Background
Tumors with high tumor mutational burden (TMB) or defects in mismatch repair (dMMR) respond well to immune checkpoint inhibitors (ICIs). TMB and DNA repair gene mutations including dMMR are closely related to the increase of neoantigens, which are recognized by immune cells to trigger an immune response. Although not a standard of care in thyroid cancer treatment, there are ongoing clinical trials for ICI use in differentiated thyroid carcinoma. However, not much has been explored concerning the neoantigen landscape and its association with immune traits in papillary thyroid cancer (PTC). We aim to analyze the immune landscape of PTC in association with neoantigen burden, TMB, and DNA repair gene mutations.

Methods
We used the PTC cohort data from The Cancer Genome Atlas (TCGA). The mutation counts and data for neoantigen prediction were acquired from TCGA mutation calling. CloudNeo pipeline was used for neoantigen prediction. TMB was calculated as the sum of missense and indel mutation counts per megabase pairs covered by whole-exome sequencing. Tumor-infiltrating immune cells were estimated using CIBERSORT.

Results
Out of the 496 PTC patients from cBioPortal, a subset of 400 patients with available mutation counts and predicted neoantigen burden was included in the study. Immune cell infiltration estimated by CIBERSORT showed macrophage M2 as the most abundant, followed by macrophage M0 and other T cells (figure 1). The TMB ranged from 0.03 to 2.05 with a median value of 0.2. Neoantigen burden ranged from 0 to 18 with a median value of 1, which is relatively low compared to the median value of 18 in non-small cell lung cancer (NSCLC) (figure 2). One or more DNA repair gene mutations were discovered in 32 patients (8%). The mutation status of repair genes was not related to TMB or neoantigen burden. TMB or neoantigen burden was not related to immune traits such as infiltration of CD8+ T cells or regulatory T cells, cytolytic activity score, and PD-L1 expression.

Conclusions
This is the first study to report the immune landscape of PTC in the context of neoantigen. The lack of association between TMB or neoantigen burden with immune traits may be due to the relatively low number of neoantigens in PTC compared to other immunogenic cancers such as NSCLC. Our results suggest that mutations in DNA repair genes or TMB are likely to have limited value in predicting response to ICI treatment in PTC.

REFERENCES

Abstract 753 Figure 1 Immune cell infiltration estimated by CIBERSORT

Abstract 753 Figure 2 Histogram of neoantigen burden

Abstract 754
DYSREGULATION OF SOLUBLE IMMUNE CHECKPOINT PROTEINS IN NEWLY – DIAGNOSED EARLY BREAST CANCER PATIENTS

Background
Checkpoint proteins regulate the immune system. Breast cancer (BC) cells exploit the up-regulation or down-regulation of these proteins to evade anti-tumor immune responses. Soluble forms of immune checkpoint molecules (ICM) can be measured in human plasma. However, their biological and clinical significance remains mostly unknown. The aim of the present analysis was to measure the levels of pre-
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 < 0.000001), CTLA-4 (p < 0.005), TIM-3 (p < 0.00006), HVEP (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