Background Checkpoint inhibitors can induce robust and durable responses in a subset of patients. Extending this benefit to more patients could be facilitated by better understanding of how interacts with immune cells with the tumor microenvironment, which is a critical barrier to control both local and systemic disease. The composition and pattern of the immune infiltrate associates with the likelihood of response to immunotherapy. Inflamed tumors that exhibit a brisk immune cell infiltrate are responsive, while those in which immune cells are completely or partially excluded are not. Transforming growth factor (TGF-β) is immunosuppressive and associated with the immune excluded phenotype.

Methods Using an immune competent mammary tumor derived transplant (mTDT) model recently developed in our lab, exhibits inflamed, excluded or deserts immune infiltrate phenotypes based on localization of CD8 lymphocytes. Using whole transcriptome deep sequencing, cytof, and PET-CT imaging, we evaluated the tumor, microenvironment, and immune pathway activation among immune infiltrate phenotypes.

Results Three distinct inflamed tumors phenotypes were identified: ‘classically’ inflamed characterized by pathway evidence of increased CD8+ T cells and decreased PD-L1 expression, inflamed tumors with pathways indicative of neovascularization and STAT3 signaling and reduced T cell mobilization, and an inflamed tumor with increased immunosuppressive myeloid phenotypes. Excluded tumors were characterized by TGFβ gene expression and pro-inflammatory cytokine signaling (e.g. TNFα, IL1β), associated with decreased leukocytes homing and increased immune cell death of cells. We visualized and quantified TGFβ activity using PET-CT imaging of 89Zr-fresolimumab, a TGFβ neutralizing antibody. TGFβ activity was significantly increased in excluded tumors compared to inflamed or desert tumors, which was supported by quantitative pathology (Perkin Elmer) of its canonical signaling target, phosphorylated SMAD2 (pSMAD2). pSMAD2 was positively correlated with PD-L1 expression in the stroma of excluded tumors. In contrast, in inflamed tumors, TGFβ activity positively correlated with increased F4/80 positive macrophages and negatively correlated with expression of PD-L1. CyTOF analysis of tumor and spleen immune phenotypes revealed increased trafficking of myeloid cells in mice bearing inflamed tumors compared to excluded and deserts.

Conclusions The immunocompetent mTDT provides a model that bridges the gap between the immune landscape and tumor microenvironment. Integration of these high-dimensional data with further studies of response to immunotherapies will help to identify tumor features that favor response to treatment or the means to convert those that are unresponsive.

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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

Geoffrey Gibney, Joanne Xu, Gino Lisi, Joseph Drabick, Ari Vander Walde, Kelsey Poorman, W. Kom, Michael Atkin. Georgetown University — LCCC, Washington, DC, USA; Caris Life Sciences, Phoenix, AZ, USA; Noris Comprehensive Cancer Center, Los Angeles, CA, USA; John Wayne Cancer Institute, Santa Monica, CA, USA; UMS Sylvester Comprehensive Cancer Center, Miami, FL, USA; Penn State Hersey Cancer Institute, Palmyra, PA, USA; West Cancer Center, Germantown, TN, USA

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 counter was used to estimate cell population abundance in the TME. Gene set enrichment analysis (GSEA) was performed on transcriptomes. 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., IFNg 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.

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

Bradley Garman, Lauren Menard, Dan Jiang, Sherif Daouti, Priyanka Mehta, Miyi Jacques, Mohsin Bollisetty, Chrisots Hatzis, Natally Manjarrez Orduno, Michaela Bowden, Justin David*, Justin David, Justin David. Bristol-Myers Squibb, Lawrenceville, NJ, USA

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 arschni-transformed median ion counts. Pairwise Wilcoxon Rank Sum tests were performed on arschni-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