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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Disclosure Information R.M. Powell: None. M.J.W. Peeters: None. A. Rachbech: None. P.T. Straten: None.

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

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10.1136/jitc-2020-ITOC7.27

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.*

Disclosure Information C.A. Reichel: None. L. Mittmann: None. J. Schaubächer: None. R. Hennel: None. G.

Zuchtriegel: None. M. Canis: None. O. Gires: None. F. Krombach: None. L. Holdt: None. S. Brandau: None. T. Vogl: None. K. Lauber: None. B. Uhl: None.

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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10.1136/jitc-2020-ITOC7.28

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

Disclosure Information I.L.M. Reijers: None. E.A. Rozeman: None. P. Dimitriadis: None. O. Krijgsman: C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; BMS. L.J.W. Bosch: None. S. Cornelissen: None. J. Bouwman: None. J.M. Versluis: None. D.

Rao: None. B. van de Wiel: None. A.J. Spillane: None. R.A. Scolyer: F. Consultant/Advisory Board; Modest; MSD, Nera-care, Myriad, Novartis. A.M. Menzies: F. Consultant/Advisory Board; Modest; BMS, MSD Oncology, Novartis, Pierre Fabre, Roche. A.C.J. van Akkooi: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Amgen, BMS, Novartis. F. Consultant/Advisory Board; Modest; Amgen, BMS, Novartis, MSD, Merck, Merck-Pfizer, 4SC. G.V. Long: F. Consultant/Advisory Board; Modest; Aduro, Amgen, BMS, Mass-Array, Pierre-Fabre, Novartis, Merck MSD, Roche. C.U. Blank: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; BMS, Novartis, Nanostring. F. Consultant/Advisory Board; Modest; BMS, MSD, Roche, Novartis, GSK, AZ, Pfizer, Lilly, Genmab, Pierre Fabre.

P01.16 EFFECTS OF THE STAT3 INHIBITORS ON SENESCENT TUMOUR CELLS

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10.1136/jitc-2020-ITOC7.29

Background Cellular senescence is the process of cell proliferation arrest. Premature cellular senescence can be induced by chemotherapy, irradiation and, under certain circumstances, by cytokines. Senescent cells produce a number of secreted proteins and growth factors that may either stimulate or inhibit cell proliferation. One of the major cytokines that play role in regulation of cellular senescence is IL-6. IL-6/STAT3 signaling pathway represent decisive regulatory factors in cellular senescence. The objective of this study was to compare the effects of the STAT3 inhibitors on senescent and proliferative tumour cells. Further, the therapeutic potential of the STAT3 inhibitors was evaluated using murine tumour models.

Materials and Methods *In vitro*, as well as *in vivo* experiments were performed using TC-1 (model for HPV16-associated tumours) TRAMP-C2 (prostate cancer) cell lines. C57BL/6Ncrl mice, 7–8 weeks old, were obtained from Velaz (Prague, Czech Republic). Experimental protocols were approved by the Institutional Animal Care Committee of the Institute of Molecular Genetics (Prague, Czech Republic). STAT3 inhibitors, namely STATTIC, BP-102 (synthesised at the University of Hradec Kralove) and their derivatives were tested for their effects on tumour cells, such as cytotoxicity, ability to inhibit STAT3 phosphorylation, cell proliferation and tumour growth in syngeneic mice.

Results We have previously demonstrated that docetaxel-induced senescence in the TC-1 and TRAMP-C2 murine tumour cell lines, which was proved by *in vitro* (detection of increased p21 expression, positive beta-galactosidase staining, and the typical SASP capable to induce 'bystander' senescence), and *in vivo* experiments, using C57BL/6 mice [1]. Both TC-1 and TRAMP-C2 cells displayed elevated IL-6 secretion and activated STAT3 signaling pathway. Therefore, we tested efficacy of the STAT3 inhibitors on these cell lines. Cytotoxic and STAT3 phosphorylation inhibitory effects of the

inhibitors were observed in both proliferating and senescent cells. Antitumor effects of selected inhibitors were evaluated.

Conclusions Collectively, STAT3 is an attractive target for therapeutic approaches in cancer treatment and we can assume that inhibition of the STAT3 pathway can be used for elimination of the pernicious effects of the senescent cells.

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Disclosure Information O. Sapega: None. R. Mikyskova: None. K. Musilek: None. J. Bieblova: None. Z. Hodny: None. M. Reinis: None.

P01.17 TIM-3/GALECTIN-9 PATHWAY CONTROLS THE ABILITY OF MALIGNANT CELLS TO ESCAPE HOST IMMUNE SURVEILLANCE. REGULATORY MECHANISMS AND THERAPEUTIC TARGETS

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10.1136/jitc-2020-ITOC7.30

Background Human cancer cells implement a variety of biochemical mechanisms which allow them to escape host immune surveillance resulting in disease progression. We have reported that the immune receptor Tim-3 and its natural ligand and possible trafficker galectin-9 determine the capability of human acute myeloid leukemia (AML) cells to evade cytotoxic immune attack.¹ Our further studies demonstrated that breast, colorectal and other human solid malignant tumour cells display high activity of this pathway² which can also be used for immune evasion. It is, however, important to understand the mechanisms which regulate the biochemical activity of Tim-3/galectin-9 pathway and expression of its components as well as the molecular basis of its capability to impair anti-cancer activity of cytotoxic lymphoid cells.

Materials and Methods In this study we used human cancer and non-malignant cell lines as well as primary human malignant tumour samples. We also used primary human T cells and natural killer (NK) cells. Western blot analysis, ELISA, quantitative real-time PCR, on-cell Western, immunohistochemistry, flow cytometry and biochemical assays were used as key instrumentals to conduct measurements.

Results We found that galectin-9 is used by human cancer cells to escape host immune surveillance. Cancer cells use various biochemical pathways to overexpress galectin-9. Regardless the biochemical background, transforming growth factor-beta (TGF-β) and transcription factor Smad-3 play crucial role in galectin-9 expression in human cancer cells. We identified the key receptors through which galectin-9 can then trigger killing of cytotoxic T lymphocytes and impairing of anti-cancer activity of natural killer cells.

Conclusions In this work, we report the biochemical mechanisms underlying overexpression of galectin-9 in human