multiple cycles. This process is completely automated through the CODEX® instrument and readily deployable on commercially available fluorescence microscopy systems. Using a 30+ antibody CODEX® panel, we compared formalin-fixed paraffin embedded (FFPE) human breast cancer tissues at different stages of disease progression with normal breast tissues. Our antibody panel was designed to detect cancer cells as well as non-malignant cells in order to comprehensively survey the tumor microenvironment and normal control tissues. Data were analyzed using the CODEX® software suite to identify key cell types and analyze spatial associations.

Results Our analyses revealed more than 20 distinct cell types in human breast cancer and normal tissues. Cell populations, biomarker expression and cellular spatial distributions differed distinctly between cancerous and normal breast tissues. Differences were robust, repeatedly observed and indicative of altered cellular milieus in normal versus cancerous breast tissues.

Conclusions Collectively, these data establish CODEX® as a readily deployable and practical tool for spatially-resolved, highly multiplexed biomarker analysis of human FFPE samples.

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P01.07 TARGETING A MEMBRANE PROXIMAL EPITOPE ON MESOTHELIN INCREASES THE TUMORICIDAL ACTIVITY OF A BISPECIFIC ANTIBODY BLOCKING CD47 ON TUMOR CELLS

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Background Mesothelin (MSLN) is recognized as a relevant tumor-associated antigen for cancer immunotherapy, because of its overexpression on various solid tumors, including mesothelioma, pancreatic, lung, gastric and ovarian carcinoma. However, an anti-MSLN monoclonal antibody (mAb), amatuximab, has demonstrated only limited efficacy in clinical trials. It has been already demonstrated that the targeting of a membrane-distal domain of an antigen with a mAb is suboptimal at inducing Fc-related effector functions. As amatuximab targets a membrane-distal domain of MSLN, we investigated whether mAbs targeting different epitopes would bestow a better efficacy. Furthermore, in order to incorporate novel modalities to enhance tumor-killing, we have paired these MSLN targeting arms with an anti-CD47 arm to generate bispecific antibodies (bsAb). Indeed, the 'don't eat me signal' CD47 is a promising target in cancer and therapeutic blockade has recently showed clinical evidence of efficacy. Therefore, we investigated the contribution of a CD47 arm and the impact of the different anti-MSLN targeting arms on the tumoricidal activities of CD47xMSLN bsAbs.

Materials and Methods A panel of anti-MSLN mAbs and CD47xMSLN biAbs carrying the same anti-CD47 arm and different anti-MSLN arms were generated and characterized for their epitope specificity. Their tumor cell killing efficacy *in vitro* and *in vivo* was analyzed using cell-based assays, xenograft models and various MSLN+ human malignant cell lines originated from different tissues (e.g., lung, gastric and hepatic origin).

Results Our data revealed that all CD47xMSLN bsAbs, regardless of the recognized MSLN epitope, showed higher activity than the corresponding anti-MSLN mAbs in tumor-cell killing assays and demonstrated superior anti-tumor activity in a xenograft model. Targeting a membrane-proximal epitope rendered an anti-MSLN mAb more effective in mediating antibody-dependent cell-mediated cytotoxicity (ADCC) but did not optimize antibody dependent cellular phagocytosis (ADCP) activity. However, targeting the membrane-proximal epitope of MSLN afforded the CD47xMSLN bsAb enhanced ADCC and ADCP activity, resulting in superior activity *in vivo*. Mechanistically, engaging a MSLN membrane proximal region with a CD47-bsAb format not only enhanced FcγR-IIIA signaling but also interestingly disrupted more efficiently the CD47/SIRPα axis, resulting in optimized phagocytosis of tumor cells. Finally, we showed that treatment with CD47xMSLN bsAb targeting membrane proximal MSLN epitope induced an accumulation of myeloid cells and NK cells in the tumor microenvironment.

Conclusions This study demonstrated that when designing antibody-based molecules, the targeted region on a tumor-associated antigen needs to be carefully considered to ensure maximal effector function. In the context of MSLN-positive solid tumors, we showed that an approach targeting a membrane-proximal epitope coupled to a CD47-blocking arm afforded an improved ADCC and ADCP profile, translating into increased *in vivo* efficacy.

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P01.08 BEYOND PD-1: CHARACTERIZATION OF NEW CHECKPOINTS RESTRICTING FUNCTION OF CYTOTOXIC LYMPHOCYTES INFILTRATING HUMAN CARCINOMA

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Background T and NK cells from human renal cell carcinoma (RCC) are functionally non-responsive. Analysis of the TCR signaling cascade required for effector function identified that proximal signaling molecules were activated whereas activation of downstream ERK was blocked. Further investigation showed increased diacylglycerol kinase alpha (DGK-α) levels in T and NK cells from the RCC tumor microenvironment (TME). These cells were refractory to stimulation showing no degranulation or IFN-γ production. Using a small molecule DGK–α inhibitor (R59022), the function of tumor-infiltrating

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lymphocytes was restored *ex vivo*. A correlation of high DGK-α and loss of function was also observed in an experimental mouse model of adoptive therapy where CAR T cells that had lost their activity after infiltrating into solid tumors were found to have increased DGK-α.[1] Blockade of the Programmed cell death protein 1 (PD-1) with monoclonal antibodies is used in the clinic enabling some patients to achieve tumor control. However, not all patients respond. DGK-α activity is positioned downstream of PD-1 and should, if overactive, curb T cell function even if PD-1 inhibition is released. Thus, we hypothesize that dual inhibition of PD-1 and DGK–α might be required to fully unleash the T cell's potential in the TME. Current DGK-α inhibitors are not suitable for clinical application. Therefore, we investigated alternative means using an RNA interference (RNAi) approach to target DGK-α alone as well as in combination with PD-1 in T and NK cells.
Material and Methods Knockdown is performed by RNAi using INTASYL™ compounds developed by Phio Pharmaceuticals. INTASYL™ compounds incorporate drug-like properties into the siRNA, resulting in enhanced uptake in the presence of serum with no need for further transfection reagents. Knockdown is analyzed by RT-qPCR and flow cytometry. Functional assays include cytotoxicity, degranulation and cytokine production in tumor mimicking environments.
Results A tumor mimicking *in vitro* system was developed which allows for the demonstration of functional restoration or prevention of functional loss of cell activity. Using T cell/tumor cell co–cultures at high tumor cell density, functional suppression could be induced in T and NK cells comparable to those observed in the TME. Testing of DGK-α targeting INTASYL™ compounds, silencing of DGK-α was observed in human U2OS osteosarcoma cells. Using a fluorescently labeled compound, highly efficient transfection of human primary immune cells was seen. Combinations of PD-1 and DGK-α targeting compounds are being tested and evaluated for synergism in experimental models.
Conclusions Strong activity of specific T and NK cells is necessary for tumor control. Dual targeting of PD-1 and DGK-α may be required to fully enable T and NK cell reactivity in the TME. Current DGK-α inhibitors do not exhibit the desirable pharmacokinetic/pharmacodynamic (PK/PD) properties for clinical development. The tested self-delivering RNAi technology represents a promising approach to targeting intracellular immune checkpoints such as DGK-α.

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P01.09 DUAL SIGNALLING PROTEIN 107 TRIGGERS INNATE AND ADAPTIVE IMMUNE RESPONSE TOWARDS TUMOUR CELLS

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Background Dual signalling protein 107 (DSP107) is a trimeric fusion protein consisting of the extracellular domains of human SIRPα and 4-1BBL. SIRPα binds to CD47, frequently overexpressed on cancer cells, and 41BBL binds to 41BB on activated T-cells. The SIRPα domain triggers the innate immune response by inhibiting the CD47/SIRPα 'don't eat me' signalling. It thus promotes phagocytosis of cancer cells by granulocytes, macrophages and dendritic cells. With its other side, 41BBL domain binds to pre-activated T cells and stimulates their expansion, cytokine production and cytolytic effector function. Our hypothesis is that augmented phagocytosis and improved co-localization of immune cells will lead to better antigen presentation towards activated T and B cells and the generation of memory T and B cells will be enforced. As result DSP107 might lead to immunity after rechallenge with the same tumour type.
Materials and Methods Primary phagocytes were incubated with stained tumour cells in presence or absence of DSP107 or/and therapeutic antibodies. Fluorescence microscopy measured uptake of tumour cells by macrophages. FACS identified primary granulocytes positive for CD11b staining and membrane dye. HT1080-41BB cells were mixed with HT1080-CD47 or HT1080-wt in presence of DSP107 and IL-8 release to supernatant was measured by ELISA. Further, primary T cells were co-cultured with αCD3Fc and fluorescent protein transduced carcinoma cells at different DSP107 concentrations.
Results The number of granulocytes that phagocyte tumour cells was increased in presence of DSP107. Further, DSP107 not only stimulated more macrophages to engulf tumour cells, but also the number of tumour cells that were taken up per phagocyte rose. Already enhanced phagocytosis of tumour cells by therapeutic antibodies (e.g. Cetuximab, Rituximab and Trastuzumab) was improved even further by DSP107. A model system showed that activation of the 41BB/41BBL axis by DSP107 was dependent on cross-linking via CD47 domain. This indicates low off-target T cell activation. Apart from the model system, DSP107 stimulated primary T cells in co-culture with carcinoma cells (transduced to express αCD3 and a fluorescent protein). Cytolytic activity against carcinoma cells was improved and outgrowth of tumour cells was reduced in a dose dependant manner.
Conclusions DSP107 blocks the CD47/SIRPα checkpoint resulting in enhanced tumour cell phagocytosis and stimulates the 41BB/41BBL axis leading to T cell mediated tumour cell killing. DSP107 is a novel bifunctional therapeutic that targets and activates both innate and adaptive anticancer immune responses. DSP107 is a first-in-class drug candidate that can

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