pretreated vs -untreated WB, suggesting a production/activation of (potentially leukemia-specific) cells after kit-stimulation. Moreover kit-pretreated WB regularly and significantly improved provision, activation as well as antileukemic and leukemia-specifically directed immune reactive cells after MLC. TEM showed exosome-like structures with a typically cup-shaped appearance without any differences between healthy and AML samples. fNTA revealed average vesicle sizes of 177 ± 23 nm (healthy) and 178 ± 17 nm (AML). Higher levels of EVs were detectable in AML samples compared to healthy controls in serum and after DC-culture, but lower levels after MLC independent of culture conditions. Interestingly, the number of EVs increased during cultivation of DC of AML and healthy samples, but not in AML-derived MLC samples.

Conclusions We will provide data in AML patients and healthy volunteers about a potential role of DCs- and MLC-derived exosomes as biomarkers in immune responses, malignant progression or as potential therapeutic targets for AML patients.

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P01.12 IMPACT OF COMPLEMENTARY SUBSTANCES ON IMMUNE CELL ACTIVITY

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Background Natural substances and micronutrients are more and more included in anti-cancer therapy. However, benefit and harm both are reported from one and the same substance. This emphasizes the urgent need for the systematic analysis of a personalized approach which patient will profit from which substance.

Materials and Methods Risk analysis was performed using PBMCs isolated from patients diagnosed with advanced solid cancer. Benefit was analyzed using 3D-microtumors directly prepared from individual patient tumors. Blood cells and cancer cells were treated with different natural substances, namely curcumin, artesunate and vitamine C, as single agents and in combination therapy with guideline-directed drugs for 72h. Impact on cell metabolic activity was measured with the Cell-Titer Glo assay. The cell phenotype was described by FACS analysis.

Results In 80% of the patients natural substances induced a slight (mean: 10.7%, range: 2.3–17.7%) metabolic inhibition of the immune cells, which was minor in comparison to the strong immunotoxicity of chemotherapeutic drugs (e.g. 5-FU, mean: 33.5%; Gemcitabine: 67.2%). Contrary, 20% of the patients revealed a stimulatory effect on PBMC depending on the basic activity and the exhaustion of the immune cells. Combination therapy revealed that natural substances were able to reduce (mean: 16.4%, range: 5.2–42.8%) immunotoxicity mediated by chemotherapy. Analysis of the 3D-microtumors indicated that natural substances can mediate an anti-cancer effect, which was most obvious in relapsed tumors heavily pretreated with chemotherapeutic drugs. In addition, natural substances were identified as chemosensitizer. For example, curcumin was found to increase efficacy of

Mitomycin C in breast cancer, Bicalutamid in prostate cancer and 5-FU combined with Cisplatin in gastric cancer.

Conclusions Complementary substances have a different effect depending on dosing, timing, cell type and cell characteristics. Therefore preclinical testing is required to identify the most effective complementary substances for the individual cancer patient analyzing both immune cells and cancer cells.

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P01.13 MERTK SIGNALING IS CRITICAL FOR T CELL PROLIFERATION AND MEMORY

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Background Overexpression of TAM receptors, including MERTK, in some cancers are integral for chemoresistance, proliferation and metastasis.¹ Our group has previously demonstrated that T cells also express MERTK and engagement of MERTK signaling is responsible for increased proliferation, functional capacity and metabolic fitness.² It is therefore important to further study the effect of MERTK inhibition on T cell function in the context of cancer treatments where MERTK inhibitors may play a role. Here we provide evidence that MERTK inhibition impacts greatly on T cell proliferation, specifically reducing phosphorylated mTOR. We have also demonstrated that MERTK expression is increased on CD8 central memory subsets during longterm expansion providing evidence that this signaling pathway may be important for sustaining T memory responses.

Materials and Methods Flow cytometric analysis was used to investigate the effect of titration of MERTK small molecule inhibitor UNC2025 on healthy donor T cells activated with CD3/CD28 dynabeads. Cell trace dye was used to track proliferation of CD4 and CD8 T cells along with markers of memory differentiation (CCR7 and CD45RO), activation (CD137) and function (IFN γ , Tnf α and IL-2). MERTK signaling was assessed using phospho flow cytometric methodology of phosphorylated mTOR, AKT, ERK1/2, p38-MAPK and STAT5. Long term cultures of donor T cells of up to 28 days were investigated for MERTK expression alongside memory differentiation.

Results We demonstrated that moderate concentrations of MERTK inhibitor reduced proliferation of activated T cells. Despite inhibition of cell division, cell size still increased 2 fold compared to resting cells and cell viability remained unchanged. Additionally, the proportion of central memory to effector memory populations and intracellular cytokine production was not impacted. Analysis of molecules involved in MERTK signaling revealed that phosphorylated mTOR was significantly modulated following the addition of MERTK inhibitor. Long term culture of CD8 T cells demonstrated MERTK was significantly increased following early and late re-stimulation, and expression of MERTK was strongly associated with central memory subsets.

Conclusions Our results demonstrate that inhibition of MERTK signaling on T cells reduces cell division where mTOR is significantly impacted. Despite this, other functional aspects, such as intracellular cytokine production remain

unchanged. Therefore, interruption of MERTK signaling on T cells has a specific effect on cell division rather than cytotoxic function on a cell by cell basis. This has potential ramifications on the use of MERTK inhibitors to treat tumors where the ability to form substantial cytotoxic T cell populations might be reduced. In addition, increased MERTK expression on central memory subsets during long term culture suggests this signaling pathway could be critical for generating memory pools of T cells and provide new avenues for the improvement of adoptive T cell therapy protocols.

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P01.14 EXCESSIVE BIOLOGICAL AGEING OF CIRCULATING NEUTROPHILS IN CANCER PROMOTES TUMOR PROGRESSION

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Background Beyond their well-established role in host defense, neutrophils are increasingly recognized to contribute to the pathogenesis of malignant tumors. Recently, ageing of mature neutrophils in the systemic circulation has been identified to be critical for these immune cells to properly unfold their anti-infectious properties. The role of neutrophil ageing in cancer is still unknown.

Material and Methods Employing syngeneic mouse models of head and neck squamous cell carcinoma (cell line SCC VII) and breast cancer (cell line 4T1), cytokine expression (by multiplex ELISA), neutrophil trafficking (by multi-channel *in vivo* microscopy and flow cytometry), and neutrophil function (*in vitro* assays) were analyzed.

Results Here, we show that signals released during early tumor growth promote excessive biological ageing of circulating neutrophils as indicated by age-related changes in their molecular repertoire. These events facilitate the accumulation of these highly reactive immune cells in malignant lesions and endow them with potent pro-tumorigenic functions. In particular, excessively aged neutrophils release neutrophil elastase which, in turn, stimulates the proliferation of cancer cells. Counteracting accelerated biological ageing of circulating neutrophils by blocking the chemokine receptor CXCR2 effectively suppressed tumor growth.

Conclusions Our experimental data uncover a potent self-sustaining mechanism of malignant tumors in fostering pro-tumorigenic phenotypic and functional changes in circulating neutrophils, thus supporting tumor progression. Interference with this aberrant process might provide a novel, already pharmacologically targetable strategy for cancer therapy. *This study was supported by Deutsche Forschungsgemeinschaft (DFG), Sonderforschungsbereich (SFB) 914.*

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P01.15 PERSONALIZED COMBINATION OF NEOADJUVANT DOMATINOSTAT, NIVOLUMAB (NIVO) AND IPILIMUMAB (IPI) IN MACROSCOPIC STAGE III MELANOMA PATIENTS STRATIFIED ACCORDING TO INTERFERON-GAMMA (IFN-GAMMA) SIGNATURE – THE DONIMI STUDY

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Background The previous OpACIN and OpACIN-neo studies investigating neoadjuvant IPI plus NIVO have demonstrated high pathologic response rates (74–78%) and favorable long-term outcomes for patients (pts) with a pathological response; at 36 and 18 months follow up only 1/71 (1.4%) responders has relapsed. In contrast, pathological non-responders have a poor prognosis; 15/23 (65.2%) have relapsed so far. This emphasizes the need for baseline biomarkers predictive of non-response and new neoadjuvant treatment combinations for these pts. In our previous studies, baseline IFN-γ signature high pts were more likely to respond to IPI plus NIVO. The DONIMI study tests the combination of NIVO ± IPI combined with a class 1 histone deacetylase inhibitor, domatinostat (DOM), according to the pts IFN-γ signature. We have developed a neoadjuvant IFN-γ signature, based on the signature previously described by Ayers *et al.*, that will be used for the first time to classify pts in this prospective trial.

Trial design This two-center investigator-initiated phase 1b study aims to assess the safety and feasibility of neoadjuvant NIVO ± DOM ± IPI in 45 stage III melanoma pts with macroscopic de-novo or recurrent disease. IFN-γ signature high pts (n=20) will be randomized (stratified by center) to Arm A (2 cycles NIVO 240 mg q3wk) or Arm B (2 cycles NIVO 240 mg q3wk + DOM 200 mg twice daily (BID), d1-14, q3wk). IFN-γ signature low pts (n=25) will be randomized to Arm C (2 cycles NIVO 240 mg q3wk + DOM 200 mg BID, d1-14, q3wk) or Arm D (2 cycles NIVO 240 mg q3wk + IPI 80 mg q3wk + DOM 200 mg once daily (OD), d1-14, q3wk). Based on safety data of the first 5 pts in arm D, the remaining pts will be treated with either a higher dosing scheme (200 mg BID, d1-14, q3wks), a lower dosing scheme (100 mg OD, d1-14, q3wks) or the same dosing scheme (200 mg OD, d1-14, q3wks). The primary endpoint is safety and feasibility. A treatment arm will be declared as not feasible if 2/5 or 3/10 patients cannot adhere to the planned time of surgery (week 6 ± 1week) due to treatment-related adverse events. Biopsies (week 0, 3), blood samples (week 0, 3, 6, 12) and feces (week 0, 3, 6) will be collected for translational research. To date, 7 patients have been enrolled.

Clinical trial information NCT04133948

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