Molecular characterization of microsatellite stable (MSS) colorectal cancer (CRC) patients with a *BRAF* ^{V600E} mutation

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INTRODUCTION

- A *BRAF*^{V600E} mutation is an unfavorable prognostic biomarker for CRC and is associated with short-lived treatment response to *BRAF* and *EGFR* blockade.
- Anti PD-1 therapies are ineffective in MSS CRC but demonstrate efficacy in combination with *BRAF* + *EGFR* inhibition for MSS *BRAF*^{V600E} CRC.
- A comprehensive characterization of MSS *BRAF*^{V600E} CRC as an immunologically distinct subpopulation of MSS CRC has not been performed.

Aim: Here, we characterize the clinicopathological and transcriptomic features of MSS *BRAF*^{V600E} CRC patients, relative to *BRAF*^{V600E} WT MSS CRC patients.

METHODS

- De-identified records of MSS CRC patients were retrospectively analyzed from the Tempus clinicogenomic database. CMS subtypes were derived using the CMScaller algorithm.
- Categorical and continuous variables were compared using chi-squared (CS) test and Wilcoxon rank sum test (W), respectively.
- Overall survival was assessed using Univariate Cox regression analysis with risk set adjustment (RSA) method.
- The association between demographical, clinicopathological, and immunological factors was independently tested within each cohort.
- The significance of these associations, stratified by BRAFV600E status, was determined using the Likelihood Ratio Test (LRT).

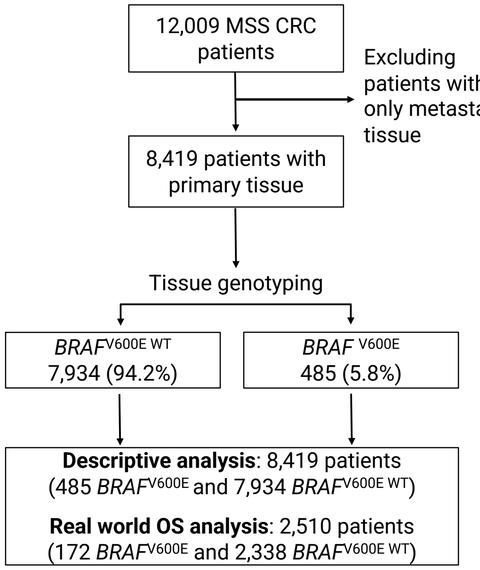

Figure 1. Flowchart depicting the inclusion of data in this study.

SUMMARY

- *BRAF*^{V600E} exhibited immune activation characteristics that were not observed in the *BRAF*$^{V600E\ WT}$ group.
- Our findings support investigation of novel immune-based therapeutic strategies of MSS *BRAF*^{V600E} CRC as an immunologically distinct subpopulation of MSS CRC.

RESULTS

Demographical differences stratified by *BRAF*^{V600E} status

		BRAF $^{V600E}_{WT}$	*BRAF* ^{V600E}	P-value
Age (median)		60 (50, 69)	65 (54, 74)	<0.001
Ethnicity	Not Hispanic or Latino	2,499 (82%)	170 (89%)	0.008
	Hispanic or Latino	551 (18%)	20 (11%)	
Gender	Female	3,268 (41%)	263 (54%)	<0.001
	Male	4,666 (59%)	222 (46%)	
Race	White	3,577 (74%)	241 (83%)	NA
	Black or African American	649 (13%)	19 (6.6%)	
	Other	368 (7.6%)	20 (6.9%)	
	Asian	218 (4.5%)	7 (2.4%)	
TNM stage	I	182 (2.3%)	8 (1.6%)	0.3
	II	683 (8.6%)	33 (6.8%)	
	III	1,856 (23%)	129 (27%)	
	IV	5,193 (65%)	313 (65%)	

Table 1. Demographics of the MSS cohort used in this study excluding unknown entries with statistical significance indicated for the difference in distributions.

BRAF^{V600E} is enriched in CMS1 and right-sided CRC

- Immune activated subtype CMS1 is significantly enriched in *BRAF*^{V600E} (CS p<0.001).
- *BRAF*^{V600E} is associated with right CRC (CS p<0.001).

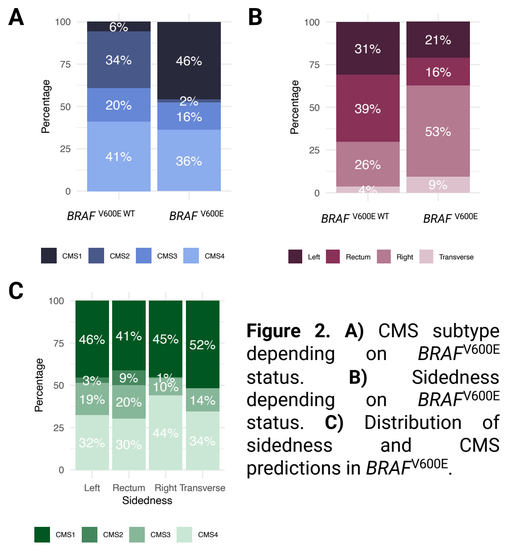

Figure 2. A) CMS subtype depending on *BRAF*^{V600E} status. **B)** Sidedness depending on *BRAF*^{V600E} status. **C)** Distribution of sidedness and CMS predictions in *BRAF*^{V600E}.

Enhanced immune activation associated with *BRAF*^{V600E}

In *BRAF*$^{V600E\ WT}$ the following immune-associated changes were observed:

- Increased PD-L1 detection (CS p=0.03).
- Altered TCR gamma Shannon entropy density (KS p< 0.001).
- Increased immune infiltration (W p<0.001), with less NK cells and Neutrophils (W p<0.001) and more CD8, regulatory T cells, Macrophages M2 and B cells (W p<0.001).

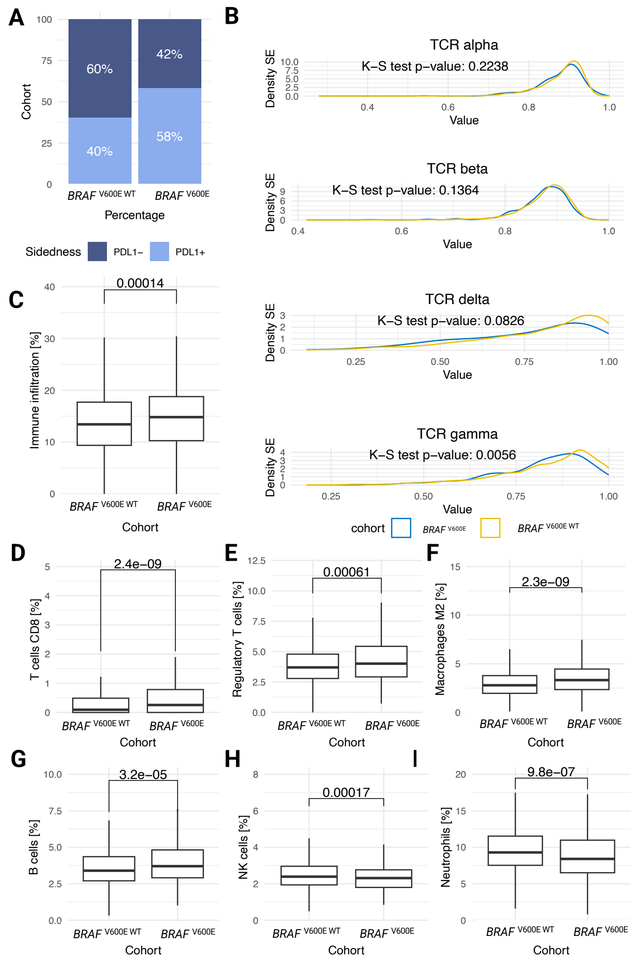

Figure 3. A) IHC based PD-L1 detection grouped by *BRAF*^{V600E} status. **B)** T-cell receptor Shannon entropy density depending on *BRAF*^{V600E} status. **C)** Percentage of immune cell infiltration in the tissue depending on *BRAF*^{V600E} status. **D-I)** Prevalence of immune cells in primary tissue depending on *BRAF*^{V600E} status.

Differential impact on overall survival (OS) by *BRAF*^{V600E} status

- In *BRAF*$^{V600E\ WT}$ significantly increased hazard ratios (HR) were observed for transverse CRC, CMS1 and CMS3 (LRT p<0.01). A lower HR was associated with diagnosis at < 65 years old (LRT p<0.0001), < median % of neutrophils, CD8 T cells and B cells (p<0.001).
- In *BRAF*^{V600E}, HR was decreased for a < median % of neutrophils in primary tissue (LRT p = 0.02).
- Stage 3 cancer was associated with a significantly decreased HR (*BRAF*^{V600E} LRT p-value = 0.044, *BRAF*$^{V600E\ WT}$ LRT p-value = 0.014).

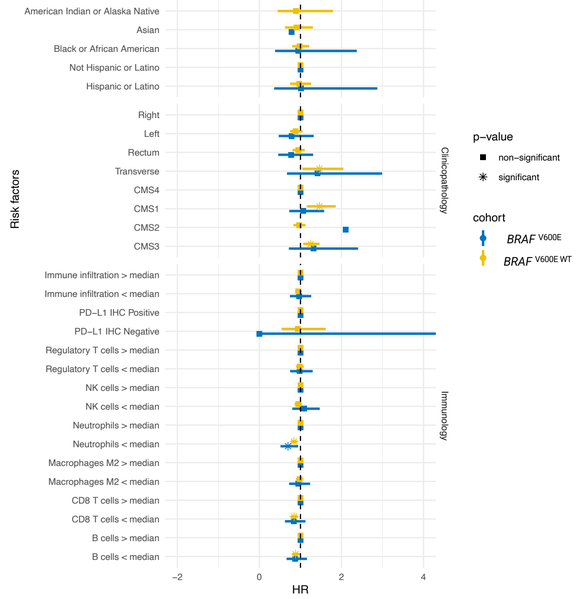

Figure 4. Univariate Cox regression analysis indicating the associations between demographics, clinicopathological, immunological factors and OS within *BRAF*^{V600E} status.

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