10TH MILDRED SCHEEL CANCER CONFERENCE

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ABSTRACT BOOKLET

Bonn, Germany
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Berliner Freiheit 2
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Deutsche Krebshilfe
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WHERE THERE'S A WILL, THERE'S A WAY

It was September 25th, 1974 when Dr. Mildred Scheel founded the Deutsche Krebshilfe (German Cancer Aid) with the intention to help people in critical health situations by care and persistent support. In terms of her vision the Mildred Scheel Cancer Conference is dedicated to strengthen oncological research with the overall aim to help cancer patients.

Mildred Scheel always wanted to help. Being the daughter of a radiologist, she developed a lively interest in medicine. She studied medicine and passed her state exam with the highest marks. in 1969 she married Walter Scheel, who was elected Federal President in 1974. As "First Lady", one of Mildred's duties was to take on a social task. At that time, to talk about cancer was a social taboo. As a physician, Mildred Scheel had seen patients suffering from cancer almost daily and was fully aware of the silence surrounding the disease and the importance of giving patients a voice.

As a consequence, she founded German Cancer Aid in September 1974. Since then, the development of German Cancer Aid has been a success story. Mildred Scheel died in 1985. Some may call it an irony of fate that she died from cancer. But her legacy, German Cancer Aid, lived on and became Germany's leading non- governmental funding organisation in the field of oncology.

The Mildred Scheel Cancer Conference stands for interdisciplinary communication in cooperation with the Deutsche Krebsgesellschaft (German Cancer Society) we invited international experts in the area of oncological research. For the tenth time clinicians, scientists and especially young scientists meet to share and discuss their experience and ideas – quite in terms of Mildred Scheel's initial idea.

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TALKS

A-207

Some thoughts about specific epigenetic changes and the origins of cancer

Stephen Baylin
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There is a current and increasing research emphasis emerging for the importance of how the cell “state” of progenitor cell origins for human cancers determines key features of resultant tumor phenotypes. In this context, the factors in determining progression of the cancers, from pre-malignant stages through initiation and later advanced stages are also critical to understand and perhaps nothing is more important than considering the role of chronic inflammation for contributing to the initial stages. Our group has been focusing on these issues making use of organoid models for normal human lung airway cells and from human and mouse colon epithelium. We are outlining the importance during early cancer, premalignant stages of the evolution of an epigenetic change, cancer -specific, abnormal gene promoter DNA methylation and associated silencing or prevention of inducibility of tumor suppressor genes (TSG’s). We are showing how the time dependent appearance of these changes, and chromatin events accompanying them creates a state for sensitizing cells to oncogenic effects of key cancer mutations before any genetic changes have ensued. At this time point introductions of single mutations result in one-step transformation of cells and their ability to form cancers in mice. We are relating this above evolution to how groups of the above silenced genes mediate the changes, to roles for chronic inflammation in the dynamics and for consequences for how immune evasion states of cancers may arise. Data will be discussed in this talk to illustrate the above study findings.

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A-209

Functional synapses between small cell lung cancer and glutamatergic neurons

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Small cell lung cancer (SCLC) is the most aggressive type of lung cancer, characterized by fast metastatic spread, early recurrence and high mortality rates. Despite comprehensive genomic characterization, no targeted therapy could be developed to date and the recent introduction of immunotherapy only led to a marginal improvement in survival. This lack of progress underscores the pressing need for a deeper understanding of SCLC. In this study, we performed an extensive *in vivo* genetic screen using the *Trp53*$^{flox/flox}$*;Rb1*$^{flox/flox}$ (RP) mouse model of SCLC and conducted an in-depth re-analysis of genetic and expression data from human patients. Across species, we identified genetic alterations and high expression of neuronal, synaptic and glutamatergic gene sets as key features of SCLC. Our data show that SCLC cells can form synaptic contacts with glutamatergic neurons *in vitro* and *in vivo* and that these contacts can mediate electrical inputs. Consistent with a tumor-supporting role of cancer-neuron interactions, the co-culture with neurons led to a marked increase in the proliferation of SCLC cell lines. Furthermore, the repression of glutamate release with riluzole and the activation of the inhibitory glutamate receptor GRM8 with DCPG resulted in improved response and survival in the RP mouse model. Our findings suggest that SCLC can hijack neuronal signaling to derive a growth advantage and provide a proof of principle for the use of neuromodulatory drugs in SCLC.

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A-212

Concepts of Mildred Scheel Early Career Centers (MSECC)

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In a position paper published in 2017, the German Cancer Aid expressed the strong concern that the increasing shortage of young scientists in cancer medicine poses a significant threat to cancer research in Germany. If this development was

not stopped, it would ultimately also have a negative impact on the care of cancer patients.

The German Cancer Aid called on the health and science policymakers to create adequate structures to strengthen young Clinician Scientists (MDs) and Medical Scientists (PhDs) in the field of cancer research at all German medical faculties with interdisciplinary cancer centers and to ensure their funding.
To initiate a corresponding development, the German Cancer Aid announced the funding program "Mildred Scheel Early Career Centers" in 2017.
In 2018, an expert committee selected 5 proposals for funding - Cologne/Bonn, Dresden, Frankfurt a. M., Hamburg and Würzburg (Cologne/Bonn was extended later to Aachen/Bonn/Cologne/Düsseldorf; ABCD and Frankfurt a.M. to Frankfurt/Marburg). With 5 years of start-up funding, concrete solutions and innovative concepts for strengthening young scientists should be implemented as models.
Each Mildred Scheel Early Career Center is funded with 10 Mio. Euros.

Prof. Christian Brandts is the spokesperson of the Mildred Scheel Early Career Network and will present an overiew of the programm and highlight the features of the different sites. In addition, two selected junior group leaders from the 'Mildred Scheel School of Oncology ABCD' (Dr. Filippo Beleggia) and from the 'Mildred Scheel Early Career Center Würzburg' (Dr. Dimitrios Papadopoulos) will present their research topics.

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A-213

Targeting imbalanced transcription programs in cancer

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Molecular therapies are rapidly transforming clinical practices. Not only do they promise improved patient outcomes, but also fewer toxicities and shorter hospital stays.
My lab has a strong interest in establishing the cancer relevance of RNA life cycle regulators. We advance personalized precision oncology by developing CRISPR-based tools and methods to discover and mechanistically dissect new RNA-based cancer vulnerabilities. When possible, we elicit multiplexed CRISPR mutagenesis directly in living tissues and tumors, thereby boosting relevance for human disease (*PNAS*, 2016; *Nature reviews cancer*, 2020; *Nature protocols*, 2022; *Nature protocols*, 2022). In collaboration with chemists and biological engineers, we develop and apply novel molecular cancer therapeutics (including small molecules, siRNA-loaded nanoparticles and peptide binders). We develop treatment biomarkers and predict patient stratification cohorts based on expected drug sensitivities. We test our findings in relevant preclinical mouse models of cancer (*Cancer Cell*, 2017) and are invested in translational efforts including Phase I/II trials to bring new precision therapies to clinic (*Science translational medicine*, 2014).

To contribute to this development, we deploy a *state-of-the-art* CRISPR screening portfolio to model genetic dependencies *in vitro* and *in vivo*. Since relevant experimental systems are typically incapable of representing genome-scale screening libraries, there is a strong need for customization, cost reduction and streamlining. Our pipeline www.CRISPR-CLUE.de now aids both us and the community in the production of fully customized CRISPR libraries (*Nucleic Acids Research*, *2020*). More recently, we have developed artificial self-fragmenting RNA molecules to guide CRISPR nucleases to multiple distinct loci, thereby enabling us to perturb multiple genes per cell and to discover new context dependent cancer vulnerabilities at precision and scale. These screens have uncovered several context-dependent RNA life cycle vulnerabilities, which we now aim to exploit therapeutically.

Our hope is that our screening efforts together with a deepened understanding of epitranscriptomal processes will foster the development of personalized therapy approaches for cancer patients.

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A-214

Genetic modification of T lymphocytes goes in vivo: Implications for cancer immunotherapy

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Gene transfer technology is constantly improving, especially with respect to *in vivo* delivery precisely into therapy-relevant cell types. T lymphocytes and tumor cells are most relevant for setting up cancer immunotherapeutic strategies. Generating chimeric antigen (CAR) T cells directly *in vivo* relies strongly on vector technology, particularly high selectivity for T lymphocytes. Using surface engineered lentiviral vectors targeted to T cell markers has provided proof-of-concept for this strategy in humanized mouse models. As alternative to lentiviral vectors, AAV vectors can be targeted to T lymphocytes or tumor cells through insertion of DARPins into exposed loop regions. Delivery of immunmodulating factors such as checkpoint inhibitors by tumor-targeted AAV vectors enables combination strategies with CAR cell therapies. Ongoing preclinical studies are evaluating the different vector platforms and will identify potential hurdles towards clinical application.

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A-215

Modeling disease with human organoids

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Techniques for culturing functional human breast epithelium in three-dimensional (3D) matrices have been championed for more than 30 years by Mina Bissell. Additionally, around a decade ago, Sasai and colleagues pioneered pluripotent stem cell (PSC)–based technology to create organoids that mirror specific parts of the central nervous system (CNS). Lancaster and Knoblich modified this technology and provided particularly notable examples of “mini-brain” structures. Although PSCs can be used to model everything ranging from tissues to organismal development, adult stem cells (ASCs) can also be isolated to generate organoid models of the primary tissues in which they reside. Specific growth factor cocktails allow long-term expansion of ASC organoids by mimicking the organ stem cell niche, as first established for mouse and human intestine and airway epithelium. The organoid structures generated from PSCs and ASCs reflect crucial tissue features in terms of overall architecture, the collection of differentiated cell types, and tissue-specific function. Organoids thus represent a model system that can be compared to traditional genetically engineered mouse models (GEMMs), cell lines, and patient-derived xenografts (PDXs).

As a definition, organoids are microscopic self-organizing, three-dimensional structures that are grown from stem cells in vitro. They recapitulate many structural and functional aspects of their in vivo counterpart organs. This versatile technology has led to the development of many novel human cancer models. It is now possible to create indefinitely expanding organoids starting from tumor tissue of individuals suffering from a range of carcinomas. Alternatively, CRISPR-based gene modification allows the engineering of organoid models of cancer through the introduction of any combination of cancer gene alterations to normal organoids. When combined with immune cells and fibroblasts, tumor organoids become models for the cancer microenvironment enabling immune-oncology applications. Emerging evidence indicates that organoids can be used to accurately predict drug responses in a personalized treatment setting. I will illustrate the current state and future prospects of the rapidly evolving organoid field through examples from my lab.

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A-217

Neoadjuvant Immunotherapy: the current revolution that will change atient management across multiple tumor types

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Neoadjuvant Immunotherapy development, at first in macroscopic lymphnode positive stage III melanoma patients, is rapidly changing management of melanoma patients, cutaneous Squamous Skin Cancer patients, MSI (DNA-Repair Deficient) ColoRectal Cancer patients. Likewise in other tumor types the neoadjuvant chemo-immunotherapy regimens of anti-PD(L)1-based / chemo / targeted therapy combo's are changing patient management of TNBC patients, Locally advanced Stage III NSCLC patients, Bladder Cancer patients, with multiple trials on the way in various additional tumor types.

This current revolution started in stage III melanoma patients and demonstrated 3 important improvements: more cures, shorter treatment cycles, less surgery.

Survival of macroscopic stage III melanoma is poor. Five-year overall survival and relapse-free survival rates for surgery alone range from 40-59% and 30-39% respectively. The current standard of care is therapeutic lymph node dissection (TLND) followed by a year of adjuvant systemic therapy. Multiple phase 2 trials have shown that neo-adjuvant immunotherapy induces major pathologic response rates (pathologic complete response: pCR, 0% viable tumor cells; near-pCR, <10% viable tumor cells), which translate into durable relapsefree survival rates. Single agent anti-PD-1 achieves 20-30% MPR, the combination of ipilimumab and nivolumab doubles the MPR rates to 50-60%. The OpACIN trial demonstrated that neoadjuvant immunotherapy induced both higher numbers and a broader repertoire of tumour-resident T-cells in peripheral blood compared with adjuvant immunotherapy. The follow up PRADO trial demonstrated that 60% of patients developed a pCR and did not need to undergo a TLND, and did not need any further adjuvant therapy. This PRADO trial clearly demonstrated that **neoadjuvant immunotherapy delivers on 3 promises: more cures, shorter treatment cycles, less surgery**. These advantages were also clearly demonstrated for neoadjuvant cemiplimab (anti-PD1) for locally advanced cSCC tumors in the face/head&neck with a 56% pCR rate, and most spectacularly for MSI Colorectal Cancers in the NICHE-1 and NICHE-2 trial @ the NKI-Amsterdam with a close to 100% pathologicCR and pathologic nearCR response rate.

In stage III melanoma very recently, the randomized phase 2 trial S1801, reported its first interim results. S1801 compared TLND, followed by 18 courses of adjuvant pembrolizumab, to 3 courses of neoadjuvant pembrolizumab, followed by surgery and 15 adjuvant doses. With a median follow up of 14 months a 23% EFS rate benefit was observed. The ongoing phase 3 NADINA trial randomizes patients between TLND + one year of adjuvant nivolumab (control arm) or 2 courses of neoadjuvant therapy with ipilimumab+nivolumab, followed by adjuvant therapy only for non-MPR patients. There is rapidly, consistent, accumulating evidence generated from all phase 1 and phase 2 trials indicating clinical superiority of neoadjuvant immunotherapy over adjuvant systemic therapy for macroscopic stage III melanoma. Therefore, payers should consider neoadjuvant immunotherapy for re-imbursement, as this approach is the best option for our patients and classify it as best medical practice.

Highlights:

- Neoadjuvant Immunotherapy has emerged as superior to adjuvant immunotherapy
- Neoadjuvant Immunotherapy is superior to neoadjuvant targeted therapy
- Neoadjuvant Immunotherapy induces greatest diversity and amplitude of T cell clones
- Neoadjuvant Immunotherapy is best medical practice for macroscopic stage III melanoma
- Neoadjuvant Immunotherapy should be considered for reimbursement
- Neoadjuvant Immunotherapy will become the new standard of care

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A-218

Senescence-associated stemness in Treatment Failure

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Senescence, the failsafe cellular program to limit oncogenic progression, paradoxically, allows acquisition of de novo senescence-associated stemness (SAS) via epigenetic reprogramming. SAS contributes to cancer stemness and aggressiveness in experimental models, and hence as well directly implicates in adverse treatment outcome and disease complications such as relapse and metastasis in human cancers. Combining highly-advanced sequencing technologies and single cell-resolution diagnostic tools, molecular characteristic unique to SAS are defined which enables prediction and detection of SAS-driven pathological events, and formulation of hypothetical treatment modules targeting SAS, to be refined in cross-species clinical-resemblance experimental platforms that ultimately aims for medical precision in the clinic.

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A-219

Collective cancer metastasis and resistance: mechanisms and vulnerabilities

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Resistance to therapeutic treatment along with metastatic progression jointly determine a fatal outcome of cancer. Cancer metastasis and therapeutic resistance are traditionally studied as separate fields using non-overlapping strategies. However, emerging evidence, including from in vivo imaging and in vitro organotypic culture, now suggests that both programs cooperate and reinforce each other in the invasion niche and persist upon metastatic evasion. To identify how invasion and resistance programs cooperate, we used intravital microscopy of orthotopic sarcoma and melanoma xenografts. We demonstrate that these tumors invade collectively and that, specifically, cells within the invasion zone acquire increased resistance to radiotherapy, rapidly normalize DNA damage, and preferentially survive. Using a candidate-based approach to identify effectors of invasion-associated resistance, we targeted β1 and αVβ3/β5 integrins, essential extracellular matrix receptors in mesenchymal tumors, which mediate cancer progression and resistance. Combining radiotherapy with β1 or αV integrin monotargeting in invading tumors led to relapse and metastasis in 40-60% of the cohort, in line with recently failed clinical trials individually targeting integrins. However, when combined, anti-β1/αV integrin dual targeting achieved relapse-free radiosensitization and prevented metastatic escape. Collectively, invading cancer cells thus withstand radiotherapy and DNA damage by β1/αVβ3/β5 integrin cross-talk, but efficient radiosensitization can be achieved by multiple integrin targeting. In ongoing work, we identify candidate mechanisms particularly engaged in collectively invading cells enhancing survival in response to radiation and/or chemotherapy. Identifying nodes shared in collective metastasis and therapy resistance signaling networks should offer new opportunities to improve anti-cancer therapy beyond current strategies, to eliminate both nodular lesions and cells in metastatic transit.

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A-221

Towards the targeted use of untargeted chemotherapy

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Genotoxic chemotherapies that are presumed to exert their effect primarily through the induction of DNA breaks may show profound mechanistic distinctions. To more closely examine the precise mechanism of diverse genotoxic chemotherapies, we have developed a genetic loss of function-based approach to characterize chemotherapeutic function in mammalian cells. Briefly, we have used phenotypic “signatures” to characterize drug action with high resolution. These robust loss of function “signatures” can be used to cluster unknown drugs into functional categories

and define mechanisms of action for uncharacterized cytotoxic agents.

A major focus of these efforts has been the study of compounds that are commonly used as components of chemotherapeutic drug regimens. For example, we found that oxaliplatin and cisplatin, two agents that are thought to exert their effects via the same mechanism of action, are mechanistically quite distinct. While cisplatin promotes cell death via inducing DNA crosslinks and a DNA damage response, oxaliplatin kills cells by promoting inhibiting ribosome synthesis and translation. Thus, platinum drugs may not function interchangeably with their derivatives in cancer regimens. This phenomenon may explain a lack of efficacy for oxaliplatin in the treatment of malignancies conventionally treated by cisplatin, and the initially unanticipated value of oxaliplatin as a treatment for colorectal cancer. This work also highlights how specific tumor cell dependencies have empirically guided standard of care treatment. These studies have also been extended to examine mechanisms of combination drug regimens in pancreatic cancer and myeloid leukemia, where our work highlights the targeted effects of established chemotherapies on the tumor microenvironment.

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A-222

Patient-on-a-chip – using multi-organ chips for patient-specific treatment selection

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The first concepts for reproducing human systemic biology called human- or body-on-a-chip, claimed that microphysiological systems (MPS) would become the relevant technology platform emulating the physiology and morphology of human organisms at the smallest biologically acceptable scale.

Today, MPS are used for safety and efficacy evaluation of compounds in preclinical research and for studying developmental processes in vitro. For these purposes different MPS designs with single- or multi-organ combinations are commercially available. These include models of the human bone marrow for testing of target-effects of large molecules, combinations of skin and liver models to study application route effects of cosmetic compounds or liver and pancreas combinations for modelling insulin-signaling. Furthermore, combination of healthy tissue and tumor models can be used to study efficacy of treatment approaches in vitro.

Different cell sources such as biopsies, cell lines or primary cell-based models have been used as a basis for such as MPS. Currently, more and more models are developed originating from induced pluripotent stem cells. These models overcome limitations of availability and predictability which are limiting factors for primary cells and cell lines. Furthermore, stem-cell derived models enable the construction of autologous multi-organ MPS where all organ models are derived from one donor.
Such autologous multi-organ MPS are the basis for developing patient-on-a-chip platforms by inducing the disease phenotype in vitro or by incorporating diseased tissue of the patient into the MPS. Ultimately, we believe that these models will be used for predictively selecting, scheduling and dosing an individual patient's personalized therapy or medicine.

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A-223

Direct RNA binding by MYCN links defective RNA processing to MYC/MAX/MXD network dynamics

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Overexpression of the MYCN oncoprotein drives the genesis of high-risk neuroblastoma, an aggressive pediatric tumor with particularly poor prognosis. MYCN binds chromatin at thousands of active promoters in an obligate heterodimer with the MAX partner protein, where it enhances productive elongation by RNA polymerase II (RNAPII). MYCN-driven transcription is antagonized by the MXD transcription repressor proteins, which compete with the former for binding to

MAX. The MXD-MAX heterodimer binds DNA at the same positions as its MYCN counterpart and silences promoters by fostering histone deacetylation. The dynamics underlying the formation of activating or repressive MAX heterodimers remain unknown.
We recently documented the interactions of MYCN with the nuclear RNA exosome, a 3'-5' exoribonucleolytic complex responsible for the degradation of a vast array of non-coding transcripts [1]. MYCN-RNA exosome interactions at promoter-proximal positions evict an inefficiently elongating RNAPII from DNA thereby limiting conflicts with progressing replication forks and safeguarding the viability of neuroblastoma cells. The interactions of MYCN with the RNA exosome, whose function is not restricted to promoters, indicated a potential DNA-independent mode of action, which could underlie the switch to repressive MXD-MAX heterodimers.
By utilizing enhanced UV crosslinking and immunoprecipitation (eCLIP) and *in vitro* binding assays we now show that MYCN binds thousands of UG-rich intronic RNA sites independently of DNA binding. Furthermore, MYCN binds typical exosome transcript targets, such as enhancer RNAs and promoter upstream transcripts (PROMPTs). Accordingly, both acute (via an auxin-inducible degron) and sustained (via shRNA) ablation of the exosome complex enhances MYCN RNA-binding. Conversely, the same conditions result in a global decrease in MYCN chromatin binding and its replacement by MNT, a member of the MXD protein family, and the SIN3A histone deacetylase complex. The RNA-bound form of MYCN has a distinct interactome enriched for RNA processing and DNA damage repair factors further indicating a distinguishable function from its DNA-bound counterpart.
We propose that a dynamic balance exists between DNA- and RNA-bound forms of MYCN. Accumulation of aberrant RNA, such as undegraded introns and PROMPTs induces the release of MYCN from chromatin, which then directs the decay of these non-coding transcripts by the RNA exosome. The vacant promoters are then occupied by MXD proteins that silence promoters and essentially prevent further transcription initiation. These findings reveal a stress response mechanism for MYCN that enhances the resilience of rapidly proliferating neuroblastoma cells.

[1] Papadopoulos, D. et al. MYCN recruits the nuclear exosome complex to RNA polymerase II to prevent transcription-replication conflicts. Mol. Cell, doi:10.1016/j.molcel.2021.11.002 (2022)

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A-220

Mechanisms underlying the synthetic lethality of PARP inhibitors with homologous recombination deficiency

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An important advance in cancer therapy in the last decade has been the development of poly ADP-ribose polymerase (PARP) inhibitors for the treatment of select ovarian and breast cancers. The clinical benefit stems from the synthetic lethality of PARP inhibitors with homologous recombination (HR) deficiency, which deficiency is prevalent in the cancer types listed above. The current model to explain the synthetic lethality is based on the observation that PARP inhibitors trap PARPs on DNA: the trapped PARPs block progression of the replisome, leading to the formation of DNA double-strand breaks (DSBs), which require HR for repair. I will discuss a novel mechanism to explain the synthetic lethality between PARP inhibitors and HR deficiencies. We show that PARP1 functions together with the proteins TIMELESS and TIPIN to protect the replisome from transcription-replication conflicts (TRCs). In the absence of any one of these proteins, TRCs evolved into DNA DSBs that required HR for repair, explaining the observed synthetic lethality. In further support of this model, when we inhibited transcription elongation, which prevents the emergence of transcription-replication conflicts, then the HR-deficient cancer cells became resistant to PARP inhibitors. Interestingly, trapping of PARPs on DNA was not required for the synthetic lethality with HR deficiency, since we could observe strong synthetic lethality simply by depleting PARP1 and PARP2 by siRNA. Our model provides a new framework for understanding the mechanism of action of PARP inhibitors in the clinic and the mechanisms by which resistance can emerge.

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A-225

Enhancer-hijacking in acute myeloid leukemia

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Loss of chromosome 7 (-7) or deletion of the long arm (del7q) are recurring chromosome abnormalities in myeloid leukemias. These aberrations are highly associated with MDS and AML (~10%). These chromosome aberrations are associated with a high risk of disease progression and inferior survival. The frequent occurrence of del7q suggests that the affected region(s) may harbour critical tumour suppressor gene(s) acting via dominant silencing mechanisms that remain elusive. Recently, *MLL5* has been proposed as a possible tumour suppressor in chromosomal region 7q22. However, there is data indicating that *MLL5* alone does not account for the properties of AML with del(7q). Mouse modelling promoted another candidate, MLL3, as a haploinsufficient tumor suppressor on 7q36, cooperating with other events (such as hyperactive RAS pathway, or p53 alterations) occurring in monosomy 7 or del(7q). Another candidate tumor suppressor gene represents EZH2 mutated in low frequencies in AML and MDS. EZH2 is a member of the PRC2 complex involved in establishing repressive the H3K27me3 mark. Here, I will describe a novel mechanism where deletions of chromosome 7q or other chromosomal rearrangements result in oncogene activation by juxtaposing hematopoetic enhancers into the vicinity of *Homo sapiens motor neuron and pancreas homeobox 1* (*MNX1*) leading to aberrant activation of this homeobox transcription factor by changing gene activation through promoter-enhancer interaction, which is regulated within structural domains of megabase scale, so-called “topologically associating domains” (TADs).

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A-224

Modelling Cancer-Microbiome Interactions with Organoids and Organs-on-Chips

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In the last decade, adult stem cell-derived organoids have become versatile tools in disease modelling and bioengineering. The possibilities to expand healthy human tissue of almost every organ and control its differentiation states makes these 3D tissue models a suitable platform for studies in diverse research areas. Among these are host-microbiome interactions, where numerous clinical associations await functional validation. Here, I highlight our recent advances in modelling cancer-microbiome interactions using organoids and organs-on-chips. I discuss approaches to expose human intestinal organoids to cancer-associated bacteria and the various read-outs which can provide mechanistic insights into their crosstalk. A particular focus is laid on genotoxic bacteria that can induce mutations in cancer genomes and on intracellular bacteria and their roles in colorectal cancer disease progression.

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A-226

CAR T cells as anticancer agents

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Cellular immunotherapy has high conceptual promise for the treatment of cancer. One advance is the use of chimeric T cell receptors (CARs) to generate T cells with engineered specificities for tumor-associated surface antigens. The translational development of CAR-based T cell therapies is most advanced in cancers derived from the B cell lineage. CAR T cell therapy of leukemia and lymphoma can generate durable remissions in a high proportion of patients with refractory disease and is currently changing treatment paradigms in these cancers.
In solid cancers, the clinical activity of CAR T cells remains limited. Tumor cells in solid cancers often express potential target antigens at low and/or variable levels, rendering CAR-mediated lysis ineffective. Selective upregulation of antigens on tumor cells by pretreatment e.g. with epigenetic agents could be an attractive approach at least for some targets. Increasing the avidity of CARs for their antigen may enhance their capacity to act against solid cancer targets but could

lead to on-target toxicities by crossreactivities with normal tissue. In addition, novel logic-gating approaches may allow safe targeting of antigens coexpressed on non-cancer cells in a tumor cell-specific context.
Beyond issues of selective antigen recognition, the microenvironment of solid cancers has emerged as a major barrier to tumor infiltration, function and persistence of therapeutic T cells. To develop effective CAR T cell therapies for solid cancer, detailed knowledge of the mechanisms that prevent T cell infiltration and activity at tumor sites is needed, along with conceptual advances that overcome immune-inhibitory barriers and antigen-negative immune escape.

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A-227

Structure-guided design of the first-in-class KMT9 inhibitor with anti-cancer activity

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Inhibition of epigenetic regulators by small molecules is an attractive strategy for cancer treatment. Recently, we characterised the role of lysine methyltransferase 9 (KMT9) in prostate, lung, and colon cancer. Our observation that the enzymatic activity was required for tumour cell proliferation identified KMT9 an attractive therapeutic target. Here, we report the development of the first-in-class, potent and selective KMT9 inhibitor (compound 4, KMI169) through structure-based drug design. KMI169 functions as a bi-substrate inhibitor targeting the SAM and substrate binding pockets of KMT9 and exhibits high potency, selectivity, and cellular target engagement. KMT9 inhibition selectively downregulates target genes involved in transcription and cell cycle regulation and impairs proliferation of tumours cells including castration- and enzalutamide-resistant prostate cancer cells. Together, KMI169 represents a valuable tool to probe cellular KMT9 functions and paves the way for the development of clinical inhibitors as therapeutic options to treat malignancies such as therapy-resistant prostate cancer.

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A-228

Diet, Microbiome and Immune System Trialogue in Liver Cancer Development and Response to Immunotherapy

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Cells alter their metabolic state in response to changes in nutrient availability through the induction of transcriptional programs that affect the levels of nutrient transporters and metabolic enzymes. Cell-intrinsic mechanisms of nutrient sensing are intimately linked to adaptive metabolic responses and play critical roles in shaping the complex and dynamic nutrient environment of a growing tumor. Moreover, changes in cellular metabolism are associated with the activation of diverse immune subsets. Elucidating how nutrient supplies affect the metabolism, signaling, and thus function of immune cells in diverse and complex immune microenvironments remains a significant challenge. Immune responses involve rapid and extensive changes in the activities of immune cells with concomitant alterations in cellular metabolism. We aim to understand how food composition, in combination with alcohol consumption, induced dysbiosis impacts host metabolism, and subsequently the development of steatohepatitis induced hepatocellular carcinoma (HCC), specifically through the axis of adaptive immune cell metabolic status. Diet induced chronic inflammation promotes a bidirectional relationship between the immune system and systemic metabolism, to further influence the effector function of immune cells. The relationship between changes in whole body metabolism and immunometabolism drives immense changes in humoral and cellular immune response, and further HCC development. Long term diet intervention was analyzed comprehensively with FC, Met-Flow, MassSpec, 16S RNA-seq, scRNA/ATAC-seq, and spatial transcriptomics. Macronutrient composition regulated tumor development through systemic metabolic reprograming and epigenetic alteration, impacting the immune cell effector function along with supporting the immuno-escape mechanism of malignant cells through regulation of their MHC machinery. In summary, we show that the regulation of nutrient sensing pathways by dietary intervention regulate immunometabolism and modulate immunoediting in life-style induced cancers.

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A-229

Self-inflicted DNA breaks allow cancer cell escape from growth limits imposed by genotoxic stress

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I will present two discoveries from my team dealing with precision medicine approaches and possibilities. The first part deals with widely used genotoxic cancer therapy, such as irradiation. These modalities operate through extensive induction of DNA breaks, yet cancer cells frequently display resistance to such interventions. Intriguingly, studies following the dynamics of radiation-induced DNA lesions have identified a temporally distinct and unexplained secondary wave of DNA breaks. Here, I will present our findings on a new pathway that allows tolerance to genotoxic stress. We uncovered that cancer cells actively and reversibly elevate levels of DNA breaks, a mechanism that acts to strengthen the G2/M cell cycle checkpoint thereby limiting premature re-entry into the cell cycle. I will explain the mechanisms underlying this response, which is distinct for cancer cells. Collectively, we demonstrate that tumor cells deploy regulated DNA breaks as a mechanism to delimit the detrimental effects of exogenous DNA double-strand breakage and ensure survival. Finally, in the second part of my presentation I will outline our new approach to determine the phenotypic impact of genetic variants including their impact on radiation and drug responses. We call our approach CRISPR-Select, which allows for precise, quantitative, and rapid analysis without the need for generation of clones or selection. It provides unique opportunities for example to identify cancer-causing or treatment-response predicting mutations.

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A-230

Aging and senescence of the adaptive immune system in colorectal cancer

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There is an increasing incidence of colorectal cancer (CRC) during aging. This has been largely attributed to cancer-cell specific mechanisms including the accumulation of mutations in gate-keeper genes. Growing evidence also proposes an age-dependent dysregulation of adaptive immunity. As adaptive immunity plays a central role for the host anti-tumor immune response in CRC, an aged immune system could be a critical determinant for CRC progression. Especially cellular senescence has been regarded as one of the central mechanisms affecting immune cell dysfunction during aging. However, molecular mechanisms and the functional relevance of senescence-associated pathways for the anti-tumor immune response against CRC are only poorly understood.
Therefore, we investigated the functional role of p21 in CD4+ T cells in murine CRC models. p21, an inhibitor of various cyclin-dependent kinases (CDKs), is considered one of the central regulators of cell-cycle progression and cellular senescence. In our studies, we observed that the activation of p21 is critical for the Th1 effector function and p21-deficiency in CD4+ T cells results in increased tumor growth. Interestingly, tumor infiltrating p21-deficient CD4+ T cells showed signs of exhaustion with a loss of co-stimulatory molecules such as CD27/CD28. In human CRC, low p21 expression in CD4+ T cells within the tumor microenvironment was associated with reduced cancer-related survival. Finally, the adoptive transfer of p21-deficient T cells pre-treated with Palbociclib, an inhibitor of the p21 targets CDK4 and CDK6, restored cytotoxic function and prevented exhaustion of T cells in murine CRC.

Taken together, our data show an important role for p21 in regulating effector function and preventing exhaustion of CD4+ T cells within the anti-tumor immune response against CRC. Furthermore, CDK inhibitors might serve as promising therapeutics to enhance adaptive anti-tumor immunity in aged patients with CRC.

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A-231

Intratumoral Senescence and Metastasis

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Lars Zender, M.D., is Professor of Medical Oncology and Director and Chairman of the Department of Medical Oncology and Pneumology at University Hospital Tuebingen, Germany. He furthermore serves as the spokesperson of the Cluster of Excellence iFIT (EXC2180) "Image-Guided and Functionally Instructed Tumor Therapies" and as the scientific director of the Comprehensive Cancer Center Tübingen-Stuttgart.
Lars Zender's scientific work especially focuses on the identification of new therapeutic targets to overcome therapy resistance in solid tumors. He developed novel mosaic (chimaeric) liver cancer mouse models, which allow to conduct high throughput functional genomic analyses (shRNA- and Crispr/Cas screens) directly in vivo. He is co-founder of the Tübingen Center for Academic Drug Discovery and Development (TüCAD2), which conducts drug development projects for prioritized functionally identified therapeutic targets. To date, three small molecule drugs from the TüCAD2 pipeline went first-in-human.
Another key aspect in the scientific work of Lars Zender is his work on cellular senescence. In particular the Zender laboratory is studying the senescence associated secretory phenotype and how senescent tumour cells and pre-cancerous cells are recognized and cleared by the immune system. Lars Zender is an ERC Consolidator Grand awardee and received many prestigious prizes, including the Gottfried Wilhelm Leibniz Prize of the German Research Foundation (DFG) and the German Cancer Award. In his lecture at the 10th Mildred Scheel Cancer Conference Lars Zender will talk about a new role of senescent cells in the process of metastasis.

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POSTER ABSTRACTS

Epigenetics

A-120

Inhibition of the CAK complex is synthetically lethal with mutation of PBRM1 in ccRCC

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Summary and Introduction
Despite advances in the development of immune checkpoint therapies for clear cell renal cell carcinoma (ccRCC), outcomes for many patients with metastatic disease remain poor. In this project we are investigating therapeutic vulnerabilities that result from mutations in the SWI/SNF complex component **PBRM1**, the second most frequently mutated gene in ccRCC. We conducted loss of representation shRNA screens using an inducible **shRNA library targeting 667 genes** that regulate epigenetic processes. We ran the screen in a renal cancer cell line 786-0 which is wildtype for PBRM1, and in parallel in an isogenic 786-0 cell line in which the PBRM1 gene was knocked out by CRISPR mutation.

Materials and Methods
Multistep procedure: From the broad epigenetic field focusing on the important players with PBRM1.
1. shRNA screen: Transducing virally a 667 genes shRNA library in the cells, turning on the knockdown with an inducible system, after 12 days of knockdown, collecting gDNA, cleaning, amplification and sequencing, we analysed the data.
2. hit validation: Transducing single shRNA, knocking down specific genes. These cells had to competitively proliferate and were quantified and validated accordingly.
3. therapeutic validation and mechanistic analysis: with proliferation and inhibition assay, cell cycle analysis, western blotting, RNA-seq, AI-supported microscopy for DNA damage pathways

Results
The screen revealed several **synthethic lethal genes**. Amongst these were **CDK7 and CCNH** which together form the CDK-activating kinase (CAK) complex that is important for cell cycle progression as well as for regulating general transcription via the TFIIH transcription factor. A number of CDK7 inhibitors are being assessed in clinical trials for a variety of cancer types. We could show in our isogenic cell lines, PBRM1 loss was associated with higher sensitivity to **CDK7 inhibition**. We are investigating the molecular relationship between PBRM1 and CAK and aim to test CDK7 inhibitors for ccRCC therapy in preclinical models.

Discussion
In a focussed shRNA screen in two isogenic cell lines with/without PBRM1, we identified two genes from the CAK complex (CDK7 and CCNH) as synthetic vulnerabilities in PBRM1 deficient RCC cells. We are currently analyzing the **molecular connection** between PBRM1 and the CAK complex, and expand the testing of CDK7 inhibitors to **preclinical models** of RCC. Several CDK7 inhibitors are currently in early clinical trials, though so far not in RCC. We hope that out data may guide further trials in RCC and help to improve treatment of patients with PBRM1 mutated RCC.

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A-125

Epigenetic downregulation of Slfn11 can be returned by HDAC-inhibition, indicating the use of ATR-inhibitors to overcome chemoresistance in ovarian cancer

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Summary and Introduction

The recurrence of ovarian cancer after a first response to antineoplastic drugs like cisplatin is often associated with resistance formation and thus a bad prognosis in clinical treatment. Here we investigate several ovarian cancer cell lines and their cisplatin-resistant subtypes on the impact of DNA helicase Slfn11, which is reported to be epigenetically downregulated in a broad spectrum of cancer types thereby mediating higher tolerance to cytostatics.
The resulting research question is whether re-expression of the DNA helicase Slfn11 induced by epigenetically active drugs is a possible way to overcome chemoresistance in ovarian cancer and how it relates to the DNA damage response pathway.

Materials and Methods
qPCR and Western blot were used to investigate the expression of genes and proteins related to the mentioned signalling pathways. Cytotoxicity was determined by MTT-assay. Flow cytometry was used for cell cycle analysis.

Results
Western blot data indicate that silencing of Slfn11 expression is not an exclusive issue explaining cisplatin resistance of cells. By addressing several epigenetic targets, highest re-expression was achieved by HDAC-inhibition thereby sensitizing cells to cisplatin. We also show that Slfn11-negative cells depend in their cisplatin sensitivity on DNA damage response pathway by highly selective inhibitors at sub-toxic concentrations. Notably, co-treatment with cisplatin highlights ATR-kinase as promising target for sensitization, while downstream proteins like CHK1 and WEE1 are less important for resistance formation in most cell lines. Western blot data reveal that ATR bypasses its described signalling pathway and activates homologous recombination also indicated by an S-Phase arrest shown by flow cytometry data.

Discussion
Our findings reveal that returning the epigenetic downregulation of Slfn11 by HDAC-inhibition is a possible way to convert the resistant phenotype of ovarian cancer cells. Its low expression could become a biomarker for the use of ATR-inhibitors in clinical therapy. ATR kinase emerges as sensitive key player and thus as promising target for overcoming chemoresistance in ovarian cancer.

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A-126

TRIM67 drives tumorigenesis in oligodendrogliomas through Rho GTPase-dependent membrane blebbing

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Summary and Introduction
IDH mutant gliomas are grouped into astrocytomas or oligodendrogliomas depending on the codeletion of chromosome arms 1p and 19q. Although the genomic alterations of IDH mutant gliomas have been well described, transcriptional changes unique to either tumor type have not been fully understood. Here, we identify Tripartite Motif Containing 67 (TRIM67), an E3 ubiquitin ligase with essential roles during neuronal development, as an oncogene distinctly upregulated in oligodendrogliomas.

Materials and Methods
We used several cell lines, including patient-derived oligodendroglioma tumorspheres, to knock down or overexpress TRIM67. We coupled high-throughput assays, including RNA sequencing, total lysate-mass spectrometry (MS), and coimmunoprecipitation (co-IP)-MS with functional assays including immunofluorescence (IF) staining, co-IP, and western blotting (WB) to assess the *in vitro* phenotype associated with TRIM67. Patient-derived oligodendroglioma tumorspheres were orthotopically implanted in mice to determine the effect of TRIM67 on tumor growth and survival.

Results
TRIM67 overexpression alters the abundance of cytoskeletal proteins and induces membrane bleb formation. TRIM67-associated blebbing was reverted with the nonmuscle class II myosin inhibitor blebbistatin and selective ROCK inhibitor fasudil. NOGO-A/Rho GTPase/ROCK2 signaling is altered upon TRIM67 ectopic expression, pointing to the underlying mechanism for TRIM67-induced blebbing. Phenotypically, TRIM67 expression resulted in higher cell motility and reduced cell adherence. In orthotopic implantation models of patient-derived oligodendrogliomas, TRIM67 accelerated tumor growth, reduced overall survival, and led to increased vimentin expression at the tumor margin.

Discussion

Taken together, our results demonstrate that upregulated TRIM67 induces blebbing-based rounded cell morphology through Rho GTPase/ROCK-mediated signaling thereby contributing to glioma pathogenesis.

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A-132

Targeting the epigenetic deregulation of heparan sulfate proteoglycan formation in breast cancer cells to affect coagulability as a metastatic trigger

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Summary and Introduction

Cancer cells display an aberrant expression profile of heparan sulfate proteoglycans (HSPGs). While cell surface HSPGs were shown to affect multiple malignant processes, such as cell proliferation or angiogenesis, their impact on the coagulability of cancer cells remains hardly investigated. In these terms, the epigenetic downregulation of 3-O-sulfotransferases appears to be a key issue in cancer to increase coagulation and thus trigger platelet activation as a crucial factor for hematogenous metastatic spread. The aim of this study is to investigate whether DNA hypermethylation as a reason to silence 3-O-sulfotransferases in breast cancer cells can be reversed by epigenetic targeting approaches using DNA-methyltransferase inhibitors (DNMTi) and if this is reflected by decreased coagulation and platelet activation.

Materials and Methods

The impact of breast cancer cell treatment with DNMTi on re-expression of 3-O-sulfotransferase was analyzed by qPCR and Western blot and functionally reflected by thrombin formation assay and platelet activation potential following light transmission aggregometry or ATP release assays.

Results

Treatment of MCF-7 and MDA-MB-231 human breast cancer cells with different DNMTi induced partially a higher expression of 3-O-sulfotransferase, which could be figured out as a critical factor in reducing coagulability by binding and activating antithrombin III. Lower levels of thrombin generation were associated with an attenuated platelet activation potential, indicated by a lower aggregation and granule release.

Discussion

Our data confirm the role of HSPGs in cancer-induced coagulability and thus malignancy. 3-O-sulfotransferase appears to be a valuable target for epigenetic pharmacological approaches and warrants further investigation.

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A-134

Polymerization of C-terminal binding proteins as new molecular principle of epigenetic regulation in treatment-refractory, metastatic prostate cancer

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Summary and Introduction

The transcriptional corepressor C-terminal binding proteins (CtBP) form redox-dependent homo-tetrameric assemblies that regulate the expression of various epithelial, pro-apoptotic and pro-oncogenic genes through their involvement in large multi-component polycomb repressive complexes. As a part of these complexes, CtBPs bind to multiple protein components with short linear PxDLS-like sequence motifs. The presence of a non-canonical tandem ALDLS motif on the putative metastasis suppressor Retinoic acid-induced 2 (RAI2) protein prompted investigation on possible implications of a dual interaction with CtBPs.

Materials and Methods

To characterize the molecular interaction between RAI2 and CtBPs, we employed an integrated approach combining biophysical and structural biology techniques. For confirmation studies, we applied VCaP cells as model of castration-resistant prostate cancer and validated the clinical implications of our findings by gene expression analysis of circulating tumor cells isolated from metastatic prostate cancer patients.

Results

Applying an integrated structural biology approach including single particle cryo-electron microscopy, we show that RAI2 induces CtBP polymerization, which results in the formation of well-ordered RAI2-mediated filaments of stagged tetrameric CtBP layers. Consistently, we detected the formation of RAI2-mediated CtBP nuclear foci in RAI2-expressing cancer cells. RAI2-mediated CtBP polymerization synchronized with the relief of CtBP corepressor activity through promoting posttranslational histone modification at the target promotor of CDKN1A. Loss of RAI2 gene expression, which we observed to be characteristic during the progression of prostate cancer to treatment-resistant subtypes, led to a marked increase of neuroendocrine markers in RAI2-depleted VCaP cells.

Discussion

Our data demonstrate a previously unknown mechanism of polycomb repressive activity regulation by multivalent short linear sequence motif-induced polymerization implied in nuclear compartmentalisation, which plays a crucial role in balancing transcriptional activities. These findings hold potential for a better diagnosis and understanding of the development of resistance to androgen deprivation therapy in castration-resistant prostate cancer patients.
Our findings serve as model for a broad spectrum of related multivalent protein/protein interactions with diverse functional outcomes and provide a blueprint for the design of tandem motif-based inhibitor compounds targeting oncogenic CtBPs.

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A-150

CTLA4 DNA methylation regulates CTLA-4 expression and predicts response to immunotherapy in head and neck squamous cell carcinoma

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Summary and Introduction

The majority of patients with recurrent or metastasized head and neck squamous cell carcinoma (HNSCC) does not benefit from immune checkpoint blockade (ICB) and a relevant portion experiences severe and persistent immune-mediated side effects. Therefore, predictive biomarkers are urgently needed to allow for a personalized treatment. In this study, we investigated DNA methylation of the immune checkpoint gene *CTLA4* with regard to its predictive value.

Materials and Methods

We analyzed *CTLA4* promoter methylation in tumors of HNSCC patients (*N* = 29) treated with ICB at the University Medical Center Bonn with regard to response to ICB and progression-free survival. We further analyzed a second cohort (*N* = 138) of patients that did not receive ICB with regard to *CTLA4* promoter methylation, CTLA-4 protein expression, and immune cell infiltrates. Finally, we tested inducibility of CTLA-4 protein expression in HNSCC cells using the DNA methyltransferase inhibitor decitabine.

Results

Lower *CTLA4* promoter methylation correlated with response to ICB and prolonged progression-free survival. We could show that not only tumor infiltrating immune cells, but also HNSCC cells harbor cytoplasmic and nuclear CTLA-4 expression. *CTLA4* promoter methylation inversely correlated with infiltrates of $CD3^+$, $CD4^+$, $CD8^+$, and $CD45^+$ immune cells. *CTLA4* methylation did not correlate with protein expression in tumors, however, decitabine treatment led to an induction of *CTLA4* mRNA and CTLA-4 protein expression in HNSCC cell lines.

Discussion

Our results indicate that *CTLA4* DNA hypomethylation is a predictive biomarker for response to ICB in HNSCC. Our study warrants further analyses of the predictive value of *CTLA4* DNA methylation in clinical trials of anti-PD-1 and/or anti-CTLA-4 immunotherapy in HNSCC.

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A-153

ICOS DNA methylation regulates melanoma cell-intrinsic ICOS expression, is associated with differentiation and predicts response to immune checkpoint blockade

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Summary and Introduction

Immune checkpoint blockade (ICB) has revolutionized treatment of advanced melanoma. However, only a minority of patients shows long-term therapy response, necessitating new therapeutic strategies. Inducible T cell costimulator ICOS is an emerging target in immuno-oncology. Aim of this study was to deepen knowledge of ICOS expression patterns and epigenetic regulation via DNA in melanoma in the context of ICB.

Materials and Methods

We comprehensively analyzed *ICOS* DNA methylation of specific CpG sites and expression patterns within melanoma cells and tumor microenvironment with regard to immune correlates, differentiation, clinical outcomes, and ICB response. Analyses included data of whole tumor samples obtained from The Cancer Genome Atlas (*N*=470) (1) and Liu et al. (*N*=121) (2), melanoma cell lines (*N*=33) from the GDSC database (3), and single cell mRNA sequencing from Tirsoh et al. (*N*=4,645 single cells) (4). Additionally, data of *N*=123 of melanoma patients treated with anti-PD-1 ICB at the University Hospital of Bonn between 2012-2022 were included. The effect of pharmacological demethylation of the A375 melanoma cell line with decitabine with regard to ICOS expression was investigated.

Results

Our study revealed sequence-contextual *ICOS* CpG methylation patterns consistent with an epigenetically regulated gene. Methylation patterns were cell type-specific for melanoma and immune cells. CpG-dependent *ICOS* methylation correlated with ICOS mRNA expression, immune infiltration, melanoma differentiation markers, prognosis, and response to ICB. ICOS mRNA and protein expression was found on infiltrating immune and melanoma cells, where a cytoplasmatic or nuclear expression was observed. Melanoma cell-intrinsic ICOS mRNA and protein expression was inducible by pharmacological demethylation with decitabine. High ICOS mRNA expression was associated with favorable overall survival (OS) in non-ICB-treated patients, and predicted response and a prolonged progression-free survival (PFS) in patients treated with anti-PD1-ICB. In contrast, *ICOS* hypomethylation was associated with poor OS in patients without ICB but predicted higher response and prolonged PFS and OS in ICB-treated patients.

Discussion

Our study identified *ICOS* DNA methylation and mRNA expression as promising prognostic and predictive biomarker for immunotherapy in melanoma and points towards an undescribed tumor-role of ICOS in melanoma.

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A-166

Role of the PPARγ/ DNMT1 axis in the regulation of β-catenin signaling in glioblastoma multiforme and implications for CIK cell therapy

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Summary and Introduction

Glioblastoma multiforme (GBM) is a highly malignant primary brain tumor with poor prognosis and resistance against established treatment modalities promoted by glioma stem cells. While the aberrant activation of the Wnt/ß-catenin pathway is known to control expression of genes conferring stem cell-like properties, the interplay of underlying molecular mechanisms remains unclear. This study aimed at characterizing upstream regulation of Wnt/ß-catenin by

focusing on the crosstalk between genomic (proliferator-activated receptor gamma: PPARγ) and epigenomic (DNA methyltransferase 1: DNMT1) mechanisms postulated to modulate this pathway in GBM and neuroblastoma.
In this study, we investigated whether PPARγ inhibition could affect the expression of DNMT1 and key oncoprotein β-Catenin. In addition, we examined a possible downregulation of long nucleotide elements (LINE-1), a surrogate marker of global methylation. Because cytokine induced killer (CIK) cell therapy has shown promising results in recent GBM clinical trials, its compatibility with the PPARγ-DNMT1 axis was addressed.

Materials and Methods
Three genetically distinct GBM (G35, 84, 233s) and one neuroblastoma cell line (WAC2) were treated with a PPARγ antagonist (GW-9662) at 100/50 µM/DMSO for 72 h. Cytotoxicity was determined using Cell Counting Kit-8. PPARγ, DNMT1, ß-catenin and LINE-1 mRNA levels were assessed by qPCR. For CiK cell generation, peripheral blood mononuclear cells were obtained from three independent donors by gradient density centrifugation. Target cells were labeled with CFSE, treated with GW-9662 and CIK cells for 24 h each and analyzed by flow cytometry. Cell viability was quantified as relative specific lysis (%).

Results
A significant downregulation of DNMT1 and ß-catenin following PPARγ inhibition was observed in WAC2 to 0.48-fold with 100uM ($p<0.01$). β-catenin levels were found to be significantly downregulated in G35 (0.93-fold), too. Of interest, PPARγ inhibition significantly reduced LINE-1 expression in both GBM (G35: 0.59-fold; 233s: 0.96-fold) and neuroblastoma (WAC2: 0.82-fold) cell lines. Relative specific lysis of all cell lines increased significantly when combined with CIK cells at different ratios to target cells, e.g., in 233s – 4% (0x), 19% (1x), 39% (5x), 58% (10x). Effect on named gene signature, cytotoxicity and relative specific lysis were positively correlated with baseline PPARγ expression.

Discussion
Interfering with the Wnt/ß-catenin allows to target glioma stem cells. Alteration of downstream genes and corresponding protein function induced by PPARγ inhibition indicated a crosstalk between genetic and epigenetic determinants in malignancies of the central nervous system. However, combination with CIK cells as sustainable and effective adoptive immunotherapy requires further attention.

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A-168

The lncRNA PAPAS suppresses breast carcinogenesis by promoting differentiation of mammary epithelial cells

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Summary and Introduction
Extensive remodeling of the female mammary epithelium during development and pregnancy has been linked to cancer susceptibility. The faithful response of mammary epithelial cells (MECs) to hormone signaling is key to avoid breast cancer development. Here we have investigated the role of the long non-coding RNA (lncRNA) PAPAS in MECs and in breast cancer. PAPAS is an antisense transcript of the genes encoding ribosomal RNA (rRNA) and adapts their activity to the cellular proliferation state. We found that the lncRNA exerts a dual function as a driver of lactogenic differentiation, and as a tumor suppressor.

Materials and Methods
PAPAS and precursor-rRNA (pre-rRNA) levels were monitored by RT-qPCR in murine MECs, breast cancer cell lines and human breast tumor tissues. Moreover, PAPAS loss-of-function in MECs was conducted by shRNA-mediated knockdown, while PAPAS gain-of-function in breast cancer cells was achieved by CRISPR activation. To assay the impact of PAPAS manipulation on the epigenetic state of rRNA genes, we performed chromatin immunoprecipitation (ChIP), while tumorigenicity of manipulated cells was analyzed in orthotopic xenograft experiments. Global changes in gene expression were assessed by RNA-Seq. To compare regulation of PAPAS synthesis between MECs and breast

cancer cells, we performed ChIPs for R-loops, RNase H1 and replication protein A (RPA).

Results
Differentiation of MECs into post-mitotic lactocytes requires PAPAS upregulation, whereas knockdown of PAPAS derepresses rRNA genes and attenuates the response to lactogenic hormones. Notably, depletion of PAPAS renders undifferentiated MECs tumorigenic. Accordingly, PAPAS levels are diminished in human breast tumors and murine mammary cancer cell lines, and restoration of PAPAS expression is sufficient to curb tumorigenicity. Transcriptome analysis revealed that elevation of PAPAS levels reverts a gene expression profile associated with breast cancer metastasis and immune evasion. Mechanistically, R-loops formed at the 3' end of rRNA genes initiate PAPAS transcription, which is counteracted by RNase H1 and RPA. In human and murine breast cancer, both RNase H1 and RPA are upregulated, providing a molecular explanation for PAPAS repression.

Discussion
Taken together, our findings show that the lncRNA PAPAS safeguards differentiation, growth control and immune surveillance of the mammary epithelium. Our mechanistic insights may help to harness restoration of R-loop-dependent PAPAS synthesis as a novel therapeutic strategy in breast cancer.

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A-194

Altered DNA methylation profiles in SF3B1 mutated CLL patients

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Summary and Introduction
Survival of cancer patients has been shown to be often affected by the mutations in splicing factor genes. Chronic lymphocytic leukemia (CLL) patients with a mutation in splicing factor 3b subunit 1 (*SF3B1*) have worse prognosis than patients without *SF3B1* mutations. Because of the close interconnection between splicing machinery and epigenome, we investigated if the *SF3B1* mutations affect the genome methylation of the CLL patients. Whereas an overall decrease in methylation was observed in CLL carcinogenesis, the interaction between the *SF3B1* mutational stage and the originating B cells epigenetic stage has not been described.

Materials and Methods
We profiled genome-wide the DNA methylation status of 27 CLL patients with and without *SF3B1* mutations using MEDIP-seq and analzyed the results with QSEA package.

Results
We identified a local hypomethylation in CLL patients with mutations in *SF3B1* at 67 250bp regions, from which most was proximal to telomeric regions. These differentially methylated regions (DMRs) showed an enrichment in signaling genes known to be important in cancer e.g., *NOTCH1*, *HTRA3*, and *BCL9L*. Moreover, although the patients with *SF3B1* mutations exclusively clustered in two out of three epigenetic stages of the originating B cells, not all the DMRs could be explained by the different methylation programming (maturation) status of the B cells. This suggested that mutations in *SF3B1* cause additional epigenetic aberrations during carcinogenesis. In addition, 12 DMRs contained a binding site for an already implicated in CLL transcription factor, which is required for a proper differentiation of B cells - IKAROS.
We identified regions specifically hypomethylated in CLL patients with *SF3B1* mutations by investigating the genome-wide methylation profiles. In agreement with previous study, some of the changes identified overlapped changes reported as differentially methylated during physiological B cell maturation.

Discussion

Despite the fact that our results indicate *SF3B1* mutations are, at least in part, associated with B cell developmental stages, a large fraction of DMRs identified in this study has not been related to B cell development. This indicates that the methylation at these regions is associated with *SF3B1* specific functions. A better understanding of the interplay between DNA methylation and splicing may pave the way for new treatment options for CLL patients with *SF3B1* mutations.

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A-199

Distinct alterations in the mutation spectrum and DNA methylation landscape are associated with primary endocrine resistance in luminal breast cancer

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Summary and Introduction
Endocrine therapy is highly effective in blocking the estrogen receptor pathway in HR+/HER2- early breast cancer (EBC). However, up to 40% of patients relapse during or after adjuvant endocrine therapy. Here, we investigated molecular mechanisms associated with resistance to endocrine therapy and developed predictive models.

Materials and Methods
In the WSG-ADAPT trial (NCT01779206), HR+/HER2- EBC patients received pre-operative short-term endocrine therapy (pET). Treatment response was determined by Ki67 before and after pET. We performed whole exome panel sequencing and Infinium MethylationEPIC-based DNA methylation analysis post-pET in a discovery cohort (n=364, responder and non-responder pairs matched for clinicopathologic features) and a validation cohort (n=270, unmatched). Statistical analysis was performed using Fisher's exact test. Differential methylation was determined using Limma. Predictive indices of endocrine resistance under both treatments were constructed using lasso penalized logistic regression. A TCGA BRCA sub-cohort was used for external validation.

Results
With a frequency of up to 32% in AI non-responders, TP53 mutations were most frequently linked with the development of resistance to endocrine therapy with tamoxifen (TAM) and aromatase inhibitors (AI). We observed a global loss of DNA methylation in TAM non-responders associated with the tumor microenvironment. Conversely, we observed an increase in methylation in AI non-responders related to developmental transcription factors, hypoxia and estrogen signaling. Increased immune cell infiltration and decreased levels of fibroblasts and endothelial cells were linked to TAM or AI resistance. Based on these findings, we developed the Predictive Endocrine ResistanCe Index (PERCI). PERCI stratified NR and R groups in both treatment groups and cohorts with high accuracy (ROC AUC TAM discovery 93.9%, validation 83%; AI discovery 98.6%, validation 76.9%). A simplified PERCI efficiently predicted progression-free survival in a TCGA BRCA subcohort (Kaplan-Meier log-rank p-value = 0.03 between low and high PERCI groups).

Discussion
By combining information about genomic alterations, patient age, TME composition and differential methylation, we developed novel predictors of resistance to TAM and AI, PERCI TAM and PERCI AI, with potential additional prognostic value. Our findings revealed differences in endocrine resistance mechanisms between tamoxifen and aromatase inhibitors, which may provide a way to overcome resistance by switching to the unaffected drug.

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Microbiome

A-195

Cancer Prevention in gastroesophageal carcinogenesis utilizing FXR agonists

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Summary and Introduction

Chronic reflux of gastric and bile acid (BA) causes inflammation at the gastroesophageal junction leading to Barrett Esophagus (BE) and Gastrioesophageal Adenocarcinoma (GEAC). Obesity and a diet rich in fat are risk factors of GEAC and are associated with gut microbial changes. Here, we explored the diet-microbiota-metabolome-axis affecting GEAC-carcinogenesis in both mice and humans. Considering that the bile acid (BA) sensor farnesoid X receptor (FXR) has been described to intervene the tumorigenic signaling, we assessed its potential as therapeutic target by using its agonist obeticholic acid (OCA).

Materials and Methods

Tissue, serum and stool samples of the mouse model of BE and GEAC (L2-IL1B), as well as of healthy, BE and GEAC diagnosed patients was collected and explored at histological, genetic, microbial and metabolic level. We then exposed L2-IL1B-derived organoids to deoxycholic acid, DCA (a secondary BA) and to OCA. Based on these results, we treated L2-IL1B mice with control or high fat diet +/- OCA.

Results

HFD accelerated tumorigenesis in L2-IL1B mice while altering the gut microbiota composition and increasing BA levels in stool and tissue. Similarly, in patients, BA levels, and associated microbial alterations correlated with disease progression. Importantly, expression FXR was upregulated in human and murine BE but downregulated in GEAC. In L2-IL1B mice, FXR knockout enhanced the dysplastic phenotype, increased serum BA levels and Lgr5 progenitor cell numbers. In organoids, treatment with OCA was protective compared to DCA and in mice, treatment with OCA ameliorated the dysplastic phenotype of the mice.

Discussion

We provide a novel concept of GEAC carcinogenesis being accelerated via the diet-microbiome-metabolome axis, leading to BA-based FXR antagonism on progenitor cells. Further, FXR activation protected against adverse effects of systemic BA in BE, suggesting that OCA may have potential as a novel approach in differentiation therapy in GEAC prevention.

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Targeted therapy

A-111

Spatial and temporal transcriptomics of SHH-medulloblastoma with chromothripsis identifies multiple genetic clones that resist treatment and lead to relapse

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Summary and Introduction

Pediatric medulloblastomas with chromothripsis are characterized by high genomic instability and dismal prognosis, as tumors are often resistant to the limited treatment options.

Using spatial transcriptomics, we set out to understand underlying molecular features leading to the aggressiveness of these tumors by comparing chromothriptic and non-chromothriptic SHH-medulloblastomas. In patient-derived xenografts of chromothriptic SHH-medulloblastoma, we investigated resistance mechanisms by spatial and temporal transcriptomic profiling over the course of treatment.

Materials and Methods

We applied spatial transcriptomics to profile 13 chromothriptic and non-chromothriptic medulloblastomas from the same molecular subgroup. To map genetic subclones to the spatial transcriptomics profiles, we leveraged single-cell DNA sequencing data and copy-number variant profiling.

Patient-derived xenografts of chromothriptic medulloblastoma were treated with carbon ion radiotherapy and PARP inhibitor and profiled by spatial transcriptomics at different time points covering the transition from the minimal residual disease stage to treatment-resistant regrown tumors.

Results

Our data reveal a higher extent of spatial intra-tumor heterogeneity, increased proliferation and stemness, but lower immune infiltration and differentiation in chromothriptic medulloblastomas. Spatial mapping of genetic subclones identifies a regionally distinct architecture and clone-specific phenotypic features.

In patient-derived xenografts of chromothriptic medulloblastoma, an ecosystem of cells from multiple genetic clones resisting treatment and leading to relapse highlights the importance of multi-clone interplay. Tumor microtubes might play a role in treatment resistance in chromothriptic medulloblastoma.

Discussion

Our findings contribute to a better understanding of the determinants of aggressiveness and the molecular makeup of chromothriptic medulloblastoma. They also suggest that resistant cells do not necessarily come from one homogenous pool of stem cells, as multiple genetic clones with distinct phenotypic compartments persist through treatment.

Finding potential mechanisms of treatment resistance could have therapeutic implications, and the identification of tumor

microtubes suggests cell network communication as a putative target.

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A-112

Functional and diagnostic relevance of phosphoproteomic pathway signatures in squamous cell carcinoma of the lung.

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Summary and Introduction
Lung cancer is the leading cause of cancer-related deaths worldwide. Fibroblast growth factor receptor 1 (FGFR1) gene amplification is one of the most common and druggable genetic alterations in squamous cell lung cancer (SQCLC), and selective tyrosine kinase inhibitors targeting FGFR1 have been developed. Phase I and II clinical trials have demonstrated the safety and activity of these inhibitors in SQCLC with disease control rates of 26-39%. Some patients have benefited from treatment for more than 14 months. However, resistance mechanisms, either naturally present in patients or acquired during treatment, have limited treatment efficacy in clinical trials.

Materials and Methods
The aim of the present project was to investigate the resistance signalling pathways of FGFR1 inhibition in FGFR1-amplified SQCLC cells. To identify signalling pathways that lead to resistance to FGFR1 inhibition, we performed a large-scale phosphoproteomic mass spectrometry analysis of SQCLC cells with FGFR1 amplification and intrinsic, pharmacological or mutationally induced resistance to FGFR1 inhibition.

Results
Using phosphoproteomic mass spectrometry, we compared FGFR1-amplified SQCLC cell lines with either an intrinsic or induced opposite response to FGFR1 inhibition. We were able to quantify 11,052 phosphosites and found a strong and significant phosphorylation of AKT1 and its downstream target PRAS40 at its activating phosphosites in the resistant cell lines. Co-inhibition of AKT and FGFR1 synergistically re-sensitised resistant lung cancer cells to FGFR1 inhibition. Furthermore, by immunohistochemical staining of phosphorylated AKT in tissue samples from SQCLC patients, we were able to demonstrate a strong intrinsic variation of AKT phosphorylation.

Discussion
In conclusion, we have used a phosphoproteomic approach to investigate the resistance signalling pathways of FGFR1 inhibition in squamous cell lung cancer. In addition to providing a large data library of resistance-associated phosphorylation patterns, we identified a common resistance pathway involving AKT activation. Studying AKT activation may help predict response to FGFR1 inhibition, and combining AKT inhibitors with FGFR1 inhibitors may pave the way to effective therapy for FGFR1-dependent lung cancer patients.

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Evaluating the RIST molecular-targeted regimen in a neuroblastoma 3D-spheroid cell culture model

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Summary and Introduction

Background: Neuroblastoma is the most common extracranial solid tumor in childhood. Despite intensive treatment regimens the outcome for patients with high-risk, relapsed and refractory neuroblastoma remains dismal and novel treatment strategies are urgently needed. Irinotecan, a topoisomerase inhibitor and the alkylating agent Temozolomide(I/T) are the current backbone to develop novel treatment options for several tumor entities. RIST represents a metronomic molecular targeted treatment strategy, combining I/T with the multikinase inhibitor Dasatinib (Srycel, S) and mTOR inhibitor Rapamycin (R). A prospective randomized phase II clinical trial testing the RIST protocol in relapsed and refractory neuroblastoma patients (NCT01467986) completed recruitment and the results are currently under evaluation. For preclinical drug testing the growth of cancer cells in form of spheroids compared to monolayer cultures is of advantage since it reproduces a wide range of avascular solid tumor characteristics, including the three-dimensional architecture and cancer stem cell (CSC) properties.

Aim: Rigorous assessment of the RIST treatment regimen in a neuroblastoma spheroid model characterized by an augmented neoplastic phenotype.

Materials and Methods

Establishment of growth conditions for spheroids in a 96-well format for five neuroblastoma cell lines. Evaluation of CSC marker expression by mRNA and protein analysis. Testing the viability of spheroids after drug- and control-treatment by applying luminescence-based assays. Assessment of RNA-binding protein La, a well-known factor promoting cancer cell plasticity, in neuroblastoma by tissue microarray analysis and patients' data mining.

Results

Compared to monolayer cultures, spheroid cultures not only showed increased expression of CSC markers CXCR4, NANOG, and BMI1, but also augmented phosphorylation of the neuroblastoma-associated La protein at threonine 389 (Thr389). Molecular targeted 'pre-treatment' reduced neoplastic signaling, CSC marker expression and the viability of spheroids. The RIST treatment significantly decreased the viability of spheroids in all neuroblastoma cell lines tested.

Discussion

The RIST protocol efficiently reduced the viability of neuroblastoma cells in a preclinical spheroid model despite the presence of increased CSC properties. Furthermore, the results underscore the importance of the neuroblastoma spheroid model for preclinical drug testing in a rigorous, robust, and efficient high-throughput format which can be valuable to identify more effective treatments for children with high-risk neuroblastoma in the future.

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The role of ADAM8 in multiple myeloma

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Summary and Introduction

Multiple myeloma (MM), the second most common hematological malignancy, arises from post-germinal center B cells. Despite a lot of progress, the disease still remains uncurable, majorly because of extensive clinical and genetic heterogeneity, underlining the need for personalized or targeted therapies. In our previous studies, we found that, among others, mutations in receptor tyrosine kinases (RTK) (e.g. Insulin-like growth factor 1 receptor (IGF1R)) and the high expression of *ADAM8* was associated with a shorter survival in MM patients. The current study therefore aimed to take a closer look into the functional role of ADAM8 in the context of important survival signaling pathways in MM (e.g. IGF1R).

Materials and Methods

Protein expression levels of ADAM8 were assessed using Western blot in 7 human MM cell lines (HMCL) (U266, AMO1, MM1.S, KMS11, L363, JJN3, KMS12BM) and IGF1R CRISPR/Cas9 knockout (KO) clones of the HMCL L363. Subsequently, an siRNA knockdown of ADAM8 was performed in 5 HMCL to determine the influence of reduced ADAM8

expression on RTK signaling effectors by Western blot.

Results
Western blots showed that catalytically active ADAM8 (isoform with metalloprotease domain) was expressed in 5 out of 7 HMCL. ADAM8 siRNA knockdown led to a strongly reduced expression of the IGF1R in all of these 5 HMCL. IGF1R activation, determined by its phosphorylation was also reduced in 3 HMCL after ADAM8 knockdown, as was AKT phosphorylation in 4 HMCL. In contrast to the observed downregulation of IGF1R upon ADAM8 siRNA knockdown, ADAM8 expression was upregulated in L363 IGF1R KO clones.

Discussion
ADAM8 appears to be closely linked to IGF1R expression levels and the PI3K/AKT signaling pathway, which is known to be crucial for the survival and proliferation of cancer cells. The fact that IGF1R KO clones upregulate ADAM8 suggests a possible feedback loop. This gives a first impression of the functional role of ADAM8 in MM and underlines its attractiveness as a potential therapeutic target. In future experiments, we will therefore assess e.g. the effect of an ADAM8 inhibitor, BK-1361, on signaling and survival in HMCL.

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A-137

The plasticity factor ZEB1 at the crossroads of intratumor heterogeneity,therapy resistance and anti-tumor immunity

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Summary and Introduction
Malignancy results from the interplay between cancer cells and the tumor microenvironment (TME). TME-derived TGFβ partially activates epithelial-mesenchymal-transition (EMT) in tumor cells via transcription factors, such as ZEB1, which promotes invasiveness, cellular plasticity and intratumor heterogeneity. ZEB1 is also heterogeneously expressed in cancer-associated fibroblasts (CAFs), but our understanding of its role there is limited. Evidence suggest crosstalk of EMT and the DNA damage response (DDR) with a strong, yet poorly understood impact on chemoresistance and immunomodulation. However, the mechanisms remain largely elusive.

Materials and Methods
We sought to determine the impact of ZEB1-driven plasticity on the DDR of tumor cells and in within the TME by employing single-cell transcriptomics and high-content microscopy across cancer cell lines and genetic mouse tumor models, aiming to expose weak spots of malignancies.

Results
We discovered a chemoresistant ZEB1 high expressing sub-population (ZEB1^{hi}) of cancer cells displaying a Zeb1-driven characteristic DNA replication stress response engaging the DDR nuclease MRE11 that benefits chemoresistance, but can be selectively targeted by MRE11 inhibition for chemosensitization in vitro and in vivo. Hence, we demonstrate that ZEB1-driven DDR plasticity is key to stress tolerance, but manifests a selective vulnerability in ZEB1^{hi} cells, highlighting the translationally relevant DDR-EMT intercept. In colorectal cancer CAFs, we show that ZEB1 is essential for CAF diversification to promote metastasis and to restrict immunogenicity. Strikingly, loss of ZEB1 in CAFs sensitizes murine CRC to immune checkpoint therapies.

Discussion
Taken together, we conclude that ZEB1 promotes malignancy and therapy resistance from different cell types. As ZEB1$^{hi/pos.}$ CAFs also display the characteristic ZEB1-dependent DDR rewiring, we now seek to simultaneously target both unfavorable cell subtypes, i.e. chemoresistant Zeb1^{hi} cancer cells and immune-dampening Zeb1$^{hi/pos.}$ CAFs to improve (chemo)therapeutic success.

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A-138

Improved rectal cancer response to radiotherapy through tumor vessel normalization induced by PFKFB3 inhibition

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Summary and Introduction
Treatment of patients with locally advanced rectal cancer (RC) is based on neoadjuvant chemoradiotherapy followed by surgery. In order to reduce the development of therapy resistance, it is necessary to further improve previous treatment approaches. Recent *in vivo* experimental studies suggested that the reduction of tumor hypoxia by tumor vessel normalization (TVN), through the inhibition of the glycolytic activator PFKFB3 could significantly improve tumor response to therapy.

Materials and Methods
We have evaluated *in vitro* and *in vivo* the effects of the PFKFB3 inhibitor 2E-3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) on cell survival, clonogenicity, migration, invasion and metabolism using colorectal cancer cells, patient-derived tumor organoids (PDO) and xenografts (PDX).

Results
3PO treatment of colorectal cancer cells increased radiation-induced cell death and reduced cancer cell invasion. Moreover, Gene Set Enrichment Analysis shows that 3PO is able to alter the metabolic status of CRC PDOs towards oxidative phosphorylation. Additionally, *in vivo* neoadjuvant treatment with 3PO induced TVN and reduced tumor hypoxia, culminating in significantly better tumor response to therapy.

Discussion
In this study, we show that rectal cancer treatment could be improved by the addition of 3PO to its treatment regime. Our results support PFKFB3 inhibition as a possible future neoadjuvant addition for rectal cancer patients.

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A-141

In vivo evaluation of rectal cancer PDXs' radio-sensitization by PFKFB3 inhibition through KAN0438757 administration

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Summary and Introduction
Rectal cancer is an important disease with about 700,000 new cases worldwide yearly. Its treatment is mainly based on radiochemotherapy and surgery, but many tumors appear to be radiotherapy (RT) resistant. Recently, the glycolysis-regulating enzyme PFKFB3 was associated with homologous recombination repair of DNA double-strand breaks, induced by ionizing radiation in cancer cells, consequently possibly contributing to RT resistance. Thus, PFKFB3 inhibition would promote radiosensitization and therapy effectiveness.
The novel molecule KAN0438757 (KAN) has been identified as a potent PFKFB3 inhibitor and is therefore a promising compound in the treatment of rectal cancer which could improve the results of RT.

Materials and Methods
Rectal cancer patient-derived tumors are transplanted into highly immunosuppressed mice (NRG strain), thereby generating patient-derived xenografts (PDXs). 25 mice engrafted with tumors from the same patient are randomized into four treatment cohorts: (i) Control, (ii) KAN, (iii) RT, and (iv) combined KAN and RT. To study the effects of KAN *in vivo*, the animals' weights and their tumor growth curves are evaluated under treatment. After performed treatment the mice are sacrificed and the PDXs are harvested to evaluate their histology. DNA damage is evaluated by immunohistochemistry (IHC) staining for pH2AX. To investigate cell death, a TUNEL assay was performed. Specific tumor-cell apoptosis is evaluated by IHC for cleaved caspase 3. Proliferation rates within the tumor tissue are tested using a BrdU staining. Hypoxia was evaluated by Pimonidazole staining.

Results

Preliminary results show that combined treatment did not significantly reduce tumor volume compared to the RT-only group. However, histological analysis shows that combined treatment led to a significantly less viable tumor areas and extensive necrosis, compared to all other treatments. Moreover, a higher expression of the DNA damage marker pH2AX was found in the combined treatment group.

Discussion

Our currently results indicate that a treatment with KAN has a radiosensitizing effect on rectal cancer PDX tumors, since extensive necrotic areas were found under combined treatment. Moreover, increased DNA damage was found in the tumors from mice that received combined KAN and RT. Further evaluation on cancer cell death and proliferation, as well as tumor vessel normalization are ongoing.

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A-142

Combination of RMC-4550 (SHP2i) and LY3214996 (ERKi) for the treatment of KRAS-mutant pancreatic cancer.

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Summary and Introduction

Mutant KRAS is present in over 90 % of pancreatic as well as 30-40 % of lung and colorectal cancers and is one of the most common oncogenic drivers. For decades, despite intensive research, most mutant KRAS isoforms, including the ones frequently associated with pancreatic ductal adenocarcinoma (PDAC), could not be targeted directly. Moreover, the targeting of single RAS downstream effectors with monotherapies was shown to induce adaptive mechanisms leading to tumor recurrence or resistance. Here we show our results of the combined inhibition of SHP2 and ERK, using the small molecule inhibitors RMC-4550 and LY3214996 respectively.

Materials and Methods

Both human and murine PDAC cells were used to investigate growth inhibition, apoptosis induction and MAPK-pathway inhibition of RMC-4550 and LY3214996 *in vitro*. We then used both endogenous (KCP) and transplantation-based mouse models to evaluate the *in vivo* efficacy and tolerability of RMC-4550 or LY3214996 monotherapy compared to different RMC-4550 + LY3214996 combination regimens.

Results

Here we show synergistic anticancer activity, superior disruption of the MAPK pathway, and significantly increased apoptosis induction compared to single-agent treatments *in vitro*. *In vivo*, we demonstrate good tolerability and efficacy of the combination, with significant tumor regression in multiple PDAC mouse models. We also show evidence that ^{18}F-FDG PET can be used to detect and predict early drug responses in animal models.

Discussion

Our pre-clinical results were used as basis for a first-in patient trial. The phase 1a/b SHERPA trial (**SH**P2 and **ER**K inhibition in **pa**ncreatic cancer, NCT04916236), is currently ongoing in patients with *KRAS*-mutant NSCLC, CRC and PDAC.

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A-152

Molecular differences of desmoplastic versus replacement histopathological growth patterns of colorectal cancer liver metastases spatially resolved

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Summary and Introduction
Different histopathological growth patterns (HGP) of colorectal cancer liver metastases (CRCLM), among which the desmoplastic HGP (dHGP) and the replacement HGP (rHGP) are the most frequent, have proven prognostic and predictive significance. In order to identify new treatment options and find non-invasive biomarkers, molecular mechanisms driving different HGPs need to be further explored.

Materials and Methods
We performed spatial RNA sequencing analysis (21,804 spots) using formalin-fixated paraffin embedded CRCLM (3 dHGP vs 3 rHGP) to obtain deep molecular insights in morphologically differing areas of the two HGPs. *In silico* findings were proven *in vitro* by using patient-derived tumor organoids (PDO).

Results
In general, cancer areas of CRCLM with predominating rHGP showed increased markers for poor prognosis, migration, invasion and epithelial mesenchymal transition. Meanwhile, dHGP cancer areas showed upregulation of genes promoting inflammatory reactions.
Importantly, a signature of increased canonical WNT signalling was identified in cancer areas of CRCLM expressing the rHGP, while cancer cells of the dHGP showed upregulation of WNT signalling antagonists of the Dickkopf (*DKK)* family.

Discussion
In this study, we propose the canonical WNT signalling pathway for further investigation as a target in treating the prognostically poorer and therapeutically more resistant rHGP of CRCLM.

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A-162

Functionalized gold nanoparticles: A promising tool for retinoblastoma treatment

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Summary and Introduction
Retinoblastoma (RB) is the most common malignant intraocular tumor in early childhood. Despite advancements in treatment, RB management remains challenging due to massive side effects and resistances against chemotherapeutic drugs like etoposide. Gold nanoparticles (AuNPs) gained significant attention as potential therapeutic agents in cancer treatment due to their unique physicochemical properties.

Materials and Methods
AuNPs are composed of a core of gold atoms with a diameter of less than 20 nm and can be easily synthesized and functionalized with various biomolecules, such as antibodies or peptides. The use of AuNPs in cancer therapy is a rapidly evolving field, with promising results in preclinical studies. By functionalizing AuNPs with hyaluronic acid (HA), the biomolecule-gold complex can be internalized by retinal cells via their CD44 receptors. We successfully used HA-AuNPs as nanocarriers to deliver therapeutic agents such as the atrial natriuretic peptide (ANP), which has been shown to reduce neovascularization in the eye and various tumor entities, to the retina, the place of origin of RB.

Results
In the study presented, we investigate the effect of ANP coupled, HA coated gold nanoparticles (ANP-HA-AuNPs) on the tumor formation potential of etoposide resistant RB cells in the chicken chorioallantoic membrane (CAM) assay. Additionally, we established an orthotopic *in vivo* RB rat eye model and investigated the impact of ANP-HA-AuNPs on

etoposide-resistant RB cell tumor growth using various nanoparticle administration routes, including non-invasive eye drops. We could show that treatment of etoposide-resistant RB cell lines with ANP-HA-AuNPs resulted in a significant decrease in tumor growth in *in ovo* CAM assays. The *in vivo* rat eye model enabled us to validate the anti-tumorigenic effect of ANP-HA-AuNPs on chemoresistant RB cell tumor growth.

Discussion
In conclusion, AuNPs have emerged as a promising tool for minimal invasive treatment of chemoresistant RB. Our results show that ANP-HA-AuNPs decrease the tumorigenic potential by diminishing tumor size, weight and tumor formation capacity *in ovo* and *in vivo*.

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A-163

Vascular heterogeneity in adjacent tissue of livers colonized by desmoplastic and replacement type colorectal cancer liver metastases

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Summary and Introduction
Different histopathological growth patterns (HGP's) of colorectal liver metastases (CRCLM) have been recognized to influcence patients' outcome. Two subgoups, the desmoplastic HGP (dHGP) and the replacing HGP (rHGP), have been shown to differ significantly in overall survival as well as in the mechanism the metastases obtain blood supply. The dHGP utilizes sprouting angiogenesis (SA) to sustain the tumor growth, whilst the rHGP relys on vessel cooption (VC), a process where pre-formed vessels are highjacked by the metastatic cells. However, the drivers of VC are still poorly understood. Moreover, blockage of the VEGF signaling pathway is effective only in metastases utilizing SA, and does not improve the survival of VC CRCLM patients.To date, prior identification of CRCLM HGP is not possible and only analysis of the surgical specimen by clinical pathology is able to identify it.
We hypothesized that the tumor microenvironment (TME), especially the local hepatic vasculature might have an influence on the development of specific HGPs and their mechanisms of blood supply.

Materials and Methods
We performed single-cell RNA sequencing analysis using dHGP and rHGP CRCLM and their adjacent normal liver, totalizing 22.419 cells.
We validated the findings using two independent data sets with the same stratification, one from a public bulk RNA sequencing database, and another one which was generated by us using spatial RNA sequencing data.

Results
We detected a distinct cell population mainly expressed in the dHGP phenotype that exhibit a set of marker genes canonically attributed to endothelial cells of liver sinusoids. Additionally, we observed a gene signature which is related to a neoangiogenic process. Curiously, this cluster co-occures next to canonical liver sinusoids and neovascular endothelial cells. This novel cluster shows a upregulated gene siganture related to endothelial homeostasis, barrier function and regulation, as well as endothelium developement. These findings were further validated in CRCLM bulk and spatial RNA sequencing data.

Discussion
Our data identifies a new endothelial cell population in the hepatic vasculature of CRCLM using SA, which may influence the blood supply of the dHGP CRCLM. Moreover, in future these findings might be used as possible predictors for CRCLM HGP.

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A-164

Molecular Tumor Board of the UMG - Molecular analyses and identification of molecular targets in gastrointestinal malignancies

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Summary and Introduction
Tumors exhibit a wide variety of molecular profiles. These genomic alterations differ from entity to entity and from tumor to tumor. The molecular profiles of individual tumors are analyzed and a group of experts from various disciplines examines options for targeted, personalized therapies resulting from the identified genetic alterations within the framework of molecular tumor boards (MTBs).

Materials and Methods
The MTB of the UMG exists since 2019. From October 2019 – December 2022, the molecular tumor profiles of 558 patients with 561 tumors were analyzed and examined for targeted therapy options in 650 case discussions. Most patients had gastrointestinal (GI, 66%) or lung (19%) tumors, but urological, gynecological, breast, head and neck, or endocrine tumors were also examined in the MTB. The group of 372 GI-patients includes tumors of pancreas (36%), colon (33%), bile (19%), stomach/oesophagus (11%), and liver (2%).
Molecular profiles of the GI-tumors are analyzed either by specialized panels covering less than 100 genes, and/or by a more comprehensive panel covering around 500 genes and an RNA analysis (e.g. TSO-500). One of the routinely used specialized panels is optimized for tumors of the pancreas, bile and liver.

Results
For 73% of the GI-patients, an analysis using a specialized panel was carried out. Overall, 41% of patients underwent analysis using a large panel, with 26% of patients undergoing this analysis exclusively. This was particularly the case for tumors of the stomach/oesophagus or colon.
A detailed analysis of the therapy recommendations for different entities resulting from the molecular analyses revealed, that the specialized panel was sufficient to generate recommendations based on clinical data (level m1a-m2c) in 51 % of cases (43% in stomach/oesophagus to 58% in bile). In addition, a therapy recommendation based on cell culture/animal experiment data (level m3) was given in an average of 20% of cases. For 15% of patients, after analysis with a specialized panel, a subsequent analysis was then carried out with a large panel (e.g. if the first analysis did not reveal a satisfactory therapeutic option). 19 of 22 patients (86%) received a therapy recommendation after advanced analysis using a large panel. For 22 of 35 patients (62%), a further therapeutic option with a better evidence was recommended.

Discussion
MTBs give patients access to innovative and personalized treatments. Even a small, specialized analysis shows a high recommendation rate for therapeutic treatments. Such specialized analyzes are cheaper than comprehensive analyzes and require less effort when evaluating and interpreting the results. It is desirable that patients have access to such analyzes more frequently and sooner than current MTB participation requirements allow.

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A-165

Molecular Tumor Board of the UMG - Follow-up of therapy recommendations

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Summary and Introduction
Molecular tumor boards (MTBs), as an important part of personalized medicine, serve to bring together the expertise of oncologists, pathologists, bioinformaticians, etc. in order to discuss an interpretation of the analysis results and make a

treatment recommendation. So far, there is little data on whether these recommendations of the MTBs are implemented in individual cancer therapies and whether patients respond to these therapies.
As part of this project, a follow-up is carried out for patients who have been presented to the MTB of the University Medical Center Göttingen (UMG), which has been active since 2019. In addition to the implementation of a specialized follow-up strategy into tumor board procedures, the MTB structures, recommendations, evidence and cost coverage by health insurance companies are analyzed and evaluated. This creates a system of knowledge-generating patient care that will support the future work of the MTB.

Materials and Methods
The establishment of a workflow for the follow-up includes both the creation of structures in a database and the collection of data from various internal data sources of the UMG (e.g. Onkostar and ixserv) as well as from external medical practices and clinics. So far, data from 70 MTB conferences held between October 2019 and December 2022 have been evaluated (n = 650 case discussions for 558 patients). Each MTB presentation of a patient is considered as a case.

Results
A customized system of different patient statuses was defined for the follow-up strategy, ranging from the MTB presentation to the subsequent therapy, the therapy response or to the death of the patient. Usually, different transient statuses are passed through until a final status (e.g. end of therapy/lost to follow-up/death of the patient) is reached. Time intervals were defined for patients in transient stages, after which the data is to be updated. In 650 evaluated cases at least one treatment recommendation was given to 493 of 558 patients (88.35%). An implementation into cancer therapy has been found in 66 (13.39%) of all patients with treatment recommendations. In total, the number of patients lost to follow-up was n = 98 (18%). In patients available to follow-up, the implementation of treatment recommendations was 16.71%. Future implementation of treatment recommendations is still possible in about 12% of patients.

Discussion
Of all 558 evaluated patients more than 88% received a treatment recommendation. In contrast to the high rate of treatment recommendations, the UMG MTB sees an implementation in only about 13% of patients. The establishment of the new systematic follow-up-system enables and confirms the findings to be in line with published insights of other German MTBs.

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A-170

Preclinical development of the endogenous CXCR4 antagonist EPI-X4 for therapy of cancer and inflammatory diseases

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Summary and Introduction
The CXCR4/CXCL12 axis plays an important role in several processes of the human body, including development, stem cell homeostasis, and immune cell function. Aberrant CXCR4/CXCL12 signaling is involved in diverse pathologies such as cancer and inflammatory diseases. The **E**ndogenous **P**eptide **I**nhibitor of C**X**CR**4** (**EPI-X4**) is a 16-amino acid residue fragment of human serum albumin, which has previously been identified in our lab. The peptide specifically binds to CXCR4, blocks CXCL12-induced signaling and migration and acts as an inverse receptor agonist. EPI-X4 is a promising candidate for the development of improved analogues for the therapy of CXCR4-associated diseases.

Materials and Methods
We optimized the antagonistic activity of EPI-X4 by combining computational approaches and rational drug design. In addition, we applied different methods to prevent enzymatic degradation and to prolong systemic circulation time *in vivo*.

Results

Compared to the wild-type peptide, these newly developed EPI-X4 derivatives have a more than 1000-fold increased anti-CXCR4 activity, are stable for several hours in blood plasma and circulation *in vivo*, and are therapeutically active in different mouse models of inflammatory diseases, e.g. topical dermatitis and eosinophilic asthma, and cancer, e.g. Waldenström's macroglobulinemia and acute myeloid leukemia. In addition, therapeutic efficacies of lead derivatives are currently being evaluated in mouse models of other CXCR4-dependent diseases like chronic lymphocytic leukemia, sepsis, and rheumatoid arthritis.

Discussion

In conclusion, our data show that optimized EPI-X4 derivatives are excellent candidates for further developement for the treatment of cancer or inflammatory diseases.

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A-171

Functional investigation of the IGF1R-PYK2 axis in Multiple Myeloma

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Summary and Introduction

Previous Next Generation Sequencing studies in our group identified point mutations in almost all primary multiple myeloma (MM) cases in a network of Receptor Tyrosine Kinases (RTKs) and their effectors. RTK mutations were associated with worse overall survival with IGF1R being among the most frequently mutated RTK. The focal adhesion kinase PYK2, which can be regulated by RTKs and shows an increased expression in MM cells compared to normal bone marrow cells, was found among the mutated effectors. Thus, we aimed to investigate the impact of IGF1R on PYK2 as well as the role of WT and mutant PYK2 on RTK-signaling in different human myeloma cell lines (HMCL).

Materials and Methods

We performed siRNA knockdowns (kd) (IGF1R, PYK2) and transfected four HMCL with $IGF1R^{WT}$ and two mutants ($IGF1R^{mut1}$/^{mut2}) and two HMCL with WT and mutant PYK2, using the Sleeping Beauty system. The impact of PYK2-kd/ko and overexpression (OE) of $IGF1R^{WT/mut1/mut2}$ and $PYK2^{WT/mut}$ on RTK signaling was investigated by western blots. Immunofluorescence was used to localize (p)IGF1R and (p)PYK2 in two HMCL before and after IGF1R-CRISPR-Cas9-knockout and in the absence/presence of IGF1. Finally, *in vitro* drug screenings with an IGF1R (linsitinib) and a PYK2 (VS-4718) inhibitor as well as a co-inhibition of VS-4718 with linsitinib or carfilzomib were performed, and the effect on HMCL viability assessed using MTT and annexin V/PI assays.

Results

IGF1R-kd reduced the activation levels of PYK2 and AKT in all and the activation levels of MEK and ERK in some of the HMCL, while PYK2-kd influenced the activation levels of AKT in only 1/4 HMCL and the activation of MEK/ERK in 3/4 HMCL. Notably, OE of $PYK2^{mut}$, however, entailed lower activation levels of IGF1R in U266 and L363 cells, though this was not seen upon OE of $PYK2^{WT}$. Moreover, the PYK2 and IGF1R activation patterns were highly similar in the four IGF1R-OE sublines and IGF1 stimulation recruited (p)PYK2 to the membrane before but not after IGF1R-ko in L363.Treatment with either linsitinib or VS-4718 affected the viability of 6/7 HMCL to varying degrees. Co-inhibition of VS-4718 with carfilzomib had an effect that was more than additive in 3/6 HMCL and had at least an additive effect in 3/6 HMCL; while a co-inhibition of VS-4718 and linsitinib had at best an additive effect in 5/6 HMCL and no effect in U266.

Discussion

In summary, the current study underlines the attractiveness of IGF1R and PYK2 as therapeutic targets and suggests a direct impact of IGF1R on PYK2 in MM.

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A-175

The Role of GSK3β in DNA Repair Mechanisms and Resistance in PDAC

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Summary and Introduction

Current studies demonstrate the existence of various molecularly and genetically defined subtypes of pancreatic cancer, but their clinical relevance and therapeutic potential are still largely unknown. An exception are tumors with gBRCA1/2 mutations, which are characterized by insufficient DNA repair and are candidates for platinum-based therapy and PARP inhibitors. The identification and characterization of novel subtypes with addressable vulnerabilities are at the center of our ongoing investigations.

Materials and Methods

Established models of pancreatic carcinogenesis, murine and human PDAC cell lines, patient-derived-cell lines (CDX) and -organoids (PDO) were used for subtype identification and characterization experiments. RNA-seq, Western-blot, qPCR, flow-cytometry, immunohistochemistry, immunofluorescence, live-cell imaging, DNA damage repair assay, BrdU and MTT studies were carried out to characterize molecular, biochemical and functional features of the novel and clinical highly relevant GSK3β;NFATc1^{high} subtype.

Results

Our studies revealed existence of a novel subtype in 15-20% of all PDAC samples that is defined by high levels of the GSK3β-NFATc1 transcription pathway. Moreover, GSK3β;NFATc1^{high} subtype correlates with disease progression, resistance and poor survival. We also showed that pharmacological or genetic inactivation of the GSK3β-NFATc1 pathway overcomes resistance in CDX and PDO through impaired homologous recombination (HR) and DNA repair. RNA-seq analyses identified GSK3β-NFATc1 regulated gene signatures involved in DNA repair, replication stress and HR, specifically BRCA1, BRCA2, Rad51. Immunofluorescence analysis confirmed NFATc1 dependent DNA repair in resistant PDAC cells, while HR repair assays showed a highly significant loss of HR activity upon disruption of the GSK3β-NFATc1 pathway. Finally, cisplatin induced resistance causes increased expression and activation of the GSK3β-NFATc1 pathway, whereas genetic silencing of NFATc1 not only increased basal and platin-induced DNA damage responses but also prevented PDAC subtype cells from recovery.

Discussion

The GSK3β;NFATc1^{high} subtype defines a highly aggressive and resistant subgroup in pancreatic cancer. Moreover, targeted inactivation of the GSK3β-NFATc1 pathway causes an “inducible BRCAness” phenotype, thus resensitizing PDAC tumors to platin-based therapy.

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A-182

Development of KRAS inhibitor-based combination therapies for PDAC

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Summary and Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a malignancy with a poor prognosis. In 90% of PDAC, mutations in the KRAS oncogene drive the disease. Although $KRAS^{G12C}$ mutations occur in only 1% of PDACs, $KRAS^{G12C}$ inhibitors show clinically meaningful anticancer activity with an acceptable toxicity profile. However, rapid resistance occurs, implicating the need to tailor $KRAS^{G12C}$ inhibitor-based combination therapies.

Materials and Methods

We used MiaPaCa2 ($KRAS^{G12C}$) cells and $KRAS^{G12C}$ mutated patient-derived organoids (PDOs) to evaluate the effectiveness of Sotorasib, a $KRAS^{G12C}$ inhibitor. Cell viability was measured using CellTiter-Glo®, and the IC_{50} was calculated using GraphPad-Prism9. Next, we performed a Sotorasib-anchored combinatorial drug screen in MiaPaCa2 cells with a compound library of 129 drugs. We analyzed dose-response curves using the R package GRmetrics and validated this data by clonogenic growth assay. Bliss synergy scores were calculated using the online software Synergy Finder. Subsequently, we treated MiaPaCa2 cells with different concentrations of Sotorasib, stained them with propidium iodide (PI) and analyzed cell cycle progression using FACS. Additionally, we analyzed protein expression by Western blot. We performed live cell analysis on 2D cell lines and PDOs using Incucyte SX5®.

Results

Our findings show that Sotorasib effectively targets KRAS in 2D cell lines and PDOs as a single compound. Sotorasib induced a cytostatic response with a cell cycle arrest in the G1 phase. In the unbiased drug screen experiment, Nintedanib (a broad-spectrum kinase inhibitor), TNO-155 (SHP2), BI3406 (SOS1), Binimetinib (MEK), Ponatinib (tyrosine kinase inhibitor), or AZD7762 (Chk1) were found to synergize with the $KRAS^{G12C}$ inhibitor. We validated the screening hits using long-term clonogenic assays and detected Bliss synergy scores in the range of 10 to 30 after 7 days of treatment.

Discussion

Our study highlights the potential of Sotorasib in $KRAS^{G12C}$ mutated PDAC. Furthermore, our data revealed Sotorasib-based combination therapies with clinical potential. However, further research is needed to determine the mechanistic underpinnings of the combination therapies.

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A-183

Exploiting genetic vulnerabilities of BAP1 loss by HDAC inhibitors

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Summary and Introduction

BAP1 is a deadly tumor suppressor gene that is frequently mutated in several cancer entities, such as renal cell carcinoma, uveal melanoma and cholangiocarcinoma. We previously discovered that BAP1 loss is associated with aggressive tumors, metastasis and poor patient survival in renal cell carcinoma and developed an immunohistochemistry test for stratifying patients. Unfortunately, there are no specific treatments for tumors with mutations in *BAP1*.

Materials and Methods

The aim of this project is the identification of synthetic lethal targets of *BAP1*-mutated tumor types using a large-scale RNA interference screen and validate the results *in vitro* in cell lines from renal cell carcinoma, uveal melanoma and cholangiocarcinoma, *in vivo* in mice and *ex vivo* in patient-derived organoids.

Results

Through bioinformatics analyses, we found a histone deacetylase (*HDAC)* as one of the most prominent hits in the synthetic lethality screen. After knockdown of *HDAC*, a decrease of proliferation and colony formation in *BAP1*-deficient cell lines from renal cell carcinoma, uveal melanoma and cholangiocarcinoma, but not in *BAP1*-competent cell lines, was found. Besides, by treating cells with the *HDAC* inhibitor quisinostat, we detected a stronger decrease of cell viability of *BAP1*-deficient cell lines compared to wild-type *BAP1* cell lines. Additionally, the knockdown of the histone deacetylase had a higher efficiency suppressing the migration of BAP1-null cells compared to *BAP1*-competent cells. Furthermore, *in vivo* studies in mice revealed an extraordinary depletion of tumor growth after knockdown of the histone deacetylase in the context of BAP1 loss, strongly suggesting that this HDAC is a true synthetic lethal interactor with BAP1 loss. To further validate the use of HDAC inhibitors in *BAP1*-mutant tumors, we generated patient-derived organoids from primary

tumors of renal cell carcinoma and uveal melanoma as pre-clinical models. We observed that the patient-derived organoids with BAP1 loss are substantially more sensitive to the *HDAC* inhibitor quisinostat.

Discussion

Pre-clinical data suggest that HDAC inhibitors might be effective as personalized medicine in clinical trials of renal cell carcinoma, uveal melanoma and/or cholangiocarcinoma after patient stratification for BAP1 loss.

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A-187

Prostate-specific membrane antigen as a vascular target for endogenous radiotherapy of triple-negative breast cancer

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Summary and Introduction

Triple-negative breast cancer (TNBC) accounts to 10-20% of all breast cancers and has a worse prognosis than other subtypes. Current therapy options are limited and the need for targeted therapies is urgent. Recently, PSMA rose attention as a potential target, as it is expressed on the tumor-associated vasculature of TNBC. Thus we aimed to analyze the PSMA expression in different TNBC including breast cancer stem cell lines, and their associated endothelial cells. In vivo, the therapeutic efficiency of one high dose and four fractionated doses of [^{177}Lu]Lu-PSMA in TNBC xenografted mice were compared.

Materials and Methods

In vitro expression analyses at gene and protein levels were done with PCR and western blotting. Endothelial cells co-cultured with different TNBC cell lines in a trans-well system and as 3D tumor spheroid model were analyzed for PSMA expression via immune stainings. Cell uptake and apoptotic effect of [^{177}Lu]Lu-PSMA in vitro were analyzed with gamma counter measurements and flow cytometry for TNBC cells alone and their respective endothelial co-cultures. TNBC xenografts were induced by orthotopically implanting MDA-MB-231 cells in immunocompromised mice. For therapy, the animals were intravenously injected with [^{177}Lu]Lu-PSMA (1x for single dose or 4x for fractionated dose) or NaCl (control). Tumor growth was monitored weekly via 2-[^{18}F]FDG microPET/CT. Ex vivo analyses included immune-, H&E, and TUNEL staining.

Results

PSMA expression was detected in 91% of the investigated cell lines, with breast cancer stem cells showing the highest level. PSMA expression was induced in all TNBC-co-cultured endothelial cells. The expression and cellular uptake of [^{177}Lu]Lu-PSMA increased significantly under hypoxic conditions. The apoptotic effect of [^{177}Lu]Lu-PSMA was the highest in BT-20 and MDA-MB-231 associated endothelial cells. The therapy study revealed significant smaller tumor volumes in both therapy groups compared to the control group. Tumor growth inhibition rates were 38% (1x [^{177}Lu]Lu-PSMA) and 30% (4x [^{177}Lu]Lu-PSMA). Immunolabeling with α-PSMA and α-CD31 antibodies revealed their co-localization on tumor-associated vasculature. H&E staining of organ sections showed no morphological abnormalities. TUNEL staining revealed a higher amount of apoptotic cells in the tumor compared to the control.

Discussion

We successfully demonstrated the presence of PSMA on TNBC associated endothelial cells and its potential of PSMA as a vascular target for an endogenous radiotherapy with [^{177}Lu]Lu-PSMA. The endothelial PSMA expression in TNBC provides the therapeutic option to reduce blood supply in very fast growing tumor burdens. Treatment of TNBC xenografted mice with [^{177}Lu]Lu-PSMA successfully and significantly (single dose: $p<0.001$; fractionated dose: $p=0.02$) inhibited tumor growth.

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A-203

Combinatorial CRISPR-CasRX screens to unravel novel effective drug combinations in breast, colon and pancreatic cancer

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Summary and Introduction
Despite progress in the development of targeted cancer therapies, drug resistance is a widespread problem. Combination therapy is becoming increasingly important as it offers the possibility to overcome resistance, reduce toxicity and expand the range of treatments. The development of CRISPR/Cas enables systematic exploration of gene dependencies and vulnerabilities. The pairwise perturbation of drug target genes in combinatorial CRISPR-screens mimics inhibitory effects of drugs to find synergistic combinations. However, the relatively small scale of these screens highlights the technical obstacles. Building on critical new technology developed by us, which increased screening scalability, we evaluated 100 000 drug target combinations and mapped genetic interactions (GI) across three cancer entities (breast, pancreas, colon).

Materials and Methods
To ensure rapid clinical translatability, we selected the gene targets of 539 drugs in late clinical stages with an intrinsic anti-tumor activity to construct a combinatorial gRNA library. We used the CRISPR/Cas13 systems, that enables RNA knockdown. We generated human cancer cell lines with stable Cas13 expression, using a transposon-based approach for delivery. After performing three combinatorial CRISPR screens per cancer entity and sequencing the CRISPR-libraries, the results were analyzed with the Orthrus pipeline to detect statistically significant GI. The resulting gene interaction networks were transformed into drug synergy landscapes based on drug-target information.

Results
We could show that synergy between genes is rare and highly context dependent. Overall, only 0.5% of tested drug target combinations showed statistically significant synergies. Whereas most of the trends are tissue-specific, some trends are universal across all three tissues, such as combinations containing tubulin inhibitors. We detected recurrent negative GI between TUBB3 and genes involved in protein homeostasis, apoptosis, DNA replication and damage, epigenetic modification and metabolism. The drug interaction networks show synergy between Tubulin inhibitors (Paclitaxel) and DNA-polymerase inhibitors (Gemcitabine), which is already approved as first-line therapy in pancreatic cancer. We also detected significant synergy between BET inhibitors and Tubulin inhibitors, where promising preclinical data in other solid tumors is available.

Discussion
Tubulin inhibitors are widely used in cancer treatment. However, the main problems with Tubulin inhibitors are high systematic toxicity and resistance. The validation of our novel combinations offers the possibility to combat resistance development. The expected outcome of this project will be comprehensive identification of GI in human cancer, creating the largest dataset concerning combinations and screened cell lines.

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Immunotherapy

A-128

Heparin reverses the immunosuppressive function of platelets towards cancer cells – An in vitro approach

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Summary and Introduction
Thromboses are considered a frequently underestimated risk and reason for mortality in metastatic tumor diseases. Increased coagulability, manifested in four- to seven-fold higher risk of thromboembolism in tumor patients is at least partially based on activation of platelets by cancer cells. Platelets were increasingly considered as immune-competent cells, thus protecting metastatic cancer cells during their hematogenous passage from the immune surveillance. Low molecular weight heparin (LMWH) is the guideline-based anticoagulant in thromboprophylaxis and treatment of cancer patients. Although there is an ongoing debate whether heparin can act anti-tumorigenic beyond anticoagulation, an impact of heparin on platelet-induced immune deregulation has not been addressed yet. *Research question* was to investigate how tumor-activated platelets induce immune-suppressive activities and whether heparin derivatives can reconstitute the immune balance under *in vitro* conditions.

Materials and Methods
Immunosuppressive effects of platelets or platelet releasates on human $CD4^+$, $CD8^+$ and NK cells, as well as interference by heparin was investigated by flow cytometry. Besides commercial heparins, synthetic polymers were tested as potential heparin mimetics.

Results
Immunosuppressive effect of platelets or tumor cell-induced platelet releasates became evident e.g. by a strongly increased differentiation of naïve $CD4^+$ cells into regulatory T cells. Notably, preincubation of platelets with heparin significantly attenuated this effect. Unfractionated heparin (UFH) was slightly more active than LMWH, but selected mimetics partly outperformed UFH in this efficiency, giving rise for structure/activity relationships. Furthermore, incubation with heparins showed reactivation of NK cell cytotoxicity in a leukemia cell approach, which was decreased in the presence of platelet releasates. To follow these effects at a molecular level, TGF-β as a major component of platelet releasates was depicted as important immunosuppressive modulator. To investigate, whether TGF-β is targeted by heparin, co-incubation of platelet releasates or TGF-β with heparins resulted in a decrease of T_{reg} differentiation. Interaction of heparin with TGF-β is still analyzed kinetically by biosensor approaches.

Discussion
LMWH and heparin mimetics can reverse the immunosuppressive activities of platelets and thus provide a novel immuno-oncological perspective for their guideline-based application in oncology.

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A-145

NLGN4X TCR T cells targeting experimental gliomas

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Summary and Introduction

A human leukocyte antigen (HLA)-A*02-restricted tumor associated antigen in the neuroligin 4 X-linked (NLGN4X) protein was found to be specifically overexpressed in human gliomas. Individualized multipeptide vaccination targeted NLGN4X and induced specific cytotoxic T cells responses in patients with newly diagnosed glioblastoma.

Materials and Methods

Post vaccination NLGN4X-tetramer-sorted T cells were subjected to single cell T cell receptor (TCR) sequencing for TCR discovery. The identified TCR was delivered to human T cells (NLGN4X-TCR-T) and functional profiling was performed by flow cytometry and in vitro cytotoxicity assays. NOD scid gamma (NSG) major histocompatibility complex (MHC) I/II knockout (KO) (NSG MHC I/II KO) mice were challenged with NLGN4X-expressing experimental gliomas and treated with intracerebroventricular injection of NLGN4X-TCR-T to assess its therapeutic potential.

Results

We apply for the first time for therapeutic use of an HLA-A*02 restricted vaccine-induced TCR that binds to the NLGN4X antigen. We show the cytotoxic and polyfunctional phenotype of NLGN4X-TCR-T in various cellular models. Intracerebroventricular delivery of NLGN4X-TCR-T prolongs survival and leads to objective radiographic responses in experimental gliomas-bearing NSG MHC I/II KO mice.

Discussion

NLGN4X-TCR-T demonstrates efficacy in a preclinical experimental glioblastoma model. On a global scale, we provide first evidence for the therapeutic retrieval of vaccine-induced human TCRs for the off-the-shelf treatment of glioblastoma patients.

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A-148

Loss of BCL11B induces the development of highly cytotoxic innate T cells out of IL-15 stimulated peripheral blood αβ CD8+ T cells

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Summary and Introduction

BCL11B, an essential transcription factor for thymopoiesis, regulates also vital processes in post-thymic lymphocytes. Increased expression of BCL11B was recently correlated with the maturation of NK cells, whereas reduced BCL11B levels were observed in native and induced T cell subsets displaying NK cell features. We show that BCL11B-depleted CD8+ T cells stimulated with IL-15 acquired remarkable innate characteristics. The induced innate CD8+ (iiT8) cells expressed multiple innate receptors, factors regulating migration and tissue homing, and showed high cytotoxic potential against leukemic and neuroblastoma tumor cells.

Materials and Methods

Human peripheral blood alpha-beta CD8+ T cells were isolated from buffy coats of healthy donors using magnetic separation. Double-strand breaks in the exon 4 of the BCL11B locus were induced *via* electroporation introduced highly specific ribonucleoprotein leading to efficient loss of BCL11B on the protein level followed by stimulation with CD3/CD28 and supplementation with IL-2 and IL-15. The phenotype of BCL11B depleted cells was determined by flow cytometry, transcriptome, and proteome analysis. Spontaneous cell-mediated cytotoxicity against leukemic K562 target cells and antibody-dependent cell-mediated cytotoxicity against CHLA-136 neuroblastoma spheroids was measured to prove functionality.

Results

Loss of BCL11B in human CD8+ T cells in combination with IL-15 stimulation induced a unique innate-like phenotype without losing T cell properties. The induced innate CD8+ T (iiT8) cells were characterized by high surface expression of the natural killer cell receptor NKp30, CD56, CD161, and the Fc receptor CD16. Key transcription factors related to an

innate phenotype were upregulated including ID2, PLZF, and HOPX. Proteome analysis revealed an enrichment of pathways related to signaling pathways relevant for migration through vascular endothelium. Additionally, iiT8 displayed high cytotoxic activity. Leukemic K562 target cells were spontaneously lysed via the NKp30/B7H6 axis and CHLA-136 neuroblastoma spheroids were lysed in the presence of an anti-GD2 antibody.

Discussion

Recent reports about the effects of BCL11B depletion in human T cells in the context of different cytokine regimes underline its crucial role as a regulator of lymphocyte innateness and go in line with our observations. However, BCL11B knock-out combined with IL-15 stimulation gives rise to iiT8 cells characterized by unique features. First, surface expression CD161 together with upregulation of migration-related pathways. Second, the ability to lyse tumor target cells in an antibody-dependent manner. The combination of the most desired T and NK cell features make the iiT8 cells the ideal
candidate for adoptive cell transfer, CAR therapy, or therapeutic antibody approaches.

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A-151

Early dynamics in circulating cell-free DNA methylation predict response to immunotherapy in metastatic melanoma

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Summary and Introduction

Despite its remarkable success, the majority of patients with metastatic melanoma do initially not respond or acquire resistance to anti-PD-1 immune checkpoint blockade (ICB). Hence, efforts are increasingly focusing on alternative treatment regimens, whose number has increased over the last years. Treatment decisions are mainly based on cross-sectional imaging, which is only valuable if applied over a relatively long period of usually 3-6 months. Predictive biomarkers might allow for a personalized treatment and eventually improve patient outcome. In this study, we analyzed circulating cell-free *SHOX2* DNA methylation (*SHOX2* ccfDNAm) in blood plasma of patients with metastatic melanoma to predict and monitor response to anti-PD-1-based ICB.

Materials and Methods

We prospectively assessed the value of *SHOX2* ccfDNAm levels in blood plasma of patients with metastatic melanoma that were treated with anti-PD-1-based ICB at the University Hospital Bonn with regard to treatment response, survival, and melanoma recurrence. In patients treated with palliative ICB (*N* = 42) samples were collected prior to and four weeks after treatment initiation, while in patients treated with adjuvant ICB (*N* = 55) samples were collected during treatment and after adjuvant treatment completion. Moreover, we assessed *SHOX2* ccfDNAm levels in *N* = 126 patients without malignant disease. *SHOX2* methylation levels and their dynamic changes were analyzed using a well-established quantitative methylation-specific PCR.

Results

SHOX2 ccfDNA was hypermethylated in blood plasma of patients with metastatic melanoma compared to patients without malignant disease. We found a significant correlation between low *SHOX2* ccfDNA methylation four weeks after palliative treatment initiation and better progression-free and overall survival. Patients who achieved *SHOX2* ccfDNAm clearance after four weeks showed favorable therapy response. In patients undergoing adjuvant anti-PD-1 treatment, *SHOX2* ccfDNAm testing allowed for recurrence detection in case of distant metastases formation.

Discussion

Our study showed that rapid dynamics in *SHOX2* ccfDNAm may be used as an early predictive biomarker in melanoma patients with anti-PD-1 immunotherapy. Thus, *SHOX2* ccfDNAm testing might harbor the potential to guide treatment planning in metastatic melanoma and supports radiological de-escalation strategies. Further studies are needed to assess the clinical applicability of *SHOX2* ccfDNAm and whether it allows for an improved clinical decision-making.

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A-169

Lysozyme expression is associated with favorable prognosis and predicts response to immune checkpoint inhibitors in melanoma

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Summary and Introduction
Immune checkpoint blockade (ICB) has led to a therapeutic breakthrough in melanoma. However, primary and acquired resistance mechanisms limit ICB efficacy. The anti-microbial enzyme lysozyme plays an important role in innate immunity and leads to immunomodulatory effects by releasing pro-inflammatory cytokines. Thus, lysozyme represents an interesting target that potentially contributes to anti-tumor immune response. We aim to study the significance of lysozyme in the context of melanoma immunogenicity and ICB response.

Materials and Methods
We comprehensively analyzed lysozyme expression with regard to overall survival (OS), progression-free survival (PFS), and ICB response. We analyzed lysozyme expression in tissues from a non-ICB melanoma cohort provided by The Cancer Genome Research Atlas Network, tissues and blood plasma from anti-PD-1 ICB cohorts, including patients treated at the University Hospital Bonn, melanoma cell lines, and single cells from melanoma tissue. Expression data were generated by whole tissue RNA-sequencing, single-cell RNA-sequencing, quantitative reverse transcription PCR (qRT-PCR), immunohistochemistry (IHC), and enzyme-linked immunosorbent assay (ELISA).

Results
We observed significant differences in lysozyme expression between tumor-infiltrating immune and melanoma cells on a single cell level. While macrophages were the main source of lysozyme expression, we also found a tumor cell-intrinsic expression. Moreover, lysozyme expression was significantly associated with favorable OS, PFS, and ICB response.

Discussion
Our findings suggest a significant role of lysozyme in the context of melanoma immunogenicity and ICB response with a hitherto undescribed tumor cell-intrinsic function.

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A-176

Generation of a Nanobody-based CAR-T Cell Platform

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Summary and Introduction
In recent years, significant advances have been made in cancer therapy. Immunotherapy, such as immune checkpoint blockade (ICB) has gained particular attention for its success in improving the body's response to tumor cells. However, not all patients respond, and some may experience recurrence following initial treatment response.
Chimeric antigen receptor (CAR) T cell therapy has emerged as an alternative to ICB and has shown excellent results for the treatment of hematological malignancies. However, its efficacy in solid tumors is limited by tumor heterogeneity, inefficient trafficking to the tumor site, and exhaustion of CAR-T cells. CAR-T cells based on nanobodies could overcome some of the limitations and may represent an emerging and novel treatment option.

Materials and Methods
CRISPIR-Cas genetic modification
Viral transduction
Flow Cytometry
Cloning
Bioluminescence Killing Assays

Results
Using the ALFA system and CRISPR-based approaches, we introduced the synthetic ALFA-tag, a highly versatile epitope tag, into the genomic sequence of murine ovarian cancer cell receptors. In addition, we have successfully

generated murine CAR-T cells containing an nanobody-ALFA (nbALFA) as the antigen recognition domain, and demonstrated their specific activation and killing capacity when co-cultured with ALFA-tagged cancer cells *in vitro.*

Discussion

In conclusion, we have developed an experimental and generic nanoCAR-T cell platform that can be applied to a variety of cancer models. Using these approaches, we aim to test antigen-dependent therapeutic efficacy as well as study resistance mechanisms in a variety of preclinical cancer mouse models in the future.

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A-178

Tumor-reactive cytotoxic CD4 T cells: Exploiting the constitutive HLA class II-positive melanoma cell phenotype

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Summary and Introduction

CD8 T cells are important cytotoxic anti-tumor effectors, but frequently lack effective activity due to functional exhaustion. Recent studies identified cytotoxic CD4 T cells among tumor-infiltrating lymphocytes. CD4 T cells recognize antigens presented on HLA class II (HLA-II) molecules. Interestingly, melanoma cells can show constitutive HLA-II (constHLA-II) expression. Based on this knowledge we asked for the capacity of CD4 T cells to kill autologous tumor cells. Moreover, we aimed to gain insights into inter- and intrapatient heterogeneity of HLA-II expression in melanoma.

Materials and Methods

Patient-derived, tumor-reactive CD4 T cells were amplified in mixed lymphocyte-tumor cultures. The T cells' phenotype and their capacity to kill autologous melanoma cells were studied applying flow cytometry and real-time cytotoxicity assays, respectively. Inter- and intra-patient HLA-II expression patterns on melanoma cells were investigated both *in vitro* (flow cytometry) and *in situ* (immunohistochemistry).

Results

Tumor antigen-specific CD4 T cells could be obtained from the peripheral blood of melanoma patients. Those T cells efficiently killed constHLA-II melanoma cells whereas co-culture with HLA-II-negative melanoma cells did not trigger cytotoxic effector functions. Accordingly, CD4 T cells upregulated production of pro-apoptotic and cytotoxic molecules in the presence of constHLA-II melanoma cells. This upregulation was blocked in the presence of anti-HLA-II antibodies. Comparative analyses of metastatic melanoma tissues and corresponding cell lines showed strong inter-patient as well as intra-patient heterogeneity in HLA-II expression, suggesting that some melanomas escape cytotoxic CD4 T cells activity by HLA-II downregulation. HLA-II-negative melanoma cells could be sensitized to cytotoxic CD4 T cells by IFNγ, inducing *de novo* expression of HLA-II molecules, though JAK1/2 mutant melanomas remained HLA-II-negative.

Discussion

We demonstrated HLA-II dependent killing of metastatic constHLA-II melanoma cells by autologous CD4 T lymphocytes, suggesting CD4 T cell-based therapies should be taking into consideration for treatment of advanced disease. Moreover, strategies should be developed that induce HLA-II expression on HLA-II-negative melanoma cells, including JAK1/2 mutants, to overcome evasion from CD4 T cell cytotoxicity.

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A-184

MDM2 inhibition enhances immune checkpoint inhibitor efficacy

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Summary and Introduction

The treatment of malignant tumors with MDM2 inhibitors reactivates p53, a major tumor suppressor, by interfering with the MDM2-p53 interaction consequently leading to p53 stabilization and p53-mediated tumor cell death. Recent reports indicate that, in addition to cell cycle arrest, DNA-repair and apoptosis induction, p53 has an immunomodulatory function. We investigated the immunostimulatory potential of MDM2 inhibitors in combination with immune checkpoint inhibitor (ICI) therapy in malignant melanoma.

Materials and Methods

The expression of pro-inflammatory and immunogenicity markers of melanoma cell lines was assessed by RT-qPCR and flow cytometry. A melanoma mouse model was established to perform gene expression analyses of melanoma tissue using a Microarry, survival studies and phenotypic characterization of tumor/metastasis-infiltrating T cells by CyTOF after treatment with MDM2 inhibitors and ICIs. We created a stable murine *Tp53* knockdown (*Tp53* KD) melanoma cell line using a retroviral *Tp53* shRNA-containing vector for *in vitro* an *in vivo* experiments. Chimeric mice with a complete hematopoietic *Il15rα* knockout (*Il15rα* KO) were generated by irradiation and transplantation of *Il15rα* KO bone marrow. Correlative studies of human melanoma gene expression data derived from The Cancer Genome Atlas were conducted to confirm our experimental findings.

Results

We could show a p53-dependent upregulation of IL-15 and MHC-II in human and murine melanoma cells after *in vitro* treatment with MDM2 inhibitors. A pro-inflammatory gene expression signature was observed in murine melanoma tissue after treatment with MDM2 inhibitor and ICI compared to ICI alone. Further, tumor-infiltrating T-cells of murine melanoma and lung metastasis had a more activated and less exhausted phenotype. This was reflected in an improved survival of melanoma-bearing mice receiving the combined treatment with MDM2 inhibitor and ICI. The effect could be reversed when *Tp53* KD melanoma cells or mice with a hematopoietic *Il15rα* KO were used. Finally, high expression of CIITA, a transcriptional activator of HLA-DR, and IL-15 was linked to IFN pathway gene expression in human melanoma and a prolonged survival of melanoma patients.

Discussion

Together this study indicates that MDM2 inhibitors enhance the anti-tumor effect of ICI by creating a pro-inflammatory milieu in the melanoma tissue leading to an improved anti-melanoma immune response. Clinical trials are warranted to investigate safety and efficacy of MDM2 inhibitors in combination with ICI in melanoma patients.

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A-188

Armoring CAR-T cells with C-C-motive receptor 8 (CCR8) and a dominant negative TGF-β receptor (DNR) to enable efficacy in solid tumor models and remodel the TME

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Summary and Introduction

Chimeric antigen receptor (CAR) T cells have shown great efficacy in treating hematological malignancies. In solid tumors CAR T cells have yet to demonstrate significant clinical efficacy. In solid tumors, CAR T cells are frequently prevented access to tumor tissue and face profound suppression at the tumor site. Our group could previously demonstrate that arming CAR T cells with C-C-motive-receptor 8 for improved tumor-directed migration along the C-C-chemokine ligand 1 - CCR8 axis and a dominant-negative receptor against TGF-β for resistance to suppression enabled activity in pancreatic cancer models. The impact of this approach on the tumor microenvironment and the transferability to other entities was however unclear. We now investigated the influence of DNR carrying CAR T cells on the tumor microenvironment and the potential of this combination for treatment of HER2-positive cancer models in conjunction with

a HER2-targeted CAR.

Materials and Methods
Primary murine and human T cells were isolated and activated. Primary human monocytes were isolated, differentiated and polarized into different subsets of macrophages. T cells were retrovirally transduced. Phenotype, activation, exhaustion and proliferation of T cells and macrophages were monitored in vitro. Cytokine production was assessed with ELISA. In vivo, survival and tumor growth of mice that were subcutaneously injected with tumor cells and treated with CAR T cells carrying either CCR8, DNR or both receptors were measured. To look at chemokine expression in tumor material, mRNA was isolated from tumor material and RT-qPCR was performed.

Results
We found that expression of CCR8 can redirect CAR T cells to the tumor and a DNR can prevent immunosuppression of CAR T cells in the tumor microenvironment. Furthermore, binding TGF-β onto the cell surface via a DNR alters the polarization of the macrophages in the tumor microenvironment towards a pro-inflammatory phenotype. The improved functionality of CAR- CCR8-DNR T cells compared to CAR T cells against the HER2 antigen could be demonstrated in vitro and in vivo in two human HER2+ tumor models.

Discussion
Equipping CAR T cells with CCR8 and DNR emerges as a strategy not only limited to certain antigens, but as a potential universal approach to render cellular therapies more effective. The modularity of this concept promises further preclinical and perhaps clinical development to improve personalized immunotherapy.

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A-193

REG3B modulates tumor growth and antitumor immunity

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Summary and Introduction
The use of checkpoint inhibitors which release immunosuppression in the tumor microenvironment is promising for the treatment of breast cancer. However, the efficacy of this treatment is in part dependent on the degree of infiltration and activation of T cells in the tumors, demonstrating the necessity to develop new therapies that enhance antitumor immunity. Previous data suggested a role of REG3B in antitumor immunity. Therefore, mammary tumors in mice overexpressing REG3B in the intestine were studied in regard to tumor growth and immune cell infiltration to investigate its potential for immunotherapy.

Materials and Methods
TS1 cells derived from MMTV-PyMT FvB mice were injected in the mammary gland of REG3B-overexpressing FvB mice, wildtype FvB mice or immunocompromised NSG mice. In some experiments, mice were treated with recombinant REG3B, anti-PD1 or isotype control. Tumors were analyzed using flow cytometry, immunohistochemistry, Western Blot or qPCR.

Results
REG3B-overexpressing mice have a changed microbiome compared to wildtype mice and displayed significantly decreased tumor growth. Additionally, tumors revealed enhanced infiltration by cytotoxic T cells and T helper cells. We also showed that REG3B itself did not enhance T cell activation, however the secretome of intestinal organoids derived from REG3B-overexpressing mice did increase the surface expression of known T cell activation markers.
We further tested treatment of mammary tumors with recombinant REG3B, which led to enlarged necrotic areas in the tumors upon therapy but no overall tumor volume reduction. Immune cells were mildly activated in the REG3B-treated tumors, and the appearance of enhanced necrosis in the treatment group was dependent on the presence of functional immune cells. Combination treatment with checkpoint inhibitors could not significantly enhance the therapeutic effect.

Discussion
In summary, this study has demonstrated that intestinal overexpression of REG3B in mice leads to significant protection from mammary tumor growth, which was most likely achieved by enhanced antitumor immunity. We hypothesize that this is mediated through an indirect mechanism either by a changed microbiome or metabolites secreted by intestinal

epithelial cells. Our findings might lead to new cancer treatments using REG3B in the future after improving the therapy protocol.

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A-198

WAPAL and RAN cooperatively regulate T cell proliferation upon allogeneic stimulus

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Summary and Introduction

Graft-versus-Host-Disease (GvHD) is a common complication after allogeneic hematopoietic stem cell transplantation. Current GvHD drugs have widespread toxicities due to general inhibition of allogeneic T cells, not being able to differentiate between the unwanted GvH and the desired graft-versus-leukemia effect. T cell phenotype modulation has not been widely studied in this context.
Wings apart-like homolog (WAPAL) and RAs-related Nuclear protein (RAN) emerged as potential regulators of GvH effects in T cells. Therefore, we designed and screened fully chemically modified siRNAs targeting WAPAL and RAN, followed by testing in mixed lymphocyte reactions.
Another therapeutic moiety, extracellular vesicles (EVs) derived from mesenchymal stem cells (MSC) have been shown to 1) facilitate siRNA delivery to difficult-to-transfect cell types and 2) to improve GvHD.
We screened 12 siRNAs per target and identified leads showing the best potency at silencing mRNA (IC50 in the range of 24 - 1000 nM) and a maximal silencing of in the range of 65%-97%) of HeLa, Jurkat and primary T cells. Cellular fractionation in primary T cells, Jurkat and in HeLa showed a predominant (70-100%) cytoplasmic mRNA localization of both targets.

Materials and Methods

Cells were treated with 1:5 dilution series of siRNA and incubated for 6 days. Silencing activity was quantified using branched DNA assay (QuantiGene). Subcellular fractionation was performed with activated T cells using REAP method. T cells were stimulated with γ-irradiated major-mismatched dendritic cells (allogeneic stimulus) or with CD2/CD3/CD28 activation beads (non-specific stimulus), treated with siRNA and incubated for 4 more days. Proliferation was analyzed using CellTrace Violet proliferation kit via flow cytometry.

Results

WAPAL silencing inhibited T cell proliferation up to 70% upon allogeneic stimulus (co-incubation with irradiated allogeneic dendritic cells). Upon formulation into EVs, the inhibitory effect could be enhanced to nearly 100%. When siRNAs against WAPAL and RAN were combined, T cell proliferation could nearly be abolished. In addition, the effect of WAPAL silencing, as well as WAPAL siRNA in EVs and WAPAL/RAN siRNA combination was specific to the allogeneic situation and did not affect T cell proliferation upon stimulation with CD2/CD3/CD28 beads.

Discussion

Data suggest that WAPAL and RAN work synergistically to regulate T cell activation/proliferation upon allogeneic stimulus. The chemical structure of the siRNAs used in this study corresponds to that of approved siRNA drugs, therefore potentially enables rapid clinical translation.

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A-202

Chemical optimization of miR-146a mimic for the treatment of Graft versus Host disease

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Summary and Introduction

Mice transplanted with miR-146a-/- T cells show more aggressive GvHD, and pretreatment of T cells a commercial miR-146a mimic (nucleofection), lower GvHD mortality and severity can be achieved. Here, we generated cholesterol-

conjugatedmiR-146a mimics spanning chemical scaffolds from miRNA-like to siRNA-like in order to identify a clinically relevant scaffold for Graft versus Host disease therapy.

Materials and Methods

We tested the impact of (1) the length of the sense strand, and (2) the complementarity of the sense strand on miRNA efficacy in reporter and in functional assays. We first cloned a fully complementary target site 4x in tandem into the 3'UTR of a dual luciferase reporter system to test an siRNA-like effect. Then, we used an in vitro model of GvHD, mixed lymphocyte reactions, to compare T cell inhibition functionality of miRNA mimics. In this model we mixed irradiated dendritic cells from different donors with T cells from a different donor.

Results

For the fully complementary target reporter setting, an siRNA-like structure led to less efficient silencing. In the functional assay, the mixed lymphocyte reaction, we saw that both a shorter or a fully complementary sense strand significantly enhanced the T cell inhibitory effect. A further enhancement could be achieved by increasing the number phosphorotioate modifications in the antisense strand. We achieved the most potent T cell inhibition (up to 80 %, IC 50 216 nM) using a chemical structure intermediate structure between miRNA and siRNA patterns.

Discussion

Collectively, we demonstrate that (1) some elements of siRNA chemical design may augment miRNA mimic efficacy in a functional setting, and (2) antisense strand metabolism is rate-limiting for miRNA functionality.

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Imaging

A-110

Retrospective analysis of long-term toxicity of moderately hypofractioned vs. conventional radiotherapy in localized prostate cancer

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Summary and Introduction
Due to a low α/β ratio, prostate cancers (PC) are known have a high sensitivity to hypofractioned radiotherapyirradiation (HRT)[1]. Especially with regards to efficacy and toxicity, HRT has been shown to yield similar outcomes to conventional radiotherapy (CRT) in localized PC. However, German long time data, comparing HRT and CRT, is rare.

Materials and Methods
A total of n=55 localized PC patients were treated with HRT between July 2016 and December 2018. After matched-pair analysis, these were retrospectively compared to n=55 CRT patients. HRT patients received a total dose of 60 Gy in over 20 fractions, whilst CRT patients were treated with a total dose of 72-78Gy inover 36-39 fractions. Intermediate- and high risk patients were treated with concomitant androgen deprivation therapy for 6 to 24 months. After radiotherapy, patients received a regular follow-up and serum PSA-levels were controlled quarterly.

Results
An interim analysis of short and mid- term toxicities, published in 2020, showed a significantly lower rate of proccititis and cystitis in the HRT group[2]. Data on long-term toxicity in HRT and CRT patients are currently being recorded and will be retrospectively analyzed.

Discussion
Large randomized studies have shown that HRT is a safe and less expensive alternative to CRT. Thus, an implementation of HRT on a wide scale is in the interest of both patients and oncologists. **Follow-up data regarding long-term toxicity is currently being recorded and will be reported at the conference.**

References:

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Cancer Models

A-116

Tumor-Intrinsic PD-L1 Exerts an Oncogenic Function through the Activation of the Wnt/β-Catenin Pathway in Human Non-Small Cell Lung Cancer

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Summary and Introduction
Programmed death ligand 1 (PD-L1) strongly inhibits T cell activation, thereby aiding tumors in escaping the immune response. PD-L1 inhibitors have proven to be effective in the treatment of different types of cancer, including non-small cell lung cancer (NSCLC). Yet, the knowledge regarding the biological function of tumor-intrinsic PD-L1 in lung cancer remains obscure.

Materials and Methods
In our study, we set the goal of determining the function of PD-L1 using overexpression (stable transfection) and knockdown (siRNA) strategies. Exosomes were isolated from the PD-L1-overexpressing cells and the metastatic related miRNA was analyzed using real-time RT-PCR. Additionally, the protein expression of PD-1 and PD-L1 was evaulated by immunohistochemistry on Tissue Microarrays (TMAs).

Results
PD-L1 silencing resulted in decreased migratory and invasive ability of tumor cells, together with attenuated colony-forming capacity. Ectopic expression of PD-L1 showed the opposite effects, along with increased activities of MAPK and Wnt/β-catenin pathways, and the upregulation of Wnt/β-catenin target genes. Additionally, overexpression of PD-L1 was associated with dysregulated cellular and exosomal miRNAs involved in tumor progression and metastasis. In primary lung tumors, immunohistochemistry revealed that both PD1 and PD-L1 were highly expressed in squamous cell carcinoma (SCC) compared to adenocarcinoma ($p = 0.045$ and $p = 0.036$, respectively). In SCC, PD1 expression was significantly associated with tumor grading ($p = 0.016$).

Discussion
Taken together, our data suggest that PD-L1 may exert an oncogenic function in NSCLC through activating Wnt/β-catenin signaling, and may act as a potential diagnostic marker for lung SCC.

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A-122

Single cell multi-omics analysis of chromothriptic medulloblastoma highlights genomic and transcriptomic consequences of genome instability

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Summary and Introduction

Chromothripsis is a form of genome instability, by which a presumably single catastrophic event leads to substantial genomic rearrangements of one or a few chromosome(s). Generally considered as an early event, chromothripsis likely plays a causative role in the development of a number of tumors. Until now, little was known about the heterogeneity of chromothripsis across different clones from the same tumor and about clonal evolution in response to treatment. We investigated clonality as well as functional consequences of chromothripsis in tumors by single cell genomic and transcriptomic analysis in human medulloblastomas with germline *TP53* mutation (Li-Fraumeni syndrome, LFS). These medulloblastomas are a clinically challenging type of childhood brain tumor associated with a dismal prognosis. As chromothripsis prevalence in these tumors reaches 100%, understanding genomic heterogeneity due to chromothripsis and its consequences on the transcriptome are essential to identify potential targets for novel therapeutic strategies for this subgroup of patients.

Materials and Methods

We combined bulk deep and single-cell DNA and RNA sequencing assays in three LFS medulloblastoma tumors and four patient-derived xenograft models, allowing us to score chromothripsis events at clonal resolution. Furthermore, we leveraged fluorescence in situ hybridization (FISH), immunofluorescence analyses and CRISPR/Cas9 mediated inactivation of candidate genes to investigate potential causes and functional consequences of chromothripsis in LFS medulloblastoma.

Results

We demonstrated the ability to detect chromothripsis events at the single-cell level in tumors, unraveling the extent of intra-tumor heterogeneity with clonal resolution. We reconstructed the order of somatic events, identified early alterations likely linked to chromothripsis and depicted the contribution of chromothripsis to malignancy. We characterized subclonal variation of chromothripsis and its effects on double-minute chromosomes, cancer drivers and putative druggable targets. Furthermore, we highlighted the causative role and the fitness consequences of specific rearrangements in neural progenitors.

Discussion

Tumors in LFS patients constitute a paradigm for the understanding of chromothripsis. Our work focusing on medulloblastoma in LFS patients can provide a roadmap from where the findings may be extended to different contexts, as the link between chromothripsis and *TP53* mutations also holds true outside the context of constitutive defects. Unravelling the extent of genomic heterogeneity will be necessary to detect actionable targets, determine the evolutionary history and defeat the evolutionary capacity of tumor cells with high genome instability.

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A-131

Do metabolic deregulation and chemoresistance share a common path in cancer cells? – Answers by in vitro-approaches using ovarian and breast cancer cells

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Summary and Introduction

One of the fundamental hallmarks of cancer is the deregulation of cancer cell metabolism. An increased energy consumption is required for tumor cell proliferation and cell cycle activity, which is matched by an intensified metabolic turnover and higher demands of nutrients. Pathological hyperglycemia, caused e.g. by diabetes mellitus is a triggering factor in these terms. Hence, diabetes and cancer are correlated in prevalence, and diabetic diseases are a bad predictor for the outcome of malignant diseases. It is not well investigated whether metabolic deregulation also occurs to a greater extent in the development of chemoresistance, a key obstacle in clinical treatment of patients.
We therefore investigate how a simulated hyperglycemic situation affects the proliferation of ovarian and breast cancer cells and their sensitivity for a cytotoxic treatment. The research is focused on the identification of molecular pathways and metabolic mechanisms involved in chemoresistance as new targets for sensitization strategies.

Materials and Methods

A2780 human ovarian cancer cells (hOCC) and a doxorubicin resistant subline were cultivated with different glucose concentrations and sensitivity to doxorubicin and a glucose metabolism inhibitor was evaluated by MTT and flow cytometry. Cellular glucose metabolism was mainly focused on hexokinase 2 analyzed by qPCR and Western blot to be

related with chemoresistance. Additionally, MDA-MB-231 breast cancer cells were investigated regarding the potential synergistic mechanism between glucose metabolism and cytostatic activities.

Results

While short term deregulation of media glucose levels do not directly impact the hOCC sensitivity to doxorubicin, a resistant subtype of A2780 cells displays a strong dependency on high glucose supply, indicated by massive upregulation of hexokinase 2 at mRNA and protein level. Notably, inhibition of glycolysis by a rather non-toxic concentration of 3-BrPA, which acts, among other targets, by inhibiting hexokinase 2, attenuates the doxorubicin resistance, resulting in a reduced required concentration of doxorubicin to achieve the same cytotoxic effect. Furthermore, a synergistic effect of doxorubicin and glucose metabolism inhibitors metformin or 3-BrPA became evident in MDA-MB-231 breast cancer cells.

Discussion

Our data provide evidence for a chemoresistance-related hypermetabolism-axis, highlighting hexokinase 2 as a promising target to affect resistance formation. Interfering with glucose metabolism appears a promising way to increase the therapeutic index of cytostatic drugs.

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A-135

Influence of tumor-associated fibroblasts and their exosomes on the development and progression of prostate cancer

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Summary and Introduction

The unique tumor microenvironment (TME) of the prostate and in particular fibroblasts are known to play an essential role in the growth and progression of prostate cancer (PCa). Earlier studies from our group have shown that co-injection of patient-derived prostate cancer-associated fibroblasts (CAFs) and non-cancer associated fibroblasts (NCAFs) with tumor cells stimulated tumor growth in xenograft models compared to the injection of tumor cells alone. Our present study aims to investigate the effect of patient-derived prostate CAFs and NCAFs on the functional aspects of tumor cells *in-vitro* and their role in epigenetic regulation.

Materials and Methods

Three patient-derived prostate normal fibroblasts (NCAFs) and CAF pairs were examined for their influence on the viability, proliferation and migration of prostate cancer cell lines LNCaP and LNCaP C4-2. Cells were co-cultured in transwells to test viability (WST-1), proliferation (BrdU) and migration. Extracellular vesicles (EV) were enriched from fibroblasts by ultracentrifugation. The expression of miRNAs isolated from cells and EVs was studied by microarray analysis and RT-qPCR.

Results

Patient-derived fibroblasts significantly increased the viability and proliferation of the tumor cells compared to the tumor cells alone. The influence of NCAFs on both viability and proliferation was more pronounced compared to CAFs on the LNCaP cells. However, the influence of the fibroblasts was vice versa on the LNCaP-C4-2 cells. The LNCaP cells exhibited no migration whereas the LNCaP C4-2 cells exhibited slight migration. The effect of different fibroblast pairs on the migration of LNCaP C4-2 cells varied among the pairs. 103 miRNAs and 6 miRNAs were found to be differentially expressed between CAFs and NCAFs and their EVs, respectively. miR-10b-5p and miR-210-3p were confirmed by RT-qPCR to be upregulated in the CAFs and their exosomes.

Discussion

Our *in-vitro* data confirmed the *in-vivo* results. Fibroblasts modulate the viability, proliferation and migration of PCa. However, the effects are heterogeneous due to patient and cell line-specific characteristics. Further studies will focus on the possible effects of fibroblasts on apoptosis in correlation to viability and invasion. CAFs and their EVs are characterized by specific miRNA expression supporting an epigenetic regulation of fibroblast characteristics and functions. Therefore, we will further investigate the mechanism of tumor progression induced by fibroblasts and their EVs via miRNA regulation.

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A-149

Modulation of urothelial carcinoma lipid metabolism to inhibit metastatic outgrowth

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Summary and Introduction
Urothelial carcinoma (UC) in one of the ten most common cancers, showing a 5-year survival rate of 6% in the metastatic stages. Current treatment options for metastatic UC are limited and the probability to develop drug resistant UC is high. Thus, there is an urgent need for the development of new therapeutic strategies for UC. A prominent characteristic for UC and other cancers is an altered lipid metabolism. On one hand altered lipid metabolism is associated with rapid growth of cancer cells and tumor formation. On the other hand, altered lipid metabolism can decrease metastatic spread and outgrowth; for example, due to the cell death mechanism ferroptosis. Thus, the aim of the study is to assess whether modulation of UC lipid metabolism can be utilized to inhibit metastatic outgrowth.

Materials and Methods
Metastatic outgrowth is difficult to visualize in mice, concurrently general mouse models for UC are lacking. In contrast in transparent and fli:GFP labeled zebrafish larvae the process of metastatic outgrowth can be visualized live at high resolution. Therefore, we established a zebrafish engraftment model utilizing human UC cell lines. Intravenous injection of fluorescently labeled UC cells are executed at two days post fertilization in zebrafish larvae. This enables us not only to quantify the metastatic growth and the localization of metastases *in vivo*, but also to test drugs in a preclinical setting. The drug concentrations used were determined as the maximum dose that was tolerated by the zebrafish larvae. The larvae were imaged one and four days after injections.

Results
Four days after injection metastatic UC colonies were found in the liver and in the human bone marrow analogous hematopoietic niche in the tail of zebrafish larvae. In addition, the cells form tumors in an anatomical region analogous to the urothelium in humans which was never described in a zebrafish xenograft model before. This model enabled us to identify two SCD (stearoyl-CoA desaturase) inhibitors that inhibited metastatic outgrowth about 38% (CI=95%, p=0,0011) and 68% (CI=95%, p=0,0002) while normal development of zebrafish larvae was not affected.

Discussion
Collectively we developed a novel simple translational UC model which allowed us to identify two inhibitors as new potential UC therapeutics. These findings agree with our data suggesting that high expression of SCD correlates with decreased UC specific survival in patients. We currently validate both SCD inhibitors in a mouse model of orthotopic bladder cancer.

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A-154

Establishment of a new patient derived retinoblastoma long-term 2D and 3D in vitro culture

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Summary and Introduction
Retinoblastoma (RB) is the most common primary intraocular childhood tumor. Primary 2D RB tumor and tumor derived stromal cell cultures are important tools to investigate the interaction of tumor microenvironment and tumor cells in RB. Therefore, it was desirable to immortalize and characterize primary retinoblastoma tumor and stroma cells to overcome their limited division rates and short life span. To bring the cells closer to their *in vivo* situation a 3D culture was established to investigate the interactions of the tumor- and stroma cells in the future.

Materials and Methods
Immortalization was performed by lentiviral transduction of the largeT-antigen or human telomerase reverse transcriptase. Afterwards the immortalized cells were characterized and compared with the native cells to determine changes in cell viability, proliferation and apoptosis levels, expression of different cell markers and tumor formation capacity as well as tumor weight and size in an *in ovo* model. A 3D culture was performed on non-adherent culture plates

and in form of hanging drops as well as in Matrigel, which imitates the extracellular matrix *in vitro*.

Results

We successfully established four immortalized RB derived cell lines, both tumor and stromal cells. In comparison to the native cells, these immortalized cells are more proliferative and display a longer life span, but also higher apoptosis rates. Comparing the expression of different cell markers in native and immortalized cells with their expression in the original tumor, we identified patient derived stromal cells with a complete expression consistency for future experiments. The immortalized cells are also able to grow on the chicken chorioallantoic membrane *in ovo.* A 3D spheroid culture was successfully established in non-adherent culture plates and as hanging drops. Grown spheroids transferred into Matrigel display a strong growth and migration potential.

Discussion

In summary, the immortalized cells can be used for further investigations on mechanisms underlying the development of retinoblastoma. In addition, the impact of stromal RB cells on therapy outcome and new therapeutic interventions can be investigated with the established 3D co-cultivation system in the future.

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A-155

EV-mediated immunomodulation of CD8+ T cells in renal cell carcinoma

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Summary and Introduction

Treatment strategies of renal cell carcinoma (RCC) involve checkpoint inhibition, a common target of which is the PD-(L)1 pathway. PD-L1 plays a crucial role in regulating tumor-directed immune responses and is often overexpressed on tumor cells. Extracellular vesicles (EVs) are an essential part of intercellular communication. Tumor-secreted (PD-L1+) EVs have been proven to have immunomodulatory effects in several tumor entities. However, the role of EVs in RCC is still unclear. The aim of this study is to evaluate possible immunomodulatory effects of RCC-derived EVs on CD8+ T cells.

Materials and Methods

EVs of 5 untreated and Interferon-γ (IFNγ) RCC cell lines were isolated by differential ultracentrifugation. Quality and quantity of the EVs were assessed by Western Blotting (WB), Nano Particle Tracking analysis, and electron microscopy. CD8+ T cells were isolated from the blood of healthy donors and co-cultivated with RCC-EVs over the course of 3 days. Proliferation and PD-1 expression of T cells was measured using flow cytometry, cyto-and chemokine secretion was analyzed by ELISA.

Results

All RCC cell lines exhibited low baseline PD-L1 expression. 4 out of 5 cell lines upregulated PD-L1 expression in cells and EVs in response to IFNγ treatment, albeit at varying levels. RCC-EVs, particularly from IFNγ-treated cells, had an antiproliferative effect on CD8+ T cells in co-cultures and induced a cell line-specific alteration of PD-1 expression. The secretion of IL-2, IL-10, CXCL10 and CCL5 by CD8+ T cells was increased by RCC-EVs, notably more so by EVs from IFNγ-treated cells.

Discussion

Cells and EVs of RCC cell lines express low levels of PD-L1, which can be upregulated by IFNγ treatment. RCC-derived EVs influence early T cell activation and proliferation patterns of CD8+ T cells. Furthermore, early results indicate that RCC-EVs induce the production of predominately pro-inflammatory cyto- and chemokines in T cells. The results support the hypothesis of a tumor-EV-mediated immunomodulation in RCC.

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A-156

Differential Immune Activation Profiles in ex vivo NSCLC

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Summary and Introduction
Due to the highly heterogenic nature of cancers, the efficiency of tumor immuno-therapies strongly depends on the microenvironment of the tumor and metastatic niches. Presently, we aim to elucidate differences in patient specific activation profiles of paired tumor and peripheral (non-tumor) lung tissue after treatment with various immune stimulants including the checkpoint inhibitor Nivolumab

Materials and Methods
Precision-cut lung (PCLS) and tumor slices (PCTLS) were prepared from NSCLC patients. We leverage histological profiling, flow cytometry, bulk RNA sequencing, and cytokine multiplex analysis to understand how PC(T)LS respond differently to anti-CD3/CD28 T cell activation, LPS stimulation, Nivolumab treatment and Nivolumab in combination with anti-CD3/CD28 after 30h. Furthermore, we are optimizing microphysiological chip systems for PC(T)LS increase overall culture duration.

Results
PC(T)LS are characterized histologically in varying proximities to the tumor tissue. These regions differ based on live, $CD45^+$ and $Ecadherin^+/Epcam^+$ cell frequencies. Because PCTLS viability is notably lower than PCLS, we are establishing microfluidic culture systems to improve overall viability and extend culture duration. While PCTLS contain a higher $CD45^+$ cell count than PCLS, further examination of T cell subsets revealed that PCTLS $CD8^+$ T cells express significantly higher levels of the exhaustion marker CD39. After treatment with anti-CD3/CD28 beads, PCLS secrete higher levels of IFNg (200-fold), IL-17 (70-fold) and IL-2 (35- fold) than PCTLS. In contrast, after LPS treatment, IL-1b expression is 10-fold higher in PCTLS than PCLS. Strikingly, Nivolumab treated PCTLS but not PCLS, secrete significantly higher levels of IFNg (12-fold) and lower levels of TGFb2 (1.5-fold) as compared to the medium control. In support of these differential responses, RNA transcriptome analysis reveal that different treatments induce microenvironment dependent immune pathway enrichments when comparing PC(T)LS.

Discussion
These results highlight differences in tissue resident immune cell activation when comparing tumor and non-tumor tissue. On one hand, antigen-independent T cell stimuli is more effective in inducing T cell related cytokine responses in PCLS. On the other hand, LPS stimulation induced a stronger macrophage related cytokine response in PCTLS. Finally, PCTLS cytokine secretion demonstrates the immune-modulatory effects of Nivolumab in tumor tissue. These findings allow for further investigation into T cell responses to immunotherapies in the in the native lung microenvironment.

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A-157

Breast cancer associated fibroblasts remodel the 3D tumor microenvironment via oxytocin - mediated contraction

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Summary and Introduction

Breast myoepithelial cells (MYO) remodel basement membrane (BM) proteins and regulate luminal epithelial cell polarity. As natural suppressors of tumor cell invasion via protease inhibition, MYO become defective and decrease in number during tumorigenesis. Upon Oxytocin binding to the Oxytocin receptor (OXTR), MYO contract via Calcium signaling resulting in lactation. Breast mesenchymal cells (MES), essential for breast gland development and wound healing are migratory and regulate matrix proteins. Cancer associated fibroblasts (CAFs) mainly stem from (myo)fibroblasts, rarely from tumor cells undergoing an epithelial mesenchymal transition and impact the 3D tumor environment (TE). Although, Oxytocin can inhibit breast cancer proliferation, there is a need to understand the impact on the 3D TE. This study explored the contractile nature of breast CAFs, MYO and MES during Oxytocin signaling and 3D TE remodeling.

Materials and Methods

1) MYO, MES (primary human control breast) and CAFs (Luminal B breast tumor) fractionation (mincing, enzymes).
2) RNAseq
3) 2D, 3D, spheroids (collagen 1.2 mg/ml), migration, invasion, forces (pa) and automated microscopy.
4) Oxytocin (100nM)
5) Live Calcium imaging (Fura-8 AM), F-actin stress fibers (phalloidin) staining and Confocal microscopy.

Results

Using RNAseq identifies significant gene expression signatures for Luminal B CAFs (n=10), normal MYO and MES (total n=6) proving CAFs stem from MES. Biomechanical collagen functional studies reveal similarities in 2D and 3D migration, invasion and spheroid-induced matrix forces, where CAFs show significantly faster migration and directional persistence ($p<0.0001$). In contrast to MES, CAFs show a gain of OXTR expression similar to MYO, thus, we tested if Oxytocin could mediate 2D and 3D contraction. Initial live cell imaging following Oxytocin treatment shows a halt of migration with cell flattening only with MYO and CAFs, supporting a contraction phenotype. Quantitative analyses *following Oxytocin cell treatment* demonstrate statistical significant key findings:1) CAFs increase coherency of F-actin aligned stress fibers; 2) only CAFs and MYO sustain cytoplasmic Calcium ion oscillation using live confocal imaging and steadily increase 2D contractile forces; 3) single CAFs in 3D collagen contract more than CAF spheroids, supporting single cell but not collective oxytocin mediated contraction, and 4) Inhibitor treatment of CAFs in 3D collagen identify Calcium pathway members like, CREB, Calmodulin kinase and a possible novel role of RhoB-kinase for contraction.

Discussion

We support Oxytocin induced contractile forces generated by CAFs stiffen and remodel the breast 3D TE regulating tumor migration and disease progression.

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A-158

Carbohydrate-restricted diets have varying effects on the liver cancer progression in mice

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Summary and Introduction

Hepatocellular carcinoma (HCC), the most common type of liver cancer, is known for strong therapy resistance and poor prognosis. Dietary approaches in cancer research have received attention increasingly in the recent years, due to their impact on tumor metabolism and potential therapeutic implications in cancer treatment. Of the various approaches, carbohydrate restriction (CR) showed tumor inhibitory effects in rodent models, possibly via limited glucose supply to tumor cells.

Materials and Methods

In our study, we used a transgenic mouse model of hepatocellular carcinoma, named ASV-B. These mice, previously described having a metabolic shift toward the Warburg effect, were subjected to two different CR regimens: a “low carb high fat” (LCHF, ketogenic diet) and a “low carb high protein” (LCHP) chow. The impact of distinct dietary CR strategies on the composition of the immune microenvironment within tumors, the bile acid profile in the blood serum, and the gut

microbiome were examined.

Results
Both diet applications showed substantial inhibition of tumor growth, however their effects on tumor microenvironment were variable. While LCHF diet resulted in significant changes of adaptive immune cells, specifically interleukin-17-producing lymphocytes, no such change was observed with LCHP diet. Additionally, we sought to investigate the systemic effects and observed that carbohydrate restricted-diet heavily influenced the microbiome composition in the gut and the bile acid composition in the serum of ASV-B mice.

Discussion
Taken together, the effect of dietary intervention in ASV-B mice is of particular interest, since these mice demonstrated resistance to different therapeutic options (sorafenib, etoposide and doxorubicin), overall suggesting that macronutrient composition, especially carbohydrate availability, is crucial for murine HCC progression. Given the safety of dietary modifications in clinical use, they may offer a promising strategy, especially in the adjuvant setting.

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A-173

The Chorioallantoic Membrane Assay as a Xenograft Model to Evaluate Head and Neck Squamous Cell Carcinoma Treatment with Antibody-Drug Conjugates

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Summary and Introduction
Besides immune checkpoint inhibitors and tyrosine kinase inhibitors, targeted therapies with antibody-drug conjugates (ADC) have improved long-term survival in various oncologic entities, such as urothelial carcinoma and triple-negative breast cancer. However, ADCs have not yet been approved for the treatment of head and neck squamous cell carcinoma (HNSCC). Meanwhile, proteomic analyses suggest that many HNSCC tumors express markers that are targets of approved ADCs.
Most HNSCC patients show little to no significant response to targeted therapies. To investigate underlying resistance mechanisms and adaption processes at the tissue level, the Chorioallantoic Membrane Assay (CAM assay) is employed.

Materials and Methods
HNSCC, SCLC, and NSCLC cell lines were screened by XTT assay and Crystal Violet assay to investigate the response of individual cell lines to treatment with Enfortumab vedotin as well as Sacituzumab govitecan (SG).
FaDu cells (HNSCC) were xenografted into the CAM assay. Three experimental groups and two control groups were formed. Two of the experimental groups were treated intravenously while the third one was treated topically with SG. One of the two control groups remained untreated, while the second was subjected to an intravenous sham treatment. Tumor xenografts were analyzed by immunohistochemistry.

Results
In vitro, the HNSCC cell lines respond differently to treatment with ADCs. Topical treatment as well as intravenous application with ADCs are feasible in the CAM assay. Immunohistochemical staining reveals differences between topical and intravenous treatment. Intravenous treatment with ADCs does not cause excess mortality in the CAM assay compared to sham intravenous treatment.

Discussion
The CAM assay-xenograft model appears to be a suitable screening model for testing ADCs in vivo. In this context, toxicological data regarding systemic tolerability can be obtained in addition to pharmacodynamic conclusions. ADCs appear to be a promising option for the treatment of HNSCC tumors. However, this must first be proven in clinical trials.

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A-180

Evaluation and targeting of the metabolic signature of human pancreatic ductal adenocarcinoma subtypes

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Summary and Introduction
Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive human tumor with a 5-year survival rate of approximately 11%, which is the lowest among all types of cancer. One of the main challenges in treating PDAC is its high level of tumor heterogeneity, which often leads to the failure of the therapy. Recent research has identified different metabolic subtypes of PDAC based on their metabolic requirements and gene expression profiles. These metabolic subtypes are associated with distinct metabolite pathways, including glycolysis, lipogenesis, and redox pathways, which have prognostic value. It is revealed that the glycolytic subtype of PDAC exhibits a more aggressive phenotype, as evidenced by a significantly shorter overall survival rate compared to the lipogenic PDAC subtype. Interestingly, these metabolic subtypes are also associated with previously identified molecular subtypes of PDAC, including classical/pancreatic progenitor (Lipogenic) and basal-like/squamous (Glycolytic) tumors. These findings suggest a correlation between metabolic signatures, molecular characteristics, and the outcomes of pancreatic cancer. Therefore, the identification of distinct metabolic profile of PDAC and investigation the role of glycolysis in pancreatic cancer has potential therapeutically implications such as development of target therapy.

Materials and Methods
In this study, we used protein-based and RNA-based assays to identified distinct metabolic profiles among different PDAC models. We also studied the role of glycolysis in PDAC therapy and explored the use of a newly developed glycolytic inhibitor (KAN0438757) in combination with a senescence inducer agent Inflachromeme (ICM).

Results
The results of the study showed that PDAC organoids and and cell lines exhibit high heterogeneity regarding their metabolic status. Interestingly, combination of KAN0438757and ICM leads to a significant reduction in tumor growth.

Discussion
Our study highlights the importance of understanding the metabolic signatures of different subtypes of PDAC and provides evidence for the potential efficacy of a novel combination therapy targeting glycolysis, senescence induction, and standard chemotherapy. These findings suggest that targeting the metabolic demands of different subtypes of PDAC could lead to more effective and personalized treatments

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A-181

Establishment of a Humanization protocol using human peripheral blood mononuclear cells in immunosuppressed NRG mice

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Summary and Introduction
Animal models remain a crucial method to study tumor biology and potential novel therapeutic approaches in cancer research. Continuous improvement of these models, such as patient-derived xenografts (PDX), allow for more realistic tumor model settings and better translation of potential therapeutic approaches into the clinic.
Colorectal cancer research also benefits from these new *in vivo* models. Despite the progress, current models have limitations that do not completely reflect the complexity of the disease and the diversity of cells involved in tumorigenesis. One of the main limitations of PDX models is the need for immunosuppressed animals. As a result, interactions between immune cells and tumor cells cannot be studied, although immune cells possess a crucial role in tumor progress. Humanization of NRG mice using human peripheral blood mononuclear cells (hPBMCs) from the same-matching patient, who donated tumor tissue can reduce this limitation. Moreover, a humanization protocol, which operates via simple intraperitoneal injection (*i.p.*) rather than a laborious intravenous injection (*i.v.*) would be favored.

Materials and Methods
Human PBMCs from a donor were injected into healthy NRG mice by *i.v.* or *i.p.* injection in different cell numbers ($1x10^6$ - $2x10^6$). Over a period of 28 days, the mice were observed and their weight measured twice a week. FACS and IHC analysis for human $CD45^+$ cells were used to evaluate the blood and the harvested organs for engraftment success and immune cell infiltration.

Results
Over the entire period, the weight of the animals remained constant and no signs of graft-versus-host disease were detected, suggesting that NRG humanization with PBMCs for the period of 28 days was safe in our current animal facility. After 7 days humanization with PBMCs from an immunosuppressed donor (long term use of prednisolone), FACS analysis showed similar results for viable circulating human $CD45^+$ cells in all groups ($1x10^6$ *i.p*. 18,62%; $1x10^6$ *i.v.* 20,17%; $2x10^6$ *i.p*. 17,3%; $2x10^6$ *i.v*. 20,2%).
Additionally, FACS analysis after 28 days humanization, showed that animals humanized *i.p*. had higher percentage of viable circulating human $CD45^+$ cells than animals humanized *i.v* ($1x10^6$ *i.p*. 35,5%; $2x10^6$ *i.p*. 46,1%; $1x10^6$ *i.v.* 15,7%; $2x10^6$*i.v.* 27,17%). Corroborating these findings, IHC analysis for human $CD45^+$ cells exhibited higher immune cell infiltration rates in organs of *i.p.* humanized mice.

Discussion
In sum, humanization of the NRG strain with *i.p.* hPBMCs is an effective and comparatively simple method to transplant human immune cells into mice to create a more realistic model for the evaluation of tumor microenvironment interactions.

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A-196

From addiction to adaptation – The NFATc1 dependent metabolic switch in pancreatic cancer during hypoxia and nutrition deficiency

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Summary and Introduction
Glutamine is an important source of energy in pancreatic cancer cells (PDAC) and involved in virtually most biosynthetic pathways. During the progression of PDAC, impaired tumor perfusion often leads to reduced glutamine and oxygen supply and therefore those cells must adjust their metabolism for survival.
Over time, PDAC cells learn to adapt to the lack of glutamine and oxygen and manage to survive, although the underlying mechanisms governing the transition from addiction to adaptation are poorly understood. Our aim is to define the role of the adaptive transcription factor "nuclear factor of activated T-Cells" (NFATc1) in hypoxia and glutamine deficiency dependent metabolic rewiring and survival mechanisms of PDAC.

Materials and Methods
Glutamine deprivation induced cellular stress responses, metabolic reprogramming and gene regulations are determined in a time-dependent manner both in murine (KPC; KNPC and NKC cells) and human pancreatic cancer lines as well as in patient-derived organoids. Consequences of cell adaptation are measured on the level of gene regulation, metabolic enzyme activities and substrate alterations, oxidative phosphorylation and cell proliferation by RNA-Seq, high performance liquid chromatography/mass spectrometry, seahorse, qPCR, western blot, immunofluorescence, MTT and cell growth studies. The glutamine-deficient subtype and the underlying metabolic and transcriptional mechanisms are confirmed in patients' tumor samples and in patient derived organoids and xenografts as well.

Results
Our ongoing study reveals significant metabolic, signaling and transcriptional changes during the process of PDAC cell adaptation to glutamine deficiency. In particular, integrated stress induced signaling and transcription pathways link glutamine-deficiency and hypoxia with metabolic rewiring in PDAC cells to determine a highly aggressive and proliferative phenotype. To be precise, within several weeks of Glutamine-Deficiency and within days of hypoxia, the activity and expression of key metabolic enzymes and transporters are adjusted and linked to the acticity of NFATc1 in concert with ATF4 and HIF1α. Further studies are currently assessing the therapeutic potential of specific interference

with cell adaptation mechanisms.

Discussion

This study contributes to a better understanding of the mechanisms in adaptation of pancreatic cancer cells to nutrient deficiency and provides a theoretical platform for novel, tailored treatment strategies to combat this cancer.

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A-197

Delineating epithelial-mesenchymal plasticity driven organoid heterogeneity to identify novel therapies in pancreatic cancer

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Summary and Introduction

Pancreatic ductal adenocarcinoma (PDAC) is characterized by strong desmoplastic reactions and pronounced inter- and intra-tumour heterogeneity, resulting in increased chemoresistance and high mortality rates. While organoid technologies have been widely used to functionalize tumour biology in the past decade, they have yet to capture the morphological diversity found in PDAC.

Materials and Methods

In this study, we aimed to generate a phenotypic organoid landscape of over 30,000 single cell-derived organoids from primary murine PDAC cells and patient-derived organoids using a branching organoid model system. To achieve this, we cultured tumour cells from distinct molecular murine PDAC subtypes, namely classical and basal-like, in three-dimensional (3D) floating collagen gels to generate clonal PDAC organoids.

Results

Branched PDAC organoids retained their transcriptional subtype and established distinct phenotypes based on the parental PDAC subtype. We identified the existence of multiple distinct organoid phenotypes derived from individual PDAC tumours, indicating distinct morphogenetic programs and intra-tumoral heterogeneity. To create a phenotypic PDAC organoid landscape, we developed a novel methodology called PHeMap (Phenotypic Heterogeneity Mapping), which employed deep convolutional neural networks to perform morphological classifications of the organoids. We further characterized distinct organoid phenotypes at the molecular level, identifying transcriptional programs, notably epithelial-to-mesenchymal plasticity, that govern the generation of intra-tumoral heterogeneity from single cells up to the organoid level. As the unlocking of phenotypic plasticity emerges as a novel cancer hallmark, we identified that mesenchymal organoids rely on plasticity mechanisms to escape treatment, whereas epithelial organoids demonstrate higher levels of heterogeneous phenotypes to evade treatment and require combinational therapy to overcome treatment resistance.

Discussion
With our organoid assay and heterogeneity mapping, we successfully captured the inherent tumour cell heterogeneity and employed this platform to target cellular and phenotypic tumour plasticity in PDAC.

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A-200

Secreted Luciferases For Ex Vivo Monitoring of Tumor Burden In Mouse Models of Advanced Ovarian Cancer

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Summary and Introduction
Ovarian cancer is one of the leading causes of cancer-related death in women. Due to the lack of screening methods for early detection and mistakable symptoms, ovarian cancer often remains undiagnosed until late metastatic disease stages. Although first-line treatments are effective, long-term therapy outcomes are still unsatisfactory and acquired resistance is a key challenge underscoring the need for new experimental models and alternative therapy strategies. In experimental mouse models of advanced ovarian cancer, quantification of intraperitoneal tumor burden is usually performed by luciferase-based bioluminescence (BLI) measurements using an intravital imaging system (IVIS) and tumor-endogenously expressed luciferases such as the firefly luciferase (ffluc). This approach, however, has several disadvantages as it not only requires repetitive anaesthesia which has increased animal welfare concerns, but also results in unreliable measurements in late-stage disease due to suboptimal substrate distribution and signal quenching resulting from ascites. Thus, this project aims at developing a novel non-invasive *in vivo* imaging platform for monitoring of advanced ovarian cancer growth using a secreted luciferase as reporter tool for quantitative assessment.

Materials and Methods
Applying the CRISPR/Cas9-based “prime-editing” technique, we generated a syngeneic murine ID8-ffluc ovarian cancer cell line harboring a human-relevant oncogenic point mutation in p53 (ID8.p53Y217C- ffluc). Furthermore, using CRISPitope, we generated cell lines expressing fusion proteins with a secreted luciferase (teLuc), an additional fluorescent protein (mNeon), as well as a defined T cell epitope (hgp100). This allowed for testing therapeutic efficacy of adoptive T cell transfer immunotherapy using epitope-specific pmel-1 TCRtg T cells. After intraperitoneal tumor cell injection into immunocompetent mice, we correlated teLuc bioluminescence activity in urine of tumor-bearing mice with tumor-endogenous ffluc activity determined by intravital imaging once a week.

Results
For *in vitro* validation, we tested for teLuc bioluminescence activity in supernatants of CRISPitope-engineered cell lines and verified that pmel-1-specific T cells recognised these ID8 cell variants. Next, we performed *in vivo* experiments and demonstrated significant correlation of teLuc activity in urine with endogenous ffluc activity and tumor burden of non-treated mice. Tumor regression upon ACT immunotherapy was also successfully assessed by the teLuc platform.

Discussion
In summary, we successfully established an innovative, non-invasive platform for intraperitoneal tumor monitoring that not only represents a major advance in animal welfare, but also facilitates the tracking of tumor growth and regression in an advanced ovarian cancer mouse model.

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A-201

The influence of Nras and Trp53 point mutations on melanoma plasticity and the tumor microenvironment

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Summary and Introduction
Oncogenic mutations promote cancer growth by activation of proto-oncogenes or inhibition of tumor suppressor genes.

Activating mutations in components of the MAPK signaling pathway are particularly frequent and play a central role in the development of melanoma. In addition, these molecular alterations may modulate cell differentiation states and thereby affect the tumor microenvironment, and influence responses to cancer immunotherapies. Therefore, we aim to study the impact of human-relevant oncogenic point mutations on melanoma cell plasticity, progression and therapy-resistance.

Materials and Methods

We used the CRISPR-Cas9-based “prime-editing” technology and introduced oncogenic point mutations in the *Trp53* and *Nras* gene in murine B16F1 melanoma cells. Successfully prime-edited melanoma cells were validated by Next generation sequencing and functionally characterized, e.g. by qRT-PCR or Western Blot analyses to determine cell differentiation states *in vitro*. In addition, we performed *in vivo* experiments to assess tumor growth kinetics and differences in the composition of the tumor immune microenvironment by flow cytometry as well as 3´mRNA sequencing.

Results

Here, we have established polyclonal B16F1 $Trp53^{Y217C}$ and B16F1 $Trp53^{Y217C}$ $Nras^{Q61K}$ melanoma cell cultures and generated a variety of monoclonal cell lines. Surprisingly, we identified pairs of differentiated ($MITF^{high}$) and dedifferentiated ($MITF^{low}$) monoclonal B16F1 $Trp53^{Y217C}$ $Nras^{Q61K}$ cell lines. Using these cells, we observed slightly reduced tumor growth and increased $CD45^{+}$ immune cell infiltration in dedifferentiated tumors when compared to differentiated melanomas upon subcutaneous implantation into immunocompetent mice. Analyses of tumor tissue and established *ex vivo* melanoma cell lines further demonstrated maintenance of differentiated as well as dedifferentiated melanoma cell states compared to *in vitro* data.

Discussion

In summary, we have generated novel and human-relevant experimental models that are highly suitable to study genetic as well as phenotypic heterogeneity in melanoma and its effects on immune regulation. In future studies, we will apply immunotherapeutic approaches to study therapeutic responses and resistance mechanisms. All in all these approaches may help to identify novel and personalised treatment strategies for melanoma in the future.

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A-206

Recapitulation of extracellular matrix in mouse PDAC organoids

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Summary and Introduction

Pancreatic adenocarcinoma (PDAC) is projected to significantly increase and become the second cause of cancer-related deaths before 2030.As a new three-dimenstional (3D) structure that mimics the complexity and function of organs *in vitro,* organoids have emerged as a promising tool for studying PDAC.Despite their advantages, organoids fail to recapitulate the desmoplastic feature of PDAC that plays a key factor in tumor progression and resistance to therapy. To address this limitation, we performed co-cultures of mouse PDAC organoids with pancreatic stellate cells (PSCs) which are the main contributors for the fibrotic PDAC phenotype.

Materials and Methods

PSCs from healthy C57BL/6 mice, and from C57BL/6 mice that developed PDAC tumors upon orthotopic KPC-cell implantation, were isolated and co-cultured with healthy pancreatic and KPC-PDAC for 6 days. Then, from day 6 until day 9, these co-cultures were incubated with peripheral blood mononuclear cells (PBMCs), which were obtained from mice stimulated with mesothelin-peptide nanovaccine. On day 1,3,6, and 9, the induction of an immunosuppressive pathway was evaluated by measuring the secretion of IL-6 in the supernatant using ELISA. After 9 days of co-culture, the co-cultures were fixed and the components of the extracellular matrix (ECM) were characterized by two-photon microscopy.Also, biophysical properties of organoids were analysed by measuring mass density, weight, and diameter.

Results

As opposed to healthy pancreatic organoids, co-cultures of PSCs with PDAC organoids led to a higher infiltration of PSCs as depicted by confocal imaging. However, this did not translate in a difference in collagen deposition or lipid droplets accumulation in comparison to healthy pancreatic organoid-PSCs co-cultures. Interestingly, although no alteration were found in the mass density of tumor organoids from co-cultures with PSCs, there was a significant

reduction of PDAC organoid weight and diameter.These findings will be further evaluated. With our co-culture model, we could observe an increase of IL-6 when tumor organoids were co-cultured with tumor-derived PSCs. Preliminary data from *in vivo* experiments using immunotherapeutic-treated PBMCs in addition to this co-culture reduced this immunosuppressive effect.

Discussion

In general, our *in vitro* PDAC organoid-PSCs co-culture model shows an alteration in tumor organoids biophysical features and an increase in immunosuppressive biochemical properties in response to PSCs addition, that allows to mimic PDAC microenvironment. Future efforts will focus on improving the visualization of ECM and on assessing the suitability of this 3D *in vitro* approach to test the efficacy and mechanism of different therapies including mesothelin-peptide nanovaccine.

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Other

A-121

Metabolomic analysis in CSF and serum of patients with malignant glioma (GlioLiq)

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Summary and Introduction
Glioblastoma (GBM) is the most common malignant tumor of the central nervous system in adults. The standard therapy includes the three columns of surgery, radiation, and alkylating chemotherapy. However, this standard therapy has remained practically unchanged for more than 15 years and effective innovative therapeutic options are still lacking. To address the unmet need, new targets are being sought that could provide a potential target structure for new therapeutic approaches. At present, cerebrospinal fluid (CSF) of GBM patients is rarely collected and respective analyses have not been given particular attention. The aim of this project is to characterize the CSF of patients with glioblastoma and other malignant gliomas in comparison to control patients. In addition to standard CSF parameters, metabolomic analyses are carried out.

Materials and Methods
Since August 2019, a collaborative project between the Department of Neurosurgery and the Department of Neurology has been recruiting patients who require surgery for malignant glioma. Patients with benign brain tumors (such as meningioma or vestibular schwannoma) or patients who need brain surgery for other reasons (e.g., hydrocephalus) serve as controls. In the course of the current work, prospectively collected serum and CSF samples from 30 patients with malignant glioma and 22 control patients were subjected to targeted metabolomic analysis by mass spectrometry (MxP Quant 500 kit, Biocrates Life Science AG; evaluation: MetIDQ and MetaboAnalyst 5.0).

Results
To date, 61 patients with malignant glioma and 37 control patients have been enrolled. Regarding standard CSF parameters (cell count, protein and lactate concentration) glioma patients did not differ significantly from the control cohort. Data sets from the targeted serum metabolome assay were analyzed by partial least squares discriminant analysis (PLS-DA). The cross-validated PLS-DA plots showed a clear separation between the glioma and control group. For six metabolites, significantly higher concentrations were found in the glioma group, whereas 113 metabolites were significantly decreased in this group. In this context, the lysophosphatidylcholine lysoPC a C18:1 was identified as a potential serum biomarker (AUC 0.761). Analysis of the CSF metabolome showed significantly elevated levels of short-chain acylcarnitine C2 (p=0.0025) and various glycerophospholipids, including PC ae C34:2 (p=0.014) in glioma patients.

Discussion
The identification of novel CSF biomarkers in malignant gliomas is of tremendous importance. This is the first study to address the metabolomic analysis of CSF in glioma-patients. Whether any of the identified metabolites can actually serve as a diagnostic biomarker needs to be investigated in further studies.

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A-143

Targeting protein tyrosine phosphatase type E (PTPRE) and miR631 –new approaches for treatment of chemoresistant retinoblastoma?

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Summary and Introduction
Retinoblastoma (RB) is the most frequent intraocular cancer in infancy and early childhood. Since chemotherapy resistance diminishes RB treatment options, the necessity for new therapeutic approaches is evident. Protein tyrosine phosphatase type E (PTPRE) is known to support oncogenesis in several cancer entities. In the study presented we

investigated PTPRE`s expression as well as its regulatory mechanisms and signaling pathways in etoposide resistant retinoblastoma.

Materials and Methods

Expression levels of PTPRE and microRNA (miR)631 were analyzed by Real-Time PCR, Western Blot and immunohistochemistry in RB cells and patients. To examine effects of a PTPRE knockdown (KD), cell viability, proliferation, apoptosis and *in ovo* CAM assays were performed. Upstream regulation of PTPRE was investigated by transient miR631 transfection, FGF receptor inhibition and PTPRE promotor methylation analyses via bisulfit conversion and sequencing. Additionally, Western Blot analyses were used to investigate the phosphorylation status of different protein kinases following PTPRE KD.

Results

Etoposide resistant RB cells and RB patients tumor tissue express increased levels of PTPRE. MiR631, by contrast, is opposingly expressed. Promotor methylation analyses revealed that PTPRE expression is not regulated by altered promotor methylation. PTPRE KD decreases cell viability and proliferation *in vitro* as well as tumor size i*n vivo* and increases caspase dependent apoptosis. Reduced PTPRE expression and induced apoptosis following miR631 overexpression indicate PTPRE regulation via this miR. FGF receptor signaling appears to be an additional mechanism of PTPRE regulation since inhibition of FGF receptor signaling leads to similar results as seen after PTPRE KD. Additionally, PTPRE KD leads to altered phosphorylation of various protein kinases, thereby potentially influencing different cell signaling pathways.

Discussion

PTPRE expression, most likely regulated by miR631, seems to enhance the tumorigenic potential of etoposide resistant RB cells. These results indicate a potential role of PTPRE and miR631 as novel targets for retinoblastoma treatment.

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A-167

The ribosomal protein L22 promotes MDM4 exon skipping

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Summary and Introduction

p53 is the most frequently mutated tumor suppressor in human cancer. It can induce cell death in response to genotoxic stress. To avoid excessive activity, its function is controlled by the negative regulators MDM2 and MDM4. MDM2 but not MDM4 functions as an E3 ubiquitin ligase acting on p53. However, like MDM2, MDM4 can inhibit the transcriptional activity of p53.

Both, MDM2 and MDM4, are overexpressed in some tumor species, resulting in oncogenic p53 inactivation as an alternative to p53 mutation. One mechanism of MDM4 overexpression is the enhanced inclusion of its exon 6. This leads to increased amounts of the transcript that encodes the functional full-length MDM4-FL protein, whereas exon 6 skipping results in an unstable transcript translated to synthesize a truncated MDM4-S protein.

Correlation analyses of mRNA levels in large tumor panels suggested that the ribosomal protein (RP)L22 might promote the skipping of MDM4 exon 6. L22 binds to a specific stem-loop structure within RNA that we identified in previous work. Strikingly, intron 6 of the MDM4 gene contains three bona fide L22-binding consensus sequences close to the splice donor site. Taken together, these previous findings raised the possibility that L22 might regulate MDM4 splicing by direct interaction with MDM4 intron 6.

We found that L22 indeed regulates the splicing of MDM4 and probably additional genes, likely by direct binding to its consensus sequences in its target RNAs, and that this is particularly relevant in the context of nucleolar stress.

Materials and Methods

- cell cultivation and treatment, siRNA-mediated knockdown
- CRISPR/Cas9-mediated knockout, genotyping, sequencing
- RNA isolation, RT-qPCR
- Western blot
- cell confluence measurement
- RNA sequencing

- RNA folding prediction

Results

We found that L22 regulates MDM4 splicing as expected, in particular in the context of nucleolar stress. Deletion of the three L22-binding consensus sequences in MDM4 intron 6 completely abrogated MDM4 splicing regulation by L22 and increased resistance to nucleolar stress. Currently, we are trying to define by what mechanisms L22 regulates MDM4 splicing. Approaches include the demonstration of L22 binding to MDM4 pre-mRNA as well as the search for L22 interaction partners.

Discussion

We show that the regulation of MDM4 splicing by L22 represents an additional way of how p53 can be activated upon nucleolar stress, besides the known inhibition of MDM2 by ribosomal proteins including L5, L11 but also L22 in this context. Moreover, we have hints that L22 acts as a more global splicing regulator, modulating the splicing of additional genes besides MDM4 via similar mechanisms not only in humans, but also in other organisms. Future investigations thereof will contribute to our understanding of nucleolar stress, cancer development and therapy.

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Identification of functional states with tumour initiating cell activity in human PDAC

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Summary and Introduction

Pancreatic ductal adenocarcinoma (PDAC) is mostly diagnosed late and is among the top leading causes of cancer-related deaths. Treatments have limited efficacy and patients present with relapse and metastasis. A better understanding of PDAC progression is urgently needed for development of effective therapies. Our group previously showed that PDAC progression is driven by a succession of transiently active tumour initiating cell (TIC) clones and not by a stable self-renewing stem cell-like population. A gene activation screen to identify TIC activity regulators and their functional testing suggests that GNB1 induces stable clonal TIC activation involving mTOR signalling. Here, we further decipher the transcriptional programs associated with transient TIC activity in PDAC.

Materials and Methods

We performed single cell RNA sequencing (scRNA-seq) of 16 primary patient-derived PDAC cultures (12 organoid, 4 semi-adherent). To help identify TIC-associated subpopulations in the scRNA-seq dataset, we used GNB1 knockdown (KD) and overexpression (OE) cultures in bulk RNA-seq, and mTOR phosphoarray to understand mTOR downstream pathway activation.

Results

Integrated scRNA-seq analysis from a total of 129,482 cells (1,335-16,129 cells per patient) identified 16 distinct clusters differing in metabolism, mTOR signalling, and proliferation, among others. Our dataset shows similar transcriptional states to published scRNA-seq of freshly dissociated PDACs and so is suitable for studying heterogeneity. We next generated a GNB1 signature based on bulk RNA-seq of GNB1 OE and KD cultures. Interestingly, the scRNA-seq cluster with the highest GNB1 signature score showed upregulation of 4EBP1/2 target genes. In line with this, mTOR phosphoarray with GNB1 OE and KD cultures showed activation of mTORC1/4EBP1/S6K. We identified another candidate cluster enriched for mTORC1 signalling together with EMT, response to hypoxia, and glycolysis. To enable prospective isolation of cells from the clusters of interest, we selected a combination of surface markers based on high cluster-specific expression. FACS-based sorting of a triple-marker-positive population is feasible and will allow its functional analysis for TIC activity and drug sensitivities.

Discussion

We have identified transcriptional states with activated mTOR that may be involved in TIC activity. Understanding the mechanisms and drivers of TIC activity in PDAC will help in development of TIC activity-targeted treatment strategies that may reduce disease progression.

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Prevention of anthracycline-induced cardiotoxicity by targeting of Rho-regulated signalling in different cardiac cell-types

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Summary and Introduction
Irreversible cardiotoxicity is the dose limiting adverse effect of doxorubicin (DOX), which is part of the first-line therapeutic regimen of breast cancer. HMG-CoA reductase inhibitors (statins) and small-molecule Rac1 inhibitors can reduce DOX-induced cardiotoxicity *in vitro* and *in vivo*. However, the relevance of different cardiac cell types and/or various Rho GTPases for the pathophysiology of anthracycline-mediated congestive heart failure is unclear. Here, we investigated the impact of various types of Rho GTPases in DOX-induced cyto- and genotoxicity by employing different primary cardiac cell types and pharmacological inhibitors of Rho-regulated signaling.

Materials and Methods
Irreversible cardiotoxicity is the dose limiting adverse effect of doxorubicin (DOX), which is part of the first-line therapeutic regimen of breast cancer. HMG-CoA reductase inhibitors (statins) and small-molecule Rac1 inhibitors can reduce DOX-induced cardiotoxicity *in vitro* and *in vivo*. However, the relevance of different cardiac cell types and/or various Rho GTPases for the pathophysiology of anthracycline-mediated congestive heart failure is unclear. Here, we investigated the impact of various types of Rho GTPases in DOX-induced cyto- and genotoxicity by employing different primary cardiac cell types and pharmacological inhibitors of Rho-regulated signaling.

Results
The level of DOX-induced DSB largely varied between the different cardiac cell types. The lowest amount of Dox-induced DSB was observed in cardiomyocytes and DSB remained unrepaired. By contrast, an efficient repair of DOX-induced DSB was observed in endothelial cells and fibroblasts. DSB formation by DOX was largely prevented by pharmacological inhibition of Rac1 and/or Cdc42, but not of RhoA, in each cell type. Moreover, the viability of all types primary cardiac cells was reduced by DOX in a dose dependent manner. Surprisingly yet, specifically the cardiomyocytes failed to respond to DOX treatment with an increase in apoptosis. Despite of a noticeable DSB reduction, Rac1 inhibition only moderately ameliorated DOX-induced cytotoxicity.

Discussion
We conclude that cardiac cell types others than cardiomyocytes are also of relevance for the pathophysiology of DOX-induced cardiotoxicity. In addition, Rac1- and Cdc42-regulated mechanisms of non-cardiomyocytes have the strongest impact on the DOX-induced DDR of the heart. Hence, pharmacological targeting of Rac1-/Cdc42-regulated pathways is anticipated as promising strategy to mitigate cardiotoxicity evoked by anthracycline-based anticancer therapy.

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DEUTSCHE KREBSHILFE – GERMAN CANCER AID

The non-profit organisation German Cancer Aid is Germany's leading non-governmental funding institution for oncology-related activities. We are based in Bonn and our main objectives are to improve care for cancer patients, to promote research and to promote social awareness of cancer by means of detailed public campaigns to enhance information and education.

German Cancer Aid was founded in 1974 and follows the theme “Help. Research. Information.”. We support research projects to improve the prevention, diagnosis, therapy, after-care, and self-help of cancer. All supported projects are of the highest scientific quality and have the potential for significant clinical impact, so that cancer patients rapidly benefit from new knowledge. We also invest in the training of future generations of scientists and medical doctors.

German Cancer Aid has grown to be an established player in the national health service and is the most important private source of funds for cancer research in Germany, with an annual investment of up to 70 million Euro each year in various oncological projects. Because of the enormous amount of scientific work in recent decades, the care for cancer patients has fundamentally improved. Nevertheless, a lot of work still must be done.

Besides scientific support, we launch campaigns, organise information events and offer detailed brochures to keep the public informed about various types of cancer. Awareness, prevention, and early detection are very important to us. We offer cancer patients advice and help. We support self-help associations and provide advanced training for those who deal with cancer patients and their daily needs.

For nearly 50 years, German Cancer Aid has been an important consultant for health and research politics. It makes a significant contribution to the improvement in the care of cancer patients throughout Germany and helps to ensure that the votes of cancer patients are born in mind.

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Please find further information about our numerous activities on our website **www.krebshilfe.de** or contact us via e-mail: **deutsche@krebshilfe.de**.