Supplementary Materials:



Supplemental Figure S1. IDO1 expression following neoadjuvant chemotherapy and radiation. Immunohistochemistry analysis reveals upregulation of IDO1 expression in a subset of PDACs following neoadjuvant chemotherapy and radiation therapy. Scale bar represents 100µm.



Supplemental Figure S2. RNA sequencing of dissected stroma of human PDAC tissue. Stroma of representative FFPE tumor sections from patients treated with GVAX were microdissected by a pathologist. RNA was purified, amplified and RNA sequencing was performed. Read count was normalized to the total prior to analysis. IDO1-high and IDO1-low expression was determined by IHC as Figure 3 for comparative analysis. Expression of IL17D, TGFb, IL4, IL5, IL10, and IL12R were assessed, respectively, as indicated (A-F) (n=14). ** p<0.01, ns: not significant, by unpaired t-test.



Supplemental Figure S3. RNA sequencing of chemokine receptors and cytokines in dissected stroma of human PDAC tissue. Stroma of representative FFPE tumor sections from patients treated with GVAX were microdissected by a pathologist. RNA was purified, amplified and RNA sequencing was performed. Read count was normalized to the total prior to analysis. IDO1-high and IDO1-low expression was determined by IHC as Figure 3 for comparative analysis. Expression of CCL2, CCL5, CXCR3, CXCL9, CXCL10 and CXCL11 were assessed, respectively, as indicated (A-F) (n=14). ** p<0.01, ns: not significant, by unpaired t-test.



Supplemental Figure S4. Gene expression of isolated CD4 cells from treated murine PDAC tumor. Mice underwent hemispleen procedure receiving $2x10^6$ Panc02 PDAC cells followed by administration of 100 mg/kg Cy on day 3 and GVAX on day 4. IDO1 inhibitor (200 µg/kg) was administered by oral gavage twice a day starting on day 3 and continuing for 13 days. Anti-PD-1 antibody (100µg) or IgG control (100µg) was given intraperitoneally starting day 5 and continuing twice a week (n=3 mice per group pooled). At day 13, mice were sacrificed and RNA was extracted from isoalted CD4+ cells. RT-PCR was performed. Expression of IL6, IL2, IL17A, IL17F, IL17B, IL17C, iL17D, IL4, TGF β , and IL10 were assessed, respectively, as indicated (A-J). * p<0.05, ** p<0.01, *** p<0.001, ns: not significant, by 1-way ANOVA.



Supplemental Figure S5. Characteristics of CD8 cells from treated murine PDAC tumor. Mice underwent hemispleen procedure receiving $2x10^6$ Panc02 PDAC cells followed by administration of 100 mg/kg Cy on day 3 and GVAX on day 4. IDO1 inhibitor (200 µg/kg) was administered by oral gavage twice a day starting on day 3 and continuing for 13 days. Anti-PD-1 antibody (100µg) or IgG control (100µg) was given intraperitoneally starting day 5 and continuing twice a week (n=3 mice per group pooled). At day 13, mice were sacrificed for flow cytometry analysis. Both (A) splenocytes and (B) tumor infiltrating lymphocytes were separately isolated and processed into single cell suspensions and stained for cell surface markers and Annexin V for the comparison of apoptosis between treatment groups. (C-D respectively as indicated) RNA was extracted from isoalted CD8+ cells. RT-PCR was performed. Expression of CXCR3, IFNγ, and EOMES were assessed. * p<0.05, *** p<0.001, ns: not significant, by 1-way ANOVA.



Supplemental Figure S6. High IDO1 expression in GVAX treated PDAC tissue was not associated with type of myeloid lineage cells. (A) High IDO1 expression was not associated with myeloid cell density nor (B) PD-L1+ CD68+ cell density nor significantly associated with (C,D) M1 (CD163⁻CD68⁺CSF1R⁺) vs M2 (CD163⁺CD68⁺CSF1R⁺) tumor associated macrophages (TAM) as determined by CD163/CD68/CSF1R expression on multiplex IHC. None significant.



Supplemental Figure S7. Myeloid flow cytometry panel for PDAC bearing mice treated with IDO1 inhibitor and GVAX. Mice underwent hemispleen procedure receiving 2x10⁶ Panc02 PDAC cells followed by administration of 100 mg/kg Cy on day 3 and GVAX on day 4. IDO1 inhibitor (200 µg/kg) was administered by oral gavage twice a day starting on day 3. Mice were sacrificed at day 13 for flow cytometry analysis. (A) Total numbers of myeloid cells: [CD3-CD11b+] on flow cytometry analysis of Panc02 hemispleen mice following treatment. (B) Total number of inflammatory monocytes: [CD3⁻CD11b⁺Ly6C^{hi}Ly6G⁻F480⁺]. (C) Total number of macrophages: [CD3⁻CD11b⁺Ly6C^{lo}Ly6G⁻F4/80⁺]. None significant.



Supplemental Figure S8. PD-L1+ expression is uncommon in monocytes and myeloid derived suppressor cells. Mice underwent hemispleen procedure receiving 2x10⁶ Panc02 PDAC cells followed by administration of 100 mg/kg Cy on day 3 and GVAX on day 4. IDO1 inhibitor (200 µg/kg) was administered by oral gavage twice a day starting on day 3. At day 13 mice were sacrificed, tumor-bearing livers processed and infiltrating immune cells stained and assayed by flow cytometry. Total number of PD-L1+ expression on (A) monocytes: [CD3⁻CD11b⁺Ly6C^{hi}Ly6G⁻F480⁺] (B) M-MDSC: [CD3⁻CD11b⁺Ly6C^{hi}Ly6G⁻] and (C) G-MDSC: [CD3⁻CD11b⁺Ly6C^{hi}Ly6G⁺] on flow cytometry analysis. None significant.