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New insights into pancreatic cancer: notes from a virtual meeting.

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On behalf of the 1st Virtual Göttingen-Munich-Marburg Pancreatic Cancer Meeting

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Conflict of Interest Statement

ND has a pending patent entitled “Simple transcriptomic signatures to determine chemosensitivity for pancreatic ductal adenocarcinoma”. All other authors declare no potential conflicts of interest.

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains a major challenge in cancer medicine. Given the increase in incidence and mortality, interdisciplinary research is necessary to translate basic knowledge into therapeutic strategies improving the outcome of patients. On the 4th and 5th of February 2021, three German pancreatic cancer research centers, the clinical research unit (CRU) 5002 from Göttingen, the collaborative research center (CRC) 1321 from Munich, and CRU325 from Marburg organized the *1st Virtual Göttingen-Munich-Marburg Pancreatic Cancer Meeting* in order to foster scientific exchange. This report summarizes current research and proceedings presented during the meeting.

Introduction

With over 400.000 related annual deaths, a five-year survival rate of 10%, and a rising incidence, pancreatic ductal adenocarcinoma (PDAC) remains a significant health burden. These characteristics illustrate the need to intensify research and to share concepts, expertise and data. Therefore, three Deutsche Forschungsgemeinschaft (DFG)-funded PDAC research consortia CRU5002, CRC1321, and CRU325 organized the *1st Virtual Göttingen-Munich-Marburg Pancreatic Cancer Meeting*. The meeting was balanced with respect to gender and career stage, and therefore, was also a forum for young scientists. 16 talks attracted over 200 international participants and were separated into four sessions: g*enome dynamics*, *tumor microenvironment*, *cell-of-origin/differentiation/subtypes and emerging therapeutic concepts* (Fig. 1*)*. This report summarizes important findings communicated during the meeting.

Genome Dynamics

Genome dynamics converges various aspects of biology ranging from regulation of the transcriptome to the DNA damage response. The close entanglement of chromatin regulatory proteins and DNA replication were addressed by **Gwen Lomberk.** She reported on the tumorigenic histone methyltransferase G9a which targets histone 3 lysine 9 (H3K9) for di-methylation, thus inducing transcriptional repression. Oncogenic KRAS induced the expression of G9a complex members. Inactivation of G9a in genetically engineered mouse models (GEMMs) reduced H3K9me2 and abrogated the formation of precursor lesions, demonstrating a crucial role of G9a in tumorigenesis. Further, Gwen Lomberk introduced a role of G9a in regulating active replication forks. Her findings are of particular interest since the disturbed progression of replication forks triggers an intra S-phase cell cycle checkpoint, activating the ataxia telangiectasia and Rad-related (ATR) - checkpoint kinase 1 (CHK1) branch of DNA-damage signaling. She showed that combined G9a and CHK1 inhibition increased single-stranded DNA breaks, caused a collapse of the replication fork, and induced cell death [1].

Recent reports have documented the existence of a PDAC continuum ranging from classical to aggressive basal-like cancers [2–4]. Such subtypes differ in the response to therapies [3], therefore underpinning the idea of patient stratification. A prerequisite of this approach is the mechanistic understanding of the subtype biology. **Shiv Singh** offered a comprehensive overview on the dynamic interactions between classical or basal-like cancer cells and the inflammatory stromal components. He provided evidence that the proinflammatory cytokine 'TNFα' is enriched in basal-like tumors. His findings demonstrate that TNFα promotes transcriptional shifts from the classical to the basal-like subtype identity, provoking de-differentiation. Since FDA-approved anti-TNF agents are available, inhibition of the TNFα-driven network might represent a strategy to reduce the aggressiveness.

Amplifications of the *MYC* oncogene are associated with a worse survival. MYC has a great value as an integrator of KRAS signaling and therapies tackling MYC have been described. Recent work has emphasized a role of MYC in remodeling the tumor microenvironment

(TME) [5,6]. In order to further dissect this relationship, ***Bastian Krenz,*** *Anneli Gebhardt*, and *Martin Eilers* used a mouse model allowing inducible MYC inactivation to mimic therapeutic intervention. He showed that the tumor response upon MYC inactivation is dependent on the immune system and illustrated novel molecular underpinnings of MYC´s crosstalk with the TME.

PDAC is initiated, driven, and maintained by mutations in *KRAS* [7,8]. However, clinical inhibition of the canonical KRAS pathway has not been successful so far. Therefore, understanding redundancy, adaption, and resistance occurring in response to inhibition is pivotal for success. A cause of MEK inhibitor (MEKi) resistance was discussed by **Pawel Mazur.** By a genetic screen, the methyltransferase SETD5 was found to confer MEKi resistance [9]. Interference with SETD5 expression increased the sensitivity towards MEKi. The SETD5 complex contains the NCOR1-HDAC3 co-repressor and the methyltransferases G9a and GLP. Mechanistically, the SETD5 complex removes the activating histone acetylation mark H3K9ac allowing G9a to methylate this residue. Genes repressed by the SETD5 complex were connected to drug and glutathione metabolism, processes conferring drug resistance. Accordingly, a triple-therapy that combines MEKi with compounds targeting the enzymatic subunits HDAC3 and G9a/GLP is efficient in pre-clinical models[9].

Microenvironment

Plasticity does not only apply to tumor cells, but also accounts for cells of the TME. **Karin Feldmann** from *Max Reichert´s* laboratory characterized the role of paired-related homeobox 1 (Prrx1) transcription factor (TF) in the TME. Prrx1 is overexpressed in cancer associated fibroblasts (CAFs), particularly in patients with basal-like cancers [10]. Using genetic models to inactivate the *Prrx1* gene in CAFs, she demonstrated that *Prrx1*-deficient CAFs were forced into the myofibroblastic (myCAF) cellular state, increasing extracellular matrix deposition and restraining tumor progression. CAFs with high Prrx1 expression shaped an immune-suppressive microenvironment, promoted tumor cell EMT, and mediated gemcitabine resistance [10]. Karin Feldmann´s data on plasticity of CAFs exemplified the promise of fibroblast re-programing as a therapy.

The value of TME reprogramming was also demonstrated by **Corinne Bousquet**. Her talk focused on the implications of the somatostatin analog SOM230. Somatostatin acts via the G-protein coupled receptors sst1-5. Previous work already demonstrated that SOM230 acts on sst1, which is selectively expressed on CAFs [11]. Activation of sst1 by SOM230 blocked AKT-mTOR signaling-dependent protein synthesis. Subsequently, the production of IL-6, which acts in a paracrine fashion to drive tumor cell plasticity and chemoresistance [11], was decreased. Comprehensive secretome analysis of CAFs suggested that SOM230 caused reduced expression of the chemokine CSF-1 (macrophage colony-stimulating factor 1) [12], which contributes to the recruitment of monocytes and their polarization into macrophages. Hence, treatment with SOM230 reduced intra-tumoral M2-like-polarized tumor-associated macrophages (TAMs) [12] and abrogated the pro-metastatic processes associated with these cells. Consistently, the combination of gemcitabine and SOM230 was sufficient to reduce tumor growth and metastasis [12].

Cellular cross-talks in PDAC were also illustrated by **Marina Pasca di Magliano**. She introduced results from a multi-omics mapping approach of the TME. This multimodal analysis pointed to a substantial inter-tumoral heterogeneity of immune infiltration [13] and confirmed the highly immune-suppressive character of PDAC. $CD8^+$ T cell exhaustion was associated with abundant expression of the immune checkpoint T cell immunoglobulin and ITIM domain (TIGIT) [13], a key inhibitor of the immune anti-tumor responses. Intriguingly, TIGIT expression levels in PDAC patients matched in tumor and blood, qualifying the immunoglobulin as a biomarker for patient stratification prior to immunotherapy, a strategy currently under evaluation (e.g. NCT04294810). Furthermore, a role of Apolipoprotein E (ApoE), which was found to be overexpressed in TAMs, was discussed. *In vivo* experiments linked *ApoE* deficiency with reduced tumor growth and suggested a causative role of T cells mediating these effects. Treatment of PDAC cells with ApoE induced NF-kappa-B-dependent CXCL1 expression, thus further emphasizing the impact of the multi-directional tumor-stroma cell communication.

Felix Picard from the group of *Magdalena Huber* presented data on the population of IL-17 producing non-canonical $CD8^+$ T cells (Tc17 cells) and illustrated their implications on the TME. Tc17 cell abundance was associated with advanced tumor stages and reduced survival. In murine models, Tc17 cells accelerated tumor growth in a paracrine manner. Culturing of quiescent pancreatic stellate cells with conditioned media from Tc17 cells directed their differentiation towards inflammatory CAFs (iCAFs), while co-culture of Tc17-induced iCAFs with PDAC cells enhanced their proliferation *ex vivo* and promoted tumor growth *in vivo*. Hence, Felix Picard demonstrated how the interplay of different cellular compartments of the TME can foster PDAC progression.

Cell-of-origin, Differentiation, Subtypes

Elisa Espinet from the laboratory of *Andreas Trumpp* showed that clustering based on DNA methylation revealed two groups with different methylation levels at genomic regions encoding repeat elements. $Methylation^{low}$ tumors showed higher expression of endogenous retroviral transcripts and a strong engagement of the dsRNA sensing machinery with subsequent activation of an interferon signature [14]. This resulted in pro-tumorigenic reprogramming of stromal cells and sensitized this subset of more aggressive tumors for JAK/STAT inhibition [14]. Interestingly, $Methylation^{low}$/$IFNsign^{high}$ and $Methylation^{high}$/$IFNsign^{low}$ PDAC cells revealed distinct lineage traits specific for ductal or acinar cells, respectively, at the methylation and transcriptional level, suggesting the existence of two distinct origins of PDAC [14].

Alexander Kleger and his team have contributed protocols allowing to differentiate human pluripotent stem cells (hPSC) towards the pancreatic lineage [15]. In his talk, he presented a protocol which promotes differentiation of hPSC into pancreatic-duct-like organoids (PDLO), which resemble human duct epithelium at various levels, including function [16]. Genetic engineering to induce $KRAS^{G12D}$ in *CDKN2A*-proficient and -deficient PDLOs was used to demonstrate the value of such model. The group not only utilized this novel platform to explore the impact of KRAS signaling on oncogene-induced senescence but further explored processes operative in intraductal papillary mucinous neoplasms (IPMNs). This was

exemplified by a *GNAS* R201C mutated mosaic culture of human bone marrow stromal-cells from a patient with McCune-Albright syndrome. In accordance with the implication of *GNAS* mutations in driving IPMN, *GNAS*$^{WT/R201}$ mutated PDLOs formed large proliferative cysts and grew as well-differentiated ducts resembling human IPMNs *in vivo*. The power of the presented protocol to model human carcinogenesis and hereditary syndromes at early stages of plasticity and dysplasia was emphasized.

Nelson Dusetti presented the efforts of the PaCaOmics clinical trial to use patient-derived models, which conserve the inter- as well as the intratumoral heterogeneity of the disease [17,18] for translational research. In PDX models, a continuum of well-differentiated to undifferentiated PDACs was observed, which related to a gradient of transcriptional markers [4]. Only the extremes in the continuum express a pure classical or basal-like profile, while tumors with both subtype features and intermediate expression of markers exist. The models were used to establish predictive signatures for tumor progression and response towards therapies. While a *Pancreatic Adenocarcinoma Molecular Gradient* (*PAMG*) is predictive for responsiveness towards the mFOLFIRINOX regimen [4], an mRNA expression signature predictive of Gemcitabine response (*GemPred*) identifies patients who benefit from adjuvant Gemcitabine [19], allowing selection of a less toxic therapy. In summary, Nelson Dusetti presented the relevance of pre-clinical patient-derived models to identify signatures predicting the clinical outcome.

Feda Hamdan from the groups of *Steven Johnsen* and *Zeynab Najafova* integrated gene expression and epigenome mapping data from PDX to identify subtype-specific enhancer programs. Complementary analysis of nascent transcription and chromatin topology identified a unique group of transcribed super-enhancers that displayed frequent interactions, and were essential for basal-like target gene expression. She identified a **b**asal-like A **s**ubtype-specific **t**ranscribed **e**nhancer **p**rogram (B-STEP) characterized by enhancer RNA (eRNA) production that is associated with higher order chromatin interactions and gene activation. Notably, RNA *in situ* hybridization-based eRNA detection represents a fast tool to identify patients with basal-like A subtypes. Feda Hamdan´s findings provide a first proof-of-

concept that subtype-specific epigenetic changes are relevant for tumor progression and can be detected at a single cell level.

Emerging Therapeutic Concepts

A mesenchymal PDAC subtype overlapping basal-like cancers was described [20]. Mesenchymal PDACs showed increased *KRAS* mRNA expression [20], which is consistent with copy number gains in murine mesenchymal [21] and human basal-like cancers [3]. **Chiara Falcomatà** from the *Dieter Saur* group, observed a marked MEKi resistance of mesenchymal PDAC cells, which can be explained by higher signaling thresholds. To define options to break MEKi resistance, a combination drug screen was conducted. Here, a strong synergism between the MEKi trametinib and the multikinase inhibitor nintedanib was observed. Combined treatment induced apoptosis *in vitro* and disease regression *in vivo*. Using single cell RNA sequencing and immunophenotyping, it was shown that these responses are paralleled by transformation of the TME. Indeed, the drug combination primes cytotoxic and effector T cells to infiltrate the tumors, thereby sensitizing mesenchymal cancers to PD-L1 inhibition. In summary, this data suggested that a combination of a MEKi with nintedanib will prime for immune-checkpoint inhibitors.

Stephan Dreyer focused on the association between DNA damage response (DDR) and replication stress to develop precision treatments. By interrogating the transcriptome and genome of primary PDAC-derived cells, Dreyer tested a novel signature of homologous recombination deficiency which predicts responses towards platinum-based chemotherapy and PARP inhibition [22]. Independent of DDR deficiency, the basal-like subtype showed an enrichment of genes indicative for replication stress. Importantly, a transcriptomic signature of replication stress qualifies as a biomarker for responses towards ATR and WEE1 inhibitors [22]. Hence, replication stress and DDR deficiency can occur independently of each other and predict for different therapies [22].

Channing Der illustrated the importance of targeting the canonical KRAS signaling. He showed that inhibition of KRAS signaling regulates metabolic processes fostering autophagic flux, thus rendering tumors dependent on autophagy. Consequently, inhibiting canonical

KRAS signaling together with autophagy, achieved through hydroxychloroquine, represents a synergistic therapy which has been successfully explored in pre-clinical models [23] and is currently under clinical investigation (NCT04386057, NCT04132505). The second approach he presented was based on a forward genetic screen combined with a drug screen [24]. Analysis of KRAS-dependent PDAC cells showed that inhibition of all RAF isoforms – ARAF, BRAF, and CRAF – impairs growth. Therefore, a sub-lethal dose of a pan-RAF inhibitor was used for a combinatorial drug screen. Interestingly, MEK and ERK inhibitors synergized with the pan-RAF inhibitor. Mechanistically, ERK inhibition prevents the negative feedback reactivation of ERK previously observed upon pharmacological interference with canonical KRAS signaling. Consequently, the combination therapy disrupted TF networks downstream of ERK, including MYC, E2F, and AP1, thus inducing apoptosis in *in vivo* models [24]. These findings support the development of novel therapeutic concepts of low-dose vertical inhibition of canonical KRAS signaling.

Jennifer Morton used genetic modelling of the disease in mice. She presented three important groups of genetic alterations: First, she showed data on the tumor suppressor *PTEN*, which signals upstream of the PI3K-AKT-mTOR pathway. Her findings associate *PTEN* loss with a rapid acceleration of pancreatic tumorigenesis and susceptibility towards mTOR inhibition [25]. Deletion of *Rictor* (rapamycin-insensitive companion of MTOR) or pharmacological inhibition of mTORC2 could significantly extend survival of *KPC* mice [26]. Dual mTORC1/2 inhibitors effectively combine with MEKi, inducing tumor regression, significant metabolic rewiring and reprogramming of the TME. Second, she presented findings regarding the lysine demethylase *KDM6a*, which is mutated in basal-like cancers. *Kdm6a*-deficient GEMMs showed a dramatic acceleration of tumorigenesis, and transcriptomic analyses link *KDM6a* to signatures associated with cell cycle control. Interestingly, downregulation of $Kras^{G12D}$-expression was observed, suggesting that loss of *KDM6A* during tumor initiation may reduce the KRAS signaling threshold in precursor lesions, enabling them to circumvent senescence in favor of rapid progression. Third, she also presented models for DDR defective subtypes, based on *Atm*- and *Brca1*-deficiency.

Treatment combining ATR- and PARP-inhibition was superior in extending mouse survival compared to single agents, demonstrating the therapeutic impact. Furthermore, *Brca1*-defcient and *Atm*-deficient models substantially differed in immune cell infiltration, underscoring the intertumoral heterogeneity and at the level of the immune phenotype.

Conclusions

The meeting consisted of presentations addressing a spectrum from basic through translational projects and provided insights into cutting edge technologies and innovative approaches, improving our overall understanding of PDAC. The need and options to target canonical KRAS signaling or downstream integrators were communicated. Possible drawbacks of toxicity associated with such strategies were discussed, but can probably be overcome by low-dose drug combinations. Combinatory regimens efficiently targeting the tumor cells will offer possibilities for immunotherapies. Considering the heterogeneity of immune infiltrates, there is also a need for personalizing such therapies. Monitoring of immune checkpoints, like TIGIT, may pave the way.

The meeting emphasized the necessity to model the various aspects of PDAC development, progression, and therapy response. The power of modelling combined with functional clinical platforms was exemplified by members of the *Cancer Research Center of Marseille* and the *PRECISION PANC* consortium. Such efforts will lead to implementation of precision oncology, which are emerging for chemotherapies or targeted therapies tackling DNA damage signaling.

The unique heterogeneity of PDAC was illustrated in all sessions. Multi-omics approaches resulted in the detection of signatures enabling subtype and therapy response prediction. Several talks highlighted the implication of multidirectional cross-signaling between different cellular compartments for progression, plasticity, and therapy. The therapeutic value of interfering with cellular and molecular crosstalk was highlighted. However, we are only at the beginning of efforts towards understanding the molecular interactions especially under therapeutic perturbance.

Although not all current topics of PDAC research were presented, the forum documented the clear progress made in the last five years (Fig. 2). Together, the meeting strongly encouraged us to increase our efforts in PDAC research in order to finally make an impact on patient outcome.

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Author names in bold designate shared co-first authorship.

Figure Legends

Figure 1: *Drivers of PDAC biology and resistance*

PDAC phenotypes are driven by highly entangled, unique features, which are characterized by: alterations in Genome dynamics, the TME, as well as the cell-of-origin the tumor derives from and the predominant molecular subtype it represents. These hallmarks of PDAC determine the development of novel therapeutic strategies for personalized and improved PDAC treatment. The *1st virtual Göttingen-Munich-Marburg Pancreatic Cancer Meeting* comprised scientific talks from all four fields of cutting-edge topics of PDAC research.

Figure 2: *Challenges and Emerging Opportunities*

The *1st virtual Göttingen-Munich-Marburg Pancreatic Cancer Meeting* revealed several areas of progress in PDAC research. Based on these findings, novel scientific challenges and therapeutic opportunities evolve. Essential driver pathways and integrators can be targeted by rational combination therapies. The highly dynamic nature of cellular cross-talks including their therapy-induced perturbance needs to be analyzed and therapeutically addressed. Advances in these fields will lead to molecular-informed, multilayer stratified, personalized treatments.