Recurrent Somatic Mutations affect Immune Cell Density in the Tumor-core

Fifty-six patients in the ccRCC cohort previously underwent whole exome DNA sequencing from which we identified recurrent somatic mutations: *BAP1* (4/56 patients, 7.1%), *KDM5C* (9/56, 16.1%), *MTOR* (4/56, 7.1%), *PBRM1* (22/56, 39.3%), *PTEN* (5/56, 8.9%), *SETD2* (8/56, 14.3%), and *VHL* (42/56, 75.0%). Patients with *MTOR* alterations had significantly lower tumor-core cell density of T-bet+ T-cells (29 vs. 13 cells/mm^2; $p < 0.05$) and CD163+ macrophages (264 vs. 47 cells/mm^2; $p < 0.05$). Patients with *SETD2* alterations had significantly lower tumor-core cell density of CD8+ T-cells (97 vs. 30 cells/mm^2; $p < 0.05$). *PBRM1* alterations were not associated with significant differences in immune cell density. Comparisons for other alterations can be found in **Supplemental Figure 2**.

RNA-seq Immune Cell Gene Expression is Inconsistently Associated with mIF Density

Bulk RNA-seq data was obtained from tumor-core samples in 92 patients with ccRCC. Correlation was determined between tumor-core cell density as determined by mIF, and xCell score; an RNA-seq based gene expression score quantifying relative expression of 64 different cell types. Relevant comparisons were made between xCell immune cell-types and corresponding mIF marked cell types.

Within the ccRCC cohort, CD8+ xCell score and CD8+ mIF cell density were strongly correlated (Spearman's R = 0.63). Moderate correlation was found between B-cell xCell score and CD-20+ mIF cell density (R = 0.42), T-reg xCell score and FOXP3+ mIF cell density (R = 0.38), and a generalized xCell macrophage score with CD-68+ mIF cell density (R = 0.34). Poor correlation was identified between M2 macrophage xCell score and CD163+ and CD206+ mIF cell density (R = -0.16), and Th1 T-cell xCell score and T-bet+ mIF cell density (R = -0.23). A correlation matrix for the ccRCC cohort is available in **Supplemental Figure 3.**

Low Angiogenesis and High T-effector Score Tumors are Infiltrated with CD8+ T-cells and CD68+ TAMs

Angiogenesis and T-effector gene signature scores, previously derived from the IMmotion150 trial data, were determined for 92 patients with ccRCC[1]. Patients with high angiogenesis scores had significantly lower mIF cell densities of CD68+ and CD8+ cells in the tumor-core ($p = 0.002$ and $p = 0.05$, respectively), stroma ($p = 0.04$ and $p = 0.05$, respectively), and tumor-stroma interface zones ($p < 0.001$ and $p = 0.001$, respectively). Patients with high T-effector scores had significantly higher tumor-core mIF cell densities of CD68+ ($p < 0.001$), CD8+ ($p = 0.002$), FOXP3+ ($p = 0.04$), and PDL1+ cells ($p = 0.02$) **Supplemental Figure 4**.

Sensitivity Analyses

Several analyses were conducted to confirm that the previous findings were robust. First, PD-L1 tumor-core density was correlated with Tumor/CD163 and Tumor/CD8 interface nK(75), confirming that these are not merely surrogate findings for PD-L1 density. Spearman's correlation coefficients for PD-L1 density were -0.014 and 0.01 for Tumor/CD163 and Tumor/CD8 interface nK(75), respectively. **Supplemental Figure 5**.

To confirm that the Tumor/CD163 interface nK(75) metric was not easily reproduced using a Tumor/Stroma density ratio, a Spearman's correlation and Kaplan Meier analysis were conducted, revealing a weak positive relationship ($R = 0.31$) and no difference in overall survival when stratified by the CD163 Tumor/Stroma density ratio (log-rank $p = 0.75$). **Supplemental Figure 6**.

To confirm that the TCGA overall survival validation was not influenced by false-stratification, the CD163 Clustering Gene Signature was divided into tertiles instead of using a median cutoff, which confirmed a stepwise association with clinical outcomes along the tertile continuum. **Supplemental Figure 7**.

Ridgeline plots can be visualized in **Supplemental Figure 8**, detailing how many samples were excluded due to the 10 cell per ROI cutoff for spatial analysis.

1. McDermott DF, Huseni MA, Atkins MB, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat Med.* 2018;24(6):749-757.