We have previously shown that RARα/β/γ pan-antagonists inhibit the growth of PC cells (at 16-34 nM)[6,7] and that ATRA stimulates transactivation of RARγ at sub-nanomolar concentrations (EC_{50} 0.3 nM), whereas much higher concentrations were required for RARα-mediated transactivation (EC_{50} 16 nM).[5] This, together with the low level of ATRA in PC, led us to hypothesize that RARγ may play a role in PC pathogenesis and that RARγ-specific antagonism may be an effective treatment. Consistent with this notion, we found that concentrations of 10^{-9} M and below of ATRA promoted survival/proliferation and opposed adipogenic differentiation of human PC cell lines by a mechanism that involves RARγ. We also found that a RARγ-specific antagonist potently induced mitochondria-dependent, but caspase-independent, cell death in PC cell lines. Lastly, we suggest that combining a RARγ-specific antagonist with a RARα-selective agonist may be an effective therapy for prostate and other cancers.

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Poster M-IV-02
Features of circulating microRNA production in acute leukemia.

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Objectives: Acute leukemia (AL) is a clonal lesion of the hematopoietic system, which is characterized by poor prognosis, recurrent course and high mortality. Most of the genetic processes in leukemia cells are controlled epigenetically, which is extremely important to consider in the absence of known genetic mutations in the patient. MicroRNAs have recently been considered as the main regulators of the activity of genes responsible for proliferation, differentiation and control of the cell cycle. The existence of circulating microRNAs included in the composition of exosomes and microvesicles, indicates their systemic effect not only on tumor cells, but also on other tissues of the body.
Materials and Methods: In this study, we analyzed the expression of microRNA-15, -16, -21, 34, -126, -128 and -210 in leukocytes and blood plasma of 20 AL patients with blastosis and 10 healthy donors. miRNA expression was quantified using a StemLoop technology and RealTimePCR with TaqMan probes compared to the expression of the reference-gene U6. Data were analyzed using t test, and a $P<0.05$ was considered statistically significant.
Results: It was shown that the expression levels of microRNA-15, -16, -21, -34 and -210 in donors blood plasma were in several times higher than in cells. The levels of expression of miRNA-126 and -128 in the blood plasma and in leukocytes were not statistically different. In contrast, the expression levels of microRNA-15, -16, -21 and-126 in blood plasma in patients with leukemia were in several times lower than in cells, but microRNA-210 had a reverse situation. The levels of expression of microRNA-34 and -128 in plasma and in leukocytes were comparable. Intracellular expression of miRNA-15, -21, -34, and -126 was significantly higher in patients than in donors, but the expression of miRNA-16, -128 and -210 did not differ. We also observed a statistically significant decrease in circulating microRNA-15, -16 and -21 and an increase in microRNA-210 in the blood plasma of patients compared with the control. The levels of circulating microRNA-34, -126 and -128 did not differ in both groups.
Conclusions: We assume that the processes of various microRNAs accumulation within blast cells in AL and their secretion into circulation are part of the overall process of leukemogenesis. However, the mechanisms controlling the extracellular production of microRNAs have not been sufficiently studied.

Poster M-IV-03

Multiplex PCR for molecular screening of microRNA expression levels for the monitoring of Glioblastoma multiforme.

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Introduction: Glioblastoma multiforme (GBM) is an extremely malignant tumor, affecting the brain tissue, characterized by severe current and poor prognosis. Treatment methods of GBM such as surgery, chemotherapy and radiotherapy, significantly worsen the quality of life. Therefore, the appointment one of these methods should take into account not only the presence of a pathological focus, but also individual features of the tumor clone functioning. MicroRNAs are small regulatory molecules that have long been in the focus of oncologists as important cancer biomarkers and in particular GBM.
Objective: To develop and evaluate semiquantitative multiplex PCR for simultaneous screening of the miRNA-15a, -21 and -128 genes expression levels in GBM patients.
Material And Methods: The study used 30 peripheral blood samples from patients with GBM and 15 control donor's samples. The total RNA was isolated from the plasma using a standard phenol-chloroform technique followed by the implementation of the StemLoop protocol for the reverse transcription reaction (RT). PCR was performed simultaneously in the same tube for three microRNAs and a reference gene (RNU6B). In the PCR, TaqMan labeled probes were used: dye Cy5 for RNU6B, 15a FAM for MicroRNA, R6G for microRNA-21, ROX for microRNA-128.
Results: The optimization of primer sequences, reagent concentrations and temperature profiles for RT and PCR allowed a successful monitoring of the relative levels of the three major microRNAs involved in the pathogenesis of GBM. The combination of four reactions in one tube made it possible to level the technical errors of the non-multiplex protocol. It was shown that an increase in the level of expression of microRNA-21 significantly reflects the progression of the tumor and vice versa. In contrast, the levels of microRNA-15a and -128 were inversely related to the severity of GBM flow.
Conclusions: The developed protocol for the simultaneous determination of the expression levels of the microRNA-15a, -21 and -128 genes can be used in monitoring the state of the tumor process with GBM. These results can be used in personalized therapy of glioblastoma

Poster M-IV-04

Treatment with a dual inhibitor of EZH2 and EHMT2 histone methyltransferase promotes differentiation of acute myeloid leukaemia.

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EZH2/KMT6 methyltransferase plays a critical role in normal hematopoiesis and its mutation or deregulated expression has been conclusively demonstrated in the pathogenesis of acute myeloid leukemia (AML)[1]. The principal function of EZH2 is to maintain specific patterns of H3K27^{me3} repressive epigenetic marks that affect the balance between self-renewal and differentiation of hematopoietic or leukemic stem cells, although non-histone targets have been identified[2-4]. Interestingly, both loss of EZH2 as well as its overexpression play roles in AML, pointing to context-dependent tumor suppressor or oncogene functions that could hamper the development of targeted therapies[1]. Recurrent inactivating EZH2 mutations are found in myeloid malignancies[1] and recent research has shown EZH2 to be downregulated in individuals with chemoresistant AML[5]. On the other hand, knockdown of EZH2 has been shown to promote differentiation of MLL/AF9 AML and reduce susceptibility to MLL/AF9-transformed leukemia *in vivo*[1]. We have shown that inhibition of another epigenetic repressor, LSD1, promoted differentiation of AML[6] and hypothesised that targeting EZH2 may lead to a similar outcome. We initially tested GSK343, an S-adenosyl methionine (SAM)-competitive inhibitor of EZH2, and were surprised to find that it did not phenocopy results obtained via EZH2 knockdown. Our analysis suggested that this may occur at least in part through induction of genes associated with a stem-cell phenotype including the Wnt pathway. We then tested a dual inhibitor of EZH2 and EHMT2 (HKMTI-1-005)[7] and found that in contrast to GSK343, HKMTI-1-005 effectively induced differentiation in AML cells, primarily via inhibition of EZH2. In agreement with the phenotypic results, transcriptomic analysis revealed that HKMTI-1-005 upregulated the expression of myeloid differentiation pathway genes with and without all-*trans*-retinoic acid (ATRA), while downregulating genes associated with maintenance of a stem cell phenotype. Consistent with the notion that it may act as a "double-edged sword" in AML, we report that EZH2 associates with the master regulator of myeloid differentiation, retinoic acid receptor alpha (RARα), in an ATRA-dependent manner. In contrast to GSK343, we found that HKMTI-1-005 promoted the interaction between EZH2 and RARα. In summary, our results strongly suggest that dual inhibition of EZH2 and EHMT2 represents a novel therapeutic approach against AML that warrants further investigation.

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Poster M-IV-05

The polyamine analog verlindamycin promotes differentiation and cell death in neuroblastoma

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Neuroblastoma (NB) is the second most common solid tumor in childhood, accounting for 8-10% of the total number of pediatric cancers and 15% of deaths.[1] Deregulated polyamine biosynthesis is a common feature of NB and drugs targeting this metabolic pathway, such as difluoromethylornithine (DFMO), are in clinical and preclinical development.[2] The polyamine analogue verlindamycin (2d) inhibits the histone demethylase LSD1, as well as homologous enzymes involved in polyamine biosynthesis such as spermine oxidase and N1-acetylpolyamine oxidase.[3] We previously demonstrated that 2d cooperated with all-*trans*-retinoic acid (ATRA) to promote differentiation of acute myeloid leukemia[4] and reasoned that this drug might be effective in NB. Consistent with this notion, we found that treatment of a panel of NB cell lines with 2d and ATRA strongly induced differentiation that was associated with reduced growth and colony formation, as well as induction of G0 arrest and apoptosis. We found that 2d/ATRA treatment targeted NB cells regardless of MYCN status and biochemical analysis revealed that when expressed, MYCN protein was strongly downregulated. This process was not transcriptionally regulated but was due to increased turnover of MYCN protein, at least in part via proteasome-dependent destruction. Here we report that in addition to its established activities, 2d effectively induces, via ribosomal frame-shifting, expression of functional antizyme (Az). Consistent with previous results describing the function of Az tumor suppressor,[5] we found that 2d treatment led to the selective targeting of ornithine decarboxylase (the rate-limiting enzyme for polyamine biosynthesis) as well as key oncoproteins such as cyclin D and Aurora A kinase. The finding that 2d treatment diminished Aurora A levels is notable in the context of MYCN expressing NB as we have previously shown that Aurora A binds MYCN, preventing its degradation.[6] Furthermore, transcriptomic analysis revealed that treatment of NB cells with 2d/ATRA led to upregulation of genes associated with neuronal differentiation and downregulation of genes linked to a stem cell phenotype and NB pathogenesis, including EZH2.[7] Retinoid-based multimodal differentiation therapy is one of few interventions that extends relapse-free survival in MYCN-associated high-risk NB[8] and the results presented here strongly argue that the potential inclusion of verlindamycin in this regimen merits further investigation.

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Poster M-IV-06

Biochemical characterization and in vivo efficacy of mutant IDH1 inhibitor HMS-101

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Mutations in isocitrate dehydrogenase 1 (IDH1) are found in 6% of AML patients. Mutant IDH produces R-2-hydroxyglutarate (R2HG), which induces histone- and DNA-hypermethylation through inhibition of epigenetic regulators, thus linking metabolism to tumorigenesis. By computational screening of over 500,000 compounds, we identified IDH1mut inhibitor HMS-101, which effectively reduced R-2HG levels in IDH1mut cells. We studied the antileukemic effect of HMS-101 in vivo, performed biochemical and structural binding studies and evaluated the molecular mechanism. HoxA9 immortalised mouse bone marrow cells overexpressing IDH1mut were transplanted in syngeneic recipients and treated with either vehicle or 1mg of HMS-101. For biochemical/structural studies IDH1mut proteins were purified from the bacterial cell lysates using Ni-NTA agarose affinity chromatography. To study the efficacy of HMS-101 in primary AML cells, we treated IDH1mut and IDH2mut cells in vitro. HMS-101 decreased R-2HG in IDH1mut cells with an IC50 of 1.6μM but not in IDH2mut cells. On pure protein, HMS-101 was able to reduce the activity of IDH1mut protein and the production of R-2HG. We then co-crystallized IDH1mut (R132H) protein with HMS-101 and confirmed that the compound binds to the active site of the enzyme, interacting with histidine and valine, disallowing the binding of NADPH to the pocket and suggesting competitive binding of HMS-101 to mutant IDH1. Lethally irradiated mice transplanted with mouse bone marrow cells expressing IDH1mut were treated with 1mg/kg HMS-101 orally (once daily). These mice showed prolonged survival and lower leukemic burden in comparison to the vehicle treated mice. To investigate the mechanism of action of HMS-101, we checked the expression of genes involved in differentiation and regulation of cell cycle between IDH1 wild-type and IDH1mut cells. There was a marked up-regulation in cell differentiation transcription factors, CEBPA and PU.1. and decrease in cell cycle regulators cyclin B1 and A2. Thus, we were able to prove that HMS-101 was able to inhibit cell cycle and induce cellular differentiation, which was confirmed biologically in IDH1 mutant cells. Thus, we established HMS-101 as a direct inhibitor of mutant IDH1 which is able to inhibit cellular proliferation and division and induce differentiation in IDH1mut cells. HMS-101 binds at a different site than other IDH1 inhibitors and will be further investigated for its differential effect on mutant IDH1.

References

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Poster M-IV-07

Inducible re-expression of KLF4 impairs growth of patient-derived ALL in mice and sensitizes towards chemotherapy

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Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor which acts either as oncogene or tumor suppressor in a tissue-dependent way. Acute lymphoblastic leukemia (ALL) requires novel treatment options and displays down-regulation of KLF4 that evolves further in cells surviving treatment. Here, we aimed at deciphering the role of KFL4 for growth behavior and treatment response in patient-derived ALL *in vivo*.
We transplanted primary tumor cells from ALL patients into severely immune-compromised mice to generate patient-derived xenografts (PDX). Lentiviral transduction allowed genetic engineering to re-introduce wildtype KLF4 or a truncated form of KLF4 which lacked the zinc-finger DNA binding domain and served as control. To molecularly mimic treatment, we introduced a Tetracycline inducible expression system in PDX ALL cells and coupled expression of the transgene to a fluorochromic marker; upon carefully titrated Doxycycline, KLF4 was re-expressed at physiological levels in an on and off manner in PDX ALL cells *in vivo*.
Re-expression of KLF4 significantly diminished spontaneous proliferation of PDX ALL cells *in vivo*. Competitive *in vivo* assays demonstrated that control cells overgrew KLF4 expressing cells within the same mouse. Re-expression of KLF4 caused cell cycle arrest and induced apoptosis accompanied by cleavage of Caspase-3 and PARP.
To investigate the effect of re-expressed KLF4 on chemosensitivity, mice were treated with the cytotoxic drug Vincristine. Vincristine eliminated KLF4 expressing cells with much higher efficiency than control cells suggesting that chemosensitivity for Vincristine was significantly increased by re-expressing KLF4.
We had recently shown that treatment reduces KLF4 expression even further, as ALL cells of children after chemotherapy express less KLF-4 compared to cells at diagnosis (Cancer Cell 2016). To revert this evolution, we re-expressed KLF4 in PDX ALL treated with chemotherapy to the disease status of minimal residual disease and found that KLF-4 inhibited tumor growth more potently in previously treated cells compared to untreated cells. Taken together, our data suggest that re-expression of KLF4 to physiological levels impairs tumor growth in ALL and sensitizes towards treatment. We conclude that existing drugs increasing KLF4 levels should be evaluated in patients with ALL, especially in the clinically relevant situation of at minimal residual disease.

Poster M-IV-08

Compensatory upregulation of neurotrophin signaling rescues neuroblastoma cells from death after KIT knockdown.

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Neuroblastoma (NB) is an embryonal tumor arising from neural crest cells and it is one of the most common malignancies of childhood. NBs are highly heterogenic and they account for nearly 15% of all childhood cancer-related deaths. Receptor tyrosine kinase KIT is expressed by considerable number of NB tumors and it is also expressed by several other cancers including leukemias. KIT positive NB cells characterize an aggressive subset of cancer stem cells and KIT inhibition results in reduced tumor growth, so it is considered as promising target for NB treatment. Still the main problem of targeted anti-cancer therapy is acquired resistance of malignant cells to the drugs. Here we report that KIT knockdown by RNA-interference in model NB cells resulted in inhibition of proliferation and induction of apoptosis. Nevertheless analysis of changes in signaling pathways based on microarray-profiled gene expression data revealed that in NB cells simultaneously with apoptosis had happened activation of growth-related pro-survival pathways. Amongst others there was an increase in expression of neurotrophins (NGF and BDNF) and their receptors (TrkA and TrkB respectively). We showed that exogenous NGF and BDNF rescued NB cells from apoptosis and partially restored their proliferation. We identified ERK2 as major component of such compensatory signaling. Inhibition of ERK2 in KIT-downregulated cells resulted in enhanced cell death, blocked compensatory increase in TrkA and TrkB expression, and prevented rescue of NB cells by neurotrophins. Inhibition of ERK also decreased effective concentrations of KIT inhibitor imatinib and improved its effect on neuroblastoma cell proliferation. Moreover prolonged treatment of neuroblastoma cells with imatinib in combination with ERK inhibitor prevented cells from acquiring resistance to imatinib. Overall we show how neuroblastoma cell can compensate KIT signaling downregulation by employing other pro-survival pathways and that these mechanisms are highly dependent on ERK signaling. The results were obtained within the Program of fundamental research for state academies for 2013-2020 years (№ 01201363823). The results on microarray gene expression profiling and shRNA experiments were obtained within the RSF grant (project No. 14-14-01089-П) and functional cell culture experiments were performed within RFBR grant (project No. 17-04-01697A).

Poster M-IV-09

Metabolic characterization of FLT3-ITD cells upon FLT3 inhibition under hypoxia

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Acute Myeloid Leukemia (AML) is a clonal disorder of the hematopoietic system caused by genetic alteration. FMS-like tyrosine kinase 3 (FLT3) mutations are present in 20-30% of AML patients and are associated with poor prognosis. Second generation FLT3 inhibitors (quizartinib (AC220), crenolanib, PLX3397, and ASP2215) demonstrated good FLT3 specificity and efficient inhibition [2]. However, the responses to FLT3 inhibitors are transient, lasting for 3 to 6 months owing to the emergence of resistance. Previously published literature suggests differential mRNA expression and protein levels of several metabolic enzymes upon FLT3 inhibition. We decided to investigate with a metabolic approach the metabolic reprograming upon FLT3 inhibition to better understand the cellular response to the drug and its correlation to drug resistance. Nuclear Magnetic Resonance (NMR) technology can provide detailed metabolic profiles of the core metabolic cycles with high reproducibility. In addition, we utilized tracer-based assay (isotope labelled substrates) to further understand the metabolic flux and connection between the different pathways. Concerning the metabolic responses to FLT3 inhibition, we see a global suppression of glycolysis and its efflux into Krebs cycle. In addition, we see a relatively constant contribution of Glutaminolysis to Krebs cycle. An increasingly interesting concept in Leukemia metabolism is the influence of bone marrow microenvironment, including oxygen levels. In order to characterize the metabolic fingerprint of FLT3-ITD cells, we performed preliminary metabolic measurements in hypoxia (1% oxygen, mimicking bone marrow niche). As expected, we observed significantly different metabolic profiles of FLT3-ITD cells in hypoxia compared to normoxia (i.e. higher glycolysis). We plan to confirm our drug -induced metabolic shifts under hypoxia, as we hypothesize that such a hypoxic environment will influence the cellular response to AC220.

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Poster M-IV-10

SAMHD1 is a Resistance Factor and Predictive Biomarker for Decitabine in Acute Myeloid Leukemia

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The hypomethylating agents (HMAs) decitabine (DAC) and azacytidine (AZA) are important therapeutics for acute myeloid leukemia (AML). In recent years, DNA-hypomethylating agents (HMAs) including the azanucleosides decitabine (DAC, 5'-aza-2-deoxycytidine) and azacytidine (AZA, 5'-azacytidine) have emerged as less toxic alternative treatment options for myelodysplastic syndrome (MDS) and older AML patients who cannot tolerate intensive chemotherapy. However, only subsets of AML patients respond effectively to DAC or AZA, which exhibit an overall response rate ranging from 10 to 70% in different clinical trials. Currently, both drugs are frequently used and considered to be equivalent, in part due to the lack of head-to-head comparisons and to an incomplete understanding of their mechanisms of action. We recently found that the dNTP triphosphohydrolase SAMHD1 hydrolyzes the triphosphate of the nucleoside analog cytarabine resulting in diminished drug potency. Here, we investigated the impact of SAMHD1 on the HMAs AZA and DAC, structurally related cytidine nucleoside analogs. We find that the bioactive metabolite DAC-TP functions as an unphysiological activator and substrate of SAMHD1, resulting in drug inactivation. In contrast, the major AZA metabolite, AZA-TP, is not recognized by SAMHD1. Genetic and pharmaco-metabolic strategies that diminished SAMHD1 expression potentiated the cytotoxicity of DAC, but not AZA, in AML cell lines, primary leukemic blasts, and AML xenotransplant models. A retrospective clinical analysis established an inverse correlation between SAMHD1 expression in leukemic blasts at diagnosis and clinical response to DAC. Thus, SAMHD1 is a DAC resistance factor, a predictive biomarker for HMA stratification, and a therapeutic target in DAC-based AML therapy.

Poster M-IV-11

Curcumin as a treatment approach for MDS/AML in human.

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Myelodysplastic syndrome (MDS) is a hematopoietic disease resulting in an ineffective hematopoiesis with pancytopenia and a reduced function of blood cells. In some cases, MDS can develop into acute myeloid leukemia (AML) which is characterized by the presence of more than 20% blasts in the bone marrow[1].
GFI1 (Growth factor independent 1) is a transcriptional repressor. One of its functions is the recruitment of histone deacetylases and histone acetyltransferases to certain genes regulating hematopoiesis[2]. A single-nucleotide polymorphism of GFI1 generates a protein that contains an asparagine (N) instead of a serine (S) at position 36 (GFI1-36N) which has a prevalence of 3% – 5% among Caucasians[3].
Previous experiments have shown that the GFI1-36N mutation as well as reduced levels of GFI1 (GFI1-KD) accelerate the initiation and progression of AML in mice due to altered histone modifications[2]. We hypothesize that the treatment of patients with low GFI1 expression or GFI1-36N mutation can be improved by using histone acetyltransferase inhibitors (HATi). One potential HATi is Curcumin, a particularly interesting substance because it is used as spice in Asia and has so far no known toxic side effects.
To analyze the effect of Curcumin on MDS/AML development, we crossed NUP98-HOXD13 mice, a mouse model mimicking the t(2;11)(q31;p15) translocation found in 1-2% of human AML patients[4], with GFI1-WT, GFI1-36N or GFI1-KD mice.
The different groups were treated with 20 mg/kg curcumin once a week from day 50 after birth. As a control, we treated mice with the same genotypes with 1 mg/kg azacitidine, which is approved as an epigenetic therapy of high risk MDS patients by inhibiting methyltransferases[5] . As soon as the mice become overt leukemic, we extracted the bone marrow cells to analyze them by flow cytometry and microscopy. Our first results show that the treatment of NUP98-HOXD13 transgenic mice with Curcumin effectively prevents the development of AML in mice expressing either GFI1-36N or with reduced expression of GFI1, while Azacytidine treatment does not.
Overall, our data indicate that Curcumin could be a potential targeted alternative therapy for MDS/AML patients with low expression of GFI1 or presence of GFI1-36N, hopefully leading to a better prognosis.

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Poster M-IV-12

Epigenomic profiling of paediatric B-cell lymphoblastic leukemia

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The lymphoid leukaemias are the most common neoplastic diseases in childhood constituting around 30% of cancers before the age of 15 years. Although a continuous increase in survival rates over recent years can be observed in paediatric ALL, children with certain genetic alterations, like chromosomal rearrangements including *KMT2A* (e.g. t(4;11)(q21;q23)) or hypodiploidy have an especially poor prognosis.
Thus, in these subgroups novel treatment targets have to be identified. Since genetic alterations in paediatric ALL are well characterised, we herein focus on the epigenomic characterization of paediatric ALL subgroups with distinct genetic make-up, especially on subgroups with poor prognosis, like *KMT2A* rearranged ALL.
To characterize the epigenome of these ALLs we analysed reference epigenomes defined by the *International Human Epigenome Consortium* (IHEC) consisting of chromatin immunoprecipitation (ChIP)-sequencing of 6 histone marks (H3K27me3, H3K36me3, H3K4me1, H3K27ac, H3K9me3), RNA-sequencing data (RNA-Seq) and whole-genome bisulfite-sequencing data (BS-Seq). In addition to the epigenomic data, also clinical data, comprehensive genomic data, characterised by means of molecular cytogenetic analyses and SNP/CGH-array data, are integrated in the analysis. As controls we use benign precursor B-cells. Data were partly generated within the EU-funded BLUEPRINT project and partly in the context of the DFG-network "Epigenomic profiling in paediatric lymphoid leukaemia- perspectives for diagnostics, prognosis and therapy".
We will present our data about the chromatin architecture of paediatric ALL, which revealed 18.692 subtype specific chromatin determinant regions, with each ALL subtype group segregating into a separate cluster by Multiple Correspondence Analysis (MCA). Moreover, DNA methylation signatures of the different subtypes will be presented in the context of transcriptional data.
First results of our work in progress already reveal that the integration of epigenomic, transcriptomic, genomic and clinical analysis in paediatric ALL will decipher subtype-specific modifications and deregulated molecular pathways.

Poster M-IV-13

Development of a haploid genetic screen for the characterization of signaling pathways mediating drug resistance in leukemia

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Acquired drug resistance is still one of the most severe problems faced by leukemia therapy. Most of the known mechanisms causing resistance against targeted therapies involve additional genetic alterations. To perform forward genetic screens for drug resistant phenotypes induced by targeted (e.g. imatinib) and non-targeted (e.g. cytarabin) anti-leukemic drugs, we generated genome wide gene trap libraries in the BCR/ABL positive haploid human chronic myeloid leukemia cell line KBM7 using a bifunctional switchable conditional gene trap/protein tagging vector. This gene trap enables not only the recovery of drug toxicity-mediating genes by selection of mutant drug-resistant clones, but in addition the subsequent identification of signal transduction pathways in which this gene is engaged by switching the gene trap vector to a protein tagging vector. Of particular advantage compared to CRISPR/Cas9 and shRNA-based resistance screens, this endogenous protein tagging strategy enables a straight-forward interactome analysis using mass spectrometry thus identifying additional targets within the cellular context of the previously identified single gene. The first forward genetic screen using imatinib resulted in several candidate genes including genes involved in the regulation of the p53 signaling pathway (MDM2, USP15) and a gene activating the HIPPO pathway (MOB3A). As a pathway controlling cell survival and proliferation the HIPPO pathway already gained a lot of attention within the recent years in diverse leukemia clinical studies, highlighting the fact that this approach is indeed capable to identify novel targets to overcome drug resistance.
Therefore, each drug resistance gene as well as their interaction partner are of potential clinical relevance and will be further analyzed in annotated leukemia patient samples available from the UCT (Universitaeres Centrum für Tumorerkrankungen), Frankfurt, which is supported by the Deutsche Krebshilfe.

Poster M-IV-14

Replicative stress triggers an apoptosis-associated loss of Wilms Tumor 1 protein in leukemic cells

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The *Wilms Tumor 1* (*WT1*) gene encodes a transcription factor that can activate or repress genes involved in differentiation, cell cycle progression, and apoptosis. Whereas *WT1* functions as a tumor suppressor gene in the pediatric renal cancer Wilms Tumor, WT1 may act as an oncogene in other types of tumors. While WT1 is expressed to a low amount in normal hematopoietic stem cells, multiple studies have reported increased WT1 expression in a significant proportion in acute myeloid and chronic myeloid leukemia cells. Moreover, a remaining expression of WT1 in the bone marrow or peripheral blood after cytotoxic chemotherapy indicates remaining leukemic clones in patients.
We determined the regulation and relevance of WT1 in leukemic cells exposed to replicative stress and DNA damage. To induce these conditions, we used the clinically relevant chemotherapeutics hydroxyurea (HU) and doxorubicin (Doxo). HU inhibits the ribonucleotide reductase and Doxo interacts with DNA by intercalation and inhibits topoisomerase II. In addition to this pharmacological approach, we genetically eliminated WT1 using various methods.
We used western blot and flow cytometry techniques to show that HU and Doxo alter the cell cycle and promote apoptosis to a variable extent in a panel of leukemic cell lines and that caspases cleave WT1 during apoptosis. Chemical inhibition of caspases as well as an overexpression of mitochondrial, anti-apoptotic BCL2 family proteins prevent the processing of WT1 and cell death in hydroxyurea-sensitive cells. Although the reduction of WT1 correlates with the pharmacological efficiency of chemotherapeutics in various leukemic cells, the elimination of WT1 by different strategies of RNA interference (RNAi) does not lead to changes in the cell cycle of HU-resistant cells. RNAi against WT1 does also not increase the extent of apoptosis and the accumulation of the replicative stress and DNA damage marker phospho-H2AX (γH2AX) in HU-resistant K562 cells exposed to HU. We could also show by immunofluorescence, that a targeted genetic depletion of WT1 in primary murine oviduct cells does not increase the levels of γH2AX.
Our findings position WT1 as a downstream target of the apoptotic process that occurs in response to cytotoxic forms of replicative stress and DNA damage. Thus, WT1 can be used as a pharmacological marker for drug efficiency. Proposed functions of WT1 upstream of the apoptotic process need to be reconsidered.

Poster M-IV-15

Identification and Characterization of Novel FLT3-Inhibitors

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FMS-like tyrosine kinase 3 (FLT3) is a member of the receptor tyrosine kinase (RTK) family III. Upon ligand binding, FLT3 activates several signalling pathways that regulate survival, differentiation and cell growth. 25% of acute myeloid leukemia (AML) cases carry FLT3-internal tandem duplications (ITD). Such mutations lead to a constitutive activation of FLT3, resistance to conventional therapy, and poor AML patient survival. Several FLT3-inhibitors (FLT3i), like the multiple kinases inhibitor Midostaurin and the more specific agent AC220, were developed. While these produce good results in the treatment of newly diagnosed AML with oncogenic FLT3, refractory AML cases with secondary FLT3 mutation are often resistant to FLT3i treatment. Thus, synthesis of new and more specific inhibitors is required.
We tested a battery of 41 novel compounds with a suspected ability to block FLT3. As cellular model system we used the human FLT3-ITD positive AML cell line MV4;11. We performed viability staining, cell cycle analysis, and Annexin V/propidium iodide staining to evaluate the efficacy of the FLT3i. Reduction of FLT3 phosphorylation was tested by immunoblotting. Eight FLT3i induced apoptosis in MV4;11 cells and blocked FLT3 phosphorylation at 10 nM and below. Two substances are at least as effective as AC220. We additionally tested murine cell line BaF/3 expressing different form of FLT3 mutations for their responses to our new FLT3i. We could show that our new FLT3i can block the phosphorylation and growth-promoting capacities of FLT3 mutations that are largely resistant to AC220. Our new FLT3i was tested in xenotransplantation model of MV4;11 injected intravenously in NOD/SCID/γ - chain (NSG)- mice. The mice tolerated our substance well and overall survival was increased after treatment.
Taken together we present a new, specific, and effective FLT3i that is able to overcome therapy-associated FLT3 mutations.

Poster M-IV-16

Acquired resistance to MDM2 inhibitors, but not to cytarabine, results in high frequency of *TP53* mutations in AML cells

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MDM2 inhibitors are currently tested in patients with acute myeloid leukaemia (AML). Resistance formation to MDM2 inhibitors including nutlin-3 was shown to be associated with *TP53* mutations or other aberrations in p53 signalling[1]. Here, we established nutlin-3-resistant sub-lines of the *TP53* wild-type AML cell lines MOLM-13, MV4-11, OCI-AML-2, OCI-AML-3 and SIG-M5 by continuous exposure to step-wise increasing drug concentrations. The nutlin-3-adapted AML sub-lines displayed 6- to 36-fold higher nutlin-3 IC_{50}s than the respective parental cell lines. 9 out of 13 MOLM-13 sub-lines, 15 out of 15 MV4-11 sub-lines, 10 out of 10 OCI-AML-2 sub-lines, 10 out of 12 OCI-AML-3 sub-lines, 4 out of 9 SIG-M5 sub-lines and 9 out of 13 nutlin-3-adapted MOLM-13 sub-lines had acquired *TP53* mutations. While nutlin-3-adapted MOLM-13, SIG-M5 and OCI-AML-3 sub-lines acquired 4 to 10 different *TP53* mutations, MV4-11 and OCI-AML-2 sub-lines acquired only one *TP53* mutation. In contrast, adaptation of MOLM-13 cells to the standard AML therapeutic cytarabine did not result in *TP53* mutations in 10 out of 10 sub-lines. These results show that nutlin-3 frequently selects for cells with *TP53* mutations, while *TP53* mutations in cells adapted to the standard AML therapeutic cytarabine are rare.

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Poster M-IV-17

SHIP1 inhibition as novel therapeutic approach in chronic lymphocytic leukemia

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Over the past decade, significant improvement in understanding the pathogenesis of chronic lymphocytic leukemia (CLL) has highlighted the importance of active B cell receptor signaling. This has revealed promising targeted treatment options, including the small molecule inhibitors targeting the phosphatidylinositol-3-kinase (PI3K) signaling pathway. Idelalisib and Duvelisib are under clinical investigation for CLL treatment, however, treatment-related toxicities are limiting their application and none of these approaches are curative. Here, we are testing a novel approach that aims to target CLL B cells selectively and simultaneously restore an appropriate immune function. The phosphatase SHIP1 negatively regulates PI3K signaling in B cells by dephosphorylating the 5`phosphate of Phosphatidylinositol (3,4,5)-trisphosphate. Our data show that in CLL, similarly to recent findings in acute lymphoblastic leukemia (Chen et al., Nature 2015), pharmacological inhibition of SHIP1 leads to rapid cell death of CLL cells. This has been tested on primary CLL patient samples and Tcl-1 driven murine CLL cells. To confirm the specificity of the observed effects, we genetically activated AKT in the Tcl-1 driven mouse model, which induced cell death in CLL cells *in vitro*. In addition to the direct effects on CLL cells, we sought to investigate the impact of SHIP1 inhibition on other immune cell functions as well as the immunogenicity of CLL cells undergoing cell death upon SHIP1 inhibition. We observed that SHIP1 inhibition lowers the activity threshold of T cells and induces immunogenic cell death of CLL cells with an increase of antigen-specific T cell proliferation. In previous studies and in striking contrast to the observed deleterious effect on the malignant CLL cells, SHIP1 inhibition has been shown to induce expansion of murine hematopoietic and mesenchymal stem cell compartments (Brooks et al., Stem cells 2014). Therefore, we propose that inhibition of SHIP1 in CLL can simultaneously induce direct and immunogenic cell death in the malignant CLL clone(s) and lower the threshold for activation of T cells.

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Brooks et al., Stem cells 2014

Poster M-IV-18

Smac mimetics induces synergistic apoptosis when combined with cytotoxic drugs, independently from TNFalpha and NFkappaB, but similarly to silencing of XIAP in ALL

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Acute lymphoblastic leukemia (ALL) suffers poor prognosis and better treatment is urgently required. Smac mimetics (SM) represent novel promising anti-cancer drugs counteracting inhibitor of apoptosis proteins (IAPs), a family including cIAP1/2 and X-linked IAP (XIAP).
SM have been primarily designed to target X-linked IAP (XIAP). Nevertheless, in SM sensitive tumor cells the effect of SM has been shown to be mediated mainly by degradation of cellular IAP (cIAP) and activation of TNFα and NFκB signaling pathways, independently of XIAP. Here we show that degradation of cIAP1/2 by SM occurred independently from SM-induced apoptosis in ALL. In ALL cell resistant against SM alone, treatment with SM resulted in significant sensitization for drugs used within standard induction therapy for childhood ALL. Cell death induction by SM combined with cytotoxic drugs was caspase dependent, but independent from neutralizing TNFα or knockdown of either RelB or NEMO.
In contrast, knockdown of XIAP using miR30 flanked shRNA expression sensitized ALL cells towards cytotoxic drugs in competitive vivo assays. We expressed different fluorochromes such that expression of blue indicated cells with control knockdown and expression of green, cells with knockdown of XIAP. We finally injected both populations into a single mouse and studied their distribution. Knockdown of XIAP in ALL cells lead to the reduction of the XIAP protein by more than 90% and remained stable over several passages, without affecting cIAP1/2 expression.
In genetically engineered PDX cells growing in mice, silencing XIAP alone significantly inhibited ALL growth and survival in fluorochrome-controlled competitive assays, indicating an essential function of XIAP for patients' ALLcells in vivo.
Taken together, our data uncover a novel prominent role for XIAP as therapeutic target in ALL, either alone or for enhancing conventional chemotherapy.

Poster M-IV-19

Epigenetic control of Hematopoiesis by Histone Arginine Methylation

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Hematopoietic differentiation is driven by transcription factors, which orchestrate a fine tuned transcriptional network. At bipotential branching points lineage decisions are made, where key transcription factors initiate cell type specific gene expression programs. These programs are stabilized by the epigenetic activity of recruited chromatin modifying cofactors. An example gives the association of the transcription factor RUNX1 with the protein arginine methyltransferase 6 (PRMT6). We demonstrated that PRMT6 is recruited by RUNX1 to target genes during differentiation. Here, PRMT6 mediates a repressive chromatin environment by establishment of a histone modification pattern with high H3R2me2a and low H3K4me3. Interestingly, the repressive RUNX1/PRMT6 complex is formed cell-type and promoter dependent. This way RUNX1 is able to initiate a specific cell type dependent gene expression program, while actively repressing the competing program. Importantly, inhibition of PRMT6 by shRNA or small molecule inhibitor leads to growth inhibition and promotion of erythropoiesis. Our data reveal that the RUNX1/PRMT6 axis could be a molecular target to facilitate enhanced erythropoiesis for regenerative medicine and may suppress cell growth in a therapeutic setting for the treatment of leukemia.

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Poster M-IV-20

Pharmacologic LSD1 inhibition perturbs human terminal granulopoiesis while preserving monopoiesis

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Introduction: Histone modifications such as methylation are severely perturbed in myeloid leukemias. We previously demonstrated the central role of the lysine-specific histone demethylase 1 (LSD1) in normal murine hematopoiesis (Sprüssel et al, Leukemia 2012). Remarkably, in human acute myeloid leukemia (AML) LSD1 expression is de-regulated and therefore represents an attractive AML therapeutic target. In fact, first generation LSD1 inhibitors were shown to induce AML cell differentiation and apoptosis in combination with all-trans-retinoic acid (ATRA) exposure. However, the exact mechanisms-of-action and hematopoietic side effects of LSD1 inhibition in normal human hematopoiesis and AML are not yet fully understood. Materials and Methods: We studied the impact of the novel LSD1 inhibitor IMG-7289 on the colony forming unit (CFU) potential of human CD34+ progenitor in vitro. Furthermore, IMG-7289 anti-AML activity was investigated with AML cell lines. Results: LSD1 inhibition with IMG-7289 fostered monopoiesis while perturbing erythro- and granulopoiesis. LSD1 inhibition in combination with ATRA completely blocked terminal human hematopoietic differentiation. However, in re-plating experiments IMG-7289-treated progenitors showed strikingly increased CFU-activity compared to carrier control-treated progenitors. Finally, IMG-7289 significantly inhibited the growth (IC50 29 nM) and leukemic colony formation of the Kasumi-1 AML cell line. Conclusion: Taken together, pharmacologic LSD1 inhibition interferes with normal human hematopoietic differentiation while preserving normal progenitor potential. IMG-7289 displays AML cytotoxicity at nanomolar concentrations in vitro.

Poster M-IV-21

Combined targeting of AKT and mTOR synergistically inhibits proliferation and tumoroid formation of primary colorectal carcinoma cells.

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Colorectal cancer (CRC) is the third most frequently diagnosed type of cancer worldwide. Surgical treatment remains the only option that promises to cure the disease, however, many CRC patients with advanced stages of colorectal cancer have to be subjected to palliative chemotherapy. The PI3K-AKT-mTOR signaling pathway is frequently activated in colorectal tumors due to mutations in the genes encoding the catalytic and regulatory subunits of PI3K i.e. *PIK3CA* and *PIK3R1*, respectively, as well as overexpression of AKT isoforms and mutations in mTOR. In addition, mutations and downregulation of PTEN which is a negative regulator of PI3K-AKT-mTOR signaling contribute to the activation of this pathway in CRC. Given its importance for proliferation and survival, the PI3K-AKT-mTOR pathway is a promising target in the treatment of cancer. In previous works, we have shown that combined inhibition of AKT and mTOR is highly synergistic in cell lines from patients with hepatocellular carcinoma and cholangiocarcinoma. In this study, we analyzed the effects of AKT inhibitor MK-2206 and mTORC1 Inhibitor RAD001 on a panel of primary colorectal carcinoma cell lines, as well as primary samples from CRC patients. Our data demonstrate that combined treatment with MK-2206 and RAD001 results in strong synergistic effects in some, but not all tested CRC cell lines. Interestingly, response to treatment varied between cell lines derived from the primary lesion and a lymph node metastasis of the same patient. Pretherapeutic treatment analysis of tumor spheroid cultures of primary tumor samples is a promising approach to assess the susceptibility of tumor cells to certain drug combinations and to determine effective treatment regimes. Combined treatment with AKT and mTOR inhibitors resulted in a synergistic inhibition of tumoroid growth in 4 out of 4 primary CRC patients samples, analyzed in a 3-dimensional tumor model.
In summary, our data demonstrate that combined treatment with AKT and mTOR inhibitors has synergistic effects on proliferation in some CRC cell lines and primary tumor cells from CRC patients. Therefore, dual treatment with AKT and mTOR inhibitors may be a promising approach for the treatment of CRC patients.

Poster M-IV-22

Integrative Network Analysis of E2F1 Interactomes Reveals Cancer Type-Specific Receptor Protein Signatures Underlying Transcription Factor-Mediated Epithelial-Mesenchymal Transition

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Cancer is a disease of subverted regulatory pathways and the E2F1 transcription factor is one of the most potent metastatic inducers. Here, we reconstructed the regulatory network around the E2F family, whose deregulation is associated with cancer progression and chemoresistance. We integrated gene expression profiles of cancer cell lines from E2F1-driven highly aggressive bladder and breast tumors, and used network analysis methods to identify the tumor type-specific core of the network. By combining logic-based network modeling, in vitro experimentation, and gene expression profiles from patient cohorts displaying tumor aggressiveness, we identified and experimentally validated distinctive, tumor type-specific signatures of receptor proteins associated to epithelial-mesenchymal transition (EMT) in bladder and breast cancer. Our integrative network-based methodology, exemplified in the case of E2F1-induced aggressive tumors, has great potential to assist in the development of cohort- as well as tumor type-specific personalized cancer therapeutics and ultimately, to fight metastasis and therapy resistance.

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Poster M-IV-23

Metabolic characterization of resistant leukemic cell lines and definition of targets associated with resistance-associated metabolic reprogramming in Acute Myeloid Leukemia

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A major obstacle for successful cancer chemotherapy is the development of resistances. Even though there are key drugs for treatment of acute myeloid leukemia, for instance, cytarabine (Ara-C) and doxorubicin (Dox), cancer cells can react to treatment and eventually become resistant to the chemotherapeutic in order to accelerate proliferation. We believe that cancer cells alter their metabolism and this metabolic reprogramming plays a key role in the development of drug resistance. Therefore, the present study aims to assess if the rewiring of cell metabolism can be a crucial part of the process through which AML cells become resistant to these chemotherapeutics and, if so, to study the metabolic mechanisms driving this adaptation in order to provide potential strategies for the treatment of drug-resistance in AML.
To answer those questions, AML resistant cell lines (THP-1 and HL-60) to Ara-C and DOX chemotherapeutics were developed. We are currently running many different experimental approaches (metabolomics, proteomics, transcriptomics) to thoroughly characterize the metabolism of the resistant cell lines and their parental (sensitive counterparts) under normoxia and hypoxia. We expect to define potential metabolic targets whose inhibition or combination with known drugs could prevent the acquisition of the resistant phenotype and inhibit the proliferation of resistant cells, therefore, forestalling therapeutic failure. For instance, our first assays showed that AraC-resistant cell lines are the most glycolytic cell lines, while Dox-resistant cell lines are the least glycolytic ones. In addition, the same pattern could be seen in both cell lines and in both oxygen conditions, being increased in hypoxia.
In conclusion, results reveal that the resistance mechanism developed by AML cells lines is associated with an important metabolic reprogramming dependent on drug inducer, that should be further studied in order to understand resistance mechanism, opening new therapeutic avenues to treat AML disease.
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Gene and Immunotherapy

Poster M-V-01

A target-specific reporter system to identify CRISPR/Cas9-induced knockouts

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Genetic engineering represents a valuable tool to analyze gene function. The clustered regulatory interspaced short palindromic repeats (CRISPR)/Cas9 technology provides the ability to create double-strand breaks (DSBs) at desired positions in the genome. These DSBs will be most likely repaired through non-homologous end joining (NHEJ), an error-prone repair mechanism, which typically results in small insertions and/or deletions (indels) at the breaking point. If the indels cause a frameshift in the genome, the function of the gene can be knocked out due to the production of truncated and degraded proteins. To characterize the resulting mutations usually single cells are used to select for successful knockout cells. However, this procedure is time and resource intensive and not applicable to primary samples that cannot survive in vitro for a longer period of time. To overcome the limitation, we generated a reporter system that allows increasing and selecting for successfully gene edited cells based on published data (Kim et al. 2011 and 2013). The reporter consists of two fluorescent proteins in a row, one of which is in frame and the other out of frame. The fluorochromes are separated by a target-specific sgRNA recognition site. Once Cas9 induces sgRNA-specific DSBs and indel formation in the recognition site, the second fluorochrome might get into frame allowing expression of the second fluorochrome and enriching successfully edited cells by flow cytometry. The reporter allows incorporation of up to ten different sgRNA target sites between both fluorochromes such that a single reporter construct can serve for up to ten different targets. The reporter system allowed enriching successfully edited cells by one order of magnitude or more. Taken together, our newly developed reporter system enables a marked increase in successfully gene-edited cells in a given cell pool which might enable Cas9-mediated gene editing in otherwise challenging settings, such as animal models *in vivo*.

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Poster M-V-02

Improvement of gene editing efficiency in hematopoietic stem cells

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Introduction: The case of successful allo-HSCT in an HIV-infected AML patient ("Berlin patient") from a CCR5Δ32 -homozygous donor has triggered development of novel approaches in HIV treatment, i.e. HSCT-based cellular therapy with site-specific genome editing to knockout the CCR5 gene. HSCs are among the most promising targets for genetic manipulations, since they comprise a multipotent progenitor population of stem cells that give rise to all types of blood cells. Therefore, new methods of HSC genome editing with engineered nucleases (ZFN, TALEN, CRISPR/Cas9) have high potential applications in gene therapy of HIV.
Aim : To improve site-specific editing of CCR5 gene in HSC by CCR5-Uco-TALEN.
Materials and methods: In-vitro transcription of mRNA for CCR5-Uco-TAL endonucleases and eGFP was performed on DNA plasmids as described. CD34+ cells were separated by MACS, resuspended in StemSpan medium supplemented with IL-6, SCF, TPO and FLT3-ligand. mRNA electroporation was performed using different concentrations of TALEN (from 50 to 200 μg) and 2.5 μg of eGFP mRNA under variable electroporation settings: 220-500V, 5-10ms, 1 pulse. Thereafter, cells were incubated at 32°C for 24 h before eGFP positivity was assessed by flow cytometry. CCR5 gene editing efficiency and CCR2 off-target activity mediated by CCR5-Uco-TALEN were determined by ddPCR. The colony-forming ability of treated HSC was evaluated by CFU assay. The number of apoptotic events was measured by flow cytometry with AnnexinV-FITC/7AAD staining. Biallelic CCR5 modifications were estimated by the ddPCR on DNA from methylcellulose colonies.
Results: We found the following electroporation conditions to be optimal for HSC: 300V, 10ms, 1 pulse. Under these conditions transfection efficiency averaged 80% and cells viability was higher than 85%. ddPCR results indicate that a TALEN mRNA concentration of 100 μg was most favorable for HSC electroporation mediating CCR5 gene editing efficiencies of 46.3±7% for CCR5 and a maximal on- to off-target ratio (CCR5 vs CCR2). The frequency of off-target events was directly associated with applied mRNA concentrations (0-10%). There was no influence of the gene-editing procedure on the ability of transfected HSC to form colonies in methylcellulose.
Conclusions: We have established an optimized protocol for CCR5 gene editing with CCR5-Uco-TALEN in HSC.

Poster M-V-03

Immunotoxins in Combination with Endosomal Escape Enhancers –A Perspective for Targeted Cancer Treatment?

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Background: The administration of chemotherapeutics is often accompanied with side effects that hinder their efficacy and hamper patients' quality of living. Among many strategies to overcome these limitations, the administration of immunotoxins (ITs) has become a promising approach. ITs consist of a targeting moiety that can bind specifically to the targeted tissue and of a toxin that is responsible for cell killing. For a smooth translation from bench to clinics, the optimization of both their poor tumor penetration and the conjugation strategy between the targeting ligand and toxin have to be addressed.
Objectives: Our aim was to develop an IT consisting of the ribosome-inactivating protein dianthin and the anti-epidermal growth factor receptor antibody panitumumab. Moreover, we intended to optimize the reaction conditions for the conjugation of dianthin to panitumumab via a chemical linker. Lastly, we aimed at analyzing the activity of the IT *in vitro* on carcinoma cell lines with respect to the co-administration of the endosomal escape enhancer SO1861.
Methods: Dianthin was expressed in *Escherichia coli* and conjugated to panitumumab via the chemical linker succinimidyl-3-(2-pyridyldithio)propionate (SPDP) after finding out optimal reaction conditions. The enzymatic activity of dianthin was proven via an adenine release assay and the cytotoxicity of dianthin and IT was examined *in vitro* on HCT-116 target and MDA-MB-453 off-target cells.
Results: A 5-fold molar excess of SPDP and a reaction time of 1 h at 22 °C were identified as optimal reaction conditions for the conjugation of dianthin to panitumumab. The enzymatic activity of the IT was reduced by 48% in comparison to dianthin. The IT achieved a half maximal inhibitory concentration (IC_{50}) of 39.83 nM while dianthin alone showed no cytotoxicity *in vitro*. In combination with SO1861, dianthin showed an IC_{50} of 1.05 nM and the IT of 0.0112 nM on the target cell line. The IT did not reveal any toxicity neither in the presence nor absence of SO1861 on off-target cells. SO1861 alone did not had any effect on the cells.
Conclusion: Panitumumab-dianthin exhibited an enhanced cytotoxicity by a factor of 3556 over dianthin when combined with SO1861 while off-target cells remained unaffected by this treatment. These observations highly enable this IT for further *in vivo* testing.
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Poster M-V-04

Fluorescent genetic barcoding for multiplex tracking of normal and malignant hematopoietic cells

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Understanding heterogeneity within phenotypically identical cells plays an increasingly important role for regenerative medicine and cancer. Despite progress in single cell sequencing methodologies, there is an urgent need for efficient experimental approaches that enable the functional interrogation of defined cell clones or cell populations *in vitro* and *in vivo*.
Building on the potential of flow cytometry for the real-time characterization of heterogeneous populations as well as on stable lentiviral gene marking, we here describe a lentiviral fluorescent genetic barcoding (FGB) vector platform for multiplexed functional characterization of hematopoietic cells over time. This FGB platform consists of six fluorescent color coding vectors, which express GFP, YFP and mKO2 either alone or in pairs, respectively. Transduction of each of the six vectors into separate wells prior to sample mixing enabled the flow cytometric deconvolution as well as the longitudinal tracking of all input populations. A similar transduction approach also supported efficient multiplexing of murine ($CD45^+EPCR^+CD48^-CD150^+$; ESLAM) and human ($CD34^+$) hematopoietic stem and progenitor cells as well as the assessment of their color coded progeny distribution within the bone marrow of recipient mice. In combination with immunophenotyping, progeny of color coded ESLAM cells sporadically even showed a lineage bias suggestive of initial clonal labeling and engraftment events. Furthermore, investigation of the prototypical Hoxa9/Meis1-dependent (H9M) murine acute myeloid leukemia model demonstrated the maintenance of color coded cell mixing ratios for the first 4-5 weeks *in vivo*, before emergence of a dominant color code. Bone marrow samples of these mice also enabled the enrichment of putative color coded leukemic stem cell populations by fluorescent activated cell sorting. Finally, co-transplantation of six CD45.1 and six CD45.2-derived color coded H9M populations individually exposed to increasing concentrations of the HDACi Entinostat facilitated a 12-fold reduction in mouse numbers required for IC_{50} (463.8nM ±141.3) determination in short term *in vivo* assays.
Together these data show the potential of FGB-mediated multiplex assays for gaining insights into the competitive behavior of normal and malignant cells in a wide variety of applications at reduced workload, processing time, and mouse utilization. Currently, the applicability of system is being evaluated for clonal tracking studies.

Poster M-V-05

Restoration of *Atm* induces lymphoma regression *in vivo* and is mediated by cell autonomous and non-cell autonomous mechanisms

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Ataxia Telangiectasia Mutated (*ATM*) plays a key role in DNA-damage response singling and therefore, protects genome stability. Germline mutations in *ATM* cause Ataxia-telangiectasia (A-T), which is characterized by severe cerebellar ataxia, and cancer predisposition. In addition, *ATM* is frequently inactivated or downregulated in a wide range of human cancers. It is unclear whether *ATM* loss acts as an instigating lesion promoting acquisition of additional oncogenic mutations, or whether continued absence of ATM is critical for preserving the cancerous state. We generated a reactivatable *Atm* allele, which displays *Atm*-knockout phenotype, however, allows restoring functional ATM kinase in living animals. We observe that *Atm* reactivation in thymic T-cell lymphomas and *Myc*-driven Non-Hodgkin's B-cell lymphomas induces regression of the tumor. Interestingly, tumor protecting effects of *Atm* reactivation could be observed both: in tumor cells and in non-malignant tumor stroma. Specifically, we find increased ability of *Atm*-proficient cells to induce apoptosis in response to exogenous DNA damage. In addition, we observe reduced numbers of activated T cells in B cell lymphomas from *Atm*-deficient recipient mice and human A-T patients. Using CD4 and CD8 T-cell depletion experiments, we show impaired T-cell mediated immune surveillance as a contributor to cancer predisposition in *Atm*-defective mice and A-T patients. Our results demonstrate that continued lack of *Atm* expression is critical for the maintenance of the oncogenic state in B- and T-cell lymphomas *in vivo*. Our data further indicate that transplantation of ATM-proficient immune cells might be considered as a therapeutic approach for treatment of cancer in A-T patients.

Poster M-V-06

TALEN-mediated knockout of the HIV co-receptor CCR5

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In 2016, worldwide about 36.7 million people were infected with the human immunodeficiency virus (HIV) with only app. half of them (39-65%) having access to antiretroviral therapy (ART). Despite great improvements in ART, HIV infections remain incurable, chronic diseases. Until today, there has been only one reported cure from HIV. The so-called "Berlin patient" received a stem cell transplantation from an HLA-matched donor homozygous for a naturally occurring 32-bp deletion in the coding region of the C-C motif chemokine receptor 5 gene (*CCR5Δ32*). Affected individuals (app. 1% of Caucasians) are highly resistant to R5-tropic HIV strains at the same time not showing health issues. Our lab recently developed a novel CCR5-specific TAL effector nuclease (CCR5-Uco-TALEN) that facilitates excellent activity in primary T cells (knockout rates > 60 %) combined with low toxicity. Lately, we confirmed the low off-target activity of our CCR5-Uco-TALEN by next-generation amplicon sequencing. Only two out of the 10 most likely predicted off-targets, *C-C motif chemokine receptor 2* (*CCR2*, exogenic) and *glypican 5* (*GPC5*, intergenic) showed TALEN activity at these sites. To reliably determine gene-editing events in CCR5-edited CD4+ T cells at the two confirmed off-target sites, we adapted our sensitive and precise GEF-ddPCR assay to screen for *CCR2* and *GPC5*. On-target activity of our CCR5-Uco-TALEN often led to an 18-bp deletion exactly between the two TALEN binding sites corresponding to the first intracellular loop of CCR5. We show here that this 18-bp deletion, as well as amino acid substitutions in that region, result in impaired protein folding or transportation causing lower or no CCR5 cell surface presence. In order to produce high numbers of CCR5-negative $CD4^+$ T cells, not only high *CCR5* gene-editing frequencies are necessary, but biallelic knockout is essential. To determine the rates of biallelic and monoallelic knockout in CCR5-Uco-TALEN treated T cells, we have established a protocol for high-resolution melting curve analysis. This method allows us to determine mono- and biallelic knockouts on a single-cell level, as well as to distinguish between hetero- and homodimeric gene-editing events. Moreover, we evaluated high-quality capped mRNA of our CCR5-Uco-TALEN produced by TriLink Biotechnologies for its gene-editing efficiency and its dosage. Altogether, our progress highly promotes the translation of our CCR5-Uco-TALEN towards clinical application.

Poster M-V-07

Use of an inducible vector to explore gene effects on reprogramming and subsequent hematopoietic differentiation

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The potential of induced pluripotent stem cells (iPSC) to generate any cell type makes iPSC highly interesting for regenerative medicine approaches, drug screening and disease modelling, such as in cancer biology. However, attempts to reprogram cancer cells were only successful in a limited number of specific cases. Therefore, it would be of interest to develop a model system to investigate potential causes of reprogramming blockades. We used a third generation all-in-one lentiviral self-inactivating (SIN) vector containing an optimized tetracycline-responsive promoter (T11) for inducible transgene expression, the minimal ubiquitous chromatin opening element (CBX3) upstream of a constitutive hPGK promoter to control transactivator (M2) expression and a puromycin resistance cassette. As a model system, Newborn Human Foreskin Fibroblasts (NuFF) were transduced with the all-in-one inducible vector designed to express enhanced green fluorescent protein (EGFP) upon induction of the T11 promoter and transduced cells were enriched via puromycin selection (1 μg/mL, 48 hours) prior to reprogramming with the established 4-in-1 lentiviral reprogramming cassette. This protocol allowed successful iPSC generation in the presence and absence of doxycycline as demonstrated by expression of the pluripotency marker TRA-1-60. Importantly, we demonstrate the necessity of the CBX3 element for inducible control of EGFP expression in iPSC clones as it was not possible to induce EGFP following reprogramming of cells transduced with similar vectors lacking the CBX3 element. Furthermore, transgene expression level was adjustable via application of different doxycycline concentrations in iPSC clones. We also demonstrate the possibility to express transgenes during different stages of hematopoietic differentiation from iPSC as shown by fluorescent microscopy and flow cytometry of embryoid bodies and the mature hematopoietic cells derived from them. Similar levels of hematopoietic differentiation were achieved in the presence or absence of doxycycline-induced EGFP expression as demonstrated by production of >80% $CD45^+$ cells upon cultivation in APEL medium supplemented with human IL-3 (25 ng/mL). In summary, our data shows the potential for reversible and temporal control of transgene expression prior to, during or after iPSC generation, rendering this system a useful tool to investigate the influence of genes on reprogramming efficiency as well as differentiation of iPSC clones.

Poster M-V-08

Clonal dynamics studied in cultured iPS cells and cell lines reveal major growth imbalances within few weeks

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Cells in a culture dish are often thought to be monoclonal and uniform. On the other hand, it is commonly accepted that in an experiment even robust cell lines should be used with similar passage numbers, because cells might "change" over time. To gain deeper insights into the behaviour of cultured cells on the clonal level we applied RGB marking, a lentiviral multi-colour cell tracking method developed in our lab. As a relevant cell type to analyse we chose human induced pluripotent stem cells (iPS) as well as two cultured cell lines and primary human fibroblasts as controls. Especially human iPS cells, which spark hopes for future tissue replacement therapies, need to be expanded from a single cell to large quantities prior to any potential use. However, long-term expansion has been suggested to compromise cell integrity and quality. Based on our labelling technique we assessed the potential reduction of clonal diversity, e.g. due to inherent growth imbalances or loss of clones during replating procedures, by flow cytometry and fluorescence microscopy. In all cell types tested, we observed a marked reduction in clonal diversity within weeks of culturing time. After 38 passages, two of three iPS cultures consisted almost entirely of one or two clones, respectively. Karyotype and function, as assessed by cardiomyocyte differentiation and tissue engineering, did not show obvious differences. In the two analysed cell lines, the first clones became distinguishable already after seven to ten weeks, depending on culture conditions. Our results reveal a quick outgrowth of clones and visualize a normally invisible and potentially undesired behaviour of cultured cells, especially when using long-term cultures.

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