Increased neutrophil infiltration and lower prevalence of tumor mutation burden and microsatellite instability are hallmarks of RAS mutant colorectal cancers

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Background:

The tumor microenvironment (TME) of colorectal cancers (CRC) is modulated by oncogenic drivers such as KRAS. The TME comprises a broad landscape of immune infiltration. What is less known is how tumor genomics associates with the immune cell landscape. The objective of this study was to characterize immune cell types in RAS wild-type (WT) and mutant (MT) CRC, and to examine the prevalence of immuno-oncologic (IO) biomarkers (e.g. tumor mutation burden (TMB), PD-L1, MSI-H/dMMR) in these tumors. We performed genomic and transcriptomic analysis to confirm associations of mutant RAS with immune infiltration of the TME conducive to metastasis vs. potential response to immunotherapies.

Methods:

A total of 7,801 CRC were analyzed using next-generation DNA sequencing (NextSeq, 592 Genes and WES, NovaSEQ), IHC, and whole transcriptome RNA sequencing (NovaSeq) (Caris Life Sciences, Phoenix, AZ). MSI/MMR was tested by FA, IHC and NGS. TMB was classified as high or low based on a cut-off of > 10 mutations per MB). Immune cell fraction was calculated by QuantiSeq (Finotello 2019, Genome Medicine). Significance was determined by X^2 and Fisher-Exact and p adjusted for multiple comparisons (q) was <0.05.

Results:

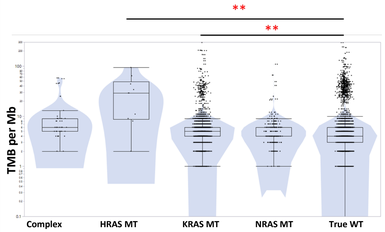
Mutant KRAS was seen in 48% of mCRC tumors; NRAS in 3.7%, HRAS in 0.1%. The distribution was similar in patients < or >= than 50 yrs. In MSS tumors, there was a significantly higher neutrophil infiltration in KRAS MT (median cell fraction 6.6% vs. 5.9%) and NRAS MT (6.9%) overall and also when individual codons were studied. B cells, M2 macrophages, CD8+ T cells, dendritic cells and fibroblasts were lower in KRAS mutant tumors; B cells and M1 macrophages are lower in NRAS (q<0.05). dMMR/MSI-H was significantly more prevalent in RAS WT (9.1%) than in KRAS (2.9%) or NRAS MT (1.8%) tumors, and highest in HRAS MT tumors (60%, q<0.05).TMB-H was more prevalent in RAS WT (11%) than KRAS (5.8%) or NRAS (5.1%) MT, and highest in HRAS MT tumors (70%, all q<0.05). In MSS tumors, KRAS MT tumors showed more TMB-H than WT (3.1% vs. 2.1%, q<0.05), especially in KRAS non 12/13/61 mutations (5.5%, vs. 2.1%, q<0.05) and G12C (4.4%, p<0.05). PD-L1 expression was studied: in MSS tumors, KRAS-G12D (10.4%) and G13 MT (11.8%) showed higher mutation rates than RAS WT tumors (q<0.05).

Results

Table 1: Patient demographics and subtype distribution

RAS mutation status in CRC	Females N	Males N	Total N	%	Median age
KRAS MT	1766	1983	3749	*48.1%*	61.0
NRAS MT	115	170	285	*3.7%*	60.0
HRAS MT	5	5	10	*0.1%*	66.5
KRAS MT/NRAS MT	8	9	17	*0.2%*	59.0
KRAS MT/HRAS MT	1	3	4	*0.1%*	61.5
True WT	1602	2134	3736	*47.9%*	62.0
Total	3497	4304	7801		

Figure 1a- TMB and RAS mutations in entire cohort.



Level	Minimum	Median	Maximum
Complex	5	**6**	58
HRAS MT	8.75	**29**	94
KRAS MT	4	**5**	296
NRAS MT	4	**4**	109
True WT	3	**4**	294

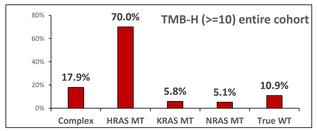
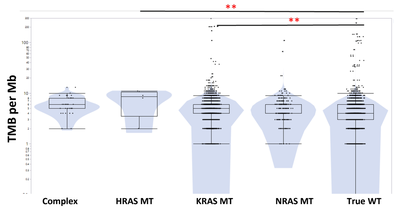


Figure 1b- TMB and RAS mutations in MSS cohort.



Level	Minimum	Median	Maximum
Complex	2	**6**	13
HRAS MT	2	**8.5**	11
KRAS MT	0	**5**	296
NRAS MT	1	**4**	109
True WT	0	**4**	294

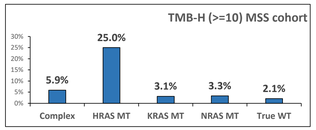


Figure 2: IO markers in RAS MT vs RAS WT. TMB-H and MSI-H/dMMR were more prevalent in RAS WT than KRAS/NRAS/HRAS MT. PD-L1 expression was less prevalent in KRAS/NRAS/HRAS MT than in RAS WT.

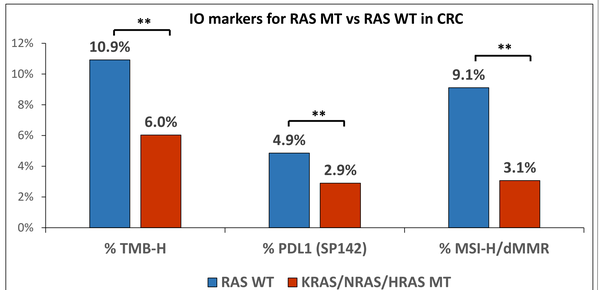
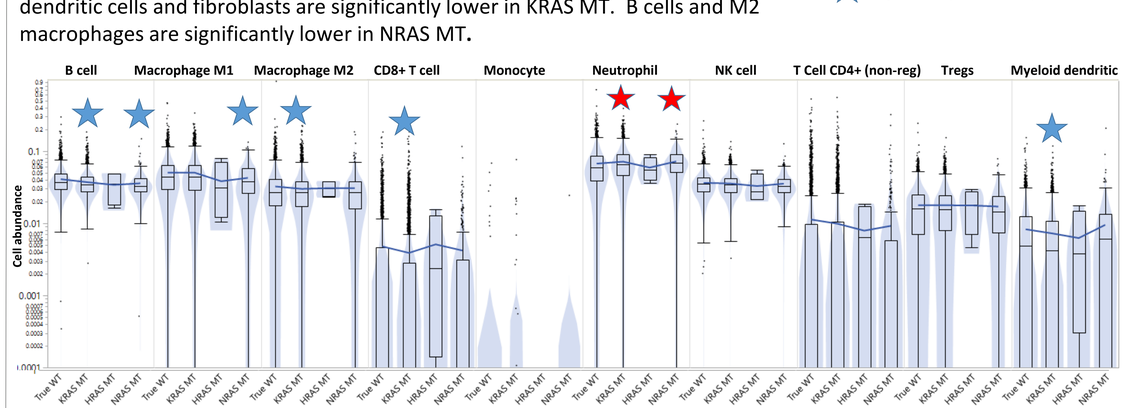
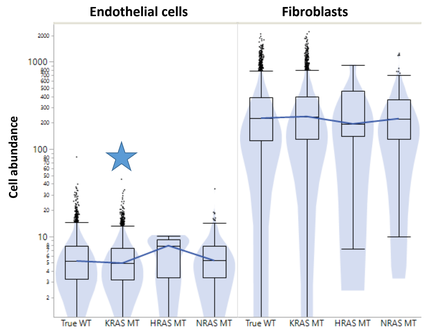


Figure 3: Lymphocyte infiltration in MSS tumors. KRAS MT and NRAS MT were significantly higher in neutrophil infiltration. B cells, M2 Macrophages, CD8+T cells, dendritic cells and fibroblasts are significantly lower in KRAS MT. B cells and M2 macrophages are significantly lower in NRAS MT.





Summary of significant immune infiltrate results compared to WT tumors		
Immune cell	KRAS MT	NRAS MT
B cell	Significantly lower	Significantly lower
Macrophage M1	N.S.	Significantly lower
Macrophage M2	Significantly lower	N.S.
CD8+ T cell	Significantly lower	N.S.
Neutrophil	Significantly higher	Significantly higher
Myeloid dendritic	Significantly lower	N.S.
Endothelial cells	Significantly lower	N.S.
Fibroblasts	N.S.	N.S.

Conclusions:

- While RAS mutations were more prevalent overall than generally reported, no significant differences in age were observed.
- TMB-H/PD-L1-H and MSI-H/dMMR is significantly higher in RAS WT than in RAS MT. In MSS tumors, RAS mutations are associated with higher TMB almost unanimously.
- KRAS and NRAS mutations are associated with increase neutrophil abundance, while HRAS shows no difference. Overall CD8+ T cells and B cells are less abundant in KRAS and NRAS mutants.
- These results demonstrate significant differences in the TME of RAS mutant CRC that identify variable susceptibilities to immuno-oncologic agents, and provide further detailed characterization of heterogeneity between RAS variants, at the molecular as well as immunogenic levels.