to anti-CTLA4, mesenchymal tumors are highly resistant. Most strikingly, in tumors arising from a mixture of both cell types, a minority population (10%) of mesenchymal cells can cross-protect the vast majority (90%) of their epithelial neighbors from immune attack.[1] However, the mechanisms underlying such cross-protection remain elusive. This is particularly important as most human breast cancers contain minority populations of such mesenchymal cells which can protect the tumor as a whole from immune attack.

Methods Using a combination of transcriptomic and CRISPR/Cas9 approaches, we first identified that mesenchymal carcinoma cells express high levels of CD73, an ecto-enzyme that catalyzes the production of adenosine. Additionally, we used digital spatial profiling to determine whether CD73 expression differs across distinct epithelial and mesenchymal sectors in mixed tumors.

Results Abrogation of CD73 from mesenchymal carcinoma cells prevented the assembly of an immunosuppressive tumor microenvironment and resulted instead in increased numbers of tumor-infiltrating lymphocytes and cross-presenting dendritic cells. Most strikingly, abrogation of CD73 sensitized previously refractory mesenchymal tumors completely to ICB. In the context of mixed tumors comprised of both epithelial and mesenchymal carcinoma cells, gradients in expression of CD73 were observed corresponding to the epithelial or mesenchymal sectors of these mixed tumors. Importantly, mixed tumors in which the minority population of mesenchymal carcinoma cells lacked the expression of CD73, were also sensitized partially to ICB. Thus, these mesenchymal carcinoma cells knocked out for CD73 could no longer protect their epithelial neighbors from immune attack.

Conclusions Taken together, our work suggests that mesenchymal carcinoma cells exert immune-suppressive effects which are also prominent in heterogeneous tumors. Furthermore, targeting the adenosinergic signaling pathway in mesenchymal carcinoma cells can potentiate the efficacy of ICB.

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233 GENE EXPRESSION ANALYSIS OF IMMUNE CHECKPOINT THERAPY IN MOUSE TUMOR MODELS REVEALS SIMILARITIES AND DIFFERENCES IN IMMUNE CELL POPULATIONS AND FUNCTIONAL PROCESSES THAT REFLECT RESPONSE TO TREATMENT

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Background Experimental therapies that target the immune system have expanded greatly in recent years due to the success of immune checkpoint inhibitory antibodies such as ipilimumab and pembrolizumab. Preclinical development of these novel immune-oncology drugs requires the availability of well characterized mouse models to evaluate therapeutic mode of action, efficacy, and safety. Syngeneic mouse tumor models provide robust systems in which to evaluate novel immune-oncology therapies. Efficacy in these models can be measured by tumor volume changes in subcutaneous implants or by impacts on survival for orthotopic implants. Mode of action can be assessed by identifying changes in the tumor microenvironment following dosing. Multiple analytical methods can be used to track changes in immune populations and activation status from flow cytometry to immunohistochemistry to gene expression analysis.

Methods We endeavored to characterize the functional tumor microenvironment changes for two syngeneic models following treatment with anti-PD-1 and anti-CTLA-4 antibodies The syngeneic models used for the study were both colon adenocarcinomas, MC38 and CT26. Mice bearing subcutaneous tumors were dosed intraperitoneally with either vehicle alone, anti-CTLA-4, anti-PD-1, or a combination of the two immune checkpoint inhibitors on days 1, 4, and 8. Tumors were harvested on day 9 and assessed for gene expression by microarray analysis. The gene expression results were evaluated for the relationship between treatment regimen and tumor volume change by expression level association, functional set enrichment analysis, and immune cell population gene set variation analysis.

Results For each of the tumor models, >10,000 genes were found to be significantly differentially expressed. Functional set enrichment analysis showed notable changes in cell cycle and mitotic markers as well as immune response markers in MC38. In contrast, CT26 showed principally changes in immune response markers. Immune cell population set analysis revealed differential impacts on numerous immune cell populations between the models which correlate with therapy induced changes in tumor growth. These include expected changes in CD8+ T-cell populations for both models but also differential changes in other populations including CD56dim NK cells, eosinophils and B cells in MC38 and neutrophils in CT26. We also conducted a genomic analysis by whole exome sequencing. Both tumor models have relatively high tumor mutational burden; CT26 TMB = 377 MB and MC38 TMB = 69/MB. These data show the value of robust bioinformatics analysis of gene expression data sets to provide insights into the mode of action and model responses to investigational immune-oncology drugs.

Conclusions N/A

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235 ANTIGEN PRESENTATION PATHWAYS PRIME MELANOMA PATIENTS FOR MORE DURABLE RESPONSE TO ANTI–PD-1 CHECKPOINT BLOCKADE THERAPY

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Background Favorable outcomes utilizing anti–PD-1 based immune therapies for unresectable melanoma patients are hypothesized to be dependent on antigen processing and presentation mechanisms. The present study utilizes multiomics to examine the contribution of antigen presentation pathways in metastatic melanoma tumors either responsive or resistant to anti-PD-1 therapy.

Methods To unveil the mechanisms that predispose unresectable, stage III/IV melanoma patients to respond to anti–PD-1-based therapies, we conducted expression proteomics as well as employed the mRNA immune oncology panel (HTG Molecular Diagnostics, Inc., Tucson, AZ). Formalin-fixed, paraffin-embedded tissues collected from 27 patients prior to anti-PD-1 therapy (previously consented and enrolled in the

Moffitt Cancer Center Total Cancer Care (TCC) protocol) were utilized in this study. For the proteomics analysis, we examined 19 FFPE samples, whereas the targeted mRNA analysis utilized 25 FFPE samples with 17 samples analyzed using both omics approaches. Robust mass spectrometry analysis used a pooled sample to optimize the number of detected peptides. The melanoma patients were selected from the database based on whether they had progression free survival (PFS) greater than 1 year (n=15) or PFS less than 6 months (n=12).

Results We identified more than 250 transcript/protein candidates that demonstrated differential expression between poor and good responders following anti-PD-1 therapy. Utilizing MetaCore software and subsequent downstream analyses of expression profiles for a knowledge-based curation of pathways and protein networks, we illustrated both the enrichment of Gene Ontology (GO) terms and specific antigen processing/presentation proteins. Of the top 10 GO processes, 7 were related to antigen processing/presentation and Major Histocompatibility Complex (MHC) presentation. Antigen processing/presentation and cytokine production/signaling via IFN-γ–mediated signaling through NF-κB and the JAK/STAT pathway interaction with iNOS were mechanistic candidates of response to anti-PD-1 therapy.

Conclusions These comparative analyses illustrated the importance of antigen processing/presentation pathways mediated by both MHC class I and II in activating the immune system to initiate and maintain the immune-based response to anti–PD-1 therapy in metastatic melanoma patients. The current study also demonstrated the value of proteogenomics in defining mechanisms of response and resistance to anti-PD-1 therapy.

Trial Registration N.A.

Ethics Approval The procurement of FFPE samples was approved under IRB-approved protocol (MCC 18583, Advarra) from patients who had received a biopsy within 1 year prior to the start of anti–PD-1 therapy. Biopsy samples were obtained from the institutional biobank (Total Cancer Cancer). The radiology reads (performed by T.R.) from this research were funded by a parent research project to Moffitt Cancer Center by Navigate BioPharma to J.G. and J.M.

Consent N.A.

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236 EVALUATION OF PD-L1 EXPRESSION IN PRIMARY LUNG TUMOR AND METASTATIC LYMPH NODES IN THE PRESENCE OF IMMUNE CELLS

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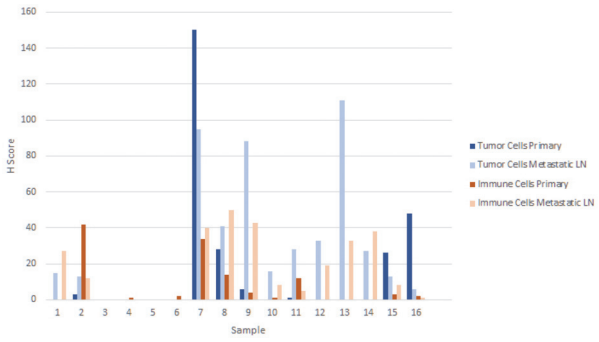
Background Immunotherapies against programmed death ligand-1 (PD-L1) have been established as an effective treatment for a subset of lung cancer patients. Even though it is critical for a successful therapy to know prevalent PD-L1 expression patterns in all affected tissues, information on matching lymph node metastases and immune cells is particularly limited. The purpose of this study was thus to evaluate comparative PD-L1 expression profiles in those tissues.

Methods FDA-approved IHC assays for PD-L1 (Dako 22C3) were performed on a lung tissue array (LC814A, US Biomax) according to manufacturer's instructions. Histopathological analysis by H-scoring was performed to determine the rate and intensity of positive tumor and immune cell staining for each of the 80 cores. The H score was calculated as follows:



Abstract 236 Figure 1 PD-L1 Staining in adenocarcinoma

A total of up to 300 cells were assessed, per specimen, at 40x high-power magnification (typically over 7–10 fields). A staining level of 0–3 was then assigned to each cell, to designate the intensity of specific positive membranous-to-cytoplasmic staining. The H score was subsequently calculated as% cells staining at level 1 (x1) +% cells staining at level 2 (x2) +% cells staining at level 3 (x3) = total H score per sample. This resulted in a maximum possible H score of 300.

Results Of the 16 adenocarcinoma tumor samples with a valid staining, 7 (44%) showed positive PD-L1 staining for tumor cells and 10 (63%) for primary immune cells. Importantly, 9 matching metastatic lymph node samples out of the 16 samples (56%) showed an increased PD-L1 H score compared to primary tumors for both tumor cells and immune cells (figure 1). Of the 15 squamous cell carcinoma samples with a valid staining, 11 (73%) showed detectable PD-L1 expression levels in the primary tumor and 12 (80%) in the primary immune cells, while 7 (47%) and 9 (60%) showed lower scores in matching metastatic lymph node tumor cells and their immune cells, respectively (figure 2). Very low or no expression of PD-L1 was detected in small cell lung cancer, as to be expected from previous studies.

Conclusions Squamous cell carcinomas and adenocarcinomas display significant heterogeneity with regard to PD-L1 expression in associated lymph node metastases. While the reasons for this frequent discordant PD-L1 expression pattern involving both tumor and immune cells need to be investigated further, our findings may help guide the proper interpretation of PD-L1 companion diagnostic test results and subsequent therapeutic decisions.

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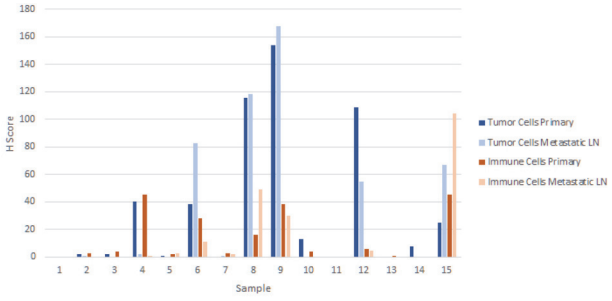

Abstract 236 Figure 2 PD-L1 Staining in squamous cell carcinoma