Investigating the clinical and molecular characteristics of class II and III BRAF mutations and their response to anti-EGFR therapy in MSS CRC: A comprehensive analysis.

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Significance and Background

- BRAF mutations represent a highly heterogeneous group of molecular alterations seen in colorectal cancer (CRC).
- Class I BRAF mutation (V600) render aggressive biology to CRC and poor response to EGFR blockade therapy.
- Currently there are limited data on clinical and molecular features of class II and III BRAF mutations and their response to EGFR blockade therapy.
- In this large comprehensive cohort study, we investigated the clinical and molecular characteristics of BRAF mutation classes and their impact on clinical outcomes in a large cohort of patients with mismatch proficient-microsatellite stable CRC.

Methods

- A total of 24,327 pMMR/MSS CRC specimens were profiled by next-generation sequencing (592-gene, NextSeq; WES, WTS NovaSeq) (Caris Life Sciences, Phoenix, AZ).
- BRAF mutations were detected by NGS and classified using published literature (Sahin et al. JCO OP 2021).
- Interferon gamma signature (Cristescu et al. 2018) and MAPK pathway activity score (MPAS) (Wagle et al 2018) were calculated using RNA expression data (TPM: Transcript per million).
- Real-world overall survival information was obtained from insurance claims and calculated from tissue collection to last contact, while post-treatment survival from first of treatment to last contact.
- Kaplan-Meier estimates were calculated for molecularly defined cohorts using Cox-proportional hazard analysis. Significance was determined as p values of <0.05.

Table 1. Demographic and clinical characteristics of patients

		MSS Class 1	MSS Class 2	MSS Class 3	MSS WT	Total	P values
Gender	Female	720 (6.73%)	60 (0.56%)	145 (1.36%)	9776 (91.36%)	10701	<0.0001
	Male	548 (4.02%)	72 (0.53%)	178 (1.31%)	12828 (94.14%)	13626	
Age	Median Age	66	64	63	62	62	<0.0001
	Age IQR	57-74	55-70	53-72	52-70	53-71	
Race	Asian or Pacific Islander	24 (3.19%)	3 (0.40%)	8 (1.06%)	718 (95.35%)	753	<0.0001
	White	840 (6.55%)	70 (0.55%)	175 (1.36%)	11744 (91.54%)	12829 (	
	Black or African American	60 (1.86%)	15 (0.47%)	43 (1.33%)	3104 (96.34%)	3222	
	Other	51 (4.36%)	4 (0.34%)	17 (1.45%)	1097 (93.84%)	1169	
	Unknown	293 (4.61%)	40 (0.63%)	80 (1.26%)	5941 (93.50%)	6354	
Ethnicity	Hispanic or Latino	119 (4.22%)	17 (0.60%)	35 (1.24%)	2651 (93.94%)	2822	<0.0001
	Not Hispanic or Latino	865 (5.82%)	75 (0.50%)	201 (1.35%)	13727 (92.33%)	14868	
	Unknown	284 (4.28%)	40 (0.60%)	87 (1.31%)	6226 (93.81%)	6637	
Sidedness	Left-sided	361 (2.64%)	59 (0.43%)	172 (1.26%)	13058 (95.66%)	13650	<0.0001
	Right-Sided	541 (10.26%)	34 (0.64%)	83 (1.57%)	4617 (87.53%)	5275	
	Transverse	130 (13.04%)	4 (0.40%)	7 (0.70%)	856 (85.86%)	997	
	Other/Unclear	236 (5.36%)	35 (0.79%)	61 (1.38%)	4073 (92.46%)	4405	
Specimen Sites	Colon	709 (7.05%)	54 (0.54%)	120 (1.19%)	9176 (91.22%)	10059	<0.0001
	Liver	186 (3.60%)	35 (0.68%)	72 (1.39%)	4873 (94.33%)	5166	
	Rectum	70 (2.14%)	12 (0.37%)	58 (1.78%)	3124 (95.71%)	3264	
	Lung	33 (2.48%)	9 (0.68%)	18 (1.35%)	1270 (95.49%)	1330	
	Peritoneum	85 (8.99%)	5 (0.53%)	16 (1.69%)	839 (88.78%)	945	
	Lymph Node	38 (5.97%)	2 (0.31%)	12 (1.88%)	585 (91.84%)	637	
	Unclear/Other	147 (5.02%)	15 (0.51%)	27 (0.92%)	2737 (93.54%)	2926	

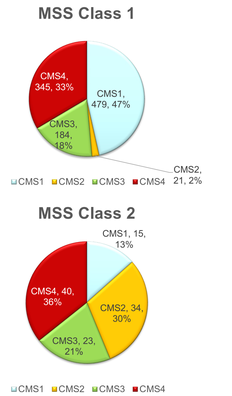


Figure 1. CMS classification of BRAF classes and BRAF WT CRC

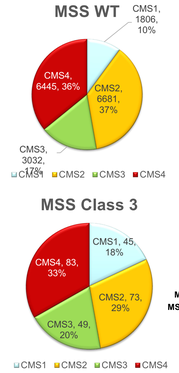
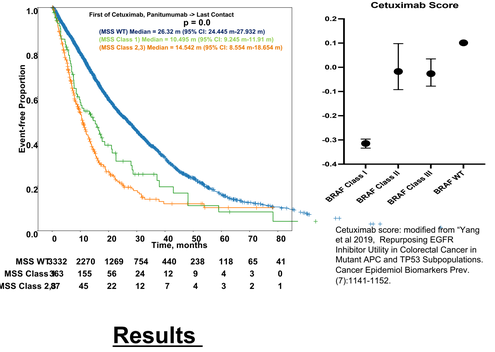
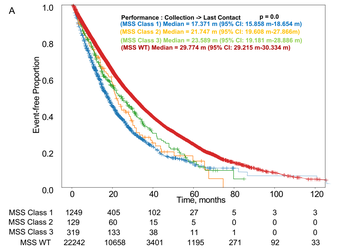


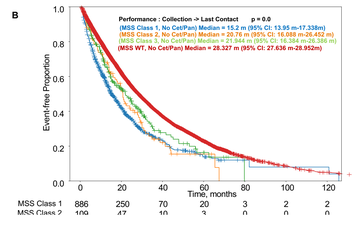
Figure 3 A) Survival outcomes of patients with BRAF distinct mutation classes and WT disease B) Cetuximab score for each BRAF class and WT.

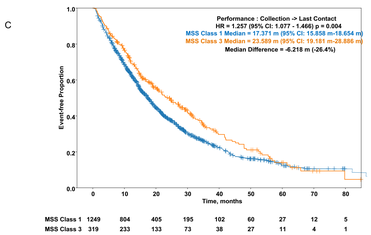


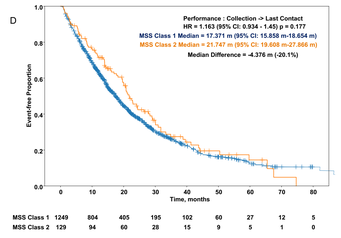
Cetuximab score: modified from "Yang et al 2019. Repurposing EGFR Inhibitor Utility in Colorectal Cancer in Mutant APC and TP53 Subpopulations. Cancer Epidemiol Biomarkers Prev. (7):1141-1152.

Figure 2. A) Overall OS outcomes of patients with BRAF WT, class I, II and IIII mutations B) those without anti-EGFR therapy, C) Class I vs III D) Class I vs II









Results

- A total of 1268, 132, and 323 patients with class I, II, and III BRAF mutations were identified. Class I BRAF mutations were significantly lower in African Americans (1.8%), and patients with class II and III had significantly higher left-sided tumors compared to patients with class I BRAF mutations (Table 1).
- Class I BRAF mutations were significantly enriched with (CMS1) (Class I, II and III: 47% vs. 13% vs. 18%) while class II and III BRAF mutations presented with more often CMS2 subtype (canonical) compared to class I (2%, 30% and 29%, p<0.05).
- Class I BRAF and KRAS/NRAS mutations were nearly mutually exclusive (0.5%), while KRAS mutation incidences were 13% and 27.4% for class II and class III (p<0.001), respectively.
- Patients with class II and III mutations had significantly better overall survival compared to patients with class I mutations (p<0.0001) and worse overall survival compared to wild-type BRAF pts. (P<0.01, Figure 1A). This was also observed among patients who did not receive anti-EGFR therapy (p<0.001, Figure 1B).
- Among patients treated with anti-EGFR, patients with class II and III BRAF mutations had significantly better post-anti-EGFR survival compared to class I BRAF mutants (14.5 months vs 10.4 months P<0.01)
- Cetuximab score was significantly lower for class I compared to Class II and III BRAF mutations (p<0.05) (Figure 3).

Conclusion

- Patients with class II and III BRAF mutated CRC present with clinically and biologically distinct diseases compared to patients with class I BRAF mutations, and they have improved outcomes compared to patients with class I, albeit worse than those with BRAF WT.
- While KRAS mutations are mutually exclusive with class I BRAF mutations, they can be concurrently seen with class II and III BRAF mutations, and class II and III BRAF mutations carry distinct CMS signatures compared to class I BRAF mutations.
- Class II and III BRAF mutations have better cetuximab scores compared to class I, and improved post-EGFR therapy survival outcomes were noted in the class II & III combined cohort.

References

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