Impact of *RAS* and *BRAF V600E* mutations on tumor immune microenvironment and associated genomic alterations in patients with microsatellite instability (MSI) or DNA mismatch repair deficient (dMMR) colorectal cancers

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INTRODUCTION

- All international guidelines currently recommend routine testing of all CRC for MSI/dMMR for Lynch Syndrome screening, prognosis information and treatment guidance
- In metastatic setting, the analysis for all *RAS* and *BRAF* mutational status is recommended to select the most appropriate treatment choice
- The impact of *RAS* and *BRAF* mutations on prognosis and treatment effect in MSI/dMMR patients is not understood in either localized or metastatic setting

METHODS

(N = 448)
Stage I-IV MSI/dMMR Colorectal Cancer patients

+

Molecular profiling with Tempus xT and xR assays*

Retrospective, De-identified Clinico-molecular Real-World Data (RWD) of patients

*Tempus xT assay - DNA-seq of 595-648 genes at 500x coverage
Tempus xR assay - whole-exome capture RNA-seq

- MSI status was determined by assessment of 44 or 239 loci by NGS
- dMMR was determined by Tempus IHC testing
- Tumor mutational burden (TMB), tumor neoantigen burden (TNB), PD-L1 positivity, immune infiltration, and canonical immune pathways (82 gene set signatures) were analyzed

Characteristic	Wildtype, N=229[1]	*RAS*^{mut}, N=100[1]	*BRAF V600E*^{mut}, N=119[1]	p-value[2]
Sex				0.003
Female	135 (59%)	46 (46%)	82 (69%)	
Male	94 (41%)	54 (54%)	37 (31%)	
Age, Median (Range)	66 (21, 85)	56 (23, 86)	73 (55, 86)	<0.001
Unknown	29	6	22	
Race				0.3
White	131 (57%)	49 (49%)	67 (56%)	
Black/African American	13 (5.7%)	5 (5.0%)	4 (3.4%)	
Asian	6 (2.6%)	0 (0%)	1 (0.8%)	
Other/Unknown	79 (34%)	46 (46%)	47 (39%)	
Ethnicity				0.005
Not Hispanic or Latino	79 (83%)	27 (73%)	37 (100%)	
Hispanic or Latino	16 (17%)	10 (27%)	0 (0%)	
Unknown	134	63	82	
Stage at Diagnosis				0.5
0	1 (0.6%)	0 (0%)	0 (0%)	
1-2	56 (34%)	19 (27%)	22 (26%)	
3-4	108 (65%)	51 (73%)	64 (74%)	
Unknown	64	30	33	

[1] n (%)
[2] Fisher's exact test; Kruskal-Wallis rank sum test; Pearson's Chi-squared test

Table 2. Overview of Cohort Demographics

RESULTS

Overview of molecular characteristics

Characteristic	wild-type, n = 229[1]	*RAS* mutant, n = 100[1]	*BRAF mutant* n=119[1]	p-value[2]
MSI-H, n (%)	217 (96%)	98 (98%)	119 (100%)	0.064
Unknown	3	0	0	
TMB-H, n (%)	206 (96%)	95 (95%)	119 (100%)	0.024
Unknown	14	0	0	
NTB, Median (IQR)	16 (12, 21)	12 (9, 19)	15 (11, 20)	0.003
Unknown	33	2	2	
†**PDL-1+, n (%)**	24 (31%)	2 (5.9%)	8 (31%)	0.013
Unknown	152	66	93	

[1] n (%)
[2] Fisher's exact test; Kruskal-Wallis rank sum test; Pearson's Chi-squared test

Table 2. Most patients were MSI-High (MSI-H) and TMB-High (TMB-H). However, double wild-type and RAS^{mut} tumors were significantly more likely to be TMB-Low, although a small minority (~4-5%). In a reduced cohort, RAS^{mut} tumors had a significantly lower median neoantigen tumor mutation burden (NTB) than BRAFV600E^{mut} or wildtype tumors. RAS^{mut} tumors were both less likely to be PD-L1 positive.

Immune-related pathways differentially expressed by RASmut & BRAF V600Emut CRC tumors

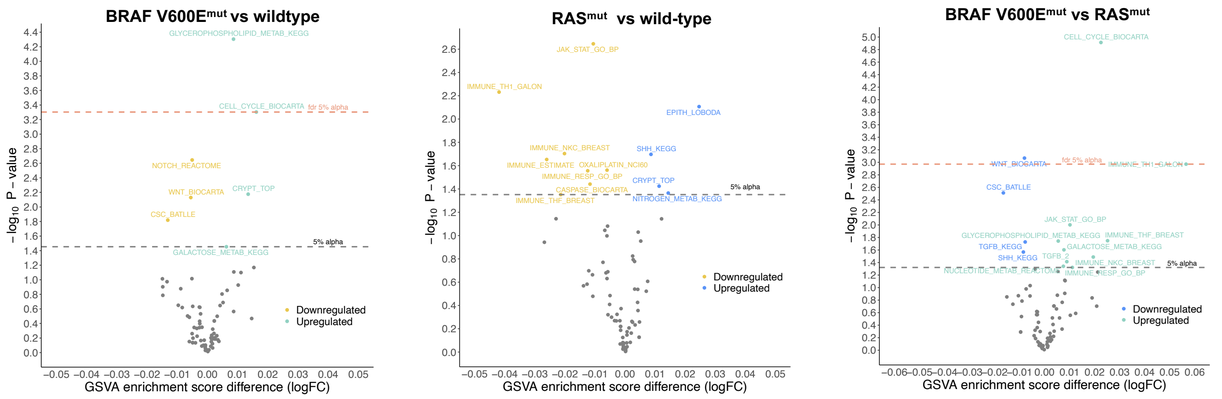

Figure 1. BRAF V600E mutated tumors showed a significant upregulation of mitotic and metabolic pathways compared with wild-type tumors. By contrast, RAS^{mut} tumors had an increased stemness (SHH pathway) and a widespread downregulation of inflammatory pathways compared with wild-type tumors. Pathway enrichment scores were computed through GSVA and compared between groups via differential expression analysis. Differentially expressed pathways (at 5% alpha level) are shown. Pathways differentially expressed after false discovery adjustment are also represented.

RAS^{mut} and *BRAF V600E*^{mut} impact on CRCs tumor immune microenvironment (TiME)

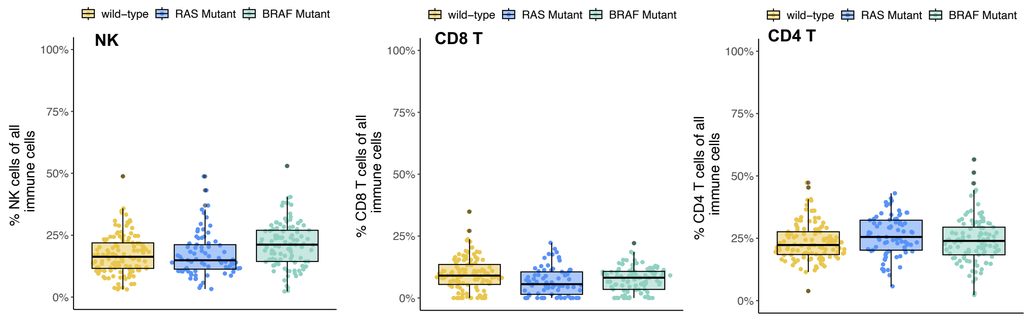

Figure 2. The proportion of natural killer (NK) cells was significantly higher in *BRAFV600E*^{mut} compared to *RAS*^{mut} and wildtype tumors (median 21% vs. 15% vs.16%, p <0.001). The proportion of cytotoxic CD8+ T cells was significantly lower in the *RAS*^{mut} compared to *BRAFV600E*^{mut} and wildtype tumors (median 6% vs. 8% vs. 9%, p=0.004). Both *RAS*^{mut}/*BRAF V600E*^{mut} tumors presented higher CD4+ helper T cell infiltrate compared with wildtype tumors (26% vs. 24% vs. 22%, p=0.037). Proportions of infiltrating immune cells were estimated through RNA-seq.

Immune checkpoint expression by *RAS*^{mut} & *BRAFV600E*^{mut} CRCs TiME

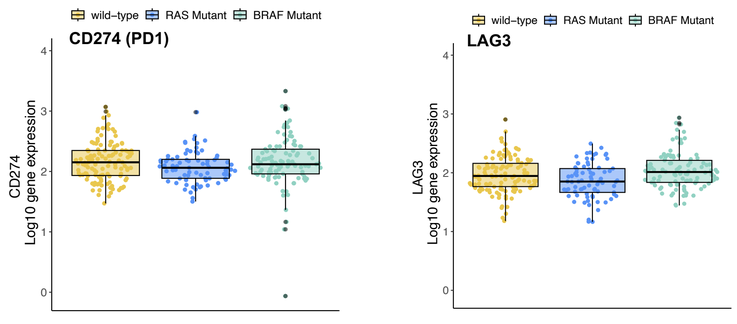

Figure 3. *RAS*^{mut} tumors tend to have lower PD1 expression compared with *BRAFV600E*^{mut} or wildtype tumors (median Log10 gene expression 2.06 vs. 2.12 vs. 2.15, p=0.058). *BRAFV600E*^{mut} tumors had higher *LAG3* expression than *RAS*^{mut} and wildtype tumors (median Log10 gene expression 2.01 vs. 1.85 vs. 1.95, p=0.003). Immune checkpoint expression by CRC tumor immune microenvironment was estimated by RNA-seq.

SUMMARY

The data discussed in this study suggest that MSI/dMMR CRC harboring *RAS* mutations are less immunogenic and appear to contain a lower tumor inflammatory profile of TIME than *RAS*^{wt} or *BRAF V600E* mutated tumors. Further analysis and validation are needed to confirm our data.

Presenting Author Declaration of Interest: No conflict of interest to declare
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