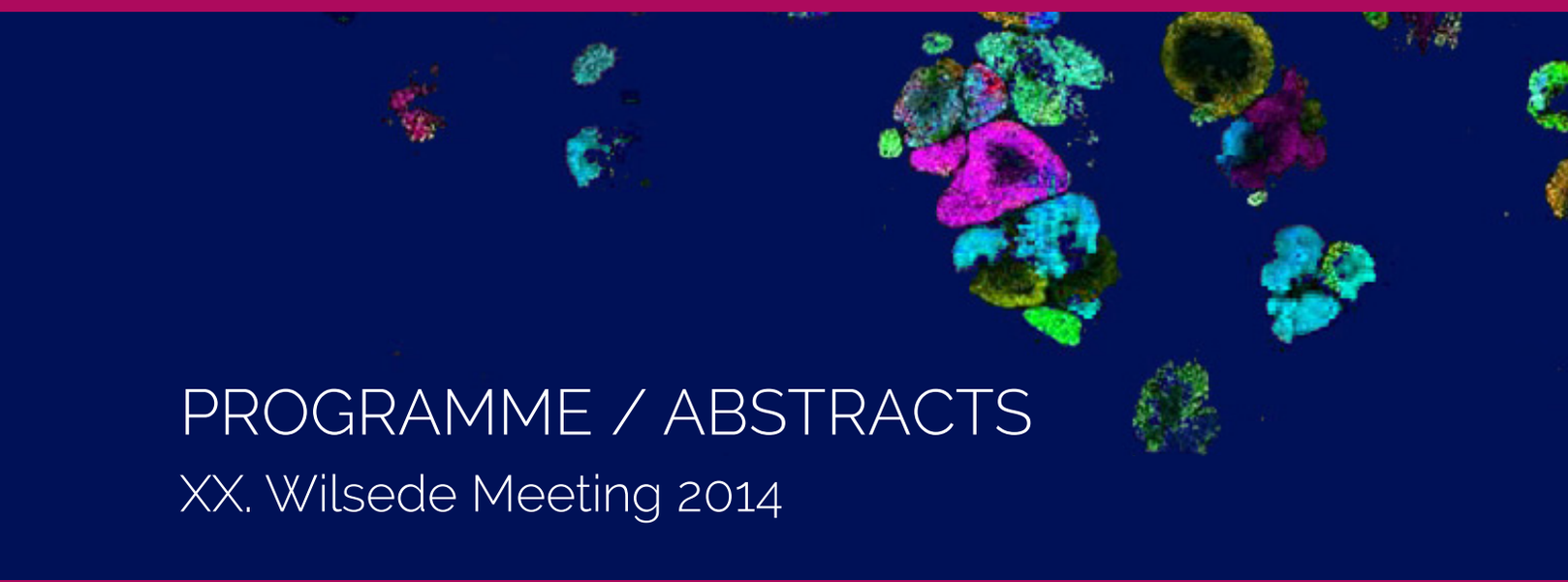


PROGRAMME / ABSTRACTS

XX. Wilsede Meeting 2014

MODERN TRENDS IN HUMAN LEUKEMIA & CANCER

June 21-24, 2014 – Wilsede, Germany

We cordially thank for supporting the Wilsede Meeting

Deutsche Krebshilfe
HELFEN. FORSCHEN. INFORMIEREN.

Universitätsklinikum Hamburg-Eppendorf

DFG Deutsche Forschungsgemeinschaft

HPI

LEUKAEMIA & LYMPHOMA RESEARCH
Beating Blood Cancers

Welcome to the XX. Wilsede Meeting on

"Modern Trends in Human Leukemia and Cancer"

As in previous years, the main purpose of the XX. Wilsede meeting is to evaluate recent developments in the field of leukemia and cancer biology. The heterogeneity of malignant cells is a central theme in this year's meeting. Also, the concepts of normal and malignant stem cells, the importance of epigenetic events and the opportunities to translated basic findings into targeted therapies will be discussed. The new advent of immunotherapeutic approaches and the great potential of tumorlytic viruses are another topic of the XX. Wilsede meeting. The impressive progress that has been made in these fields 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 decision 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 meeting.

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. Emer. Rolf Neth, who was the initiator and driving force of the Wilsede meetings, as well as Prof. Emer. Axel Zander, who carried the baton from 1994 to the present, deserve special thanks. In the last more than 10 years, Dr. Carol Stocking was one, if not the main person to keep this 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

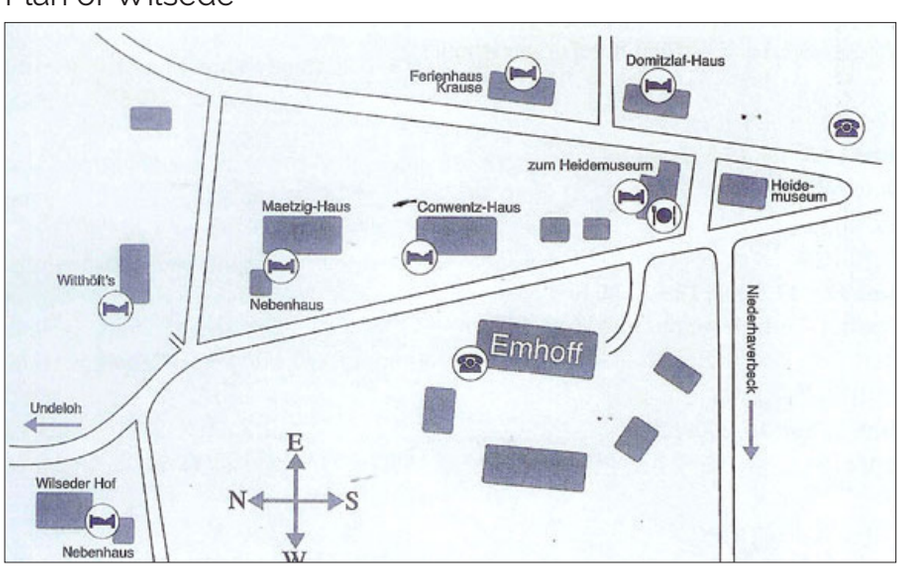
Wilsede

... 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 Harnburg and Hanover, 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: Modem 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 wastobe pulled down, but the NVP, together with the Lower Saxony Authority for Preservation of Historical Monuments, succeeded to place the building under the Preservation Act. About one third of the building was reconstructed under the direction of the architect Prof. Maetzig using the original techniques, e.g. grooved planks instead of nails. The former hallway, cowsheds and stables were rebuilt into a large meeting room and the "Dönz", the former living room, is today the kitchen. The shape of the lamps is to remind the visitor of the old pitch torches. The architectural style of the Ernhoff is said to be the oldest of the moorland region and it is especially typical for the southem part of this region. Nowadays buildings of this kind have almost completely disappeared due to disrepair and deterioration, making Ernhoff doubly precious.

Plan of Wilsede



Contents

Scientific and Organizing Committee

Prof. Dr. Konstanze Döhner, Ulm (DE)
Prof. Dr. med. Boris Fehse, Hamburg (DE)
Prof. Dr. Stephan Hahn, Bochum (DE)
Prof. Dr. Wolfgang Janni, Ulm (DE)
Prof. Dr. rer. nat. Holger Kalthoff, Kiel (DE)
Prof. Dr. med. Nicolaus Kröger, Hamburg (DE)
Prof. Dr. med. Ralf Küppers, Essen (DE)
Prof. Dr. med. Martin Trepel, Hamburg (DE)

Abstract reviewer

Prof. Dr. med. Thomas Brabletz, Freiburg (DE)
Prof. Dr. Christian Buchholz, Langen (DE)
Prof. Dr. med. Konstanze Döhner, Ulm (DE)
Prof. Dr. Christine S. Falk, Hannover (DE)
Prof. Dr. med. Walter Fiedler, Hamburg (DE)
Prof. Dr. med. Stephan Hahn, Bochum (DE)
Prof. Dr. med. Wolfgang Janni, Ulm (DE)
Prof. Dr. Manfred Jücker, Hamburg (DE)
Prof. Dr. rer. nat. Holger Kalthoff, Kiel (DE)
Prof. Dr. med. Nicolaus Kröger, Hamburg (DE)
Prof. Dr. rer. nat. Ralf Küppers, Essen (DE)
Univ.-Prof. Dr. Dorothee von Laer, Innsbruck (A)
PD Dr. med. Dr. rer. nat. Sonja Loges, Hamburg (DE)
Prof. Dr. med. Carsten Müller-Tidow, Halle (DE)
Prof. Dr. Jürgen Ruland, München (DE)
Dr. Carol Stocking-Harbers, Hamburg (DE)
Prof. Dr. med. Martin Trepel, Hamburg (DE)

Main-Sponsor

Deutsche Krebshilfe e.V.

Co-Sponsors

Deutsche Forschungsgemeinschaft (DFG)
Leukemia & Lymphoma Research
Heinrich Pette Institute, Leibnitz Institute for Experimental Virology (HPI)
University Medical Center Hamburg-Eppendorf (UKE)

Meeting Office/Contact

CSi Hamburg GmbH
Conferences | Symposia | Incentives
Falkenried 88
20251 Hamburg, Germany
Phone: +49 40 30770300

Registration Hamburg

June 21, 2014 – 08.30 to 14.00
Campus Lehre, Building N55
University Medical Center Hamburg-Eppendorf (UKE)

On-site Registration · Emhoff/Wilsede

Contact:
Alexandra Werner (+49 157 35730004),
Antje Blömeke (+49 157 35730006)

June 21, 2014	14.00 to 17.00
June 22–23, 2014	08.00 to 18.00
June 24, 2014	08.00 to 12.00

Arrival (June 21, 2014)

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

From 08.30: Registration and pick up of 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 21, 2014 (Arrival)

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

22.30 from Wilsede to Undeloh

June 22 & 23, 2014

08.00 from Undeloh to Wilsede

22.30 from Wilsede to Undeloh

June 24, 2014 (Departure)

08.00 from Undeloh to Wilsede

12.00 from Wilsede to Undeloh
13.00 from Wilsede to Undeloh
14.00 from Wilsede to Undeloh

Undeloh: parking lot near "Undeloher Hof"

Bicycles ("rent-a-bike")

A deposit of € 10,- is required and will be refunded after return of the bike.
Undeloh: "Hotel Heiderose"
Wilsede: "Wilseder Hof"

Departure (June 24, 2014)

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

Departures 12.30, 13.30 and 14.30

Attire of the meeting: Casual

Wilsede

Zum Heidemuseum · 29646 Bispingen/Wilsede
Phone: +49 4175-217, Telefax: +49 4175-8318
Contact: Klaus Parpart

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

Wilseder Hof · 29646 Bispingen/Wilsede
Phone: +49 4175-311, Telefax: +49 4175-80 00 92
Contact: Stefan Wischhof

Witthöfts Gästehaus · 29646 Bispingen/Wilsede
Phone: +49 4175-545, Telefax: +49 4175-82 11
Contact: Dr. Uta Büttinghaus

Undeloh

Witte's Hotel · 21274 Undeloh
Phone: +49 4189-81 33 60, Telefax: +49 4189-629
Contact: Wolf-Dieter Hennig

Undeloher Hof · 21274 Undeloh
Phone: +49 4189-81 89 10, Telefax: +49 4189-468
Contact: Inge Brunkhorst

- Landhaus Undeloher Hof
- Haus Uhlchen

Hotel Heiderose · 21274 Undeloh
Contact: Ullrich Wischhof
Phone: +49 4189-311, Telefax: +49 4189-314

Breakfast

June 22-24, 2014

Guests accommodated in

Wilsede: Guests staying at Conventz-Maetzig-Domitzlaff-House and Witthöfts Gästehaus: at Gasthof „Zum Heidemuseum“ (starting at 7.00)
For guests staying at Hotel Wilseder-Hof: at their hotel
Undeloh: at their hotel or guesthouse

Welcome Reception and Dinner

June 21, 2014 19.30
Hotel Wilseder Hof

Lunch

June 22, 2014 12.30-14.00
June 23, 2014 12.15-14.00
Gasthof “Zum Heidemuseum”

June 24, 2014 packed Lunch

Barbecue/Farewell Dinner

June 22 and 23, 2014 19.00
Emhoff

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, June 22, 2014 · 16:00-18:00

Heterogeneity (I), Targeted therapies (III), Others (VII)

#

S-I-01 · S-I-02 · S-I-03 · S-I-04 · S-I-05 · S-I-06 · S-I-08 · S-I-09

S-III-01 · S-III-02 · S-III-03 · S-III-04 · S-III-05 · S-III-06 · S-III-07

S-III-08 · S-III-09 · S-III-10 · S-III-11 · S-III-12 · S-III-13

S-VII-01 · S-VII-02 · S-VII-03 · S-VII-04 · S-VII-05 · S-VII-06 · S-VII-07 · S-VII-08

Poster Session II = Monday, June 23, 2014 · 16:30-18:30

Stem cells (II), Gene Therapy (IV), Leukemogenesis (V), Epigenetics (VI)

#

S-I-07

M-II-01 · M-II-02 · M-II-03 · M-II-04 · M-II-05 · M-II-06 · M-II-07 · M-II-08 · M-II-09 · M-II-10 · M-II-11

M-II-12 · M-IV-01 · M-IV-02 · M-IV-03

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-V-09 · M-V-10

M-VI-01 · M-VI-02 · M-VI-03 · M-VI-04 · M-VI-05 · M-VI-06

M = Monday *S = Sunday* *Roman numerals = Topic*

Location

Conventz-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 no later than 1 hour before the start of the session.

Saturday · June 21, 2014

08.30 – 14.30	**Registration** at UKE N55 (Hamburg) and in Wilsede	
16.30 – 16.45	**Welcome Address** Boris Fehse & Nicolaus Kröger, Hamburg (DE)	

Session I: Heterogeneity of malignancies & metastasis

Chairs: Ralf Küppers, Essen (DE); Carol Stocking, Hamburg (DE)

16.45-17.10	**Molecular and genetic profiling of B-cell lymphomas - from basic research to new diagnostic tools** *Wolfram Klapper, Kiel (DE)*	Speaker 01
17.10-17.35	**Novel molecular concepts of leukemic transformation in chromosome 3q21;3q26 rearranged AML** *Ruud Delwel, Rotterdam (DE)*	Speaker 02
17.35-18.00	**Clinical implications of clonal heterogeneity in multiple myeloma** *Marc S. Raab, Heidelberg (DE)*	Speaker 03
18.00-18.15	**Characterization of oncogenes on chromosome 21 identified by shRNA-based viability screening (short talk)** *Jan-Henning Klusmann, Hannover (DE)*	Speaker 04
18.15-19.00	**Special Lecture** **Myeloproliferative Neoplasms – New Tails and a Point of Order** *Tony Green, Cambridge (UK)*	Speaker 05
19.15	**Welcome Reception and Dinner** Wilseder Hof	

Sunday · June 22, 2014

Session II: Stem cells in homeostasis and disease

Chairs: Walter Fiedler; Axel Zander, Hamburg (DE)

09.00-09.25	**Consequence of WT1 Expression and Mutation in Acute Myeloid Leukemia** *Carol Stocking, Hamburg (DE)*	Speaker 06
09.25-09.50	**Activating FLT3-ITD receptor mutations in AML are associated with a specific epigenetic signature composed of a discrete subset RUNX1-bound DNaseI hypersensitive sites enriched for AP-1 and C/EBP motifs** *Peter Cockerill, Birmingham (GB)*	Speaker 07
09.50-10.05	**Using genome-wide binding profiles of transcription factors to reconstruct transcriptional networks in normal hematopoietic stem/progenitor and leukaemic cells (short talk)** *Dominik Beck, Sydney (AU)*	Speaker 08
10.05-10.20	**The transcriptional regulator Fuse Binding Protein 1 (FUBP1) is essential for fetal and adult hematopoietic stem cell self - renewal (short talk)** *Martin Zörnig, Frankfurt (DE)*	Speaker 09
10.20-10.50	Coffee break	
10.50-11.15	**Two consecutive developmental stages of long-term hematopoietic stem cells** *Claudia Waskow, Dresden (DE)*	Speaker 10
11.15-11.30	**Induced pluripotent stem cell model of severe congenital neutropenia with gene deficiency (short talk)** *Tatsuya Morishima, Hannover (DE) + Kyoto (JP)*	Speaker 11
11.30-11.45	**Novel all-in-one LeGO-SWITCH vectors: a versatile tool for the analysis of oncogene function and tet-controlled expression from lentiviral vectors (short talk)** *Stefan Horn, Hamburg (DE)*	Speaker 12

Sunday · June 22, 2014

Session III: Targeted therapies – what is the right cell, what is the best molecule?

Chairs: Holger Kalthoff, Kiel (DE); Martin Trepel, Hamburg (DE)

11.45-12.10	**Targeting signal transduction in AML** *Hubert Serve, Frankfurt (DE)*	Speaker 13
12.10-12.25	**Opposing regulation of BIM and BCL2 gene expression controls glucocorticoid induced apoptosis of paediatric acute lymphoblastic leukaemia cells (short talk)** *Duohui Jing, Sydney (AU)*	Speaker 14
12.30-14.00	**Lunch (Heidemuseum) & Poster viewing**	
14.00-14.25	**Oncogenes and feedback in acute lymphoblastic leukemia** *Markus Müschen, San Francisco (US)*	Speaker 15
14.25-14.50	**Aberrant chromatin in programming in core-binding factor acute myeloid leukemia** *Constanze Bonifer, Birmingham (GB)*	Speaker 16
14.50-15.15	**Mutated IDH1 as a therapeutic target** *Michael Heuser, Hannover (DE)*	Speaker 17
15.15-16.00	Coffee break	
16.00-18.00	**POSTER SESSION I** **(Heterogeneity, Targeted therapies, Others)** *Chairs: Holger Kalthoff, Kiel (DE); Ralf Küppers, Essen (DE); Nicolaus Kröger, Hamburg (DE)*	
19.00	Barbecue and Live Music Emhoff	

Monday · June 23, 2014

Session IV: Adoptive Immunotherapy

Chairs: Christine S. Falk, Hannover (DE); Nicolaus Kröger, Hamburg (DE)

09.00-09.25	**Of Cars and Trucks: T cells with extraordinary Performance** *Hinrich Abken, Cologne (DE)*	Speaker 18
09.25-09.50	**Engineering of chimeric antigen receptor (CAR)-modified T cells for adoptive immunotherapy of cancer – emerging opportunities and challenges** *Michael Hudecek, Würzburg (DE)*	Speaker 19
09.50-10.15	**Cancer therapy using tumor-infiltrating lymphocytes (TIL) for adoptive transfer; can it get any better?** *Per Thor Straten, Herlev (DK)*	Speaker 20
10.20-10.45	Coffee break	
10.45-11.10	**Cell therapy for leukemia crosses the activity threshold** *Stephan Grupp, Phildelphia (US)*	Speaker 21
11.10-11.35	**Targeting tumors by alloreactive T-cells** *Fred Falkenburg, Leiden (NL)*	Speaker 22
11.35-12.00	**T Cell Engineering for Immunotherapy of Cancer** *Hans Stauss, London (UK)*	Speaker 23
12.00-12.15	**TALEN-mediated TCR knockout (short talk)** *Belinda Berdien, Hamburg (DE)*	Speaker 24
12.15-14.00	Lunch (Heidemuseum) & Poster viewing	
14.00-14.20	**Funding Opportunities for Young Scientists and Clinicians – German Cancer Aid** *Thomas Brabletz – German Cancer Aid, Erlangen (DE)*	

Monday · June 23, 2014

Session V: Gene Therapy: Oncolytic viruses on their way to the clinics

Chairs: Boris Fehse, Hamburg (DE); Dorothee von Laer, Innsbruck (AT)

14.20-14.45	**Tumor and tumor stem cell targeted oncolytic viruses** *Christian Buchholz, Langen (DE)*	Speaker 25
14.45-15.10	**An oncolytic Rhabdovirus for the treatment of brain cancer, melanoma and prostate cancer** *Dorothee von Laer, Innsbruck (AT)*	Speaker 26
15.10-15.35	**Pexa-Vec (JX-594), an Oncolytic and Immunotherapeutic Vaccinia Virus, for the Treatment of Patients with Advanced Tumors** *Caroline Breitbach, San Francisco (US)*	Speaker 27
15.35-16.00	**Engineering Measles Virus for Cancer Therapy** *Guy Ungerechts, Heidelberg (DE)*	Speaker 28
16.00-16.30	Coffee break	
16.30-18.30	**POSTER SESSION II** **(Stem cells, Epigenetics, Leukemogenesis, Gene Therapy)** *Chairs: Thomas Brabletz, Erlangen (DE);* *Christine S. Falk, Hannover (DE); Boris Fehse, Hamburg (DE)*	
19:00	Farewell Dinner Emhoff	

Tuesday · June 24, 2014

Session VI: Epigenetics and cell ageing

Chairs: Thomas Brabletz, Erlangen (DE); Daniel G. Tenen, Singapore (SG)

09.00-09.25	**Epigenetic Changes in Myelodysplastic Syndrome (MDS) Focus on BCOR** *Phil Koeffler, Los Angeles (US)*	Speaker 29
09.25-09.50	**Micro RNAs, EMT and Cancer Stem Cells** *Thomas Brabletz, Erlangen (DE)*	Speaker 30
09.50-10.15	**Stem cell aging and rejuvenation** *Hartmut Geiger, Ulm (DE)*	Speaker 31
10.15-10.45	Coffee break	
10.45-11.10	**B-Cell Differentation and neoplastic transformation under the perspective of the epignome** *José I Martín-Sobero, Barcelona (ES)*	Speaker 32
11.10-11.35	**Leukemia initiating cell formation requires myeloid differentiation** *Daniel G. Tenen, Singapore (SG)*	Speaker 33
11.35-11.50	**A transgenic mouse model demonstrating the oncogenic role of mutations in the polycomb-group gene EZH2 in lymphomagenesis (short talk)** *Tobias Berg, Frankfurt (DE)*	Speaker 34
11.50-12.00	Concluding remarks	
12.00	**Departure (packed Lunch)**	

We thank you for your active participation and wish you a pleasant and safe trip home.
Hope to see you again for the Wilsede Meeting in June 2016!

SPEAKER ABSTRACTS

HETEROGENEITY OF MALIGNANCIES & METASTASIS

Speaker 1

Molecular and genetic profiling of B-cell lymphomas-from basic research to new diagnostic tools

Wolfram Klapper [1], Reiner Siebert[2] on behalf of the Deutsche Krebshilfe Netzwerk MMML, the BMBF funded International Cancer Genome Consortium Project MMML-Seq (ICGC MMML-Seq), the BMBF joint projects MMML-MYC-SYS and MMML-Demonstrators

[1] Department of Pathology, Hematopathology Section and Lymph Node Registry, University of Kiel, Germany
[2] Institute of Human Genetics, University of Kiel, Germany

The majority of lymphatic neoplasms including Burkitt Lymphoma, Follicular Lymphoma and Diffuse large B-cell Lymphoma are supposed to derive from germinal center B-cells. Germinal centers represent a unique scenario in which physiologically mutations are introduced into the genome of B-cells and, thus, are an Achilles' heel of neoplastic transformation. Due to their common cellular origin germinal center lymphomas share morphological and immunophenotypical features and to some extent have the capability to transform from one to the other entity. However, these lymphomas differ strikingly in their clinical presentation (like patient age and aggressiveness) and consequently their therapy.
Within joint projects summerized in this presentation, germinal center derived lymphomas are being comprehensively analyzed e.g. by gene expression and genome sequencing.
The clinical translation is performed in close collaboration with the German collaborative clinical study groups and help to identify new diagnostic and prognostic subgroups of lymphomas as well as potential new therapeutic approaches.

Speaker 2

Novel molecular concepts of leukemic transformation in chromosome 3q21;q26 rearranged AML

Ruud Delwel

Dept. of Hematology, ErasmusMC, Rotterdam, The Netherlands

Chromosomal rearrangements without gene fusions have been implicated in leukemogenesis by causing deregulation of proto-oncogenes via relocation of cryptic regulatory DNA elements. ML with inv(3)/t(3;3) is associated with aberrant expression of the stem-cell regulator EVI1. Applying functional genomics and genome-engineering, we demonstrate that both 3q rearrangements reposition a distal GATA2 enhancer to ectopically activate EVI1 and simultaneously confer GATA2 functional haploinsufficiency, previously identified as the cause of sporadic familial AML/MDS and MonoMac/Emberger syndromes.
Genomic excision of the ectopic enhancer restored EVI1 silencing and led to growth inhibition and differentiation of AML cells, which could be replicated by pharmacologic BET inhibition. Our data show that structural rearrangements involving single chromosomal repositioning of enhancers can cause deregulation of two unrelated distal genes, with cancer as the outcome.

Speaker 3

Clinical and Therapeutic Implications of Clonal Heterogeneity in Multiple Myeloma

Marc S. Raab

Max Eder Group Experimental Therapies for Hematologic Malignancies, German Cancer Research Center (DKFZ) and University of Heidelberg, Heidelberg, Germany

Virtually all cancer entities comprise a mixture of clones, a feature termed intra-clonal heterogeneity, that compete for spatial and nutritional resources in a fashion that leads to disease progression and therapy resistance. This process of competition resembles the schema proposed by Darwin to explain the origin of the species, and applying these evolutionary biology concepts to cancer has the potential to improve our treatment strategies. Multiple myeloma (MM) has a unique set of characteristics that makes it a perfect model in which to study the presence of intra-clonal heterogeneity and its impact on therapy. Novel therapies have improved the outcome of MM patients, increasing both progression-free and overall survival. Current therapy comprises an induction, consolidation and maintenance phases and it is important to consider how these components of MM therapy are affected by the presence of intra-clonal heterogeneity. In this evolutionary context therapy can be considered as a selective pressure differentially acting on the myeloma clones and impacting on their chances of survival. Current knowledge of intra-clonal heterogeneity, as well as its impact on the different components of MM treatment and strategies to overcome drug resistance caused by clonal evolution are discussed.

Speaker 4

Characterization of oncogenes on chromosome 21 identified by shRNA-based viability screening

Lena Stachorski, Aliaksandra Maroz, Veera Raghavan Thangapandi, Dirk Reinhardt, Jan-Henning Klusmann

Hannover Medical School Pediatric Hematology and Oncology, Carl-Neuberg-Str. 1, Hannover 30625, Germany

Children with trisomy 21 (Down syndrome, DS) are predisposed to develop acute megakaryoblastic leukemia (DS-AMKL) as well as the antecedent transient leukemia (DS-TL). Mutations in the transcription factor GATA1 have been found in nearly all children with DS-AMKL and DS-TL, but not in other malignancies. Recent whole genome sequencing efforts suggested that the interplay of trisomy 21 and GATA1s-mutation is sufficient to cause DS-TL.

To elucidate the pathogenesis of trisomy 21-associated leukemia, we integrated an RNAi viability screening and a proteomics approach to create an oncogenic gene network on hsa21 around GATA1s. shRNA-mediated knock-down of 42 genes conferred a profound selective growth disadvantage in DS-AMKL cell lines (CMK and CMY). 31 candidate genes are located in chromosomal region 21q22.1-21q22.3. 11 (out of 14 tested) were overexpressed in DS-AMKL compared to non-DS-AMKL. A secondary functional validation screening showed that the potential oncogenes participate in different cellular processes affecting proliferation, cell viability, apoptosis or differentiation. Gain- and loss-of-function studies of 11 selected candidates in CD34+ hematopoietic stem and progenitor cells (HSPCs) uncovered their regulatory function in megakaryopoiesis erythropoiesis and myelopoiesis. Knockdown of four genes (USP25, BACH1, U2AF1 and C21orf33) inhibited megakaryocytic and erythroid in vitro differentiation, while enhancing myeloid differentiation. Inversely, ectopic expression of six genes (U2AF1, C21orf33, IFNGR2, WDR4 or GABPA) resulted in a switch from erythroid to megakaryocytic differentiation.
Using an in vivo biotinylation approach to study the protein-protein interaction in DS-AMKL cells, we could show that GATA1 forms protein-complexes with hsa21-oncogenes. Several interactions are perturbed in N-terminal truncated GATA1s.
Thus, we propose a complex interactive network located on hsa21 around GATA1 positively regulating megakaryopoiesis. Deregulation of this network might result in synergistic effects on hematopoietic differentiation, which promotes transformation of GATA1s-mutated fetal hematopoietic progenitor cells.

SPECIAL LECTURE

Speaker 5

Myeloproliferative Neoplasms – New Tails and a Point of Order

Tony Green

Department of Haematology, Cambridge Institute of Medical Research
and Wellcome Trust/MRC Stem Cell Institute, University of Cambridge, UK

The human myeloproliferative neoplasms (MPNs) are a spectrum of clonal haematological malignancies which arise in the haematopoietic stem cell compartment. These disorders are experimentally tractable, permit clonal analysis and provide a window on the earliest stages of tumorigenesis. A single somatic gain-of-function mutation in JAK2 is present in most MPN patients. This observation emphasised the significance of the JAK signalling pathway which plays a key role in stem cell biology and has now been implicated in many human malignancies. The Green lab is focussing on the pathogenesis of the MPNs and particularly on the molecular and cellular consequences of JAK2 mutations. These studies have had rapid clinical impact with new approaches to both diagnosis and therapy. Additional highlights of general relevance for stem cell and cancer biology include unexpected insights into chromatin biology, clonal evolution and haematopoietic stem cell function. Most recently, our discovery of CALR mutations in the majority of JAK2 unmutated MPN patients has identified a new cancer gene and revealed an unexpected link with endoplasmic reticulum biology.

STEM CELLS IN HOMEOSTASIS AND DISEASE

Speaker 6

Consequence of WT1 Expression and Mutation in Acute Myeloid Leukemia

Maike Schwieger[1], Kira Behrens[1], Claudia Reichardt[2], Marion Ziegler[1], Ulla Bergholz[1], Christoph Englert[2], Carol Stocking[1]

[1] Heinrich-Pette-Institute, Leibniz-Institute for Experimental Virology, Martinistrasse 52, Hamburg, Germany
[2] Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany

The Wilms' tumor (WT1) zinc-finger transcription factor was first identified as a tumor-suppressor in spontaneous Wilms' tumor of the kidney, in which both WT1 alleles are mutated. In contrast, high levels of WT1 gene expression in up to 75% of acute myeloid leukemia (AML) have led to the hypothesis that WT1 is an oncoprotein in the hematopoietic compartment. Recent analysis has demonstrated that the WT1 gene is one of the most common targets of mutations in AML, with circa 12% of all AML samples containing WT1 mutations, of which 80% are heterozygous inactivating or null mutations, predictive of a haploinsufficient tumor suppressor. To resolve this conundrum of WT1 function in leukemogenesis, we have utilized three independent mouse models to determine 1) the normal function of Wt1 in hematopoiesis, 2) the pattern of Wt1 gene expression during proliferation and differentiation, and 3) the oncogenic potential of Wt1 in collaboration with a weak oncoprotein such as RUNX1/ETO. In addition, we have used these different systems to identify direct target genes of Wt1 in the early myeloid compartment and AML. Taken together our results demonstrate an important function of Wt1 in maintenance of the hematopoietic stem cell function during stress, in promoting proliferation at the expense of differentiation, and in synergizing with RUNX1/ETO to induce AML. Potential target genes of Wt1 that mediate these different functions have been identified and include regulators of apoptosis, adhesion and chemokine production. Our results clearly demonstrate the oncogenic function of WT1 in AML, which is consistent with the high expression levels of either wildtype or mutated oncogenic isoforms of WT1. In contrast, the majority of WT1 mutations in AML leads to reduced absolute WT1 protein levels, a characteristic that must also carry a selective advantage in the evolving leukemia. In view of the highly antigenic properties of Wt1, we postulate that this advantage is conferred by the ability of these cells to escape immune surveillance. Thus a fine balance of WT1 expression levels and isoform production, which is regulated by diverse mechanisms, is critical for leukemia progression.

*Equal contribution

Speaker 7

Activating FLT3-ITD receptor mutations in AML are associated with a specific epigenetic signature composed of a discrete subset RUNX1-bound DNaseI hypersensitive sites enriched for AP-1 and C/EBP motifs.

Pierre Cauchy[1], Sally James[2], Jason Piper[3], Anetta Ptasinska1, Joaquin Zacarias-Cabeza[1], Maria-Rosaria Imperato[1], Martina Canestraro[1], Salam Assi[2], Maarten Hoogenkamp[1], David Westhead[2], Sascha Ott[3], Constanze Bonifer[1]; Peter Cockerill[1]

1 University of Birmingham, U.K.
2 University of Leeds, U.K.
3 University of Warwick, UK

Acute myeloid leukemia (AML) with a FLT3 internal tandem duplication (FLT3/ITD) mutation is an aggressive hematologic malignancy with a high rate of relapse. This mutation converts a normally inducible cytokine receptor to a constitutively active receptor that activates MAPK and JAK/STAT pathways. To obtain mechanistic insights into how the epigenome is reprogrammed in this type of AML, we have employed genome-wide mapping of DNaseI Hypersensitive Sites (DHSs). This approach identifies active regions of the genome where potential enhancer and promoter elements exist as open regions of chromatin that are occupied by transcription factors. We have also developed genome-wide footprinting methods to predict occupancy of transcription factor binding sites.

We compared karyotypically normal (KN) AML bearing FLT3-ITD mutations to KN AMLs lacking this mutation, and to normal CD34+ peripheral blood stem cells (PBSCs). A detailed analysis of chromatin accessibility and transcription factor occupancy in FLT3-ITD AML revealed the existence of about 1,000 specific DHSs that were consistently enriched in the FLT3-ITD AML samples compared to normal CD34+ cells and other KN AMLs. These sites were enriched for DNA motifs for several transcription factors including RUNX1, AP-1, and C/EBP, that were predicted to be preferentially occupied by DNaseI footprinting. A RUNX1 ChIP analysis further confirmed that these specific DHSs bound RUNX1. This is partly mediated by an increase in RUNX1 levels because we saw a parallel 2 fold increase in the average level of RUNX1 mRNA expression in FLT3-ITD AML. However, the principle actions of FLT3-ITD at the level of the genome are likely to be via the MAPK dependent activation of factors such as AP-1. Many of the AP-1 motifs we identified were found by DNaseI footprinting to be occupied in FLT3-ITD+ AML but not in normal PBSCs. This activation of inducible factors and DHSs in AML is also probably the main mechanism leading to the AML-specific binding of RUNX1 to many additional sites that are not occupied in normal cells. In contrast, we found no enrichment for motifs for STAT or IRF family proteins that may also respond to FLT3 signalling. In summary, our data suggests that RUNX1 together with inducible factors plays a major role in reprogramming gene expression in FLT3-ITD AML.

Speaker 8

Using genome-wide binding profiles of transcription factors to reconstruct transcriptional networks in normal hematopoietic stem/progenitor and leukaemic cells.

Dominik Beck, Diego Chacon, Julie Thoms, Dilmi Perera, Ashwin Unnikrishnan, Jason Wong, John Pimanda

Lowy Cancer Research Centre and the Prince of Wales Clinical School, UNSW Australia, Sydney Australia

Genome-wide combinatorial binding patterns for key transcription factors (TFs) have not been reported for primary human hematopoietic stem and progenitor cells (HSPCs), and have constrained analysis of the global architecture of molecular circuits controlling these cells. Here we provide high-resolution genome-wide binding maps for a heptad of key TFs (FLI1, ERG, GATA2, RUNX1, SCL, LYL1, and LMO2) in human CD34+ HSPCs, together with quantitative RNA and microRNA expression profiles.

First, we catalog binding of TFs at coding genes and microRNA promoters, and report that combinatorial binding of all 7 TFs is favored and associated with differential expression of genes and microRNA in HSPCs. We uncover a previously unrecognized association between FLI1 and RUNX1 pairing, establish a correlation between the density of histone modifications that mark active enhancers and the number of overlapping TFs at a peak, demonstrate bivalent histone marks at promoters of heptad target genes that are poised for later expression, and identify complex relationships between specific microRNAs and coding genes regulated by the heptad. These data reveal the power of integrating multifactor sequencing of chromatin immunoprecipitates with coding and noncoding gene expression to identify regulatory circuits controlling cell identity.

Second, for the power of these integrated data sets to be fully harnessed by experimental scientists we have built the user-friendly database BloodChiP (http://www.med.unsw.edu.au/CRCWeb.nsfpage/BloodChIP). The database has at its core the genome-wide binding profiles of the heptad TFs in human HSPCs and we have further integrated publicly available binding profiles in normal differentiated and leukaemic cells. These ChIP-seq profiles were complemented with data sets of chromatin marks and gene expression in the same cells. BloodChIP supports exploration and visualization of combinatorial TF binding at a particular locus in human CD34+ HSPCs and other normal and leukaemic cells or retrieval of target gene sets for user-defined combinations of TFs across one or more cell types. All queries can be exported into external sites to construct TF–gene and protein–protein networks and to evaluate the association of genes with cellular processes and tissue expression. In conclusion, we provide insights into the combinatorial regulation of seven key hematopoietic TFs in human CD34+ HSPCs and provide important resource investigate these profiles in normal differentiated and leukaemic cells.

Speaker 9

The transcriptional regulator Fuse Binding Protein 1 (FUBP1) is essential for fetal and adult hematopoietic stem cell self renewal

Katharina Gerlach[1], Uta Rabenhorst[2], Frederic Thalheimer[3], Marek Kijonka[1], Michael Rieger[3], Martin Zörnig[1]

1 Georg-Speyer-Haus, Paul-Ehrlich-Strasse 42-44, 60596 Frankfurt, Germany
2 Karolinska Institute Department of Biosciences and Nutrition, NOVUM, Huddinge, Sweden
3 Goethe-University Hospital Frankfurt/Main Department for Hematology/Oncology, 60596 Frankfurt, Germany

Hematopoietic stem cells (HSCs) are defined by their ability to replenish all blood cell types and to self-renew, thereby enabling sustained hematopoiesis throughout life. Although our insights how self-renewal and multilineage differentiation are controlled at the molecular level has increased during the last decade, more research is needed to fully understand the complex interplay between the molecules required for HSC function.

We identified the transcriptional regulator FUBP1 as an essential factor necessary for hematopoietic stem cell (HSC) self-renewal. Homozygous Fubp1 gene trap embryos (Fubp1null/null) lacking FUBP1 activity, die in utero at day E15.5 and exhibit an anemic phenotype. The total fetal liver cell count and the number of long-term repopulating HSCs (LT-HSCs) are severely reduced in the homozygous embryos. Competitive transplantation experiments with E15.5-derived fetal liver cells (Fubp1null/null vs. Fubp1+/+) and with lentivirally transduced adult LT-HSCs (Fubp1 vs. scrambled shRNA) revealed a significantly lower engraftment of FUBP1-deficient cells in primary transplants and no engraftment in secondary transplants. These data confirm that FUBP1 is a central player in the regulation of fetal as well as adult HSC self-renewal.

Single cell tracking of adult Fubp1 knockdown LT-HSCs demonstrated significantly prolonged generation times and increased apoptosis rates. Ex vivo culturing of these cells confirmed a drastic expansion defect as a consequence of FUBP1 inactivation.

To elucidate the mechanisms by which FUBP1 controls the self-renewal capacity of LT-HSCs, we investigated the transcriptional network regulated by FUBP1 with Affimetrix gene profiling arrays and via quantitative real-time PCR. Our results indicate that FUBP1, amongst others, inhibits the expression of the cell cycle inhibitor p21 and the pro-apoptotic Noxa gene in adult LT-HSCs. We are also interested in the upstream regulation of FUBP1 in LT-HSCs and try to identify HSC-relevant transcription factors (TFs) contoling Fubp1 gene expression. ChIP experiments using murine bone marrow mononuclear cells demonstrated binding of TAL1/SCL within the Fubp1 promoter region. The ongoing studies will further our understanding of how FUBP1 is implicated in the regulatory network that controls the development and maintenance of the hematopoietic system.

Speaker 10

Two consecutive developmental stages of long-term hematopoietic stem cells

Tatyana Grinenko[1], Kathrin Arndt[1], Melanie Portz[1], Nicole Mende[1], Marko Günther[1], Kadriye Nehir Cosgun[1], Dimitra Alexopoulou[2], Naharajan Lakshmanaperumal[3], Ian Henry[3], Andreas Dahl[2]; Claudia Waskow[2]

1 Regeneration in Hematopoiesis, Center for Regenerative Therapies Dresden, Germany
2 Deep Sequencing Group SFB655, Biotechnology Center, Dresden University of Technology, Dresden, Germany
3 Bioinformatics Service, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Long-term hematopoietic stem cells (LT-HSCs) are well known to differ in their clonal expansion capacities after transplantation. By analysing the cellular output after transplantation of stem cells differing in surface expression levels of the Kit receptor, we show that LT-HSCs can be systematically subdivided into two subtypes with distinct reconstitution behaviour. LT-HSCs expressing intermediate levels of Kit receptor (Kitint) are quiescent in situ but proliferate extensively after transplantation and therefore repopulate large parts of the recipient's hematopoietic system. In contrast, metabolically active LT-HSCs expressing high levels of Kit receptor (Kithi) display more limited expansion capacities and show reduced but robust levels of repopulation after transfer. Transplantation into secondary and tertiary recipient mice show maintenance of efficient repopulation capacities of Kitint but not of Kithi LT-HSCs, meaning that these stem cell subtypes are consecutive developmental stages at the top of the hematopoietic hierarchy and Kitint LT-HSCs contain the most potent clonal expansion capacity. Initiation of differentiation is marked by the transit from Kitint to Kithi HSCs, both of which precede any other known stem cell population.

Speaker 11

Induced pluripotent stem cell model of severe congenital neutropenia with gene deficiency

Tatsuya Morishima[1,2], Ken-Ichiro Watanabe[2], Akira Niwa[3], Hodeyo Hirai[4], Satoshi Saida[2], Takayuki Tanaka[3], Itaru Kato [2], Katsutsugu Umeda[2], Hidefumi Hiramatsu [2], Megumu K Saito[3], Kousaku Matsubara [5], Souichi Adachi[6], Masao Kobayashi [7], Tatsutoshi Nakahata[3], Toshio Heike[2], Karl Welte[1], Julia Skokowa [1]

1 Hannover Medical School Department of Molecular Hematopoiesis, Hannover, Germany
2 Graduate School of Medicine, Kyoto University Department of Pediatrics, Kyoto Japan
3 Center for iPS Cell Research and Application, Kyoto University Department of Clinical Application, Kyoto Japan
4 Kyoto University Hospital Department of Transfusion Medicine and Cell Therapy, Kyoto Japan
5 Nishi-Kobe Medical Center Department of Pediatrics, Kobe Japan
6 Graduate School of Medicine, Kyoto University Human Health Sciences, Kyoto Japan
7 Hiroshima University Graduate School of Biomedical Sciences Department of Pediatrics, Hiroshima Japan

Congenital neutropenia (CN) is a rare inherited disorder of hematopoiesis with a 20% risk to develop acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). Recently, we reported the data from next-generation DNA deep sequencing of leukemia-causing candidate genes in CN patients who developed AML or MDS. This report shows that 64.5% of the patients demonstrated mutations within the RUNX1 gene and the vast majority of patients with RUNX1 mutations (75%) also revealed earlier acquired CSF3R mutations. The high frequency of RUNX1 mutations in CN patients who developed leukemia and the high incidence of associated RUNX1 and CSF3R mutations unveil a unique molecular pathway of leukemia development.
Induced pluripotent stem (iPS) cells are reprogrammed somatic cells with embryonic stem (ES) cell-like characteristics produced by the introduction of specific transcription factors. It is believed that iPS cell technology, which generates disease-specific pluripotent stem cells in combination with directed cell differentiation, will contribute enormously to patient-oriented research, including disease pathophysiology. To investigate the mechanisms of leukemia development from CN patients, we first worked on generating CN patient-derived iPS cells which can replicate the disease presentation.
We generated iPS cell lines using skin fibroblasts obtained from an SCN patient with HAX1 gene deficiency by retroviral transduction of OCT3/4, SOX2, KLF4 and cMYC genes. Next, we differentiate them into neutrophils in vitro using a serum- and feeder-free monolayer culture system. These HAX1 deficiency patient-derived iPS cell lines replicated the hematological phenotype in the patient such as maturation arrest, defective granulopoiesis and apoptotic predisposition due to low mitochondrial membrane potential in vitro. Furthermore, we corrected for the HAX1 gene deficiency in patient-derived iPS cells by lentiviral transduction with HAX1 cDNA and successfully reserved the disease presentation in gene-corrected cells.
These results shows our culture system combined with lentiviral gene transduction will serve as a useful tool to facilitate disease modeling for CN. We are now investigating the mechanisms of leukemia development from CN patients harboring different CN-specific mutations (ELANE, HAX1, G6PC3) using this system.

Speaker 12

Novel all-in-one LeGO-SWITCH vectors: a versatile tool for the analysis of oncogene function and tet-controlled expression from lentiviral vectors

Stefan Horn, Jill-Sandra Kant, Johannes Polke, Almut Uhde, Rüdiger Klapdor, Boris Fehse

Clinic for Stem Cell Transplantation, University Medical Centre Hamburg-Eppendorf Research Dept. Cell and Gene Therapy, Martinistrasse 52, 20246 Hamburg, Germany

Lentiviral vectors (LVV) have been widely used in basic research and gene therapy for the efficient delivery of genetic material and stable integration into the host genome. While most LVV available confer constitutive transgene expression, a time- and dose-dependent regulation of expression has proven highly beneficial for numerous research applications.

Recently, we have developed novel all-in-one lentiviral vectors (LeGO-SWITCH) by introducing the tet-inducible KRAB-domain containing tTR fusion repressor into the LeGO vector platform. The new vectors mediated controlled expression of genes and shRNAs with high-level induction and low background even in bulk cultures of various cell lines and primary mesenchymal stromal cells (MSCs).

To further evaluate the functionality of the LeGO-SWITCH vectors, we analysed the conditional expression of a human oncogene (PIK3CA) that encodes the catalytic subunit of class IA PI3-kinases (PI3K). We demonstrate tet-controlled expression of the wild-type PI3K and a kinase domain mutant (H1047R) in haematopoietic cells (Ba/F3). Induced expression of the mutant, but not the wild-type PI3K resulted in a strictly tet-dependent activation of the PI3K/AKT signalling cascade. In addition, LeGO-SWITCH vectors allowed for time- and dose-dependent oncogene expression and subsequent pathway activation. We further demonstrate tet-controlled regulation of relevant cellular processes, including proliferation and survival, and conditional cell transformation by tet-regulated induction of factor-independent growth. The highly selective cell transformation initiated by mutant PI3K enabled us to screen for non-controllable cells that were able to grow factor-independently even in the absence of induction. Importantly, non-regulable cells were only found after seeding extraordinary high numbers of cells, and were detected with a more than 10.000-fold lower frequency as compared to inducible control cells. By sequencing regulation-relevant parts of the proviral genomes in selected cell clones we were able to identify rare, but defined point mutations within the tet-dependent regulator fusion protein as well as uncommon recombination events within the tetracycline-response element (TRE) as possible molecular escape mechanisms.

In conclusion, the novel LeGO-SWITCH vectors allow for controlled and titratable production of an oncoprotein of interest and thus offer new possibilities to investigate oncogene functions by conditional expression.

TARGETED THERAPIES – WHAT IS THE RIGHT CELL, WHAT IS THE BEST MOLECULE?

Speaker 13

Targeting Signal Transduction in AML

Hubert Serve[1,2,3], Sebastian Mohr[1,2,3], Jasmin Corso[4], Sebastian Wagner[1,2,3], Christof Schaab[5,6], Tobias Berg[1,2,3], Ivana Metzler[1], Gesine Bug[1], Heike Pfeifer[1], Oliver G. Ottmann[1,2,3], Björn Steffen[1], Christian Brandts[1,2,3], Henning Urlaub[4], Thomas Oellerich[1,2,3]

1 Goethe University, Department of Medicine, Hematology/Oncology, Frankfurt, Germany
2 German Cancer Consortium (DKTK), Frankfurt/Mainz Site, Frankfurt, Germany
3 DKFZ, Heidelberg, Germany
4 Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany
5 Evotec (München) GmbH, Am Klopferspitz 19a, 82152 Martinsried, Germany
6 Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

Next generation sequencing revealed that AML is the result of a dynamic process, where multiple driver mutations coincide in branching hematopoietic subclones. One important class of mutations alters the function of proteins that are in involved in signal transduction. These mutations are thought to help the AML bone marrow to highjack the physiological communication of hematopoietic progenitor cells with their microenvironment. Most frequent in this class are mutations in the receptor tyrosine kinase FLT3 that confer an adverse prognosis to the afflicted patients. Several FLT3-specific kinase inhibitors are being clinically evaluated, with mixed success. While there is no doubt FLT3 inhibitors induce biological effects and clinically meaningful responses, so far no data have been presented that prove that FLT3 inhibition can provide long term benefits for AML patients. This may be due to several mechanisms: (1) under the treatment with FLT3 inhibitors, gatekeeper mutations that hinder binding of the inhibitor to the kinase rapidly occur; (2) we and others have shown that in AML blasts, extracellular stimuli activate intracellular kinases that confer resistance to FLT3 inhibition. Namely, the kinases Btk and Syk are activated by TLR9 and integrin receptors, respectively and potentiate the activation of STAT3/5, NFkappaB and Myc pathways. The discovery of targetable FLT3 cooperation partners that mitigate the effects of FLT3 inhibition in AML is one promising result of signal transduction analyses by quantitative mass spectrometry in AML model cell lines and in primary AML samples. Also, this methodology may be developed into a tool to monitor protein expression and phosphorylation events in primary patient samples before, during and after kinase inhibitor treatment, to use this information to newly interpret drug responsiveness and therapy resistance and to react accordingly by combining drugs for treatment. Taken together, targeting signal transduction in AML holds promise, if rational combination of signal inhibition and molecular monitoring of phosphorylation events before and during therapy are used to outwit the event of alternative and redundant pathways.

Speaker 14

Opposing regulation of BIM and BCL2 gene expression controls glucocorticoid-induced apoptosis of paediatric acute lymphoblastic leukaemia cells

Duohui Jing[1,2], Vivek A Bhadri[1], Chintanu Sarmah[1], Dominik Beck[3], Jason Wong[3], Kathy Knezevic [3], John Pimanda[3,4], Richard B. Lock[1,2]

[1] Children's Cancer Institute Australia for Medical Research, UNSW, High street, Sydney 2031, Australia
[2] School of Women's and Children's Health, UNSW, Sydney 2031, Australia
[3] Lowy Cancer Research Centre and the Prince of Wales Clinical School, UNSW, Sydney 2031, Australia
[4] Prince of Wales Hospital Department of Haematology, Sydney 2031, Australia

Glucocorticoids are critical components of combination chemotherapy regimens used to treat paediatric acute lymphoblastic leukaemia (ALL), although resistance is frequently encountered at relapse. The pro-apoptotic BIM protein is an important mediator of glucocorticoid-induced apoptosis of normal and malignant lymphoid cells, while the anti-apoptotic BCL-2 confers resistance. The purpose of this study was to gain a greater understanding of the role of BIM and BCL2 in dexamethasone-induced apoptosis of paediatric ALL patient-derived xenografts (PDXs) that were inherently sensitive or resistant to glucocorticoids. NOD/SCID mice engrafted with PDXs were treated with dexamethasone (15 mg/kg IP) or vehicle control, and spleen-derived human leukaemia cells harvested 8 hours thereafter. Downstream effectors of the glucocorticoid receptor (GR) were analysed, by gene expression profiling, and transcriptional regulators were studied by chromatin immunoprecipitation (ChIP) and ChIP-seq analyses. Microarray analysis of gene expression showed that KLF13 and MYB gene expression changes were significantly greater in a group of 5 dexamethasone-sensitive PDXs compared with 5 resistant PDXs (FDR<0.05). ChIP revealed that, following dexamethasone treatment, the GR bound to the KLF13 promoter resulting in increased KLF13 expression in sensitive, but not resistant, PDXs. Next, we demonstrated by ChIP that KLF13 bound to the MYB promoter in competition with SP1 resulting in inhibition of MYB expression only in sensitive xenografts. Since MYB is a known activator of the BCL2 gene, impaired KLF13 induction in dexamethasone-resistant PDXs resulted in maintained BCL2 expression and inhibition of dexamethasone-induced apoptosis. This was in direct contrast to dexamethasone-sensitive xenografts, in which BCL2 expression was switched off. ChIP-seq analysis revealed a novel GR binding site in a BIM intragenic region (IGR) in dexamethasone-sensitive, but not resistant, PDXs. Upon GR binding, acetylated histone (H3K9Ac) and RNA Pol II bound to the BIM promoter, resulting in BIM promoter activation. This appears to be the first evidence that the GR may directly activate BIM via distal regulation, and that the absence of GR binding at the BIM IGR is responsible for glucocorticoid resistance. In summary, we have identified novel mechanisms of opposing BIM and BCL2 gene regulation that control glucocorticoid-induced apoptosis in paediatric ALL.

Speaker 15

Permissive feedback enables oncogenic signaling in pre-B cells and represents a therapeutic target in acute lymphoblastic leukemia

Seyedmehdi Shojaee[1], Zhengshan Chen[1], Hilde Schjerven[1], Maike Buchner[1], Rahul Nahar[1], Srividya Swaminathan[1], Huimin Geng[1], Lai N Chan[1], Lars Klemm[1], Wolf-Karsten Hofmann[2], Yi Hua Qiu[3], Nianxiang Zhang[4], Kevin R. Coombes[4], Elisabeth Paietta[5], Jeffery Molkentin[6], H Phillip Koeffler[7,8], Cheryl L. Willman[9], Stephen P. Hunger[10], Ari Melnick[11], Steven M. Kornblau[3], Warren Alexander[12],
Markus Müschen[1]

1 Department of Laboratory Medicine, University of California San Francisco, San Francisco CA 94143
2 III. Medizinische Klinik, Medizinische Fakultät Mannheim, Universität Heidelberg, Germany
3 Department of Leukemia and 4Department of Bioinformatics and Computational Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA
5 Albert Einstein College of Medicine, Bronx, NY 10466, USA
6 Howard Hughes Medical Institute and Cincinnati Children's Hospital, University of Cincinnati, Cincinnati, OH 45247, USA
7 Division of Hematology and Oncology, Cedars Sinai Medical Center, Los Angeles, CA 90095
8 Cancer Science Institute of Singapore, National University of Singapore, Singapore
9 University of New Mexico Cancer Center, Albuquerque, NM
10 Children's Hospital of Colorado, Aurora, CO 80045, USA
11 Departments of Medicine and Pharmacology, Weill Cornell Medical College, New York, NY 10065, USA
12 Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Targeted therapy of cancer typically focuses on the development of agents that will inactivate a transforming oncogene. In this study, we tested the concept that besides the oncogene itself, factors that enable permissiveness of a normal cell to oncogenic signaling represent a novel class of therapeutic targets. This hypothesis was based on our finding that acute activation of oncogenes in pre-B cells typically results in immediate cell death in the vast majority of cells. Only a small minority of cells is capable of adapting quickly enough to evade cell death and to subsequently undergo oncogenic transformation. Permissiveness to oncogenic signaling was correlated with strong upregulation of negative feedback regulators of MAPK (DUSP6, SPRY2, ETV5) and STAT5 (CISH, SOCS2, SOCS3) signaling. Genetic deletion of the sprouty family Ras inhibitor Spry2, the MAPK phosphatase Dusp6 or Etv5, a transcriptional activator of SPRY2/DUSP6, decreased robustness of negative feedback control and compromised oncogenic transformation of pre-B cells. Likewise, ablation of the suppressors of cytokine signaling (SOCS) family molecules Cish, Socs2 and Socs3 reversed permissiveness of pre-B cells to oncogenic signaling. Interestingly, IKZF1, a critical tumor suppressor in pre-B ALL, binds directly to transcriptionally represses DUSP6, SPRY2, ETV5, CISH, SOCS2, and SOCS3 promoters, hence lowering the threshold of maximum allowable oncogene signaling strength. Interstingly, a small molecule inhibitor of DUSP6 acutely subverted negative feedback regulation and selectively induced cell death in pre-B ALL cells. In addition, small molecule inhibition of DUSP6 was sufficient to overcome conventional mechanisms of drug-resistance in pre-B ALL and had strong selective activity on drug-resistant patient-derived pre-B ALL cells that were injected into NOD/SCID transplant recipient mice. These findings identify permissive negative feedback control of oncogenic signaling as a previously unrecognized vulnerability of pre-B ALL cells and a new class of potential therapeutic targets.

Speaker 16

Aberrant chromatin programming in core-binding-factor acute myeloid leukemia

Anetta Ptasinska[1], Salam A. Assi[1,2], Jason Piper[6], Pierre Cauchy[1], Daniel G. Tenen[4], Anna Pickin[1], Peter N. Cockerill[1], David R. Westhead[2], Sascha Ott[6], Olaf Heidenreich[3], Constanze Bonifer[1]

1 School of Cancer Sciences; College of Medical and Dental Sciences; University of Birmingham; Birmingham, UK
2 Faculty of Biological Sciences, University of Leeds, Leeds, and UK
3 Northern Institute of Cancer Research, University of Newcastle, Newcastle upon Tyne, UK
4 Cancer Science Institute, National University of Singapore, Republic of Singapore
5 Systems Biology Center, University of Warwick

Our lab studies core-binding-factor acute myeloid leukemia (AML) to address the question of how leukaemic transcription factors reprogram the epigenome of normal myeloid progenitor cells. The t(8;21) translocation fuses the DNA binding domain of the hematopoietic master regulator RUNX1 to the ETO protein. The resultant RUNX1/ETO fusion protein is a leukaemia-initiating transcription factor that interferes with RUNX1 function. The result of this interference is a block in differentiation and, after the acquisition of additional mutations, the development of acute myeloid leukemia (AML).

In our previous work we determined genome-wide binding sites of RUNX1/ETO and RUNX1. We also measured open chromatin regions, histone acetylation and RNA-Polymerase II binding as well as global gene expression. Our work demonstrates that selective removal of RUNX1/ETO leads to a widespread reversal of aberrant epigenetic reprogramming, including alterations in histone modification patterns and RNA-Polymerase II occupancy as well as inhibition of self-renewal and the induction of myeloid differentiation (Ptasinska et al., 2012, Leukemia). Here we use a novel digital footprinting methodology and ChIP-sequencing to identify the core RUNX1/ETO responsive transcriptional network of t(8;21) cells. We show that the transcriptional programme underlying leukemic propagation is regulated by a dynamic equilibrium between RUNX1/ETO and RUNX1 complexes, which bind in a mutually exclusive fashion to identical genomic sites and have distinct preferences for co-activator and co-repressor binding. Perturbation of this equilibrium by RUNX1/ETO depletion results in a global reassembly of transcription factor complexes within pre-existing open chromatin and the formation of a new C/EBP-dominated transcriptional network driving myeloid differentiation. Our work demonstrates on a system-wide level that the block in myeloid differentiation in t(8;21) is caused by the dynamic balance between RUNX1/ETO and RUNX1 activities and the repression of C/EBP and highlights the core targets of transcriptional reprogramming in t(8;21) AML.

Speaker 17

Mutated IDH1 as a therapeutic target

Michael Heuser

Hannover Medical School Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

Mutations in the metabolic enzymes isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) are frequently found in glioma, acute myeloid leukemia (AML), melanoma, thyroid cancer and chondrosarcoma patients. Mutant IDH produces 2-hydroxyglutarate (2HG), which induces histone- and DNA-hypermethylation through inhibition of epigenetic regulators. We investigated the role of mutant IDH1 using the mouse transplantation assay. Mutant IDH1 alone did not transform hematopoietic cells during 5 months of observation. However, mutant IDH1 greatly accelerated onset of myeloproliferative disease (MPD)-like myeloid leukemia in mice in cooperation with HoxA9 with a mean latency of 83 days compared to cells expressing HoxA9 and wildtype IDH1 or a control vector (167 and 210 days, respectively, P=.001). Mutant IDH1 accelerated cell cycle transition through repression of cyclin-dependent-kinase inhibitors Cdkn2a and Cdkn2b, and activated MAP-kinase signaling. By computational screening, we identified an inhibitor of mutant IDH1, which inhibited mutant IDH1 cells and lowered 2HG levels in vitro, and efficiently blocked colony formation of AML cells from IDH1 mutated patients but not of normal CD34+ bone marrow cells. These data demonstrate that mutant IDH1 has oncogenic activity in vivo and suggest that it is a promising therapeutic target in human AML cells.

GENE THERAPY & IMMUNOTHERAPY

Speaker 18

Of CARs and TRUCKs: engineered T cells to target cancer

Hinrich Abken

Center for Molecular Medicine Cologne (CMMC), University of Cologne, and Clinic I for Internal Medicine, University Hospital Cologne, Cologne, Germany

Adoptive therapy with antigen-specific T cells is achieving impressive efficacy in early phase trials, in particular in hematologic malignancies, strongly supporting the notion that the immune system can control cancer. A current strategy of favor is based on ex vivo engineered T cells which are redirected by a chimeric antigen receptor (CAR) and recognize target cells in a pre-defined fashion by an antibody-derived binding domain. Such CAR T cells can substantially reduce the tumor burden as long as the targeted antigen is present on the cancer cells. However, loss of MHC or immunogenic antigens make cancer cells invisible to cytotoxic T cells and may contribute to deadly tumor relapses. We present an effective cell-based strategy to attack antigen-loss cancer cell variants by combining antigen-directed T cell therapy with the inducible release of a cytokine. Cytotoxic T cells were engineered to release inducible IL-12 upon CAR engagement in the targeted tumor lesion, which in turn attracts an innate immune cell response towards those cancer cells that are invisible to CAR T cells. Such TRUCKs, T cells redirected for universal cytokine-mediated killing, exhibited remarkable efficacy against solid tumors with diverse cancer cell phenotypes. CAR IL-12 T cells produced accumulation of activated macrophages in the targeted lesion that was crucial to the anti-tumor response. Inducible cytokine supplementation by CAR redirected T cells allows to target otherwise inaccessible tumor lesions, in a manner associated with reduced systemic cytokine toxicity, by recruiting and activating innate immune cells for a pro-inflammatory response.

Speaker 19

Engineering of chimeric antigen receptor (CAR)-modified T cells for adoptive immunotherapy of cancer – emerging opportunities and challenges

Michael Hudecek[1], Stanley R. Riddell[2,3], Hermann Einsele[1]

1 Medizinische Klinik und Poliklinik II, Universitätsklinikum Würzburg, Germany
2 Institute for Advanced Study, Technical University of Munich, Germany
3 Program in Immunology, Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, USA

Adoptive immunotherapy with T cells that were modified by gene-transfer to express a tumor-targeting chimeric antigen receptor (CAR) is being investigated as a novel and transformative way for treating cancer. CARs are synthetic receptors with an extracellular antigen-binding domain derived from the VH/VL chains of an antibody, an intracellular signaling domain – most commonly CD3zeta in cis with a co-stimulatory domain such as CD28 or 4-1BB, and recognize surface molecules independent from HLA. The CAR-transgene can be inserted into autologous T cells to provide a personalized tumor-reactive T-cell product for an individual patient. Pilot clinical trials at centers in the US have demonstrated the curative potential of this approach with dramatic and durable complete anti-tumor responses in a subset of patients with chemo-radiotherapy refractory CD19+ B-cell acute and chronic leukemia (ALL/CLL) that received T cells modified with a CAR specific for the B-lineage marker CD19. Importantly, clinical responses correlated with engraftment and persistence of CAR T cells following adoptive transfer. An ongoing effort in the field is to identify and validate alternative tumor antigens to extend applications of CAR T-cell therapy. Our group has developed a CAR specific for the ROR1 molecule that is expressed on several hematologic malignancies and epithelial cancers, including triple-negative breast cancer and demonstrated the ability of ROR1-specific CAR T cells to confer anti-tumor reactivity in pre-clinical models. We are in the process of establishing the GMP manufacturing process for CAR T cells and preparing clinical trials to implement this powerful new therapeutic modality at our institution.

Supported by: Deutsche Krebshilfe e.V., Max Eder Program

Speaker 20

Cancer therapy using tumor infiltrating lymphocytes (TIL) for adoptive transfer; can it get any better?

Per thor Straten

Center for Cancer Immune Therapy, Department of Haematology,
Copenhagen University Hospital Herlev, Denmark

Adoptive cell transfer (ACT) using tumor infiltrating lymphocytes (TIL) has achieved impressive clinical results in several single institution phase I/II clinical trials performed outside Europe, and holds promise to enter the mainstream of standard melanoma care in the near future. At our Institution we have an ongoing phase I/II study, and a European phase III study is being planned together with collaborators in Amsterdam and Manchester. However, despite unprecedented response rates in TIL based ACT, some patients do not respond or response is temporary. Moreover, for some patients, TILs cannot be grown; a feature that may be even more relevant if TIL based ACT is considered for other solid cancers. Thus, there is a need to improve this therapy both in terms of the cellular source, characterization of markers for response, as well as modifying the therapy for higher response rates and more complete and lasting responses. We have conducted a comprehensive monitoring of the patients included in the trial at our institution to achieve more insight into the biology of TIL therapy. Moreover, we are studying if T cells for TIL therapy could be improved in their capacity to home to tumor sites and retain functional capacity in a hostile tumor microenvironment.

Speaker 21

Cell therapy for leukemia crosses the activity threshold

Stephan Grupp

Division of Oncology, Departments of Pediatrics and Pathology, Children's Hospital of Philadelphia and Perelman Scholl of Medicine and the University of Pennsylvania

Chimeric antigen receptors (CARs) combine antigen recognition and intracellular signaling domains into a single chimeric protein. We previously reported on CTL019 anti CD19 CAR cells with up to 100,000x in vivo proliferation, durable anti-tumor activity, and prolonged persistence, including a sustained CR in 1 of 2 pts with ALL (Grupp, et al. NEJM 2013). More recently, we have reported on 30 pts (25 children and 5 adults) with relapsed, refractory ALL treated with CTL019.

T cells were lentivirally transduced with a CAR composed of an anti-CD19 scFv plus 4-1BB/CD3 signaling domains. The pediatric CTL019 dose range was 107 to 108 cells/kg with a transduction efficiency (TE) of 11–45%. Two thirds of pts had relapsed ALL after a prior allogeneic SCT. T cells were collected from the patient regardless of prior SCT status. 22 children 5 adults with CD19+ ALL were treated. One child had T cell ALL aberrantly expressing CD19. All but 3 pts had active disease immediately prior to CTL019 infusion. A median of 3.7×106 CTL019 cells/kg (0.7–18×106/kg) were infused. 27/30 pts (90%) achieved a CR, including the pt with CD19+ T cell ALL. 6 month OS is 78% and EFS 67% in the cohort. Recurrences have included 3 pts who developed CD19(-) ALL. All pts developed some degree of delayed cytokine release syndrome (CRS), some severe. Cytokine analysis showed marked increases of IL-6 and IFN. Treatment for CRS that included significant hypotension or respiratory instability was required in ~30% of pts with the IL-6 receptor antagonist tocilizumab, with rapid resolution of CRS. No GVHD has been seen in pts who were s/p allo SCT, although the cells collected from pts s/p allo SCT were median 100% donor origin. B cell aplasia was seen up to 24mo.

Summary: CTL019 cells are T cells genetically engineered to express an anti-CD19 scFv coupled to CD3 signaling and 4-1BB costimulatory domains. These cells can undergo robust in-vivo expansion and can persist for >18mo in pts with relapsed ALL. CTL019 therapy is associated with a significant CRS that responds rapidly to IL-6-targeted anti-cytokine treatment. This approach has promise as a salvage therapy for patients who relapse after allo SCT, and collection of tolerized cells from the recipient appears to have a low risk of GVHD. CTL019 cells can induce potent and durable responses for patients with relapsed/refractory ALL.

Speaker 22

Targeting Tumors by Alloreactive T cells

J. H. Frederik Falkenburg

Hematology, Leiden University Medical Center, The Netherlands

Adoptive T cell therapy in the context of allogeneic stem cell transplantation (SCT) allows the exploration of cellular immunotherapy strategies to control hematological cancers. After engraftment of donor hematopoiesis in the patient, T cells derived from the same donor are capable of recognizing polymorphic antigens disparate between donor and recipient, and can elicit both graft versus host disease (GVHD) and graft versus tumor (GVT) reactivity. T cell responses against polymorphic antigens expressed on normal non-hematopoietic tissues of the recipient are responsible for GVHD. In contrast, donor derived T cells directed against polymorphic antigens preferentially expressed on cells of hematopoietic origin can attack normal and malignant hematopoietic cells of recipient origin while preserving donor hematopoiesis and sparing of patient derived non-hematopoietic tissues. Donor derived CD8 T cells directed against antigens recognized in the context of HLA class-I molecules are known to exhibit cytotoxicity against target tissues. Since HLA class-I molecules are broadly expressed on all nucleated cells, CD8 T cells recognizing HLA class-I bound peptides derived from hematopoiesis specific proteins are preferential candidates for specific anti tumor reactivity after transplantation. However, not all T cell reactivity against antigens expressed on non hematopoietic tissues will lead to severe GVHD. The cellular activation state influenced by inflammatory circumstances in the non hematopoietic tissues plays a role in the ability of T cells to damage these tissues. Since HLA class-II molecules show a restricted tissue distribution, CD4 T cells reacting with antigens presented in HLA class-II may also lead to relatively specific GVT responses. T cells recognizing antigens in the context of HLA-DQ and HLA-DP have been found in patients associated with anti tumor effects with limited GVHD. However, under inflammatory circumstances upregulation of HLA class-II molecules on non-hematopoietic tissues can result in GVHD. These results illustrate that both the specificity of the T cell responses, and the inflammatory environment will determine the balance between GVHD and GVT reactivity after stem cell transplantation. Skewing the T cell response towards hematopoiesis restricted antigens by in vitro selection of T cells with defined specificity and controlling the inflammatory conditions in the patients may allow the separation of GVT from GVHD.

Speaker 23

T Cell Engineering for Immunotherapy of Cancer

Hans J. Stauss

Institute of Immunity and Transplantation, University College London, United Kingdom

Adoptive T cell transfer has been used to control cancer growth and prevent disease from viral reactivation in immunosuppressed patients. The selection of antigen-specific T cell populations with appropriate effector function remains a major bottleneck for T cell therapy. The transfer of genes encoding TCR chains and regulatory molecules provides an opportunity to reliably produce therapeutic T cells of desired specificity and function.

We have isolated HLA-A2-restricted T cell receptor (TCR) specific for Epstein-Barr Virus (EBV) and cytomegalovirus (CMV) to test their function in CD8+ T cells and also in CD4+ helper T cells. Retroviral transfer of the TCR genes generated CD4+ T cells that bound HLA-A2/peptide multimers and produced cytokines when stimulated with MHC class II-deficient cells presenting the relevant viral peptides in the context of HLA-A2. Peptide titration revealed that CD4+ T cells had a 10-fold lower avidity than CD8+ T cells expressing the same TCR. The impaired avidity of CD4+ T cells was corrected by transferring simultaneously TCR and CD8 genes. The CD8 co-receptor did not alter the cytokine signature of CD4+ T cells, which remained distinct from that of CD8+ T cells. Using the xenogeneic NOD/SCID in vivo model, we show that human CD4+ T cells expressing TCR and CD8 protected efficiently against the growth of tumours expressing the TCR-recognised EBV or CMV antigens. Together, we describe a robust approach for generating therapeutic CD4+ T cells capable of providing MHC class I-restricted immunity against MHC class II-negative tumours in vivo.

Speaker 24

TALEN-mediated editing of endogenous T-cell receptors to improve efficiency and safety of T-lymphocyte reprogramming

Belinda Berdien[1], Ulrike Mock[1], Djordje Atanackovic[2], Boris Fehse[1]

[1] Dept. of Stem Cell Transplantation, UCCH, University Medical Centre (UMC) Hamburg-Eppendorf Research Dept. Cell and Gene Therapy, Martinistraße 52, 20246 Hamburg, Germany
[2] UMC Hamburg-Eppedorf Dept. of Oncology/Haematology, Martinistraße 52, 20246 Hamburg, Germany

The use of reprogrammed T lymphocytes for adoptive immunotherapy has shown great promise in various malignant diseases. To redirect T cells, transgenic T-cell receptors (TCRs) or chimaeric antigen receptors (CARs) are introduced, in most cases using integrating (retro-)viral vectors. The presence of an endogenous TCR conferring a T cell's reactivity against a defined antigen may impair efficacy of TCR transgenesis, but also cause safety problems owing potential mispairings and/or expansion of auto-/alloreactive clones. Genome editing mediating complete shut-off of the endogenous TCR chains has been proposed to solve these problems. To this end, we designed novel TCR- and TCR--specific pairs of TAL-effector nucleases (TALENs). We also developed an optimised mRNA-electroporation protocol for effective TALEN-delivery into T lymphocytes. Based thereon we were able to knockout both TCR chains with so far unmatched efficiencies in Jurkat (TCR-: 59.7 ± 4.0%, TCR-: 37.4 ± 7.3%) as well as primary T cells (TCR-: 58.0 ± 15.0%, TCR-: 41.0 ± 17.6%).

We finally applied a successive knockout strategy for the endogenous TCR chains followed by subsequent lentiviral transduction of the respective chains of an Influenza-virus (Flu-) specific model TCR. We show that this approach led to complete editing of Jurkat as well as primary T cells with strongly improved expression and functionality of transgenic TCRs. In conclusion, we suppose that our new technique for efficient editing of endogenous TCRs may be very useful in the context of T-cell reprogramming for adoptive immunotherapy.

LEUKOMOGENESIS

Speaker 25

Tumor and tumor stem cell targeted oncolytic viruses

Christian J. Buchholz

Paul-Ehrlich-Institut, Molecular Biotechnology and Gene Therapy, 63225 Langen, Germany

Restricting delivery of oncolytic viruses and their infection and propagation to the relevant cell type is a key issue in virotherapy. Virus tropism is determined at different stages during the viral life cycle. Of these, attachment to the cell surface receptor is the primary step not only for virus infection but also for vector mediated gene delivery. Vectors with a restricted tropism, ideally highly specific just for the cell type of interest for the given therapeutic application, are expected to improve safety and efficacy. Altering receptor usage of a viral vector is a complex protein engineering task, which was first accomplished for oncolytic measles viruses (MVs).

We described a CD133-targeted oncolytic measles virus (MV) as a promising approach to specifically eliminate CD133+ cancer initiating cells (CICs). Selective entry was mediated by an engineered MV hemagglutinin (H). The H protein was blinded for its native receptors and displayed a CD133-specific single-chain antibody fragment (scFv) as targeting domain. De novo generated CD133-targeted MV (MV-141.7), was absolutely specific for CD133+ cells and showed potent oncolytic activity against glioma, hepatocellular carcinoma and colon cancer. Most interestingly, the oncolytic activity of CD133-specific viruses was substantially enhanced compared with the unmodified MV-NSe. So far differences in the density of MV receptor and CD133 as well as differences in spreading kinetics have been excluded to be causative. Ongoing efforts aim at exploring the underlying mechanism.

Recently, we have transferred the entry targeting strategy to AAV vectors by mutating the heparan-sulfate proteoglycan (HPSG) binding site in the capsid proteins and simultaneously fusing a designed ankyrin repeat proteins (DARPins) with high affinity for the tumor antigens Her2/neu or EpCAM to the VP2 capsid protein. Remarkably, a single i.v. injection of such tumor targeted AAV vectors is sufficient to track 80% of all tumor sites in a metastatic mouse model and to extend survival considerably longer than upon state-of-the art anti-cancer medication. Moreover, ongoing studies suggest that this approach enables the detection of few tumor cells in human blood.

Speaker 26

An oncolytic Rhabdovirus for the treatment of brain cancer, melanoma and prostate cancer

Dorothee von Laer[1], Alexander Muik[2], Lawton J. Stubbert[3], Roza Z. Jahedi[4], Yvonne Geiß[2], Catherine Dold[1], Reinhard Tober[1], Ursula Dietrich[2], Hrvoje Miletic[4,5], John C. Bell[3]

1 Division of Virology, Innsbruck Medical University, Innsbruck, Austria
2 Georg-Speyer-Haus, Frankfurt am Main, Germany
3 Ottawa Hospital Research Institute, Centre for Innovative Cancer Research, Ottawa, Canada
4 Department of Biomedicine, University of Bergen, Bergen, Norway
5 Department of Pathology, Haukeland University Hospital, Bergen, Norway

Background:
Oncolytic viruses destroy cancer cells specifically without harming normal tissues and are a highly promising new class of anti-cancer therapeutics. The Vesicular Stomatitis Virus (VSV) is an extremely potent oncolytic agent; however, clinical application has been limited by its devastating neurotoxicity and the rapid induction of a neutralizing antibody response. Here, we sought to exploit the many virtues of replication competent VSV as an anticancer therapeutic, while at the same time mitigating its propensity to infect and destroy normal brain cells.

Material and methods:
We removed the VSV glycoprotein G as key neurovirulence deteminant and replaced it with the arenavirus glycoprotein LCMV-GP thereby generating a replicating therapeutic, rVSV(GP). Here, we analyzed safety and efficacy of this novel virus in vitro and in vivo.

Results:
While there are no doses at which wildtype VSV (wtVSV) can be safely introduced into rodent brains, we found that our chimeric strain rVSV(GP) caused no significant neurotoxicity even at doses of 108 plaque forming units injected directly into the brains of rodents. In addition, rVSV(GP) was a much safer virus when delivered systemically compared to its parental rVSV strain. rVSV(GP), however, was significantly more potent against a spectrum of human cancer cell lines than current oncolytic virus candidates. Furthermore, it retained rVSVs potent oncolytic activity in both syngeneic and xenogeneic orthotopic human glioblastoma models as well as in a syngeneic CT26 colon carcinoma brain metastasis model. Most importantly, in contrast to wtVSV, rVSV(GP) was not inactivated by human serum complement and did not induce a neutralizing antibody response in mice. The lack of neutralizing antibody induction allowed rVSV(GP) to access and replicate within tumour tissue of pre-immunized animals. Thus, rVSV(GP) is the first oncolytic virus that has the potential to fully retain therapeutic efficacy upon repeated therapeutic application.

Conclusions:
Taken together, rVSV(GP) is an extremely promising new oncolytic virus platform, which does not show any of the major drawbacks that have limited clinical efficacy of oncolytic viruses so far.

Speaker 27

Pexa-Vec (JX-594), an Oncolytic and Immunotherapeutic Vaccinia Virus, for the Treatment of Patients with Advanced Tumors

Caroline J. Breitbach[1], James Burke1, Anne Moon[1], Tae-Ho Hwang[2], John C. Bell[1,3], David H. Kirn[1]

[1] Jennerex Inc., San Francisco, CA, USA
[2] Pusan National University, Busan, South Korea
[3] Ottawa Hospital Research Institute & University of Ottawa, Ottawa, ON, CA

Pexa-Vec (pexastimogene devacirepvec; JX-594) is a cancer-targeted oncolytic and immunotherapeutic vaccinia virus engineered to selectively replicate in and destroy cancer cells with cell cycle abnormalities and epidermal growth factor receptor (EGFR)/ras pathway activation. Direct oncolysis plus granulocyte macrophage – colony stimulating factor (GM-CSF) expression also stimulates tumor vascular shutdown and anti-tumoral immunity. Over 300 patients with advanced cancers, including hepatocellular carcinoma and colorectal cancer, have been treated by intravenous (IV) and/or intratumoral (IT) injections to date. Treatment with Pexa-Vec has been generally well-tolerated to date, with acute, transient flu-like symptoms being the most common treatment-emergent adverse events. Anti-tumoral activity has been observed on multiple Phase 1 and 2 trials in patients with hepatocellular carcinoma, renal cell carcinoma and colorectal cancer.

Speaker 28

Oncolytic Measles Virus in Combination withImmune Checkpoint Blockade

Christine E. Engeland[1], Christian Grossardt[1], Sascha Bossow[1], Ivan Shevchenko[2], Viktor Umansky[2], Dirk Jäger[3], Christof von Kalle[1], Guy Ungerechts[1,3]

1 Department of Translational Oncology, National Center for Tumor Diseases and German Cancer Research Center, Im Neuenheimer Feld 460, Heidelberg, Germany
2 German Cancer Research Center, Clincal Cooperation Unit Dermato-Oncology
3 Department of Medical Oncology, National Center for Tumor Diseases and University Hospital, Ruprecht-Karls-University, Im Neuenheimer Feld 460, Heidelberg, Germany

Phase I trials with oncolytic measles viruses (MV) based upon the live attenuated vaccine strain have been initiated with first signs of clinical efficacy and no dose-limiting toxicities. The blockade of the CTLA-4 and PD-1/PD-L1 checkpoints is a novel paradigm in immunotherapy of cancer. The recent success of these antibodies against malignant melanoma and a number of other tumor entities has been celebrated as a breakthrough in oncology. However, not all patients experience tumor remission. The combination of oncolytic virotherapy with these immunotherapeutics promises benefits, allowing for tumor reduction by direct cell lysis, tumor vaccination effects and enhanced tumor-specific T cell responses. Combination therapies including immune checkpoint modulators are leading-edge in cancer therapy. With the recent advances in the development of oncolytic viruses, the combination of virotherapy with immune checkpoint blockade is highly warranted. In a preclinical study, we have combined oncolytic virotherapy with immune checkpoint blockade. Combination treatment of oncolytic MV with antibodies against CTLA-4 and PD-L1 was studied in murine tumor models. Oncolytic efficacy was addressed in a xenograft model of human melanoma. The combination treatment was equally efficient in this model, with high rates of complete tumor remission (> 80%). Immunotherapeutic effects were assessed in a fully immunocompetent murine model of malignant melanoma. Here, therapeutic benefits in terms of tumor growth and median overall survival were observed for animals treated with MV in combination with anti-CTLA-4 and anti-PD-L1, respectively, compared to controls receiving an antibody isotype control. Functional aspects of immunomodulation were assessed by analyses of tumor-infiltrating lymphocytes. Tumors treated with MV and anti-CTLA-4 or PD-L1 showed increased levels of tumor-infiltrating CD3+ T cells and decreased levels of intratumoral FoxP3+ regulatory T cells. In tumors treated with MV and anti-PD-L1, the frequency of activated CD8+ cytotoxic T cells expressing IFN-g was also markedly increased. Furthermore we could demonstrate highly effective MV-mediated lysis of tumor cells in primary tissue from melanoma patients.

Based on the current results, we are developing a Phase I study of anti-PD-L1 antibody in combination with MV in patients with PD-L1 positive locally advanced or metastatic solid tumors.

EPIGENETICS AND CELL AGEING

Speaker 29

Epigenetic Changes in Myelodysplastic Syndrome (MDS) Focus on BCOR

Qi Cao[1], Markus Muschen[2], Sigal Gery[1], H. Phillip Koeffler[1,3,4]

[1] Cedars-Sinai Medical Center, Los Angeles, CA (USA)
[2] Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, USA
[3] Cancer Science Institute of Singapore, National University of Singapore, Singapore
[4] National University Cancer Institute, Singapore

Myelodysplastic syndromes (MDS) are a clonal hematopoietic disorder with limited therapeutic options. Our whole exome and candidate gene sequencing of 1,400 MDS samples (spearheaded by laboratories of Torsten Hafferlach and Seishi Ogawa) identified frequent driver mutations including BCOR. The gene is located on the X chromosome and 5-10% of MDS patients have nonsense mutations of the gene. BCOR is an epigenetic repressor which has not been studied in detail in MDS.

BCOR is expressed in early hematopoietic stem cells (HSC); levels decrease with progressive myeloid differentiation. Preliminary experiments using a BCOR conditional knockout (KO) cellular model suggested that the high levels of BCOR in hematopoietic stem cell is associated with their quiescence. We used the conditional BCOR KO murine model to study its role in hematopoiesis including proliferation and differentiation, as well as identifying target genes of BCOR. Studies were also done with both MDS samples having nonsense BCOR mutations, as well as silencing (shRNA) BCOR in human acute myeloid leukemia cell lines. Further studies showed that loss of BCOR had a profound effect on the polycomb complex.

Speaker 30

MicroRNAs, EMT AND CANCER STEM CELLS

Thomas Brabletz

Dept. of Visceral Surgery and Comprehensive Cancer Center, Univ. Freiburg, Germany

We have shown, that in particular tumor cells at the invasive front of common adenocarcinomas undergo an epithelial-mesenchymal transition (EMT) and aberrantly express EMT-associated transcriptional repressors, like ZEB1. The amount of such cells strongly correlates with metastasis formation and poor clinical outcome. Strikingly, metastases show again a re-differentiated phenotype, indicating a mesenchymal-epithelial re-transition (MET) and a support a regulatory role of the tumor environment for malignant tumor progression.

We described that the EMT-activator ZEB1 is a crucial promoter of metastasis and demonstrated that ZEB1 inhibits expression of cell polarity factors and the microRNA-200 family, whose members are strong inducers of epithelial differentiation. These results indicate that ZEB1 triggers a microRNA-mediated feedback-loop, which stabilizes EMT and promotes dissemination of cancer cells. Moreover we detected that in addition ZEB1 is necessary for the tumor initiating capacity of pancreatic, breast and colorectal cancer cells. ZEB1 inhibits expression of miR-200c, miR-203 and miR-183, which cooperate to suppress expression of stem cell factors, as demonstrated for the polycomb repressor Bmi1.

We propose that ZEB1 links EMT-activation and stemness-maintenance by suppressing stemness-inhibiting microRNAs and thereby is a promoter of mobile, migrating cancer stem cells. Notably, these cells also acquired a drug-resistance phenotype. Thus, targeting the ZEB1 – miR-200 feedback loop might be a promising treatment option for fatal tumors, such as pancreatic cancer.

Speaker 31

Stem Cell Aging and Rejuvenation

Maria Carolina Florian[1], Gina Marka[1], Karin Soller[1], Yi Zheng[2], Hartmut Geiger[1,2]

[1] Institute of Molecular Medicine and Stem Cell Aging, University of Ulm, Ulm, Germany
[2] Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, OH, USA

Hematopoietic stem cell (HSC) function declines upon aging and it is associated with impaired hematopoiesis in the elderly. The cellular molecular mechanisms of stem cell aging are still poorly understood, precluding rational approaches to ameliorate stem cell aging and thus tissue attrition with age. Stem cell aging is not fixed as we demonstrate a critical mechanistic role of the activity of the small RhoGTPase Cdc42 in HSC aging and identify it as a target to pharmacologically rejuvenate stem cell intrinsic age-associated phenotypes of LT-HSCs via inhibition of Cdc42 activity. Cytoplasmatic as well as nuclear polarity in young and aged LT-HSCs with respect to the proteins Cdc42, tubulin and AcH4K16 is regulated by Cdc42 activity (aged LT-HSCs are apolar). These data support a novel concept in which aging-associated changes in LT-HSC self-renewal and differentiation are regulated by changes in stem cell polarity. We also demonstrate an unexpected shift from canonical to non-canonical Wnt signalling due to elevated expression of Wnt5a in aged HSCs that causes activation of Cdc42 upon aging. Aged HSCs present with a strongly reduced level and primarily cytoplasmatic localization of -catenin in the majority of the cells. A stem cell intrinsic reduction of the aging associated elevated Wnt5a expression via a knock-down approach functionally rejuvenated aged HSCs, as the frequency of polarized chronologically aged HSCs with respect to distribution of both Cdc42 and tubulin is increased (younger), while they also show a high expression level and nuclear localization of -catenin, indicative of a reversion to active canonical Wnt signalling and an overall phenotype more similar to young HSCs. Additional novel data indicate that apolarity correlates with the mode of stem cell division, so that upon aging HSCs generate more frequently daughter cells similar to each other through a symmetric mode of division.

Speaker 32

B-cell differentiation and neoplastic transformation under the perspective of the epigenome

José I. Martín-Subero

Department of anatomic Pathology, Pharmacology and Microbiology, University of Barcelona, Spain
Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

DNA methylation is an epigenetic mark playing an important role in regulatory and developmental processes, both in the context of physiological and pathological conditions. During the last decades, this epigenetic mark has been the subject of multiple studies in the field of hematological neoplasms, being tumor suppressor gene silencing through promoter hypermethylation the most widely reported finding. The recent development of unbiased tools allowing us to quantify methylation levels throughout the genome are now modifying our perception on the role(s) of DNA methylation during cell differentiation and neoplastic transformation. In the context of the BLUEPRINT Project and the chronic lymphocytic leukemia (CLL) Genome Project, we have performed a detailed analysis of the DNA methylome during the complete B-cell differentiation process (including 10 cell subpopulations) and in a large series of CLLs. Both normal B-cell differentiation and CLL leukemogenesis involve a massive reconfiguration of the DNA methylome, affecting both functional domains such as enhancers, and apparently non-functional regions like heterochromatin and polycomb-repressed regions. The results derived from these analysis indicate that the role of DNA methylation is more nuanced than previously accepted, being its functions dependent on both genomic and chromatin context.

Speaker 33

Leukemia initiating cell formation requires myeloid differentiation

Daniel G. Tenen[1,2], *Min Ye*[2]

[1] Cancer Science Institute, National University of Singapore
[2] Harvard Stem Cell Institute, Boston, MA USA

Leukemia initiating cells (LICs) are thought to be responsible for leukemia initiation, maintenance and recurrence. Elimination of the LIC compartment is an essential and potentially sufficient therapy for leukemia. Recent evidence in solid tumors reinforce the functional role for cancer initiating cells in endogenous tumor development. In leukemia, LICs can arise from self-renewing hematopoietic stem cells (HSCs) and also from committed progenitors. However, it remains unclear how leukemia-associated oncogenes instruct LIC formation from these different cells. Using an acute myelogenous leukemia (AML) mouse model induced by the fusion oncoprotein MLL-AF9, we examined the formation of LICs derived from either hematopoietic stem cells (HSC) or more differentiated common myeloid progenitors. We demonstrated that regardless of the initiating cell type, myelomonocytic differentiation through the granulocyte macrophage progenitor (GMP) stage is critical for LIC generation, and the formation of LIC undergoes processes that recapitulate the hierarchies of normal hematopoiesis. Blocking myeloid differentiation through disrupting a lineage-restricted transcription factor eliminates both normal granulocyte formation and AML development, and restoring myeloid differentiation "rescues" AML formation. Our findings define the process of cancer cell differentiation and identify myelomonocytic specification through the GMP stage as a critical step in LIC formation. Narrowing down the specific stage through which malignant transformation occurs provides entry points for therapeutic intervention.

Speaker 34

A transgenic mouse model demonstrating the oncogenic role of mutations in the polycomb-group gene EZH2 in lymphomagenesis

Tobias Berg[1], Silvia Thoene[2], Damian Yap[3], Tracee Wee[2], Nathalie Schoeler[2], Patty Rosten[2], Emilia Lim[4], Misha Bilenky[4], Andrew J. Mungall[4], Thomas Oellerich[1], Sherry Lee[3], Courteney K Lai[2], Patricia Umlandt[4], Anisa Salmi[2], Harry Chang[2], Lisa Yue[2], David Lai[2], Grace Cheng[4], Ryan D. Morin[4], Martin Hirst[4], Hubert Serve[1], Marco A. Marra[4], Gregg B. Morin[2], Randy D. Gascoyne[5], Samuel A. Aparicio[3], R. Keith Humphries[2]

1 University of Frankfurt Department of Medicine - Hematology/Oncology, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany
2 BC Cancer Agency Terry-Fox-Laboratory, 675 10th Ave W, Vancouver V5Z 1L3, Canada
3 BC Cancer Agency Molecular Oncology, 675 10th Ave W, Vancouver V5Z 1L3, Canada
4 BC Cancer Agency Canada's Michael Smith Genome Sciences Centre, 675 10th Ave W, Vancouver 60590, Canada
5 BC Cancer Agency Centre for Lymphoid Cancer, 675 10th Ave W, Vancouver V5Z 1L3, Canada

The histone methyltransferase EZH2 is frequently mutated in germinal center-derived diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL). To further characterize these EZH2 mutations in lymphomagenesis, we generated a mouse line where EZH2Y641F is expressed from a lymphocyte-specific promoter. Spleen cells isolated from the transgenic mice displayed a global increase in tri-methylated H3K27 and showed a small, but significant increase in the fraction of germinal-center (GC) B cells (GL7+FAS+) in aged EZH2Y641F transgenic mice. There was an increase from a mean of 0.792 % in WT mice to a mean of 1.412% GL7+FAS+ cells in EZH2Y641F mice (p=0.003, n=5, age 60-62 weeks). The mice did not show an increased tendency to develop lymphoma. As EZH2 mutations often coincide with other mutations in lymphoma we combined the expression of EZH2Y641F by crossing EZH2Y641F transgenic mice with Eμ-Myc transgenic mice. In this combination we observed a dramatic acceleration of lymphoma development. The lymphomas show histological features of high-grade disease with a shift towards a more mature B cell phenotype. In-depth immunophenotyping showed that the accumulating cell type is IgDlo, CD21-, CD23- and partially expresses AA4.1. This marker combination is consistent with the transitional stage (T1) of B cell development. As demonstrated by combining RNAseq and ChIPseq, EZH2Y641F also led to gene expression and epigenetic changes involving important pathways in B cell regulation and function. We also observed an increase in the proliferation rate of splenic B cells in Eμ-Myc/EZH2Y641Fmice. While cell-cycle genes were not specifically enriched in the global analysis, there were several of these genes that were found deregulated (including e. g. CyclinD1).

In summary, EZH2Y641F can enhance the fraction of GC B cells in the spleen and collaborates with Myc to accelerate lymphomagenesis demonstrating a cooperative role of EZH2 mutations in oncogenesis. We were able to identify several key pathways that may be contributing to the acceleration of lymphoma development observed with EZH2Y641F and may also be important for the understanding of pathogenesis of EZH2 mutated lymphoma.

POSTER ABSTRACTS

M = Monday *S = Sunday* *Roman Numerals = Topic*

HETEROGENEITY OF MALIGNANCIES & METASTASIS

Poster S-I-01

Elevated levels of soluble human leukocyte antigen-E are associated with advanced disease stage and early treatment requirement in chronic lymphocytic leukemia

Bettina Wagner[1], Fabiola da Silva Nardi[1], Sabine Schramm[1], Thomas Kraemer[2], Christina Bade-Döding[2], Jan Dürig[3], Peter A. Horn[1], Ulrich Dührsen[3], Holger Nückel[3], Vera Rebmann[1]

[1] University Hospital Essen Institute for Transfusion Medicine, Virchowstr. 179, 45147 Essen, Germany
[2] MH Hannover Institute for Transfusion Medicine, Carl-Neuberg-Straße 1, 30625 Hannover, Germany
[3] University Hospital Essen Department of Hematology, Hufelandstr. 55, 45147 Essen, Germany

Soluble HLA-E (sHLA-E) molecules are able to prevent NK cell lysis suggesting a crucial role in cancer immune escape mechanisms. In this prospective study sHLA-E levels and HLA-E genotypes were analyzed in 50 chronic lymphocytic leukemia (CLL) patients and compared to 35 healthy controls. HLA-E genotyping did not differ between patients and controls. The sHLA-E levels (mean +/- SEM pg/ml) of controls (1222+/-101) were similar to levels of CLL patients with Binet stage A (1115+/-92). However, sHLA-E increased significantly (p=0.01) with higher disease stage. The 5-year-follow-up after blood sampling showed that patients with sHLA-E levels <1716 pg/ml (N=37) had a signific antly improved progression-free survival than patients with sHLA-E >1716 pg/ml (53 vs. 10 months, p=0.01, Hazard Ratio: 0.34). Serial determination of sHLA-E revealed that sHLA-E levels significantly (p=0.01, N=11) decreased after therapy from 1773 to 1039 pg/ml. This provides first evidence that HLA-E may be implicated in disease progression of CLL.

POSTER ABSTRACTS
Heterogeneity of malignancies & metastasis

Poster S-I-02

Atypical cell populations associated with acquired resistance to cytostatics and cancer stem cell features. The role of mitochondria in nuclear encapsulation

David Diaz-Carballo

Universitätsklinikum Marienhospital Onkologie, Düngelstr. 33, 44623 Herne, Germany

Until recently, acquired resistance to cytostatics has mostly been attributed to biochemical mechanisms like decreased intake and/or increased efflux of therapeutics, enhanced DNA repair, and altered activity or deregulation of target proteins. Although these mechanisms have been widely investigated, little is known about membrane barriers responsible for the chemical imperviousness of cell compartments and cellular segregation in cytostatic-treated tumors. In highly heterogeneous cross-resistant and radiorefractory cell populations selected by exposure to anticancer agents, we found a number of atypical recurrent cell types in i) tumor cell cultures of different embryonic origins, ii) mouse xenografts, and iii) paraffin sections from patient tumors. The first type, named spiral cells, is marked by a spiral arrangement of nuclei. The second type, monastery cells is characterized by prominent walls inside which daughter cells can be seen maturing amidst a rich mitochondrial environment. The third type, called pregnant cells, is a giant cell with a syncytium-like morphology, a main nucleus and many endoreplicative functional progeny cells. A rare fourth cell type identified in leukemia was christened shepherd cells, as it was always associated with clusters of smaller cells. Alongside morphologic peculiarities, these populations presented cancer stem cell (CSC) markers, aberrant signaling pathways, and a set of deregulated miRNAs known to confer both stem-cell phenotypes and highly aggressive tumor behavior. Furthermore, a portion of resistant tumor cells displayed nuclear encapsulation via mitochondrial aggregation in the nuclear perimeter in response to cytostatic insults, probably conferring imperviousness to drugs and long periods of dormancy until nuclear eclosion takes place. This phenomenon was correlated with an increase in both intracellular and intercellular mitochondrial traffic as well as the uptake of free extracellular mitochondria. All these cellular disorders could in fact be found in untreated tumor cells but were more pronounced in resistant entities, suggesting a natural mechanism of cell survival triggered by chemical injury, or a primitive strategy to ensure stemming, self-renewal and differentiation under adverse conditions, a fact that may play a significant role in chemotherapy outcomes.

Poster S-I-03

Assessment of Clonality in BcrAbl-induced Leukaemia by Genetic Barcodes

Doreen Winkelmann[1], Lars Thielecke[2], Tim Aranyossy[1], Andreas Dahl[3], Ingo Roeder[2], Ingmar Glauche[2], Boris Fehse[1], Kerstin Cornils[1]

[1] University Medical Center Hamburg-Eppendorf, Clinic for Stem Cell Transplantation Research Department Cell and Gene Therapy, Martinistr. 52, 20246 Hamburg, Germany
[2] Dresden University of Technology, Faculty of Medicine Carl Gustav Carus Institute for Medical Informatics and Biometry, Fetscherstr. 74, 01307 Dresden, Germany
[3] Dresden University of Technology, Biotechnology Center (Biotec) Deep Sequencing Group SFB 655, Fetscherstraße 105, 01307 Dresden, Germany

Introduction:
Genetic marking by integrating viral vectors is a powerful tool to investigate the fate of cells. Genetic barcodes represent an expression-independent method for tracking single clones based on the introduction of unique sequences in cell. Barcodes can be identified by PCR and allow quantification of clonal contributions by (next-generation) sequencing. Determination of clonal contribution in a population facilitates the assessment of clonal dynamics of a marked population over time. We here used this method to analyse clonal competition in a BcrAbl-transduced cell line in vitro as well as in the development of BcrAbl-induced leukemia in mice.

Methods:
We constructed a -retroviral vector encoding BcrAbl in conjunction with GFP. In this vector, our BC32-sequence flanked by sequences for Illumina-Adaptors was introduced to establish a -GFP-BC32-BcrAbl-vector plasmid library. -retroviral vectors were generated and used to transduce the murine, IL-3-dependent cell line Ba/F3. After IL-3 withdrawal, cells were cultured for 52 days; samples were taken every 2-3 days to follow the clonal dynamics in vitro. 10.000 GFP-positive cells were transplanted into female Balb/C mice without conditioning. Diseased mice were sacrificed about 20 days after transplantation and haematopoietic organs were analysed for BC32-content.

Results:
The complexity of the plasmid library was about 8 x 104 different BCs. Transduction rate of Ba/F3-cells was low (2,9% GFP-positive) corresponding to 2.900 different BCs in the initial culture. After IL-3 withdrawal, the percentage of GFP-positive increased to 60%, but remained stable at this level. GFP-positive, but also negative cells were found to contain various amounts of different BC32. Transplantation of GFP-positive cells led to overt leukaemia in six recipient mice. Investigation of the BC-sequences showed that leukaemia was monoclonal in all animals at the final stage, and that it was caused by only two different BcrAbl clones. Vector insertion-site analysis revealed integrations in the proximity of tumour suppressor genes in both clones, which might have contributed to their eventual prevalence.

Conclusion: Our -GFP-BC32-BcrAbl vector is a suitable tool to follow-up clonal competition in vitro and in vivo even in the absence of reporter-gene expression. Marking with barcoded vectors can be expected to provide novel insights in the clonal progression of malignant diseases.

POSTER ABSTRACTS
Heterogeneity of malignancies & metastasis

Poster S-I-04

On the trace of hidden cells: Application of mathematical modeling to predict the outcome of individual CML patients under TKI treatment

Tino Rothe[1], Matthias Horn[2], Andreas Hochhaus[3], Ingmar Glauc he[1], Ingo Röder[1]

[1] TU Dresden Medizinische Fakultät Carl Gustav Carus Institut für Medizinische Informatik und Biometrie, Fetscherstr. 74, 01307 Dresden, Germany
[2] Universität Leipzig Institut für Medizinische Informatik, Statistik und Epidemiologie, Härtelstraße 16-18, 04107 Leipzig, Germany
[3] Universitätsklinikum Jena Klinik für Innere Medizin II, Bachstraße 18, 07743 Jena, Germany

Introduction:
Treatment of CML patient with Tyrosine-Kinase-Inhibitors (TKI, such as Imatinib) induces a characteristic biphasic decline in tumor load, quantified by BCR-ABL transcript levels in peripheral blood cells. This decline is qualitatively similar for most patients, however a distinct patient-specific heterogeneity of the response dynamics remains. Although many patients reach complete molecular remission, a residual disease is retained in the majority of patients, which appears causative for rapid relapse upon treatment cessation. Robust estimation of residual disease level for individual patients is a major challenge.

Methods:
We use computer simulations to estimate residual leukemic stem cell levels based on the patients treatment response in peripheral blood. Therefore, we apply a bi-exponential regression model (Stein et.al. 2013) to estimate four characteristic parameters for each patient's time course (gradients and intercepts of both declines). We use statistical and dynamical modeling to obtain an optimal mapping of these statistical parameters onto a set of model parameters that simulates the individual response of a given patient.

Results:
We identified several parameters of the simulation model that can account for the observed patient heterogeneity. It appears that the cytotoxicity of the TKI as well as the drug's influence on the balance between leukaemic stem cell activation and quiescence predominantly influence the individual patient response. We furthermore investigate whether differences within the normal, non-leukaemic stem cell population are suited to explain inter-patient heterogeneity. We report on our results to efficiently map the statistical parameters describing the patient's response to TKI treatment onto a set of dynamic model parameters that recapitulates this behavior in silico.

Conclusions:
Computer simulations of individual patient responses under TKI treatment are a promising tool to predict residual disease levels in CML. They allow quantitative risk estimations for combination therapies or treatments cessations.

References:

Stein et al.: Rapid initial decline in BCR-ABL1 is associated with superior responses to second-line nilotinib in patients with chronic-phase chronic myeloid leukemia. BMC Cancer 2013 13:173.

Poster S-I-05

Cooperativity of RUNX1 and CSF3R mutations in the development of leukemia in severe congenital neutropenia: a unique pathway in myeloid leukemogenesis

Julia Skokowa[1], *Doris Steinmann*[2], *Karl Welte*[1]

[1] Julia Skokowa Molecular Hematopoiesis, MHH, Carl-Neuberg Str. 1, 30625 Hannover, Germany
[2] Doris Steinemann Institute of Cell and Molecular Pathology, MHH, Carl-Neuberg Str. 1, 30625 Hannover, Germany
[3] Karl Welte Molecular Hematopoiesis, MHH, Carl-Neuberg Str. 1, 30625 Hannover, Germany

Severe congenital neutropenia (CN) is a pre-leukemic bone marrow failure syndrome with a 20% risk of evolving into leukemia or MDS. Patterns of acquisition of leukemia-associated mutations were investigated using next-generation deep-sequencing in 31 CN patients who developed leukemia or MDS. Twenty (64.5%) of the 31 patients had mutations in RUNX1. A majority of patients with RUNX1 mutations (80.5%) also had acquired CSF3R mutations. In contrast to their high frequency in CN patients who developed leukemia or MDS, RUNX1 mutations were found in only 9 of 307 (2.9%) patients with de novo pediatric AML. A sequential analysis at stages prior to overt leukemia revealed RUNX1 mutations to be late events in leukemic transformation. Single-cell analyses in two patients showed that RUNX1 and CSF3R mutations were present in the same malignant clone. Functional studies demonstrated elevated G-CSF-induced proliferation with diminished myeloid differentiation of hematopoietic CD34+ cells co-expressing mutated forms of RUNX1 and CSF3R. The high frequency of cooperating RUNX1 and CSF3R mutations in CN patients suggests a novel molecular pathway of leukemogenesis: mutations in the hematopoietic cytokine receptor (GCSFR) in combination with the second mutations in the downstream hematopoietic transcription fator (RUNX1). The detection of both RUNX1 and CSF3R mutations could be used as a marker for identifying CN patients with a high risk of progressing to leukemia or MDS.

POSTER ABSTRACTS
Heterogeneity of malignancies & metastasis

Poster S-I-06

A scarce subgroup of cells in patient-derived ALL xenografts is drug-resistant, low-cycling and self-renewing

Catarina Castro-Alves[1], *Sebastian Tiedt*[1], *Irmela Jeremias*[1,2]

[1] Helmholtz Zentrum München Gene Vectors - Group Apoptosis, Marchioninistraße 25, 81377 München, Germany
[2] Dr von Haunersches Kinderspital, Ludwig Maximilians Universität Department of Oncology, München, Germany

The prognosis of patients with acute lymphoblastic leukemia (ALL) depends on the ability of chemotherapy to effectively eliminate ALL cells. Unfortunately in many patients, a subgroup of ALL cells is treatment resistant ultimately inducing relapse. To improve treatment outcome, a better understanding and targeting of drug-resistant ALL cells is required.

The aim of this work was to characterize treatment-resistant cells derived from patients with ALL.

Towards this aim, we used the individualized xenograft mouse model of ALL and developed a method to isolate minimal numbers of patient-derived xenograft (PDX) cells from mouse bone marrow. Thereby, ALL PDX cells were lentivirally transduced to express NGFR, a red fluorochrome and luciferase. Luciferase enabled in vivo imaging. MACS selection targeting NGFR, followed by FACS sorting targeting the red fluorochrome, enabled cell enrichment of more than factor 10.000 with minor cell losses. In order to address the proliferative behavior of cells, they were labeled with CFSE. This revealed a rare subpopulation of in vivo long-term label-retaining cells (LRCs) in all three ALL samples tested. Re-transplantation of LRCs into secondary recipients showed that these LRCs could convert into non-LRCs, i.e. rapid proliferating cells, and vice-versa, suggesting that non-cell autonomous mechanisms are at least partly responsible for the growth behavior of ALL cells in vivo.

LRCs and non-LRCs had the same leukemia-initiating cell frequencies, in contrast to what we initially hypothesized. Furthermore, in vivo treatment with cytotoxic drugs led to an overall reduction in tumor burden but analysis of the two sorted populations showed that LRCs were highly resistant to chemotherapeutic drugs in contrast to non-LRCs. However, when LRCs and non-LRCs were isolated and submitted to drug treatment in vitro, both subpopulations were sensitive to treatment, which suggests again a role for external factors in determining the response of leukemia cells to drugs in vivo (e.g. protection conferred by the niche).

In summary, genetic engineering of ALL xenograft cells enabled developing a very sensitive enrichment and isolation procedure. We identified a small subpopulation of in vivo long-term resting and drug resistant cells in patient-derived ALL cells. These cells might be important targets for therapy and should be considered when evaluating novel therapeutic approaches to treat ALL.

Poster S-I-07

Efficient RGB-marking of mouse models of metastasizing colorectal and liver tumours

Pierre Abramowski[1], Daniel Wicklein[2], Olga Kraus [3], Kristoffer Riecken[1], Udo Schumacher[2], Hartmut Schlüter[3], Boris Fehse[1]

[1] University Medical Centre Hamburg-Eppendorf, Oncological Centre, Clinic for Stem Cell Transplantation Research Department Cell and Gene Therapy, Martinistr. 52, 20246 Hamburg, Germany
[2] University Medical Centre Hamburg-Eppendorf Department of Anatomy and Experimental Morphology, Martinistr. 52, 20246 Hamburg, Germany
[3] University Medical Centre Hamburg-Eppendorf Mass Spectrometric Proteomics, Institute of Clinical Chemistry, Martinistr. 52, 20246 Hamburg, Germany

High morbidity and mortality associated with cancer constitute a challenging problem and emphasize the necessity to develop novel treatment strategies. Metastatic outgrowth represents the most lethal but yet not sufficiently understood facet of cancer. The identification of markers differentially expressed on the surface of parental and metastatic cancer cells may allow to specifically target metastatic cells and to advance anti-tumour therapies. We focus on one frequent tumour entity with systemic dissemination, namely colorectal cancer, in a mouse model.

Red-green-blue (RGB)-marking allows tracking of cells endowed with metastatic properties. In the colorectal cancer model, the human cell line HT29 was transduced with lentiviral vectors encoding mCherry, Venus, or Cerulean. Different combinations of inserted vectors led to individual cell marking by the generation of numerous colours. Transduced cells were selected by puromycin. 1x106 RGB-labelled cells were transplanted s.c. into each recipient Pfp/Rag2-/- mouse. After 4 weeks blood and bone marrow were analyzed by flow cytometry. Primary tumour tissues and lungs were prepared for fluorescence microscopy and histology. Single-cell sorting was used to generate clones of cancer cells derived from the primary tumours as well as metastases. For protein expression analysis, ex-vivo expanded cancer cells were subjected to 2D-gelelectrophoresis.

RGB-labelled HT29 cells reliably gave rise to tumours in Pfp/Rag2-/- mice. Fluorescent protein-expressing cells were found in the blood and bone marrow of affected mice. Flow cytometry and fluorescence microscopy point to a polyclonal composition of primary tumours. In contrast, cancer cells from lungs appeared to be of a rather oligoclonal origin.

RGB-labelled cells found in primary tumours and lungs indicate systemic tumour dissemination with a tendency of declining polyclonality as cancer progresses, i.e. from primary tumour engraftment to its metastatic spread to distant organs. To define mechanistic properties necessary for metastatic engraftment both primary as well as lung-derived cancer cells will be subjected to mass spectrometry. Moreover, clonality of metastases will be quantified by genetic barcoding of HT29 cells prior to transplantation into recipient mice.

POSTER ABSTRACTS
Heterogeneity of malignancies & metastasis

Poster S-I-08

Generation of in vivo murine models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing

Dirk Heckl[1,2], Monika Kowalczyk[3], David Yudovich[2], Rishi Puram[2], Marie McConkey[2], Aviv Regev[3]

[1] Hannover Medical School Experimental Hematology, Carl-Neuberg-Str.1, 30625 Hannover, Germany
[2] Brigham&Women's Hostpital Hematology, 1 Blackfan Circle, Boston, MA 02115, United States
[3] Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, United States

Recent genome sequencing efforts have demonstrated that human malignancies commonly bear mutations in four or more driver genes.1 Murine models of these recurrent mutations have yielded insights into the biology and treatment of cancer, but it has not yet been possible to engineer primary cells that recapitulate the genetic complexity of human disease. Mice bearing combinations of three or more genetic lesions are exponentially more difficult to generate through breeding. Recent advances in genome editing technologies now offer the potential to overcome these limitations.2-4 Using a lentiviral small guide RNA (sgRNA):Cas9 delivery system we modified murine hematopoietic stem cells (HSCs) in vivo at up to five genomic loci, leading to clonal outgrowth and myeloid malignancy. Myeloid malignancy triggered by genome editing presented with 3-5 modified genes and up to 10 loci. Lentivirus-delivered sgRNA:Cas9 genome editing is a powerful tool for the engineering of in vivo cancer models appropriately reflecting the complexity of human disease.

References

1. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. The New England journal of medicine 368, 2059-2074 (2013).
2. Jinek, M. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337, 816-821 (2012).
3. Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819-823 (2013).
4. Mali, P. et al. RNA-guided human genome engineering via Cas9. Science 339, 823-826 (2013).

Poster S-I-09

RGB-Marking for multi-color clonal cell tracking in stem cells and tumors

Kristoffer Riecken[1], Diego Gomez-Nicola[2], V. Hugh Perry[2], Boris Fehse[1]

[1] UMC Hamburg-Eppendorf Res. Dept. Cell and Gene Therapy, Martinistrasse 52, 20246 Hamburg, Germany
[2] University of Southampton Centre for Biological Sciences, Tremona Road, Southampton, SO16 6YD, Great Britain

Transplantation of stem or tumor cells represents a standard technique in regenerative medicine and cancer research. Stable cell marking with genetically encoded fluorescent proteins is a convenient method to track transplanted cells in vivo. Specific marking of single cells with unique labels rather than whole tissues with the same label represents a promising strategy to follow-up the fate of individual cells and their progeny.

RGB marking developed in our lab is a technology that allows to stably stain individual cells with a characteristic color hue. Using this method we have shown that essentially all perceivable colors can be generated, and that clonal colors remain stable over time, in all daughter cells, even upon (re)transplantation. The RGB color mixing principle is broadly known from computer or TV screens. Here, RGB colors originate from mixing three fluorescent proteins in the basic colors red, green and blue (RGB) at different but very constant amounts in individual cells, simultaneously transduced by three lentiviral LeGO vectors. Individually marked clones can be tracked to assess the behavior of cells in different situations, e.g. loss of clonality during tumor progression, clonality of metastases, or numbers of engrafted stem cells within a transplant.

We have now established novel vectors and methods to further improve applicability of RGB marking. LeGO vectors with drug-selectable fluorescent markers allow for convenient protocols to obtain pure, RGB-marked cell populations in a short time frame. We also explored different vector types, utilized various promoters and incorporated novel fluorescent proteins to identify optimal RGB-marking strategies for distinct experimental settings. We were able to drastically reduce the time needed for image acquisition of RGB-marked cells, all colors of RGB marking become directly visible at the oculars of the microscope. With this "RGB marking live", pictures and even movies of RGB-marked, moving cells become possible. Similarly, time-lapse imaging enables in-vitro cell tracking over prolonged periods of time. Finally we investigated the possibility to apply RGB vectors in vivo to stain cells in situ. This approach allowed us to follow newly generated cells and track their migration without needing transgenic animals or ex-vivo handling of (stem) cells.

In conclusion we have established further strategies of RGB marking for various applications in vitro and in vivo.

STEM CELLS IN HOMEOSTASIS AND DISEASE

Poster M-II-01

Cytochrome P450 and drug metabolism in the stem cell niche

Salvador Alfonso, Meng Su, Rick Jones, Gabriel Ghiaur

Johns Hopkins University ONcology, 1650 Orleans St, Baltimore, MD 21287, United States

Although it is clear that the bone marrow (BM) microenvironment or niche regulates normal and leukemia hematopoietic stem cells (HSCs) homeostasis, there is limited knowledge detailing the mechanisms involved. We recently found that the BM niche protects human HSCs from the pro-differentiation effects of retinoic acid (RA) via expression of CYP26, a cytochrome P450 (CYP), RA-metabolizing enzyme1. We found that unopposed RA signaling resulted in HSC loss, while inhibition of RA signaling expanded SCID-repopulating cells (SRCs). More so, BM stroma and not human HSCs, expressed CYP26. While BM stroma maintained human SRCs in culture, inhibition of stroma CYP26 resulted in complete loss of SRCs. Even though the majority of AML blasts are sensitive to RA in vitro, RA based therapies have no clinical impact on AML other than acute promyelocytic leukemia2. Thus, we hypothesized that differences between in vitro and in vivo sensitivity to RA may be explained by the BM niche's expression of CYP26. Using a variety of AML cell lines (NB4, HL60, KG1, OCI-AML3, Kasumi) and leukemia stem cells (LSCs) from patients, we show that BM stroma prevented RA-induced differentiation, which could be overcome by inhibition of stromal CYP26. Emerging data shows that the stem cell niche is a protective environment for LSCs3, but the processes responsible for this protection are unclear. Although CYP enzymes have been implicated as systemic mechanisms of drug resistance (via hepatic inactivation), their role within the BM microenvironment has never been studied. Using a qPCR array platform, we find that BM-derived stroma cells express most CYP drug metabolizing enzymes. We further find that CYP3A4, which is thought to inactivate more than 50% of all chemotherapeutics, is expressed by BM stroma but not normal or malignant stem cells. Using shRNA mediated knockdown or pharmacological inhibitors, we find that stroma-based resistance of AML and multiple myeloma cells to etoposide and bortezomib (two classical substrates of CYP3A4), respectively, depends on CYP3A4 activity. Taken together, our studies uncover a novel mechanism of microenvironment-mediated drug resistance and provide further proof that the BM niche may be a drug-free sanctuary for malignant cells. Targeting stromal drug-metabolizing enzymes holds promise for eliminating minimal residual disease and improving outcome in AML and other hematologic malignancies.

References

1 - Ghiaur G et al. PNAS 2013
2 - Petrie K et al. Curr Opinion Hem 2009
3 - Krause DS et al. Cytometry B Clin Cytom 2013

Poster M-II-02

HIF prolyl hydroxylase 2 (PHD2) is a critical regulator of hematopoietic stem cell maintenance during steady-state and stress

Rashim Pal Singh

Medical faculty TUD Department of Clinical Pathobiochemistry, Fetscherstraße 74, Dresden 01307, Germany

Hypoxia is a prominent feature in the maintenance of hematopoietic stem cell (HSC) quiescence and multipotency. Hypoxia-inducible factor (HIF) prolyl hydroxylase domain proteins (PHDs) serve as oxygen sensors and may therefore regulate this system. Here, we describe a mouse line with conditional loss of HIF prolyl hydroxylase 2 (PHD2) in very early hematopoietic precursors that results in self-renewal of multipotent progenitors under steady-state conditions in a HIF1- and SMAD7-dependent manner. Competitive bone marrow (BM) transplantations show decreased peripheral and central chimerism of PHD2-deficient cells but not of the most primitive progenitors. Conversely, in whole BM transfer, PHD2-deficient HSCs replenish the entire hematopoietic system and display an enhanced self-renewal capacity reliant on HIF1. Taken together, our results demonstrate that loss of PHD2 controls the maintenance of the HSC compartment under physiological conditions and causes the outcompetition of PHD2-deficient hematopoietic cells by their wild-type counterparts during stress while promoting the self-renewal of very early hematopoietic progenitors.

References

Blood June 27, 2013 vol. 121 no. 26 5158-5166

POSTER ABSTRACTS
Stem cells in homeostasis and disease

Poster M-II-03

Sustained chronic Epo exposure induces hematopoetic progenitors

Marta Murray

Medical Techincum TUD Institute of Pathology, Fiedlerstrssase 27, 01307 Dresden, Germany

Recombinant human erythropoetin (Epo) has been used for treating patients with anemia of numerous causes, including chronic kidney failure, infections and gastrointestinal disease. Previously, certain cancer patients received erythropoiesis-stimulating agents that eventually led to significantly worse overall survival. It is becoming increasingly clear that Epo has pleiotropic effects, inside and outside the hematopoietic system. Hence, it is imperative to better understand the ill-defined role of Epo on the primitive hematopoietic system. Utilizing a transgenic mouse model (Epo-Tg6), we studied detailed effects of sustained Epo exposure on the hematopoietic system of the mouse. The absolute amount of CD34+ hematopoietic stem cells (homeostatic HSCs) and multipotent progenitors (MPP2-4) are significantly induced in mature Epo transgenic mice (Epo-Tg6) compared to their WT littermates. We show that this induction is directly related to inhibition of apoptosis of these cells. Moreover, this feature of Epo is unique from its known proliferation and differentiation activity on erythroid progenitors such as megakaryocyte erythrocyte progenitors.

Poster M-II-04

Alterations in mesenchymal stromal precursor cells from the bone marrow of the acute myeloid and lymphoid leukemia patients: newly diagnosed, before and after allogeneic hematopoietic stem cell transplantation

Irina Shipounova[1], Natalia Petinaty[1], Ekaterina Zezina[2], Alexey Bigildeev[1], Nina Drize[1], Tamara Sorokina[3], Larisa Kuzmina[3], Elena Parovichnikova[3], Valery Savchenko[4]

[1] National Research Hematological Center Physiology of Hematopoiesis Lab, Novy Zykovsky Pr.,4, 125167 Moscow, Russia
[2] Lomonosov Moscow State University Faculty of Biology, sub department of Immunology, Vorob'evy gory 1, Moscow, Russia
[3] National Research Hematological Center High Dose chemotherapy, Hematopoiesis Depressions and Bone Marrow Transplantation Department, Novy Zykovsky Pr., 4, 125167 Moscow, Russia
[4] National Research Hematological Center Director, Novy Zykovsky Pr.,4, 125167 Moscow, Russia

Hematopiesis is maintained in close contact with bone marrow (BM) stroma. In leukemia patients malignant hematopoietic cells as well as high dose chemotherapy probably affect the hematopoietic microenvironment. The goal of this study was to analyze the alterations in human multipotent mesenchymal stromal cells (MSC) and fibroblast colony forming units (CFU-F), derived from the BM of acute myeloid and lymphoid leukemia (AML and ALL) patients.

17 newly diagnosed and 35 patients with allogeneic hematopoietic stem cell transplantation (alloHSCT) were involved in the study after informed consent. BM was aspirated at the day of diagnosis, before the conditioning and during 1 year after the alloHSCT. MSC and CFU-F were cultured in standard conditions. The relative expression level (REL) of genes was measured by RT2 Profiler PCR Array and TaqMan qRT-PCR. MSC and CFU-F from 50 donors of BM for alloHSCT were used as a control.

MSC production and CFU-F concentration in the BM of all patients at the moment of diagnosis were decreased. Before alloHSCT these parameters did not differ from donors but after alloHSCT decreased significantly during the next year. Gene expression analysis revealed that in MSC at the moment of AML diagnosis the REL of FGF2, IL1B, IL6, JAG1, PDGFB, VCAM1, VEGFA decreased more than 2 fold. Prior to alloHSCT the REL of IL6 and IL1B were still very low, after the alloHSCT the REL of IL6, JAG1, PDGFB increased, IL1B stayed at the low level at least for 6 months. In MSC at the moment of ALL diagnosis the REL of IFNG, IGF1, IL6, KITLG, NES, PTPRC, TBX5 decreased more than 5 fold, while REL of CSF3, ICAM1, IL1B, ITGAX – increased more than 5 fold. REL of IL6, IDO1, and were elevated prior to alloHSTC and stayed at such level for a year. Prior to alloHSCT the REL of FGFR2, PDGFRA, SDF1 and FGF2 were very low, after the alloHSCT the REL of JAG1, FGF2, TGFB1 slightly increased, while FGFR2, PDGFRB, SDF1 stayed low for 1 year

During the leukemia development malignant cells alter MSC leading to the decrease in their proliferative ability, in the REL of many regulatory genes and in the number of CFU-F in the BM. Chemotherapy used for remission induction did not affect stromal precursor cells. Conditioning regiments used for the alloHSCT significantly damage both types of studied stromal precursors, and the effect lasted at least for 1 year. So, both leukemic cells and chemotherapy affect BM hematopoietic microenvironment.

POSTER ABSTRACTS
Stem cells in homeostasis and disease

Poster M-II-05

Interleukin-1 beta improves the ability of multipotent mesenchymal stromal cells to maintain hematopoietic precursor cells by up-regulation of genes encoding adhesion molecules and cytokines

Alexey Bigildeev[1], Ekaterina Zezina[2], Irina Shipounova[1], Dmitry Kostushev[3], Nina Drize[1]

[1] National Research Hematological Center Physiology of Hematopoiesis laboratory, Noviy Zikovskiy proezd, 125167 Moscow, Russia
[2] Lomonosov Moscow State University Biology department, sub department Molecular immunology, Leninskie gory, 1, 125167 Moscow, Russia
[3] I.M. Sechenov First Moscow State Medical University Pharmaceutical faculty, Bolshaya Pirogovskaya st. 2-4, 119991 Moscow, Russia

The interactions of stromal microenvironment and hematopoietic stem cells within bone marrow (BM) niches play a pivotal role in the maintenance of stable hematopoiesis. Multipotent mesenchymal stromal cells (MSCs) have been shown to maintain hematopoietic progenitor cells (HPCs). Thus MSCs can be used as a model to study the interactions between stromal cells and HPCs. It was shown previously that Interleukin 1 beta (IL-1 beta) stimulates the ability of MSCs to maintain HPCs in vitro.

The aim of this study was to investigate the mechanism of IL-1 beta effect on the ability of MSCs to support HPCs in vitro.

MSCs were isolated from the BM of 26 donors (13 males and 13 females) ranging in age from 18 to 56 years (median: 32). The samples were collected after informed consent during aspiration of BM for allogeneic transplantation. MSCs were cultivated in standard conditions in aMEM supplemented with 10% fetal bovine serum ±4 pg/ml IL-1 beta, and maintenance of HPCs on MSC layers was estimated using cobblestone area forming cell (CAFC 1-2wk) assay. The gene expression was analyzed in MSCs from 5 donors using RT2 Profiler PCR Array (Qiagen). The expression of several genes was evaluated on larger cohort of donors (n=26) by means of qRT-PCR.

Treatment of MSCs with IL-1 beta resulted in enhanced ability of these cells to maintain HPCs. The frequency of CAFC 1-2wk increased in 1.57±0.13 fold ($p<0.01$). Screening gene expression analysis revealed that the genes encoding adhesion molecules (ITGB1, ITGA6, ITGAX, JAG1 and ICAM1), growth factors (LIF, IGF1, IL1B, IL6, TGFB1, TGFB3), membrane-bound molecules (CD-13, -90, -105, -73) and differentiation markers (BMP-2, -6, -9, -14, COL1A, SOX9, BGLAP, PPARG) were up-regulated in treated with IL-1 beta MSCs.

The results of screening were partially confirmed by qRT-PCR: the expression level of ICAM1 (3.08±0.84, $p=0.01$), IL1B (7.9±2.6, $p=0.01$) and IL6 (2.0±0.3) was up-regulated, while JAG1 (0.74±0.4, $p=0.03$) was down-regulated and no differences were detected in IGF1, SOX9, BGLAP, PPARG in IL1 beta treated MSCs. Further verification of screening results is necessary. We also revealed that SDF1 was down-regulated (0.75±0.1, $p=0.05$) and FGF2 was up-regulated, although not statistically significant in IL-1 beta treated MSCs.

IL-1 beta enchances the ability of MSCs to maintain HPCs in vitro, and this effect could be explained by modulation of the expression of genes encoding adhesion molecules and cytokines.

Poster M-II-06

Activating c-KIT mutations confer oncogenic cooperativity and rescue RUNX1/ETO-induced DNA-damage and apoptosis in human primary CD34+ hematopoietic progenitors

Christian Wichmann[1,2], Isabell Quagliano-Lo Coco[1], Linping Chen-Wichmann[2], Andrea Kinner[1], Christian Brandts[3], Reinhard Henschler[2], Manuel Grez[1]

[1] Georg-Speyer-Haus, Paul-Ehrlich-Strasse 42, 60596 Frankfurt, Germany
[2] Ludwig Maximilian University Hospital, AöR Department of Transfusion Medicine, Cell Therapeutics and Hemostaseology, Max-Lebsche Platz 32, 81377 Munich, Germany
[3] Universitätsklinikum Frankfurt Med. Klinik II – Hämatologie/Onkologie, Theodor-Stern-Kai 7, 60595 Frankfurt, Germany

The RUNX1/ETO (RE) fusion protein, which originates from the t(8;21) chromosomal rearrangement, is one of the most frequent translocation products found in de novo acute myeloid leukemia (AML). In RE leukemias, activated forms of the c-KIT tyrosine kinase receptor are frequently found, thereby suggesting oncogenic cooperativity between these oncoproteins in the development and maintenance of t(8;21) malignancies. In this report, we show that activated c-KIT cooperates with RE to expand human CD34+ hematopoietic progenitors ex vivo. CD34+ cells expressing both oncogenes resemble the AML-M2 myeloblastic cell phenotype, in contrast to RE-expressing cells which largely undergo granulocytic differentiation. Oncogenic c-KIT amplifies RE-depended clonogenic growth and protects cells from exhaustion. Activated c-KIT reverts RE-induced DNA-damage and apoptosis. In the presence of activated c-KIT, RE-downregulated DNA-repair genes are re-expressed leading to an enhancement of DNA-repair efficiency via homologous recombination. Together, our results provide new mechanistic insight into RE and c-KIT oncogenic cooperativity and suggest that augmented DNA-repair accounts for the increased chemoresistance observed in t(8;21)-positive AML patients with activated c-KIT mutations. This cell protective mechanism might represent a new therapeutic target, as RE cells with activated c-KIT are highly sensitive to pharmacological inhibitors of DNA-repair.

POSTER ABSTRACTS
Stem cells in homeostasis and disease

Poster M-II-07

PU.1 cooperates with IRF4 and IRF8 to suppress pre-B cell leukemia

Sebastian Carotta[1,2], Swee Pang[1], Stephen Nutt[1]

[1] WEHI Molecular Medicine, 1G Royal Pde, Melbourne 3052, Australia
[2] Boehringer Ingelheim RCV Oncology, Dr Boerhinger Strasse, 1120 Wien, Austria

Content:
The Ets family transcription factor PU.1 and the interferon regulatory factor (IRF)4 and IRF8 regulate gene expression by binding to composite DNA sequences known as Ets/interferon consensus elements (EICE). While all three factors are expressed from the onset of B cell development, single deficiency of these factors in B cell progenitors only mildly impacts on bone marrow B-lymphopoiesis. Here we tested whether PU.1 cooperates with IRF factors in regulating early B cell development. Lack of PU.1 and IRF4 resulted in a block in development at the pre-B cell stage. The combined deletion of PU.1 and IRF8 reduced recirculating B cell numbers. Strikingly however, all PU.1/IRF4 and a high proportion of PU.1/IRF8 deficient mice developed pre-B cell acute lymphoblastic leukemia (B-ALL) associated with reduced expression of the established B-lineage tumor suppressor genes Ikaros, Spi-B and BLNK. These genes are directly regulated by PU.1/IRF4/IRF8 and restoration of Ikaros or Spi-B expression inhibited leukemic cell growth. In summary, we demonstrate that PU.1, IRF4 and IRF8 cooperate to regulate early B cell development and to prevent pre-B-ALL formation.

Poster M-II-08

Assessment of the functional activity of the bone marrow early progenitor cells of patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors

Denys Bilko[1], Ilona Zhaleiko[1], Iryna Dyagil[2], Nadja Bilko hoch[1]

[1] Zhaleiko Ilona CMCR. NaUKMA, [2] Skovorody str. Building [2], office 106, 04070 Kiev, Ukraine
[2] Dyagil Iryna Radiation oncohematology and transplantation, Prospekt Pobedy 119/112, 03115 Kiev, Ukraine
[3] Bilko Nadija CMCR. NaUKMA, [2] Skovorody str. Building [2], office 106, 04070 Kiev, Ukraine

Leukemic SC in chronic myeloid leukemia (LSC) originate from HSC as a result of t9;22 chromosomal translocation. At this stage HSC and LSC are nearly indistinguishable by function, morphology or phenotype, while numbers of more differentiated LSC progenies compared to HSC are slightly altered. Drugs for targeted therapy of CML are tyrosine kinase inhibitors (TKI), imatinib and nilotinib. It is believed that these drugs are effective only for rapidly proliferating cells, while LSC are less prone to TKI and could be the cause of relapse after the end of the therapy. The aim of our study was investigation of functional activity of LSC and precursor cells during the therapy with TKI in search of possible prognostic factors.

Methods:
52 patients treated with TKI were studied. Clinical and hematological parameters and percentage of Ph+ metaphases in bone marrow cells were used to confirm CML and success of TKI therapy. Depending on the response to TKI, patients were divided into 3 groups: thouse getting hydroxycarbamide as alternative therapy, patients with optimum response to treatment (no Ph+ cells) and patients with suboptimal response with incomplete molecular response.

Results:
Analysis of the functional activity of HSC and progenitor cells of patients with CML in CFU assay in vitro showed same average CFU-GM in patients with no response to TKI and patients who received carboxy carbamide. In patients with optimal response to nilotynib, the number of colonies was two times lower, compared to patients treated with imatinib. Analysis of the colonies indicated, that for patients with an optimal response to nilotinib, numbers of differentiated cells (blasts, promyelocytes, myelocytes and metamyelocytes) were significantly lower, compared to patients who had optimal response to imatinib. This is probably due to the fact that nilotinib eliminates LSC and early progenitor cells in CML. A relationship was shown between the SI, which is calculated at diagnosis and numbers of colonies in CFU assay in vitro for patients treated with TKI during dynamic observations. Higher SI value correlated with greater numbers of colonies in culture. Dependence between SI and numbers of clusters have also been identified

Conclusions:
Results suggests important prognostic criteria based on CFU assay in vitro, which are based on changes in early progenitors in CML. Analysis of function of LSC and progenitors may allow revelation of mechanisms of resistance and disease progression.

References

Diachenko M. Dyagil I, Perechrestenko T, Bilko N., Bone marrow progenitor cells functional activity and proliferation potential in CML patients undergoing TKI treatment. European applied Sciences Journal #1/1-2013., pp 9-13.

Poster M-II-09

Identification of a prognostic gene expression signature for aza response in mds and cmml patients

Ashwin Unnikrishnan[1], Dominik Beck[1], Arjun Verma[1], Magnus Tobiasson[2], Austin Kulasekararaj[3], Sally Galbraith[4], Jake Olilvier[4], Susan Wilson[4], Andrea Pellagatti[5], Jacqueline Boultwood[5], Moritz Gerstung[6], Elli Papaemmanuil[6], Peter Campbell[6], Robert Lindeman[7], Kevin Lynch[8], Ghulam Mufti[3], Eva Hellstrom-Lindberg[2], Jason Wong[1], John Pimanda[1,7]

[1] Lowy Cancer Research Centre UNSW Australia Prince of Wales Clinical School, Sydney 2052, Australia
[2] Karolinska Institutet Department of Medicine, Center for Hematology and Regenerative Medicine, Stockholm, Sweden
[3] King's College London School of Medicine Department of Haematological Medicine, London, Great Britain
[4] UNSW Australia School of Mathematics and Statistics, Sydney 2052, Australia
[5] University of Oxford Nuffield Department of Clinical Laboratory Sciences, Oxford, Great Britain
[6] Wellcome Trust Genome Campus Wellcome Trust Sanger Institute, Hinxton, Great Britain
[7] Prince of Wales Hospital Haematology Department, High Street, Randwick 2031, Australia
[8] Celgene Corporation, North Ryde Australia

Myelodysplastic syndrome (MDS) and chronic myelomonocytic leukaemia (CMML) are haematological disorders that develop in haematopoietic stem or progenitor cells (HSPCs) and are characterised by ineffective haematopoiesis. 5'-Azacitidine (AZA) is a DNA demethylating agent that is effective in treating MDS and CMML. However, response rates are less than 50% and the basis for poor response is currently unknown. A patient's potential to respond cannot be currently determined until after multiple cycles of AZA treatment and alternative treatment options for poor responders are limited. To address these fundamental questions, we enrolled patients on a compassionate access program prior to the listing of AZA on the pharmaceuticals benefit scheme in Australia. We have collected bone marrow from 18 patients (10 MDS, 8 CMML) at seven different stages of treatment, starting from before treatment until after six cycles of AZA treatment, and isolated high-purity HSPCs at each stage. 10 of these patients (5 MDS and 5 CMML) responded well to AZA while 8 were poorer responders. We performed next-generation sequencing (RNA-seq) of these HSPCs in an effort to identify the basis of poor response to AZA therapy. Analysis of the RNA-seq data from pre-treatment HSPCs has strikingly revealed the differential expression of 1148 genes between good and poor responders to future AZA therapy. Using a Fluidigm nanofluidic system, we have validated the differential expression of the top 92 genes within this set, both in our cohort and in pre-treatment CD34+ cells from two independent cohorts totalling 67 AZA-treated patients, from the U.K. and Sweden. Using an independent dataset of MDS patients with CD34+ gene expression and long-term survival, we have validated that our gene signature does not simply segregate patients based on disease severity or poor overall survival. We have subsequently further narrowed our prognostic gene signature down to 13 genes. Our findings have immediate clinical utility to prospectively identify best responders AZA therapy. Given that the detection of gene mutations is easier to perform in a diagnostic laboratory, we are investigating correlatory links between our gene signature and established driver mutations. In addition, pathway analyses of the differentially expressed genes indicates that the HSPCs of poor responders have decreased cell cycle progression and increased signalling through integrin and mTOR/AKT pathways. The discovery of these deregulated pathways in poor responders offers the potential for future combination therapies targeting these pathways together with AZA to effect good response in poor responders to AZA monotherapy.

Poster M-II-10

Unravelling the subclonal architecture of adult B-ALL at a single cell level

Fabian Lang[1], Bartosch Wojcik[1,2], Susanne Badura[1], Anja Vogel[1], Tamra Tesanovic[1], Martin Ruthardt[1], Oliver G Ottmann[1], Michael A Rieger[2]

[1] Goethe-University Hospital Frankfurt Department for Hematology/Oncology, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany
[2] Goethe-University Hospital Frankfurt LOEWE Center for Cell and Gene Therapy Frankfurt and Department for Hematology/Oncology, Theodor-Stern Kai 7, 60590 Frankfurt/M, Germany

The existence of a leukemic hierarchy and a stable phenotype of leukemia initiating (stem) cells (LICs) in acute lymphoblastic leukemia (ALL) remain controversial[1]. Despite the proven subclonal composition[2] of the disease, correlations of functionally defined subclones and their molecular composition, that may be unrelated to their marker phenotype, require stable maintenance of cellular identity in culture systems. Since cell lines do not represent the heterogeneity of the disease and there is a difficulty to culture primary ALL patient material, solving questions regarding plasticity of marker expression and a leukemic stem-cell driven hierarchy are clearly hampered.

We established a patient-derived cell culture system[3] with a stable karyotype for 6 months to isolate distinct clonal subpopulations from the same patient. Within these patient-derived ALL cultures, we identified single cell clones, showing both stable and plastic surface marker profiles. Some subclones are highly leukemogenic in immunocompromised NSG mice, and others, showing the same prospective phenotype, have low or no leukemogenic potential in vivo. In depth molecular (quantitative proteomics and sequencing) and functional characterization (videomicroscopy-based cell tracking) and their correlation will shed light on the clonal architecture, genealogy and plasticity of adult ALL. Further, new surface markers and potential drug targets are defined for diagnosis and therapy of aggressive ALL subpopulations with high leukemogenic potential facilitating rational development of strategies targeting LICs to overcome treatment resistance and relapse in adult ALL.

References

1. Cobaldea C, Sanchez-Garcia. B-cell acute lymphoblastic leukaemia: towards understanding its cellular origin. Bioessays. 2009 Jun;31(6):600-9.
2. Anderson K1, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, Kempski H, Moorman AV, Titley I, Swansbury J, Kearney L, Enver T, Greaves M. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011 Jan 20;469(7330):356-61. Epub 2010 Dec 15.
3. Nijmejer BA, Szuhai K, Goselink HM, van Schie ML, van der Nurg, de Jong D, Marijt EW, Ottmann OG, Willemze R, Falkenburg JH. Long-term culture of primary human lymphoblastic leukemia cells in the absence of serum or hematopoietic growth factors. Exp Hematol. 2009 Mar;37(3):376-85. Epub 2009 Jan 9.

POSTER ABSTRACTS
Stem cells in homeostasis and disease

Poster M-II-11

The critical role of Runx1 in myelopoiesis and leukemogenesis

Kira Behrens, Birte Niebuhr, Ursula Müller, Carol Stocking

Heinrich-Pette-Institute Retroviral Pathogenesis, Martinistrasse 52, 20251 Hamburg, Germany

The Runx1 transcription factor is among the most frequently mutated genes in myeloid leukemia. Strikingly, >20% of all AML patients harbor mutations within the RUNX1 gene locus. Nevertheless the precise function of Runx1 in myelopoiesis has not been fully elucidated and only a few target genes have been identified. To further dissect the role of Runx1 in myeloid differentiation, we examined the effect of Runx1-deficiency or over-expression in granulocyte-monocyte-progenitors (GMP). Similar to the LSK compartment, the GMP population is greatly expanded in Runx1 KO bone marrow as compared to wildtype C57BL/6 controls. Strikingly, in vitro assays demonstrated impaired differentiation towards both the G and M lineage in Runx1 KO, with little or no mature forms observed after one week. We conclude that in the absence of Runx1, myeloid differentiation is restrained but not blocked, and may therefore partly account for the increased levels of myeloid progenitors observed in vivo.

To identify Runx1 target genes gene, expression analysis of Runx1 wildtype and KO GMPs as well as primary KO GMPs genetically engineered to overexpress RUNX1 was performed. A total of 36 reciprocally regulated genes were identified, many of which encode adhesion factors important for retaining HSPC in the bone marrow niche. Genome wide DNA-binding analysis confirmed Runx1 binding to regulatory regions of target genes and furthermore revealed cooperative binding of Runx1 with several other TFs implicated in complex networks regulating self-renewal and differentiation of early hematopoietic progenitors. Nevertheless, Runx1 deficiency did not influence transcription of all Runx1 bound genes, potentially reflecting its diverse functions in synergy with other TFs, ranging for a pivotal recruiting factor to a minor scaffold protein.

To analyze whether the impairment of myeloid differentiation induced by Runx1 deficiency together with a strong proliferation signal is sufficient to induce leukemia in vivo, FLT3-ITD was introduced into Runx1 KO bone marrow. Interestingly recipient mice developed a myeloproliferative neoplasia, whereas the reconstitution of Runx1 in this system led to an aggressive leukemia. We postulate that Runx1 inactivation induces a pre-leukemic state, most likely due to the accumulation of early myeloid progenitors with impaired differentiation potential, and that further mutations in synergy with Runx1 complete the block in differentiation and/or allow survival of the aberrant blasts.

Poster M-II-12

Skirmish in the Stem Cell Niche: Competitive Interactions of Tumor Cells for Limited Space

Astrid Beerlage, Konstanze Geiger, Jan Zieger, Gerd Klein

University medical clinic Tübingen Section for Transplantation Immunology and Immunohematology, Center for Medical Research, Waldhörnlestraße 22, Tübingen 72074, Germany

Introduction:
Breast and prostate cancer preferentially metastasize to the bone. Previous studies in mice strongly suggested that tumor cells can bind to similar structures in the bone marrow as hematopoietic stem cells do [1]. In our in vitro studies we try to answer the question whether different tumor cells are able to actively displace hematopoietic stem cells from cellular or extracellular components of the human endosteal stem cell niche.

Methods:
Adhesion forces of tumor cells and hematopoietic stem cells to different extracellular matrix components and cell-cell-interaction forces were measured by single cell force spectroscopy. Maximum detachment force between the cells and the substrates were determined. To measure competitive cell-cell adhesion, co-incubation and dislodgment assays were performed. The following cell lines were studied: the prostate cancer cell line PC3, the breast cancer cell line MDA-MB-231 and the CD 34+ cell line KG1a as a surrogate for hematopoietic progenitor cells. CD34+ CD38- hematopoietic stem and progenitor cells were isolated from umbilical cord blood. For the analysis of niche-related cells bone marrow-derived mesenchymal stem cells, the stromal cell line HS-5 and the osteoblastic Cal72 cell line were used.

Results: The strongest adhesion forces to extracellular matrix components were measured for the laminin isoforms LM-511 and LM-521 and for collagen type IV, three typical extracellular matrix components in the niche. A preferential adhesion of KG1a cells was found for fibronectin, whereas a stronger binding of the prostate and breast cancer cells was observed for netrin-1. Adhesion of the PC3 cells to the osteoblastic CAL72 cells was significantly weaker than adhesion of KG1a or MDA-MB-231 cells to CAL72. Adhesion of CD-34+CD38- to CAL72 was comparable to PC3 adhesion. Neither MDA-MB-231 nor PC3 were able to actively dislodge KG1a from their binding to Cal-72. Co-incubation of MDA-MB-231 or PC3 showed a slight decrease of KG1a adhesion.

Discussion:
The observed cell-matrix interactions were significantly stronger than the analyzed cell-cell interactions. This suggests a higher impact of integrin-mediated matrix adhesion compared to the adhesion forces mediated by cell surface adhesion molecules. Our studies support the hypothesis that tumor cells might occupy vacant places in the hematopoietic stem cell niche rather than actively dislodge hematopoietic stem cells from their places within the niche.

References

[1] Shiozawa et al., J. Clin. Invest. 121:1298-312, 2011

TARGETED THERAPIES – WHAT IS THE RIGHT CELL, WHAT IS THE BEST MOLECULE?

Poster S-III-01

A novel role of RUNX1 in the response to radiation and chemotherapy

Daniel Speidel[1,2], Melissa Abas[1]

[1] Children's Medical Research Institute, 214 Hawkesbury Road, Westmead 2145, Australia
[2] University of Sydney Sydney Medical School, Sydney 2006, Australia

We describe a novel mode of regulation and function of RUNX1 that appears important in the context of radiation and chemotherapy and may explain the association between RUNX1 mutations and chemotherapy resistance.

The transcription factor RUNX1 has a well documented and crucial role in blood cell differentiation processes and hematopoietic stem cell renewal. Its function is also frequently impaired in leukemia and MDS, and this has clearly been linked to the development of the disease.

In addition to contributing to cancer development recent clinical studies with AML, ALL and MDS patients have revealed a strong association between RUNX1 mutations and chemotherapy resistance/ poor prognosis. The molecular mechanisms underlying this relationship are not understood.

Here we show that RUNX1 expression is strongly upregulated by several (but not all) genotoxic agents, including ionizing radiation and cytarabine, in vivo and in vitro. Moreover, high intracellular RUNX1 levels confer a strong growth disadvantage and induce apoptosis in a variety of cell lines. Thus, RUNX1 contributes directly to the DNA damage response of hematopoietic cells and restricts their growth. Significantly, a mutated version of RUNX1 with a defective RUNT domain – the region in which almost all patient-derived missense mutations are located – is also attenuated in its apoptotic and growth restricting function.

Our data suggest that RUNX1 plays an important but so far unrecognized direct role in the response of normal and malignant hemaotopoietic cells to cytotoxic therapy. The findings provide an explanation for the link between RUNX1 mutations and chemotherapy resistance but may also help to design therapies that overcome mutant RUNX1 related resistance.

Poster S-III-02

Targeting of AML1-ETO fusion gene activates both pro-apoptotic and proliferation signaling in acute leukemia cells

Pavel Spirin[1], Timofey Lebedev[1], Natalia Orlova[1], Anton Buzdin[2], Carol Stocking[3], Vladimir Prassolov[1]

[1] Engelhardt Institute of Molecular Biology Russian Academy of Sciences Cell Biology, Vavilova, 119991 Moscow, Russia
[2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry group for genomic regulation of cell signaling systems, Miklukho-Maklaya, 117997 Moscow, Russia
[3] The Heinrich Pette Institute, Leibniz Institute for Experimental Virology Retroviral pathogenesis, Martinistraße, 20251 Hamburg, Germany

The t(8;21)(q22;q22) rearrangement represents the most common chromosomal translocation in acute myeloid leukemia (AML). It results in a transcript encoding for the fusion protein AML1-ETO (AE) with transcription factor activity. AE is considered to be an attractive target for treating t(8;21) leukemia. However, AE expression alone is insufficient to cause transformation, and thus the potential of such therapy remains unclear. Several genes are deregulated in AML cells, including KIT, which encodes a tyrosine kinase receptor. Previously we showed that AML cells transduced with shRNA vector targeting AE (shAE) mRNAs have a dramatic decrease in growth rate, which is caused by induction of apoptosis and deregulation of the cell cycle. A reduction in KIT mRNA levels was also observed in AE silenced cells, but silencing KIT expression reduced cell growth but did not induce apoptosis. Here we performed transcription profiling of cells that escape cell death and revealed activation of a number of signaling pathways involved in cell survival and proliferation. We examined 68 regulatory pathways most frequently associated with cancer. Among the 68 cancer-associated pathways, thirty-seven are known to have a positive impact on cell survival and proliferation; fifteen, a negative impact; and the remaining sixteen are neutral or ambiguous. We identified that in shAE leukemic cells the 40 of 68 (59%) cancer-associated pathways were upregulated and only 3 of 68 (4%) were downregulated compared to the control shSCR cells. Our data demonstrates that most of these pathways are implicated in survival of leukemic cells after AE repression. Among the positive proliferation and survival regulator pathways (promitotic pathways), 78% were upregulated. However, for the negative proliferation and survival regulator pathways (proapoptotic pathways), only 5/15 (33%) were upregulated, 2/15 (13%) were downregulated and the remaining 54% were intact. This clearly indicates that the persistent shAE cells have an enhanced proportion of intracellular regulatory pathways promoting cell survival and proliferation. We provide clues that the inhibition of AE protein or gene alone may be insufficient to treat the leukemic cells. Also our data suggests that the ERK2 (MAPK1) protein could mediate activation of 23 out of 29 (79%) of these upregulated pathways and thus may be regarded as the key player in establishing the t(8;21)-positive leukemic cells resistant to AE suppression.

POSTER ABSTRACTS
Targeted therapies – what is the right cell, what is the best molecule?

Poster S-III-03

Following the progression of LMO2-mediated T cell leukaemia

Katia Ruggero[1], Helio Pais[1], Osama Al-Assar[1], Jennifer Chambers[1], Victoria Weston[2], Pamela Kearns[2], Terence Rabbitts[1]

[1] University of Oxford Weatherall Institute of Molecular Medicine, Oxford, OX3 9DS, Great Britain;
[2] Birmingham University School of Cancer Sciences, Institute for Biomedical Research West, Birmingham, Great Britain

T-cell acute leukemias are frequently caused by chromosomal translocations involving oncogenic transcription factors. LMO2 typifies this class of molecule and is recurrent ectopically expressed in T-ALL. We are interested in the discovery of molecular perturbations that drive oncogenesis in LMO2-directed T-ALL, from cancer initiating cell to overt disease. In order to identify LMO2-induced genes that arise in the cancer initiating cells, we exploited a mouse model transgenic for LMO2 expression in the T cell compartment, that represent a faithful model for human T-ALL. Mice ectopically expressing LMO2 in the thymus developed T-ALL after a long asymptomatic pre-leukaemic phase, likely after the acquisition of one or more secondary mutations. Our mouse model thus provides the opportunity to study the initiating phase of leukaemogenesis for which there is no detectable stage in humans. The asymptomatic phase in the LMO2-transgenic mouse is characterized by a T cell differentiation blockade. Transgenic animals show an early accumulation of double negative (DN) T cells in the thymus at the DN2/3 stage. These cells posses self-renewal properties and are considered the leukaemia initiating cells in this T-ALL model.

Using Next Generation Deep Sequencing (RNA-seq) we analyzed gene expression changes in pre-leukemic DN1, 2, 3 & 4 thymic cells of LMO2-transgenic and wild-type mice. Bioinformatics analysis of sequencing data revealed genes differentially upregulated and downregualted in DN subpopulations in the LMO2-transgenic mice. In parallel, we have developed a database for interrogating the mRNA spectrum encoding cell surface proteins (the so-called surfaceome) and employed this to identify new markers of early T cell neoplasia and potential new therapeutic targets aberrantly expressed in LMO2 neoplasias. Gene expression perturbations observed in mouse pre-leukemic thymic cells were compared to the RNA-seq gene profiling obtained for mouse leukemia arising in the LMO2 transgenic mice, as well as in human T-ALLs harbouring LMO2 translocation. This comprehensive analysis allowed us to identify surface proteins whose expression is aberrant in the LMO2-dependent transgenic model and in human T-ALL with LMO2 chromosomal translocations, as well as new potential therapeutic targets expressed at early stage in the LMO2-induced cancer initiating cells that may provide a novel and effective way to eradicate the disease associated with cancer initiating cells.

Poster S-III-04

The Hedgehog pathway mediator GLI represents a negative prognostic marker in acute myeloid leukemia and its inhibition mediates anti-leukemic effects in vitro

Jasmin Wellbrock[1], Emily Latuske[1], Julian Köhler[1], Katharina Wagner[2], Hauke Stamm[1], Eik Vettorazzi[3], Gabi Vohwinkel[1], Marianne Klokow[4], Roswitha Kuhling-Thees[2], Sonja Loges[1], Michael Amling[4], Manfred Jücker[5], Carsten Bokemeyer[1], Michael Heuser[2], Jürgen Krauter[2], Walter Fiedler[1]

1 University Medical Center Hamburg-Eppendorf Department of Hematology and Oncology, Martinistrasse 52, 20246 Hamburg, Germany
2 Hannover Medical School Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
3 University Medical Center Hamburg-Eppendorf Department of Medical Biometry and Epidemiology, Martinistrasse 52, 20246 Hamburg, Germany
4 University Medical Center Hamburg-Eppendorf Department of Osteology and Biomechanics, Martinistrasse 52, 20246 Hamburg, Germany
5 University Medical Center Hamburg-Eppendorf Center for Experimental Medicine, Institute of Biochemistry and Signal Transduction, Martinistrasse 52, 20246 Hamburg, Germany

Leukemic stem cells depend on signals provided by bone marrow (BM) niche cells. Due to its function in stem cell biology, we investigated expression and prognostic impact of the Hedgehog pathway in acute myeloid leukemia (AML).
Pre-treatment samples from 104 patients with newly diagnosed AML (AMLSG 07-04 trial) were analyzed by quantitative PCR. Expression of receptors SMO and PTCH1 and downstream transcription factors GLI1, GLI2 and GLI3 was found in 69%, 41%, 73%, 20% and 26% of cases, respectively. However, no expression of Hedgehog ligands was observed. GLI2 expression had a significant influence on event-free, relapse-free and overall survival (p=0.037, p=0.026 and p=0.013, respectively). We could verify our findings using microarray-based gene expression data of a second, independent patient cohort published by Verhaak et al (n=290 pts; Haematologica 2009; 94). The negative prognostic impact of GLI2 expression on overall and event-free survival could be confirmed (p=0.003 for overall and p=0.007 for event-free survival, respectively; no data available for relapse-free survival). Furthermore, within this cohort, also GLI1 had a negative prognostic impact (p<0.001 for both event-free and overall survival). Although Hedgehog ligands were not expressed in AML bone marrow aspirates, Desert Hedgehog (DHH) plasma levels were significantly increased in AML patients compared to healthy subjects (p=0.002). Provision of Desert Hedgehog could be ascribed to BM niche cells including endothelial cells which showed elevated DHH expression levels compared to the whole cell BM compartment. Next, we investigated the effect of GLI inhibition in vitro using the specific GLI1/2 inhibitor GANT61. GANT61 mediated significant anti-leukemic effects on several AML cell lines as well as freshly isolated primary AML cells (n=10 pts) as shown by reduced proliferation and colony-formation capacities and induction of apoptosis. These observations could be confirmed via targeted knockdown of GLI2 and GLI1 in UKE-1 and OCI-AML5 cells using shRNA contructs. In both cell lines, the GLI knockdown resulted in reduction of cell growth and induction of apoptosis. In conclusion, GLI expression is a negative prognostic factor in AML that mirrors activated Hedgehog signaling induced by paracrine stimulation through BM niche cells. Inhibition of Hedgehog signaling by blocking GLI activity might represent a promising therapeutic approach for AML treatment.

POSTER ABSTRACTS
Targeted therapies – what is the right cell, what is the best molecule?

Poster S-III-05

Prevention of Minimal Residual Disease in Ph+ ALL

Iris Appelmann[1], Cory D. Rillahan[1], Elisa de Stanchina[2], Gregory Carbonetti[2], Chong Chen[1], Scott W. Lowe[3], Charles J. Sherr[4]

1 Memorial Sloan Kettering Cancer Center Cancer Biology and Genetics Program, 415 East 68th Street, New York, New York 10065, United States
2 Memorial Sloan Kettering Cancer Center Antitumor Assessment Core Facility, 415 East 68th Street, New York, New York 10065, United States
3 HHMI, Memorial Sloan Kettering Cancer Center Cancer Biology and Genetics Program, 415 East 68th Street, New York, New York 10065, United States
4 HHMI, St. Jude Children's Research Hospital Department of Tumor Cell Biology, 262 Danny Thomas Place, Memphis, Tennessee 38105, United States

Aggressive Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) that genetically and phenotypically mimics the human disease can be induced by the introduction of cultured BCR-ABL(p185)-expressing Arf-null pre/pro-B cells into healthy, unconditioned syngeneic mice. Only 20 polyclonal donor cells are sufficient to induce lethal ALL within 30 days of their IV administration, indicating that BCR-ABL expression and Arf inactivation are sufficient to guarantee leukemogenesis in healthy recipient animals. Leukemic mice enter transient remission in response to treatment with potent second generation tyrosine kinase inhibitors (TKI) such as dasatinib (SprycelTM). However, like human patients with Ph+ ALL, the continuously treated animals ultimately relapse with the emergence of leukemic clones containing clinically relevant BCR-ABL mutations, the nature of which depends upon the intensity of TKI treatment. Premature withdrawal of dasatinib when animals are in remission results in re-emergence of leukemia; surprisingly, leukemic B cells recovered from these animals lack BCR-ABL mutations and remain sensitive to dasatinib ex vivo. Hence, minimal residual disease depends upon salutary signaling within the hematopoietic microenvironment. In agreement, the response to TKI therapy can be significantly improved by abrogating cytokine signaling through a knockdown of the common gamma chain of the cytokine receptor. Administration of the Janus kinase (JAK) inhibitor ruxolitinib (Jakafi™) mimics this response. Although ruxolitinib demonstrated no anti-leukemic activity of its own, the overall survival of leukemic mice inoculated with 200,000 p185+ Arf-/- pre/pro-B cells was significantly extended after administration of a targeted combination therapy of ruxolitinib and dasatinib in comparison to mice treated with dasatinib alone. Addition of dexamethasone further reduced the leukemic burden, prevented CNS relapse, and led to prolonged survival. This implicates prevention of minimal residual disease and relapse by a non-toxic combination of targeted treatments. These studies have provided a rationale for a Phase I/II clinical trial employing these agents, particularly in older patients who are ineligible for bone marrow transplantation or do not tolerate cytotoxic chemotherapy.

Poster S-III-06

A mechanistic rationale for targeting the unfolded protein response in pre-B acute lymphoblastic leukemia

Behzad Kharabi Masouleh[1,2], Huimin Geng[1], Christian Hurtz[1], Lai N. Chan[1], Aaron C. Logan[3], Mi Sook Chang[4], Chuanxin Huang[5], S Swaminathan[1], Haibo Sun[6], Valeria Cazzaniga[1,7], G. Cazzaniga[7], Elisabeth Paietta[8], Ari Melnick[5], H.P. Koeffler[6], Markus Müschen[1,3]

1 University of California San Francisco Department of Laboratory Medicine, San Francisco, CA United States
2 RWTH Aachen University Medical School 3Department of Oncology, Hematology and Stem Cell Transplantation, Aachen, Germany
3 University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, United States
4 Children's Hospital Los Angeles, Los Angeles, United States
5 Weill Cornell Medical College Departments of Medicine and Pharmacology, New York, United States; 6Cedars Sinai Medical Center, Los Angeles, United States
7 Università di Milano-Bicocca, Ospedale S. Gerardo Centro Ricerca Tettamanti Clinica Pediatrica, Monza, Italy
8 Albert Einstein College of Medicine, New York, United States

BACKGROUND: The unfolded protein response (UPR), a stress-induced pathway emanating from the endoplasmic reticulum (ER), regulates expression and activity of molecules including HSPA5, ERN1, PRDM1 and XBP1. These molecules are required for terminal differentiation of B cells into plasma cells and highly expressed in plasma cell-derived multiple myeloma.

RESULTS: We report the unexpected finding that their promoters are hypermethylated and not expressed in normal pre-B cells, whereas pre-B cell acute lymphoblastic leukemia (ALL) cells express them at very high levels. XBP1 expression was most striking and linked to BCR-ABL1 kinase activity and transcriptionally activated by STAT5, ERK and AKT and repressed by BACH2 and BCL6. Furthermore, high expression of XBP1 predicted poor overall (OS) and relapse-free survival (RFS) in two high risk pre-B ALL clinical trials (COG P9906 and ECOG E2993). A multivariate analysis showed that high expression of XBP1 is an independent predictor of poor outcome regardless of established risk factors such as white blood cell count (WBC) and minimal residual disease (MRD) (COG P9906). In addition, high levels of XBP1 also correlated with a positive MRD status. To study the function of UPR molecules in physiological pre-B and malignantly transformed pre-B ALL cells, we developed genetic mouse models for inducible, Cre-mediated deletion of Hspa5, Prdm1 and Xbp1 which consistently induced cellular stress and cell death in normal pre-B cells and in pre-B cell acute lymphoblastic leukemia (ALL) driven by BCR-ABL1 and NRASG12D. Furthermore, Xbp1-deletion resulted in cellular senescence and cell cycle arrest owing to increased levels of p53, p21 and Arf. Similarly, in an in vivo setting, Xbp1-deletion substantially prolonged survival of NOD-SCID transplant recipient mice (n=7; p=0.007). Mechanistically, deletion of Xbp1 leads to increased expression of the pro-apoptotic molecule and stress MAP kinases p38 and JNK.

Clinical relevance: To investigate the potential clinical relevance of these findings, we used recently identified small-molecule inhibitors for XBP1 activation1,2. Small-molecule inhibition of XBP1 activation overall mimicked the genetic findings inducing cell death in multiple patient-derived pre-B ALL cases and caused reduction of leukemia-initiating cells in patient-derived pre-B ALL cells in vivo and significantly prolonged survival of leukemia-bearing NOD-SCI mice.

Conclusions: Collectively, these studies reveal that pre-B ALL cells are uniquely vulnerable to ER stress and identify the UPR pathway and its downstream effector XBP1 as novel therapeutic targets to overcome drug-resistance in pre-B ALL.

References

1. Papandreou I et al. (2011) Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. Blood 117:1311–1314.
2. Kriss CL et al. (2012) Overexpression of TCL1 activates the endoplasmic reticulum stress response: a novel mechanism of leukemic progression in mice. Blood 120:1027–1038.

POSTER ABSTRACTS
Targeted therapies – what is the right cell, what is the best molecule?

Poster S-III-07

Predicting the unseen - Optimization of CML treatment using mathematical models

Ingmar Glauche[1], Matthias Horn[2], Tino Rothe[1], Martin Müller[3], Andreas Hochhaus[4], Markus Loeffler[2], Ingo Roeder[1]

1 Technische Universität Dresden Institute for Medical Informatics and Biometry, Dresden, Germany ;
2 University of Leipzig Institute for Medical Informatics, Statistics and Epidemiology, Leipzig, Germany ;
3 Universitätsklinikum Mannheim, Mannheim, Germany ;
4 Universitätsklinikum Jena Klinik für Innere Medizin II, Jena, Germany

Content
Molecular response to tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) is characterized by a biphasic decline of BCR-ABL transcript levels. Although qualitatively similar for most patients, there is a considerable patient-specific heterogeneity in treatment response. For the decision whether or not treatment cessation is a save option for a particular patient, the number of residual leukemic stem cells (LSC) is one of the most important parameters. Because this number is clinically unobservable, we suggest mathematical modeling and computer simulation to predict the stem cell dynamics. Whereas on one hand we apply the modeling approach to assess overall treatment benefits for a potential combination of TKIs with stem cell activating drugs such as Interferon-a, we on the other hand adapt the model to individual patient data to estimate the patient-specific risk of molecular relapse upon treatment cessation. Parameter estimation and validation was performed using long-term follow-up data from different clinical trials on Imatinib (IM) therapy ("IRIS", "CML IV"). Our model predicts that a subset of patients (14%) can achieve complete eradication of LSCs within less than 15 years of IM treatment. Furthermore, the model prognosticates that 31% of the patients will remain in deep molecular remission following treatment stop after a fixed period of two years in MR(5.0), while 69% are expected to relapse. Instead of a applying such a fixed rule we propose to use model-predicted residual LSC numbers to assess the patient-specific risk of molecular relapse upon treatment discontinuation. Application of the suggested rule for deciding about the time point of treatment cessation is predicted to result in a significant reduction in rate of molecular relapse. The proposed model-based procedure allows for a better appraisal of individual prognosis of CML patients with direct impact on clinical decision-making.

Poster S-III-08

Antigenic modulation limits the key effector cell mechanisms employed by Type I anti-CD20 mAb

Tom Tipton, Stephen Beers, Mark Cragg

LLR Antibody and Vaccine Group, University of Southampton, Southampton, SO16 6YD, Great Britain

Following the success of rituximab in the treatment of B cell malignancies, two other anti-CD20 monoclonal antibodies (mAb) ofatumumab and obinutuzumab are now entering clinical use. Ofatumumab has an enhanced capacity for complement dependent cytotoxicity (CDC) whereas obinutuzumab, a type II mAb, lacking the ability to redistribute into lipid rafts, is glycoengineered for augmented antibody dependent cellular-cytotoxicity (ADCC). Through the use of gamma chain knockout mice, FcR-mediated effector mechanisms have been shown to be critical for effective anti-CD20 therapy with both type I and II mAb. However, we recently showed that type I mAb such as rituximab have a propensity to undergo rapid antigenic modulation, whereby antibody and antigen are internalised by the target cell in an FcRIIb facilitated fashion. Here we assessed the key FcR effector mechanisms affected, comparing type I and II antibodies of various isotypes in ADCC and antibody dependent cellular phagocytosis (ADCP) assays using both mouse and human systems. Rituximab and ofatumumab depleted hCD20 B-cells in the mouse less effectively than glyco-engineered and WT forms of obinutuzumab, particularly when human IgG1 (hIgG1) mAb were compared. Interestingly, hIgG1 mAb were ineffective in ADCC assays with mouse effectors, whereas ADCP was equivalent for m2a and hIgG1 isotypes. However, rituximab and ofatumumab's ability to elicit both ADCC and ADCP was greatly impaired by antigenic modulation, whereas type II antibodies remained unaffected. These data demonstrate that both ADCP and ADCC are severely impaired by antigenic modulation and that ADCP is the main effector mechanism employed by the mouse in vivo. This work provides support for the use of obinutuzumab over rituximab or ofatumumab in the treatment of B cell malignancies and that future anti CD20 therapeutics should focus on augmenting ADCP rather than ADCC.

POSTER ABSTRACTS
Targeted therapies – what is the right cell, what is the best molecule?

Poster S-III-09

Defining the role of AKT isoforms in regulating oncogene-mediated transformation of hematopoietic cells

Stefan Horn[1], Florian Ewald[2], Nicole Grabinski[2], Boris Fehse[1], Manfred Jücker[2]

[1] Clinic for Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf Research Dept. Cell and Gene Therapy, Martinistrasse 52, 20246 Hamburg, Germany
[2] University Medical Center Hamburg-Eppendorf Dept. of Biochemistry and Signal Transduction, Martinistrasse 52, 20246 Hamburg, Germany

Human leukemogenesis is characterized by the initiation and evolution of aberrant cell clones which undergo unregulated growth and resist cell death as well as immune control mechanisms.

The serine/threonine kinase AKT/proteinkinase B is involved in the regulation of several important cellular processes that include glucose metabolism, growth, proliferation, survival and cell migration. AKT constitutes a family of three conserved isoforms (AKT1, AKT2 and AKT3) sharing a high degree of structural and sequence homologies and functional overlap. Dysregulation of the phosphoinositide 3-kinase (PI3K)-AKT pathway is commonly found in solid tumors as well as hematological neoplasia. In this study we determined the relevance of AKT isoforms for in vitro transformation and growth-factor independent proliferation and survival of hematopoietic cells.

We demonstrate that all three AKT isoforms were strongly activated in Ba/F3 cells expressing activating point mutations in the catalytic subunit of class IA PI3K (E542K or H1047R). Silencing of single AKT isoforms by isoform-specific shRNAs resulted in reduced levels of phospho-AKT in conjunction with the subsequent dephosphorylation of AKT downstream targets, including GSK-3, mTOR and FoxO3a. Whereas cells expressing mutant PI3K were highly sensitive to MK-2206, an inhibitor that targets all three AKT isoforms, down-regulation of AKT1 and AKT2, but not AKT3 strongly inhibited factor-independent proliferation and cell survival. Furthermore, only the knockdown of AKT1 and AKT2 led to an altered cell cycle regulation upon growth-factor withdrawal. In agreement with these results, AKT1 or AKT2 silencing specifically reduced the potential of oncogenic PI3K mutants and of the leukemia-associated neurotrophic tyrosine kinase receptor type 1 mutant TrkA to confer factor-independent growth to Ba/F3 as well as FDC-P1 cells by at least 1 log whereas knockdown of AKT3 only slightly affected hematopoietic cell transformation.

Our data show that AKT is critically involved in oncogene-mediated anti-apoptotic and mitogenic signalling in hematopoietic cells and that AKT isoforms have differential roles as positive regulators in hematopoietic cell transformation. Defining the relevance of AKT isoforms as potential drivers of uncontrolled leukemic growth should facilitate the identification of optimal therapeutic strategies for the treatment of hematological malignancies.

Poster S-III-10

Hyperactivation of mTORC1 and mTORC2 by multiple oncogenic events causes addiction to cap-dependent mRNA translation in T-cell leukemia

Adrian Schwarzer[1], Helmut Holtmann[2], Johannes Zuber[3], Doris Steinemann[4], Brigitte Schlegelberger[4], Zhixiong Li[1], Axel Schambach[1], Christopher Baum[1]

[1] Hannover Medical School Institute of Experimental Hematology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2] Hannover Medical School Institute of Biochemistry, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[3] Research Institute of Molecular Pathology (IMP), Dr. von Bohr Gasse 7, 1030 Vienna, Austria
[4] Hannover Medical School Institute of Cell and Molecular Pathology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

High activation of the PI3K-AKT-mTOR pathway is characteristic for T-cell acute lymphoblastic leukemia (T-ALL). The activity of the master regulator of this pathway, PTEN, is often impaired in T-ALL. However, experimental evidence suggests that input from receptor tyrosine kinases (RTKs) is required for sustained mTOR activation, even in the absence of PTEN. We previously reported the expression of Neurotrophin receptor tyrosine kinases (TRKs) and their ligands in human T-ALL samples. Here we aimed to dissect the downstream signaling cascades of TRK-induced T-ALL in a murine model and show that T-ALLs induced by deregulated receptor tyrosine kinase signaling acquire activating mutations in Notch1 and lose PTEN and the homeodomain transcription factor Cux1 during clonal evolution. These events cooperated to induce a gradual shift from MAPK signaling towards hyperactivation of the PI3K-mTOR pathway. We dissected the role of both mTOR complexes and found that the depletion of mTORC1 or mTORC2 reduced the growth of blasts, but was not sufficient to induce apoptosis. In contrast, knockdown of the mTOR downstream effector eIF4E caused a striking cytotoxic effect, demonstrating critical addiction to cap-dependent translation. Although high mTORC2-AKT activation is commonly associated with drug-resistance, we demonstrate that a strong mTORC2-AKT activation generates a specific susceptibility to 4EGI-1, an inhibitor of cap-dependent translation. To decipher the mechanism of 4EGI-1, we performed a genome-wide analysis of mRNAs that are translationally regulated by 4EGI-1 in T-ALL. 4EGI-1 reduced the ribosomal occupancy of mRNAs involved in translation, mitochondria and cell cycle progression, such as Cyclins and ribosomal proteins. Polysome profiling in vivo recapitulated the in vitro results, showing that 4EGI-1E caused a global decrease in the ribosomal occupancy of cellular mRNAs. Network analysis suggested c-Myc as a central node within the depleted mRNAs. Abrogation of c-myc expression and induction of apoptosis were observed as soon as 4 hours after injection of 4EGI-1E (1.5 mg) into leukemia bearing animals. Depletion of T-ALL blasts from the bone marrow was achieved after 5 days of daily 4EGI-1E treatment, T-ALL (DMSO: 39% (SD 18%) vs. 4EGI-1E 0.54% (SD 0.62%) p=0.003), with limited effects on normal hematopoiesis. These data suggest that disrupting the eIF4E-eIF4G interaction constitutes a promising therapy strategy in mTOR-deregulated T-cell leukemia.

POSTER ABSTRACTS
Targeted therapies – what is the right cell, what is the best molecule?

Poster S-III-11

Activation of TRKB receptor in murine hematopoietic stem/progenitor cells induced lymphoblastic leukemia and systemic mastocytosis

Min Yang[1,2], Kezhi Huang[1], Guntram Büsche[3], Arnold Ganser[2], Zhixiong Li[1]

[1] Hannover medical School Exp. Hematology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2] Hannover Medical School Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[3] Hannover Medical School Institute of Pathology, Carl-Neuber-Str. 1, 30625 Hannover, Germany

Dysregulation of TRK (tropomyosin-related kinase) receptors has been reported to be involved in many human diseases including cancer, and neurological diseases. Recent data suggest that TRK signalling might contribute to the pathogenesis of mastocytosis by autocrine and paracrine loops. We have recently demonstrated a potential role of neurotrophins and TRK signalling in leukemia development. Now we demonstrate that activation of TRKB by its ligand BDNF (brain-derived neurotrophic factor) in murine hematopoietic stem/progenitor cells also efficiently induces a disease with striking similarities to human systemic mastocytosis (SM) in vivo.

In the present study, 37 C57BL/6J mice were transplanted with retrovially gene-modified primary hematopoietic stem/progenitor cells (TRKB/BDNF=17, TRKB=8, BDNF=5, tCD34=7) in 3 independent experiments. The experiments were terminated after an observation time of up to 454 days (mean 354 days). Interestingly, lymphoblastic leukemia was diagnosed in 5 animals, whereas the other 12 animals unexpectedly developed SM, affecting mainly spleen, liver, and bone marrow with multifocal compact mast cell infiltrates. Mast cells demonstrated mainly features of mature hypergranular mast cells, expressing TRKB, BDNF, c-Kit, FcRI and CD25 by flow cytometry. Most SM animals followed an indolent course. In contrast, leukemic mice survived <6 months after transplantation. At the final analysis, most of SM animals had normal blood counts; only 2 animals showed slight enlargement of spleen. In contrast, no animals with TRKB alone, BDNF alone or tCD34 showed mastocytosis or other hematological malignancies.These data strongly suggest that activation of TRKB by BDNF (autocrine or paracrine) is important to promote mastocytosis. Importantly, TRKB activation in our model seemed to be more potent than Kit D816V in transgenic mice for induction of SM (incidence: 71% vs. 29%), whereas retroviral-mediated expression of Kit D816V even failed to induce SM in transplanted animals.

Interestingly, C3H/HeJ animals (n=5) transplanted with TRKB and BDNF modified 32D cells (murine myeloid progenitors) developed only myeloid leukemia with no sign of increased mastocyte numbers. The fact that mastocytosis is induced only when TRKB is activated in hematopoietic stem/progenitor cells strongly supports the accepted view that mast cells are derived from hematopoietic stem cells.

In summary, we provide the first direct evidence for induction of mastocytosis by activation of TRKB in hematopoietic stem/progenitor cells in vivo. Our data indicate an important role of TRKB in the pathogenesis of mastocytosis and acute leukemia.

References

Chen CC, Grimbaldeston MA, Tsai M, Weissman IL, Galli SJ. Identification of mast cell progenitors in adult mice. Proc Natl Acad Sci U S A. 2005;102:11408-11413.
Li Z, Beutel G, Rhein M, et al. High-affinity neurotrophin receptors and ligands promote leukemogenesis. Blood. 2009;113:2028-2037.
Peng WM, Maintz L, Allam JP, et al. Increased circulating levels of neurotrophins and elevated expression of their high-affinity receptors on skin and gut mast cells in mastocytosis. Blood. 2013;122:1779-1788.
Xiang Z, Kreisel F, Cain J, Colson A, Tomasson MH. Neoplasia driven by mutant c-KIT is mediated by intracellular, not plasma membrane, receptor signaling. Mol Cell Biol. 2007;27:267-282.
Zappulla JP, Dubreuil P, Desbois S, et al. Mastocytosis in mice expressing human Kit receptor with the activating Asp816Val mutation. J Exp Med. 2005;202:1635-1641.

Poster S-III-12

The receptor tyrosine kinase ron in metastatic ewing sarcoma: a novel therapeutic target?

Carolin Schleithoff[1,2], Amelie Tillmanns[1], Birgit Lechtape[1], Christiane Schaefer[1], Georg Hempel[2], Heribert Jürgens[1], Uta Dirksen[1], Jenny Potratz[1]

[1] Universitätsklinikum Münster Pädiatrische Hämatologie-Onkologie, Albert-Schweitzer Campus A1, 48149 Münster, Germany
[2] Westfälische Wilhelms-Universität Institut für Pharmazeutische und Medizinische Chemie, Corrensstrasse 48, 48149 Münster, Germany

Objective:
Due to poor prognosis of Ewing sarcoma patients with metastatic disease novel treatment options are urgently needed; a prerequisite for therapeutic development is a better understanding of metastasis biology. Therapeutic targeting of receptor tyrosine kinases (RTKs) has improved prognosis in many cancers. However, it is emerging that RTKs act as networks to bypass targeted inhibition. Our previous work suggested the "pro-invasive" RTK RON (recepteur d'origine nantais) as a possible factor of resistance to IGF1R (insulin-like growth factor 1 receptor) targeted therapies in pediatric sarcomas. Our objective therefore is to analyze and target RON function in pediatric sarcomas.

Results:
RON is expressed in Ewing sarcomas. mRNA expression in primary tumours of 6 patients with metastases was significantly higher than in 15 patients with localized disease. RON protein was found constitutively phosphorylated, i.e. activated, as were downstream signalling elements AKT and ERK. To assess RON as a potential therapeutic target, effects of a RON specific antibody on viability of sarcoma cell lines determined in monolayer and 3D cultures. No effects were observed, neither alone nor in addition to an IGF1R antibody, independent of baseline IGF1R antibody sensitivity. This prompted us to investigate RON variants. A variant that lacks the extracellular antibody binding domain but retains the tyrosine kinase domain is described. Preliminary data suggest its presence in Ewing sarcoma. In keeping, a small molecule inhibitor of the RON tyrosine kinase domain showed some activity. Other RON variants are currently being investigated.

Conclusions:
RON is expressed and activated in Ewing sarcomas. RON short-form or splicing variants challenge experimental and therapeutic targeting strategies. Given the clinical relevance of metastasis, the lack of metastasis-directed targeting strategies and the ongoing development of multi-tyrosine kinase inhibitors, further studies are warranted.

Acknowledgements:

The project is supported by the Deutsche Krebshilfe.

POSTER ABSTRACTS
Targeted therapies – what is the right cell, what is the best molecule?

Poster S-III-13

Pooled shRNA screen to identify tumour cell-specific therapeutic targets in Ewing Sarcoma

Christiane Schaefer, Carolin Schleithoff, Birgit Lechtape, Heribert Jürgens, Uta Dirksen, Jenny Potratz

Universitätsklinikum Münster, Pädiatrische Hämatologie und Onkologie, Albert-Schweitzer-Campus 1, 48149 Münster, Germany

Objective:
In Ewing sarcoma patients treatment toxicity is severe, for high-risk patients prognosis remains poor; therefore novel therapeutic targets and treatment approaches should be explored and developed. Ewing sarcoma exhibits a characteristic chromosomal translocation, leading to the oncogenic transcription factor EWS-FLI. To further understand the role of EWS-FLI mediated oncogenic transformation, we aim to identify synthetic lethal proteins in this tumour cell-specific environment.

Methods:
We are establishing a pooled shRNA screening approach as a tool for functional identification of potential target genes. A pooled lentiviral shRNA kinase library will be transduced. Depletion or enrichment of individual shRNAs will be quantified by next generation sequencing. shRNAs that are depleted from the pool constitute potential targets.

Results:
To model the tumour cell-specific background, we are using an A673 Ewing sarcoma cell line that is stably transduced with a shRNA against the endogenously expressed EWS-FLI (A673-iEF = EWS-FLI off) or control (A673-iERG = EWS-FLI on). With pooled screening technology it is crucial to assure integration of 1 shRNA per cell. Utilizing self-generated and purchased lentiviral particles, including shRNA sequences against Lamin A/C, non-silencing control or EG5, we performed explicit virus titrations and were able to validate effective protein knockdown by single shRNA intergration, at a multiplicity of infection (MOI) of 0.3. Puromycin selection of transduced cells and culture conditions are being optimized. Furthermore we developed a real-time PCR approach to identify and quantify shRNAs from test-pools to thereby model pool deconvolution.

Conclusion:
We successfully optimized the key variables of a pooled shRNA screening approach in a Ewing sarcoma tumour cell-specific environment. We now aim to identify proteins/genes that are essential for Ewing sarcoma cell survival specifically in the presence of EWS-FLI oncogene, to provide novel molecular targets for treatment of this malignancy.

Acknowledements:

A project within the Translational Sarcoma Research Network (TranSaRNet) supported by the BMBF (FKZ 01GM0869).
The A673-iEF cell line model is kindly provided by S. Lessnick (Huntsman Cancer Institute; University of Utah).

GENE THERAPY & IMMUNOTHERAPY

Poster M-IV-01

Attenuated measles virus controls pediatric acute B-lineage lymphoblastic leukemia in NOD/SCID mice

Nike Lühl[1], Felix Zirngibl[1], Carmen Dorneburg[1], Jiwu Wei[2], Meike Dahlhaus[1], Thomas Barth[3], Lüder Meyer[1], Manon Queudeville[1], Sarah Eckhoff[1], Klaus-Michael Debatin[1], Christian Beltinger[1]

[1] University Medical Center Ulm Department of Pediatrics and Adolescent Medicine, Ulm, Germany ;
[2] Nanjing University Jiangsu Key Laboratory of Molecular Medicine, School of Medicine, Nanjing China;
[3] University Medical Center Ulm Department of Pathology, Ulm, Germany

Novel therapies are needed for pediatric acute lymphoblastic leukemia resistant to conventional therapy. While emerging data suggest leukemias as possible targets of oncolytic attenuated measles virus, it is unknown whether measles virus can eradicate disseminated leukemia, in particular pediatric acute lymphoblastic leukemia. We evaluated the efficacy of attenuated measles virus against a large panel of pediatric xenografted and native primary acute lymphoblastic leukemia ex vivo, and against four different acute lymphoblastic leukemia xenografts of B-lineage in nonobese diabetic/severe combined immunodeficient mice. Ex vivo, attenuated measles virus readily spread among and effectively killed leukemia cells while sparing normal human blood cells and their progenitors. In immunodeficient mice with disseminated acute lymphoblastic leukemia a few intravenous injections of attenuated measles virus sufficed to eradicate leukemic blasts in the hematopoetic system and to control central nervous system disease resulting in long-term survival in three of the four xenografted B-lineage leukemias. Differential sensitivity of leukemia cells did not require increased expression of the measles entry receptors CD150 or CD46 nor absence of the anti-viral retinoic acid-inducible gene I/melanoma differentiation associated gene-5 /interferon pathway. Attenuated oncolytic measles virus is dramatically effective against pediatric B-lineage acute lymphoblastic leukemia in the preclinical setting warranting further investigations towards clinical translation.

POSTER ABSTRACTS
Gene Therapy & Immunotherapy

Poster M-IV-02

Protection of murine hematopoietic cells from combined cytarabine/ anthracycline therapy utilizing cytidine deaminase (CDD) and multidrug resistance gene 1 (MDR1) gene transfer

Sebastian Brennig[1,2], Nico Lachmann[1,2], Ruhi Phaltane[1,2], Axel Schambach[2,3], Thomas Moritz[1,2]

[1] Reprogramming and Gene Therapy Group, REBIRTH Cluster of Excellence Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover, Germany
[2] Institute of Experimental Hematology Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover, Germany
[3] Division of Pediatric Hematology/Oncology, Boston Children's Hospital Harvard Medical School, Boston, United States

Myeloprotective gene therapy utilizing drug resistance (CTX-R) gene transfer has been proven to efficiently protect hematopoietic cells from the side effects of anti-cancer treatment. Given that anti-cancer chemotherapy usually is applied as a combination of multiple agents, simultaneous delivery of CTX-R genes represents a logical strategy. To prove this concept in the context of antileukemic therapy we have evaluated combined expression of cytidine deaminase (CDD) and multidrug resistance gene 1 (MDR1) in hematopoietic cells to obtain protection against cytosine arabinoside (Ara-C) as well as daunorubicin (DNR), two highly effective drugs in acute leukemias. Lentiviral vectors with a spleen focus forming virus (SFFV) or elongation factor 1 short (EFS) promoter expressing (i) a human CDD-cDNA (SFFV.CDD or EFS.CDD), (ii) a human codon-optimized MDR1-cDNA (SFFV.MDR1 or EFS.MDR1), or (iii) both CTX-R genes in combination (SFFV.CDD.2A.MDR1) were utilized. Initially, murine 32D cells were transduced and subsequently treated with Ara-C and/or DNR. SFFV.MDR1 and EFS.MDR1 transduced cells were completely protected from 125nM DNR (non-transduced cells died at 25-50nM) while susceptible to Ara-C, whereas SFFV.CDD and EFS.CDD gene-modified cells were protected from Ara-C (≥2000 nM; non-transduced cells died from 500nM onwards) but lacked DNR resistance. Cells co-transduced with SFFV.MDR1 and SFFV.CDD or EFS.CDD were protected against Ara-C (>2000nM) and DNR (≥125nM) when given alone. Even more important, co-transduced cells were protected against combined Ara-C/DNR treatment (≥2000/200nM). SFFV.CDD.2A.MDR1 gene-modified cells were protected from DNR doses of 125nM and Ara-C up to 1000nM, thus Ara-C resistance was reduced 2-fold in comparison to co-transduced cells. Furthermore, SFFV.CDD transduced primary murine hematopoietic lin- cells were protected for up to 600nM Ara-C in clongenic assays, with no colonies detected for control cells from 100nM onwards. Additionally, SFFV.MDR1 transduced lin- cells demonstrated successful colony formation in methylcellulose-based clonogenic assay in the presence of 30nM DNR (no colonies in GFP control). Taken together, our data demonstrate protection of murine hematopoietic cells against Ara-C and DNR by gene transfer of CDD and MDR1, respectively. Moreover, simultaneous expression of CDD and MDR1 conferred resistance to combined Ara-C/DNR chemotherapy, a strategy potentially applicable in antileukemic therapy.

Poster M-IV-03

Optimized lentiviral vector for improved MGMTP140K-mediated myeloprotection and in vivo selection in anti-cancer therapy

Ruhi Phaltane[1,2], Nico Lachmann[1,2], Sebastian Brennig[1,2], Mania Ackermann[1,2], Ute Modlich[2,3], Thomas Moritz[1,2]

[1] Reprogramming and Gene Therapy Group, REBIRTH Cluster of Excellence Hannover Medical School, 30625 Hannover, Germany
[2] Institute of Experimental Hematology Hannover Medical School, 30625 Hannover, Germany
[3] LOEWE Research Group Genetic Modifications in Stem Cells, Paul-Ehrlich-Institute, 63225 Langen, Germany

Notwithstanding recent successes, insertional mutagenesis and variegation of transgene expression still represent considerable obstacles to hematopoietic stem cell (HSC) gene therapy. This also applies to O6-methylguanine DNA methyltransferase (MGMTP140K)-mediated myeloprotection a concept recently proven clinically effective in the context of glioblastoma therapy. Safe and efficient transgene expression is particularly relevant for drug-resistance gene-mediated chemoprotection and in vivo selection approaches due to the associated mutagenic potential of cytotoxic drugs and the proliferative stress induced. Here we evaluate a lentiviral vector expressing the MGMTP140K-cDNA from a combined promoter utilizing an ubiquitous chromatin opening element sequence (A2UCOE) in combination with the human phosphoglycerate kinase (PGK) promoter to direct stable MGMTP140K expression while preventing promoter silencing and positional effect variegation. Lentiviral vectors with the PGK promoter alone and the spleen focus-forming virus (SFFV) promoter to drive MGMTP140K expression served as controls. In our murine in vivo chemoselection model employing four doses of chemotherapy, the A2UCOE.PGK and SFFV constructs allowed for significant myeloprotection and stable selection of transgenic hematopoietic cells in the peripheral blood. In contrast, only transient enrichment and myelotoxicity was observed for the PGK group. Similarly, analysis of the bone marrow four months after transplantation demonstrated significant selection of GFP+ cells for the A2UCOE.PGK and SFFV groups on comparison of treated and non-treated cohorts (71.5±3.7% vs. 19.7±6.1%; 45.5±10.7% vs.11.9±5.5%, $p<0.05$ respectively) whereas only moderate enrichment was seen for the PGK cohort (14.5±7.6% vs. 10.5±6.5%, $p=0.48$). Moreover, the efficient myeloprotection and chemoselection observed in the A2UCOE.PGK cohort was achieved with less vector insertions compared to the SFFV group (2.2±0.2 vs. 3.3±0.8). Thus, the A2UCOE.PGK promoter allowed for sustained vector copy number-related transgene expression throughout the experiment indicating an increased resistance to silencing which also was confirmed by CpG methylation studies of the PGK promoter. Here, our data support a potential role of the A2UCOE.PGK in future MGMT-based myeloprotection and chemoselection strategies in HSC gene and cancer therapy.

LEUKEMOGENESIS

Poster M-V-01

Hoxa9-induced leukemogenesis depends on post-transcriptional regulation

Christian Bach[1,2], Biljana Culjkovic-Kraljacic[3], Yuta Mishima[1], Robert Welner[1], Katherine L. Borden[3], Daniel G. Tenen[1,4]

[1] Harvard Medical School Harvard Stem Cell Institute, 330 Brookline Ave, Boston, MA 02115, United States
[2] University Hospital Erlangen Hematology / Oncology, Ulmenweg 18, 91054 Erlangen, Germany
[3] Université de Montréal Institute for Research in Immunology and Cancer, 2900 Edouard Mont Petit Boulevard, Montreal Quebec H3T 1J4, Canada
[4] National University of Singapore Cancer Science Institute of Singapore, Singapore Singapore

High expression levels of the homeobox gene HOXA9 are associated with adverse outcome in acute myeloid leukemia (AML) patients. In addition to acting as transcription factors, some homeobox proteins, including HOXA9, have been demonstrated to modulate posttranscriptional regulators, like the eukaryotic translation initiation factor 4e (EIF4E). Despite the paramount importance of tightly regulated EIF4E-activity in hematopoietic cells, however, the relevance of its interaction with HOXA9 for leukemogenesis is unknown. Therefore, our aim was to assess the impact of post-transcriptional regulation by the HOXA9-EIF4E interaction on leukemogenesis.

To address this, we created an eIF4e interaction mutant of Hoxa9 (= Hoxa9YL) by introducing point mutations into the Hoxa9 cDNA. Importantly, Hoxa9YL retained interaction with the functionally important Hoxa9 cofactor Meis1 and DNA binding, suggesting that we specifically abrogated eIF4e interaction without interfering with other relevant aspects of Hoxa9 function. Interestingly, primary murine bone marrow cells transduced with Hoxa9YL showed a marked reduction of growth transformation compared to normal Hoxa9. To corroborate this finding in vivo, we tested Hoxa9YL in the well documented retroviral Hoxa9/Meis1 (A9/M) leukemia model, by transplanting Hoxa9YL/Meis1 (YL/M) transduced lineage-/Sca-1+/c-Kit+ (KSL) cells into recipient mice. Importantly, median onset of leukemia was increased significantly from appr. 90 days (A9/M) to >200 days (YL/M). Notably, 2 out of 11 mice in the YL/M group failed to develop AML altogether, suggesting that interaction with eIF4e is a key mechanism of Hoxa9-mediated transformation. Subsequently, we addressed compensatory upregulation of eIF4e activity in YL/M leukemias as a potential mechanism of leukemia development in the absence of Hoxa9-eIF4e interaction. However, we observed no significant difference in total gene expression or protein levels of eIF4e, activated eIF4e, and the Hoxa9 antagonist Hhex between A9/M and YL/M leukemias. Importantly, delayed disease onset was preserved in secondary recipients of YL/M leukemic bone marrow, consistent with persisting addiction of Hoxa9-induced leukemogenesis to Hoxa9-eIF4e interaction.

Taken together, our data suggests that post-transcriptional regulation by interaction with eIF4e is a critical component of Hoxa9-mediated leukemogenesis, providing a potential therapeutic target for a poor prognostic subgroup of AML.

Poster M-V-02

A case of novel cryptic t(11;11)(p15.4;q24.2) and cryptic ins(4;11)(q21.3;q23.3) in adult B-cell acute lymphoplastic leukemia (B-ALL)

Moneeb Othman

Institute of Human Genetics University Hospital Jena, kollegiengasse 10, 07743 Jena, Germany

Aims:
To characterize chromosomal breakpoint which were yet not reported in acute leukemia (AL) and to correlate detected new aberrations with a potential prognostic and therapeutic relevance in AL. Here one case with a novel cryptic t(11;11)(p15.4;q24.2) and cryptic ins(4;11)(q21.3;q23.3) from a collection of over 70 studied cases is presented.

Methods:
A 69 year old female diagnosed with B-cell acute lymphoblastic leukemia (B-ALL) and a normal karyotype after standard GTG-banding was studied retrospectively in detail by molecular cytogenetic and molecular approaches, i.e. multitude multicolor banding (mMCB), and suited locus-specific probes. Also multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA probemix P377-A1 for Hematological Malignancies Kit from MRC-Holland was performed. High resolution array-comparative genomic was used to detect the copy number aberrations.

Results:
Cytogenetic analysis revealed a normal female karyotype 46,XX. mMCB analysis identified a cytogenetically balanced translocation t(11;11)(p15.4;q24.2). FISH studies performed on both interphase nuclei and metaphases used dual color LSI probe for MLL revealed, an insertion of MLL into 4q21 as an additional abnormality in this karyotype. No aberrations and/or copy number variations were detected by MLPA and aCGH.

Conclusions:
B-ALL is the most common malignancy in pediatric patients and the leading cause of cancer-related death in children and young adults. The case presented here is, the first report of cryptic t(11;11)(p15.4;q24.2) and cryptic ins(4;11)(q21.3;q23.3) in adult B-cell ALL due to insertion of 5 MLL sequences into chromosome 4 and consequently encoding a MLL-AF4 fusion transcript and associated with dismal prognosis.

Supported in parts by DAAD.

POSTER ABSTRACTS
Leukomogenesis

Poster M-V-03

Mutational Analysis of the APL Oncoprotein PML-RAR Alpha Using a Liquid Culture Immortalization Assay Reveals an Essential Role for the PML RING Finger

Katharina Korf, Alexander Haschke, Thomas Sternsdorf

Research Institute Children's Cancer Center and Clinic of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20251 Hamburg, Germany

Although Acute Promyelocytic Leukemia (APL) is nowadays a curable disease, the mechanisms of leukemogenesis remain unclear. APL is characterized by chromosomal translocations involving the Retinoic Acid Receptor Alpha (RARa). 95-99% of APL cases carry a t(15;17) translocation, leading to a fusion of the promyelocytic leukemia protein (PML) with RARa (PML-RARa). In our previous study (Sternsdorf et al., 2006) we were able to demonstrate that RARa fusion partners act by promoting self-association which mechanistically renders RARa oncogenic. Even entirely heterologous dimerization domains are leukemogenic when fused to RARa, however, at the expense of a drastic (~10 fold) reduction in leukemia penetrance (Sternsdorf et al., 2006; Brown et al., 1997). We have systematically analyzed critical determinants of the APL oncoprotein PML-RARa using a newly established in vitro assay (Liquid Culture Immortalization). Remarkably, we found immortalizing activity of PML-RARa highly variable in different mouse strains. We identified the PML RING-finger domain and SUMOylation to be essential for leukemogenic activity using FVB mice. In addition, we analyzed DNA binding, homomerization and RXR interaction, all of which were relevant for full oncogenicity to varying degrees. By traditional transduction/transplantation in vivo mouse experiments we could demonstrate that the results correlate very well with the data we obtained using our in vitro assay.

Besides analyzing immortalization, we also looked at the nuclear localization of the PML-RARa mutants, and their ability to disrupt PML nuclear bodies (PML-NBs) using U2OS cells. We discovered that all of the immortalizing mutants also disrupt the PML-NBs, suggesting a link between disruption of PML-NBs and immortalization.

References

Sternsdorf, T., Phan, V. T., Maunakea, M. L., Ocampo, C. B., Sohal, J.,Silletto, A., Galimi, F., Le Beau, M. M., Evans, R. M., and Kogan, S. C. (2006).
Forced retinoic acid receptor alpha homodimers prime mice for APL-like leukemia. Cancer Cell 9, 81-94.

Brown, D., Kogan, S., Lagasse, E., Weissman, I., Alcalay, M., Pelicci, P. G., Atwater, S., and Bishop, J. M. (1997).
A PMLRARalpha transgene initiates murine acute promyelocytic leukemia. Proc Natl Acad Sci U S A 94, 2551-2556.

Poster M-V-04

Syk and Btk induce cell survival and proliferation in AML cells – by activating distinct signaling pathways and transcriptional programs

Thomas Oellerich[1], Sebastian Mohr[1], Julia Beck[2], Jasmin Corso[3], Marc Oellerich[4], Silvia Münch[1], Johannes Wicht[1], Carmen Döbele[1], Gesine Bug[1], Hannibal Bohnenberger[5], Christina Perske[5], Ekkehard Schütz[2], Henning Urlaub [3,6], Hubert Serve [1]

1 Johann Wolfgang Goethe University Hematology/Oncology, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany
2 Chronix Biomedical, 5941 Optical Court Suite 203E, San Jose, 95138, United States
3 Max Planck Institute for Biophysical Chemistry Bioanalytical Mass Spectrometry Group, Fassberg 11, 37077 Goettingen, Germany
4 Johann Wolfgang Goethe University Cardiology, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany
5 Georg August University Pathology, Robert-Koch-Straße 40, 37075 Goettingen, Germany
6 University Medical Center Bioanalytics, Robert-Koch-Straße 40, 37075 Göttingen, Germany

Constitutive activation of certain kinase-dependent signal transduction pathways is critical for the pathogenesis of acute myeloid leukemia (AML). Beside FLT3-ITD the spleen tyrosine kinase (Syk) induces cell survival and proliferation in a high proportion of AML blasts, but the underlying molecular events of Syk signaling have not been investigated so far. By using quantitative proteomic techniques, we have identified a multi-protein complex that is nucleated by constitutively active Syk in AML cells. This complex differs from the B lymphoid Syk interactome regarding several proteins, in particular the b2-integrin receptor Mac-1 and also the oncogenic transcription factors STAT3 and STAT5. Our functional studies in primary AML cell cultures revealed that tonic signals derived from the integrin-associated Fc-g chain lead to Syk-dependent activation of STAT3 and STAT5 that in turn induces AML cell proliferation. Moreover, stimulation of Mac-1 intensifies the constitutive Syk-mediated STAT3/5 activation in AML cells, a scenario likely to take place in the bone marrow niche. In accordance with these findings, we observed that b2-integrins including Mac-1 induce proliferation of AML cells in an AML cell/stroma coculture model. To identify further potential drug targets we screened AML bone marrow biopsies regarding their kinase expression- and activation profiles and found Btk to be expressed and activated in 26 out of 30 analyzed AML cases. Functional analyses revealed that genetic as well as pharmacological targeting of Btk diminished AML cell expansion and induced apoptosis. By phosphoproteomic analysis and transcriptome sequencing we identified distinct survival-inducing pathways in FLT3-ITD-positive and –negative AML cells that are operational up- as well as downstream of Btk. Taken together, we identified oncogenic signaling pathways driven by either Syk or Btk that might serve as therapeutic targets of AML in the future.

POSTER ABSTRACTS
Leukomogenesis

Poster M-V-05

Genetic engineering in patient-derived AML cells enables highly sensitive in vivo imaging in mice on genetically diverse samples

Binje Vick[1,2,3], Maja Rothenberg[4], Nadine Sandhöfer[5,2,3], Michela Carlet[1], Cornelia Finkenzeller[1], Christina Krupka[6,4], Michaela Grunert[1], Selim Corbacioglu[7], Martin Ebinger[8,2,3], Maya André[8,9], Wolfgang Hiddemann[2,4,5], Marion Subklewe[6,4], Klaus H. Metzeler[4,5], Karsten Spiekermann[2,4,5], Irmela Jeremias[1,2,10]

1 Helmholtz Zentrum München Group Apoptosis, Department of Gene Vectors, Marchioninistraße 25, 81377 München, Germany
2 German Cancer Consortium (DKTK), Heidelberg, Germany
3 German Cancer Research Center (DKFZ), Heidelberg, Germany
4 Ludwig-Maximilians-Universität (LMU) Department of Internal Medicine III, München, Germany
5 Helmholtz Zentrum München Clinical Cooperation Group Leukemia, München, Germany
6 Helmholtz Zentrum München Clinical Cooperation Group Immunotherapy, München, Germany
7 University of Regensburg Department of Pediatrics, Regensburg, Germany
8 University Children's Hospital, Eberhard Karls Universität Department of Pediatric Hematology/Oncology, Tübingen, Germany
9 University Children's Hospital (UKBB) Department of Pediatric Intensive Care Medicine, Basel, Switzerland
10 Dr von Haunersches Kinderspital Department of Oncology, München, Germany

Treatment of AML requires novel therapeutic strategies which at best target the complex biology of AML. Towards this aim, preclinical research is required which depends on adequate model systems. Established cell lines often harbor non-physiologic characteristics, and in vitro studies lack the in vivo interplay. To mimic the disease more closely, patient-derived AML cells are engrafted in immuno-compromised NSG mice.

Here, we improved the individualized mouse model of AML. Serial transplantation of patient-derived xenografted (PDX) cells was stably feasible in 6 of 12 samples with constant growth characteristics and immunophenotype. Lentiviral transduction resulted in transgenic (t-PDX) cells stably expressing the transgene. To quality control our model, we characterized PDX cells after engraftment, serial transplantation, and molecular manipulation by targeted deep sequencing of 43 genes known to be recurrently mutated in myeloid malignancies. Targeted re-sequencing revealed that clonal and subclonal recurrent mutations of the primary specimens were largely preserved in the PDX samples tested, even after serial transplantation and molecular engineering. However, certain minor subclones had an engraftment and/or growth advantage, becoming the major clone or getting lost upon xenotransplantation or cell manipulation.

Mice harboring t-PDX cells expressing recombinant codon-optimized firefly luciferase were repetitively monitored by bioluminescence imaging (BLI). BLI was highly sensitive and reliably detecting as low as 1 t-PDX cell within more than 10,000 mouse bone marrow cells and thereby visualizing the clinically important stage of minimal disease. Growth of t-PDX cells in mice over time was exponential. Analysis of limiting dilution transplantation assays by BLI enabled convenient quantification of leukemia stem cells within as little asfive weeks.

Taken together, patients' AML cells growing in mice closely mimic the heterogeneity of the disease. Molecular manipulation and BLI on serially transplanted PDX AML cells facilitate reliable disease monitoring and preclinical in vivo trials. The technical improvements will allow detailed preclinical studies on patient-derived AML cells of diverse genetic backgrounds to study biology and therapy of AML in the future.

Poster M-V-06

Established cell lines of human acute leukemia reveal orthotopic organ distribution upon growth in immunocompromised mice

Michaela Grunert[1], *Michela Carlet*[1], *Irmela Jeremias*[1,2]

[1] Helmholtz Zentrum München, German Research Center for Environmental Health Department of Gene Vectors, Marchioninistr. 25, 81377 München, Germany
[2] Dr von Haunersches Kinderspital, Ludwig MaximiliansUniversität (LMU) Department of Oncology, L indwurmstr. 4, 80337 München, Germany

Cell lines established from patients with acute leukemia (AL) represent an important tool for preclinical studies on biology and therapy of AL. Established cell lines (i) grow in vitro over many years in contrast to primary tumor cells which suffer poor in vitro growth; (ii) are functionally well characterized; (iii) may harbor a specific genetic background of interest; (iv) allow rapid molecular manipulation among others. Accomplishing in vitro studies, established cell lines might be used for preclinical in vivo studies using xeno-transplantation into immunocompromised mice. The usefulness of using established cell lines for preclinical in vivo trials also depends on their capability to grow orthotopically in mice.

Here, we compared the systemic growth behavior of AL cell lines in NSG mice using bioluminescence in vivo imaging (BLI) to monitor cell growth. 6 AML, 4 B-ALL and 2 T-ALL cell lines were engineered to express luciferase and injected intravenously with 5x104 to 107 cells/mouse and animals were monitored for leukemic growth using BLI. All (100%) cell lines engrafted in mice, although certain cell lines required long incubation time and/or high numbers of injected cells showing slow growth in vivo. Overall, 9 / 12 (75%) cell lines showed reliable tumor growth in mice.

We next compared the in vivo behavior of established cell lines to cells which were established from fresh patients' samples and are called patient-derived xenograft (PDX) cells. In contrast to established cell lines, PDX cells do not grow in vitro and depend on transplantation into mice.

Cell distribution throughout the murine body was highly similar between established cell lines and PDX cells and resembling organ distribution known form leukemia patients. In contrast to the majority of PDX cells, established cell lines were less well tolerated and mice showed clinical signs of the leukemic disease already at low tumor burden. In limiting dilution transplantation assays, more cells were required for engraftment of established cell lines compared to PDX cells, although established cell lines are thought to consist exclusively of leukemia initiating cells in contrast to PDX cells.

We conclude that most established cell lines grow systemically in mice. Using BLI might overcome the requirement of using subcutaneous cell injections. BLI offers the chance to study e.g. antitumor effects of novel therapeutics in established AL cell lines under the influence of a more physiologic microenvironment.

Poster M-V-07

GABPalpha modulates imatinib sensitivity in vitro and is positively correlated with BCR ABL/ABL ratio and PRKD2 in human CML

Georgi Manukjan, Tim Ripperger, Laura Santer, Nils von Neuhoff, Brigitte Schlegelberger, Doris Steinemann

Hannover Medical School Institute of Cell and Molecular Pathology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

Chronic myeloid leukemia (CML) is a malignant hematological disorder characterized by the reciprocal translocation t(9;22) leading to a constitutively active ABL kinase as the driver of the disease. Consequently, highly effective specific tyrosine kinase inhibitors (TKI) were developed for tailored treatment. In many cases, drug resistance is still observed typically initiated by distinct point mutations in the BCR-ABL fusion gene. However, further mechanisms driving disease progression and inhibiting treatment response are under debate.
The GA-binding protein (GABP) consists of the two unrelated subunits GABP and GABP.1 GABP belongs to the E26 transformation-specific (ETS) domain transcription factor family and bears the DNA-binding domain. GABP contains the transcriptional activation as well as the nuclear-localization signal domains. GABP is known as a crucial transcription factor in hematopoiesis.2,3Its involvement in CML was recently shown in independent mouse studies. Loss of GABP in BCR-ABL-transfected hematopoietic cells led to prolonged survival of recipient mice, which suffered from CML-like myeloproliferative disease.4 Similar observations have been made in GABP knockout mice.5
We analyzed dosage effects of GABP on the response to the first-line TKI imatinib in human CML. For this, stable knock-down as well as ectopic over-expression of GABP were established in the model cell line K562. Down-regulation of GABP resulted in significantly elevated imatinib sensitivity below the reported IC50. In contrast, enhanced intracellular GABP levels led to a protective effect against imatinib treatment shown in proliferation and colony-forming capacity assays. Furthermore, we analyzed the putative GABP target genes PRKD2 (protein kinase D2) and RAC2 (Rho-GTPase RAS-related C3 botulinum substrate 2) discussed as crucial factors in CML pathogenesis. In K562 cells, stable knock-down and ectopic over-expression of GABP led to reduced and increased expression of both target genes, respectively.
In addition, we screened GABPA mRNA expression in 72 untreated CML patients at the time of diagnosis. We detected a positive correlation between BCR ABL/ABL ratio and GABPA transcript levels. Furthermore, we observed a correlation between PRKD2 and GABPA expressions in primary CML samples. In summary, these findings support the potential relevance of GABP in human CML that was only reported in murine CML models until now. Further studies are required to elucidate the role of GABP in CML initiation and progression as well as in resistance to TKI therapy.

References

1. Rosmarin AG, Resendes KK, Yang Z, McMillan JN, Fleming SL. GA-binding protein transcription factor: a review of GABP as an integrator of intracellular signaling and protein-protein interactions. Blood Cells Mol Dis. 2004;32(1):143-154.
2. Yang ZF, Drumea K, Cormier J, Wang J, Zhu X, Rosmarin AG. GABP transcription factor is required for myeloid differentiation, in part, through its control of Gfi-1 expression. Blood. 2011;118(8):2243-2253.
3. Yu S, Cui K, Jothi R, et al. GABP controls a critical transcription regulatory module that is essential for maintenance and differentiation of hematopoietic stem/progenitor cells. Blood. 2011;117(7):2166-2178.
4. Yang ZF, Zhang H, Ma L, et al. GABP transcription factor is required for development of chronic myelogenous leukemia via its control of PRKD2. Proc Natl Acad Sci U S A. 2013;110(6):2312-2317.
5. Yu S, Jing X, Colgan John D, Zhao D-M, Xue H-H. Targeting Tetramer-Forming GABP Isoforms Impairs Self-Renewal of Hematopoietic and Leukemic Stem Cells. Cell Stem Cell. 2012;11(2):207-219.

Poster M-V-08

The MADS transcription factors Mef2c and Mef2d regulate early B-cell differentiation

Julia Preukschas[1], Ludmilla Unrau[1], Friderike Hauschildt[1], Meike Fischer[1], Ursula Müller[1], Daniela Indenbirken[2], Malik Alawi[2], Carol Stocking[1]

[1]Heinrich-Pette-Institute Retroviral Pathogenesis, Martinistr. 52, 20251 Hamburg, Germany
[2]Heinrich-Pette-Institute Virus Genomics, Martinistr. 52, 20251 Hamburg, Germany

Acute leukemias are characterised by a block in differentiation of hematopoietic cells caused by deregulated gene expression. In acute lymphoblastic leukemia (ALL), dysregulation of MEF2C and translocations of the MEF2D gene have been reported. The role of these transcription factors in muscle and cardiovascular development is well investigated, but little is known with regard to the function of Mef2c and Mef2d in lymphopoiesis or in ALL.

To investigate the importance of these highly conserved transcription factors, mice were generated in which both Mef2c and Mef2d were inactivated at the level of common lymphoid progenitors (CLP). Histological and cytological analysis of these mice revealed the absence of B-cell zones in lymph nodes and spleen, whereas the T-cell zone remains intact. Immature and mature B-cells were barely detectable in the bone marrow of knockout mice and extensive FACS analysis revealed a differentiation block between proB- and preB-cells.

RNAseq analysis showed that a double knockout of Mef2c and Mef2d lead to a deregulation of approx. 500 genes, in particular strongly reduced levels of transcripts encoding several transcription factors, including Klf2 and members of the AP1 family. Chromatin immunoprecipitation experiments revealed direct binding of Mef2c/d to regulatory elements of the Klf2, Jun and Fos loci. Furthermore, we could show that Mef2c/d are activated by phosphorylation during preBCR signalling, correlating with an upregulation of Klf2 and Jun mRNA expression. In vitro experiments indicate that the block in B-cell differentiation of Lin- Mef2c/d knockout cells can be abolished by an overexpression of Klf2.

These results demonstrate the important function of Mef2c and Mef2d and their target gene Klf2 in early B-cell differentiation and help to explain the impact of a dysregulation of Mef2c and Mef2d in acute lymphoblastic leukemias.

POSTER ABSTRACTS
Leukomogenesis

Poster M-V-09

Endogenous Retrovirus Induces Leukemia in a Xenograft Mouse Model for Primary Myelofibrosis

Ioanna Triviai[1,2], Marion Ziegler[1], Ulla Bergholz[1], Andrew J. Oler[3], Thomas Stübig[2], Vladimir Prassolov[4], Boris Fehse[2], Christine A. Kozak[5], Nicolaus Kröger[2], Carol Stocking [1]

1 Heinrich-Pette-Institute Leibniz Institute of Experimental Virology, Martinistrasse 52, 20251 Hamburg, Germany
2 University Medical Center Hamburg-Eppendorf Department of Stem Cell Transplantation, Martinistrasse 52, 20246 Hamburg, Germany
3 National Institute of Allergy and Infectious Diseases Computational Biology Section, Bioinformatics and Computational Biosciences Branch, 31 Center Drive, Bethesda, MD 20892, United States
4 Engelhardt Institute of Molecular Biology, Vavilov St. 32, 119991 Moscow, Russia
5 National Institute of Allergy and Infectious Diseases Laboratory of Molecular Microbiology, 4 Center Drive, Bethesda, MD 20892, United States

In studies designed to define neoplastic stem cells of Primary Myelofibrosis (PMF), a myeloproliferative neoplasm characterized by profound disruption of the hematopoietic microenvironment, we developed a xenotransplant mouse model that recapitulates both chronic and acute stages of the disease. During our analysis of various PMF patient derived stem and progenitor cells in the specific experimental setting, we observed a high frequency of Acute Myeloid Leukemia (AML) induction in NSG mice. AML was of mouse origin, confined to PMF-xenografted mice, and contained multiple clonal integrations of ecotropic murine leukemia virus (E-MuLV). Sequence analysis confirmed that E-MuLV originated from the Emv30 provirus and that no recombination events were necessary for virus replication or AML induction. Acquired pathogenicity is thus likely attributable to PMF-mediated paracrine stimulation of the myeloid compartment in xenografted mice. These proliferating cells serve as targets for retroviral transformation, as evidenced by integration into the Evi1 locus – a hotspot for retroviral-induced myeloid leukemia. In conclusion, this study corroborates a critical role of paracrine stimulation in PMF disease progression, underlines the importance of target cell numbers in MuLV pathogenicity, and mandates awareness of active MuLV replication in interpreting results from severely immunodeficient NOD mice.

Poster M-V-10

HOXB4 promotes formation and hematopoietic transition of hemogenic endothelium cells

Nadine Teichweyde, Peter A. Horn, Hannes Klump

University Hospital Essen Institute for Transfusion Medicine, Virchowstraße 179, 45147 Essen, Germany

During embryogenesis blood stem cells emerge from a unique population of endothelial cells termed hemogenic endothelium (HE). HE cells are a rare, temporary population found in restricted anatomical sites, such as the ventral floor of the dorsal aorta from which the first definitive hematopoietic cells arise. Development of these specialized endothelial cells and their subsequent hematopoietic activity, in vivo, depends on direct cell-to-cell signaling, the balanced presence of certain soluble growth factors, and mechanical forces within the vasculature. Runx1 is a pivotal transcription factor necessary for the endothelial-to-hematopoietic-transition (EHT) and marks definitive hematopoietic cells once the endothelial program is down-regulated which itself is driven by the homeodomain transcription factor HOXA3. HOXB4, in contrast, is well known to enhance hematopoietic development, at least during mouse ES-cell differentiation, in vitro. However, the underlying mechanisms are still ill-defined. Therefore, we investigated its influence on major fate decisions during different stages of hematopoietic specification, in vitro. HOXB4 mediated a significant increase in the number of endothelial-structures which expressed EPCR, VE-Cadherin, Flk1, CD31 and took up Dil-ac-LDL suggesting that it might act at the hemogenic endothelium stage. Therefore, we employed a Runx1-/- ESC-line allowing for Doxycycline-inducible Runx1 expression and expressing a tamoxifen-inducible form of HOXB4. Induction of HOXB4 alone promoted the formation of endothelial structures which, upon addition of Dox, initiated EHT. The formation of hematopoietic cells was accompanied by the upregulation of genes necessary for hematopoiesis, such as Gfi1, Gfi1b and Pu.1. Our results strongly suggest that HOXB4 promotes the development and expansion of hemogenic endothelium cells thus providing the unique opportunity to investigate the immediate process of hematopoiesis at the clonal level in a controlled fashion.

EPIGENETICS AND CELL AGEING

Poster M-VI-01

Activating FLT3-ITD receptor mutations in AML are associated with a specific epigenetic signature composed of a discrete subset RUNX1-bound DNaseI hypersensitive sites enriched for AP-1 and C/EBP motifs

Pierre Cauchy[1], Sally James[2], Jason Piper[3], Anetta Ptasinska[1], Joaquin Zacarias-Cabeza[1], Maria Rosaria Imperato[1], Martina Canestraro[1], Salam Assi[1,4], Maarten Hoogenkamp[1], David Westhead[4], Sascha Ott[3], Constanze Bonifer[1], Peter Cockerill[1]

1 University of Birmingham, U.K Institute of Biomedical Research, Birmingham, B15 2TT, Great Britain
2 University of Leeds Institute for Cancer Studies and Pathology, Leeds, LS9 7TF, Great Britain
3 University of Warwick Warwick Systems Biology Centre, Senate House, Room 327, Coventry, CV4 7AL, Great Britain
4 University of Leeds Institute of Molecular and Cellular Biology, Leeds, LS9 7TF, Great Britain

Acute myeloid leukemia (AML) with a FLT3 internal tandem duplication (FLT3/ITD) mutation is an aggressive hematologic malignancy with a high rate of relapse. This mutation converts a normally inducible cytokine receptor to a constitutively active receptor that activates MAPK and JAK/STAT pathways. To obtain mechanistic insights into how the epigenome is reprogrammed in this type of AML, we have employed genome-wide mapping of DNaseI Hypersensitive Sites (DHSs). This approach identifies active regions of the genome where potential enhancer and promoter elements exist as open regions of chromatin that are occupied by transcription factors. We have also developed genome-wide footprinting methods to predict occupancy of transcription factor binding sites.

We compared karyotypically normal (KN) AML bearing FLT3-ITD mutations to KN AMLs lacking this mutation, and to normal CD34+ peripheral blood stem cells (PBSCs). A detailed analysis of chromatin accessibility and transcription factor occupancy in FLT3-ITD AML revealed the existence of about 1,000 specific DHSs that were consistently enriched in the FLT3-ITD AML samples compared to normal CD34+ cells and other KN AMLs. These sites were enriched for DNA motifs for several transcription factors including RUNX1, AP-1, and C/EBP, that were predicted to be preferentially occupied by DNaseI footprinting. A RUNX1 ChIP analysis further confirmed that these specific DHSs bound RUNX1. This is partly mediated by an increase in RUNX1 levels because we saw a parallel 2 fold increase in the average level of RUNX1 mRNA expression in FLT3-ITD AML. However, the principle actions of FLT3-ITD at the level of the genome are likely to be via the MAPK dependent activation of factors such as AP-1. Many of the AP-1 motifs we identified were found by DNaseI footprinting to be occupied in FLT3-ITD+ AML but not in normal PBSCs. This activation of inducible factors and DHSs in AML is also probably the main mechanism leading to the AML-specific binding of RUNX1 to many additional sites that are not occupied in normal cells. In contrast, we found no enrichment for motifs for STAT or IRF family proteins that may also respond to FLT3 signalling. In summary, our data suggests that RUNX1 together with inducible factors plays a major role in reprogramming gene expression in FLT3-ITD AML.

Poster M-VI-02

Stress-induced exit from quiescence induces de novo DNA damage in hematopoietic stem cells and results in bone marrow failure in the absence of a functional Fanconi anemia signaling pathway.

Dagmar Walter[1], Amelie Lier[1], Anja Geiselhart[2], Sina Hunstscha[1], David Brocks[1], Irem Bayindir[1], Paul Kashutnig[2], Katja Müdder[3], Tim Holland-Letz[4], Peter Schmezer[5], Steven Lane[6], Marieke Essers[7,8], David Williams[9,10,11], Andreas Trumpp[3,12], Michael Milsom[1,2]

1 Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM) Experimental Hematology, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
2 German Cancer Research Center (DKFZ) Experimental Hematology, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
3 Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM) Stem Cells and Cancer, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
4 German Cancer Research Center (DKFZ) Division of Biostatistics, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
5 German Cancer Research Center (DKFZ) Division of Epigenomics and Cancer Risk, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
6 University of Queensland QIMR Berghofer Medical Research Institute, Brisbane Australia
7 Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM) Hematopoietic Stem Cells and Stress, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
8 German Cancer Research Center (DKFZ) Hematopoietic Stem Cells and Stress, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
9 Boston Children's Hospital Hematology/Oncology, Boston, MA United States
10 Harvard Medical School, Boston, MA United States
11 Dana-Faber Cancer Institute, Boston, MA United States
12 German Cancer Research Center (DKFZ) Stem Cells and Cancer, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

The accumulation of DNA damage in hematopoietic stem cells (HSCs) is a hallmark of aging and is thought to drive age-related tissue degeneration and malignant transformation. Accordingly, a number of accelerated aging syndromes are associated with defective DNA repair and genomic instability, including the inherited bone marrow failure (BMF) syndrome Fanconi anemia (FA). While the physiologic source of DNA damage in normal or FA HSCs remains unclear, long-term quiescence has been proposed to preserve the genomic stability of HSCs during aging. We hypothesised that stress-induced exit from quiescence might comprise a situation of high proliferative stress, that results in elevated DNA damage in HSCs. We treated wild type (WT) C57BL/6J mice with a range of stress-stimuli that provoke HSCs into exiting their homeostatic quiescent state (polyI:polyC; Interferon-; G-CSF; TPO; and serial bleeding). All stimuli precipitated de novo DNA damage in HSCs in vivo and resulted in the depletion of functional HSCs. Stress-induced HSC exit from quiescence resulted in elevated mitochondrial metabolism and a 50% increase in 8-Oxo-dG lesions on DNA. Retroviral overexpression of the reactive oxygen species (ROS)-detoxifying enzymes catalase and SOD2 completely rescued stress-induced DNA damage in HSCs in vivo, demonstrating that mitochondrial ROS directly precipitated DNA damage. The FA DNA repair pathway was activated in response to stress-induced DNA damage and mice with a loss of function in this pathway (Fanca-/-) had 2-fold higher levels of DNA damage in HSC compared to WT ($p<0.05$). This suggests that the FA pathway resolves stress-induced DNA damage in HSCs in vivo. Indeed, further rounds of polyI:polyC treatment led to the onset of severe aplastic anemia in all Fanca-/- mice but not in any of the WT pI:pC-treated controls, nor in the age matched PBS-treated Fanca-/- mice ($p<0.01$). BMF was characterized by profound neutropenia, thrombocytopenia and anemia and a >60% reduction in bone marrow cellularity, corresponding to an almost complete depletion of all HSCs as well as multipotent and committed progenitors. Our data strongly implicates stress-induced exit from quiescence as a cause of physiologic DNA damage in HSCs in vivo and provides a novel link between stress hematopoiesis, DNA damage, HSC aging and BMF.

POSTER ABSTRACTS
Epigenetics and cell ageing

Poster M-VI-03

The Acute Promyelocytic Leukemia-Oncoprotein PML-RARalpha Blocks Senescence and Disrupts the ATRX/Daxx -Complex to Promote Leukemia.

Katharina Korf[1], Harald Wodrich[2], Alexander Haschke[1], Ronald M. Evans[3], Thomas Sternsdorf[1]

1 Forschungsinstitut Kinderkrebszentrum Hamburg, Martinistr.52, 20251 Hamburg, Germany
2 University of Bordeaux SEGALEN Microbiologie Fondamental et Pathogénicité, 146 rue Leo Seignat, 33000 Bordeaux, France
3 The Salk Institutefor Biological Studies GEL-E, 10010 N. Torrey Pines Rd., La Jolla, California 92186-5800, United States

Although Acute Promyelocytic Leukemia (APL) has become a curable disease due to in-depth understanding of the underlying molecular processes, its investigation has provided unique and valuable insights into the processes involved in leukemogenesis. Therefore we use it as a model system. 99% of APL-patients express a PML-RAR fusion protein. While involvement of RAR has proven indispensable for oncogenicity, the role of the PML domain is far less clear. In our previous study (Sternsdorf et al., Cancer Cell, 2006) we found that substitution of PML with heterologous self-interaction domains suffices to induce leukemias, but drastically decreases oncogenic potency of the resulting fusion proteins. In this study, we have chosen the inverse strategy: we have modified the PML domain to create a more active artificial model oncoprotein by adapting PR to its biological environment: As the typical model organism for APL studies is the mouse, we have replaced the human PML domain with the murine PML domain. This oncoprotein (mPR) creates APL-type leukemias in mice with higher penetrance and shorter latency than its human counterpart, hPR. We have used this system to study immediate early effects of expression of the model RAR fusion oncoprotein.

While we found proliferating murine bone marrow cells go into senescence ex vivo, expression of mPR prevents this and robustly immortalizes murine bone marrow from every mouse strain tested so far. Senescence-associated upregulation of the cell-cycle regulators p21 and p19 was efficiently blocked by mPR expression. In mouse cells, mPR exhibits higher potency in disrupting the PML-associated Daxx/ATRX complex than hPR. Knockdown of ATRX, but not Daxx ameliorated ATRA-induced growth suppression and p21 upregulation in the human APL model cell line NB4. These data suggest, that PML-RAR promotes leukemogenesis by disrupting the Daxx/ATRX complex, which assembles at PML nuclear bodies during the onset of senescence. As this complex serves as a histone chaperone for the H3.3 histone variant [3], our observations link H3.3 predisposition and cellular senescence with leukemogenesis. Recent reports about mutations in Daxx, ATRX and H3.3 in various tumors confirm the relevance of these factors in carcinogenesis [1, 2].

References

1. Yuen, B.T. and P.S. Knoepfler, Histone H3.3 Mutations: A Variant Path to Cancer. Cancer Cell, 2013. 24(5): p. 567-574.
2. Schwartzentruber, J., A. Korshunov, X.Y. Liu, D.T. Jones, E. Pfaff, K. Jacob, D. Sturm, A.M. Fontebasso, D.A. Quang, M. Tonjes, V. Hovestadt, S. Albrecht, M. Kool, A. Nantel, C. Konermann, A. Lindroth, N. Jager, T. Rausch, M. Ryzhova, J.O. Korbel, T. Hielscher, P. Hauser, M. Garami, A. Klekner, L. Bognar, M. Ebinger, M.U. Schuhmann, W. Scheurlen, A. Pekrun, M.C. Fruhwald, W. Roggendorf, C. Kramm, M. Durken, J. Atkinson, P. Lepage, A. Montpetit, M. Zakrzewska, K. Zakrzewski, P.P. Liberski, Z. Dong, P. Siegel, A.E. Kulozik, M. Zapatka, A. Guha, D. Malkin, J. Felsberg, G. Reifenberger, A. von Deimling, K. Ichimura, V.P. Collins, H. Witt, T. Milde, O. Witt, C. Zhang, P. Castelo-Branco, P. Lichter, D. Faury, U. Tabori, C. Plass, J. Majewski, S.M. Pfister, and N. Jabado, Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature, 2012. 482(7384): p. 226-31.
3. Lewis, P.W., S.J. Elsaesser, K.M. Noh, S.C. Stadler, and C.D. Allis, Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. Proc Natl Acad Sci U S A, 2010. 107(32): p. 14075-80.

Poster M-VI-04

LncRNA HemAtlas defines blood lineage-specific lincRNA biomarkers and ceRNA modules in the hematopoietic system

Stephan Emmrich, Franziska Schmidt, Ramesh Chandra Pandey, Dirk Reinhardt, Jan-Henning Klusmann

Hannover Medical School Pediatric Hematology & Oncology, Carl-Neuberg-Str. 1, Hannover 30625, Germany

Long non-coding RNAs (lncRNAs) recently emerged as central regulators of chromatin and gene expression. We compiled a comprehensive lncRNA HemAtlas in human blood cells by sampling RNA from differentiated granulocytes, monocytes, erythroid precursors, in vitro maturated megakaryocytes, CD4-T and CD8-T cells, NK cells, B cells and stem cells (human CD34+ cord blood hematopoietic stem and progenitor cells [CB-HSPCs]) and subjected them to microarray analysis of mRNA and lncRNA expression.

The complete microarray profiling of the differentiated cells was performed on two separate array platforms. Cross-platform integration yielded a total of 4523 probe sets, which clearly distinguished myeloid lineage identity. Of those, >1000 lncRNAs were more than 10-fold differentially expressed between human blood lineages. Thus, a core fraction of lncRNAs is modulated during differentiation. Hierarchical clustering determined the top 50 lineage-specific lncRNAs for each blood cell lineage in both species.

For human megakaryocytes, erythroid cells, monocytes, granulocytes and HSPCs we validated four lincRNA candidates, respectively, to be specifically expressed by qRT-PCR. Knockdown of monocyte-specific lincRNA LINCMONO1 resulted in more than 50% reduction of myeloid colony-forming units (CFUs) in colony assays. Likewise, the total CFU counts were reduced more than 50% upon knockdown of megakaryocyte-specific LINCMEGA1. Knockdown of all four granulocyte-specific lincRNAs perturbed granulocytic in vitro differentiation as assessed by the percentage of CD66b+/CD13+ granulocytes and cellular morphology (MGG-stained cytospins). Erythroid-specific transcript LINCERY1 showed 25 to 50% reduction in burst-forming units in colony assays and decreased proliferation in liquid culture differentiation.

Integration of miRNA:lncRNA datasets yielded direct and inversely-correlating expression of gene pairs that form competing endogenous RNA (ceRNA) modules for lineage specification. One ceRNA node in granulocytes was functionally validated by miRNA inhibition and overexpression experiments inferring coding-non-coding transcript competition for the miRNAs.

Thus, our study provides a global human hematopoietic lncRNA expression resource and defines blood-lineage specific lncRNA marker and regulator genes. Moreover, we provide evidence for an extensive ceRNA network during hematopoiesis and outline the consequence on miRNA:lncRNA interaction during granulopoiesis.

Poster M-VI-05

Regulation of RUNX1 activity by the protein arginine methyltransferase 6.

Olga N Kuvardina[1], Julia Herglotz[2], Stefanie Herkt[1], Stephan Kolodziej[1], Jörn Lausen[1]

[1] Georg-Speyer-Haus Institute for tumor biology and experimental therapy, Paul-Ehrlich-Strasse 42-44, 60596 Frankfurt, Germany
[2] Heinrich-Pette-Institute Leibniz Institute for Experimental Virology, Martinistraße 52, 20251 Hamburg, Germany

The transcription factor RUNX1 (AML1, acute myeloid leukaemia 1)) is crucial for the development of definitive hematopoietic stem cells (HSC). RUNX1 activity is altered in human leukaemia by mutation, deletion and chromosomal translocation. Furthermore, haploinsufficiency of RUNX1 leads to familial platelet disorder in humans (FPD/AML). The recruitment of chromatin modifying co-factors by RUNX1 can lead to alterations in chromatin structure and mediate epigenetic changes of gene expression.

We could show that RUNX1 interacts with the protein arginine methyltransferase 6 (PRMT6). Our results demonstrate that PRMT6 is able to methylate histone tails and contributes to the histone code that is determining the epigenetic state of the given locus. We are examining the molecular influence of PRMT6 on RUNX1 and investigate if PRMT6 can be a molecular target for a therapeutic intervention of RUNX1 dependent leukaemia.

References:

Herglotz, J.; Kuvardina, O.N.; Kolodziej, S.; Kumar, A.; Hussong, H.; Grez, M.; Lausen, J. Histone arginine methylation keeps RUNX1 target genes in a repressed but poised state in human hematopoietic progenitor cells. 2013, Oncogene, 32(20): 2565-75.
Lausen, J. Contributions of the Histone Arginine Methyltransferase PRMT6 to the Epigenetic Function of RUNX1. 2013, Crit. Rev. Eukaryot Gene Expr.: 23(3):265-74. Review

Poster M-VI-06

Genome-wide methylation profiling by MCIp-Seq reveals aberrant DNA methylation patterns in childhood MDS with GATA2-deficiency

Sandra Pohl[1], Daniel Heudobler[1], Claudia Gebhard[1], Charlotte Niemeyer[2], Marcin Wlodarski[2], Michael Rehli[1]

[1] University Hospital Regensburg Internal Medicine III, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany
[2] University of Freiburg Pediatric Hematology and Oncology, Mathildenstraße 1, 79106 Freiburg, Germany

Myelodysplastic syndromes comprise a heterogeneous group of clonal hematopoietic disorders characterized by bone marrow failure, clonal and ineffective hematopoiesis as well as an increased risk to develop AML. While de novo MDS is predominantly found in elderly patients, childhood MDS is rather rare and often associated with genetic disorders and inherited bone marrow failure syndromes. Recently, germline mutations in the hematopoietic transcription factor GATA2 were identified in familial cases of MDS. Epigenetic alterations represent a major pathogenetic mechanism in MDS and have been extensively researched in adults, while genome-wide studies on deregulated epigenetic processes in childhood MDS are still lacking. The goal of the presented study was to analyze aberrant DNA methylation in familiar childhood MDS by genome-wide methylation profiling. So we obtained DNA methylation profiles across the genome in a cohort of childhood MDS samples as well as monocytes from healthy control donors using methyl-CpG-immunoprecipitation combined with next-generation sequencing. The MDS cohort consisted of 35 patients (17 female, 18 male; median age at diagnosis: 10.2 years; range 4-19) carrying different mutations in GATA2 (except for two siblings with an identical mutation).

Our analysis showed that these 35 MDS patients exhibit methylation profiles distinct from healthy controls. Based on the degree of methylation, MDS patients could be clustered into subgroups with distinctive patterns. The subgroup with the highest methylation degree was exclusively composed of patients with advanced morphological subtype (RAEB or RAEB-t) at diagnosis and aneuploidic karyotypes: monosomy 7 or trisomy 8. Mutations in GATA2 were seen in patients with low or intermediate methylation degree, showing only a conditional correlation. Moreover we could observe a significant overrepresentation of monosomy 7 and ASXL1 mutations in patients with intermediate methylation degree. Computational analyses of DNA methylation data revealed different clusters of regions associated with specific epigenetic signatures in hematopoietic (HSCs) and embryonic stem cells (ESCs). We present the first genome-wide analyses of aberrant DNA methylation in familiar childhood MDS sharing predisposing mutations in GATA2 as well as without GATA2 mutations. Our data suggest that DNA methylation changes might be associated with morphologic subtype and disease progression but the correlation with GATA2 has to be elucidated.

OTHERS

Poster S-VII-01

Case report: Erdheim-Chester disease (ECD) presenting with isolated bone lesions treated with combination of alfa-interferon and zoledronic acid

Mikhail Zarayskiy[1], Galina Salogub[1,2], Ekaterina Bulycheva[1,2], Vadim Baykov[1], Eugene Shlyakhto[1,2]

[1] First Pavlov State Medical University, Saint-Petersburg, Russia
[2] Federal Heart, Blood and Endocrinology named VA Almazov, Saint-Petersburg, Russia

A 28 years old man presented with complaints of pain in lower back and ribs. He has a history of Hodgkin lymphoma at the age of 9 with complete remission. His present complaints started at the age of 23, and 3 years later the osteolytic bone lesions were found on CT. No data of tuberculous ostititis was obtained. He underwent a surgical resection of the S1 and spinal fusion; 6 months later the tunnel resection and radiothermoablation in thoracic vertebra were performed. Histological investigation revealed the foci of fibrosis with xanthome and multinuclear giant cells. These cells were CD68+, CD1a-, less than 5% of them were S100+, factor XIIIa was expressed in 10-15% of the cells. Such characteristics led us to the idea of ECD. At the PET-scan we found multiple foci of increased FDG uptake in vertebra, ribs, pubic and iliac bones. Additional examination revealed autoimmune thyroiditis, other systems and organs were otherwise well. The BRAF V600E+ mutation was detected in bone marrow and peripheral blood, but not in the FNAB of thyroid gland.

We started the therapy with alfa-interferon (Inf) 3 million U 3 times/week and zoledronic acid (4 mg/month). This therapy is now well tolerated with only moderate flu-like syndrome, controlled by paracetamol, and mild neutropenia, which does not require CSF. Six months after the start of the therapy our patient is pain free and his overall quality of life has now much increased. PET-scan was revealed in improvement in the course of disease.

Our case report represents an early diagnosed ECD with unusual spine and pelvis involvement and without extraskeletal manifestations. It is of interest, that the patient had a lymphoproliferative disease in his childhood, but the relationship between two diseases remains to be elucidated. An early diagnosis is important in order to start the therapy, which may improve prognosis and survival. As it was shown that Inf enhances the terminal differentiation of histiocytes and dendritic cells and many clinical cases have shown its efficacy, administration of this drug in our patient seemed to be a logical promising treatment. Due to a lack of other systemic involvement we also combined Inf with the aminobisphosphonate, based on good clinical outcomes in previously described patients receiving this therapy. Our clinical case shows a very good response and tolerability of this treatment combination, implying further investigation of its safety and duration of response.

Poster S-VII-02

Screening analysis of gene mutations ERBB2, EGFR, FGFR3, BRAF, PIK3CA, KRAS and GATA3 using the protocol PCR-HRMC

Antonina Artemeva, Mikhail Zarayskiy, Aleksey Sazanov, Aleksandr Ginzburg, Kseniya Dvoreckaya, Elena Kiseleva, Irina Habarova, Vladimir Davyidenko, Vladimir Emanuel

First Pavlov State Medical University of St-Petersburg, Lev Tolstoy 6-8, 197022 Saint-Petersburg, Russia

Introduction:
Detection of the genetic mutations in biopsies obtained from cancer patients plays a crucial role in the diagnostics of different tumor types. The introduction of various modifications of standard molecular genetic techniques such as protocol of HRMC (high-resolution „melting curve") should be in high demand in the clinical-diagnostic process. Despite of medical advances, there is the steady increase of colorectal cancer's morbidity in the world, the mortality from which is at the second place among malignant tumors in all localizations. In this regard, there are 2 extremely important aspects: the problem of early tumor detection and the research of molecular characteristics of identified tumors that would help to facilitate the diagnosis, prognosis and options of treatment.

Aim. Development of the sensitive protocol for screening mutations in patients with colorectal cancer.

Materials and methods:
There were selected 7 samples of the colon cancer and 6 samples of the rectal cancer for the research. All samples were obtained intraoperatively or endoscopically from the focus of the tumor and had histological description. There were two cell lines: SW837 (rectal adenocarcinoma) and Caco-2 (colon adenocarcinoma) as positive control. The algorithm included: a) PCR in presence of the EvaGreen dye with primers, flanked the mutable regions of genes ERBB2, EGFR, FGFR3, BRAF, PIK3CA, KRAS and GATA3, c) HRMC protocol (0,1C/1 sec). The fact of mutation was recorded in identifying complementary peaks with a melting point lower than shows the normal amplicons.

Results:
The investigation of samples, it was shown that: (a) in Caco-2 were identified mutations in genes ERBB2 and KRAS, (b) similar damages were revealed in samples of tumor, but in addition to them the same mutations identified in the genes FGFR3, BRAF GATA3, (c) in the SW837 were identified mutations in genes ERBB2, EGFR, BRAF and KRAS. Besides this mutations have been identified in the genes PIK3CA and GATA3 in samples from rectal tumors. Samples with mutations have been selectively sequenced.

Conclusion:
Thus, it is shown that, a) the protocol is sensitive and reproducible for the detection of mutations in routine PCR laboratories, b) adenocarcinoma of the rectum and colon has specific profiles mutations that may need to be considered when choosing therapy and observation in the postoperative period.

Poster S-VII-03

Structural insight into the interactions of CTNNBL1 with the spliceosome

Febe van Maldegem, Sarah Maslen, Chris Johnson, Anita Chandra, Mark Skehel, Michael Neuberger, Cristina Rada

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, CB2 0QH, Great Britain

We've previously shown that the DNA mutator AID, the enzyme essential for somatic hypermutation and class switch recombination but also implicated in lymphomagenesis, interacts with CTNNBL1. CTNNBL1 knockout mice were embryonic lethal, but lineage specific ablation of CTNNBL1 in B cells resulted in a slow to exit from quiescence upon activation and reduced class switching. Targeted inactivation of CTNNBL1 in DT40 B cells also considerably diminished IgV diversification, altogether suggesting a role for CTNNBL1 in the regulation of AID activity.

CTNNBL1 is an armadillo-repeat protein that associates with the CDC5L/Prp19 complex of the spliceosome. Structurally CTNNBL1 is quite distinct from its closest homologue karyopherin-a, and while interaction with CDC5L also occurs through its NLS region, mutant analysis demonstrates that the mode of binding by CTNBBL1 is different from that of karyopherin-a with its cargos. Moreover, interaction with CWC15, another member of the CDC5L/Prp19 complex, does not seem to be dependent on charge like is the case for NLSes. In this work we have mapped the interactions of CTNNBL1, CWC15 and CDC5L within this subcomplex and explored the relevance of these interactions in vivo.

* FvM was supported by a Wilsede travel fellowship from the Leukaemia and Lymphoma Research, UK
** This work was supported by a grant from the Leukaemia and Lymphoma Research, UK

Poster S-VII-04

Maintenance of respiration capacity is a predictor of drug resistance in cancer cells

Katharina Henkenius, Melanie Maerken, Tom Kaiser, Larissa Greif, Miriam Rehberger, Stefan Metzelder, Cornelia Brendel, Andreas Neubauer

Philipps University Marburg and Medical Center of the Universities Giessen and Marburg Hematology, Oncology and Immunology, Baldingerstrasse, 35033 Marburg, Germany

Inherent or acquired drug resistance is the major obstacle in cancer therapy. An individual prediction of response to cancer therapy would be highly appreciated. It has been reported that the mitochondrial function of cancer cells is a crucially important factor for the treatment response in different cancer entities (Chonghaile et al. 2011, Vo et al. 2012). However, the method of BCL-2 peptide priming described by these investigators is rather complicated and error-prone in our hand. Therefore we sought to determine mitochondrial function by analyzing cellular respiration. We established three different therapy-resistant human cancer cell lines (MV4-11, HL-60 and NCl-H82). Resistant MV4-11 cells that possess an activating FLT3 internal tandem duplication mutation were obtained by chronic exposure to Sorafenib, a tyrosine kinase inhibitor, resistant HL-60 cells by chronic exposure to the chemotherapeutic substance Arabinofuranosyl Cytidine (Ara-C) and resistant NCl-H82 cells by chronic exposure to Etoposid (VP-16) with increasing dose levels. Cellular respiration was measured by examining their oxygen consumption rate (OCR) with the XF96 extracellular Flux Analyzer. At the time of OCR measurement (after 24 hours incubation), viability and membrane potential of the cells were concomitantly determined by flow cytometry using 4', 6-diamidino-2-phenylindole (DAPI) or Tetramethylrhodamine, ethyl ester (TMRE), respectively. It was clearly obvious that resistant cancer cells do not change their respiration capacity under the influence of tyrosine kinase inhibitors, chemotherapeutics or topoisomerase inhibitors, respectively, whereas the respiration of sensitive cancer cells decreases strongly. Moreover, we found a metabolic shift towards glycolysis in resistant cancer cells under cytotoxic agent treatment. In contrast sensitive cancer cells were metabolically more inactive after treatment. This decline in respiration and glycolysis of sensitive cancer cells occurred far before the onset of apoptosis and energy metabolism, which also lead to decreased cellular functions. These data indicate cellular respiration as an early, sensitive and reliable surrogate parameter of mitochondrial function that might be able to measure prospectively sensitivity or resistance of primary cancer cells to cancer therapy.

POSTER ABSTRACTS
Others

Poster S-VII-05

Effect of heparin and chemotherapy on global haemostasis tests during blood stem cell mobilization in multiple myeloma patients

Marina Gracheva[1], Evdokiya Urnova[2], Elena Sinauridze[3], Ivan Tarandovskiy[3], Olga Pokrovskaya[2], Larisa Mendeleeva[2], Valery Savchenko[2], Fazoil Ataullakhanov[1,3,4], Anna Balandina[1,4]

1 Federal Scientific Clinical Centre of Pediatric Hematology, Oncology and Immunology named after Dmitry Laboratory of Biophysics, 1 Samory Mashela Street, 117198 Moscow, Russia
2 National Research Center for Hematology Department of Chemotherapy of Hemoblastoses and Bone Marrow Transplantation, Novozykovskij proezd., 4a, 125167 Moscow, Russia
3 National Research Center for Hematology Laboratory of Physical Biochemistry of Blood, Novozykovskij proezd., 4a, 125167 Moscow, Russia
4 Center for Theoretical Problems of Physicochemical Pharmacology RAS, Kosygin st., 4, 119991 Moscow, Russia

Background:
Patients with Multiple Myeloma (MM) have an increased risk of venous thromboembolism (VTE) during chemotherapy (CT). To prevent thrombotic complications during MM treatment anticoagulant therapy is carried on but despite of aPTT using for preventive heparin therapy monitoring still in some cases VTE occurs [1]. Global tests have proven to be more sensitive to heparin than aPTT[2,3]. We used global tests for laboratory control of haemostasis in MM patients for subsequent adjustment of anticoagulant therapy to be carried on. Objectives: To assess effectiveness of anticoagulant therapy and protrombotic changes in haemostatic state of patients with MM using global haemostasis tests during blood stem cell (BSC) mobilization on preventive anticoagulant therapy and after CT.

Methods:
34 MM patients with very good partial or complete remissions before and after CT during BSC mobilization were enrolled in this study. They got cyclophosphamide CT (4 g/m2), G-CSF (5 ug/kg/day) injections accompanied with continuous preventive heparin therapy (500 IU/hour). Global tests Thromboelastography (TEG), Thrombin Generation Test (TGT), Thrombodynamics (TD) were used to assess haemostasis state: 1 – before BSC mobilization, 2 – on continuous heparin infusion before CT, 3 – after CT.

Results:
ll used global tests detect significant (*p<0.05) changes toward hypocoagulability in response to preventive unfractionated heparin therapy (500 IU/h): alpha in TEG was 27±11°vs.41±10° before heparin infusion (mean±SD), Amax in TGT was 152±90 nM vs. 231±60nM, Vst was 19±7 um/min vs. 29±6 um/min. Resistance to heparin was revealed at least by one global test in 20% of patients. Amax in TGT and Vst in TD were significantly increased after chemotherapy to values 192±74 nM, and 23±8 um/min respectively (normal ranges are Amax= 206±34 nM and Vst=25±3 um/min). TEG and clotting tests revealed no changes after CT. Hypercoagulability was detected at least by one global test in 60% of patients after chemotherapy despite of heparin treatment. Conclusions: In MM patients global coagulation tests TEG, TGT and TD are sensitive to preventive heparin therapy. TGT and TD revealed procoagulant changes after CT during BSC mobilization.

The work was supported by RFBR grants 14-04-00670, 12-04-00652, 14-04-31055, 12-04-00438,13-04-00401.

References

1. Kristinsson SY. 2010; 437-44. Doi: 10.1182/asheducation-2010.1.437.
2. Al Dieri R et.al. 2004 Aug; 2(8):1395-401.
3. Lee BY et.al. World J Surg. 1980 May; 4(3):323-30.

Poster S-VII-06

The role of LMO2 in the onset of haematopoiesis

Vesna Stanulovic, Pierre Cauchy, Constanze Bonifer, Maarten Hoogenkamp

University of Birmingham Institute of Biomedical Research, Vincent Drive, Birmingham, B15 2TT, Great Britain

LMO2 was originally identified due to its role in T cell acute lymphoblastic leukaemia (T-ALL) after genomic translocations, bringing the T cell receptor genes in close proximity of its promoter. This leads to overexpression of LMO2 in T-cell progenitors, resulting in the development of T-ALL. In addition LMO2 is overexpressed in a significant proportion of B-ALL and AML. It is therefore not surprising that LMO2 has an essential role in normal haematopoieis as well. It is a protein that does not bind DNA directly, but is a core component of a DNA binding complexes, where LMO2 bridges between the haematopoietic transcription factors Tal1/E2a and GATA, and binds to Ldb1. The latter has been shown to be required for long range interactions between regulatory elements such as enhancers and promoters.

It has been shown that LMO2 knock-out (LMO2-/-) mice die during embryonic development due to a complete lack of yolk sac erythropoiesis. Subsequent experiments with chimaeric mice showed that LMO2-/- cells also cannot contribute to definitive haematopoiesis. Our current research utilises an in vitro mouse embryonic stem cell (mES) differentiation system where mES cells are differentiated into haemangioblasts, which are Flk-1 (VEGF receptor 2) positive cells of mesodermal origin, and further into haemogenic endothelium (HE), which is specified endothelium that gives rise to the definitive haematopoietic progenitor cells. Gene expression analysis showed us that LMO2 becomes expressed at the Flk-1+ stage and remains expressed into the haematopoietic progenitors. We have found that LMO2-/- mES cells can differentiate into haemangioblasts, but fail to produce HE. We are now using this system to elucidate the role of LMO2 at this block in haematopoietic differentiation. We are combining genome wide DNaseI hypersensitivity analysis to recognise active regulatory regions, ChIP-sequencing to determine direct LMO2 targets and RNA-sequencing to understand the effects on the gene transcript level. In addition we are trying to identify the proteins that are in the LMO2 complex by mass spectrometry. Our poster presents the latest integrated analysis of these data.

Poster S-VII-07

Advances in small animal radiotherapy – From bedside to bench and backward

Thorsten Frenzel[1,2], Carsten Grohmann[1], Kristoffer Riecken[3], Siekmann Jordana[1], Andreas Krüll[2], Boris Fehse[3], Udo Schumacher[1]

[1] Universitätsklinikum Hamburg-Eppendorf Zentrum für Experimentelle Medizin, Institut für Anatomie und Experimentelle Morphologie, Martinistraße 52, 20246 Hamburg, Germany
[2] Universitätsklinikum Hamburg-Eppendorf Ambulanzzentrum des UKE GmbH, Bereich Strahlentherapie, Martinistraße 52, 20246 Hamburg, Germany
[3] Universitätsklinikum Hamburg-Eppendorf Klinik für Stammzelltransplantation, Forschungsabteilung Zell- und Gentherapie, Martinistraße 52, 20246 Hamburg, Germany

In a mouse model for xenograft tumors we analyzed the impact of radiation therapy (RT), radio-chemotherapy (RChT), and chemotherapy (ChT) on the number of metastasis in the blood, bone marrow or lungs of the animals. RGB-marking of the tumor cells prior to transplantation allows cell tracking and clonality analyses based on fluorescence.

Methods and Materials:
A partial body RT device for mice has been created based on an industrial X-ray tube. Four cell lines of two tumor entities were selected for transplantation: small cell bronchial carcinoma (SCID: OH1, H69) and prostate cancer (pfp/rag2: LNCaP, PC3). After s.c. injection, these cell lines develop a local tumor node as well as spontaneous metastases in SCID or pfp/rag2 mice. Five groups of mice were used per cell line: control group 1 (prior to therapy), RT, RChT, ChT, control group 2 (after therapy). ALU-PCR was used to quantify the number of tumor cells in blood, lungs, and bone marrow of the animals. Histological examinations of primary tumor and lungs were done.

Results:
Our X-ray tube allows precise partial body irradiation of mice. The analysis of the first RGB-marked primary tumors of 14 SCID mice (OH1) showed that RT decreases the number of fluorescent cells in the primary tumor and more macrophages show up at the tumor site. Dose finding studies lead to the following regimen, starting when small tumors are palpable: - RT: 5 x 10 Gy (d1-5)- ChT: SCID mice Cisplatin d1 6 mg/kg. pfp/rag2 mice Docetaxel 20 mg/kg d1,8,15 Preliminary results of 25 pfp/rag2 mice (5 per subgroup) injected with LNCaP cells showed tumor cells in the blood or in the bone marrow only in the groups with no therapy. Most lung metastases were found in animals without therapy, but also fewer ones in treated animals, best therapy was: RChT.

Discussion and Conclusion:
We created a precise instrument for partial body RT of mice. We are very close to the treatment settings used in human patients: human tumor cell lines, metastasis like in humans, same radiation doses and chemotherapeutic regimes like for human patients. Our preliminary results demonstrate an influence of the therapy on the development of distant metastasis, which will be further analyzed. So far more than 200 of 400 mice have been treated.

Acknowledgements

This project was supported by the "Deutsche Krebshilfe Mildred-Scheel-Doktorandenprogramm" and the "UCCH 1/3-Stipendium".

References

Frenzel T, Grohmann C, Schumacher U, Krüll A. Partial body irradiation of small laboratory animals with an industrial X-ray tube. ZMedPhys. 2014 (in press)

Poster S-VII-08

Triphenylphosphonium salts with varying length of the hydrophobic tail induce cell cycle arrest and apoptosis in hematopoietic cells.

Nadezhda Bilko
NaUKMA. Kiev, Ukraine

Effectiveness of the complete eradication of the malignant clone during hematopoietic stem cell transplantation is an important factor for determining effectiveness of the cell therapy and long term event free survival. Induction of apoptosis is a preferred mode of cell eradication, therefore search for the optimal and specific inducers of apoptosis, selective for the metabolically active and rapidly dividing cells is an important direction of research. In the current work we investigated apoptosis inducing properties of tri phenyl phosphonium salts, modified with the varying length of the hydrophobic tails that ranged from 6 to 16 carbons. It was shown that the compound with the longest saturated hydrophobic tail that contained sixteen carbons showed most effective induction of the apoptosis at low micromolar concentration, while high nanomolar concentration were able to induce arrest of the cell division that resulted in significant reduction in the CFU activity, when compared to the control. Micromorphological SEM analysis of the cells exposed to the low doses of tri phenyl prosphonium salts indicated presumable damage to the mitochondria, but this needs to be further confirmed. Metabolically active cells seem to be more susceptible to the effects of the investigated compound and prone to the induction of apoptosis and damage to the mitochondria would suggest possible induction pathway, based on the cytochrome C release and caspase cascade induction. Further studies of the effects of these compounds in vitro on normal and pathological hematopoietic cells will provide more information required for determination of the mechanisms of action and may result in development of the specific apoptosis inducing targeted therapy.

Keywords: Hematopoietic cells, apoptosis, phosphonium salts

LIST OF PARTICIPANTS

All information pursuant to the registration form

A

Abken, Hinrich
Universität zu Köln
Klinik I für Innere Medizin und
Zentrum für Molekulare Medizin Köln
Robert-Koch-Str. 21
50931 Cologne – Germany

Abramowski, Pierre
Universitätsklinikum Hamburg-Eppendorf
Onkologisches Zentrum
Martinistraße 52
20246 Hamburg – Germany

Appelmann, Iris
Universitätsklinik der RWTH Aachen
Klinik für Onkologie,
Hämatologie und Stammzelltransplantation
Pauwelstraße 30
52074 Aachen – Germany

Aranyossy, Tim
University Medical Centre Hamburg-Eppendorf Forschungsabteilung Zell- und Gentherapie
Martinistraße 52
20246 Hamburg – Germany

Araujo Cruz, Michelle Maria
Medizinische Hochschule Hannover
Dept. Hematology and Oncology
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Artemeva, Antonina
First Pavlov State Medical University
L. Tolstoy Street 6-8
197022 St. Petersburg – Russia

B

Bach, Christian
University Hospital Erlangen
Hematology/Oncology
Ulmenweg 18
91054 Erlangen – Germany

Barkhatov, Ildar
R. M. Gorbacheva
Memorial Institute of Children Oncology,
Hematology and Transplantation
Rentgen Str. 12
199022 St. Petersburg – Russia

Beck, Dominik
Prince of Wales Clinical School
Lowy Cancer Research Centre
PO Box 81 NSW
2031 Randwick – Australia

Beerlage, Astrid
University Medical Clinic of Tübingen
Section for Transplantation, Immunology
and Immunohematology
Waldhörnlestraße 22
72074 Tübingen – Germany

Behrens, Kira
Heinrich-Pette-Institut
Martinistraße 52
20251 Hamburg – Germany

Beltinger, Christian
University Medical Center Ulm
Dept. of Pediatric & Adoloscent Medicine
Eythstraße 24
89075 Ulm – Germany

Berdien, Belinda
Universitätsklinikum Hamburg-Eppendorf
Martinistraße 52
20246 Hamburg – Germany

Berg, Tobias
Klinikum der Johann Wolfgang Goethe-Universität
University Cancer Center (UCT) Frankfurt
Theodor-Stern-Kai 7
60590 Frankfurt – Germany

Bigildeev, Aleksei
Ministry of Health
Hematological Scientific Centre
Noviy Zikovskiy
125167 Moscow – Russia

Bilko, Nadiia
National University of Kyiv Mohyla Academy
Skovorody Str. 2
Kyiv – Ukraine

Bilko, Denys
National University of Kyiv Mohyla Academy
Skovorody Str. 2
Kyiv – Ukraine

Bonifer, Constanze
University of Birmingham
College of Medical and Dental Sciences
Institute of Biomedical Research
School of Cancer Sciences
B15 2TT Birmingham – Great Britain

Brabletz, Thomas
University of Freiburg
Medical Center
Dept. of Visceral Surgery
Comprehensive Cancer Center
Hugstetter Str. 55
79095 Freiburg – Germany

Braig, Friederike
Universitätsklinikum Hamburg-Eppendorf
2. Medizinische Klinik
Martinistraße 52
20246 Hamburg – Germany

Brandts, Christian
Klinikum der
Johann Wolfgang Goethe-Universität
University Cancer Center (UCT) Frankfurt
Theodor-Stern-Kai 7
60590 Frankfurt – Germany

Breitbach, Caroline
Jennerex Inc. Clincial & Translational Research
450 Sansome St, Apt 406
CA 94109 San Francisco – USA

Brennig, Sebastian
Hannover Medical School
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Buchholz, Christian
Paul-Ehrlich-Institut
Molecular Biotechnology and Gene Therapy
Paul-Ehrlich-Str. 51-59
63225 Langen – Germany

C

Carotta, Sebastian
Boehringer Ingelheim RCV GmbH & Co KG
Dr.-Boehringer-Gasse 5-11
1121 Wien – Austria

Cauchy, Pierre
University of Birmingham
College of Medical and Dental Sciences
Vincent Drive
B15 2TT Birmingham – United Kingdom

Chaturvedi, Anuhar
Medizinische Hochschule Hannover
Dept. Hematology and Oncology
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Cockerill, Peter
University of Birmingham
School of Immunity and Infection
Institute of Biomedical Research
College of Medical and Dental Sciences
B15 2TT Birmingham – Great Britain

Cornils, Kerstin
Universitätsklinikum Hamburg-Eppendorf
Martinistraße 52
20246 Hamburg – Germany

D

Delwel, Ruud
Erasmus University Medical Center
Department of Hematology
Doctor Molewaterplein 50-60
Rotterdam – The Netherlands

Diaz-Carballo, David
Marienhospital Herne
Institute for Molecular Oncology
Düngelstraße 33
44623 Herne – Germany

E

Emmrich, Stephan
Hannover Medical School
Pediatric Hematology & Oncology
Carl-Neuberg-Straße 1
30625 Hannover – Germany

F

Falk, Christine S.
Medizinische Hochschule Hannover
Integriertes Forschungs-
und Behandlungszentrum Transplantation
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Falkenburg, J. H. Fred
Leids Universitair Medisch Centrum
Albinusdreef 2
2333 ZA Leiden – The Netherlands

Fehse, Boris
Universitätsklinikum Hamburg Eppendorf
Interdisziplinäre Klinik und Poliklinik
für Stammzelltransplantation
Martinistraße 52
20246 Hamburg – Germany

Fiedler, Walter
Universitätsklinikum Hamburg Eppendorf
II. Medizinische Klinik und Poliklinik
Martinistraße 52
20246 Hamburg – Germany

Fischer, Stefanie
Universitätsklinikum Hamburg Eppendorf
Interdisziplinäre Klinik und Poliklinik für
Stammzelltransplantation
Martinstraße 52
20246 Hamburg – Germany

Frenzel, Thorsten
Universitätsklinikum Hamburg-Eppendorf
Klinik für Strahlentherapie und Radioonkologie
Martinistraße 52
20246 Hamburg – Germany

G

Geiger, Hartmut
Universität Ulm
Institut für Molekulare Medizin
James-Franck-Ring 11c
89081 Ulm – Germany

Ghiaur, Gabriel
John Hopkins University
1650 Orleans Street
21287 Baltimore – USA

Glauche, Ingmar
Universitätsklinikum Carl Gustav Carus
Institut für Medizinische Informatik und Biometrie
Fetscherstraße 74
01307 Dresden – Germany

LIST OF PARTICIPANTS

All information pursuant to the registration form

Goparaju, Ramya
Medizinische Hochschule Hannover
Dept. Hematology and Oncology
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Gracheva, Marina
Samory Mashela Street
117198 Moscow – Russia

Green, Tony
University of Cambridge
Department of Haemotology
Cambridge Institute of Medical Research
Hills Road
CB20XY Cambridge – United Kingdom

Grez, Manuel
Georg-Speyer-Haus
Institut für Tumorbiologie
und experimentelle Therapie
Paul-Ehrlich-Straße 42
60596 Frankfurt – Germany

Grunert, Michaela
Dr. von Haunersches Kinderspital
Lindwurmstraße 4
80337 München – Germany

Grupp, Stephan
Children's Hospital of Philadelphia Oncology/BMT
CTRB 3006 3501
Civic Center Blvd.
PA 19104-4318 Philadelphia – USA

H

Hansmann, Martin-Leo
Klinikum der
Johann Wolfgang Goethe-Universität
Institut für Pathologie
Theodor-Stern-Kai 7
60590 Frankfurt – Germany

Heckl, Dirk
Hannover Medical School
Experimental Hematology
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Heinrichs, Stefan
University Hospital Essen
Institute for Transfusion Medicine
Virchowstraße 179
54147 Essen – Germany

Henkenius, Katharina
Philipps-Universität Marburg
Baldingerstraße 1
35043 Marburg – Germany

Heuser, Michael
Medizinische Hochschule Hannover
Hämatologie, Hämostaseologie, Onkologie
und Stammzelltransplantation
Carl-Neuberg-Str. 1
30625 Hannover – Germany

Hoogenkamp, Maarten
University of Birmingham
Institute of Biomedical Research
Vincent Drive
B15 2TT Birmingham – United Kingdom

Horn, Stefan
University Medical Centre Hamburg-Eppendorf Clinic
for Stem Cell Transplantation
Martinistraße 52
20246 Hamburg – Germany

Huang, Kezhi
Hannover Medical School
Institute of Experimental Hematology
Carl-Neuberg-Straße
10625 Hannover – Germany

Hudecek, Michael
Universitätsklinikum Würzburg
Medizinische Klinik II – Hämatologie
Oberdürrbacherstrasse 6
97080 Würzburg – Germany

J

Jeremias, Irmela
Dr. von Haunersches Kinderspital
Abteilung Hämatologie und Onkologie
Lindwurmstraße 4
80337 München – Germany

Jing, Duohui
Children's Cancer
Institute Australia for Medical Research
Lowy Cancer Research Centre
PO Box 81
NSW 2031 Randwick, Sydney – Australia

K

Kalthoff, Holger
UKSH, Campus Kiel
Institut für Experimentelle Tumorforschung (IET)
Sektion Molekulare Onkologie
Arnold-Heller-Straße 3 (Haus 17)
24105 Kiel – Germany

Kant, Jill-Sandra
University Medical Centre Hamburg-Eppendorf
Forschungsabteilung Zell- und Gentherapie
Martinistraße 52
20246 Hamburg – Germany

Khan, Anjum
University College London
Institute of Immunity and Transplantation
Royal Free Hospital
NUB 2QG London – United Kingdom

Kharabi Masouleh, Behzad
Universitätsklinik der RWTH Aachen
Klinik für Onkologie, Hämatologie und
Stammzelltransplantation
Pauwelstraße 30 52074 Aachen – Germany

Klapper, Wolfram
Universitätsklinikum Schleswig-Holstein,
Campus Kiel, Sektion Hämatopathologie
und Lymphknotenregister
Michaelisstr. 11
24105 Kiel – Germany

Klein, Jacqueline
Marienhospital Herne
Institute for Molecular Oncology
Düngelstraße 33
44623 Herne – Germany

Kloos, Arnold
Medizinische Hochschule Hannover
Hämatologie, Hämostaseologie,
Onkologie und Stammzelltransplantation
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Klump, Hannes
University Hospital Essen
Institute for Transfusion Medicine
Virchowstraße 179
54147 Essen – Germany

Klusmann, Jan-Henning
Medizinische Hochschule Hannover
Hämatologie, Hämostaseologie,
Onkologie und Stammzelltransplantation
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Koeffler, Phillip
Centre for Translational Medicine
14 Medical Drive #12-01
117599 Singapore – Singapore

Korf, Katharina
Research Institute
Childrens Cancer Center Hamburg
Martinistraße 52
20251 Hamburg – Germany

Kröger, Nicolaus
Universitätsklinikum Hamburg Eppendorf
Interdisziplinäre Klinik und Poliklinik
für Stammzelltransplantation
Martinistraße 52
20246 Hamburg – Germany

Küppers, Ralf
Universitätsklinikum Essen
Institut für Zellbiologie
Virchowstraße 173
45122 Essen – Germany

L

Laer von, Dorothee
Medizinische Universität Innsbruck
Sektion für Virologie
Peter-Mayr-Straße 4b
6020 Innsbruck – Austria

Lang, Fabian
Klinikum der
Johann Wolfgang Goethe-Universität Frankfurt
Abt. für Hämatologie und Onkologie
Theodor-Stern-Kai 7
60590 Frankfurt – Germany

Latuske, Emily-Marie
Universitätsklinikum Hamburg-Eppendorf
Medizinische Klinik und Poliklinik,
Onkologie-Hämatologie
Martinistraße 52
20246 Hamburg – Germany

Lausen (Kuvardina), Olga N.
Georg-Speyer-Haus
Paul-Ehrlich-Straße 42-44
60596 Frankfurt – Germany

Lebedev, Timofey
Engelhardt Institute of Molecular Biology
Russian Academy of Sciences
32, Vaviola Str.
119991 Moscow – Russia

Li, Zhixiong
Hannover Medical School
Institute of Experimental Hematology
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Lioznov, Michael
medac GmbH
Vosshagen 89
22880 Wedel – Germany

Lock, Richard B.
Children's Cancer
Institute Australia for Medical Research
Lowy Cancer Research Centre
PO Box 81
NSW 2031 Randwick, ----Sydney – Australia

M

Maerken, Melanie
Philipps-Universität Marburg
Baldingerstraße 1
35043 Marburg – Germany

Maldegem van, Febe
MRC Laboratory of Molecular Biology
Francis Crick Avenue
CB2 0QH Cambridge – United Kingdom

LIST OF PARTICIPANTS

All information pursuant to the registration form

Manukjan, Georgi
Medizinische Hochschule Hannover
Institut für Zell- und Molekularpathologie
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Martín-Subero, José
University of Barcelona
Centre Esther Koplowitz
C/Roselló 153, 2nd Floor
08036 Barcelona – Spain

März, Manuela
Universitätsklinikum Hamburg-Eppendorf
2. Medizinische Klinik
Martinistraße 52
20246 Hamburg – Germany

Masqué-Soler, Neus
Universitätsklinikum Schleswig-Holstein,
Campus Kiel
Institut für Pathologie, Hämatopathologie
Sektion und Lymphknotenregister
Arnold-Heller-Straße 3
24105 Kiel – Germany

Melchert, Anna-Lena
Universitätsklinikum Hamburg Eppendorf
Interdisziplinäre Klinik und Poliklinik für
Stammzelltransplantation
Martinstraße 52
20246 Hamburg – Germany

Milsom, Michael
HI-STEM gGmbH
im Deutschen Krebsforschungszentrum DKFZ
Im Neuenheimer Feld 280
69120 Heidelberg – Germany

Mock, Ulrike
University Medical Centre Hamburg-Eppendorf Clinic
for Stem Cell Transplantation
Martinistraße 52
20246 Hamburg – Germany

Mohr, Sebastian
Klinikum der
Johann Wolfgang Goethe-Universität
Molekulare Hämatologie/Onkologie
Theodor-Stern-Kai 7
60590 Frankfurt – Germany

Morishima, Tatsuya
Hannover Medical School
Abt. Molekulare Hämatopoese
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Murray, Marta
Technische Universität Dresden
Department of Pathobiochemistry
Fetscherstraße 74
01307 Dresden – Germany

Müschen, Markus
University of California San Francisco UCSF
Comprehensive Cancer Center
901 Bush Street CA
94109 San Francisco – USA

O

Oberle, Anna
Universitätsklinikum Hamburg-Eppendorf
2. Medizinische Klinik
Martinistraße 52
20246 Hamburg – Germany

Oellerich, Thomas
Klinikum der
Johann Wolfgang Goethe-Universität
Molekulare Hämatologie/Onkologie
Theodor-Stern-Kai 7
60590 Frankfurt – Germany

Othman, Moneeb
Friedrich-Schiller-Universität Jena
Institute of Human Genetics
Kollegiengasse 10
07743 Jena – Germany

P

Pal Singh, Rashim
Technische Universität Dresden
Department of Pathobiochemistry
Fetscherstraße 74
01307 Dresden – Germany

Panagiota, Viktoria
Medizinische Hochschule Hannover Hämatologie, Hämostaseologie, Onkologie und
Stammzelltransplantation
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Pancho Yanza, Gabriela
Universitätsklinikum Hamburg-Eppendorf
AG Fehse
Martinistraße 52
20246 Hamburg – Germany

Phaltane, Ruhi
Hannover Medical School
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Pimanda, John
University-Lowy Cancer Research Centre
UNSW, Sydney Hospital
Medical Professorial Unit
Edmund Blacket Building
The Prince of Wales Hospital
2052 Randwick, Sydney – Australia

Pohl, Sandra
Universitätsklinikum Regensburg
Franz-Josef-Strauss-Allee 11
93053 Regensburg – Germany

Preukschas, Julia
Heinrich-Pette-Institut
Martinistraße 52
20251 Hamburg – Germany

R

Raab, Marc S.
German Cancer Research Center (DKFZ)
Max-Eder Unit
Im Neuenheimer Feld 280
69120 Heidelberg – Germany

Riecken, Kristoffer
Universitätsklinikum Hamburg-Eppendorf
Martinistraße 52
20246 Hamburg – Germany

Rothe, Tino
Universitätsklinikum Carl Gustav Carus
Institut für Medizinische Informatik
und Biometrie
Fetscherstraße 74
01307 Dresden – Germany

Ruggero, Katia
University of Oxford
John Radcliffe Hospital
OX3 9DS Oxford – United Kingdom

Schaefer, Christiane
Universitätsklinikum Münster
Klinik für Kinder- und Jugendmedizin –
Pädiatrische Hämatologie und Onkologie
Albert-Schweizer Campus A1
48149 Münster – Germany

Schleithoff, Carolin
Universitätsklinikum Münster
Klinik für Kinder- und Jugendmedizin –
Pädiatrische Hämatologie und Onkologie
Albert-Schweizer Campus A1
48149 Münster – Germany

Schwarzer, Adrian
Medizinische Hochschule Hannover
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Serve, Hubert
Klinikum der
Johann-Wolfgang Goethe-Universität
Medizinischen Klinik II: Hämatologie und
Onkologie, Rheumatologie, Infektiologie,
Therapie der HIV-Erkrankung
Theodor-Stern-Kai 7
60590 Frankfurt am Main – Germany

Serwe, Matthias
Deutsche Krebshilfe e.V.
Buschstraße 32
53113 Bonn – Germany

Shipounova, Irina
National Hematology Reasearch Centre
Physiology of Hematopoiesis
Novy Zykovsky Pr. 4
125167 Moscow – Russia

Skokowa, Julia
Medizinische Hochschule Hannover
Abt. Molekulare Hämatopoese
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Skorupa, Alexandra
Novartis AG
Roonstraße 25
90327 Nürnberg – Germany

Sonntag, Tanja
Universitätsklinikum Hamburg Eppendorf
Interdisziplinäre Klinik und Poliklinik für
Stammzelltransplantation
Martinstraße 52
20246 Hamburg – Germany

Speidel, Daniel
Children's Medical Institute
Locked Bag 23
2145 NSW Wentworthville – Australia

Spirin, Pavel
Engelhardt Institute of Molecular Biology
Vavilova Str. 32
119991 Moscow – Russia

Stamm, Hauke
Universitätsklinikum Hamburg-Eppendorf
Medizinische Klinik und Poliklinik,
Onkologie-Hämatologie
Martinistraße 52
20246 Hamburg – Germany

Stauss, Hans
UCL Medical School Royal Free Hospital
Department of Immunology
Rowland Hill Street
NW3 2PF London – United Kingdom

Sternsdorf, Thomas
Research Institute
Childrens Cancer Center Hamburg
Martinistraße 52
20251 Hamburg – Germany

Stocking, Carol
Heinrich-Pette-Institut
Forschungsgruppe Molekulare Pathologie
Martinistraße 52
20246 Hamburg – Germany

Straten, Per Thor
Copenhagen University Hospital
Herlev Center for Cancer
Immune Therapy (CCIT)
Catherine Boothvej 1
2650 Hvidore – Denmark

LIST OF PARTICIPANTS

All information pursuant to the registration form

T

Tenen, Daniel G.
National University of Singapore
Cancer Science Institute of Singapore
MD6 Center for Translational Medicine
Yong Loo Lin School of Medicine
Woodland Road
MA 02130 Boston – USA

Thakur, Mayank
Charité Universitätsmedizin Berlin
Institute for Larboratory Medicine
Forschungshaus Forum IV
Augustenburgerplatz 1
13353 Berlin – Germany

Thiele, Benjamin
Universitätsklinikum Hamburg-Eppendorf
2. Medizinische Klinik
Martinistraße 52
20246 Hamburg – Germany

Thoenissen, Gabriela
Universitätsklinikum Hamburg-Eppendorf
2. Medizinische Klinik
Martinistraße 52
20146 Hamburg – Germany

Tipton, Tom
University of Southampton
Antibody and Vaccines Group
SO16 6YD Southhampton – United Kingdom

Trepel, Martin
Universitätsklinikum Hamburg Eppendorf
II. Medizinischen Klinik
Martinistraße 52
20246 Hamburg – Germany

Triviai, Ioanna
Universitätsklinikum Hamburg-Eppendorf
Clinic for Stem Cell Transplantation
Martinistraße 52
20246 Hamburg – Germany

U

Uhde, Almut
Universitätsklinikum Hamburg Eppendorf
Interdisziplinäre Klinik und Poliklinik für
Stammzelltransplantation
Martinstraße 52
20246 Hamburg – Germany

Ungerechts, Guy
Deutsches Krebsforschungszentrum
Abteilung Translationale Onkologie
Im Neuenheimer Feld 280
69120 Heidelberg – Germany

V

Vick, Binje
Helmholtz Zentrum München
Research Unit Gene Vectors
Marchioninistraße 25
81377 München – Germany

W

Wagner, Bettina
University Hospital Essen Institute for
Transfusion Medicine
Virchowstraße 179
54147 Essen – Germany

Waskow, Claudia
Technische Universität Dresden
Center for Regenerative Therapies (CRTD)
Fiedlerstr. 42
01307 Dresden – Germany

Wellbrock, Jasmin
Universitätsklinikum Hamburg-Eppendorf
Martinistraße 52
20246 Hamburg – Germany

Welte, Karl H.
Medizinische Hochschule Hannover
Abt. Molekulare Hämatopoese
Carl-Neuberg-Straße 1
30625 Hannover – Germany

Wichmann, Christian
Medizinische Fakultät der LMU München
Abteilung für Transfusionsmedizin,
Zelltherapeutika und Hämostaseologie
Max-Lebsch Platz 32
81377 München – Germany

Wojcik, Bartosch
Klinikum der
Johann Wolfgang Goethe-Universität Frankfurt
Abt. für Hämatologie und Onkologie
Theodor-Stern-Kai 7
60590 Frankfurt – Germany

Z

Zander, Axel R.
Sana Kliniken Lübeck Hämatologisch/
Onkologische Ambulanz
Kronsforder Allee 71-73
23560 Lübeck – Germany

Zaraiskii, Mikhail
First Pavlov State Medical University
L. Tolstoy Street 6-8
197022 St. Petersburg – Russia

Zörnig, Martin
Georg-Speyer-Haus
Paul-Ehrlich-Straße 42-44
60596 Frankfurt – Germany

Cover: RGB-marked tumours in the liver [© Kristoffer Riecken, Boris Fehse]