Impact of RAS Mutations on Immunologic Characteristics of the Tumor Microenvironment in Patients With Microsatellite Instability-High or Mismatch-Repair–Deficient Colorectal Cancer

Abstract 3067

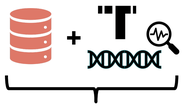
Mohamed E. Salem[1], Thierry Andre[2], Sherif El-Refai[3], Christopher Thompson[3], Elizabeth Mauer[3], Scott Kopetz[4], Josep Tabernero[5], Frank Sinicrope[6], Jeanne Tie[7], Thomas J George[8], Eric Van Cutsem[9], Sara Lonardi[10], Michael J Overman[2], and David Foureau[1]

[1]Levine Cancer Institute, Charlotte, NC, USA, [2]Assistance Publique Hospitaux de Paris, Paris, France, [3]Tempus Labs Inc, Chicago, IL, USA, [4]MD Anderson, Houston, TX, USA, [5]Vall d'Hebron University Hospital, Barcelona, Spain, [6]Mayo Clinic, Rochester, MN, [7]Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [8]University of Florida, Gainesville, FL, USA, [9]University of Leuven, Belgium, [10]Instituto Oncologico Veneto, Italy

INTRODUCTION

- The KEYNOTE-177 trial demonstrated pembrolizumab's superiority over first-line chemotherapy in MSI-H/dMMR colorectal cancer (CRC), but patients with KRAS or NRAS mutations did not show the same benefit.
- The impact of RAS mutations on the tumor immune microenvironment (TIME) of microsatellite instability-high (MSI-H)/mismatch-repair-deficient (dMMR) CRCs has not been well characterized.
- In this study, we evaluated the relationship between *RAS* mutations and the TIME in a real-world cohort of patients with MSI-H/dMMR CRC.

METHODS



De-identified records of patients (N=463) with CRC sequenced by the Tempus xT next-generation sequencing (NGS) assay were retrospectively analyzed

Loci analyzed, IHC — MSI-H was determined by assessment of 239 loci and dMMR by IHC

NGS, IHC data — Tumor mutational burden (TMB), neoantigen tumor burden (NTB, ScanNeo), PD-L1 expression, immune infiltration, canonical immune pathways (82 gene set signatures)

Overview of Demographics

Characteristic	Overall, N=463	*RAS*^{WT}, n=353	*RAS*^{mut}, n=110
***Gender, n (%)**			
Female	271 (59%)	219 (62%)	52 (47%)
Unknown	1	1	0
***Age, Median (IQR)**	69 (57, 78)	71 (62, 79)	57 (47, 69)
Unknown	128	106	22
Race, n (%)			
White	228 (83%)	182 (83%)	46 (79%)
Black/African American	21 (7.6%)	15 (6.9%)	6 (10%)
Asian	3 (1.1%)	3 (1.4%)	0 (0%)
Other	24 (8.7%)	18 (8.3%)	6 (10.3%)
Unknown	187	135	52
***Stage, n (%)**			
Stage I	19 (4.9%)	17 (5.7%)	2 (2.3%)
Stage II	83 (22%)	69 (23%)	14 (16%)
Stage III	90 (23%)	76 (26%)	14 (16%)
Stage IV	192 (50%)	135 (45%)	57 (66%)
Unknown	79	56	23

**Indicates significance by RAS^{mut} status following Pearson's Chi-squared test, Fisher's exact test, or Wilcoxon rank-sum test; Age reflects data at diagnosis; Stage reflects data available closest to biopsy collection. ‡ Percentages reflect number of patients for each metric out of all patients with reported data for that respective metric (total less missing population).*

Table 1. Description of the patient cohort analyzed.

SUMMARY

- In a cohort of 463 patients with MSI-H/dMMR CRC, **TIME of *RAS*^{mut} tumors had lower neoantigen production and lower tumor inflammation than *RAS*^{WT} tumors**.
- Overall, these data suggest that **MSI-H/dMMR *RAS*^{mut} CRCs are less immunogenic** and **contain a TIME that may be less sensitive to immune checkpoint blockade** compared to MSI-H/dMMR *RAS*^{wt} CRCs.

RESULTS

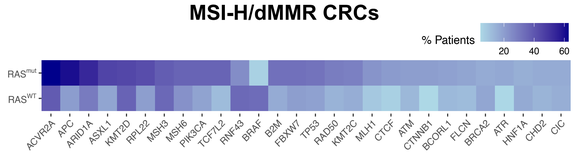
Molecular Characteristics

Characteristic	Overall, N=463	*RAS*^{WT}, n=353	*RAS*^{mut}, n=110	p-Value
MSI-H, n (%)	446 (97%)	340 (97%)	106 (97%)	0.8
Unknown	5	4	1	
TMB-H, n (%)	432 (96%)	328 (97%)	104 (95%)	0.2
Unknown	15	15	0	
***NTB, Median (IQR)**	15 (10, 20)	16 (12, 20)	12 (9, 18)	<0.001
Unknown	39	36	3	
†PDL-1+, n (%)	39 (27%)	33 (31%)	6 (15%)	0.058
Unknown	318	247	71	

**Indicates significance by RAS status following Pearson's Chi-squared test, Fisher's exact test, or Wilcoxon rank-sum test.*
†PD-L1 Status was only available for patients whose samples were assessed in-house by immunohistochemical staining.
‡ Percentages reflect number of patients for each metric out of all patients with reported data for that respective metric (total less missing population).

Table 2. *RAS*^{mut} tumors had a similar TMB but display significantly lower median NTB than *RAS*^{WT} (p<0.001). In a reduced cohort of patient tumors that underwent internal IHC, RAS^{mut} tumors demonstrated a trend towards less PD-L1 positivity, albeit non-significant in the reduced cohort.

Genomic Differences Between *RAS*^{mut} and *RAS*^{WT} MSI-H/dMMR CRCs



**Comparisons were made by Pearson's Chi-squared tests with false-discovery rate correction for multiple comparisons. Percentages reflect the proportion of patients in each group with a Pathogenic or Likely-Pathogenic somatic short variant.*

Figure 1. Significant differences were observed in genomic alterations co-occurring with *RAS*^{mut} compared to *RAS*^{WT}, including *MLH1* (23% vs. 8.8%, q<0.001), *MSH6* (36% vs. 24%, q=0.017), *APC* (60% vs. 20%, q<0.001), *ARID1A* (54% vs 30%, q<0.001), *PIK3CA* (36% vs 19%, q<0.001), and *TP53* (19% vs. 32% vs 19%, q=0.014).

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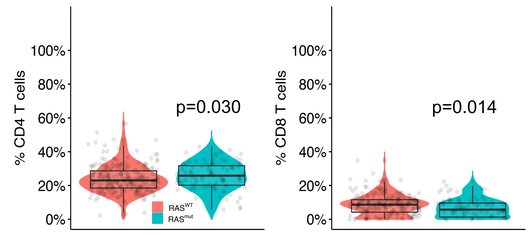
Correspondence: Mohamed.Salem@atriumhealth.org

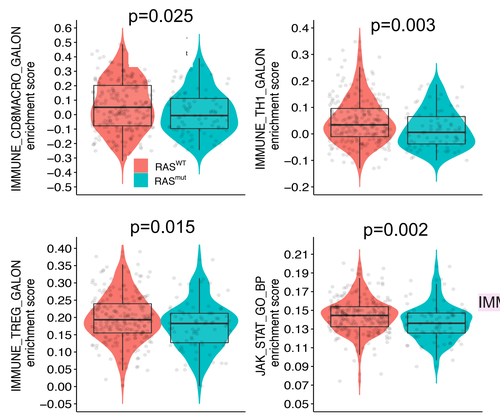
RAS^{mut} Impacts Tumor CRCs Immune Microenvironment



**Infiltrating immunocytes were compared by Wilcoxon rank-sum tests.*
**N_{Total} patient tumors with RNA-Seq. data available= 327.*

Figure 2. The proportions of immune cells present in each tumor were estimated from RNA-seq data. Compared to MSI-H/dMMR *RAS*^{WT}, MSI-H/dMMR *RAS*^{mut} CRCs had lower percentage of CD8+ T cell but higher percentage of CD4+ T cell infiltration (p<0.05).

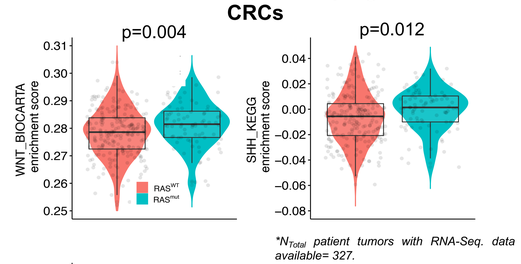
Tumor Inflammation Decreases in *RAS*^{mut} CRCs



**N_{Total} patient tumors with RNA-Seq. data available= 327.*

Figure 3. Tumor inflammation is mainly downregulated (**see Figure 5**) in *RAS*^{mut} tumors, including key pathways shown above: cytokine signaling (JAK-STAT, TH1), and adaptive immune events (CD8+ T cell, Tregs).

Cancer Stem Cell Pathways Are Upregulated *RAS*^{mut} CRCs



**N_{Total} patient tumors with RNA-Seq. data available= 327.*

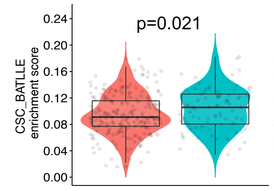


Figure 4. Cancer Stem Cell pathways showed significant upregulation (**see Figure 5**), including Sonic Hedgehog and WNT /Catenin signaling pathways

**Unadjusted for false discovery.*

Immune-related Pathways Differentially Expressed by *RAS*^{mut} Status

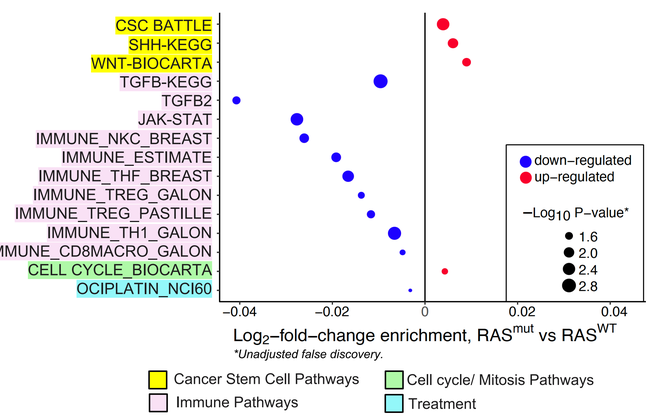


Figure 5. Pathway enrichment scores computed through GSVA (Gene Set Variation Analysis) were compared between RAS^{mut} and RAS^{WT} via differential expression analysis. Differentially expressed pathways (at 5% alpha level) are shown. No pathways were obtained when controlled for false discovery however, these serve as hypothesis-generating findings.