treatment ICM in newly diagnosed BC patients (pts) and compare them to healthy controls.

Methods Soluble forms of ICM, as well as cytokines and chemokines, were measured using Multiplex® bead array and ELISA technologies. Plasma samples from 98 BC pts and 45 healthy controls were analyzed for each protein. Data was prospectively obtained. Measured levels were compared between BC pts and healthy controls using a non-parametric test (Mann-Whitney).

Results Soluble stimulatory molecules GITR ($p < 0.000002$), GITRL ($p < 0.007$), CD27 ($p < 0.002$), CD28 ($p < 0.003$), CD40 ($p < 0.003$), CD80 ($p < 0.009$), ICOS ($p < 0.0006$) as well as inhibitory molecules PD-L1 ($p < 0.0000001$), CTLA-4 ($p < 0.005$), TIM-3 ($p < 0.00006$), HVEM ($p < 0.00002$) and TLR-2 ($p < 0.05$) levels were significantly lower in early BC pts compared to healthy controls. When analyzed according to BC characteristics (TNBC vs. non-TNBC, tumor size, stage, nodal status and age) no significant difference was detected between the soluble levels of these ICM and between the different subsets. Additionally, serum levels of CXCL5 ($p < 0.000001$), CCL23 ($p < 0.04$), IL-16 ($p < 0.00005$), interferon-a ($p < 0.03$) and IL1-RA ($p < 0.03$) were significantly lower compared to healthy controls. Serum CX3CL1 or fractalkine ($p < 0.024465$) was significantly higher compared to healthy controls.

Conclusions In the current study, we identified low levels of both stimulatory and inhibitory soluble immune checkpoint molecules in newly diagnosed, non-metastatic BC pts compared to healthy controls. These results indicate that early BC is associated with a down-regulation of both soluble stimulatory and inhibitory immune-checkpoint pathways. Newly diagnosed early BC pts have a generalized immune-suppression independent of subtype and stage, which, to our knowledge, is the first study to describe soluble immune checkpoints in early BC pts.

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Trial Registration N/A

Ethics Approval The study was approved by The Research Ethics Committee, Faculty Health Sciences, University of Pretoria, approval number 517/2017.

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755 CXCR1 AND CXCR2 CHEMOKINE RECEPTOR AGONISTS PRODUCED BY TUMORS INDUCE NEUTROPHIL EXTRACELLULAR TRAPS THAT INTERFERE WITH IMMUNE CYTOTOXICITY

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Background Neutrophils are expanded and abundant in an important fraction (up to 35% of patients) in cancer-bearing hosts. When neutrophils are expanded, they usually promote exert immunomodulatory functions promoting tumor progression and the generation of metastases. Neutrophils can undergo a specialized form of cell death called NETosis that is characterized by the extrusion of their DNA to contain infections. In cancer NETs have been described to promote metastases in mouse models. IL-8, a CXCR1/2 ligand clinically targeted by blocking antibodies, has been described to induce NETosis and is upregulated in many cancer patients. Our hypothesis is that chemokines secreted by cancer cells can mediate NETosis in tumor associated neutrophils and that NETs can be one of the immunomodulatory mechanisms provided by tumor associated neutrophils.

Methods NETosis induction of peripheral neutrophils and granulocytic myeloid derived suppressor cells by different chemotactic stimuli, tumor cell supernatants and cocultures upon CXCR1/2 blockade. NET immunodetection in mouse models and xenograft tumors upon CXCR1/2 blockade. In vitro tumor cytotoxicity assays in the presence/absence of NETs, and videomicroscopy studies in vitro and by intravital imaging to test NETs inhibition of immune cytotoxicity by immune-cell/target-cell inhibition. Tumor growth studies and metastases models in the presence of NETosis inhibitors and in combination with checkpoint blockade in mouse cancer models.

Results Under the influence of CXCR1 and CXCR2 chemokine receptor agonists and other chemotactic factors produced by tumors, neutrophils, and granulocytic myeloid-derived suppressor cells (MDSCs) from cancer patients extrude their neutrophil extracellular traps (NETs). In our hands, CXCR1 and CXCR2 agonists proved to be the major mediators of cancer-promoted NETosis. NETs wrap and coat tumor cells and shield them from cytotoxicity, as mediated by CD8+ T cells and natural killer (NK) cells, by obstructing contact between immune cells and the surrounding target cells. Tumor cells protected from cytotoxicity by NETs underlie successful cancer metastases in mice and the immunotherapeutic synergy of protein arginine deiminase 4 (PAD4) inhibitors, which curtail NETosis with immune checkpoint inhibitors. Intravital microscopy provides evidence of neutrophil NETs interfering cytolytic cytotoxic T lymphocytes (CTLs) and NK cell contacts with tumor cells.

Conclusions CXCR1 and 2 are the main receptors mediating NETosis of tumor associated neutrophils in our in-vitro and in vivo systems expressing high levels of CXCR1 and 2 ligands. NETs limit cancer cell cytotoxicity by impeding contacts with cancer cells.

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756 ASSESSMENT OF THE IMMUNE CHECKPOINT LANDSCAPE IN HEAD AND NECK SQUAMOUS CELL CARCINOMA BY SINGLE-CELL RNA SEQUENCING AND MULTISPECTRAL IMAGING

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Background Resistance to the current generation of immunotherapies is mediated by complex relations between stromal, cancer and immune cells found within the tumor microenvironment (TME). Development of more efficacious drugs is predicated on improved understanding of these multi-spatial interactions. With emergence of new immune checkpoint receptor (ICR)-targeting therapies, a better understanding of topological expression of immune checkpoint ligand (ICL) on suppressive cell types in the TME may allow for improved strategies to treat cancer patients.

Methods Single cell RNA sequencing (scRNAseq) was performed from head and neck squamous cell carcinoma (HNSCC) specimens (n=18) with matched blood from treatment-naïve patients. Immune and non-immune cells were enriched from tumor cell suspensions. Novel transcriptomic

cell-to-cell interactions were predicted between heterogeneous cell populations. Histologic inflammation was corroborated with scRNAseq and multiplex flow cytometry. Cell type-specific PD-L1 contributions within the TME were quantified using multispectral imaging.

Results Major cell type clusters (immune, epithelial, fibroblast and endothelial cells) were identified. Expression patterns for PD-1, TIGIT, LAG-3 and TIM-3 ligands were evaluated on these suppressive TME cell types. By modeling receptor-ligand interactions between CD8+ T cells and the rest of the major TME cell types, CD8+ T cells were predicted to form more ICR-ICL interactions with tumor-associated macrophages (TAMs) than with any other cell type. With focus on LGALS9/galectin-9 and CD274/PD-L1, flow cytometric analyses validated the scRNAseq observation that both ligands were expressed on TAMs from both inflamed and non-inflamed tumors. Furthermore, flow cytometry and multispectral imaging analyses implicated macrophages as one of the major contributors of CD274/PD-L1 within the TME.

Conclusions Our data suggest that in the setting of HNSCC, TAMs are one of the major contributors of ICL in the HNSCC TME. Strategies that selective target this immunosuppressive population may be necessary to break tolerance to PD-1-targeting therapies.

Ethics Approval The study was approved by the UPMC Hillman Cancer's Ethics Board, approval number 99-069.

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Late-breaking abstracts

Biomarkers, immune monitoring, and novel technologies

757 INTRATUMORAL DELIVERY CD40 AGONIST ANTIBODY VIA NOVEL NANOFLUIDIC DRUG-ELUTING SEED REDUCED TUMOR BURDEN OF MURINE PANCREATIC DUCTAL ADENOCARCINOMA

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Background Pancreatic adenocarcinoma (PDAC) is associated with extremely poor prognosis and a 5-year survival rate of 10% and remains a lethal malignancy. Surgical resection and combination with chemoradiotherapy are the current standard-of-care options, may improve long-term survival in localized disease; however, the majority of patients are diagnosed at advanced stage. The incorporation of immunotherapy in the treatment algorithm convenes a new era for PDAC treatment. Several immunotherapy approaches have been investigating for treating PDAC such as checkpoint inhibitors, vaccines, adoptive cell therapy, and so on. Immunotherapy has been shown as a promising therapeutic method for many cancer types; however, the complexity and immunosuppressive of the solid tumor microenvironment (TME) results in limited treatment efficacy for PDAC.

Methods To sensitize the TME in response to immunotherapy, we developed an implantable intratumoral drug delivery device, Nanofluidic Drug-Eluting Seed (NDES) can be injected via a minimally invasive trocar system that feasible for the clinical setting. NDES has shown efficiently delivered immunotherapy to murine breast cancer model and reduced tumor burden and showed low liver inflammation compared to the intraperitoneal delivery approach in the previous study.[1] [2] Here, we utilized NDES for the sustained intratumoral delivery of the CD40 antibody. We compared the efficacy of NDES against intraperitoneal and intratumoral administration, which mimics conventional systemic treatment. Tumor growth was investigated for treatment efficacy. Local and systemic immune responses were assessed via flow cytometry.

Results NDES delivered CD40 significantly reduced tumor burden, some even achieved tumor clearance. Local NDES CD40 delivery approach showed a systemic increase of CD8+ and CD4+ T cells in the tumor-draining lymph node and spleen by flow cytometry. Furthermore, NDES CD40 treated mice showed an increase of CD8+ and CD4+ central memory T cells locally and systemically. We also investigated the combination with radiotherapy, no significant difference in tumor burden was observed when compared to single-agent CD40 antibody. The results indicated CD40 promotes TME response and improved treatment efficacy.

Conclusions These immunological responses demonstrate 'cold' to 'hot' tumor transformation, which translated to tumor burden reduction. Overall, NDES delivery strategy offers promise for enhancing therapeutic index and transforming the landscape of PDAC tumor therapy.

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758 IDENTIFICATION OF TUMOR ANTIGEN-SPECIFIC T CELLS IN THE PERIPHERAL BLOOD OF COLORECTAL CANCER PATIENTS

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Background Interactions between the immune system and the tumor are now recognized as key determinants of clinical outcome in colorectal cancer (CRC). Immune landscapes have been extensively studied within resected primary tumors and immune markers, such as T cells, have been found to be associated with CRC patients' survival. Little is known about the immune profile of cells in peripheral blood. We hypothesize that the functional status of T cells, characterized by their response to CRC tumor-associated antigens (TAAs), can be monitored in the peripheral blood of patients and that they have prognostic relevance in CRC.

Methods In vitro T cell responses to pools of overlapping peptides representing the TAAs MUC-1, hTERT, NY-ESO-1 and CEA were assessed by analyzing IFN-gamma and TNF-alpha production by CD8+ T cells using flow cytometry, in 5 stage II-III CRC patients just prior to surgical resection and 3 healthy age- and sex-matched controls.

Results T cells responding to MUC-1, hTERT, NY-ESO-1 and CEA were present in 3, 3, 1 and 5 CRC patients, respectively, whereas only one response to TAAs (MUC-1) was found in