



EKFS CANCER SYMPOSIUM 2017

► Exploiting Tumor-Specific
Vulnerabilities for Improved
Cancer Treatment ◀

ABSTRACT BOOK

15-17 NOVEMBER 2017

Alte Mensa Göttingen, Germany

www.ekfs-cancersymposium-2017.de

Dear Colleagues,

We are delighted to welcome you to the EKFS Cancer Symposium!

The scholars of our Else Kröner-Fresenius Training Program for Clinician Scientists have brought together a unique set of speakers to address one of the most urgent questions in cancer research. Having learned about the molecular traits specific to cancer cells, this important question at hand is how we can exploit this knowledge to identify and target their vulnerabilities. Since there are multiple answers to this question, we will address it from different perspectives, ranging from genome dynamics through tumor-stroma-interactions all the way to immunotherapy.

The meeting constitutes the highlight of a three-year curriculum to train young scientists at the intersection of basic cancer research and clinical applications. With the help of the Else Kröner-Fresenius Foundation, we have inaugurated a program structure to support the most committed and talented young colleagues who are brave enough to combine clinical work and basic research on gastrointestinal tumors.

Besides vibrant scientific interactions, we also hope that you will enjoy Göttingen, one of the most traditional German university towns, with several dozens of Nobel laureates having been affiliated with it. The Göttingen spirit of highly interactive and innovative scientific discussions should be felt during the days to come.

October 15, 2017



MATTHIAS DOBBELSTEIN

Chairman and Director

Institute of Molecular Oncology, University Medical Center Göttingen



MICHAEL GHADIMI

Director and Chairman

Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen

Dear Speakers, Dear Visitors,

The EKFS scholars would also like to welcome you to our symposium with the title “Exploiting Tumor-Specific Vulnerabilities for Improved Cancer Treatment”!

For the past two to three years, we were given the opportunity to “learn science”. Not only “part-time science”, as many of us know it as common practice all over German university hospitals, but “real” science – full time, in a lab of our own choice, with a project designed by ourselves, under the constant supervision of experienced researchers as well as experienced clinicians. For all of us, this has had a big impact, not only on our careers as scientists but also on the way we act as clinicians – the way we look at research, the way we read papers and trials, the way we think. It has gotten clear to all of us that basic research and clinics are not two different worlds, as which they are sadly still treated very often, but should act together synergistically: to solve actual medical problems and cure diseases hand in hand.

The exploitation of tumor-specific vulnerabilities, on which we focused our research projects, can show all of us how well these interactions can work. Small-molecule inhibitors targeting cancer specific vulnerabilities are more and more entering patients’ treatment. Many cancer therapies which used to be standard for decades are now getting outdated. We are very happy to participate in this transition!

In this symposium, many aspects of this field are highlighted. We are extremely happy to have brought together experts from all cancer relevant areas and from different countries here in Göttingen to discuss research for the upcoming three days. We hope all of you will feel a bit of the spirit we felt in the past years and are still feeling now – some of us back in the clinic, some still in the lab. Especially, we want to highlight our poster session which will give room to our own work as well as to the work of many other young scientists like us. We are looking forward to your input!

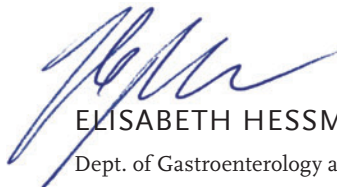
October 19, 2017

For the EKFS scholars



OLIVER HAHN

Department of Urology



ELISABETH HESSMANN

Dept. of Gastroenterology and Gastrointestinal Oncology

ABSTRACTS

EKFS SYMPOSIUM 2017

PROGRAM OVERVIEW 8

TALKS 10

- T01** *Targeting the DNA damage response for personalized cancer therapy* 11
- T02** *Synthetic Lethality – BRCA and beyond* 12
- T03** **SARTORIUS LECTURE** *GNAS drives tumorigenesis via PKA signaling and reprogramming lipid metabolism* 13
- T04** *Transcription-replication interference in hyperproliferative cancer cells* 14
- T05** *MYC-dependent control of RNA Polymerase function during the cell cycle* 15
- T06** *Oncolytic viruses and checkpoint inhibition close the cancer immunity cycle* 16
- T07** *Barrier function and colon cancer biology* 17
- T08** *Upregulation of HGF/c-MET signaling as a resistance mechanism in cancer immunotherapy* 18
- T09** *Using the patient's immune system to treat cancer* 19
- T10** *Calcineurin in intestinal tumor development* 20
- T11** *Targeting Tumour Hypoxic Response* 21
- T12** *Cellular plasticity in cancer: driving force and therapeutic target* 22
- T13** *Dissecting cancer Evolution: Patterns, dynamics and clinical implications* 23
- T14** *Germline determinants of the somatic mutation landscape in 2,642 cancer genomes* 24
- T15** **SARTORIUS LECTURE** *Modulation of transcription through disruption of BET complexes* 25
- T16** *Targeting Tumour Hypoxic Response* 26
- T17** *Drugging transcription: Progress and potential for treating human diseases* 27
- T18** *Angiogenesis revisited: role and (therapeutic) implications of endothelial metabolism* 28
- T19** *KDM4 inhibition targets breast cancer stem cells* 29

OVERVIEW: FLASH TALKS 30

POSTER SESSION I: TUMOR EPIGENETICS 32

- P01** *The role of CHD1 in androgen independent Castration-resistant Prostate Cancer* 33
- P02** *Expression of polycomb proteins in carcinogenesis of colorectal cancer – the bright side of EZH2* 34
- P03** *The Role of the BAF Complex in Wnt-mediated Transcriptional Regulation in Colorectal Cancer* 35
- P04** *The role of BET inhibition in chemo-resistance and re-sensitization in pancreatic ductal adenocarcinoma* 36
- P05** *Transcriptomic and epigenetic impact of anti-androgens on prostate cancer models* 37
- P06** *Bromodomain Testis-Specific Protein BRDT is Expressed in a Subset of Esophageal Squamous Cell Carcinomas and Controls Expression of Differentiation-Associated Genes* 38
- P07** *Functional and Mechanistic Implications of ARID1A-deficiency in PDAC Development and Progression* 39
- P08** *Cooperation of MDM2 and polycomb repressor complexes in chromatin modification* 40
- P09** *Unravelling epigenetic mechanisms of CAF-chemotherapy resistance in mammary carcinoma* 41
- P10** *The histone methyltransferase DOT1L is required for proper DNA damage response DNA repair and modulates chemotherapy responsiveness* 42
- P11** *EZH2 inhibition as a possible new therapeutic approach for Castration-resistant Prostate Cancer harboring a CHD1 deletion* 43
- P12** *Investigating the role of EZH2 in mediating chemoresistance in PDAC* 44
- P13** *Role of the E3 ubiquitin ligase complex RNF20/RNF40 in HER2-positive breast cancer* 45
- P14** *The USP22 deubiquitinase and its importance for HER2 breast cancer biology* 46
- P15** *Epigenetic mRNA modifications of stress-responsive transcripts enable cellular survival in pancreatic cancer* 47

POSTER SESSION II: TUMOR ENVIRONMENT & METASTASIS 48

- P16** *Comparative proteomics reveals a diagnostic signature for head-and-neck cancer metastasis in the lung* 49
- P17** *Hodgkin lymphoma secreted factors influence monocyte/macrophage polarization and function* 50
- P18** *Mitochondrial calcium uniporter-mediated redox signaling controls melanoma pathobiology* 51
- P19** *Functional characterization of NFATc1 in the formation of PDAC-associated cancer cachexia* 52

- P20** *Role of CC531 rat colorectal cancer cell line in translational research – comparison to two human colorectal cancer cell lines upon treatment with irinotecan* 53
- P21** *The role of TMX oxidoreductases in melanoma growth and invasion* 54
- P22** *Knockdown of Integrin α V drastically reduces tumor growth, metastasis and intraperitoneal carcinomatosis formation in a xenograft model of human pancreatic adenocarcinoma* 55
- P23** *Synergistic inhibition of tumor formation by depletion of Integrin β 4 on tumor cells and of endothelial selectins in tumor stroma: Analyses on the underlying mechanisms* 56
- P24** *In vitro study of human cancer cell adhesion to recombinant human vs. murine E- and P-selectin under dynamic vs. static binding condition* 57
- P25** *Drug scavenging of TAMs in pancreatic cancer* 58
- P26** *Cytosolic 5'-Nucleotidase 1A Drives Chemotherapeutic Resistance to Gemcitabine in Pancreatic Cancer* 59
- P27** *Role of fibroblast derived SPARC in a genetically engineered mouse model of pancreatic cancer* 60
- P28** *Disarming tumors from TGFbeta shield by radiation-induced Neutralization of TGFbeta in tumor environment* 61
- P29** *Hepato-arterial infusion of nanaoliposomal irinotecan in an orthotopic preclinical model of colorectal liver metastases – Enhanced effect in combination with embolization particles* 62

POSTER SESSION III: TUMOR IMMUNOLOGY, REPLICATIVE STRESS & DNA DAMAGE 64

- P30** *Impact of bypassed mRNA quality control on DNA damage responses and progression of pancreatic cancer* 65
- P31** *p53 activity results in replication fork processivity* 66
- P32** *Centrosome integrity as a determinant of replication stress* 67
- P33** *The impact of the integrated stress response on DNA replication* 68
- P34** *Role of the GR in intestinal epithelial cells for the development of colitis-associated colorectal cancer* 69
- P35** *Myeloid-specific calcineurin as a regulator of intestinal tumor development through the control of immune checkpoint proteins* 70
- P36** *Integrated genetic profiles of T-PLL implicate a TCL1/ATM-centered model of aberrant DNA damage responses* 71
- P37** *The implication of Kv10.1 in the regulation of G2/M transition* 72
- P38** *RNF40 loss dampens NFkB activity in colorectal cancer cells and reduces colitis burden in mice* 73
- P39** *Characterization of a novel TCL1A-mediated function in CLL – its impact on mitotic checkpoint proteins* 74

- P40** Nuclear NFATc1 signaling in steatosis and inflammation of the liver 75
- P41** Targeting Macrophage Migration Inhibitory Factor (MIF) in colorectal cancer inhibits proliferation and angiogenesis 76

POSTER SESSION IV: TUMOR GENETICS & SIGNALING 78

- P42** Lack of cooperativity in targeting Mdm2 and CDK4 in the treatment of liposarcoma cells 79
- P43** Smad4-deficiency in PDAC favors EMT and stemness 80
- P44** The β -catenin/CBP-antagonist ICG-001 inhibits pediatric glioma tumorigenicity in a Wnt-independent manner 81
- P45** Simultaneous inhibition of Smoothed and PI3K induces apoptosis in RMS-bearing mice 82
- P46** Towards novel strategies of targeting p53 related vulnerabilities in T-PLL cells 83
- P47** Interaction of signaling pathways in Burkitt lymphoma 84
- P48** T-PLL cells resemble memory-type T-cells with aberrant effector functions implicating a leukemogenic cooperation of TCL1A with TCR signaling 85
- P49** GSK3- β in pancreatic cancer - oncogene or tumorsuppressor? 86
- P50** Towards subgroup stratification using patient-specific pathways 87
- P51** Non-platinum based halogenated compounds – a novel therapeutic approach in pancreatic cancer? 88
- P52** A Novel Role for ROBO3 in the Pancreatic Cancer Plasticity 89
- P53** Targeted therapy of castration resistant prostate cancer upon development of androgen receptor splice variants 90
- P54** Heat sensitivity modulation in prostate cancer (PCA) in vitro: The impact of common PCA therapeutics 91
- P55** CD31 Expression Determines Redox Status and Chemoresistance in Human Angiosarcomas 92
- P56** Established colorectal cancers depends on mutp53 driving epithelial STAT3-mediated proliferation and invasion 93

LIST OF PRESENTERS (alphabetically) 94

PROGRAM OVERVIEW

WEDNESDAY, NOVEMBER 15th 2017

Until 14:45 **ARRIVAL, REGISTRATION & WELCOME COFFEE**

14:45 **WELCOME NOTE AND OPENING**

SESSION I **Therapy Stratification** *Chairs: Oliver Hahn & Philipp Ströbel*

15:00 – 15:30 **T01:** Christian Reinhardt Cologne, Germany

Targeting the DNA damage response for personalized cancer therapy

15:30 – 16:00 **T02:** Christopher Lord London, UK

Synthetic Lethality – BRCA and beyond

16:00 – 16:30 **T03:** Nabeel Bardeesy Boston, USA

Targeting alternative drivers in KRAS mutant pancreatic cancer

16:30 – 16:45 **COFFEE BREAK**

SESSION II **Tumor Genetics I**

Chairs: Hanibal Bohnenberger & Matthias Dobbstein

16:45 – 17:15 **T04:** Matthias Altmeyer Zürich, Switzerland

Transcription-replication interference in hyperproliferative cancer cells

17:15 – 17:45 **T05:** Martin Eilers Würzburg, Germany

MYC-dependent control of RNA Polymerase function during the cell cycle

THURSDAY, NOVEMBER 16th 2017

SESSION III **Tumor Immunology** *Chairs: Anne Kauffels & Michael Schön*

9:00 – 9:30 **T06:** Florian Kühnel Hannover, Germany

Oncolytic viruses and checkpoint inhibition close the cancer immunity cycle

9:30 – 10:00 **T07:** Stefan Fichtner-Feigl Freiburg, Germany

Barrier function and colon cancer biology

10:00 – 10:30 **COFFEE BREAK**

10:30 – 11:00 **T08:** Thomas Tüting Magdeburg, Germany

Upregulation of HGF/c-MET signaling as a resistance mechanism in cancer immunotherapy

11:00 – 11:30 **T09:** Dirk Jäger Heidelberg, Germany

Using the patient's immune system to treat cancer

11:30 – 13:00 **Flash Talks and Poster Session**

13:00 – 14:00 **LUNCH BREAK**

SESSION IV Tumor Environment & Metastasis

Chairs: Lena-Christin Conradi & Elisabeth Zeisberg

- 14:00 – 14:30 **T10:** Sebastian Zeißig Dresden, Germany
Calcineurin in intestinal tumor development
- 14:30 – 15:00 **T11:** Alan McIntyre Nottingham, UK
Targeting molecular adaptation in the hypoxic tumor microenvironment
- 15:00 – 15:30 **T12:** Thomas Brabletz Erlangen, Germany
Cellular plasticity in cancer: driving force and therapeutic target
- 15:30 – 16:00 **COFFEE BREAK**

SESSION V Tumor Genetics II

Chairs: Ramona Schulz-Heddergott & Bernd Wollnik

- 16:00 – 16:30 **T13:** Marco Gerlinger London, UK
Dissecting cancer Evolution: Patterns, dynamics and clinical implications
- 16:30 – 17:00 **T14:** Jan Korbel Heidelberg, Germany
Germline determinants of the somatic mutation landscape in 2,642 whole cancer genomes
- 19:00 **“GET TOGETHER“** in the Haus am See Göttingen (only with separate registration!)
DINNER & ENTERTAINMENT
(bus transfer leaves from the conference venue and the hotel “Central” at 18:30)

FRIDAY, NOVEMBER 17th 2017

SESSION VI Tumor Epigenetics I

Chairs: Elisabeth Hessmann & Steven A. Johnsen

- 9:00 – 9:30 **T15:** Panagis Filippakopoulos Oxford, UK
Modulation of transcription through disruption of BET complexes
- 9:30 – 10:00 **T16:** Udo Oppermann Oxford, UK
Inhibiting histone demethylases to target metabolic dependencies in multiple myeloma
- 10:00 – 10:30 **T17:** Rab Prinjha Stevenage, UK
Drugging transcription: Progress and potential for treating human diseases

SESSION VII Tumor Epigenetics II & Tumor Metabolism

Chairs: Volker Ellenrieder & Robert Götze

- 10:30 – 11:00 **T18:** Peter Carmeliet Leuven, Belgium
Angiogenesis revisited: Role and therapeutic implication of endothelial metabolism
- 11:00 – 11:30 **T19:** Roland Schüle Freiburg, Germany
KDM4 inhibition targets breast cancer stem cells
- 11:30 **CONCLUDING REMARKS & FAREWELL**



TALKS

TARGETING THE DNA DAMAGE RESPONSE FOR PERSONALIZED CANCER THERAPY

Christian Reinhardt

Department I of Internal Medicine, University Hospital Cologne, Germany;
Cologne Graduate School of Ageing Research, University of Cologne, Germany

In response to genotoxic damage, cells activate a complex, kinase-driven signaling network to arrest the cell cycle, activate DNA repair, or, if the extent of damage is beyond repair capacity, to induce apoptotic cell death. Mutations within this signaling network, which is commonly referred to as the DNA damage response (DDR), are among the most frequently occurring genomic aberrations in human cancer. While these mutations are clearly selected during cancer development, they may also be associated with actionable vulnerabilities. We employ autochthonous mouse models that mimic cancer-specific aberrations in the DDR to develop novel therapeutic strategies that leverage the mutation-associated vulnerabilities. We specifically use *Kras*-driven models of lung adenocarcinoma, as well as *Tcl1*-driven models of chronic lymphocytic leukemia to show that *Atm*-deficiency is associated with sensitivity against PARP1 inhibitors. We further employ models of small cell lung cancer to show that these tumors depend of functional ATR and CHK1 activity. Lastly, we demonstrate that combined inhibition of the checkpoint kinases CHK1 and MK2 displays synergistic anti-tumor activity in *Kras*- and *Braf*-mutant cancer. These efforts may ultimately pave the way for a clinical validation the activity of compounds interfering with components of the DDR in a cancer genotype-stratified fashion.

T02

SYNTHETIC LETHALITY – BRCA AND BEYOND

Christopher J. Lord

The CRUK Gene Function Laboratory and The Breast Cancer Now Toby Robins Breast Cancer Research Centre, The Institute of Cancer Research, London, UK

T03 Sartorius Lecture:

GNAS DRIVES TUMORIGENESIS VIA PKA SIGNALING AND REPROGRAMMING LIPID METABOLISM

Krushna C. Patra^{1,2}, Yasutaka Kato^{1,2}, Yusuke Mizukami¹, Nabeel Bardeesy^{1,2}

1 Cancer Center, Massachusetts General Hospital, Boston, MA, USA

2 Department of Medicine, Harvard Medical School, Boston, MA, USA

G-protein α (GNAS) mediates receptor-stimulated cAMP signaling, a conserved pathway that integrates nutritional and hormonal cues with regulation of cellular metabolism and other processes. In many tissues GNAS-cAMP signaling is required to maintain anti-proliferative states via inactivation of key oncogenic mediators. Paradoxically, constitutively activating mutations in GNAS and other pathway components arise in multiple tumor types, although the mechanisms by which oncogenic GNAS incites tumorigenesis, its interplay with other cancer gene mutations, and its roles in the growth of fully formed cancers remain elusive. We investigated the functions of activated GNASR201C in liver and pancreatic tumorigenesis where GNAS is frequently concurrently mutated with KRAS. By developing genetically engineered mouse models (GEMMs), we show that inducible expression of GNASR201C cooperates with KRASG12D to drive tumor initiation, and remains critical for tumor maintenance. Proteomic, metabolomic, and functional studies demonstrate that tumor maintenance requires GNASR201C-mediated activation of protein kinase A (PKA)-dependent network, which reprograms cellular metabolism, potentiating lipid remodeling and fatty acid oxidation, to sustain tumor growth. Notably, comparative examination of these pathways in KRAS mutant tumor organoids and allografts with and without GNAS mutations reveals that distinct signaling and metabolic circuits support the pathogenesis of the different tumor subtypes. Thus, our studies uncover oncogenic mechanisms and metabolic targets in cAMP/PKA-driven tumorigenesis and demonstrate unanticipated metabolic heterogeneity among KRAS-mutant cancers.

TRANSCRIPTION-REPLICATION INTERFERENCE IN HYPERPROLIFERATIVE CANCER CELLS

Matthias Altmeyer

Department of Molecular Mechanisms of Disease, University of Zurich, Switzerland

The cellular mechanisms that safeguard genome integrity are often subverted in cancer. Cancer genome sequencing has uncovered many recurrently mutated cancer genes, yet their biological functions are often poorly understood. Based on the prominent role of the genome integrity maintenance network as anti-cancer barrier we devised the hypothesis that among newly emerging cancer genes some may have important functions for genome stability. To identify novel cancer-related genome caretakers we employed a convergent multi-screening approach coupled to quantitative image-based cytometry and ranked candidate genes according to tailor-made multivariate read-outs reflecting cell viability, proliferative capacity, replisome integrity, and levels of DNA damage signaling. These analyses revealed multiple new regulators of replication stress resilience, many of which have a role in transcription-related processes. Among the candidate hits we identified components of the mRNA cleavage and polyadenylation complex. Phenotypic and functional characterization revealed that deregulation of this complex sensitizes cells to mild persistent and acute replication stress, impairs replication fork speed and origin activation timing, and leads to reduced survival under elevated replication stress conditions. Interestingly, we observed enhanced levels of RNA Polymerase II on chromatin and concomitantly increased pre-mRNA levels without significant induction of DNA-RNA hybrids (R-loops). Consistently, overexpression of RNAaseH1 only marginally alleviated replication stress hypersensitivity, while transcriptional inhibition rescued both replication fork speed and replication stress sensitivity. Taken together, our results link the cleavage and polyadenylation machinery to replication stress resilience and suggest that deregulated RNA Pol II processivity can lead to transcription-replication conflicts independent of R-loop formation.

T 05

MYC-DEPENDENT CONTROL OF RNA POLYMERASE FUNCTION DURING THE CELL CYCLE

Martin Eilers

Theodor Boveri Institute and Comprehensive Cancer Center Mainfranken,
Biocenter, University of Würzburg, Würzburg, Germany

ONCOLYTIC VIRUSES AND CHECKPOINT INHIBITION CLOSE THE CANCER IMMUNITY CYCLE

Florian Kühnel

Clinic for Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, Germany

Oncolytic viruses have shown first promising therapeutic outcomes in clinical trials. Initially developed for selective tumor infection and potent lysis of cancer cells, oncolytic viruses have now been recognized as agents with multi-faceted antitumor activities. Oncolytic virus infection is an effective inflammatory stimulus thus interfering with the tolerogenic character of the tumor microenvironment. Accordingly, viral oncolysis provides suitable preconditions for concomitant antitumoral DC-vaccination. We showed in syngeneic tumor models in mice, that administration of a tumor-targeted DC-vaccines elicited antitumoral CD8 T cell responses when administered during oncolytic tumor inflammation, but not in the presence of an intact tumor. We found that Tregs were activated and required for the therapeutic success of this treatment. Moreover, viral oncolysis results in effective cross presentation of tumor antigen. Oncolytic virotherapy is therefore an important trigger for priming antitumoral immune responses which covers the full antigenic spectrum of a tumor and consequently serves as a suitable vaccination in situ. These properties also qualify oncolytic viruses as a basis for simultaneous checkpoint inhibition. We investigated the outcome of viral oncolysis and PD-1 checkpoint inhibition on the spectrum of neoantigen-specific CD8 T-cell responses. In a syngeneic lung adenocarcinoma model (CMT64) in mice we have analysed potential neoantigens by next generation sequencing and neopeptide prediction. We found that the combination of oncolysis and PD-1 triggered a broad spectrum of neopeptide-specific T-cell responses, abrogated systemic resistance to PD-1-immunotherapy and improved elimination of disseminated lung tumors. Due to these multipronged immunomodulatory properties, oncolytic viruses represent an excellent tool to initiate the cancer immunity cycle.

BARRIER FUNCTION AND COLON CANCER BIOLOGY

Stefan Fichtner-Feigl

Department of General and Visceral Surgery, University Medical Center Freiburg, Germany

Colorectal cancer (CRC) is the third most frequent tumor entity worldwide and the second leading cause of cancer-related death. Development of metastasis is a concern for patients and clinicians alike as metastasis may be fatal, causing mass-effect and meddling with homeostasis. Approximately 30% of CRC patients show metastasis already at diagnosis and another 25-30% of CRC patients develop metastasis metachronously. The molecular and cellular events during the process of metastasis are incompletely understood. Inflammation and immunity are important determinants of tumorigenesis, impacting cancer development from initiation, promotion, progression and metastasis. Germline-encoded pattern recognition receptors (PRRs) are among critical effectors of the inflammatory responses are that, upon sensing microbial- or danger-associated molecular patterns, elicit an inflammatory response to restore homeostasis. Therefore, the microbiome has emerged as a major regulator of colorectal carcinogenesis. Recently, we have shown that microbial-associated molecular patterns are able to regulate the expression of mucins and antimicrobial peptides specifically in cancer cells leading to either tumor barrier stabilization or tumor barrier breach at the luminal surface and exclusion or invasion of bacteria inside the tumors, respectively. This means that bacteria can regulate the tumor barrier independent from the intestinal barrier. An important regulator of intestinal inflammatory responses is IRAK-M, a negative regulator of TLR signaling. We investigate the compartment-specific impact of IRAK-M on colorectal carcinogenesis using a mouse model. We demonstrate that IRAK-M is expressed in tumor cells due to combined TLR and Wnt activation. Tumor cell-intrinsic IRAK-M is responsible for regulation of microbial colonization of tumors and STAT3 protein stability in tumor cells, leading to tumor cell proliferation. IRAK-M expression in human CRCs is associated with poor prognosis. These results suggest that IRAK-M may be a potential therapeutic target for CRC treatment.

References Kesselring R, Glaesner J, Hiergeist A, Naschberger E, Neumann H, Brunner SM, Wege AK, Seebauer C, Köhl G, Merkl S, Croner RS, Hackl C, Stürzl M, Neurath MF, Gessner A, Schlitt HJ, Geissler EK, Fichtner-Feigl S: IRAK-M Expression in Tumor Cells Supports Colorectal Cancer Progression through Reduction of Antimicrobial Defense and Stabilization of STAT3. *Cancer Cell*. 2016 May 9;29(5):684-696.

UPREGULATION OF HGF/C-MET SIGNALING AS A RESISTANCE MECHANISM IN CANCER IMMUNOTHERAPY

Thomas Tüting

Laboratory of Experimental Dermatology, Department of Dermatology,
University of Magdeburg, Germany

The HGF/c-MET signaling axis is well known for its ability to promote cell proliferation, survival and migration in development, tissue regeneration and cancer. Recently, upregulation of HGF/c-Met signaling has also been described as an adaptive resistance mechanism to targeted inhibition of Braf-driven MAPK signaling in patients with advanced metastatic melanoma. Using experimental mouse melanoma models we discovered that cytotoxic cancer immunotherapies also activate the HGF/MET signaling axis both systemically and locally in the tumor microenvironment. HGF/MET signaling drives the reactive mobilization and recruitment of neutrophils from the bone marrow into tumor tissue and draining lymph nodes. In the context of T-cell-inflamed microenvironments neutrophils rapidly acquire immunosuppressive capacities and limit T-cell expansion and effector functions. Our work suggests that HGF/c-MET signaling may represent a common pathway supporting therapy resistance in cancer and provides a scientific rationale to investigate adjuvant c-Met inhibition as a strategy to improve cancer immunotherapies.

USING THE PATIENT'S IMMUNE SYSTEM TO TREAT CANCER

Dirk Jäger

Department of Medical Oncology, National Center for Tumor Diseases (NCT),
Heidelberg University Hospital, Germany

Our immune system is amazingly potent in recognizing and killing of tumor cell or cells that have the potential of malignant transformation. In established tumor, in most patient an anti-tumor immune response is still detectable but the balance between tumor growth and immune response is imbalanced. Tumors use numerous strategies to escape anti-tumor immunity. Our group is focusing on the characterization and better understanding of such mechanisms with the overall goal to specifically target such escape mechanisms in patients. Our work in colorectal cancer has identified a pathway creating an immune suppressive tumor environment mediated by macrophage populations. This pathway is targetable by CCR 5 inhibition. We now test CCR 5 inhibition in combination with checkpoint inhibition in several prospective clinical trials.

CALCINEURIN IN INTESTINAL TUMOR DEVELOPMENT

Sebastian Zeißig

Department of Medicine I and Center for Regenerative Therapies,
University Medical Center Dresden, Technical University Dresden, Germany

Colorectal carcinogenesis is a gradual process based on somatic mutations in tumor suppressor genes and oncogenes. Environmental factors such as the microbiota contribute to this process and modulate colorectal cancer (CRC) progression. As such, defects in intestinal barrier dysfunction at sites of intestinal tumors facilitate bacterial translocation into tumors, which leads to bacterial recognition by toll-like receptors (TLRs) and the activation of inflammation-associated pathways (e.g. STAT3, NF- κ B) that support tumor proliferation and inhibit tumor cell death. We have recently described that calcineurin, a phosphatase which activates transcription factors of the family of nuclear factor of activated T cells (NFAT), is not only expressed in bone marrow-derived immune cells but also in intestinal epithelial cells. Alterations in microbial stratification and composition lead to tumor-specific activation of this pathway, which promotes the proliferation of tumor cells and inhibits cell death through NFAT-dependent transcriptional regulation of tumor stem cells. This work revealed a novel inflammation-associated pathway, which is active in intestinal tumor cells and promotes tumor development in a microbiota-dependent manner. Further work demonstrated that calcineurin and NFAT exhibit cell-specific roles in CRC with promotion of tumor growth in intestinal epithelial cells and inhibition of tumor development through calcineurin-dependent cytotoxic responses of CD8⁺ T cells. In conclusion, calcineurin and NFAT are cell-specific regulators of intestinal tumor development.

TARGETING TUMOUR HYPOXIC RESPONSE

da Motta LL, Ledaki I, Purshouse K, Haider S, De Bastiani MA, Baban D, Morotti M, Steers G, Wigfield S, Bridges E, Li JL, Knapp S, Ebner D, Klamt F, Harris AL, McIntyre A¹

¹ Cancer Biology Unit, Division of Cancer and Stem Cells, School of Medicine, University of Nottingham, UK

Regions of hypoxia (low oxygen) in solid tumours are associated clinically with chemotherapy and radiotherapy resistance and poor patient outcome. Hypoxia drives molecular adaptation of triple negative breast cancer (TNBC), through the hypoxia inducible factor (HIF1 α and HIF2 α) transcription factors, which are stabilised in low oxygen. HIF proteins upregulate genes which drive many of the hallmarks of cancer (such as metabolic adaptation, angiogenesis and metastasis) including carbonic anhydrase 9 (CA9) and vascular endothelial growth factor A (VEGF-A). We investigated if the transcriptional adaptation to hypoxia in TNBC, can be modulated epigenetically by the bromodomain and extra-terminal (BET) proteins. BET proteins regulate transcription by selectively recognizing acetylated lysine residues on chromatin and recruiting transcription factors and other epigenetic regulators. BET inhibitors are being utilised in clinical trials in multiple tumour types, but their impact on hypoxic response has not been previously investigated. The BET inhibitor JQ1 significantly modulated 44% of hypoxia-induced genes, including CA9 and VEGF-A. JQ1 prevented HIF binding to the HRE at the CA9 promoter, but did not alter HIF expression. Binding of JQ1 target BRD4 to the promoters of CA9 and VEGF was increased in response to hypoxia as was relevant acetylation. JQ1 reduced TNBC growth *in vitro* and *in vivo* and inhibited xenograft vascularisation. These findings identify that BETi can inhibit molecular adaptation to hypoxia.

Acknowledgements *This work was funded by grants from Cancer Research UK (ALH), Breast Cancer Research Foundation (ALH), Breast Cancer Now (AM), Oxford NIHR Biomedical Research Centre, and the CRUK Oxford Centre (ALH) CAPES Foundation/Brazil (LLDM).*

CELLULAR PLASTICITY IN CANCER: DRIVING FORCE AND THERAPEUTIC TARGET

Thomas Brabletz

Dept. of Experimental Medicine 1, Nikolaus-Fiebiger Center for Molecular Medicine,
Univ. Erlangen, Germany

We have shown, that in particular tumor cells at the invasive front undergo a partial epithelial-mesenchymal transition (EMT) and aberrantly express EMT-associated transcription factors (EMT-TFs). The amount of such cancer cells strongly correlates with metastasis formation and poor clinical outcome in human cancers. Strikingly, metastases show a mesenchymal-epithelial re-transition (MET) with a re-differentiated phenotype, indicating high cancer cell plasticity and supporting a regulatory role of the tumor environment. We described that the EMT-TF ZEB1 is a crucial determinant of cellular plasticity. At molecular level, ZEB1 is linked in a double negative feedback loop with the miR-200 family and miR-203, which are strong inducers of epithelial differentiation. Thus aberrant ZEB1 expression stabilizes EMT and stemness, thereby promoting dissemination, metastasis and drug resistance of cancer cells. We have validated the findings, by showing that a depletion of ZEB1 in the KPC-mouse model of pancreatic cancer counteracts tumor cell plasticity and metastasis. Moreover we detected that ZEB1 controls the Notch pathway and directly cooperates with the Hippo-pathway effector YAP in driving aggressive cancer types. We determined epigenetic modifications conferred by ZEB1, screened for epigenetic drug to restore expression of its silenced target genes and to subsequently overcome therapy resistance. Despite their potent tumor-promoting effects, EMT-TFs are rarely mutated in cancer. This is likely due to the necessity for a transient expression and the associated plasticity of cancer cells, underscoring the important role of non-mutated genes in cancer progression.

T 13

DISSECTING CANCER EVOLUTION: PATTERNS, DYNAMICS AND CLINICAL IMPLICATIONS

Marco Gerlinger

Centre for Evolution and Cancer, Division of Molecular Pathology,
The Institute of Cancer Research, London, UK

GERMLINE DETERMINANTS OF THE SOMATIC MUTATION LANDSCAPE IN 2,642 CANCER GENOMES

Jan O. Korbel

Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Cancers develop through somatic mutations; however, germline genetic variation contributes to cancer risk via diverse mechanisms including by modulating mutational processes. Within the Pan Cancer Analysis of Whole Genomes (PCAWG) project, we discovered and phased 88 million single nucleotide variants, short insertions/deletions, and large structural variants in whole genomes from 2,642 cancer patients, and employed this resource to investigate germline determinants of somatic mutation across 39 cancer types. We describe over 100 germline L1 retrotransposons mediating somatic retrotransposition activity in cancer. Furthermore, rare damaging germline mutations in genes involved in DNA repair, DNA replication, and cell cycle associate with a variety of somatic mutation processes. We implicate mutations in the DNA glycosylase MBD4 with an elevated rate of C>T mutations at CpG dinucleotides, resulting in the genetic modulation of a widespread mutational process. Genome-wide association analysis reveals common genetic variation within the APOBEC3 gene cluster modulating mutations attributed to APOBEC cytidine deaminases in multiple cancer types. Analysis of somatic structural variation additionally exposed complex rearrangement patterns including duplications and template insertion cycles in BRCA1-deficient cancers. Our study underscores the notable impact rare and common germline variants have on cancer mutational landscapes.

T15 Sartorius Lecture:

MODULATION OF TRANSCRIPTION THROUGH DISRUPTION OF BET COMPLEXES

Panagis Filippakopoulos

Structural Genomics Consortium, Nuffield Department of Clinical Medicine,
University of Oxford, UK

Transcriptional programs are often deregulated in disease, offering opportunities for therapeutic intervention. One of the most promising over recent years is through targeting epigenetic readers of the bromo and extra-terminal (BET) family. Despite the successful translation of BET-inhibitors into the clinic, emergence of toxicity and resistance necessitate better understanding of the underpinning biology, in order to develop safer therapeutics. I will discuss how BETs, which offer a recruitment platform for large complexes via their modular architecture, contribute to the assembly of the transcriptional machinery. For example, employing proteomics we find BETs linked to large complexes associated both with transcriptional activation as well as suppression of key survival programmes. We furthermore identify shared and distinct structural determinants leading to complex assembly and demonstrate *in vitro* and in cells that it is possible to target specific BET-complex component interactions leading to complex perturbation. Our data point towards an under-appreciated role of modularity within BETs in linking transcriptional attenuators to chromatin, revealing protein-specific contributions into complex assembly and informing into potential combination therapies providing novel points for intervention in a clinical setting.

TARGETING TUMOUR HYPOXIC RESPONSE

Udo Oppermann

Botnar Research Center, University of Oxford, UK

The dynamic interplay between histone methyltransferases and demethylases provides an important layer in tuning transcriptional responses and programs and collectively, these chromatin modifications are fundamentally involved in proliferation, stem cell self renewal and differentiation. Methylation of histone H₃ at lysine residue 4 (H₃K₄) is implicated in activation of transcription, whereas lysine methylation of histone H₃ at residue 27 (H₃K₂₇) is correlated with repression of transcription.

The H₃K₄ methylation state found at transcriptional start sites is the result of the interplay between SET domain containing methyltransferases such as *mixed lineage leukemia* (MLL) and histone demethylase members of the JARID1 (KDM5) family of 2-oxoglutarate and Fe²⁺ dependent oxygenases. On the other hand, the methyltransferase *enhancer of zeste homolog 2* (EZH2) catalyzes the S-adenosylmethionine dependent trimethylation of H₃K₂₇, which recruits polycomb repressive complex leading to gene silencing. The histone demethylases UTX (KDM6A) and Jmjd3 (KDM6B) catalyse demethylation of methylated H₃K₂₇ residues. Both types of demethylases, namely KDM5B and KDM6A are implicated in myeloma as shown in multiple genetic analyses.

Potent inhibitors of KDM5 and KDM6 enzymes were used to interrogate histone methylation biology and anti-proliferative responses in multiple myeloma. Inhibition of KDM5 enzymes leads to an expected increase of H₃K₄me₃ levels and enrichment of cell cycle regulator genes resulting in a concomitant cell cycle arrest in a subset of myeloma cell lines. In contrast, treatment with a KDM6 inhibitor leads to an apoptotic response in myeloma systems. KDM6 inhibition counteracts Myc-driven metabolic dependencies and impairs glutamine utilisation in a unique way resulting in selected amino acid depletion, eliciting the integrated stress response (ISR) via GCN2-dependent phosphorylation of eIF2 α , ATF4 activation and induction of pro-apoptotic genes.

DRUGGING TRANSCRIPTION: PROGRESS AND POTENTIAL FOR TREATING HUMAN DISEASES

Rabinder Prinjha

Epinova DPU, Immuno-inflammation Therapy Area Unit, GlaxoSmithKline, Stevenage, UK

Epigenetics is being acknowledged as one of the most exciting areas of scientific progress in drug discovery with a critical role in driving cancer initiation and progression. Small molecule inhibitors of BET Bromodomain proteins have emerged as an exciting new class of compounds for the potential treatment of a range of cancers and immune-mediated diseases. I'll describe the discovery, characterization and progress of these inhibitors at the epigenetic, proteomic, transcriptional and cellular level. I'll describe how we generated and tested hypotheses for potential therapeutic applications in multiple therapeutic areas. I'll illustrate how and why we chose specific tumour types for the ongoing clinical studies with I-BET and emerging insights

ANGIOGENESIS REVISITED: ROLE AND (THERAPEUTIC) IMPLICATIONS OF ENDOTHELIAL METABOLISM

Peter Carmeliet

Laboratory of Angiogenesis and Vascular Metabolism,
VIB-KU Leuven Center for Cancer Biology (CCB), Department of Oncology KU Leuven, Belgium

The past 40 years of research in the angiogenesis field have focused on identifying genetic signals such as VEGF and Notch, which determine vessel sprouting. However, the role and therapeutic potential of targeting endothelial cell (EC) metabolism have been largely overlooked. We have recently reported that ECs are glycolysis addicted and that glycolysis importantly co-determine vessel sprouting downstream of VEGF and other pro-angiogenic signals. In addition, we documented that ECs are rather unique in utilizing fatty acid-derived carbons for the de novo synthesis of deoxyribonucleotides for DNA synthesis during EC proliferation when vessels sprout. Moreover, targeting (blocking) glycolysis and fatty acid oxidation inhibit pathological angiogenesis and induce tumor vessel normalization (thereby reducing metastasis and improving chemotherapy), suggesting that these metabolic pathways are new targets for anti-angiogenic drug development without evoking systemic side effects. Furthermore, lymphatic ECs differ from other EC subtypes in their metabolic requirements for lymphangiogenesis. Since many of these metabolic targets are pharmacologically druggable, these metabolic pathways represent a new promising target for therapeutic anti-angiogenesis.

References:

- B.W. Wong et al. *Nature* 542: 49-54 (2017)
- S. Schoors et al. *Nature* 520: 192-97, (2015)
- S. Schoors et al. *Cell Metab* 19: 37-48, (2014)
- B. Ghesquière et al. *Nature* 511: 167-76 (2014)
- K. De Bock et al. *Cell* 154: 651-63 (2013)
- K. De Bock et al. *Cell Metab.* 18: 634-47 (2013)

KDM4 INHIBITION TARGETS BREAST CANCER STEM CELLS

Roland Schüle

Department of Urology and Central Clinical Research,
Freiburg University Medical Center, Freiburg, Germany

Breast cancer is the leading cause of cancer death among women worldwide. During breast cancer therapy, classical treatments fail to address resistant cancer stem cell populations. Cancer stem cell traits and cancer progression are often associated with alterations of epigenetic regulators such as the lysine demethylases 4 (KDM4s). Here we describe a drug-like KDM4 inhibitor (QC6352) with unique preclinical characteristics. QC6352 is an orally available, selective and potent KDM4 inhibitor. To validate the anti-tumor properties of QC6352 under conditions recapitulating patient tumors, we established a method to isolate and propagate breast cancer stem cells (BCSCs) from individual triple-negative patient tumors after neoadjuvant chemotherapy. Limiting dilution orthotopic xenografts of these BCSCs faithfully regenerate original patient tumor histology and gene expression. QC6352 blocks proliferation, sphere formation and xenograft tumor growth of BCSCs. Importantly, QC6352 abrogates expression of epidermal growth factor receptor (EGFR), a driver of therapy-resistant triple-negative breast cancer cells, via inhibition of the KDM4A demethylase activity. Taken together, we present a unique BCSC culture system as a basis for therapeutic compound identification and demonstrate that KDM4 inhibition is a new therapeutic strategy for the treatment of triple-negative breast cancer.

OVERVIEW: FLASH TALKS

- 1** **Sen M**, Hamdan FH, Wang X, Johnsen SA
The Role of the BAF Complex in Wnt-mediated Transcriptional Regulation in Colorectal Cancer
▶ see **P03**
- 2** Zimmermann K, Gibhardt C, Stanisz H, Zhang X, Capello S, Watters A, Shannan B, Rösch A, Herlyn M. Hoth M, Vultur A, **Bogeski I**
Mitochondrial calcium uniporter-mediated redox signaling controls melanoma pathobiology
▶ see **P18**
- 3** Twomey E, **Reichardt S**
Role of the GR in intestinal epithelial cells for the development of colitis-associated colorectal cancer
▶ see **P34**
- 4** **Baumgart SJ**, Nevedomskaya E, Lesche R, Seidel H, Haendler B
Transcriptomic and epigenetic impact of anti-androgens on prostate cancer models
▶ see **P05**
- 5** **Arlt A**, von Bonin F, Pukrop T, Wilting J, Trümper L, Kube D
Hodgkin lymphoma secreted factors influence monocyte/macrophage polarization and function
▶ see **P17**

- 6 Wicklein D, Kemper M, Gebauer F, Maar H, Lange T, Samatov T, Galatenko V, Tonevitsky A, Tachezy M, Bockhorn M, Izbicki J, Schumacher U**
Knockdown of Integrin α V drastically reduces tumor growth, metastasis and intraperitoneal carcinomatosis formation in a xenograft model of human pancreatic adenocarcinoma
▶ see **P22**
- 7 Peuker K, Strigli A, Južnić L, Matthiesen L, Koch M, Hampe J, Zeissig S**
Myeloid-specific calcineurin as a regulator of intestinal tumor development through the control of immune checkpoint proteins
▶ see **P35**
- 8 Schrader A, Crispatzu G, Oberbeck S, Mayer P, Pützer S, von Jan J, Vasyutina E, Warner K, Weit N, Pflug N, Braun T, Andersson EI, Yadav B, Riabinska A, Ventura Ferreira MS, Beier F, Altmüller J, Lanasa M, Herling CD, Haferlach T, Stilgenbauer S, Hopfinger G, Peifer M, Brümmendorf TH, Nürnberg P, Elenitoba-Johnson KSJ, Zha S, Hallek M, Reinhardt HC, Stern MH, Mustjoki S, Newrzela S, Frommolt P, Herling M**
Integrated genetic profiles of T-PLL implicate TCL1/ATM-centered model of aberrant DNA damage response
▶ see **P36**
- 9 Geyer N, Ridzewski R, Dullin C, Fulda S, Rosenberger A, Hahn H**
Simultaneous inhibition of Smoothed and PI3K induces apoptosis in RMS-bearing mice
▶ see **P45**
- 10 Starzonek S, Maar H, Wicklein D, Schumacher U, Lange T**
In vitro study of human cancer cell adhesion to recombinant human vs. Murine E- and P-selection under dynamic vs. Static binding conditions
▶ see **P24**

POSTER SESSION I: TUMOR EPIGENETICS

Chairs: Rab Prinjha & Panagis Filippakopoulos

P01

The role of CHD1 in androgen independent Castration-resistant Prostate Cancer

Pal M¹ Baumgart SJ² Böcker SJ² Kari V² Johnsen SA² Hahn O^{2,3}

1 Indian Institute of Science Education and Research Kolkata, India **2** Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany **3** Department of Urology, University Medical Center Göttingen, Germany

Androgen deprivation therapy (ADT) is still the principal treatment for advanced prostate cancer, but most tumors inevitably become castration-resistant (CRPC). Resistance to ADT is frequently associated with the emergence of androgen-independent splice variants of the androgen receptor (AR variants) that lack the AR ligand binding domain and are constitutively active. Interestingly, deletion as well as inactivating mutations of chromodomain helicase DNA binding protein 1 (CHD1) gene has been identified in both localized and metastatic castration-resistant tumors. However, the role of CHD1 in gene regulation in the progression of CRPC remains elusive till date. Stable CHD1 depleted LNCaP cells (androgen-sensitive human prostate adenocarcinoma cells) showed enhanced relative growth in steroid hormone depleted media compared to control cells, an effect which could be reversed by addition of androgens. Transfection of LNCaP cells with CHD1 targeting siRNA lead to an upregulated mRNA expression of the constitutive AR splice variant AR-V7. Additionally, mRNA sequencing revealed a previously unexplored role of low CHD1 levels in prostate cancer bone metastasis. CHD1-knockout CRISPR clones were also made to further investigate the hypothesis. Unveiling the interplay between CHD1 and the androgen receptor signaling may, therefore, pave the path towards developing a targeted prostate cancer therapy.

P02

Expression of polycomb proteins in carcinogenesis of colorectal cancer – the bright side of EZH2

Bremer SCB¹ Conradi LC² Kramer F³ Kellner C¹ Hasselluhn MC¹ Reutlinger K¹ Gaedcke J²
Ghadimi M² Ströbel P⁴ Ellenrieder V¹ Heßmann E¹ Bohnenberger H⁴

1 Clinic for Gastroenterology und Gastrointestinal Oncology, University Medical Center Göttingen, Germany — **2** Clinic for General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany — **3** Institute of Medical Statistics, University Medical Center Göttingen, Germany — **4** Institute of Pathology, University Medical Center Göttingen, Germany

Colorectal cancer (CRC) is the leading malignancy in the gastrointestinal tract. Although great advances have been made in treatment of CRC the understanding of the molecular mechanisms of CRC development remains unsatisfying.

Our focus is the function of Enhancer of Zeste Homologue 2 (EZH2) in development and progression of CRC. EZH2 is the catalytic subdomain of the Polycomb Repressor Complex 2 (PRC2). This methyltransferase is well characterized in different malignancies and clearly associated with tumor progression and poorer outcome in the majority of cancer entities.

We investigated the protein (n=272, TMA and single slides, University Medical Center Göttingen) and mRNA (n=217, cancer biopsies, patients of KFO179, University Medical Center Göttingen) expressions of EZH2 in CRC precursor lesions and human colorectal cancer by immunohistochemical stainings and microarray analysis, respectively. EZH2 expression peaked in high grade intraepithelial neoplasia (HG-IEN). Interestingly, in comparison to premalignant lesions EZH2 expression was remarkably downregulated in CRC. Additionally, we found a significant better outcome in those CRC patients with high EZH2 expression in cancer cells. We could confirm these association both on protein and on mRNA level. Therefore, in contrast to different other malignancies, EZH2 seems to play a tumorsuppressive function in CRC and might serve as a marker protein for HG-IEN lesions. Our data indicate, that EZH2 inhibition, that is currently evaluated as a therapeutic strategy in cancer treatment in several clinical trials might promote CRC development and progression. Hence, patients should undergo colonoscopy and eventual IEN resection before the initiation of EZH2 inhibition.

P03

The Role of the BAF Complex in Wnt-mediated Transcriptional Regulation in Colorectal Cancer

Sen M¹ Hamdan FH¹ Wang X¹ Johnsen SA¹

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

Epigenetic regulation of the dynamic structure of chromatin strikes a balance between DNA packaging and accessibility to regulate gene expression. It is therefore not surprising that mutations in these regulators lead to major disruptions in cellular homeostasis and are a common occurrence in cancer. Recent genome-wide sequencing studies have revealed a close association between the epigenome and cancer. In these studies, subunits of the mammalian BAF complex, an ATP-dependent chromatin remodeler, were found to be mutated in over 20% of all human cancers.

The tumor suppressive role of the BAF complex and the effect of altered chromatin dynamics on the development of cancer is established in several cancers like rhabdoid tumors and ovarian cancer. Very interestingly, a recent publication described the pivotal role of BAF250a in driving colorectal cancer (CRC) wherein its inactivation alone led to the formation of invasive adenocarcinomas in mice. BAF250a is also the most frequently mutated chromatin regulator in human CRC.

Surprisingly, in contrast to an expected tumor suppressive role of BAF250a in CRC, we observe that the knockout of BAF250a in CRC cell lines leads to impaired proliferation. Importantly, one of the most commonly occurring mutations in CRC is in the APC gene which leads to hyperactive Wnt signaling. Interestingly, in cases where APC mutations co-occur with BAF250a loss, tumor formation is diminished in mice. Thus, it is interesting to explore the molecular mechanisms by which the BAF complex regulates transcription in the context of Wnt signaling mediated gene expression in CRC. From this, we will gain insights into the interplay of the BAF complex with other transcription factors which could activate or repress regulatory elements of genes that are relevant in the pathogenesis of CRC.

P04

The role of BET inhibition in chemo-resistance and re-sensitization in pancreatic ductal adenocarcinoma

Hamdan FH¹ Kutschat AP¹ Sen M¹ Wang X¹ Johnsen SA¹

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

While the mortality rates of cancer are generally declining, pancreatic cancer persists to be an exception with a 5-year-survival rate of less than 7%. As late diagnosis and resistance to conventional therapies are major contributors to high mortality rates, unconventional treatments are investigated to improve the prognosis of pancreatic cancer patients. Recent findings showed that inhibition of the Bromodomain and Extraterminal Domain (BET) family of proteins is effective, both alone and in combination with conventional chemotherapy, in decreasing pancreatic tumor growth in patient-derived xenografts.

We established L3.6 pancreatic cells that are resistant to Paclitaxel and Gemcitabine with a half maximal inhibitory concentration (IC₅₀) of 100x and 50x of normal cells, respectively. Intriguingly, we report that low concentrations of the BET inhibitor, JQ1, not only sensitize cells to these chemotherapeutic agents, but also induce significant re-sensitization of chemo-resistance.

In order to investigate the mechanisms by which BET inhibition induces chemo-sensitization to agents with different mechanisms of action, we investigated the differential genomic localization of BRD4, the most well-studied member of the BET family, and the active transcription marker H3K27ac in resistant and sensitive cells. Interestingly, recent studies implied that BET family members play an important role in regulating gene transcription through modulation of the 3D chromatin architecture which modulates the interaction of enhancers and their target genes. Accordingly, we aim to identify specific BET-driven enhancers that are gained in resistant cells and perturbed through BET inhibition, hence mediating chemo-sensitization. These enhancers can effectively contribute to resistance and may provide us with unconventional biomarkers. Eventually, this study can elucidate the potential role of BET inhibitors as effective adjuvant therapies in resistant pancreatic cancer.

P05

Transcriptomic and epigenetic impact of anti-androgens on prostate cancer models

Baumgart SJ¹ Nevedomskaya E¹ Lesche R¹ Seidel H¹ Haendler B¹

¹ Drug Discovery, Therapeutic Research Group Oncology, Bayer AG, Berlin, Germany

Prostate cancer is mainly driven by androgen receptor (AR) signaling and clinically addressed by castration, AR antagonists and androgen synthesis inhibitors. These therapies are effective for a limited amount of time after which resistance usually occurs. Resistance mechanisms center on AR signaling and include AR amplification mutations and splice variants, and elevated intra-tumoral androgen levels. Comparing the gene expression programs elicited by AR agonists and antagonists, and the overarching epigenetic modifications, will be essential for a better understanding of their molecular mode of action. Here we compared the impact of the androgen R1881 and of different AR antagonists on the VCaP prostate cancer model by generating transcriptomic profiles. The R1881-stimulated cells were treated with 0.5 or 2 μ M antagonist for 8 or 22 hours. The major differences between samples were observed according to the treatment with androgen or anti-androgens. Principal component analysis showed that anti-androgen treatments had similar effects at the 8-hour time point but that some differences emerged at the 22-hour time point. In the second part of the study, ChIP-seq was used to determine the H3K27 acetylation profiles of LNCaP cells treated with R1881, or with R1881 and an AR antagonist. Here we found a strong impact of androgen treatment on the H3K27ac profiles of numerous genes involved in hormonal regulation. Importantly, both up- and down-regulation of H3K27ac was observed following R1881 treatment, and this was reverted by additional treatment with an AR antagonist. In conclusion, anti-androgens strongly revert the effects of androgen, both at the level of gene expression and of epigenetic modification. Comparison between AR antagonists shows some differences in the overall impact on gene regulation patterns and efforts are currently ongoing to confirm and expand on these data by testing more prostate cancer models and analyzing additional histone modifications.

P06

Bromodomain Testis-Specific Protein BRDT is Expressed in a Subset of Esophageal Squamous Cell Carcinomas and Controls Expression of Differentiation-Associated Genes

Wang X¹ Hamdan F¹ Sen M¹ Johnsen SA¹

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

Esophageal cancer is one of the most malignant cancers, ranking as the sixth leading cause of cancer-related deaths worldwide. The poor survival rate and prognosis highlight the limitations in the biological understanding of esophageal cancer and the urgent need for identification of novel targeted molecular therapies. Recently, large-scale genomic analyses have revealed the extensive alternations of epigenetic regulators which may be used as a basis for developing new “epigenetic drugs”.

BRDT, bromodomain testis-specific protein, is a member of the bromodomain and extra-terminal (BET) family of epigenetic reader proteins. BET proteins can regulate gene expression by recognizing acetylated lysines, thus playing important roles in both normal development and disease progression. Inhibition of BET proteins has emerged as a potential therapy for many types of cancer. In normal human tissues, BRDT is exclusively highly expressed in testes where it drives the meiotic and post-meiotic gene expression to promote spermatogenesis.

We have identified BRDT to be expressed in over 20% of esophageal squamous cell carcinoma (ESCC), a predominant subtype of esophageal cancer. Knockdown of BRDT does not affect cell proliferation but leads to alterations in the expression of differentiation markers. In addition, RNA-seq following BRDT knockdown also supports a role of BRDT in cell differentiation. Depletion of BRDT does not alter the cellular response to BET inhibition. Surprisingly, we also identified BRDT transcripts encoding two truncated isoforms lacking the first bromodomain, which could potentially alter its epigenetic reader function. Together with genome-wide occupancy profile of BRDT, we aim to reveal the functional roles of BRDT in ESCC development and potentially identify therapeutic targets to improve current therapies for ESCC.

P07

Functional and Mechanistic Implications of ARID1A-deficiency in PDAC Development and Progression

Zhang Z¹ Hasselluhn MC¹ Kopp W¹ Hamdan FH² Sen M² Schmidt G¹ Johnsen SA²
Hessmann E¹

¹ Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany ² Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

With a 5-year survival rate below 5-7% and characteristics such as early onset of metastasis and frequent relapse, pancreatic ductal adenocarcinoma (PDAC) is one of the most dismal malignancies and is on track to become the second leading cause of cancer-related death by 2030. Epigenetic alterations are considered to be an emerging mechanism of PDAC progression. Consequently, whole genome sequencing revealed that a number of inactivating mutations of chromatin modifying or remodeling proteins exist in the PDAC genome. ARID1A (AT-rich interactive domain-containing protein 1A), the most frequently mutated subunit of the SWI/SNF (Switch/Sucrose Non-Fermentable) chromatin-remodeling complex in PDAC, alters the chromatin accessibility by facilitating the specific binding activity of SWI/SNF. Recently, ARID1A has been described as a tumor suppressor gene as inactivation of ARID1A has been discovered in a broad spectrum of cancers. However, the exact implications of ARID1A deficiency and PDAC development and progression remain elusive. Here, we aim at assessing the role of ARID1A in PDAC development and progression and at detecting the mechanistic consequences of ARID1A deficiency on the chromatin landscape in PDAC. We find that ARID1A depletion promotes proliferation and leads to alterations in EMT and stemness capacities of human PDAC cells. Remarkably, pancreas-specific ARID1A deficiency results in the occurrence of precursor lesions even independent on oncogenic activation of KRAS. Moreover, ARID1A deficiency cooperates with KRAS to accelerate progression towards PDAC by leading to decreased acinar cell population, acinar-to-ductal metaplasia and enhanced pancreatic infiltration with immune cells. Together, our data demonstrate a role of ARID1A deficiency in initiating precursor lesions and promoting PDAC progression.

P08

Cooperation of MDM2 and polycomb repressor complexes in chromatin modification

Rodewald S¹ Wienken M¹ Dickmanns A¹ Bierwirth C¹ Döbelstein M¹

¹ Institute of Molecular Oncology, University Medical Center Göttingen, Germany

MDM2 is an E3 ubiquitin ligase best known for antagonizing the tumor suppressor p53. However, recent findings highlight another, p53-independent function of MDM2 as a chromatin modifier. MDM2 precipitates with components of the polycomb repressor complex 2 (PRC2) and 1 (PRC1), indicating a physical interaction of MDM2 with PRC complexes. Furthermore, it enhances the trimethylation of histone 3 at lysine 27 (H3K27me3), by PRC2, as well as the mono-ubiquitination of histone 2A at lysine 119 (H2AK119ub1), typically mediated by PRC1, at polycomb protein target genes. Thus, MDM2 contributes to the repression of such genes that are numerous key regulatory genes in differentiation and to the maintenance of cell stemness. The simultaneous loss of MDM2 and RNF2, the enzymatic subunit of PRC1, leads to a reduction in cell proliferation, accompanied by reduced H2AK119ub1 levels, as well as to changes in global gene expression in pancreatic cancer cells. The mechanisms underlying these synthetic effects will be further investigated in order to evaluate its druggability for cancer cell elimination.

P09

Unravelling epigenetic mechanisms of CAF-chemotherapy resistance in mammary carcinoma

Mieczkowska I¹ Schmidt GE^{1,2} Reinhardt O³ Kosinsky RL¹ Velychko T¹ Alves F³ Wegwitz F¹

1 Clinic for General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany **2** Clinic for Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany **3** Molecular Imaging in Oncology, Tandemgroup Max Planck Institut for Experimental Medicine und University Medical Center Göttingen, Germany

Tumor recurrence and chemotherapy resistance remain a major cause of morbidity and mortality in cancer patients including breast cancer. In our previous work, we have shown that treatment of murine triple negative WAP-T mammary carcinomas with CAF-chemotherapy (cyclophosphamide/adriamycin/5FU) aggravates tumor cell phenotype, resulting in enhanced dissemination. Epithelial-mesenchymal plasticity and stem cells properties were thereby strongly linked to tumor cell survival. We now optimized our system to study the mechanisms allowing tumor cells to escape conventional chemotherapeutic treatments and to investigate processes responsible for the establishment of long-term chemoresistance. We thereby identified key epigenetic factors that are likely to play a central role in these processes. Specifically, a reduction of Polycomb (PcG) repressive complexes function was found here to represent an attractive potential epigenetic mechanism underlying increased epithelial to mesenchymal plasticity and stem cell characteristics during therapy survival. We focused therefore our investigations on the role played by the Polycomb repressive complexes 1 and 2 (PRC1 and PRC2) in the acquisition/repression of such properties. We furthermore took advantage of this new knowledge to assess the potential therapeutic benefit of treatments combining conventional chemotherapy with epigenetic inhibitors compensating reduction of PcG complex activity *in vitro* and *in vivo*.

P 10

The histone methyltransferase DOT1L is required for proper DNA damage response DNA repair and modulates chemotherapy responsiveness

Kari V¹ Raul SK¹ Wegwitz F¹ Kitz J² Kramer F³ Henck J¹ Mansour WY^{4,5} Eggert J¹
Spitzner M¹ Najafova Z¹ Grade M¹ Gaedcke J¹ Johnsen SA¹

1 Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany
2 Institute of Pathology, University Medical Center Göttingen, Germany **3** Institute of Medical Statistics, University Medical Center Göttingen, Germany **4** Department of Tumor Biology, National Cancer Institute, Cairo University, Egypt **5** Laboratory of Radiobiology and Experimental Radiooncology, University Medical Center Hamburg-Eppendorf, Germany

Disruptor of telomeric silencing 1-like (DOT1L) is a non-SET containing methyltransferases known to catalyze mono-, di-, and tri-methylation of histone 3 on lysine-79 (H3K79me). The role of DOT1L-mediated H3K79me has been implicated in many biological functions including gene transcription, heterochromatin formation and DNA repair. Recent studies have uncovered the role of DOT1L in the initiation and progression of leukemia and other solid tumors. The development and availability of small molecule inhibitors for DOT1L makes it more interesting to study its therapeutic relevance in the cancers. In the current study we uncovered the role of DOT1L in DNA double strand break (DSB) response and repair in colorectal cancer cells. Our results indicate that DOT1L is required for a proper DNA damage response by regulating the phosphorylation of histone H2AX variant (γ H2AX) and repair of DNA DSBs via homologous recombination (HR). Importantly, our results show that small molecule inhibitors of DOT1L synergistically act with other available chemotherapeutics agents in colorectal cancer cells. Further, the classification of CRC patients based on the H3K79me₃ levels indicate that the low levels of H3K79me₃ correlates with the poor prognosis. Our results suggest that DOT1L mediated H3K79me can be used as a predictive prognostic marker and to stratify HR defective CRC tumors for targeted therapy such as PARP inhibitors.

P11

EZH2 inhibition as a possible new therapeutic approach for Castration-resistant Prostate Cancer harboring a CHD1 deletion

Böcker SJ¹ Baumgart SJ¹ Roth A² Kari V¹ Johnsen SA¹ Hahn O^{1,2}

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

² Department of Urology, University Medical Center Göttingen, Germany

Therapy of metastasized prostate cancer today largely depends on androgen deprivation therapy (ADT). Despite initial response, tumors inevitably reach castration resistance (CRPC), a stage at which chemotherapy and second-generation antiandrogens like Abiraterone and Enzalutamide represent the main remaining therapeutic options. Acquired resistance to these drugs is an emerging problem, raising a need for new approaches. Depletion of chromodomain helicase DNA binding protein 1 (CHD1) seems to be more prevalent in CRPC compared to earlier tumor stages. In our work, we identified a correlation between androgen independent growth and CHD1 depletion in LNCaP prostate cancer cells. Using RNA sequencing, we showed a correlation between CHD1 knockdown and upregulation of Enhancer of Zeste Homolog 2 (EZH2) which could be confirmed on protein level. Treatment with 1 μ M JQ-EZ (an EZH2 inhibitor) showed a stronger effect on the proliferation of CHD1 knockdown cells compared to controls. To further characterize this association as well as the genome wide binding profiles, we performed Chromatin Immunoprecipitation followed by Next-Generation Sequencing (ChIPseq) for CHD1 and EZH2 as well as for the histone modifications H3K4me3, H3K27me3 and H3K27ac in normal FBS as well as in Charcoal-Stripped Serum (CSS). We hereby propose a newly discovered synthetic lethality which, in the future, might be exploited for treatment of prostate cancer, e.g. in patients ineligible for chemotherapeutics.

P 12

Investigating the role of EZH2 in mediating chemoresistance in PDAC

Patil S¹ Witte H¹ Najafova Z² Bohnenberger H³ Kopp W¹ Neesse A¹ Ellenrieder V¹
Johnsen SA² Hessmann E¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany **—2** Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany **—3** Institute of Pathology, University Medical Center Göttingen, Germany

Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most aggressive malignancies where the current standard chemotherapies and other targeted approaches are not effective. Therapeutic resistance is mediated by the plastic nature of PDAC cells which allows them to reprogram themselves to evade therapy. Comprehensive analyses show that the reprogramming capacity of tumor cells is predominantly controlled at the level of chromatin. Polycomb Repressor Complex (PRC) 2 member EZH2, a histone methyltransferase, is one of the epigenetic players that has been linked to tumor cell plasticity. As cellular plasticity and chemoresistance is highly intertwined, EZH2 might represent a critical inducer of chemoresistance in PDAC.

Aim: To explore the functional and mechanistic implications of EZH2-activity in mediating chemoresistance in PDAC and to investigate its role as a potential target for PDAC treatment.

Methods: The role of EZH2 in PDAC cell plasticity and chemoresistance is evaluated *in vitro* in murine and human primary PDAC cells upon genetic (CRISPR/Cas9) or pharmacological (EPZ 6438) EZH2 inhibition by diverse functional assays (e.g. sphere- and BrdU-assays). *In vivo* studies comprise orthotopic transplantation of EZH2-wildtype and deficient PDAC cells in mice. RNA- and ChIP-Seq analyses are performed to identify EZH2-dependent gene signatures.

Results: EZH2 is highly expressed in murine and human PDAC. Preliminary data suggests EZH2 as a strong promoter of PDAC dedifferentiation and link it with a stemness-associated, highly proliferative phenotype. In contrast, genetic and pharmacological inhibition of EZH2 activity abrogates PDAC cell proliferation and chemoresistance and induces gene signatures that favor a more differentiated cellular status.

Conclusion: Our data suggests that EZH2 blockade represents a promising strategy to overcome chemoresistance in PDAC.

P13

Role of the E3 ubiquitin ligase complex RNF20/RNF40 in HER2-positive breast cancer

Prokakis E¹ Kosinsky RL¹ Bedi U¹ Nagarajan S¹ Najafova Z¹ Mansouri A² Wegwitz F¹
Johnsen SA¹

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

² Molecular Developmental Genetics, Max Planck Institute for Biophysics and Molecular Biosciences, Göttingen, Germany

The HER2 (Human Epidermal Growth Factor Receptor 2) -positive subtype of breast cancer has a high tendency to develop drug resistance. Patients harboring resistant disease frequently develop distant metastases and ultimately succumb to it. Monoubiquitination at lysine 120 of H2B (H2Bub1) is an epigenetic modification whose gradual loss has been linked to progression in many cancer types. For this reason, tumor suppressor functions have been hypothesized for the H2Bub1 pathway, in particular for RNF20 and RNF40, the two catalytic subunits of the E3 ubiquitin ligase complex responsible for monoubiquitination of H2B. In this project, we studied the consequences of H2Bub1 pathway impairment through loss of RNF40 during mammary gland development as well as in HER2-positive breast cancer. We combined *in vivo* and *in vitro* approaches using a mammary epithelial cell-specific (MMTV-cre) mouse model of Rnf40 deletion. For initiation of HER2-driven mammary carcinogenesis, we introduced a further transgene, MMTV-HER2. Our *in vivo* investigations surprisingly showed that tissue-specific deletion of Rnf40 reduces mammary stem cells properties in the normal breast and impaired HER2-driven mammary carcinogenesis. To gain more insight into the underlying mechanisms, we performed knockdown of RNF40 or RNF20 in the human HER2-positive HCC1954 and SKBR3 cell lines. Subsequently, we performed functional assays as well as whole transcriptome analyses (mRNA sequencing). We observed thereby that the impaired oncogenic properties observed after RNF20/RNF40 silencing are not caused by interfering with the HER2 signaling pathway. Instead, our analyses rather point at an involvement of the stem cell-specific transcription factor SOX2, in parallel to an involvement of the RHO/RAC/CDC42 pathway in this phenomenon. Together, our results point at a tumor supportive, rather than oncogenic role of the H2Bub1 pathway in HER2 breast cancer.

P 14

The USP22 deubiquitinase and its importance for HER2 breast cancer biology

Wegwitz F¹ Prokakis E¹ Kosinsky RL¹ Bedi U¹ Gorsler T¹ Nagarajan S¹ Mansouri A² Johnsen SA¹

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

² Molecular Developmental Genetics, Max Planck Institute for Biophysics and Molecular Biosciences, Göttingen, Germany

The Ubiquitin-Specific Protease 22 (USP22) is a deubiquitinating enzyme (DUBs) and a component of the mammalian SAGA transcriptional co-activating complex. USP22 was discovered to be part of a so called “death from cancer” signature predicting therapy failure in cancer patients. This enzyme mediates deubiquitination of several target proteins, including histone H2A and H2B. In this study, we explored the biological role of USP22 in development and homeostasis of the normal breast as well as the impact of USP22 loss in HER2-driven mammary carcinomas *in vitro* and *in vivo*. We observed a reduction of breast epithelial tissue branching upon USP22 deletion, pointing at decreased proliferation potential. Furthermore, HER2-driven mammary tumorigenesis was strongly impaired upon USP22 deletion. We validated these observations *in vitro* via siRNA mediated USP22 knockdown in normal immortalized human mammary epithelial cells (MCF10A) and in HER2-positive human breast cancer cell lines (HCC1954 and SKBR3). To gain further mechanistic insights, we performed high throughput mRNA sequencing. We identified a significant downregulation of different HSP90 (Heat Shock Protein-90) genes as well as of EZH2 (Enhancer of Zeste Homologue-2) as potential target genes controlling proliferation properties. Interestingly, protein levels of the c-Myc proto-oncogene, known to drive HSP90 and EZH2 transcription, were strongly decreased upon USP22 silencing. HSP90 is an important factor increasing resistance of tumor cells to various cellular and cytotoxic stress. Consistently, we observed that loss of USP22 renders tumor cells more sensitive to cellular stress, as well as HSP90 inhibitor treatment. Together, our data point at a great potential for USP22 as a prognostic marker and potential anti-cancer therapeutic target.

P15

Epigenetic mRNA modifications of stress-responsive transcripts enable cellular survival in pancreatic cancer

Tampe B¹ Tampe D¹ Zeisberg M¹

¹ Department of Nephrology and Rheumatology, University Medical Center Göttingen, Germany

Pancreatic ductal adenocarcinoma (PDAC) is characterized by enhanced cellular stress by the hypoxic microenvironment, oxidative stress and constant exposure to potentially genotoxic cellular metabolites (i.e. hydrogen peroxide, hydroxyl radicals and superoxide anions). Cellular stress induces stress-responsive transcripts important for cellular survival. Molecular mechanisms ensuring rapid translation of stress-responsive transcripts in cancer cells to resist cellular stress remains elusive. Here, we provide evidence for epigenetic modifications of mRNA molecules ensuring rapid translation of stress-responsive transcripts. In response to cellular stress, these transcripts are shuttled into the cytoplasm along with Tet methylcytosine deoxygenase 3 (TET3). For the first time, we report that nuclear export of TET3 requires presence of a distinct domain exclusively present in TET3 among Tet enzymes. Cytoplasmic TET3 targets distinct UCCUC mRNA motifs and generates mRNA molecules harbouring hydroxymethylcytosine (5hmrC) upon cellular stress. 5hmrC potentiates translation of stress-responsive transcripts and ensures rapid protein synthesis. Therefore, TET3 and its subcellular shuttling might be promising therapeutical targets to mitigate survival of cancer cells under stress conditions.

POSTER SESSION II: TUMOR ENVIRONMENT & METASTASIS

Chairs: Sebastian Zeißig & Alan McIntyre

Comparative proteomics reveals a diagnostic signature for head-and-neck cancer metastasis in the lung

Bohnenberger H¹ Kaderali L² Yepes D³ Plessmann U⁴ Merkelbach-Bruse S⁵ Emmert A⁶ Yao S¹ Hoffmann J¹ Reuter-Jessen K¹ Dröge LH⁷ Lois AM¹ Biggemann L⁸ Walter R³ Comoglio F⁹ Pan KT⁴ Küffer S¹ Bremmer F¹ Kitz J¹ Sitte M¹⁰ Hinterthaler M⁶ Brandts C² Sebastian M² Beißbarth T¹⁰ Lotz J⁸ Schildhaus HU¹ Wolff H¹¹ Danner B⁶ Canis M^{12,13} Büttner R⁵ Serve H^{3,14} Ströbel P¹ Urlaub H¹⁵ Oellerich T^{3,14}

1 Institute of Pathology, University Medical Center Göttingen, Germany **2** Institute of Bioinformatics, University Medicine Greifswald, Germany **3** Dept. of Medicine II, Hematology/Oncology, Goethe University, Frankfurt, Germany **4** Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany **5** Institute of Pathology, University Hospital Cologne, Germany **6** Dept. of Thoracic Surgery, University Medical Center Göttingen, Germany **7** Dept. of Radiooncology, University Medical Center Göttingen, Germany **8** Institute of Radiology, University Medical Center Göttingen, Germany **9** Dept. of Haematology, University of Cambridge; Cambridge Institute for Medical Research, Wellcome Trust/MRC Stem Cell Institute, Cambridge, UK **10** Institute of Medical Statistics, University Medical Center Göttingen, Germany **11** University Medical Center Göttingen, Germany **12** Dept. of Otorhinolaryngology, University Medical Center Göttingen, Germany **13** Dept. of Otorhinolaryngology, University Hospital Munich, Ludwig-Maximilian-University München, Germany **14** German Cancer Research Center and German Cancer Consortium, Heidelberg, Germany **15** Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry; Bioanalytics, Georg August University, Göttingen, Germany

Head and neck squamous cell carcinoma (HNSCC) affects more than half a million patients every year and patients with smoking history can develop both lung metastases and secondary primary lung cancer during the course of their disease. Discrimination between lung metastasis and secondary primary lung cancer with squamous cell histology (SQCLC) is of clinical importance for HNSCC patients, because the resulting therapeutic procedures can range from curative treatment for SQCLC patients to palliative treatment for patients with metastatic HNSCC. Despite the clinical importance of discrimination, reliable diagnostic biomarkers are still lacking due to shared morphological and genomic features. / In this study we characterized a clinically and genetically well-characterized cohort of SQCLC and HNSCC by quantitative mass-spectrometry-based proteomics. In average we quantified 2250 proteins per sample in 44 SQCLC and 30 HNSCC formalin-fixed tissue samples from patients that developed undetermined lung tumors with squamous cell histology in the course of their disease. We reasoned that this proteomic approach, rather than genomic and transcriptomic studies, may identify a suitable biomarker panel, and moreover may provide insights into tumor cell functions. Using supervised machine learning we identified a proteomic signature for the classification of squamous cell carcinomas as either SQCLC or HNSCC with a diagnostic accuracy of 96%. Applying this signature on undetermined pulmonary squamous cell carcinomas leads to a significant prognostic separation. / Taken together, this study represents a proteomic resource for HNSCC and SQCLC with quantitative protein expression data for 6196 proteins and provides a diagnostic proteomic signature for classification for undetermined secondary lung tumors in HNSCC patients.

P 17

Hodgkin lymphoma secreted factors influence monocyte/macrophage polarization and function

Arlt A¹ von Bonin F¹ Pukrop T³ Wilting J² Trümper L¹ Kube D¹

1 Clinic of Hematology and Medical Oncology, University Medical Center Göttingen, Germany **2** Institute of Anatomy and Cell Biology, University Medical Center Göttingen, Germany **3** Clinic for Internal Medicine III, University Medical Center Regensburg, Germany

Interactions between tumor cells and the microenvironment crucially regulate disease progression, including tumor cell survival, metastasis and therapy resistance. A paradigmatic microenvironment-controlled cancer entity is classical Hodgkin lymphoma (HL). The malignant Hodgkin-Reed-Sternberg (HRS) cells account for less than 1% of the disease-related cells. HRS cells highly depend on signaling cross-talk with non-transformed neighboring cells, and thus HL perfectly serves as a model to analyze tumor-stroma interactions.

Tumor-associated macrophages (TAMs) have often been assigned as tumor-supportive microenvironmental cells, able to sustain proliferation and angiogenesis and to suppress anti-tumor immune responses. Therefore, the aim of our study is the characterization of mutual interactions between HRS cells and monocyte-derived macrophages.

Primary human monocytes isolated from peripheral blood were differentiated *in vitro* in the presence of HL-conditioned supernatant in comparison to colony-stimulating factor 1 (CSF1/M-CSF). Cell surface protein expression measured by flow cytometry revealed that HL-derived factors strongly support monocyte differentiation into macrophages, specifically the M2-related subtype. These macrophages are characterized by strong expression of CD40, CD163, CD206, and adhesion molecules. Functional analyses revealed high endocytic activity of HL-conditioned macrophages. In addition, we used chick chorioallantoic membrane assays to study the role of the M2-related macrophages for lymphoma behavior *in vivo*. We found that the presence of macrophages increases the probability of lymphogenic dissemination of HRS cells.

Our observations support a model in which HRS cells are able to induce monocyte differentiation into M2-related macrophages, which then modulate the lymphoma microenvironment. Further molecular and functional studies are in progress to characterize mutual interactions between TAMs and HRS cells more deeply.

P18

Mitochondrial calcium uniporter-mediated redox signaling controls melanoma pathobiology

Zimmermann K¹ Gibhardt C¹ Stanisz H² Zhang X¹ Capello S¹ Watters A³ Shannan B⁴
Rösch A⁴ Herlyn M³ Hoth M⁵ Vultur A¹ Bogeski I¹

1 Molecular Physiology, Institute of Cardiovascular Physiology, University Medical Center Göttingen, Germany **2** Dermatology, Allergology and Venereology, University Medical Center Göttingen, Germany **3** Wistar Institute, Philadelphia, USA **4** Dermatology, Allergology and Venereology, University of Essen, Essen, Germany **5** Biophysics, University of Saarland, Homburg, Germany

Melanoma is the deadliest of skin cancers and has a tendency to metastasize to distant organs, thereby causing a significant decrease in life expectancy. Previous studies demonstrated that mitochondria play an important role in melanoma pathobiology and drug resistance. Moreover, calcium and redox signaling have been shown to be important factors in melanoma growth and invasion. A major route for Ca²⁺ transfer across the inner mitochondrial membrane involves the Mitochondrial Calcium Uniporter (MCU) complex. However, how and if the MCU complex affects melanoma biology is currently unknown. Here, we show that all regulating components of the MCU complex are expressed to varying degrees in human-derived primary and metastatic tumor lesions. Ca²⁺ imaging showed that MCU controls mitochondrial Ca²⁺ levels in melanoma cells. Knockdown of MCU strongly reduced mitochondrial Ca²⁺ uptake and mitochondrial H₂O₂ production. Conversely, over-expression of MCU elevated mitochondrial Ca²⁺ concentration and enhanced the cellular oxidative burden. In addition, MCU down-regulation promoted melanoma cell migration and invasion and suppressed tumor growth in 2D and/or 3D matrix-supported melanoma cultures. Antioxidant treatment mimicked while pro-oxidants eliminated the MCU knockdown-induced melanoma migration, suggesting that MCU-controlled mitochondrial ROS production is a determinant of melanoma metastatic spread. *In vivo*, MCU-silenced tumors exhibit reduced primary tumor volumes as shown using a melanoma xenograft mouse model. Moreover, the number and the tumor mass of metastatic lesions in the lungs of the MCU-silenced xenografts were increased. Proteomic analysis identifies additional MCU-controlled signaling pathways which could affect melanoma growth and metastasis. In summary, our results demonstrate that MCU controls melanoma tumor cell behavior through calcium and redox-related mechanisms.

P19

Functional characterization of NFATc1 in the formation of PDAC-associated cancer cachexia

Spyropoulou D¹ Saul D² Goetze R¹ Hasselluhn MC¹ Kellner C¹ Kopp W¹ Patil S¹
Spitalieri J¹ Zhang Z¹ Ellenrieder V¹ Hessmann E¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany **2** Department of Trauma Surgery, Plastic and Reconstructive Surgery, University Medical Center Göttingen, Germany

Approx. 80% of PDAC-patients develop the wasting syndrome cachexia, which is defined by a loss of body mass and characterized by systemic inflammation.

Cachexia is associated with worse postoperative outcome, less response to chemotherapy and decreased survival. We hypothesize that overexpression of the oncogenic transcription factor “nuclear factor of activated T-Cells” (NFATc1) contributes to tumor cachexia by production of inflammatory cytokines. To determine the body weight development, we observed KrasG12D; p53R172H; pdx1Cre (KPC)-mice with varying NFATc1 expression and NFATc1; KrasG12D; pdx1Cre- (NKC)-mice with constitutive NFATc1 activation upon tumor formation. The occurrence of cachexia in terminally sick tumor bearing mice was correlated with blood levels of cytokines, muscle atrophy, cardiac impairment and tumor phenotype. The tissue was histologically analyzed concerning metastasis, immune cell - and cytokine infiltration and NFATc1-expression. Primary tumor cells (PTC) and cancer associated fibroblasts (CAF) were isolated and cell-free supernatants (CFS) of PTC alone and co-cultured CAFs were analyzed following NFATc1 knockdown.

We detected a severe loss of body weight in NKC-mice, while KPC-mice were classified regarding their NFATc1-expression into NFATc1high with an elevated weight loss and NFATc1low with no appreciable body weight loss.

NFATc1high-tissue showed a pronounced infiltration of immune cells and high cytokine-concentrations. Accordingly, levels of proinflammatory cytokines were elevated in mice-serum as well as in CFS in a NFATc1-dependent manner.

Together, our findings suggest that NFATc1 serves as a driver of cachexia in PDAC by controlling cytokine- and chemokine-expression and -secretion.

Orthotopic syngenic transplantation of NFATc1 pro- and deficient KPC cells into immune-competent mice will further elucidate the causative role of NFATc1 in the development of cachexia.

P20

Role of CC531 rat colorectal cancer cell line in translational research – comparison to two human colorectal cancer cell lines upon treatment with irinotecan

Nowack H¹ Spitzner M¹ Sperling J¹ Ghadimi M¹ Kauffels-Sprenger A¹

¹ Department of General, Visceral and Paediatric Surgery, University Medical Center Göttingen, Germany

Introduction: Our aim was to compare the cellular viability and proliferation of the CC531 rat colorectal cancer cell line to two human colorectal cancer cell lines (SW480 and SW837) upon treatment with irinotecan *in vitro*. Additionally, we compared protein expression of CC531 *in vitro* and *in vivo*.

Material and methods: All three cell lines were treated with increasing doses of irinotecan for 24, 48 and 72hrs *in vitro*. Colony formation assays and cellular viability assays were performed in triplicates. Additionally, CC531 cells treated *in vitro* with irinotecan for 72hrs were lysed to analyze markers for proliferation (PCNA) and apoptosis (Caspase-3) by western blot. Also, CC531 liver metastases from animals treated *in vivo* with irinotecan were analyzed immunohistochemically for the same markers.

Results: *In vitro* cellular viability was comparable in all cell lines. Colony formation assays revealed a significantly reduced plating efficiency and surviving fraction of SW837 compared to CC531 and SW480 ($p < 0.001$). With increasing doses of irinotecan Caspase-3 expression also increased in CC531 cells *in vitro*. Immunohistochemistry of tumors treated *in vivo* also showed enhanced Caspase-3 expression compared to untreated metastases. PCNA-expression was reduced upon treatment *in vivo*, but showed an increase *in vitro*.

Conclusions: *In vitro* behavior of CC531 cells upon treatment with irinotecan is comparable to that of human colorectal cancer cell lines. In our opinion, CC531 cells and the corresponding rodent model for colorectal liver metastases are feasible for translational research on colorectal cancer *in vitro* and *in vivo*.

P 21

The role of TMX oxidoreductases in melanoma growth and invasion

Zhang X¹ Vultur AM¹ Bogeski I¹

¹ Institut für Herz- und Kreislaufphysiologie, Universitätsmedizin Göttingen, Germany

Calcium and redox signals are essential regulators of melanoma pathobiology. However, information regarding molecular players involved is scarce. Here, we examined the role of endoplasmic reticulum (ER)-based protein disulfide isomerases (PDI) family members thioredoxin-related transmembrane proteins 1 and 3 (TMX1, TMX3) in melanoma. Our results show that TMX1 and TMX3 are upregulated in human melanoma samples. TMX1 downregulation inhibited melanoma cell proliferation and migration *in vitro* and tumor growth *in vivo*. Moreover, TMX1-silencing led to inhibition of NFAT1 nuclear translocation, a transcription factor present in melanoma but absent in healthy melanocytes. TMX1-silenced melanoma cells displayed an enhanced mitochondrial calcium uptake and subsequent increase in intracellular H₂O₂ levels which were responsible for NFAT1 inhibition via oxidation of calcineurin. Antioxidant treatment reversed the TMX1-induced NFAT1 inhibition. Electron microscopy of TMX1-silenced cells depicted an altered mitochondrial morphology and distances between mitochondria and ER and plasma membrane and thereby provided evidence regarding the molecular mechanism leading to TMX1-induced inhibition of NFAT1 activity and thus melanoma growth and invasion. In summary, our study identified a novel TMX1-NFAT1 signaling axis that regulates melanoma pathobiology in a calcium and redox dependent manner. TMX1 and NFAT1 represent potential novel therapeutic targets as well as biomarkers of aggressive melanoma disease.

P22

Knockdown of Integrin αV drastically reduces tumor growth, metastasis and intraperitoneal carcinomatosis formation in a xenograft model of human pancreatic adenocarcinoma

Wicklein D¹ Kemper M^{1,2} Gebauer F³ Maar H¹ Lange T¹ Samatov T⁴ Galatenko V⁴
Tonevitsky A⁴ Tachezy M² Bockhorn M² Izbicki J² Schumacher U¹

1 Institute of Anatomy and Experimental Morphology and University Cancer Center Hamburg (UCCH), University Medical-Center Hamburg-Eppendorf, Germany **2** Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, University of Hamburg, Germany **3** Department of General, Visceral and Tumor Surgery University Medical Center Cologne, University of Cologne, Germany **4** SRC Bioclinicum, Moscow, Russia

Intraperitoneal carcinomatosis is a common form of progression in abdominal cancers, which leads to a dismal prognosis for the affected patients. New therapeutical strategies for selective inhibition of the mechanisms underlying the development of intraperitoneal carcinomatosis are thus desperately needed.

Crucial in this regard is the question how to inhibit the interactions of tumor cells with the peritoneum, especially with the mesothelial cells forming its squamous epithelium.

We could show that E- and P-selectin are essential for intraperitoneal carcinomatosis formation by human pancreatic adenocarcinoma (PDAC) cells in a xenograft model.

Using microarray analysis, we could demonstrate an overexpression of integrin αV (ITGAV) in the few carcinomatoses which still developed in the selectin-deficient animals and tumor multi-array analysis including tumors of 138 PDAC patients revealed high ITGAV expression to be correlated with shortened survival. As ITGAV is a crucial activator of TGF β , we used a human PDAC cell line with a deletion of the *SMAD4* gene leading to dysfunctional canonical TGF β signaling and another cell line which is *SMAD4* wild-type for a PDAC xenograft model in immunodeficient mice. In this model, RNAi-mediated knockdown of ITGAV in the PDAC cells led to a drastic decrease in the cells' ability to form tumors and metastasize in a PDAC xenograft, interestingly for both cell lines, indicating the effects to be at least in part TGF β independent. Subsequent microarray analysis showed that 22 genes were concordantly regulated in the *SMAD4* positive and negative tumors. Additionally, ITGAV knockdown reduced intraperitoneal carcinomatosis formation in a xenograft model and this positive effect was cumulative with the reducing effect of E- and P-selectin deficiency in the animals.

These results demonstrate that ITGAV, especially in combination with E- and P-selectin, could be a new target molecule for therapeutic intervention in abdominal cancers.

P 23

Synergistic inhibition of tumor formation by depletion of Integrin $\beta 4$ on tumor cells and of endothelial selectins in tumor stroma: Analyses on the underlying mechanisms

Hanika S¹ Labitzky V¹ Schetler D¹ Kirchner L¹ Gebauer F^{1,2} Wicklein D¹ Maar H¹ Gosau T¹
Schröder-Schwarz J¹ Stürken C¹ Linder S³ Riecken K⁴ Schumacher U¹ Lange T¹

1 Institute of Anatomy and Experimental Morphology, University Medical Center Hamburg-Eppendorf, Germany **2** Department of General, Visceral and Thoracic Surgery, Hamburg, Germany **3** Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Germany **4** Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, Hamburg, Germany

Metastases account for 90% of cancer-related deaths. However, the single steps of metastasis formation are not fully understood. As the survival of cancer cells is particularly limited in the circulation, extravasation appears to be one critical step of metastasis. Prior to extravasation, tumor cells attach to the vascular endothelium by imitating the leukocytes adhesion cascade, which is mediated by cell adhesion proteins like selectins and integrins. Indeed, upregulation of Integrin $\beta 4$ (ITGB4) is associated with invasion and metastasis in various tumors.

We could show that knock-down (KD) of ITGB4 in prostatic PC-3, pancreatic PaCa5061 and ovarian SKOV3 cancer cells surprisingly affects their growth as xenograft primary tumors. In particular, tumor growth was inhibited when ITGB4-KD was combined with E-/P-selectin deficiency of the mice. The aim of this study was to investigate the molecular mechanisms underlying this synergistic growth retardation. We suggest that the loss of ITGB4 forces the induction of anoikis that must be overcome during tumor formation. IHC analyses of pH2A.X, Bim and Feulgen stain revealed increased apoptosis in ITGB4-KD tumors. To recapitulate the early events of tumor development, PC-3 cells were analyzed on day 2, 4, 8 and 16 after s.c. injection into immunodeficient control and E-/P-selectin-KO mice. Interestingly, CD45 and CD11b staining indicated a strong immune cell infiltration of ITGB4-KD tumors in wildtype mice. On the contrary, leukocytes were mainly located at the outer tumor periphery of both ITGB4-KD and control tumors in E-/P-selectin-KO mice. Using 3D invasion assays, we could show a higher invasion rate of human macrophages after indirect co-culture with ITGB4-KD tumor cells compared to control cells.

Hence, we hypothesize that ITGB4-KD tumors depend on pro-survival signals from tumor-associated leukocytes to overcome anoikis and that tumor-associated leukocytes facilitate endothelial selectins for tumor infiltration.

P24

In vitro study of human cancer cell adhesion to recombinant human vs. murine E- and P-selectin under dynamic vs. static binding condition

Starzonek S¹ Maar H¹ Wicklein D¹ Schumacher U¹ Lange T¹

¹ Institute of Anatomy and Experimental Morphology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

One critical step of metastasis formation is the interaction of endothelial E- and P-selectins with carbohydrate ligands on circulating tumor cells (CTCs), prior to their extravasation into distant organs. However, it is still unclear whether these interactions take place only under dynamic (blood flow-dependent) or also under static conditions and whether species-specific differences in the ligands of human vs. murine E- and P-selectins exist. This question is relevant since metastasis is widely studied in xenograft models.

Therefore, we analyzed potential differences in the functional ligands of human vs. murine E- and P-selectin under dynamic vs. static conditions. Three groups of human cancer cell lines were compared after different treatments, including cleavage of sialic acid residues or glycoproteins or inhibition of O- or N-glycosylation. The cells expressed either both canonical E-selectin ligands sialyl-Lewis A and X (sLeA/X), or sLeX only, or none of them.

We observed that static tumor cell adhesion to E-selectin required the presence of the aforementioned canonical ligands. Other (non-canonical) ligands must exist that are functional under dynamic conditions only. Murine selectins were more commonly and more strongly bound than human selectins suggesting a lower selectivity and higher diversity of ligands for murine selectins. Cleaving sialic acid residues and inhibiting O-glycosylation significantly impaired static binding of human E-selectin. However, the majority of the tested cell lines showed glycoprotein-independent selectin binding so that glycolipids must be considered as ligands as well. Most treatments affected either dynamic or static selectin binding again indicating different classes of ligands for static vs. dynamic binding.

Summarized, the molecular interaction between CTCs and selectins is more complex than widely assumed. Our findings encourage future studies on static vs. dynamic selectin binding in more physiologic metastasis assays.

Drug scavenging of TAMs in pancreatic cancer

Buchholz SM¹ Patzak MS¹ Richards FM² Jodrell DJ² Griesmann H³ Michl P³ Buchholz M⁴
Ellenrieder V¹ Neesse A¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany **2** Cancer Research UK Cambridge Institute, Li Ka Shing Centre, University of Cambridge, United Kingdom **3** Department of Internal Medicine I, Martin-Luther University Halle/Wittenberg, Halle, Germany **4** Clinic for Gastroenterology, Endocrinology and Metabolism, Philipps-University, Marburg, Germany

Background and Aim: The tumor microenvironment (TME) is known for mediating chemoresistance in pancreatic cancer. Tumor-associated macrophages (TAMs) are essential components of the TME and may play important roles in therapeutic resistance. Here, we investigate gemcitabine metabolism of TAMs in murine and human model systems.

Design: We differentiated and polarized the human THP-1 monocyte cell line into M1- (using phorbol myristate acetate (PMA), LPS, IF- γ) and M2-macrophages (PMA, IL4, IL13). Additionally, we isolated primary monocytes from B1-6 wildtype mice and differentiated them using M-CSF. Gemcitabine treated human (L3.6, BxPC3) and murine (KPC) tumor cells as well as TAMs were subjected to LC-MS/MS analysis. Co-culture assays were conducted to study the impact of macrophages on tumor cell viability using gemcitabine, 5-FU and paclitaxel. Expression analysis of gemcitabine-metabolism key-enzymes (CDA, DCK, Nt5c1A, (h)ENT1, (h)ENT2) was performed by Western blot.

Results: Using LC-MS/MS, we detected up to 4-fold increased concentrations of the active gemcitabine metabolite dFdCTP in M1- and M2-macrophages compared to tumor cells. Co-culture experiments showed that tumor cells treated with supernatant from gemcitabine treated macrophages were 1.5 to 6 fold more viable compared to the control ($p < 0.05$). For 5-FU and Paclitaxel no significant difference was observed. Nt5c1A dephosphorylates dFdCTP to its native prodrug (dFdC). Interestingly, it was highly expressed in tumor cells but not expressed in TAMs, providing a potential rationale for the intracellular accumulation of dFdCTP.

Conclusion: Our findings suggest that macrophages specifically activate and entrap gemcitabine intracellularly making it unavailable for tumor cells. This drug scavenging effect may contribute to the clinical failure of gemcitabine in PDAC. Ongoing *in vivo* studies focus on the effect of pharmacological inhibition of macrophages in genetically engineered mice.

P26

Cytosolic 5'-Nucleotidase 1A Drives Chemotherapeutic Resistance to Gemcitabine in Pancreatic Cancer

Patzak MS¹ Hessmann E¹ Kari V² Kitz J³ Richards FM⁴ Jodrell DI⁴ Johnsen SA²
Ellenrieder V¹ Nesses A¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany **2** Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany **3** Institute of Pathology, University Medical Center Göttingen, Germany **4** Cancer Research UK Cambridge Institute, University of Cambridge, United Kingdom

Background and Aim: The nucleoside analogue gemcitabine is administered as prodrug (dFdC), and intracellularly activated to its cytotoxic triphosphate (dFdCTP). Cytosolic 5'-nucleotidase 1A (Nt5c1A) dephosphorylates gemcitabine monophosphate to the prodrug dFdC. We found Nt5c1A to be overexpressed in murine and human pancreatic cancer cells. This study aims to elucidate the role of Nt5c1A in chemotherapeutic resistance in pancreatic cancer.

Design: Expression levels of Nt5c1A in stromal and epithelial cells in human pancreatic cancer were investigated using a tissue microarray (TMA, n=73). Moreover, Nt5c1A overexpressing murine pancreatic stellate cells (PSCs) were established. Liquid chromatography- tandem mass spectrometry (LC-MS/MS) analysis was performed on gemcitabine treated cell samples. We performed co-culture and viability assays of transfected PSCs and tumor cells to further investigate the implications of Nt5c1A overexpression in PSCs on gemcitabine sensitivity and availability.

Results: Nt5c1A was robustly expressed (score 2 and 3) in the epithelial compartment of more than 45% of all tissue samples, whereas stromal Nt5c1A was not detected in almost 90% of all patient samples in the TMA. Additionally, we revealed significantly decreased levels of cytotoxic dFdCTP in our stably transfected PSCs *in vitro* using LC-MS/MS ($p < 0.02$). Conditioned medium of transfected PSCs preincubated with gemcitabine increased cytotoxicity towards two murine cancer cell lines by 25% ($p < 0.05$).

Conclusions: Our findings support the hypothesis of Nt5c1A as a key player of chemotherapeutic resistance in pancreatic cancer cells. Co-culture of fibroblasts with recombinant overexpression of Nt5c1A and tumor cells significantly increased gemcitabine availability towards tumor cells. Further experiments will focus on the regulation of Nt5c1A expression and orthotopic mouse models will be used to investigate its impact on gemcitabine resistance.

P 27

Role of fibroblast derived SPARC in a genetically engineered mouse model of pancreatic cancer

Ramu I¹ Goetze R¹ Patzak M¹ Ströbel P² Sipos B³ Hessmann E¹ Ellenrieder V¹ Neesse A¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany
2 Institute of Pathology, University Medical Center Göttingen, Germany **3** Institute of Pathology and Neuropathology, University Clinic Tübingen, Germany

Background and Aim: SPARC (Secreted Protein Acidic and Rich in Cysteine) is a matricellular protein, secreted by cancer associated fibroblasts during PanIN progression and in the activated stroma subtype of PDA.

Experimental design: To study the role of SPARC during PanIN progression and PDA development the following mouse models were generated: LSL-KrasG12D;SPARC^{-/-};p48-Cre (KSC), and LSL-KrasG12D;SPARC^{+/+};p48-Cre (KC). Both models were extensively characterized by immunohistochemical and biochemical methods at defined time points and for survival. Primary cell lines from CAFs and tumor cells were generated. Co-culture assays and drug delivery assays were conducted.

Results: Early (3-4 months, n=7-10 mice for each genotype) and late (7-8 months, n=7-10 mice for each genotype) PanIN and ADM development was not dramatically affected by genetic SPARC ablation. However, collagen and hyaluronan expression was significantly reduced in preneoplastic tissues and tumors from KSC mice. Despite the reduction of several ECM proteins, interim analysis strongly suggests that tumor incidence was increased in KSC mice, and tumor related survival was significantly shortened in KSC mice (p<0.02). Viability of CAFs *in vitro* and α -SMA expression *in vivo* was not altered by SPARC expression. Upon TGF- β stimulation, SPARC was strongly induced via pSMAD2/3 in SPARC^{+/+} CAFs.

Conclusion: Interim analysis surprisingly suggests a tumor restraining role of stromal derived SPARC in KSC mice. RNA seq analysis and detailed pharmacokinetic drug delivery assays in isolated CAFs as well as bulk tumor tissues are ongoing to identify the exact mechanism and preclinical relevance of stromal derived SPARC in the KrasG12D mouse model.

P28

Disarming tumors from TGFbeta shield by radiation-induced Neutralization of TGFbeta in tumor environment

Guhlich M¹ Schirmer MA¹

¹ Department of Radiation Oncology, University Medical Center Göttingen, Germany

TGF β (transforming growth factor beta)-signaling exerts an important role in cell response to ionizing radiation. In humans, three isoforms of the TGF β -ligands are known: TGF β 1, TGF β 2 and TGF β 3. These ligands were shown to also affect dedifferentiation, invasion and metastasis of cancer cells. Blocking TGF β -signaling results in a stronger tumor cell eradication by the innate immune system. Besides pharmacologically approaches, this can be achieved via a strong expression of TGFBR3, the TGF β type three receptor gene. As TGF β -signaling is important for various biological systems, a local approach within the tumor could be beneficial in proceeding the fight against cancer. The aim of this project is therefore to establish a localized, radiation-induced neutralization of TGF β -ligands within the tumor environment via overexpression of TGFBR3. In order to reach this goal, we are working on creating a radio-responsive promoter.

P 29

Hepato-arterial infusion of nanaoliposomal irinotecan in an orthotopic preclinical model of colorectal liver metastases – Enhanced effect in combination with embolization particles

Kauffels-Sprenger A¹ Nowack H¹ Ghadimi M¹ Sperling J¹

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

Introduction: Our aim was to evaluate hepato-arterial infusion of nanoliposomal irinotecan (nal-IRI) with regard to effects on tumor size and protein expression of markers for proliferation, DNA-damage and apoptosis. Additionally, the effect of combination with embolization particles (Embocept® S) was tested.

Material and Methods: Male adult WAG/Rij rats received subcapsular tumor implantation of CC531 rat colonic adenocarcinoma cells in the left liver lobe. Tumor size was assessed ten days after implantation via transabdominal ultrasound and animals underwent hepato-arterial infusion. The control group received NaCl 0.9% (SHAM). The treatment groups either received nal-IRI (HAI nal-IRI), Embocept® S (HAI Embo) or combined treatment of Embocept® S and nal-IRI (HAI Embo+nal-IRI). Three days after treatment tumor size was analyzed before animals were sacrificed.

Results: SHAM showed an increase in tumor size of 87.25% compared to a slight increase of 10.46% in HAI Embo. HAI nal-IRI showed a decrease in tumor size of -23.27%. HAI Embo+nal-IRI led to a decrease in tumor size of -28.21%. All treatment groups showed a significantly reduced tumor growth or tumor size compared to SHAM ($p < 0.05$). Immunohistochemical analysis of PCNA revealed similar values in all four groups. Expression of CASPASE-3 was enhanced in both HAI nal-IRI and HAI Embo+nal-IRI, with expression in HAI Embo+nal-IRI being significantly higher in comparison to SHAM ($p < 0.05$). γ H2AX expression was also increased in HAI nal-IRI and HAI Embo+nal-IRI.

Conclusions: HAI with Embocept® S or nal-IRI alone led to a significantly reduced tumor growth compared to SHAM-treated animals. This effect is even more pronounced, when both agents are given in combination. Expression of apoptosis marker CASPASE-3 was significantly increased after combined hepato-arterial infusion. We herein proved that hepato-arterial infusion with nal-IRI was effective in our preclinical orthotopic model of colorectal liver metastases.

POSTER SESSION III: TUMOR IMMUNOLOGY, REPLICATIVE STRESS & DNA DAMAGE

Chairs: Matthias Altmeyer & Thomas Tüting

P30

Impact of bypassed mRNA quality control on DNA damage responses and progression of pancreatic cancer

Tampe D¹ Tampe B¹ Zeisberg M¹

¹ Department of Nephrology and Rheumatology, University Medical Center Göttingen, Germany

Pancreatic ductal adenocarcinoma (PDAC) is characterized by abundant tumour stroma with activated cancer-associated fibroblasts (CAFs), associated with disease progression and poor outcome. Enhanced replicative stress, the hypoxic microenvironment, oxidative stress and constant exposure to potentially genotoxic cellular metabolites (i.e. hydrogen peroxide, hydroxyl radicals and superoxide anions) cause DNA damage, and DNA double-strand breaks are abundant within CAFs. Molecular mechanisms enabling CAFs to remain susceptible to proliferative stimuli despite abundant DNA damage are not yet understood. The fact that CAFs do not return back to an unproliferative state upon DNA damage suggests that they utilize modified DNA damage responses as a prerequisite for stromal expansion and PDAC progression. Based on publicly available exon array datasets, PDACs accumulate aberrant variants of stress-responsive transcripts, including *EYA1*. *EYA1* is primarily known as tyrosine phosphatases that impact DNA damage repair responses by dephosphorylation of their sole phosphotyrosine substrate histone γ H2A.X. Here, we provide evidence that cellular stress induces heat-shock factor 1 (HSF1), associated with bypassed mRNA quality control to ensure rapid translation and cellular survival. However, bypass of mRNA quality control generates a distinct *EYA1* variant lacking exon 11 (*EYA1A Δ 11*) in an HSF1-dependent manner. We provide evidence that *EYA1A Δ 11* mediates accelerating dephosphorylation of its substrate γ H2A.X, modulating DNA damage cell fate decision. This is ultimately associated with accelerated γ H2A.X clearance and perpetuated CAF proliferation. Thus, at the cost of accuracy, CAFs use a simplified mRNA export route for heat-shock transcripts, guaranteeing their rapid translation and enabling cell survival under extreme conditions. Therefore, HSF1 and bypassed mRNA quality control could provide therapeutical targets to normalize activated CAFs and tumour stroma.

P 31

p53 activity results in replication fork processivity

Klusmann I¹ Dobbelstein M¹

¹ Institute of Molecular Oncology, University Medical Center Göttingen, Germany

The tumor suppressor protein p53 is well known for its role in maintaining a stable genome by preventing the propagation of damage onto successive generations of cells. Depending on the extent of damage experienced by a cell, p53 modulates cell cycle arrest to allow for DNA repair or induces apoptosis in case the damage is too severe to be repaired. The currently known functions of p53, commonly referred to as the “guardian of the genome”, imply action only after the damage has occurred within a cell. We challenge this view by providing evidence pointing to a more direct and earlier role for p53 in protecting a cellular genome from damage.

p53 activation enhances the processivity of DNA replication and reduces replicative stress, as monitored by multi-label fiber assays, whereas removal of p53 reduces fork progression. (Klusmann et al. 2016) This was observed in tumour-derived U2OS cells, but also in murine embryonic fibroblasts with heterozygous or homozygous p53 deletion, and in freshly isolated thymocytes from mice with differential p53 status. We are currently evaluating by which mechanisms MDM2, a p53 target gene, contributes to replication fork processivity. In particular, we are investigating whether a chromatin modifying function of MDM2, previously identified by our lab (Wienken et al. 2016), affects replication forks.

p53 helps to protect the genome during S phase by preventing replication fork stalling. These results expand p53’s tumor-suppressive functions, adding to the ex-post model (elimination of damaged cells) an ex-ante activity, i.e. the prevention of DNA damage during replication.

Klusmann, Ina et al. 2016. “p53 Activity Results in DNA Replication Fork Processivity.” Cell Reports 17(7): 1845–57.

Wienken, Magdalena et al. 2016. “MDM2 Associates with Polycomb Repressor Complex 2 and Enhances Stemness-Promoting Chromatin Modifications Independent of p53.” Molecular Cell 61(1): 68–83.

P32

Centrosome integrity as a determinant of replication stress

Tayeh Z¹ Dobbstein M¹

¹ Institute of Molecular Oncology, University Medical Center Göttingen, Germany

In most proliferating cells, centrioles are being duplicated exactly once per cell cycle as well as for DNA replication which happens once every cell cycle. In diploid cells, the centrosome usually includes not more than two centrioles and since the number of centrosomes in the cell is determined by the number of centrioles, cells have developed effective mechanisms to control centriole formation and to tightly coordinate this process with DNA replication. Both processes have to be completed before the mitosis happens. Accumulation of mutations in the S phase may halt the replication fork and or make it collapse. If the replication fork is being halt a specific signaling cascade will be triggered accordingly, starting from unwinding the DNA which allows the replication protein A RPA to coat the ssDNA, and leading to activation of ATR by ATR mediated protein ATRIP. The activation of ATR or ATRIP will phosphorylate the CHK1, and that's what it's being called replicative stress. Severe deficiency in ATR gene can also give rise to a very rare medical disorder known as Seckel syndrome. Our preliminary results show that the depletion of components such as CEP152, SASS6 and PLK4 in synchronized H1299 cells leads to impair the progression of the replication fork, in which this impairment can be rescued by the depletion of MK2 which elucidate the mechanism of how disruption of centrosomes could influence DNA replication. Our immunoblot analysis indicated that the activities of the kinases pChk1, pMK2, p-P38 and pATR/ ATM were altered upon depletion of centrosomal proteins. This suggests a role of these kinases in the crosstalk between centrosomes and DNA replication, but it requires further investigations to dissect the kinase functions in detail.

P33

The impact of the integrated stress response on DNA replication

Choo J¹ Dobbstein M¹

¹ Institute of Molecular Oncology, University Medical Center Göttingen, Germany

Cells are constantly subjected to a multitude of stresses (nutrient deprivation, hypoxia, viral infection) that have to be dealt with properly to ensure survival. Healthy and tumour cells can respond to these stimuli by activating various signaling pathways, one of which being the integrated stress response (ISR). ISR can be activated by multiple kinases (PKR-like ER kinase, PERK; double-stranded RNA-dependent protein kinase, PKR; heme-regulated eIF2 α kinase, HRI; and general control non-repressible 2, GCN2), which converge to phosphorylate eukaryotic initiation factor 2 alpha (eIF2 α) at Serine 51. Phosphorylated eIF2 α inhibits cap-dependent initiation of protein translation, and therefore inhibits global protein translation but increases the translation of stress-responsive mRNAs that help the cells respond to the specific stress stimuli. The activating transcription factor 4 (ATF4) is one of the major proteins actively translated during the induction of ISR.

Many studies have revealed the importance of the ISR in maintaining the survival of tumour cells in harsh conditions and also conferring cytoprotective effects against standard chemotherapeutics. However, most of these rely on the transcriptional activity of ATF4 whereas the relationship between ISR and DNA replication has not been extensively studied.

Preliminary results suggest that the activation of ISR with a variety of ISR inducers hampers with DNA replication. Following this, we aim to understand the following:

1. How does the activation of ISR lead to an impairment of DNA replication?
2. What is the relationship between a reduced DNA replication progression and the chemosensitivity of a cell?

Understanding how tumour cells hijack the ISR to survive would enable better targeting of these cells during treatment. We hypothesize that inhibiting the ISR in combination with replicative stress-inducing chemotherapeutics could synergistically enhance the targeted elimination of tumour cells.

P34

Role of the GR in intestinal epithelial cells for the development of colitis-associated colorectal cancer

Twomey E¹ Reichardt S¹

¹ Institute for Cellular and Molecular Immunology, University Medical Center Göttingen, Germany

Ulcerative Colitis (UC) is a chronic inflammatory disease of modern society that has been constantly increasing during the last centuries. Symptoms include diarrhea, weight loss and abdominal pain. Moreover, UC is a risk factor for development of colorectal cancer (CRC). The gold standard for treatment of UC is systemic application of glucocorticoids (GC), which exert their function by binding to the ubiquitously expressed glucocorticoid receptor (GR). In view of the pivotal role that intestinal epithelial cells (IEC) play for the pathogenesis of colitis, we aimed to elucidate the impact of the GR in this cell type for development of colitis-associated CRC. Initially we observed that dextran sodium sulfate (DSS)-induced colitis was more severe in mice with an inducible knock-out of the GR in IEC. Cytokine secretion of the pro-inflammatory cytokines TNF- α , IL-1 β and IL-6 in the distal part of the colon was higher than in the proximal part, which is in line with the fact that inflammation in UC spreads from the rectal part of the colon. However, there was no difference between mutant and wildtype mice. Analysis of gene expression in the colon confirmed that pro-inflammatory cytokines were similarly upregulated after DSS treatment in mice of both genotypes. In contrast, expression of CXCL1 and CXCL3, two chemokines typically released from enterocytes during inflammation, was increased in mice lacking the GR in IECs compared to wildtype controls after DSS treatment. When we additionally administered AOM once at the beginning of the DSS treatment as a model of CRC, we found significantly more and larger tumors in mutant mice than in wildtype controls. Collectively, our findings suggest an important role of IEC-derived chemokines and their suppression by GC for the modulation of DSS-induced colitis and colitis-associated CRC.

P35

Myeloid-specific calcineurin as a regulator of intestinal tumor development through the control of immune checkpoint proteins

Peuker K¹ Strigli A¹ Južnić L¹ Matthiesen L¹ Koch M¹ Hampe J² Zeissig S¹

1 Center for Regenerative Therapies, Technical University Dresden, Germany **2** Medical Department I, University Hospital Dresden, Germany

Colorectal cancer development is characterized by sequential accumulation of somatic mutations, which promote epithelial proliferation and subsequently tumor invasion. Recent evidence revealed that inflammation-associated pathways such as those of calcineurin and nuclear factor of activated T cells (NFAT) are active in the transformed intestinal epithelium and promote tumor development. Similar observations have been made for other inflammation-associated pathways including that of NF- κ B and have shown that activation of this pathway in epithelial and myeloid cells synergistically promotes tumorigenesis (Greten, Cell 2004). We therefore investigated whether calcineurin is also active in myeloid cells and contributes to intestinal tumor development.

To investigate the role of myeloid calcineurin, we crossed mice with myeloid-specific deletion of calcineurin into the ApcMin/+ model of genetically-induced intestinal tumor development. Mice with myeloid-specific calcineurin deletion exhibited reduced tumor multiplicity and size compared to wildtype littermates. Mechanistic studies revealed decreased proliferation as well as increased apoptotic tumor cells in these mice. Further, the expression of tumor-promoting myeloid IL-6 was decreased in mice with calcineurin deletion, while the expression of IFN- γ , a cytokine associated with negative regulation of intestinal tumor development, was increased. In addition, co-inhibitory proteins of the CD28/B7 family were downregulated in their expression in intestinal tumors of mice with myeloid calcineurin deletion, suggesting a central role for myeloid calcineurin in licensing T cell responses to intestinal tumors. Blocking experiments showed that IL-6, IFN- γ and co-inhibitory molecules indeed contributed to regulation of tumor growth in a myeloid calcineurin-dependent manner.

Together, these data demonstrate a critical role for myeloid calcineurin in the regulation of intestinal tumor development.

P36

Integrated genetic profiles of T-PLL implicate a TCL1/ATM-centered model of aberrant DNA damage responses

Schrader A^{1,2,3,4} Crispatzu G^{1,2,3} Oberbeck S^{1,2,3} Mayer P^{1,2,3} Pützer S^{1,2,3} von Jan J^{1,2,3} Vasyutina E^{1,2,3} Warner K^{1,2,5} Weit N^{1,2,3} Pflug N¹ Braun T^{1,2,3} Andersson EI⁶ Yadav B⁶ Riabinska A^{1,2} Ventura Ferreira MS⁷ Beier F⁷ Altmüller J⁸ Lanasa M⁹ Herling CD^{1,2} Haferlach T¹⁰ Stilgenbauer S¹¹ Hopfinger G¹² Peifer M¹³ Brümmendorf TH⁷ Nürnberg P⁸ Elenitoba-Johnson KSJ¹⁴ Zha S¹⁵ Hallek M^{1,2} Reinhardt HC^{1,2} Stern MH¹⁶ Mustjoki S⁶ Newrzela S⁵ Frommolt P¹⁷ Herling M^{1,2,3}

1 Department I of Internal Medicine, Center for Integrated Oncology (CIO) Köln-Bonn, University of Cologne (UoC), Germany **2** Excellence Cluster for Cellular Stress Response and Aging-Associated Diseases (CECAD), UoC, Germany **3** CMMC, Center for Molecular Medicine University of Cologne (UoC), Germany **4** Scholarship holder “Deutsche José Carreras Leukämie-Stiftung e.V.”, Germany **5** Senckenberg Institute of Pathology, Goethe-University, Frankfurt/M., Germany **6** Hematology Research Unit Helsinki, Department of Medicine and Clinical Chemistry, University of Helsinki and Helsinki University Central Hospital (HUCH), Helsinki, Finland **7** Department of Hematology, Oncology, and Stem Cell Transplantation, RWTH Aachen University Medical School, Aachen, Germany **8** Cologne Center for Genomics, UoC, Germany, Institute of Human Genetics, University of Cologne (UoC), Germany **9** Duke University Medical Center, Durham, NC, USA **10** MLL Munich Leukemia Laboratory, Munich, Germany **11** Department III of Internal Medicine, University Hospital Ulm, Germany **12** Department of Internal Medicine I, Bone Marrow Transplantation Unit, Medical University of Vienna, Vienna, Austria **13** Department of Translational Genomics, UoC, Germany **14** Department of Pathology and Laboratory Medicine, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA **15** Institute for Cancer Genetics, Department of Pathology and Cell Biology, Herbert Irving Comprehensive Cancer Center, Division of Pediatric Oncology, Department of Pediatrics, Columbia University Medical Center, Columbia University, New York, USA **16** INSERM U830, Institut Curie, PSL Research University, Paris, France **17** Bioinformatics Core Facility, CECAD, UoC, Germany

T-cell prolymphocytic leukemia (T-PLL) is a rare and poor-prognostic mature T-cell malignancy. To address its incomplete molecular concept, we integrated large-scale profiling data of alterations in gene expression, allelic copy number (CN), and nucleotide sequences in 111 well-characterized patients. The dominant alteration of T-PLL's molecular make-up is a unique and functionally synergistic combination of TCL1-overexpression and damaging ATM lesions. We also identified novel tumor-specific hot-spots for CN variability, fusion molecules, transcript variants, and progression-associated dynamics. The overall lesional spectrum of T-PLL is mainly annotated to axes of DNA damage responses, of cytokine signaling, and histone modulation. The chromosomal complexity of T-PLL is determined by a specific phenotype of impaired proximal DNA damage processing, telomere attrition, and abrogated cell death execution. Despite frequently identified ATM mutations and genomic losses, specific targeting of factors in potentially synthetic lethal relationships to ATM through small molecule inhibitors did not affect T-PLL cell viability in the context of DNA damage. Based on the most current lesions, we established a model of T-PLL evolution resolved for pivotal genetic alterations integrated with landmarks of cellular dysfunctions.

P37

The implication of Kv10.1 in the regulation of G₂/M transition

Movsisyan N¹ Urrego D¹ Pardo LA¹

¹ Oncophysiology Group, Max Planck Institute of Experimental Medicine, Göttingen, Germany

Kv10.1, also known as Eag1 (Ether-à-go-go1), is a voltage-gated potassium channel normally found in the central nervous system. However, ectopic expression of Kv10.1 has been found in over 70% of all human tumour tissues and its overexpression correlates with poor prognosis. Kv10.1 is known to be important for cell proliferation, cell volume control and angiogenesis, and it is specifically expressed during the G₂/M phase of the cell cycle. Nevertheless, the mechanism of Kv10.1-mediated cell cycle regulation – particularly at the G₂/M transition – is still unknown. The G₂/M phase progression is regulated by two major checkpoints: DNA damage-induced checkpoint (G₂/M transition) and spindle assembly checkpoint (M-phase). Therefore, in the scopes of the study we investigate possible activation of DNA damage response, formation and alignment of the mitotic spindle and its dynamics upon downregulation of Kv10.1. In this study, we show that siRNA-mediated downregulation of the channel leads to an increased polymerization rate of mitotic microtubule plus ends. In addition, a tendency of DNA response pathway activation is observed in Kv10.1 deficient HeLa cells at the G₂/M border. However, downregulation of the channel causes no significant changes in the DNA damage level. Thus, DNA damage response components might be involved in the microtubule plus end growth regulation independently of DNA damage, which may lead to activation of spindle assembly checkpoint and result in extension of the G₂/M phase. Overall, understanding the mechanism of the Kv10.1 role in cell cycle regulation can help to design novel therapeutic approaches for treatment of Kv10.1-positive cancers.

P38

RNF40 loss dampens NFκB activity in colorectal cancer cells and reduces colitis burden in mice

Kosinsky RL¹ Chua RL¹ Qui M¹ Mehlich D¹ Saul D² Mansouri A³ Wegwitz F¹ Johnsen SA¹

¹ Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

² Department of Trauma, Orthopedics and Reconstructive Surgery, University Medical Center Göttingen, Germany

³ Department of Molecular Cell Biology, Max-Planck Institute for Biophysical Chemistry, RG Molecular Cell Differentiation, Göttingen, Germany

Inflammation is considered a key factor underlying colorectal cancer (CRC). Consistently, patients with inflammatory bowel diseases have a three-fold increased risk of tumorigenesis. Mechanistically, the NFκB transcription factor family is activated during inflammation, leading to expression of anti-apoptotic factors and immunoregulatory cytokines. In addition, the loss of the epigenetic modifier RNF20 was recently linked with inflammation-associated CRC. Generally, the RING finger protein RNF20 forms an obligate heterodimer with RNF40, which is recruited by the adapter protein WAC to RNA Polymerase II during transcriptional elongation. Moreover, RNF20/RNF40 promote transcription by monoubiquitinating histone H2B at lysine 120.

We aimed to investigate the function of RNF40 in inflammation and hypothesized that due to its high similarity to RNF20, RNF40 depletion would increase inflammatory signaling as well as tumorigenic potential of CRC cells. Surprisingly, the colon-specific loss of Rnf40 exerted a protective effect on mice during acute colitis. In contrast, wild type animals were characterized by severe epithelial damage in the colon as well as systemic inflammation. *In vitro* studies in several CRC cell lines demonstrated that the tumorigenic potential (i.e. proliferation, clonogenic potential, ability to grow anchorage-independently) of these cells decreased after RNF40 depletion. Transcriptome-wide studies and gene ontology analyses revealed altered expression of genes related to chromosome segregation and replication potential following RNF40 loss. Importantly, we observed decreased induction of several NFκB target genes, including several cytokines and transcription factors, as well as decreased nuclear localization of NFκB in TNFα-treated CRC cells after RNF40 depletion. Together, these findings suggest that RNF40 loss exerts a protective effect during inflammation by reducing nuclear NFκB activity and thereby decreased expression of inflammatory cytokines.

P 39

Characterization of a novel TCL1A-mediated function in CLL – its impact on mitotic checkpoint proteins

Vasyutina E^{1,2} Aszyk C^{1,2} Herling M^{1,2}

1 Laboratory of Lymphocyte Signaling and Oncoproteome, Department I of Internal Medicine, Center for Integrated Oncology Cologne-Bonn, Germany **2** Excellence Cluster for Cellular Stress Response in Aging-Associated Diseases, University of Cologne, Germany

T-cell leukemia/lymphoma 1A (TCL1) is a lymphoid proto-oncogene whose aberrant up-regulation and prognostic impact has been reported for a variety of mature T- and B-cell leukemias. We previously demonstrated TCL1 to be overexpressed in the majority of cases of CLL. There it acts as a sensitizer in B-cell receptor signaling and as a predictor of inferior therapy response. At the molecular level, TCL1 was shown to physically interact with the kinases AKT and ATM, both molecules that govern cell survival and the DNA damage responses. However, the spectrum of molecular and cell-biological consequences of TCL1 dysregulation is not fully represented by its currently recognized effectors.

Mass spectrometric analysis of TCL1 protein complexes has identified molecules of the mitotic checkpoint complex (MCC), namely CDC20, CDK1, MAD2, and BUB3, as novel TCL1-interacting partners. TCL1 overexpression abrogated normal cell cycle progression in cultured cells and in murine TCL1-tg lymphocytes. The TCL1-imprinted cellular phenotype was consistent with the role of TCL1 in cell cycle control and DNA damage pathways, as we observed pronounced cellular aneuploidy upon enforced expression of TCL1 malignant B-cells. In line with altered cellular phenotype, constitutive TCL1 influenced the expression of key cell cycle proteins (CDC20, Cyclin A2, etc.) and activation of the DNA damage response (impaired p53 phosphorylation). Mechanistically, our data indicate that TCL1 promotes the interaction of CDC20 with its inhibitor mitotic arrest deficient 2 (MAD2) and subsequently destabilize CDC20 protein thus contributing to a faster tumor expansion, pronounced cellular aneuploidy and shorter survival of the animals.

Overall, we conclude that TCL1 can affect proper cell cycle progression in CLL by interacting with MCC components and thereby regulating the protein composition of the MCC.

P40

Nuclear NFATc1 signaling in steatosis and inflammation of the liver

Latif MU¹ Hannheide S¹ Reutlinger K¹ Kitz J² Ellenrieder V¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany **— 2** Institute of Pathology, University Medical Center Göttingen, Germany

Introduction and Objectives: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver diseases worldwide, with prevalence of 20% - 30% in western countries. High fat diet and metabolic liver disorder are the primary causes leading to NAFLD. It initiates with lipid deposition in hepatocytes (steatosis) and progresses to nonalcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis and HCC. Aberrant NFAT signaling (usually over expression or hyperactivity) inducing inflammation and tumor development in other organs e.g. pancreas has already been documented. This study intends to investigate NFATc1 regulated gene signatures in NAFLD and inflammation using *in vitro* and *in vivo* models, e.g. the Thioacetamide (TAA) induced fibrosis-cirrhosis and western diet induced NAFLD/NASH. Adipogenesis, inflammation, fibrosis, cirrhosis and cytokine signaling mechanisms were analyzed using Western Blot, RT-PCR, IHC, IF, TUNEL assay, HE staining, and Cytokine Assay, respectively.

Results and Conclusion: NFATc1 is overexpressed in hepatocytes following treatment with western diet and TAA both *in vivo* and *in vitro*. NFATc1 induction causes DNA damage and apoptosis in hepatocytes, as indicated by TUNEL assay, increased phosphorylation of p53 at serine 15 and induction of cleaved caspase 3 in mice tissue lysate. Similar effects were found upon transfection of constitutively active NFATc1 in AML12 cells and mice pretreated with either western diet or TAA. Aberrant expression of NFATc1 induces cytokine signaling, progressive hepatic inflammation and accelerates deposition of extracellular matrix. We also observed a remarkable progression of liver fibrosis in our mice models. Together, our ongoing study suggests a role for NFATc1 in liver damage and proposes a model in which NFATc1 induction drives inflammation and fibrosis presumably via regulation of specific cytokine gene signatures.

P 41

Targeting Macrophage Migration Inhibitory Factor (MIF) in colorectal cancer inhibits proliferation and angiogenesis

Klemke L^{1,2} Witt DI,2, Stark N¹ Schulz-Heddergott R¹

1 Institute of Molecular Oncology, University Medical Center Göttingen, Germany **2** equally contributing authors

Macrophage migration inhibitory factor (MIF), originally identified as a pro-inflammatory cytokine, is highly elevated in many human cancer types, including colorectal cancer (CRC). MIF acquires tumor-prone gain-of-function correlating it with tumor aggressiveness and poor clinical prognosis. Genetic depletion of MIF in mouse cancer models (e.g. breast, skin, pancreas) results in significant inhibition of tumor growth. Recently we identified that MIF in cancer cells is highly stabilized through the Heat Shock Protein 90 machinery (HSP90). Thus, MIF is a HSP90 client. Pharmacological inhibition of Hsp90 results in MIF degradation in several types of cancer cells. This provides a new way to inhibit MIF function.

To test whether MIF improves tumor growth in CRC, mice are tested in a colorectal cancer model (chemical AOM/DSS model). We analyze whether a genetic MIF depletion or an acute MIF withdrawal through Hsp90 inhibition is required for tumor maintenance and invasion. And indeed, pharmacological as well as genetic MIF depletion impairs tumor growth through reduction of tumor cell proliferation and inhibition of angiogenesis. Mechanistically, MIF depletion reduces the cell cycle control as well as the p38-VEGF/IL8 axis.

Thus, expression of stabilized MIF is essential for tumor progression and maintenance in CRC and offer an important druggable target for therapy.

POSTER SESSION IV: TUMOR GENETICS & SIGNALING

Chairs: Nabeel Bardeesy & Marco Gerlinger

P42

Lack of cooperativity in targeting Mdm2 and CDK4 in the treatment of liposarcoma cells

Sriraman A¹ Dobbstein M¹

¹ Institute of Molecular Oncology, University Medical Center Göttingen, Germany

Liposarcoma almost invariably contain an amplification of q13-15 on chromosome 12, which entails the oncogene MDM2 (100% frequency of amplification) and the gene encoding the cell-cycle dependent kinase CDK4 (frequency of amplification – 90%). MDM2 is an E3 ubiquitin ligase that mediates its ubiquitination activity towards the tumor suppressor protein p53 via its RING finger domain. CDK4 on the other hand, is bound to cyclin D, and this complex can phosphorylate retinoblastoma (Rb) protein, allowing the E2F1 transcription factor to mediate the G1 to S transition of the cell cycle. Co-amplification of MDM2 and CDK4 has been proposed to act as a ‘driving factor’ for initiating tumors. Currently, there are three studies in clinical trials involving CDK4 inhibitor – PD0332991 (NCT01209598) and LEE011 (NCT02571829) alone and in combination with MDM2 inhibitor HDM201 (NCT02343172) for treating liposarcoma patients. Therefore, we sought to investigate the impact of MDM2 and CDK4 inhibition on well-differentiated liposarcoma cells using Nutlin-3 as the MDM2 antagonist and PD0332991 (palbociclib) as CDK4/6 inhibitor. Both inhibitors reduced cell viability when applied individually. However, we observed drug antagonism upon co-treatment with Nutlin and PD0332991, followed by assays for cell viability. Upon immunoblot analysis and quantitative RT-PCR, we observed that p53 and expression of its target genes were reduced upon co-treatment with Nutlin and PD0332991, in comparison with Nutlin alone. Similar results were observed upon knockdown of MDM2 and CDK4 by siRNA, in comparison with MDM2 depletion alone. These results suggest that the combination of the two drugs does not result in additive or even synergistic effects on liposarcoma cells with respect to viability and to p53 activity.

P43

Smad4-deficiency in PDAC favors EMT and stemness

Hasselluhn MC¹ Bohnenberger H² Johnsen SA³ Ellenrieder V¹ Hessmann E¹

1 Clinic for Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany
2 Institute of Pathology, University Medical Center Göttingen, Germany **3** Clinic for General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany

Pancreatic ductal adenocarcinoma (PDAC) is a highly deadly disease with its incidence rate nearly equal to its mortality rate. Even though there has been intense research about the mechanisms behind PDAC evolution, this has not led to any new therapeutic strategies replacing Gemcitabine as method of choice. Therefore, new approaches focus on the elucidation of synthetic lethality strategies to target specific PDAC subtypes.

PDAC is characterized by a vast genetic heterogeneity. Nevertheless, there are a few marker mutations preparing the ground for the metastatic and highly invasive disease. Apart from oncogenic KRAS activation which occurs in 90 % of all patients, loss of Smad4 is a common event in PDAC development. SMAD4 is a crucial member of the TGF β signaling pathway, building transcription complexes with receptor SMADs. The SMAD4-deficient PDAC subtype is described as very aggressive and metastasis-promoting, but the molecular mechanisms behind remain unclear.

CRISPR/Cas9-mediated genome editing was established to create SMAD4 WT and SMAD4-deficient clones from murine and human PDAC cell lines. Interestingly, SMAD4 deficiency was associated with an alteration of cell morphology and phenotype, while the TGF β responsiveness remained the same. We identified increased stem cell characteristics of SMAD4-deficient clones as assessed by sphere and soft agar assays. Importantly, this was associated with decreased Gemcitabine sensitivity, offering SMAD4-deficient PDAC subtypes a loophole to evade cell death. Meanwhile, studies with the PARP inhibitor Olaparib demonstrated increased responsiveness of SMAD4-deficient clones.

By elucidating the molecular mechanisms behind SMAD4-deficient Gemcitabine resistance and Olaparib sensitivity, we aim to lay the foundation for personalized medicine. Targeting PDAC by specific and combinatory chemotherapeutic strategies based on vulnerabilities of SMAD4-deficient cancer subtypes gives hope to 50 % of all PDAC patients.

P44

The β -catenin/CBP-antagonist ICG-001 inhibits pediatric glioma tumorigenicity in a Wnt-independent manner

Wiese M¹ Walther N¹ Diederichs C¹ Schill F¹ Monecke S² Salinas G³ Sturm D⁴
Pffister SM^{4,5} Dressel R² Johnsen SA⁶ Kramm CM¹

1 Division of Pediatric Hematology and Oncology, Department of Child and Adolescent Health, University Medical Center Göttingen, Germany **—2** Institute of Cellular and Molecular Immunology, University Medical Center Göttingen, Germany **—3** Transcriptome and Genome Analysis Laboratory (TAL), Department of Developmental Biochemistry, University Medical Center Göttingen, Germany **—4** Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), Heidelberg, Germany **—5** Department of Pediatric Hematology and Oncology, Heidelberg University Hospital, Heidelberg, Germany **—6** Department of General, Visceral, and Pediatric Surgery, University Medical Center Göttingen, Germany

Pediatric high-grade gliomas (pedHGG) belong to the most aggressive cancers in children with a poor prognosis due to a lack of efficient therapeutic strategies. The β -catenin/Wnt-signaling pathway was shown to hold promising potential as a treatment target in adult high grade gliomas by abrogating tumor cell invasion and the acquisition of stem cell-like characteristics. Since pedHGG differ from their adult counterparts genetically and biologically we aimed to investigate the effects of β -catenin/Wnt-signaling pathway-inhibition by the β -catenin/CBP antagonist ICG-001 in pedHGG cell lines. In contrast to adult HGG, pedHGG cells displayed minimal detectable canonical Wnt-signaling activity. Nevertheless, low doses of ICG-001 inhibited cell migration/invasion, tumorsphere- and colony formation, proliferation *in vitro* as well as tumor growth *in vivo/ovo*, suggesting that ICG-001 affects pedHGG tumor cell characteristics independent of β catenin/Wnt signaling. RNA-sequencing analyses support a Wnt/ β -catenin-independent effect of ICG-001 on target gene transcription, revealing strong effects on genes involved in cellular metabolic/biosynthetic processes and cell cycle progression. Among these, high mRNA expression of cell cycle regulator JDP2 was found to confer a better prognosis for pedHGG patients. In conclusion, ICG-001 might offer an effective treatment option for pedHGG patients functioning to regulate cell phenotype and gene expression programs in absence of Wnt/ β -catenin signaling-activity.

P 45

Simultaneous inhibition of Smoothed and PI3K induces apoptosis in RMS-bearing mice

Geyer N¹ Ridzewski R¹ Dullin C^{2,3} Fulda S⁴ Rosenberger A⁵ Hahn H¹

1 Department of Human Genetics, University Medical Center Göttingen, Germany **2** Institute for Diagnostic and Interventional Radiology, University Medical Center Göttingen, Germany **3** Italian Synchrotron Light Source 'Electra', Trieste, Italy **4** Institute for Experimental Cancer Research in Pediatrics, J.W. Goethe University Hospital Frankfurt, Germany **5** Department of Genetic Epidemiology, University Medical Center Göttingen, Germany

Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children with a 3-year overall survival for recurrent disease <30%. RMS is currently treated with surgery or radiotherapy and chemotherapy including vincristine, actinomycin and cyclophosphamide (VAC). We are searching for a targeted therapy that is i) more efficient and ii) circumvents severe side effects of VAC treatment.

RMS show aberrant activation of the Hedgehog (HH) signaling cascade that is therefore a potential candidate to target this tumor entity. The Smoothed (SMO) inhibitors Vismodegib and Sonidegib are approved for advanced basal cell carcinoma, which show high HH activity due to mutations in e.g. the HH receptor PATCHED1 (PTCH). We here show that combined inhibition of HH and PI3K/AKT/mTOR signaling can significantly reduce tumor growth in the Ptch+/- mouse model for RMS, in which HH signaling is active.

Results: Whereas Vismodegib treatment of RMS-bearing animals stopped tumor growth, Sonidegib induced tumor shrinkage. The addition of Pictilisib was not superior to single treatment. HhAntag in combination with Pictilisib significantly reduced tumor size. In murine primary RMS cells, the combination of Vismodegib, Sonidegib or HhAntag with Pictilisib evoked synergistic anti-proliferative effects. On a molecular level, SMO or PI3K inhibitors reduced Hh and P13k/Akt signaling activity, respectively, and the combination of the drugs strengthened this effect.

Conclusion and Perspectives: SMO inhibitors evoke a heterogeneous response regarding tumor growth in the RMS *in vivo* model. Strikingly, Sonidegib single treatment and HhAntag plus Pictilisib treatment evoke a significant decrease in tumor size. Therefore, SMO indeed is a potential target for RMS therapy and the addition of Pictilisib might be beneficial for therapy outcome, depending on the SMO inhibitor investigated. In the future, we will unravel the mechanism underlying this drug-dependent tumor growth reduction.

P46

Towards novel strategies of targeting p53 related vulnerabilities in T-PLL cells

von Jan J^{1,2} Andersson E³ Schrader A^{1,2,4} Yadav B³ Mayer P^{1,2} Mustjoki S³ Herling M^{1,2}

1 Department I of Internal Medicine, Center for Integrated Oncology (CIO) Köln-Bonn, University of Cologne (UoC), Germany **2** Excellence Cluster for Cellular Stress Response and Aging-Associated Diseases (CECAD), UoC, Germany **3** Hematology Research Unit Helsinki, University of Helsinki and Department of Hematology, Helsinki University Hospital Comprehensive Cancer Center, Finland **4** Scholarship holder “Deutsche José Carreras Leukämie-Stiftung e.V.”, Germany

T-cell prolymphocytic leukemia (T-PLL) is a mature T-cell neoplasm commonly presenting at an aggressive phase associated with a marked chemotherapy resistance. Nucleosides and alkylators show very limited therapeutic activity. Although the monoclonal antibody alemtuzumab induces high response rates, virtually all patients relapse and the mostly elderly individuals are often not eligible for allogeneic stem cell transplantation. Overall, the treatment options for T-PLL are scarce and its general prognosis with average survival times of <3 yrs. remains poor. We could show, although TP53 mutations in T-PLL are rare, T-PLL cells fail to generate a distal p-p53 response upon γ -irradiation. Based on the confirmed expression of wildtype p53 protein in T-PLL, we conclude that it is retained in its inactive state by respective regulatory factors (e.g. MDM2). An ex vivo drug screen (306 substances, n=39 cases) confirmed the apoptotic potential of different p53 re-activating agents. Thus, we tested in more detail the potential of p53 reactivators as possible novel therapeutic approaches. Idasanutlin activated the repressed p53 in T-PLL cells upon γ -irradiation as shown by p-p53Ser15 immunoblots. This effect was enhanced by combination treatment with the alkylating agent Bendamustine or the HDAC inhibitor Panobinostat. Flow cytometry based evaluations of the pro-apoptotic potential of different p53 re-activating agents identified Idasanutlin and Panobionstat as most effective single substances. In a small pilot *in vivo* study, we confirm the efficacy of Idasanutlin against murine T-PLL, however, we observe severe toxicities. A second ex vivo screen testing for respective synergies (n=13 T-PLL cases) identified the combination of Idasanutlin with Panobionstat as most effective. Based on these first promising results, we plan to expand the portfolio of p53 reactivators towards non-toxic compounds, and to further evaluate functional mechanisms around p53 reactivation in T-PLL.

P 47

Interaction of signaling pathways in Burkitt lymphoma

Rausch I¹ Hand E¹ Kutz H² Klinger B³ Witzel F³ Sieber A³ Kieser A² Beissbarth T⁴
Spang R⁵ Blüthgen N³ Kube D¹

1 Clinic of Haematology and Medical Oncology, University Medical Centre Göttingen, Germany **2** Helmholtz Center Munich, Research Unit Gene Vectors, Signal Transduction Group, München, Germany **3** Charité – Universitätsmedizin Berlin, Institute of Pathology, Berlin, Germany **4** Department of Medical Statistics, University Medical Centre Göttingen, Germany **5** Institute for Functional Genomics, University of Regensburg, Germany

Understanding the structure and interplay of cellular signaling pathways is one of the great challenges in molecular biology. Recently we described a Boolean Nested Effect Model based on global gene expression data and accurately reconstructed signal flows in simulated data and then resolved B cell receptor (BCR) signaling in the BL-2 lymphoma cell line.

The aim of the current study is to further estimate the response of lymphoma tumour cells to drugs, when different microenvironments are present. To this end, we will integrate pathway knowledge and experimental data and generate a semi-quantitative model that interconnects the activation of effector pathways. The models will be founded on protein expression and kinase activity data from cellular assays where the flow of signaling is interrupted by small molecule inhibitors.

We have generated a systematic perturbation data set comprising of 15 phospho-signals in 32 defined contexts in triplicate measurements of BCR signaling in the BL-2 lymphoma cell line by interventions in MEK, PI3K-AKT, p38 MAPK, JNK, NF- κ B and BTK pathways. The analyses were done using bead-based ELISA that allows high-throughput protein measurements. Key results were further confirmed by kinase activity assays. These data are used to generate network models based on Modular Response Analysis.

The result of this semi-quantitative model suggests that the signaling network downstream of the BCR in a lymphoma cell line contains hitherto unknown links that are now verified and mechanistically investigated. These links include a negative influence of p38 MAPK on MEK/ERK signaling.

P48

T-PLL cells resemble memory-type T-cells with aberrant effector functions implicating a leukemogenic cooperation of TCL1A with TCR signaling

Oberbeck S^{*1,2} Warner K^{1,3,2} Schrader A^{*1,2,4} Crispatzu G^{1,2} Mayer P^{1,2} Pützer S^{1,2}
Diebner HH⁵ Stilgenbauer S⁶ Hopfinger G⁷ Dürig J⁸ Haferlach T⁹ Lanasa M¹⁰ Roeder J⁵
Hallek M^{1,2} Mougiakakos D¹¹ von Bergwelt-Baildon M¹ Brüggemann M¹² Newrzela S⁴
Abken H¹ Herling M^{1,2}

1 Department I of Internal Medicine, Center for Integrated Oncology (CIO) Köln-Bonn, University of Cologne (UoC), Germany **2** Excellence Cluster for Cellular Stress Response and Aging-Associated Diseases (CECAD), UoC, Germany **3** Senckenberg Institute of Pathology, Goethe-University, Frankfurt/M., Germany **4** Scholarship holder “Deutsche José Carreras Leukämie-Stiftung e.V.”, Germany **5** Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Institute for Medical Informatics and Biometry Dresden, Germany **6** Department III of Internal Medicine, University Hospital Ulm, Germany **7** Department of Internal Medicine I, Bone Marrow Transplantation Unit, Medical University of Vienna, Vienna, Austria **8** Clinic for Hematology, University Hospital Essen, Essen, Germany **9** MLL Munich Leukemia Laboratory, Munich, Germany **10** Duke University Medical Center, Durham, NC, USA **11** Department of Medicine 5, Haematology and Oncology, University Hospital Erlangen, Germany **12** Medical Department II of Hematology and Oncology, University Hospitals of Schleswig Holstein, Campus Kiel * presenting author

The pathogenesis of the rare & aggressive T-cell prolymphocytic leukemia (T-PLL) is poorly understood, which particularly applies to a mechanistic concept around its hallmark oncogene TCL1A. Existing data implicate TCL1A as an enhancer of AKT, a central node in T-cell receptor (TCR) signaling. Role of TCR activation in T-PLL pathogenesis is not known. To clarify which physiological T-cell subset T-PLL cells most resemble, we performed gene expression profiling and immunophenotyping of primary T-PLL & healthy-donor T-cells. Gene signature showed high similarity of T-PLL cells to (central) memory- (CM) over naïve-T-cells. Surface markers revealed a memory-type differentiation & predominant CM stages. High basal activation correlated with inferior clinical outcomes. In parallel, T-PLL cells lost expression of negative-regulatory TCR-coreceptors & showed a robust resistance to stimulation-induced cell death & agonistic CD95 ligation. TCR-derived signals (phosphokinase induction, IL2 release) were enhanced *in vitro* by TCL1A. A mouse model with TCL1A-initiated protracted development of T-PLL revealed congruent findings with the aberrant T-cell phenotype of human T-PLL. TCL1A+ T-cells of this model, equipped with monoclonal epitope-defined TCRs against ovalbumine or a chimeric-antigen-receptor against carcinoembryonic antigen, gained a preleukemic growth advantage in upon pulsed or continuous low-level stimulation. Overall, we establish T-PLL cells to resemble antigen-experienced memory T-cells and to retain functional effector responses upon TCR stimulation. Nevertheless, Interleukin-2-inducible T-cell kinase targeting by BMS509744 or PRN694 did not effectively induce apoptosis *in vitro*. Loss of activation regulators underlie an activated phenotype & resistance to death-inducing signals. TCL1A proactively enhances TCR responses. We postulate that this leukemogenic cooperation drives accumulation of memory-type cells that utilize amplified low-level cognate antigen input.

P 49

GSK3- β in pancreatic cancer – oncogene or tumorsuppressor?

Augustin L¹ Brunner M¹ Schmidt G¹ Reutlinger K¹ Hessmann E¹ Neesse A¹ Ellenrieder V¹

¹ Dept. of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany

GSK3- β , expressed in every human cell and a hot topic in targeted Parkinson therapy, is overexpressed in many malignancies. During the last year our lab has investigated the role of GSK3- β in early carcinogenesis. A more recent series of experiments, we could show that GSK3- β is overexpressed in different pancreatic cancer cell lines (Panc1, L3.6, KPCbl6, NKC). Furthermore, we could show that different ways of GSK3- β inhibition, be it via inhibitors or si-RNA, alters the histone modifications in these cell lines, thus postulating a significant effect of GSK3- β on gene expression in these cell lines. On top of that, both pharmacological inhibition and si-RNA treatment of these cell lines, lead to a significant reduction in growth and cell cycle arrest and to a relevant impact on target gene expression like pGS or β -Catenin.

Interestingly, these findings differ from cell line to cell line, therefore postulating a different role of GSK3- β in different pancreatic cancer cell lines. This suggests that GSK3- β is a key player in pancreatic cancer heterogeneity and implies a molecular stratification of pancreatic cancer patients before treatment in the future, to determine whether a treatment with specific GSK3- β inhibitors like Tideglusib, currently in Phase-II-trials in Parkinson therapy, could be beneficial for the outcome of these patients.

P50

Towards subgroup stratification using patient-specific pathways

Kramer F

Department of Medical Statistics, University Medical Center Göttingen, Germany

In modern Systems Medicine approaches the aim is to look at increasingly complex interactions of complete signaling pathways in order to get a more holistic view for individualized treatment decisions. Individualized treatment decisions and newly developed specialized drugs warrant the need to broaden the focus in individualized medicine from singular biomarkers to pathways. On the other hand, pathway databases offer vast amounts of knowledge on biological networks, freely available and encoded in semi-structured formats [BCSo6, SAK + 09]. The efficient re-use of pathway knowledge and its integration into bioinformatic analyses enables new insights for researchers in systems medicine.

However, the vast amount of published data on molecular interactions makes it increasingly challenging for life science researchers to find and extract the most relevant information. Our idea is to compose an analysis pipeline in order to enable patient-specific systems medicine analyses. The poster will present a workflow for integrating pathway information and omics data featuring condensing of knowledge and tailoring data towards a sub-group stratification of patients according to specific outcomes.

P 51

Non-platinum based halogenated compounds – a novel therapeutic approach in pancreatic cancer?

Goetze R¹ Buchholz SM¹ Schirmer MA² Guhlich M² Ellenrieder V¹ Ou N³ Zhang Q³ Lu QB³ Neeße A¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany — **2** Department of Radiation Oncology, University Medical Center Göttingen, Germany — **3** Department of Physics and Astronomy, University of Waterloo, Ontario, Canada

Introduction: Pancreatic ductal adenocarcinoma (PDA) is highly resistance to standard chemo- and radiotherapy. Recently, *in vitro* and *in vivo* studies discovered a new class of non-platinum-based halogenated molecules that selectively kill cancer cells. For squamous cell carcinomas cytotoxic potential as well as synergistic effects with radiotherapy have been shown. Here, we present first data of the compound 1,2-Diamino-4,5-dibromobenzene (DAB) in PDA.

Methods: Human and murine pancreatic cancer cell lines were treated with different concentrations of DAB and ionizing radiation followed by MTT assays. For clonogenic assay cells were treated with 25µM DAB and 2Gy. *In vivo*, tumor bearing KPC mice were treated over 10d either with DAB, Gemcitabine or combination of both. At day 7 and day 10 tumor volume development was evaluated by high resolution ultrasound.

Results: Cell viability was significantly reduced in all human pancreatic cancer cell lines with GI₅₀ values between 20-150µM. Comparable effects were obtained for murine cell lines. In colony formation assays in human BxPC₃ cell line a 15fold decrease upon 25µM DAB treatment was observed (72,5±7,5 colonies vs. 547,5±31,5 colonies, p=0,004). However, no synergistic effects could be achieved by combining irradiation and DAB treatment *in vitro*. Pilot studies showed very good tolerability of DAB in mice. Upon DAB treatment the tumor volume in KPC mice was not significantly reduced compared to gemcitabine (1,476mm³±0,1135mm³ vs. 1,337 mm³±0,1917mm³). Furthermore, combination of gemcitabine and DAB did not show any effect on tumor volume reduction.

Conclusion: DAB was effective in killing human and murine pancreatic cancer cells *in vitro*. Synergistic effects in combination with ionizing radiation were not achieved. *In vivo*, effects were comparable with gemcitabine monotherapy, but combination of both drugs was not synergistic. Ongoing studies will investigate the delivery of DAB in stroma rich tumors of the KPC model.

A Novel Role for ROBO3 in the Pancreatic Cancer Plasticity

Krebs N¹ Önder S¹ Xu X² Güntner JM³ Ellenrieder V¹ Singh SK¹

1 Department of Gastroenterology and Gastrointestinal Oncology, University Medical Center Göttingen, Germany **—2** Department of Cardiology and Pneumology, University Medical Center Göttingen, Germany **—3** Max-Planck Institute for Experimental Medicine and University Medical Center, Department of Hematology and Medical Oncology, Göttingen, Germany

Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal human malignancies with dismal 5-years survival rate below 7%, and it is estimated to become the second leading cause of cancer-related death by end of 2030. Pancreatic tumor heterogeneity is one of the key hallmark in disease progression and treatment. Recently, based on whole genome sequencing and transcriptome profiling our understanding of pancreatic intratumoral heterogeneity and tumor cell plasticity have significantly improved. Based on molecular sub-classification two most relevant ‘subtypes’ of PDA such as ‘Classical’ and ‘Quasi-Mesenchymal’ (Q-M) have been identified. In the precise classification, Q-M is linked to high grade tumors, associated with mesenchymal transcriptome signatures with poor prognosis in PDAC patients. Recently, it has been shown that ROBO3, a roundabout axon guidance receptor 3, is potentially involved in pancreatic carcinogenesis. Furthermore, high expression of ROBO3 is associated with poor survival of pancreatic cancer patients. However, the molecular and cellular mechanisms involved in the regulation of ROBO3 signaling in pancreatic cancer progression remain elusive. We investigated the role of ROBO3 in the progression of pancreatic cancer plasticity. Briefly, we show that ROBO3 is highly expressed in the invasive ‘Quasi-Mesenchymal’ (QM) cell lines as compared to ‘Classical’ (CL) cell lines. Importantly, ROBO3 was highly expressed in the orthotopic implanted pancreatic tumor as well as liver metastasized tissues from ‘Quasi-Mesenchymal’ (MiaPaCa2) cell line. Functionally, CRISPR/dCas9-mediated knockdown of ROBO3 in Quasi-Mesenchymal (MiaPaCa2) cells results in reduced expression of EMT-regulated genes (e.g. ZEB1) and cellular invasion rate of ‘Quasi-Mesenchymal’ cells. In conclusion, our studies highlight a novel role for the ROBO3-signalling pathway in promoting pancreatic cancer plasticity and provide a plausible target for inhibiting pancreatic cancer invasion and metastases.

P 53

Targeted therapy of castration resistant prostate cancer upon development of androgen receptor splice variants

Lammers K¹ Striepe M¹ Hahn O¹ Thelen P¹

¹ Department of Urology, University Medical Center Göttingen, Germany

Anti-hormonal therapy is pivotal for castration-resistant prostate cancer (CRPC). Particularly, second line therapies with abiraterone (Abi) and enzalutamide have recently proven to be successful. Unfortunately, tumor recurrence and the development of CRPC eventually occur in most cases. Very recent studies demonstrated the function of androgen receptor (AR) all-N-terminal splice variants (AR-Vs), facilitating complete androgen-independent prostate cancer. Therefore, new agents such as EPI-001 (EPI) targeting the AR-N-terminus are now considered.

In this study, the representative mCRPC cell line VCaP was treated continuously with Abi (5 μ M) or Abi-withdrawal to trigger and reset AR-Vs function as therapy resistance. Before this experimental background, cells were treated with 0, 1, 5, or 10 μ M EPI. Expression analyses for AR related genes were performed by qRT-PCR and Western Blot. Tumor cell viability and proliferation were assessed by Alamar blue and BrdU assays.

EPI treatments alone could not achieve the same antiandrogen effect on VCaP cells as Abi, evident from expression of androgen regulated genes such as PSA and proliferation. EPI treatments also could not considerably complement Abi treatment efficacy. Moreover, upon Abi withdrawal EPI treatments had a stimulatory effect on canonical AR signaling and elevated expression of steroidogenesis genes such as AKR1C3 occurred. PSA expression and cell viability were elevated accordingly.

This study aimed to target the remaining therapy resistance from AR-Vs developing under efficient Abi or enzalutamide treatments. These results demonstrate constraints of combinatory treatments, most probably arising from feedback mechanisms. Therefore, further reasoned concepts to optimize efficient AR-targeted therapies are warranted.

P54

Heat sensitivity modulation in prostate cancer (PCA) in vitro: The impact of common PCA therapeutics

Heining F¹ Toepperwien J¹ Thelen P¹ Mansour J² Duensing S² Krause P³ Pahernik S⁴
Trojan L¹ Popeneciu IV^{1,2} Hahn O¹

1 Department of Urology, University Medical Center Göttingen, Germany **—2** Department of Urology, Heidelberg University Hospital, Heidelberg, Germany **—3** Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany **—4** Department of Urology, Klinikum Nürnberg, Germany

High Intensity Focused Ultrasound (HIFU) is one emerging alternative to surgery and radiotherapy for prostate cancer (PCA) treatment. The possible impact of common PCA therapeutics on this new form of therapy remains unclear. Using a new cell culture based approach we investigated the possible impact of PCA therapeutics on heat sensitivity. Cell lines representative for local through metastatic PCA were treated with a thermocycler temperature gradient. Metabolic activity was then measured via MTT assay. Individual heat response profiles were generated. Determined cutoff values were verified with flow cytometry (FACS) using a PI/Annexin V assay for apoptotic activity. Pre-treatment for 48h with PCA therapeutics Finasterid and Bicalutamid as well as with chemotherapeutics such as Docetaxel and Mitoxantrone were applied in order to measure the influence on heat sensitivity. VCaP cells were re-sensitized to androgen metabolism using testosterone boost before heat treatment to investigate the role of castration resistance in this system (established VCaP derivative rVCaP). All cell lines showed a decrease in metabolic activity at temperatures corresponding to clinical HIFU treatment. In most cells, metabolic activity increased until 51.3 °C before then decreasing <20%. A significant decrease was evident at 53.9 °C. In VCaP, 22rv1, DU145 and BPH1, the activity level decreased to about 20% whereas in PC3 cells, metabolic activity disappeared almost completely. FACS analysis confirmed the loss of metabolic activity was indeed due to induction of apoptosis. Pre-treatment with Bicalutamid and Finasterid did not lead to a change in heat response whereas treatment with chemotherapeutics unexpectedly drove the cells into a more heat resistant state. Heat response did not differ between rVCaP and the original VCaP. In the future, this system might be used to investigate potential heat sensitizing drugs and thereby make HIFU a more eligible treatment option in manifold cases.

P 55

CD31 Expression Determines Redox Status and Chemoresistance in Human Angiosarcomas

Venkataramani V^{1,2,3} Küffer S³ Cheung K⁴ Jiang X² Trümper L¹ Wulf GG¹ Ströbel P³

1 Department of Hematology and Medical Oncology, University Medical Center Göttingen, Germany **2** Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA **3** Institute of Pathology, University Medical Center Göttingen, Germany **4** William Harvey Research Institute, Barts, UK and The London School of Medicine and Dentistry, Queen Mary University of London, UK

Angiosarcomas (AS) are soft tissue sarcomas with endothelial differentiation and vasoformative capacity. Most AS show strong constitutive expression of the endothelial adhesion receptor CD31/PECAM-1 pointing to an important role of this molecule. However, the biological function of CD31 in AS is unknown. The expression levels of CD31 in AS cells and its effects on cell viability, colony formation and chemoresistance was evaluated in human AS clinical samples and in cell lines through isolation of CD31^{high} and CD31^{low} cell subsets. The redox-regulatory CD31 function linked to YAP signaling was determined using a CD31 blocking antibody and siRNA approach and was further validated in CD31-knockout endothelial cells. We found that most AS contain a small CD31^{low} cell population. CD31^{low} cells had lost part of their endothelial properties, were more tumorigenic and chemoresistant than CD31^{high} cells due to more efficient reactive oxygen species (ROS) detoxification. Active down-regulation of CD31 resulted in loss of endothelial tube formation, nuclear accumulation of YAP, and YAP-dependent induction of antioxidative enzymes. Addition of pazopanib, a known enhancer of proteasomal YAP degradation re-sensitized CD31^{low} cells for doxorubicin resulting in growth suppression and induction of apoptosis.

P56

Established colorectal cancers depends on mutp53 driving epithelial STAT3-mediated proliferation and invasion

Schulz-Heddergott R¹ Edmunds SJ^{1,2} Stark N¹ Li J⁵ Conradi LC³ Bohnenberger H⁴
Dobbelstein M¹ Moll UM^{1,5}

1 Institute of Molecular Oncology, University Medical Center Göttingen, Germany **2** Medical Protein Science, Lund University, Sweden **3** Department of General, Visceral, and Pediatric Surgery, University Medical Center Göttingen, Germany **4** Department of Pathology, University Medical Center Göttingen, Germany **5** Department of Pathology, Stony Brook University, USA

Missense mutations in p53 (mutp53) are the second most frequent alterations in sporadic colorectal cancer (CRC). They generate HSP90-dependent highly stabilized aberrant proteins with abrogated tumor suppressor functions that often acquire new oncogenic gain-of-functions (GOFs).

We report that the mutp53 R248Q (Q) allele, a mutational hotspot in human CRC, exerts *in vivo* GOF activity in genetic and chemically-induced mouse models of CRC. Such tumors become dependent on continued expression of stabilized mutp53 for tumor growth and invasion. Thus, in established CRC tumors Cre-mediated genetic ablation of the floxQ allele markedly inhibits tumor proliferation and invasiveness (> 50%) by suppressing STAT3-mediated signaling of malignant epithelial cells. Furthermore, treating mice with established tumors with the Hsp90 inhibitor 17AAG has a dramatic anti-tumoral effect. These proof-of-principle data identify mutp53 as a promising drug target in human colorectal cancer.

LIST OF PRESENTERS *(alphabetically)*

Altmeyer, Matthias	Zürich, Switzerland	T04
Arlt, Annekatriin	Göttingen, Germany	P17
Augustin, Lioba	Göttingen, Germany	P49
Bardeesy, Nabeel	Boston, MA, USA	T03
Baumgart, Simon	Berlin, Germany	P05
Bogeski, Ivan	Göttingen, Germany	P18
Bohnenberger, Hannibal	Göttingen, Germany	P16
Brabletz, Thomas	Erlangen, Germany	T12
Bremer, Sebastian	Göttingen, Germany	P02
Buchholz, Sören Matthias	Göttingen, Germany	P25
Carmeliet, Peter	Leuven, Belgium	T18
Choo, Josephine	Göttingen, Germany	P33
Eilers, Martin	Würzburg, Germany	T05
Fichtner-Feigl, Stefan	Freiburg, Germany	T07
Filippakopoulos, Panagis	Oxford, UK	T15
Gerlinger, Marco	London, UK	T13
Geyer, Natalie	Göttingen, Germany	P45
Goetze, Robert-Günther	Göttingen, Germany	P51
Guhlich, Manuel	Göttingen, Germany	P28
Hahn, Oliver	Göttingen, Germany	P11
Hamdan, Feda	Göttingen, Germany	P04
Hanika, Sandra	Hamburg, Germany	P23
Hasselluhn, Marie Christine	Göttingen, Germany	P43
Heining, Franziska	Göttingen, Germany	P54
Jäger, Dirk	Heidelberg, Germany	T09
Kari, Vijayalakshmi	Göttingen, Germany	P10
Kauffels-Sprenger, Anne	Göttingen, Germany	P29
Klemke, Luisa	Göttingen, Germany	P41
Klusmann, Ina	Göttingen, Germany	P31
Korbel, Jan O.	Heidelberg, Germany	T14
Kosinsky, Robyn	Göttingen, Germany	P38
Kramer, Frank	Göttingen, Germany	P50
Krebs, Niklas	Göttingen, Germany	P52
Kühnel, Florian	Hannover, Germany	T06
Lammers, Kira	Göttingen, Germany	P53
Latif, Muhammad Umair	Göttingen, Germany	P40

Lord, Christopher J.	London, UK	T02
McIntyre, Alan	Nottingham, UK	T11
Mieczkowska, Iga	Göttingen, Germany	P09
Movsisyan, Naira	Göttingen, Germany	P37
Nowack, Hannah	Göttingen, Germany	P20
Oberbeck, Sebastian	Köln, Germany	P48
Oppemann, Udo	Oxford, UK	T16
Pal, Mrinmoy	Kolkata, India	P01
Patil, Shilpa	Göttingen, Germany	P12
Patzak, Melanie	Göttingen, Germany	P26
Peuker, Kenneth	Dresden, Germany	P35
Prinjha, Rabinder	Stevenage, UK	T17
Prokakis, Evangelos	Göttingen, Germany	P13
Ramu, Iswarya	Göttingen, Germany	P27
Rausch, Isabell	Göttingen, Germany	P47
Reichardt, Sybille	Göttingen, Germany	P34
Reinhardt, Christian	Köln, Germany	T01
Rodewald, Sabrina	Göttingen, Germany	P08
Schrader, Alexandra	Köln, Germany	P36
Schüle, Roland	Freiburg, Germany	T19
Schulz-Heddergott, Ramona	Göttingen, Germany	P56
Sen, Madhobi	Göttingen, Germany	P03
Spyropoulou, Dimitra	Göttingen, Germany	P19
Sriraman, Anusha	Göttingen, Germany	P42
Starzonek, Sarah	Hamburg, Germany	P24
Tampe, Björn	Göttingen, Germany	P15
Tampe, Desiree	Göttingen, Germany	P30
Tayeh, Zainab	Göttingen, Germany	P32
Tüting, Thomas	Magdeburg, Germany	T08
Vasyutina, Elena	Köln, Germany	P39
Venkataramani, Vivek	Göttingen, Germany	P55
von Jan, Jana	Köln, Germany	P46
Wang, Xin	Göttingen, Germany	P06
Wegwitz, Florian	Göttingen, Germany	P14
Wicklein, Daniel	Hamburg, Germany	P22
Wiese, Maria	Göttingen, Germany	P44
Zeißig, Sebastian	Dresden, Germany	T10
Zhang, Xin	Göttingen, Germany	P21
Zhang, Zhe	Göttingen, Germany	P07



CONFERENCE DINNER

on Thursday, November 16th

After a hopefully inspiring day with a lot of excellent scientific talks and discussions we would like to invite you to our conference dinner where we will have the opportunity to deepen our scientific exchange and to spend an enjoyable time together with excellent food, drinks and music.

PLEASE NOTE: *due to limited seats at the restaurant, registration and payment in advance was compulsory.*

We will meet in the “Haus am See” which is located next to the idyllic “Kiessee” in the southern part of Göttingen.

► www.tr-hausamsee.de

PRIVATE BUSES will be available for transfer to the “Kiessee” from the Leinehotel and conference venue and back:

Departure time Wilhelmsplatz: 18:30

Departure time Leinehotel: approx. 18:40

Departure time Haus am See: 23:00

Departure time Haus am See: 00:30

We are looking forward to spending a joyful evening with you!

GÖTTINGEN



Gänseliesel

PKW

Groner



Bahnhof

Berliner Straße

Unter-Masch-Str.

Obere-Masch-Str.

Am Leinekanal

P

Untere Karspüle

Obere Karspüle

Ritterplan

Goetheallee

Prinzenstr.

Stumpfkeil

Weender Straße

Jüdenstraße

Theaterstr.

Burgstr.

Berliner Straße

Geiststraße

Neustadt

Papendiek

Paulinerstr.

Gotmarstr.

Wilhelmsplatz

Alte Mensa

Barfüßerstr.

Rote Str.

Groner-Tor-Str.

Groner Straße

Zindelstr.

Weender Straße

Mauerstr.

Lange-Geismar-Str.

Kurze Str.

Kurze-Geismar-Str.



sartorius

