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BASAL CELL CARCINOMA DEMONSTRATES A T-CELL EXCLUSION IMMUNE PHENOTYPE IN CONTRAST TO OTHER ANTI-PD-1 THERAPY RESPONSIVE CUTANEOUS MALIGNANCIES

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Background Basal cell carcinoma (BCC) is considered an immunogenic tumor based on the high tumor mutational burden (TMB), increased incidence in immunocompromised patients, and responsiveness to imiquimod, a toll-like receptor agonist therapy. However, anti-PD-1 immunotherapy response rates in patients with advanced BCC appear less than that seen with other advanced cutaneous malignancies. Molecular profiles of BCC tumors were analyzed to determine immune phenotypes and resistance mechanisms in comparison to other anti-PD-1 therapy responsive cutaneous malignancies.

Methods Next generation sequencing on DNA (NGS; NextSeq and Novaseq), PD-L1 immunohistochemistry (SP-142 and 28–8 antibody clones, cutoff >5% tumor staining) and mRNA gene expression level (Whole Transcriptome Sequencing, NovaSeq) data from BCC (N=69), melanoma (N=914), and cutaneous squamous cell carcinoma (SCC) tumors (N=165) at Caris Life Sciences (Phoenix, AZ) were analyzed. Tumor mutational burden (TMB) was calculated by counting all non-synonymous missense mutations that had not been previously described as germline alterations. Microenvironment cell population counter¹ was used to estimate cell population abundance in the TME. Gene set enrichment analysis (GSEA) was performed on transcriptomes.² Statistical significance was set at P value or false discovery rate (FDR) < 0.05.

Results Of the 69 BCC tumors with NGS data, the most frequent mutations were in PTCH1 (82%), P53 (73%) and ARID1A (42%); additional relevant mutations included SMO (18%), JAK1 (9%), PI3KCA (6%), APC (4%), and CTNNB1 (3%). TMB was significantly greater in BCC compared to melanoma (median 30.5 vs 12 mut/Mb, P<0.0001) and similar to SCC (median 29.5 mut/Mb, P=0.9389). PD-L1 positivity was 1/23 (4%) in BCC, 215/831 (26%) in melanoma, and 81/147 (56%) in SCC. Interferon gamma and T-effector immune gene analyses showed significantly lower expression in BCC compared to melanoma and SCC (e.g., IFN γ

TPM=0.26 (BCC) vs 0.65 and 0.58 (melanoma, SCC, both P<0.01). BCC demonstrated the lowest CD-8 T-cell fractions and the highest neutrophil and cancer associated fibroblast (CAF) fractions compared to melanoma and SCC. Angiogenesis and TGF-beta gene sets were enriched in BCC compared to melanoma (NES=1.5, FDR=0.046 and NES=1.35, FDR=0.055, respectively), but not compared to SCC (NES=0.90, FDR=0.57 and NES=0.94, FDR=0.60, respectively).

Conclusions While BCC tumors demonstrated a high TMB, a markedly lower level of adaptive anti-tumor immunity in comparison to other cutaneous malignancies was observed. T-cell exclusion mechanisms mediated through CAFs and desmoplasia, with upregulation of TGF-beta and angiogenic signaling, may play a role. Further investigation into abrogation of these mechanisms is warranted to develop improved anti-PD-1 based therapies for BCC.

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IMMUNE CELL PROFILING ACROSS SOLID TUMOR TYPES BY MASS CYTOMETRY REVEALS TUMOR ENRICHMENT OF PD-1+/LAG-3+ CD8 MEMORY T CELLS THAT EXHIBIT TUMOR-REACTIVE YET DYSFUNCTIONAL FEATURES

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Background Characterization of human immune responses by profiling immune cells from patients is critical for the successful development of immuno-oncology agents and is useful to understand mechanism-of-action, identify pharmacodynamic/response biomarkers, and guide patient selection strategies. Extensive immune cell heterogeneity necessitates comprehensive high parameter immunophenotyping to yield these actionable insights.

Methods Cytometry by time-of-flight (CyTOF) was performed on homogenates from commercially procured tumors (n=28) and matched PBMCs (n=7) from patients with various solid tumors (colon (n=10), endometrial (n=9), kidney (n=4), liver (n=2), skin (n=1), lung (n=1), and gastro-intestinal (n=1)). Two antibody panels, recognizing a total of 18 lineage and 31 target proteins, were used to profile marker expression among the major lymphocyte and myeloid lineages. Data were analyzed using manual gating and non-linear dimensionality reduction (tSNE and UMAP), and expression was measured by frequency (% gate) and arcsinh-transformed median ion counts. Pairwise Wilcoxon Rank Sum tests were performed on arcsinh-transformed median ion counts to determine statistically significant differences in marker expression, and P values were adjusted using Benjamini-Hochberg correction (p<0.05 considered statistically significant). Cell subpopulation percentages were compared using unpaired two-sided T-tests. Sample populations with less than 150 events were excluded from