Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Independent clinicopathological correlates of BRAF V600E immunohistochemistry-positive colorectal cancer: a retrospective cohort study

Man Man Han
Tianjian Laboratory of Advanced Biomedical Sciences, School of Convergence Medicine, Zhengzhou University

Siqi Lan
West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University

Zhigang Mao
West China Hospital, Sichuan University

Deng Tang
West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University

Yali Song
West China Hospital, Sichuan University

Si Chen
West China Hospital, Sichuan University

Yuemei Chen
West China Hospital, Sichuan University

Mi Su
West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University

Xu Dong Zhang
xu.zhang@zzu.edu.cn

Tianjian Laboratory of Advanced Biomedical Sciences, School of Convergence Medicine, Zhengzhou University

Yufang Wang
West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University

Research Article

Keywords: colorectal cancer, BRAF V600E, immunohistochemistry, mismatch repair deficiency, clinicopathological phenotype, tumor differentiation, tumor heterogeneity, body mass index

Posted Date: November 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11751748/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

BRAF V600E-positive colorectal cancer (CRC) is commonly regarded as a distinctive clinicopathological subtype associated with mismatch repair deficiency (dMMR), poor differentiation, and other adverse pathological features. However, whether this phenotype is consistent across clinical and pathological contexts remains unclear. We aimed to reassess the clinicopathological characteristics of BRAF V600E immunohistochemistry (IHC)-positive CRC and determine which associations remain independent after adjustment for potential confounders.

Methods

We retrospectively analyzed 1,130 patients with CRC treated at a single institution. Clinicopathological variables, BRAF V600E IHC status, mismatch repair status, and immunohistochemical markers including MLH1, MSH2, MSH6, PMS2, Ki67, HER2, CDX2, and CK20 were evaluated. BRAF V600E IHC-positive and IHC-negative tumors were compared overall and in prespecified subgroups defined by age of onset, sex, tumor differentiation, and tumor location. Multivariable Firth penalized logistic regression was used to assess independent associations of BRAF V600E IHC positivity with dMMR, perineural invasion, vascular invasion, and poor differentiation.

Results

BRAF V600E IHC positivity was detected in 105 of 1,130 CRCs (9.3%). In the overall cohort, BRAF V600E IHC positivity was associated with lower body mass index, poor differentiation, and dMMR. Poor differentiation occurred in 31.4% of BRAF V600E IHC-positive tumors versus 19.9% of IHC-negative tumors, while dMMR was observed in 14.3% versus 6.2%, respectively. After multivariable adjustment, BRAF V600E IHC positivity remained independently associated with dMMR (odds ratio 2.229, 95% confidence interval 1.083–4.378; P = 0.030), but not with poor differentiation, perineural invasion, or vascular invasion. Exploratory subgroup analyses showed different patterns of association across age, sex, tumor differentiation, and tumor location strata.

Conclusions

In this cohort, dMMR was the most robust independent correlate of BRAF V600E IHC positivity. Other clinicopathological associations were attenuated after multivariable adjustment and varied across exploratory strata. These findings support interpretation of BRAF V600E IHC-positive CRC within its broader molecular and pathological context.

Background

Colorectal cancer (CRC) is a biologically heterogeneous disease characterized by diverse molecular alterations, pathological features, and clinical behaviors [1]. It is the second most commonly diagnosed malignancy and the fourth leading cause of cancer death in China, with an estimated 517,100 new cases and 240,000 deaths in 2022 [2]. Among the molecular events involved in colorectal tumorigenesis, the BRAF V600E mutation is of particular interest because it results in constitutive activation of the mitogen-activated protein kinase (MAPK) pathway and has been linked to the serrated neoplasia pathway, CpG island methylator phenotype, MLH1 promoter methylation, and mismatch repair deficiency (dMMR) [3]. In clinical practice, assessment of BRAF V600E contributes to molecular classification, interpretation of dMMR tumors, prognostic stratification, and treatment selection in selected settings [4, 5]. Intriguingly, while the frequency of BRAF V600E in Western cohorts is 8.8% in The Cancer Genome Atlas (TCGA) and 10.8% in the Memorial Sloan Kettering Cancer Center (MSKCC) dataset, a recent large Chinese cohort identified this variant in only 3.2% of patients ($P < 0.001$ versus both TCGA and MSKCC cohorts). Moreover, this lower frequency was consistent across disease stages [6].

BRAF V600E-mutant CRC has often been described as a distinctive clinicopathological subtype. Previous studies have reported associations with proximal colon location, poor differentiation, mucinous or high-grade histology, older age, female sex, and dMMR [7, 8]. This has contributed to the conventional view that BRAF V600E defines a relatively recognizable CRC phenotype. However, many of these features frequently coexist, and it remains uncertain whether they represent independent characteristics of BRAF V600E itself or reflect the broader molecular and pathological context in which the alteration occurs [9]. In particular, the close relationship between BRAF V600E and dMMR raises the possibility that some pathological features commonly attributed to BRAF V600E-positive CRC may be overgeneralized [3].

Accumulating evidence suggests that BRAF V600E-mutant CRC may not represent a single homogeneous entity, but rather a spectrum of tumors with variable biological and clinicopathological characteristics [9]. Associations between BRAF V600E and tumor differentiation, invasive pathological features, and immunophenotype may differ according to patient age, sex, tumor location, and other clinicopathological variables [7, 10]. A reassessment of clinicopathological associations in molecularly defined subgroups may help clarify which associations remain independent after multivariable adjustment, particularly in relation to MMR status.

In this study, we conducted a real-world clinicopathological assessment in a cohort of 1,130 CRC patients from West China Hospital, where BRAF V600E and MMR status are routinely evaluated by immunohistochemistry (IHC). Specifically, we compared BRAF V600E IHC-positive and BRAF V600E IHC-negative tumors overall and across prespecified subgroups, and used multivariable Firth-penalized logistic regression to evaluate which associations persist after adjustment. Our aim was to reassess the clinicopathological phenotype associated with BRAF V600E IHC positivity and identify independent associations for outcomes of interest, with subgroup analyses treated as exploratory.

Methods

Study population

This retrospective study was conducted at West China Hospital, Sichuan University. Clinicopathological data from patients diagnosed with CRC at West China Hospital between November 2008 and June 2023 were

consecutively retrieved from institutional medical and pathology records. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of West China Hospital, Sichuan University (No. 2023 – 958). The requirement for individual informed consent was waived because of the retrospective design.

Case selection

Inclusion criteria were: (1) histologically confirmed colorectal adenocarcinoma; (2) surgical treatment at West China Hospital between November 2008 and June 2023; and (3) availability of the predefined core clinicopathological variables required for analysis, namely BRAF V600E status, mismatch repair (MMR) status, TNM stage, tumor differentiation, tumor location, and vascular invasion and perineural invasion (PNI) status.

Exclusion criteria were: (1) non-adenocarcinoma histology of the colorectal tumor, such as neuroendocrine neoplasm, lymphoma, melanoma, gastrointestinal stromal tumor, or metastatic disease from another primary malignancy; (2) multiple synchronous primary colorectal tumors/lesions; and (3) missing data on any core variable listed above.

Unavailability of ancillary immunohistochemical markers (Ki67, HER2, CDX2, or CK20) was not an exclusion criterion. Cases without available staining were retained with the corresponding marker coded as “Unknown” and included in analyses (with “Unknown” treated as a separate category in regression models).

A total of 1,130 CRCs met the inclusion criteria.

Variable definitions

Tumor stage was classified according to the AJCC TNM staging system, 8th edition (AJCC 8th) [11], as adopted in our institution’s clinical and pathology record documentation. Tumor differentiation was graded according to the WHO classification as well differentiated (G1), moderately differentiated (G2), or poorly differentiated (G3). Because the number of G1 tumors was small (n = 23, 2.0%), G1 and G2 tumors were combined into a non-poorly differentiated group for selected comparative analyses.

Tumor location was classified as colon or rectum. Age at diagnosis was dichotomized as early-onset CRC, defined as diagnosis before 50 years of age, and late-onset CRC, defined as diagnosis at or after 50 years of age.

Immunohistochemical evaluation and scoring

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tumor sections according to institutional protocols, with appropriate antibody-specific positive and negative tissue controls run in parallel.

BRAF V600E was assessed using the anti-BRAF V600E (VE1) mouse monoclonal antibody (Ventana Medical Systems, Tucson, AZ; #760–5095). Specific granular cytoplasmic staining in tumor cells was evaluated; purely nuclear staining was regarded as nonspecific and not counted. Tumors were classified as BRAF V600E-positive when diffuse, unequivocal cytoplasmic staining of at least weak intensity was present in the majority of tumor

cells (conventionally ≥ 80%); staining confined to only scattered individual tumor cells, or weak background-like staining, was classified as negative or indeterminate; molecular verification is recommended in such cases. Known BRAF V600E-mutant melanoma and known BRAF wild-type appendix tissue were used as positive and negative controls, respectively, on the same staining run. Because BRAF V600E status was assessed by IHC, this classification represents IHC-detected BRAF V600E-associated protein expression rather than a molecularly confirmed mutation.

Mismatch repair proteins MLH1 (Zhongshan Golden Bridge; #ZM-0154), MSH2 (#ZA-0622), MSH6 (#ZA-0541), and PMS2 (#ZA-0542) were evaluated by nuclear staining only. Retained expression was defined as moderate-to-strong nuclear staining in tumor cells comparable in intensity to the internal control, in ≥ 10% of tumor cells. Loss of expression was defined as complete absence of tumor cell nuclear staining in the presence of well-stained internal control cells (lymphocytes, stromal fibroblasts, and adjacent non-neoplastic intestinal epithelium). Sections in which internal control cells showed no staining were considered invalid and were not reported. Tumors showing loss of one or more MMR proteins were classified as dMMR; tumors retaining all four proteins were classified as pMMR.

Ki67 (Zhongshan Golden Bridge; #ZM-0167) was scored on nuclear staining only; nonspecific cytoplasmic staining was disregarded. The proliferation index was assessed in tumor hotspot areas, the regions of densest nuclear positivity, avoiding necrotic, fibrotic, and inflamed areas, and restricted to invasive carcinoma, by manual counting of 500–1,000 tumor cell nuclei. Tumors were categorized using the institutional three-tier scheme as low (< 20%), intermediate (20–40%), or high (> 40%). Because no universal Ki67 cutoff exists, the scoring method (hotspot counting) and the institutional categories are reported explicitly.

HER2 (Roche; #790–4493) was scored on membrane staining, which in colorectal carcinoma typically shows a basolateral or lateral pattern. Staining was graded as follows: 0, no membrane staining or weak membrane staining in < 10% of tumor cells; 1+, faint, barely perceptible segmental membrane staining in ≥ 10% of tumor cells; 2+, weak-to-moderate basolateral, lateral, or complete membrane staining in ≥ 10% of tumor cells; 3+, strong basolateral, lateral, or complete membrane staining in ≥ 10% of tumor cells recognizable at low magnification. Tumors scored 0 or 1 + were classified as negative, 2 + as equivocal (requiring in situ hybridization for resolution), and 3 + as positive. In biopsy specimens, positivity required clusters of at least five stained tumor cells. Only invasive adenocarcinoma was evaluated.

CDX2 (Zhongshan Golden Bridge; #ZA-0520) was evaluated by nuclear staining and classified as positive when ≥ 5% of tumor cells showed nuclear staining; staining in < 5% of tumor cells, or complete absence of nuclear staining, was classified as negative (loss). CK20 (Zhongshan Golden Bridge; #ZA-0574) was evaluated by specific cytoplasmic/membranous staining and classified as positive when ≥ 5% of tumor cells were stained; tumors with < 5% or no specific staining were classified as negative.

All immunohistochemical stains were independently evaluated and scored by two pathologists, with disagreements resolved by consensus. For Ki67, HER2, CDX2, and CK20, tumors without available staining were coded as "Unknown" and were retained in all analyses as a separate category (the reference category in regression models).

Statistics

All statistical analyses were performed using R version 4.3.1 [12]. Normality of continuous variables was assessed using the Shapiro-Wilk test and the Kolmogorov-Smirnov test; nonparametric methods were applied accordingly (Table S1). Continuous variables are presented as median (interquartile range [IQR]) and were compared between BRAF V600E IHC-positive and BRAF V600E IHC-negative groups using the Wilcoxon rank-sum test. Categorical variables are presented as frequencies (percentages) and were compared using Pearson's chi-squared test, with continuity correction for 2 × 2 tables. Fisher's exact test was used when expected cell counts were small or when contingency tables contained sparse cells.

No formal adjustment for multiple comparisons was applied because the subgroup analyses were exploratory. All tests were two-sided, and $P < 0.05$ was considered statistically significant. Exploratory subgroup analyses were descriptive, and we did not perform formal statistical interaction testing to quantify effect modification.

Multivariable logistic regression analysis

To determine whether the observed associations between BRAF V600E and selected clinicopathological features were independent of potential confounders, four multivariable Firth penalized logistic regression models were constructed for dMMR, perineural invasion, vascular invasion, and poor differentiation. Firth penalized regression was used to reduce small-sample bias and to address potential separation.

Covariates were selected a priori based on clinicopathological relevance and included sex, age of onset (early-onset vs. late-onset CRC), BMI (continuous, per 1 kg/m²), T stage, N stage, tumor differentiation (G3 vs. G1/G2), MMR status (dMMR vs. pMMR), tumor location (colon vs. rectum), BRAF V600E status, and selected immunohistochemical markers where applicable. The corresponding outcome variable was excluded from each model to avoid circularity. No data-driven variable selection was performed. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs) and P-values. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC-ROC) with 95% confidence intervals, and multicollinearity was evaluated using variance inflation factors derived from standard logistic regression models.

Use of large language models

ChatGPT (OpenAI) was used solely to assist with language editing and manuscript formatting. The authors reviewed and verified all content and take full responsibility for the final manuscript.

Results

Patient characteristics

A total of 1,130 patients with CRC were included in the study. The baseline clinicopathological characteristics of the cohort are summarized in Table 1. The median age at diagnosis was 58 years (IQR, 49–69), and 652 patients (57.7%) were male. The median BMI was 22.9 kg/m² (IQR, 20.9–25.2). Tumors were located in the colon in 571 patients (50.5%) and in the rectum in 559 patients (49.5%). Most tumors were moderately differentiated (G2, 77.0%), whereas 237 (21.0%) were poorly differentiated (G3) and 23 (2.0%) were well differentiated (G1). Vascular invasion and PNI were identified in 163 (14.4%) and 401 (35.5%) cases, respectively. Regarding stage, 20.3% of patients had stage I disease, 39.9% had stage II disease, 39.0% had

stage III disease, and 0.8% had stage IV disease. dMMR was observed in 79 tumors (7.0%), and BRAF V600E IHC positivity was detected in 105 tumors (9.3%) (Table 1). The number of BRAF V600E IHC-positive cases in each subgroup is provided in Tables 3–6.

Table 1
General characteristics of the study population

Total (n = 1130)			
Age		**Tumor location**	
Median (IQR)	58 (49–69)	Colon	571 (50.5%)
Sex		Rectum	559 (49.5%)
Male	652 (57.7%)	**Mismatch repair status**	
Female	478 (42.3%)	dMMR	79 (7.0%)
BMI		pMMR	1051 (93.0%)
Median (IQR)	22.9 (20.9–25.2)	**BRAF V600E**	
Tumor differentiation		-	1025 (90.7%)
G1	23 (2.0%)	+	105 (9.3%)
G2	870 (77.0%)	**MLH1**	
G3	237 (21.0%)	-	53 (4.7%)
Inflammatory status		+	1077 (95.3%)
Yes	82 (7.3%)	**MSH2**	
No	1048 (92.7%)	-	16 (1.4%)
Vascular invasion		+	1114 (98.6%)
Yes	163 (14.4%)	**MSH6**	
No	967 (85.6%)	-	10 (0.9%)
Perineural invasion		+	1120 (99.1%)
Yes	401 (35.5%)	**PMS2**	
No	729 (64.5%)	-	60 (5.3%)
TNM Stage		+	1070 (94.7%)
	229 (20.3%)	**Ki67**	
	451 (39.9%)	Unknown	216 (19.1%)
	441 (39.0%)	Low (< 20%)	15 (1.3%)
	9 (0.8%)	Intermediate (20–40%)	53 (4.7%)

Continuous variables are presented as median (interquartile range); categorical variables are presented as frequency (percentage).

Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Total (n = 1130)			
Age		**Tumor location**	
T Stage		High (> 40%)	846 (74.9%)
T1	72 (6.4%)	**HER2**	
T2	204 (18.1%)	Unknown	353 (31.2%)
T3	687 (60.8%)	Negative (-/1+)	700 (61.9%)
T4	167 (14.8%)	Equivocal (2+)	70 (6.2%)
N Stage		Positive (3+)	7 (0.6%)
N0	685 (60.6%)	**CDX2**	
N1	321 (28.4%)	Unknown	173 (15.3%)
N2	124 (11%)	Negative	16 (1.4%)
M stage		Positive	941 (83.3%)
M0	1121 (99.2%)	**CK20**	
M1	9 (0.8%)	Unknown	209 (18.5%)
		Negative	30 (2.7%)
		Positive	891 (78.8%)

Continuous variables are presented as median (interquartile range); categorical variables are presented as frequency (percentage).

Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Table 3

BRAF V600E IHC-positive versus BRAF V600E IHC-negative colorectal cancer stratified by tumor differentiation

Tumor differentiation	G1 + G2 differentiated CRC			G3 differentiated CRC		
	BRAF V600E (-)	BRAF V600E (+)	P-value	BRAF V600E (-)	BRAF V600E (+)	P-value
N	821	72		204	33	
BMI			0.024			0.473
Median (IQR)	23 (21.1–25.2)	21.8 (20.1–24.3)		22.6 (20.7–25.1)	22 (20.2–24.4)	
Perineural invasion			0.045			1
No	547 (66.6%)	39 (54.2%)		123 (60.3%)	20 (60.6%)	
Yes	274 (33.4%)	33 (45.8%)		81 (39.7%)	13 (39.4%)	
Tumor location			0.891			0.029
Colon	386 (47%)	35 (48.6%)		123 (60.3%)	27 (81.8%)	
Rectum	435 (53%)	37 (51.4%)		81 (39.7%)	6 (18.2%)	

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells. All tests were two-sided. A P value < 0.05 was considered statistically significant.

Abbreviations: BMI, body mass index; IQR, interquartile range.

Table 6
BRAF V600E IHC-positive versus BRAF V600E IHC-negative colorectal cancer stratified by tumor location

Tumor Location	Colon cancer			Rectal cancer		
	BRAF V600E (-)	BRAF V600E (+)	P-value	BRAF V600E (-)	BRAF V600E (+)	P-value
N	509	62		516	43	
BMI			0.05			0.244
Median (IQR)	22.9 (21-25.3)	21.8 (20.5–23.9)		23.1 (21.1–25.1)	22.8 (19.5–24.9)	
Tumor differentiation			0.002			0.933
G1 + G2	386 (75.8%)	35 (56.5%)		435 (84.3%)	37 (86%)	
G3	123 (24.2%)	27 (43.5%)		81 (15.7%)	6 (14%)	
Mismatch repair status			0.11			0.026
pMMR	451 (88.6%)	50 (80.6%)		510 (98.8%)	40 (93%)	
dMMR	58 (11.4%)	12 (19.4%)		6 (1.2%)	3 (7%)	

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells. All tests were two-sided. A P value < 0.05 was considered statistically significant.

Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Overall associations of BRAF V600E IHC positivity with clinicopathological features

Complete univariable comparisons between BRAF V600E IHC-positive and BRAF V600E IHC-negative tumors are presented in Table 2, including both statistically significant and non-significant associations. Compared with BRAF V600E IHC-negative tumors, BRAF V600E IHC-positive tumors were associated with lower BMI, poorer differentiation, and dMMR. The median BMI was 22.0 kg/m² in the BRAF V600E IHC-positive group and 23.0 kg/m² in the BRAF V600E IHC-negative group (Table 2). Poor differentiation was present in 31.4% of BRAF V600E IHC-positive tumors compared with 19.9% of BRAF V600E IHC-negative tumors, whereas dMMR was observed in 14.3% and 6.2% of tumors, respectively (Table 2). No statistically significant differences were observed between the two groups with respect to age, sex, vascular invasion, PNI, T stage, N stage, M stage, TNM stage, tumor location, or the other immunohistochemical markers examined.

Table 2
Clinicopathological characteristics of BRAF V600E IHC-positive versus BRAF V600E IHC-negative colorectal cancer

BMI	**BRAF V600E (-)**	**BRAF V600E (+)**	**P-value**
			0.020
Median (IQR)	23 (21-25.2)	22 (20.1–24.4)	
Age			0.757
Median (IQR)	58 (49–69)	58 (48–69)	
Sex			0.666
Male	594 (58%)	58 (55.2%)	
Female	431 (42%)	47 (44.8%)	
Tumor differentiation			0.008
G1 + G2	821 (80.1%)	72 (68.6%)	
G3	204 (19.9%)	33 (31.4%)	
Inflammatory status			0.658
No	949 (92.6%)	99 (94.3%)	
Yes	76 (7.4%)	6 (5.7%)	
Vascular invasion			0.118
No	883 (86.1%)	84 (80%)	
Yes	142 (13.9%)	21 (20%)	
Perineural invasion			0.078
No	670 (65.4%)	59 (56.2%)	
Yes	355 (34.6%)	46 (43.8%)	
T Stage			0.491
T1	66 (6.4%)	6 (5.7%)	
T2	189 (18.4%)	15 (14.3%)	
T3	623 (60.8%)	64 (61%)	
T4	147 (14.3%)	20 (19%)	

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells.

All tests were two-sided. A P value < 0.05 was considered statistically significant. Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

BMI	BRAF V600E (-)	BRAF V600E (+)	P-value
			0.020
N Stage			0.195
N0	626 (61.1%)	59 (56.2%)	
N1	292 (28.5%)	29 (27.6%)	
N2	107 (10.4%)	17 (16.2%)	
M stage			0.586
M0	1017 (99.2%)	104 (99%)	
M1	8 (0.8%)	1 (1%)	
TNM Stage			0.23
	215 (21%)	14 (13.3%)	
	407 (39.7%)	44 (41.9%)	
	395 (38.5%)	46 (43.8%)	
	8 (0.8%)	1 (1%)	
Tumor location			0.084
Colon	509 (49.7%)	62 (59%)	
Rectum	516 (50.3%)	43 (41%)	
Mismatch repair status			0.004
pMMR	961 (93.8%)	90 (85.7%)	
dMMR	64 (6.2%)	15 (14.3%)	
MLH1			0.144
-	45 (4.4%)	8 (7.6%)	
+	980 (95.6%)	97 (92.4%)	
MSH2			0.053
-	12 (1.2%)	4 (3.8%)	
+	1013 (98.8%)	101 (96.2%)	
MSH6			0.236

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells.

All tests were two-sided. A P value < 0.05 was considered statistically significant. Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

BMI	BRAF V600E (-)	BRAF V600E (+)	P-value
			0.020
-	8 (0.8%)	2 (1.9%)	
+	1017 (99.2%)	103 (98.1%)	
PMS2			0.073
-	50 (4.9%)	10 (9.5%)	
+	975 (95.1%)	95 (90.5%)	
Ki67			0.635
Unknown	196 (19.1%)	20 (19%)	
Low (< 20%)	15 (1.5%)	0 (0%)	
Intermediate (20–40%)	50 (4.9%)	3 (2.9%)	
High (> 40%)	764 (74.5%)	82 (78.1%)	
HER2			0.49
Unknown	315 (30.7%)	38 (36.2%)	
Negative (-/1+)	639 (62.3%)	61 (58.1%)	
Equivocal (2+)	65 (6.3%)	5 (4.8%)	
Positive (3+)	6 (0.6%)	1 (1%)	
CDX2			0.187
Unknown	163 (15.9%)	10 (9.5%)	
Negative	15 (1.5%)	1 (1%)	
Positive	847 (82.6%)	94 (89.5%)	
CK20			0.262
Unknown	195 (19%)	14 (13.3%)	
Negative	26 (2.5%)	4 (3.8%)	
Positive	804 (78.4%)	87 (82.9%)	

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells.

All tests were two-sided. A P value < 0.05 was considered statistically significant. Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Associations of BRAF V600E IHC positivity according to tumor differentiation

The stratified analysis by tumor differentiation is presented in Table 3. In the G1/G2 subgroup, BRAF V600E IHC-positive tumors were associated with lower BMI and a higher frequency of PNI than BRAF V600E IHC-negative tumors (Table 3). The median BMI was 21.8 kg/m² in the BRAF V600E IHC-positive group and 23.0 kg/m² in the BRAF V600E IHC-negative group, and PNI was present in 45.8% and 33.4% of tumors, respectively (Table 3). No significant differences were observed between the two groups in dMMR, vascular invasion, tumor location, or other clinicopathological variables (Table S2).

In the G3 subgroup, BRAF V600E IHC positivity was associated with tumor location. Colon tumors accounted for 81.8% of BRAF V600E IHC-positive cases compared with 60.3% of BRAF V600E IHC-negative cases (Table 3). No other significant differences were identified in the poorly differentiated subgroup (Table S2).

Associations of BRAF V600E IHC positivity according to age of onset

The age-stratified analysis is shown in Table 4. In late-onset CRC, BRAF V600E IHC-positive tumors were associated with lower BMI, poor differentiation, PNI, dMMR, and more frequent loss of MLH1, MSH2, and PMS2 expression (Table 4). The median BMI was 21.8 kg/m² in the BRAF V600E IHC-positive group and 23.2 kg/m² in the BRAF V600E IHC-negative group. Poor differentiation was present in 30.3% and 18.5% of tumors, respectively, and PNI in 46.1% and 33.7%, respectively (Table 4). dMMR was observed in 15.8% of BRAF V600E IHC-positive tumors compared with 4.8% of BRAF V600E IHC-negative tumors (Table 4).

Table 4

BRAF V600E IHC-positive versus BRAF V600E IHC-negative colorectal cancer stratified by age of onset

Age	LOCRC			EOCRC		
	BRAF V600E (-)	BRAF V600E (+)	P-value	BRAF V600E (-)	BRAF V600E (+)	P-value
N	757	76		268	29	
BMI			0.005			0.828
Median (IQR)	23.2 (21.3–25.5)	21.8 (20-24.1)		22.2 (20.2–24.6)	22.1 (20.4–24.9)	
Tumor differentiation			0.021			0.304
G1 + G2	617 (81.5%)	53 (69.7%)		204 (76.1%)	19 (65.5%)	
G3	140 (18.5%)	23 (30.3%)		64 (23.9%)	10 (34.5%)	
Perineural invasion			0.042			1
No	502 (66.3%)	41 (53.9%)		168 (62.7%)	18 (62.1%)	
Yes	255 (33.7%)	35 (46.1%)		100 (37.3%)	11 (37.9%)	
Tumor location			0.793			0.007
Colon	381 (50.3%)	40 (52.6%)		128 (47.8%)	22 (75.9%)	
Rectum	376 (49.7%)	36 (47.4%)		140 (52.2%)	7 (24.1%)	
Mismatch repair status			< 0.001			1
pMMR	721 (95.2%)	64 (84.2%)		240 (89.6%)	26 (89.7%)	
dMMR	36 (4.8%)	12 (15.8%)		28 (10.4%)	3 (10.3%)	
MLH1			0.01			0.234
-	27 (3.6%)	8 (10.5%)		18 (6.7%)	0 (0%)	
+	730 (96.4%)	68 (89.5%)		250 (93.3%)	29 (100%)	
MSH2			0.029			0.565
-	5 (0.7%)	3 (3.9%)		7 (2.6%)	1 (3.4%)	
+	752 (99.3%)	73 (96.1%)		261 (97.4%)	28 (96.6%)	
PMS2			0.017			1

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells. All tests were two-sided. A P value < 0.05 was considered statistically significant.

Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Age	LOCRC			EOCRC		
	BRAF V600E (-)	BRAF V600E (+)	P-value	BRAF V600E (-)	BRAF V600E (+)	P-value
-	30 (4%)	8 (10.5%)		20 (7.5%)	2 (6.9%)	
+	727 (96%)	68 (89.5%)		248 (92.5%)	27 (93.1%)	

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells. All tests were two-sided. A P value < 0.05 was considered statistically significant.

Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

In early-onset CRC, the only significant association with BRAF V600E IHC positivity was tumor location (Table 4). Colon tumors accounted for 75.9% of BRAF V600E IHC-positive cases compared with 47.8% of BRAF V600E IHC-negative cases. No significant differences were observed for BMI, tumor differentiation, dMMR, or the other clinicopathological variables examined (Table S3).

Associations of BRAF V600E IHC positivity according to sex

Sex-stratified analysis is summarized in Table 5. In male patients, BRAF V600E IHC positivity was associated with poor differentiation and dMMR (Table 5). Poor differentiation was observed in 32.8% of BRAF V600E IHC-positive tumors and 17.2% of BRAF V600E IHC-negative tumors, while dMMR was present in 13.8% and 5.1% of tumors, respectively (Table 5). No other statistically significant differences were identified in male patients (Table S4).

Table 5
Sex-specific differences between BRAF V600E IHC-positive and BRAF V600E IHC-negative colorectal cancer

Sex	Male			Female		
	BRAF V600E (-)	BRAF V600E (+)	P-value	BRAF V600E (-)	BRAF V600E (+)	P-value
N	594	58		431	47	
BMI			0.34			0.018
Median (IQR)	23.6 (21.6–25.6)	23 (21-25.8)		22.2 (20-24.3)	21.1 (19.2–22.9)	
Tumor differentiation			0.006			0.453
G1 + G2	492 (82.8%)	39 (67.2%)		329 (76.3%)	33 (70.2%)	
G3	102 (17.2%)	19 (32.8%)		102 (23.7%)	14 (29.8%)	
Vascular invasion			1			0.018
No	517 (87%)	51 (87.9%)		366 (84.9%)	33 (70.2%)	
Yes	77 (13%)	7 (12.1%)		65 (15.1%)	14 (29.8%)	
Perineural invasion			0.767			0.037
No	396 (66.7%)	37 (63.8%)		274 (63.6%)	22 (46.8%)	
Yes	198 (33.3%)	21 (36.2%)		157 (36.4%)	25 (53.2%)	
Mismatch repair status			0.014			0.105
pMMR	564 (94.9%)	50 (86.2%)		397 (92.1%)	40 (85.1%)	
dMMR	30 (5.1%)	8 (13.8%)		34 (7.9%)	7 (14.9%)	

Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using Pearson's chi-squared test (with continuity correction for 2 × 2 tables) or Fisher's exact test when expected cell counts were small or when contingency tables contained sparse cells. All tests were two-sided. A P value < 0.05 was considered statistically significant.

Abbreviations: BMI, body mass index; IQR, interquartile range; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

In female patients, BRAF V600E IHC positivity was associated with lower BMI, vascular invasion, and PNI (Table 5). The median BMI was 21.1 kg/m² in the BRAF V600E IHC-positive group and 22.2 kg/m² in the BRAF V600E IHC-negative group. Vascular invasion was present in 29.8% of BRAF V600E IHC-positive tumors compared with 15.1% of BRAF V600E IHC-negative tumors, and PNI in 53.2% compared with 36.4%, respectively (Table 5). No significant differences were observed with respect to dMMR, tumor differentiation, or the other variables examined (Table S4).

Associations of BRAF V600E IHC positivity according to tumor location

The location-stratified analysis is shown in Table 6. Among colon cancers, BRAF V600E IHC positivity was associated with lower BMI and poor differentiation (Table 6). The median BMI was 21.8 kg/m² in BRAF V600E IHC-positive colon tumors and 22.9 kg/m² in BRAF V600E IHC-negative colon tumors. Poor differentiation was present in 43.5% of BRAF V600E IHC-positive colon tumors compared with 24.2% of BRAF V600E IHC-negative colon tumors (Table 6). No other significant differences were observed in colon cancer (Table S5).

Among rectal cancers, BRAF V600E IHC positivity was associated with dMMR (Table 6). dMMR was observed in 7.0% of BRAF V600E IHC-positive rectal tumors compared with 1.2% of BRAF V600E IHC-negative rectal tumors. No other significant differences were identified in rectal cancer (Table S5).

Independent associations with dMMR, PNI, vascular invasion, and poor differentiation

To determine whether the univariable associations remained significant after adjustment for potential confounders, four multivariable Firth penalized logistic regression models were constructed for dMMR, PNI, vascular invasion, and poor differentiation. The model results are presented in Table 7 and Fig. 1, and model discrimination is shown in Fig. 2.

Table 7
Multivariable logistic regression analyses

Variable	Model 1 dMMR	P-value	Model 2 Perineural invasion	P-value	Model 3 Vascular invasion	P-value	Model 4 Poor differentiation	P-value
BRAF V600E+ (vs. -)	2.229 (1.083–4.378)	0.030	1.432 (0.912–2.246)	0.119	1.296 (0.729–2.226)	0.368	1.451 (0.89–2.326)	0.134
Female (vs. Male)	1.535 (0.91–2.601)	0.108	1.18 (0.892–1.561)	0.246	1.22 (0.843–1.763)	0.291	1.251 (0.912–1.713)	0.164
Early-onset (vs. Late-onset)	2.052 (1.196–3.489)	0.009	0.986 (0.725–1.337)	0.926	1.153 (0.774–1.698)	0.479	1.066 (0.755–1.493)	0.715
BMI (per 1 kg/m²)	1.005 (0.926–1.088)	0.907	0.992 (0.951–1.035)	0.721	1.03 (0.973–1.089)	0.307	1.005 (0.958–1.055)	0.828
T1 (vs. T3)	0.911 (0.247–2.711)	0.876	0.033 (0.004–0.123)	< .001	0.868 (0.271–2.211)	0.784	0.106 (0.012–0.405)	< .001
T2 (vs. T3)	0.869 (0.358–1.907)	0.737	0.127 (0.072–0.21)	< .001	1.021 (0.575–1.756)	0.941	0.573 (0.34–0.932)	0.024
T4 (vs. T3)	0.675 (0.313–1.37)	0.284	1.253 (0.87–1.806)	0.226	2.031 (1.284–3.192)	0.003	1.95 (1.318–2.875)	< .001
N1 (vs. N0)	0.346 (0.169–0.664)	0.001	2.204 (1.628–2.99)	< .001	3.815 (2.493–5.899)	< .001	1.512 (1.055–2.163)	0.024
N2 (vs. N0)	0.149 (0.038–0.427)	< .001	2.734 (1.78–4.228)	< .001	8.727 (5.199–14.786)	< .001	3.193 (2.039–4.989)	< .001
Poor differentiation (vs. Non-poor)	4.151 (2.399–7.216)	< .001	0.837 (0.596–1.17)	0.299	1.139 (0.74–1.732)	0.549		

Firth penalized logistic regression was used to reduce small-sample bias and to address potential separation. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). A P value < 0.05 was considered statistically significant (bold type indicates significance).

Reference categories: BRAF V600E IHC–negative, male, late-onset (≥ 50 years), T3 stage, N0 stage, non-poor differentiation (G1/G2), rectal tumor, unknown Ki67, unknown HER2, unknown CDX2, unknown CK20, and pMMR.

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; PNI, perineural invasion.

Variable	Model 1 dMMR	P-value	Model 2 Perineural invasion	P-value	Model 3 Vascular invasion	P-value	Model 4 Poor differentiation	P-value
Colon (vs. Rectum)	6.806 (3.407–15.11)	< .001	0.771 (0.576–1.031)	0.0792	0.834 (0.563–1.232)	0.363	1.292 (0.923–1.814)	0.136
Ki67 Low (< 20%) (vs. Unknown)	6.708 (0.936–35.727)	0.057	1.861 (0.574–5.861)	0.293	0.227 (0.002–1.956)	0.217	0.406 (0.073–1.545)	0.199
Ki67 Intermediate (20–40%) (vs. Unknown)	0.431 (0.076–1.654)	0.236	1.15 (0.541–2.384)	0.712	0.396 (0.098–1.228)	0.114	0.584 (0.256–1.258)	0.173
Ki67 High (> 40%) (vs. Unknown)	0.65 (0.347–1.257)	0.196	2.156 (1.469–3.204)	< .001	1.763 (1.073–2.995)	0.025	0.59 (0.403–0.868)	0.008
HER2 Negative (-/1+) (vs. Unknown)	0.57 (0.314–1.042)	0.067	0.783 (0.572–1.07)	0.125	1.079 (0.714–1.645)	0.720	1.107 (0.77–1.602)	0.584
HER2 Equivocal (2+) (vs. Unknown)	1.392 (0.528–3.386)	0.488	0.79 (0.429–1.431)	0.439	1.113 (0.478–2.391)	0.794	1.182 (0.6–2.246)	0.620
HER2 Positive (3+) (vs. Unknown)	0.389 (0.003–4.783)	0.514	0.943 (0.187–4.721)	0.941	3.089 (0.454–15.932)	0.227	3.171 (0.658–14.209)	0.142
CDX-2 Negative (vs. Unknown)	1.117 (0.121–8.178)	0.918	1.591 (0.346–7.315)	0.548	2.324 (0.377–12.549)	0.352	0.511 (0.089–2.361)	0.402
CDX-2 Positive (vs. Unknown)	1.208 (0.3–4.465)	0.783	1.369 (0.616–3.02)	0.438	0.778 (0.195–2.626)	0.699	0.911 (0.392–2.055)	0.825
CK20 Negative (vs. Unknown)	12.119 (2.629–63.274)	0.001	1.302 (0.396–4.286)	0.663	2.752 (0.613–13.527)	0.188	1.603 (0.488–5.282)	0.435
CK20 Positive (vs. Unknown)	0.988 (0.314–3.641)	0.984	0.948 (0.453–2.016)	0.888	2.171 (0.687–8.644)	0.198	1.05 (0.491–2.353)	0.902

Firth penalized logistic regression was used to reduce small-sample bias and to address potential separation. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). A P value < 0.05 was considered statistically significant (bold type indicates significance).

Reference categories: BRAF V600E IHC–negative, male, late-onset (≥ 50 years), T3 stage, N0 stage, non-poor differentiation (G1/G2), rectal tumor, unknown Ki67, unknown HER2, unknown CDX2, unknown CK20, and pMMR.

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; PNI, perineural invasion.

Variable	Model 1 dMMR	P-value	Model 2 Perineural invasion	P-value	Model 3 Vascular invasion	P-value	Model 4 Poor differentiation	P-value
dMMR (vs. pMMR)	-	-	0.849 (0.47–1.497)	0.575	1.56 (0.712–3.204)	0.256	4.388 (2.558–7.561)	< .001
Firth penalized logistic regression was used to reduce small-sample bias and to address potential separation. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). A P value < 0.05 was considered statistically significant (bold type indicates significance).								
Reference categories: BRAF V600E IHC–negative, male, late-onset (≥ 50 years), T3 stage, N0 stage, non-poor differentiation (G1/G2), rectal tumor, unknown Ki67, unknown HER2, unknown CDX2, unknown CK20, and pMMR.								
Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; PNI, perineural invasion.								

In the model with dMMR as the outcome, BRAF V600E IHC positivity remained independently associated with dMMR (odds ratio, 2.229; 95% confidence interval, 1.083–4.378; P = 0.030) (Table 7 and Fig. 1). Other independent predictors of dMMR included early-onset CRC, poor differentiation, colon location, and CK20 negativity, with colon location and poor differentiation showing particularly strong associations (Table 7 and Fig. 1).

By contrast, BRAF V600E IHC positivity was not independently associated with PNI, vascular invasion, or poor differentiation in the corresponding multivariable models (Table 7 and Fig. 1). PNI was independently associated with nodal involvement and high Ki67 expression, whereas T1 and T2 tumors were less likely than T3 tumors to exhibit PNI (Table 7 and Fig. 1). Vascular invasion was independently associated with T4 stage, nodal involvement, and high Ki67 expression (Table 7 and Fig. 1). Poor differentiation was independently associated with T4 stage, nodal involvement, and dMMR, whereas T1/T2 stage and high Ki67 expression were inversely associated with poor differentiation. (Table 7 and Fig. 1).

Taken together, these analyses showed that the clinicopathological associations of BRAF V600E IHC positivity were variable across CRC subsets. Although BRAF V600E IHC positivity was associated with several clinicopathological features in univariable/stratified analyses, dMMR was the only feature that remained independently associated after multivariable adjustment. The subgroup analyses suggested exploratory variability across age of onset, sex, tumor differentiation, and tumor location; however, formal interaction testing was not performed.

Discussion

In this cohort of 1,130 patients with colorectal cancer, BRAF V600E IHC positivity was detected in 9.3% of tumors, consistent with prior reports [13]. The main finding of this study was not simply confirmation of the known association between BRAF V600E and dMMR, but the observation that the broader clinicopathological phenotype commonly attributed to BRAF V600E CRC was not consistently retained as independent associations [9]. Although BRAF V600E IHC-positivity was associated with lower BMI, poor differentiation, and dMMR in the overall analysis, only the association with dMMR remained significant after multivariable

adjustment [5]. Clinicopathological correlates of BRAF V600E IHC positivity additionally showed exploratory variability across subgroups defined by age of onset, sex, tumor differentiation, and tumor location [7]. These findings suggest that commonly attributed clinicopathological correlations of BRAF V600E are not consistently retained as independent associations after multivariable adjustment. In our cohort, dMMR was the most robust independent correlate, whereas other associations were attenuated and varied in exploratory subgroup analyses.

The association between BRAF V600E and dMMR is well established and is typically interpreted in the context of the serrated neoplasia pathway, CpG island methylator phenotype, MLH1 inactivation, and sporadic mismatch repair-deficient tumorigenesis [3, 10]. Our results are consistent with this framework [7]. dMMR was more frequent in BRAF V600E IHC-positive tumors than in BRAF V600E-negative tumors and remained independently associated with BRAF V600E IHC positivity after adjustment. This indicates that dMMR is the most robust clinicopathological correlate of BRAF V600E IHC positivity in our cohort [7, 14]. By contrast, poor differentiation, perineural invasion, and vascular invasion were not independently associated with BRAF V600E IHC positivity [15]. These findings suggest that pathological features often attributed to BRAF V600E IHC-positive CRC may partly reflect accompanying factors such as dMMR, tumor stage, nodal involvement, proliferative activity, and anatomical context rather than an independent effect of BRAF V600E itself [10, 15].

The subgroup analyses further supported this interpretation. In late-onset CRC, BRAF V600E IHC positivity was associated with lower BMI, poor differentiation, PNI, dMMR, and loss of MLH1, MSH2, and PMS2 expression [16]. In early-onset CRC, the main significant association was tumor location, whereas poor differentiation and dMMR were not significantly enriched [7]. Additional heterogeneity was observed in analyses stratified by sex and tumor location, with different associated features identified across subgroups [10]. Although these findings should be interpreted cautiously because of limited sample size in some strata and the absence of multiple-comparison adjustment, they indicate exploratory variability in clinicopathological associations across strata [9].

One potentially interesting observation was the association between BRAF V600E IHC positivity and lower BMI. This pattern was observed in the overall cohort and in several subgroup analyses. However, the cross-sectional nature of this retrospective analysis precludes causal inference, and the biological basis for this observation remains uncertain. Given the limited and inconsistent literature on anthropometric factors in BRAF-defined CRC subsets, this result should be regarded as hypothesis-generating [17–19].

Overall, our findings support a more nuanced interpretation of BRAF V600E IHC-positive CRC. While BRAF V600E IHC positivity showed a robust independent association with dMMR, other clinicopathological features commonly linked to the canonical BRAF V600E-associated phenotype were not consistently retained after adjustment and showed exploratory variability across subgroups. These results suggest that BRAF V600E IHC positivity should be interpreted in the context of co-occurring molecular features, particularly MMR status. Nevertheless, BRAF V600E status in this study was determined by IHC and used as a surrogate for molecular mutation, rather than being confirmed by sequencing. Although BRAF V600E IHC positivity has been reported to correlate with molecular testing in CRC, IHC classification may be influenced by assay- and interpretation-dependent factors, which can lead to discordance with sequencing results [20]. Future studies incorporating sequencing-based confirmation would strengthen the generalizability and support more causal mechanistic interpretations of the observed clinicopathological associations. Moreover, although BRAF V600E is biologically

linked to the serrated neoplasia pathway and the MLH1 methylation/CIMP axis, as well as to dMMR and MSI, our study did not include KRAS/NRAS mutational status, CIMP assessment, MLH1 promoter methylation, or MSI testing. Therefore, we cannot determine whether the observed attenuation of several clinicopathological associations after multivariable adjustment reflects differences in these upstream molecular processes, in addition to the effect of dMMR status itself. This limitation may reduce the granularity with which we interpret molecular-context-related heterogeneity, and future studies incorporating comprehensive molecular profiling, including MSI and CIMP/MLH1 methylation, are needed to refine mechanistic interpretation.

Conclusions

Overall, our findings support a more nuanced interpretation of BRAF V600E in CRC. While BRAF V600E IHC positivity showed a robust independent association with dMMR, other clinicopathological features often described as part of a canonical BRAF V600E-associated phenotype were not consistently retained as independent correlates after multivariable adjustment and showed variability in exploratory subgroup analyses. Because formal interaction testing was not performed, our findings should be interpreted as evidence of which associations persist after adjustment, rather than definitive statistical evidence of effect modification. Further studies incorporating interaction testing and comprehensive molecular profiling are needed to clarify the biological basis of heterogeneity.

Abbreviations

CRC: Colorectal cancer

dMMR: Deficient mismatch repair

pMMR: Proficient mismatch repair

BMI: Body mass index

PNI: Perineural invasion

EOCRC: Early-onset colorectal cancer

LOCRC: Late-onset colorectal cancer

G1: Well differentiated

G2: Moderately differentiated

G3: Poorly differentiated

IHC: Immunohistochemistry

IQR: Interquartile range

OR: odds ratio

CI: Confidence interval

AUC: Area under the curve

ROC: Receiver operating characteristic

HL: Hosmer–Lemeshow goodness-of-fit test

VIF: Variance inflation factor.

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of West China Hospital, Sichuan University (No. 2023-958). The requirement for informed consent was waived by the Ethics Committee of West China Hospital, Sichuan University due to the retrospective study design and the use of de-identified data.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form that could identify the patient.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by grants from the National Natural Science Foundation of China (Grant Nos. 82173104 and 81871233).

Authors' contributions

MMH, SL, ZM, YW and XDZ contributed to the conceptualization and methodology of the study. MMH, SL, ZM, DT, YS, SC, YMC and MS performed the formal analysis and investigation. MMH, SL, ZM and XDZ wrote the original draft of the manuscript. YW and XDZ reviewed and edited the manuscript. ZM, XDZ and YW provided resources. XDZ and YW supervised the study. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nat Rev Cancer. 2017;17(4):268.
2. Han B, Zheng R, Zeng H, Wang S, Sun K, Chen R, Li L, Wei W, He J. Cancer incidence and mortality in China, 2022. J Natl Cancer Cent. 2024;4(1):47–53.
3. Satorres C, Garcia-Campos M, Bustamante-Balen M. Molecular features of the serrated pathway to colorectal cancer: current knowledge and future directions. Gut Liver. 2021;15(1):31–43.
4. Kopetz S, Yoshino T, Van Cutsem E, Eng C, Kim TW, Wasan HS, Desai J, Ciardiello F, Yaeger R, Maughan TS, et al. Encorafenib, cetuximab and chemotherapy in BRAF-mutant colorectal cancer: a randomized phase 3 trial. Nat Med. 2025;31(3):901–8.
5. Grothey A, Fakih M, Tabernero J. Management of BRAF-mutant metastatic colorectal cancer: a review of treatment options and evidence-based guidelines. Ann Oncol. 2021;32(8):959–67.
6. Yang W, Duan Y, Huang X, Song W, Qian J, Zeng Y, Guo T, Wu Y, Li H, Zou X et al. Distinct clinical and genomic profiles of BRAF mutation subtypes in Chinese colorectal cancer: a retrospective cohort study with cross-population validation. Cell Oncol (Dordr). 2026;49(2).
7. Hitchen N, Wong HL, Wong R, Shapiro J, Burge M, Nott L, Lee B, Lim SH, Wong SF, Caird S, et al. Real-world characteristics and outcomes of patients with BRAF(V600E)-mutant metastatic colorectal cancer in Australia: the COALA project. ESMO Real World Data Digit Oncol. 2025;9:100178.
8. Caputo F, Santini C, Bardasi C, Cerma K, Casadei-Gardini A, Spallanzani A, Andrikou K, Cascinu S, Gelsomino F. BRAF-mutated colorectal cancer: clinical and molecular insights. Int J Mol Sci. 2019;20(21).
9. Barras D, Missiaglia E, Wirapati P, Sieber OM, Jorissen RN, Love C, Molloy PL, Jones IT, McLaughlin S, Gibbs P, et al. BRAF V600E mutant colorectal cancer subtypes based on gene expression. Clin Cancer Res. 2017;23(1):104–15.
10. Alig AHS, Modest DP, Stintzing S, Heinrich K, Geissler M, von Fischer L, Decker T, Vehling-Kaiser U, Held S, Moosmann N, et al. Impact of primary tumor sidedness and sex on prognosis and anti-epidermal growth factor receptor antibody efficacy in BRAF-mutant metastatic colorectal cancer: a pooled analysis of AIO studies FIRE-1, CIOX, FIRE-3, XELAVIRI, and VOLFI. ESMO Open. 2024;9(9):103677.
11. Amin MB, Edge SB, Greene FL, et al. editors. AJCC cancer staging manual. 8th ed. Chicago: American Joint Committee on Cancer, Springer;; 2017.
12. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2023.
13. Sanz-Garcia E, Argiles G, Elez E, Tabernero J. BRAF mutant colorectal cancer: prognosis, treatment, and new perspectives. Ann Oncol. 2017;28(11):2648–57.
14. Parsons MT, Buchanan DD, Thompson B, Young JP, Spurdle AB. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. J Med Genet. 2012;49(3):151–7.
15. Fanelli GN, Dal Pozzo CA, Depetris I, Schirripa M, Brignola S, Biason P, Balistreri M, Dal Santo L, Lonardi S, Munari G, et al. The heterogeneous clinical and pathological landscapes of metastatic BRAF-mutated colorectal cancer. Cancer Cell Int. 2020;20:30.
16. Christenson ES, Tsai HL, Le DT, Jaffee EM, Dudley J, Xian RR, Gocke CD, Eshleman JR, Lin MT. Colorectal cancer in patients of advanced age is associated with increased incidence of BRAF p.V600E mutation and

mismatch repair deficiency. Front Oncol. 2023;13:1193259.

17. Brandstedt J, Wangefjord S, Nodin B, Eberhard J, Sundstrom M, Manjer J, Jirstrom K. Associations of anthropometric factors with KRAS and BRAF mutation status of primary colorectal cancer in men and women: a cohort study. PLoS ONE. 2014;9(2):e98964.
18. Carr PR, Amitay EL, Jansen L, Alwers E, Roth W, Herpel E, Kloor M, Schneider M, Blaker H, Chang-Claude J, et al. Association of BMI and major molecular pathological markers of colorectal cancer in men and women. Am J Clin Nutr. 2020;111(3):562–9.
19. Murphy N, Newton CC, Song M, Papadimitriou N, Hoffmeister M, Phipps AI, Harrison TA, Newcomb PA, Aglago EK, Berndt SI, et al. Body mass index and molecular subtypes of colorectal cancer. J Natl Cancer Inst. 2023;115(2):165–73.
20. Grillo F, Paudice M, Pigozzi S, Dono M, Lastraioli S, Lugaresi M, Bozzano S, Tognoni C, Ali M, Sciallero S, et al. BRAF(V600E) immunohistochemistry can reliably substitute BRAF molecular testing in the Lynch syndrome screening algorithm in colorectal cancer. Histopathology. 2024;84(5):877–87.

Figures

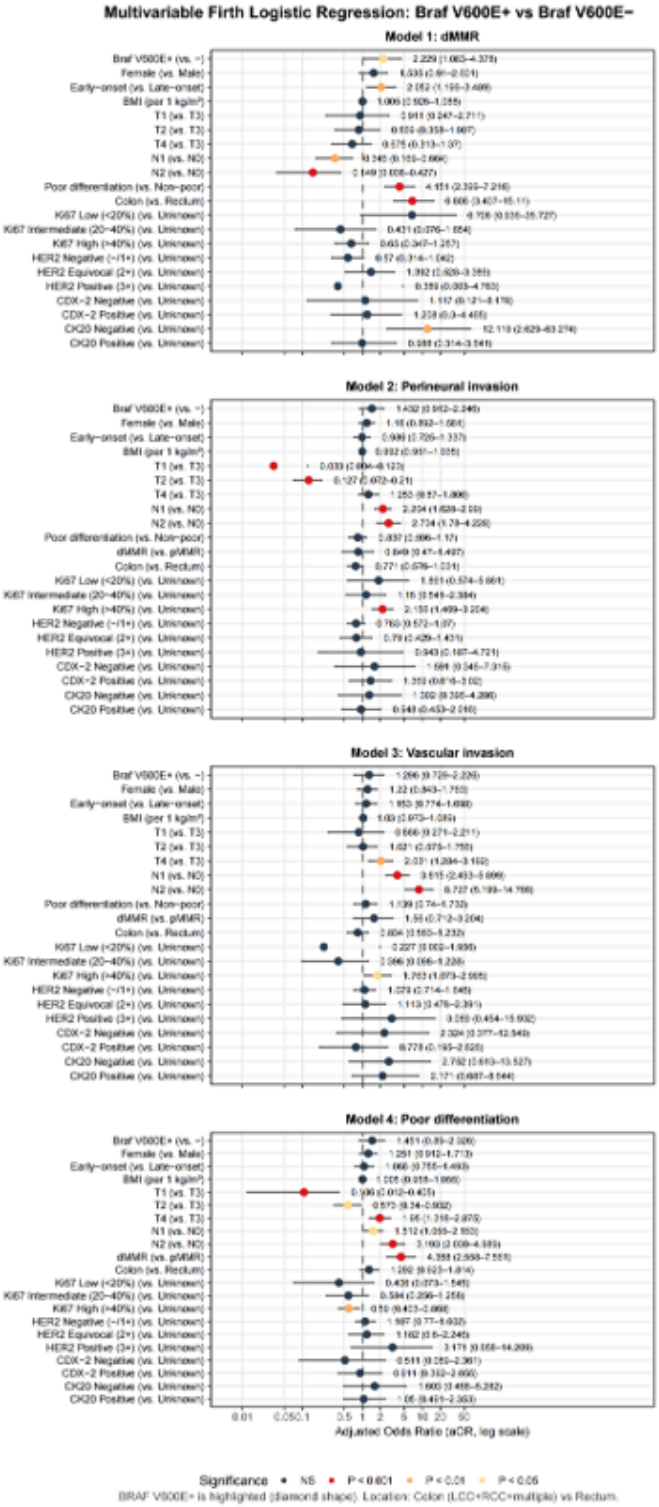

Figure 1

Forest plot of multivariable Firth penalized logistic regression analyses. Forest plot showing odds ratios and 95% confidence intervals for variables included in the multivariable Firth penalized logistic regression models. Separate models were constructed for dMMR, PNI, vascular invasion, and poor differentiation. BRAF V600E IHC positivity was independently associated with dMMR but not with PNI, vascular invasion, or poor differentiation.

Points are colored according to statistical significance: red, P < 0.001; orange, P < 0.01; yellow, P < 0.05; dark gray, not significant.

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; EOCRC, early-onset colorectal cancer; LOCRC, late-onset colorectal cancer

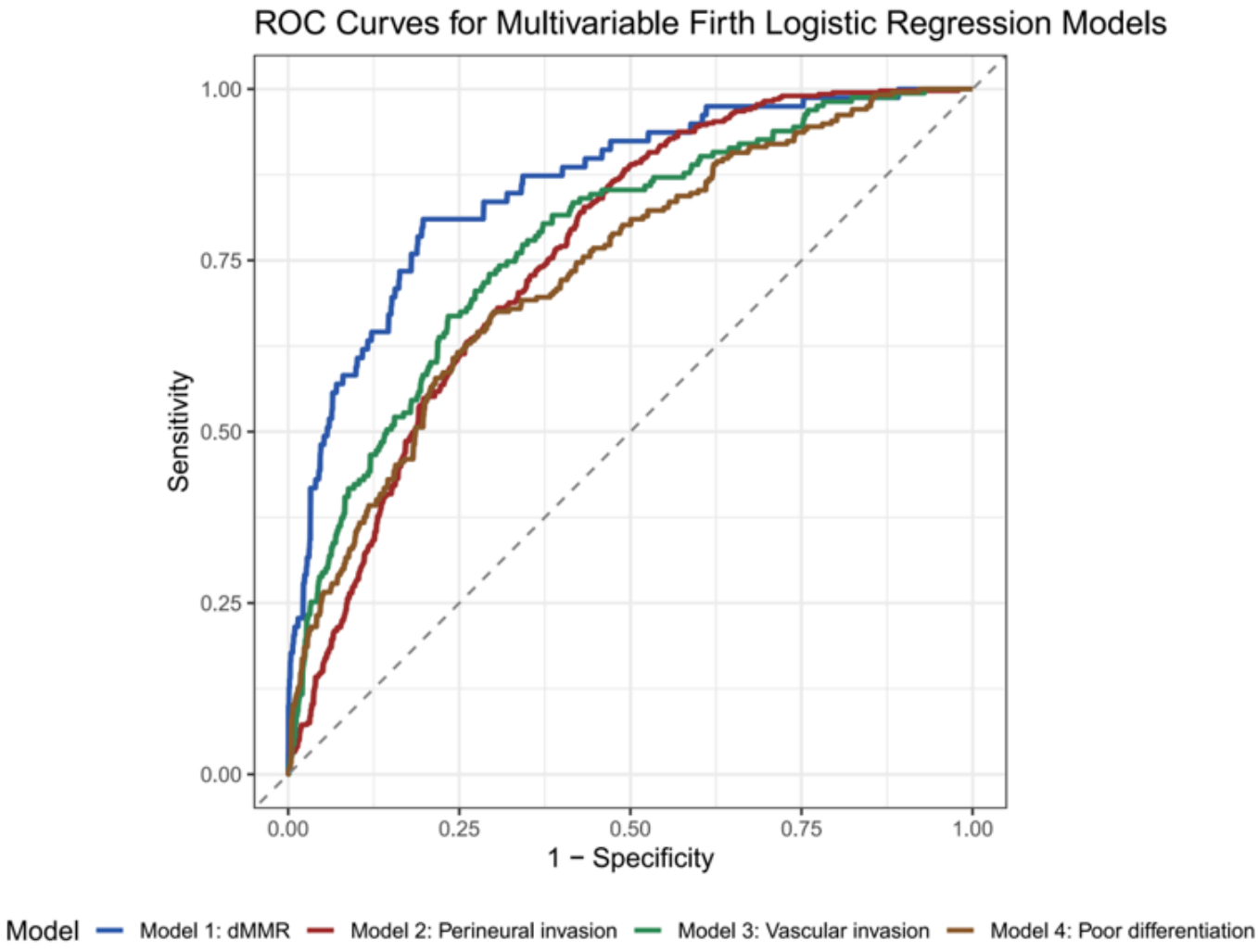

Figure 2

Receiver operating characteristic curves for the four multivariable Firth logistic regression models. Receiver operating characteristic curves showing the predictive performance of the four multivariable Firth logistic regression models for dMMR, PNI, vascular invasion, and poor differentiation. AUC, area under the curve; HL, Hosmer–Lemeshow goodness-of-fit test; VIF, variance inflation factor. Model discrimination and calibration: Model 1 (dMMR), AUC 0.857 (95% CI 0.814–0.901), HL χ^2 = 5.10, P = 0.747; Model 2 (perineural invasion), AUC 0.759 (95% CI 0.732–0.787), HL χ^2 = 6.67, P = 0.572; Model 3 (vascular invasion), AUC 0.777 (95% CI 0.739–0.815), HL χ^2 = 5.83, P = 0.666; Model 4 (poor differentiation), AUC 0.737 (95% CI 0.701–0.772), HL χ^2 = 7.28, P = 0.507. All models showed acceptable discrimination (AUC > 0.70) and good calibration (HL P > 0.05). No multicollinearity was detected (maximum VIF 1.48 across the shared covariate structure of the four models).

Abbreviations: dMMR, deficient mismatch repair; AUC, area under the curve; HL, Hosmer–Lemeshow goodness-of-fit test; VIF, variance inflation factor.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.pdf