# The tumor microenvironment and immune infiltration landscape of *KRAS* mutant pancreatic ductal adenocarcinoma (PDAC) compared to colorectal adenocarcinoma (CRC).

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## Background

The composition of the tumor microenvironment (TME) in PDACs is more heavily driven by mutant (mt) *KRAS* than any other cancer. How genomic alterations of PDAC including *KRAS* status affect the immune cell (IC) landscape remains unclear. Thus, we characterized IC types and the prevalence of immuno-oncologic (IO) biomarkers in PDAC by genomic and transcriptomic analysis and investigated associations of mt *KRAS* with IC estimates in the TME. Our findings were compared to our previous study in CRC.

## Methods

A total of 4,142 PDAC and 3,727 CRC with *KRAS-mts* were analyzed using next-generation DNA sequencing (NextSeq, 592 gene panel or NovaSeq, WES), IHC, and whole transcriptome RNA sequencing (NovaSeq) (Caris Life Sciences, Phoenix, AZ). MSI/MMR was tested by FA, IHC and NGS. TMB-H was classified based on a cut-off of ≥10 mutations per MB. ICs were estimated by QuantiSeq (Finotello 2019, *Genome Medicine*) or MCP counter (Betcht 2016, *Genome Biology*). Significance was determined by $X^2$ and Fisher-Exact and p-adjusted for multiple comparisons (q < 0.05).

## Results

**Table 1: patient demographics**

| PDAC | | | | |
|---|---|---|---|---|
| | Male | Female | Total (%) | Median age |
| *KRAS* MT | 2205 | 1937 | **4142 (81.7%)** | 68 |
| *KRAS* WT | 506 | 424 | **930 (18.3%)** | 67 |

| CRC | | | | |
|---|---|---|---|---|
| | Male | Female | Total (%) | Median age |
| KRAS MT | 1969 | 1758 | **3727 (49.9%)** | 61 |
| KRAS WT | 2134 | 1602 | **3736 (50.1%)** | 62 |

**Figure 1a –** KRAS mutational distribution in PDAC

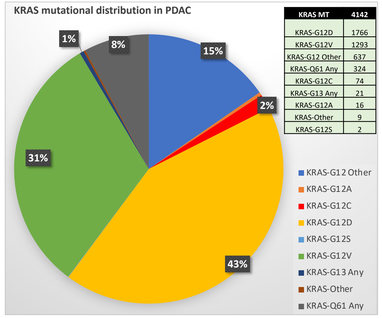



**Figure 1b –** KRAS mutational distribution in CRC

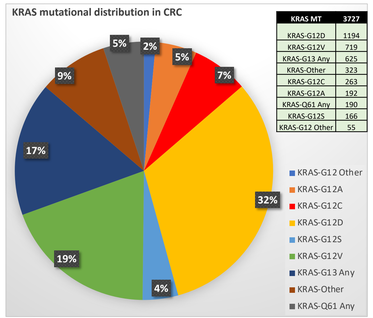



**Figure 2 –** IO markers in *KRAS* mt vs WT PDAC

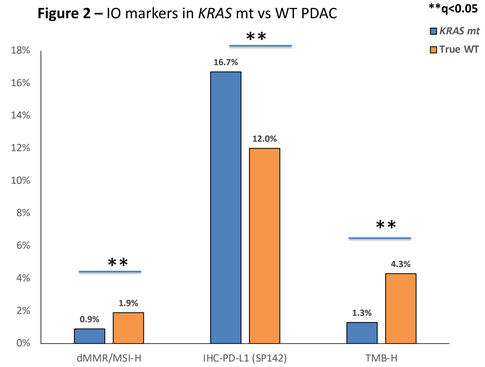



**Figure 3 –** Immune cell environment of *KRAS* mt PDAC comparted to WT. Arrows show significant increase/decrease of infiltration in *KRAS* mt compared to WT tumors.

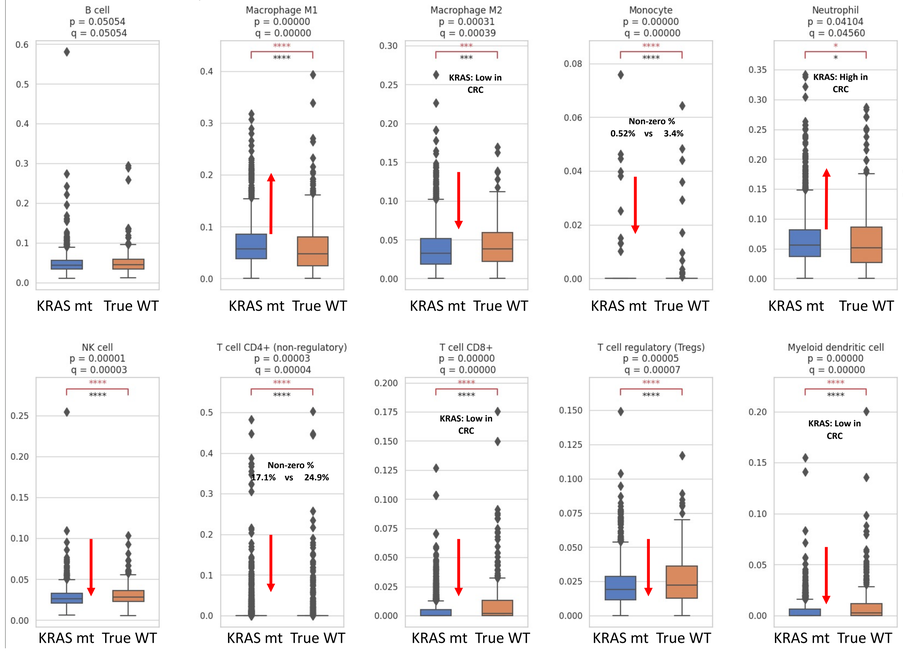
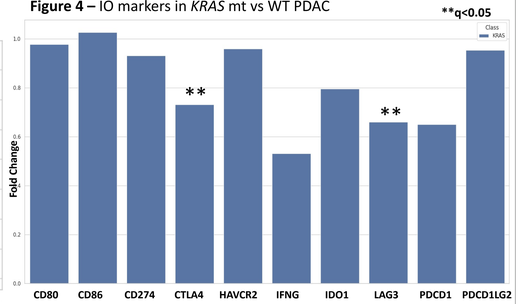



**Figure 4 –** IO markers in *KRAS* mt vs WT PDAC



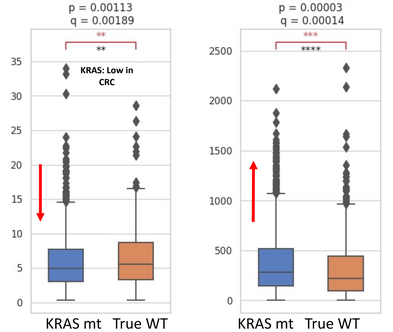




**Table 2 –** IO marker median expression for *KRAS* mt subtypes compared to *KRAS* WT. Bold/highlighted values represent q<0.05 compared to WT turmos.

| Gene | True WT N=900 | G12X N=623 | G12A N=16 | G12C N=73 | G12D N=1726 | G12S N=2 | G12V N=1269 | G13X N=21 | Other N=35 | Q61X N=319 |
|---|---|---|---|---|---|---|---|---|---|---|
| *CD80* | 6.49 | 5.81 | 6.90 | 6.47 | **6.76** | 4.29 | 6.13 | 7.95 | 6.51 | 5.86 |
| *CD86* | 9.40 | 9.12 | 11.66 | 9.29 | **10.00** | 5.11 | 9.59 | 11.30 | 9.22 | 8.99 |
| *CD274* | 5.87 | 4.73 | 5.69 | 5.86 | 5.86 | 12.50 | 5.33 | 7.31 | 6.54 | 5.10 |
| *CTLA4* | 2.48 | **1.69** | 2.60 | 1.81 | 1.85 | 2.06 | **1.85** | 2.46 | 1.87 | 1.63 |
| *HAVCR2* | 22.22 | 19.64 | 26.51 | 20.01 | **22.04** | 4.91 | 21.60 | 25.23 | 20.72 | 19.68 |
| *IFNG* | 0.80 | **0.39** | 0.42 | 0.39 | **0.44** | 0.19 | **0.43** | 0.57 | 0.52 | **0.37** |
| *IDO1* | 5.79 | 4.05 | 4.42 | 3.67 | 5.18 | 7.45 | 4.33 | 5.85 | 5.83 | 3.64 |
| *LAG3* | 1.32 | **0.78** | 0.93 | 0.95 | **0.89** | 2.63 | **0.89** | 0.83 | 1.11 | **0.78** |
| *PDCD1* | 1.06 | **0.67** | 0.84 | 0.75 | **0.72** | 0.48 | **0.67** | 0.73 | 0.67 | **0.64** |
| *PDCD1LG2* | 1.35 | 1.13 | 1.52 | 1.22 | 1.36 | 1.30 | 1.28 | 1.48 | 1.10 | 1.24 |

## Conclusions

- *KRAS* mutants were seen in 81% of PDAC and 48% of CRC.
- G12D was the most common *KRAS* variant and was seen in 43% of PDAC and 32% of CRC while *KRAS* G12C variant comprised 2% of PDAC and 7% of CRC.
- For IO related markers: In PDAC, *KRAS* mt were associated with lower prevalence of MSI-H/dMMR when compared to *KRAS* WT (0.9% vs 1.9%, *p*=0.027). PDL1 expression was significantly lower in *KRAS* wt (12%) compared to *G12D* (19%) and *G13X* (33%), similar to previous observations in CRC. However, when considering TMB, in PDAC, *G12D* (1%), *G12V* (1%) and *Q61* (1%) mutations had significantly lower TMB-H than *RAS* wt tumors (4%); in contradiction to CRC.
- The TME of *KRAS* mt PDAC showed significantly higher infiltration with M1 macrophages and cancer-associated fibroblasts (CAFs), as well as lower M2 macrophages, $CD4^+$ & $CD8^+$ T cells, T-reg, NK, myeloid dendritic and endothelial cells compared to *KRAS* wt (CRC showed similar but more pronounced in PDAC).
- Immune regulatory markers such as CTLA-4 and LAG3 are downregulated in *KRAS* mt PDAC (significant in *KRAS* mutants harboring G12D, G12V, Q61 and some rare variants.
- These results demonstrate that the TME of PDAC and CRC shows immune-cold features. Tailored immunotherapeutic strategies would have to overcome these barriers in *KRAS* mt PDAC and CRC, possibly in combination with molecularly targeted treatment strategies.