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Prognostic Impact of Deficient DNA Mismatch Repair and Mutations in *KRAS*, and *BRAF*^{V600E} in Patients with Lymph Node-Positive Colon Cancer

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Abstract

While tumor stage remains the key determinant of colorectal cancer (CRC) prognosis and treatment, there is considerable stage-independent variability in clinical outcome. Molecular markers hold promise for explaining variations in clinical behavior, and may identify patient subsets with differential efficacy and survival after adjuvant chemotherapy which is standard of care for patients with lymph node-positive, i.e., stage III, colon cancer. An increased understanding of the molecular evolution and progression of CRC has identified two major pathways of tumorigenesis that are characterized by chromosomal instability or microsatellite instability (MSI). MSI is a consequence of deficient DNA mismatch repair (MMR) that is generally due to epigenetic inactivation of *MLH1* in tumors that often carry mutations in oncogenic *BRAF*^{V600E}. Activating *BRAF*^{V600E} and *KRAS* mutations are mutually exclusive and in this article, we review the current status of these mutations and MMR status as prognostic biomarkers in stage III colon cancers.

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Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

Aziz Zaanan declares that he has no conflict of interest.

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Keywords

Colorectal cancer; Microsatellite instability; DNA Mismatch repair; *BRAF*; *KRAS*; Adjuvant Chemotherapy; 5-fluorouracil; oxaliplatin; Biomarker; Prognosis; Predictive

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and its incidence in women is second only to breast cancer worldwide [1]. Colorectal carcinogenesis is a multistep process characterized by activation of oncogenes and loss of tumor suppressor genes. Two major pathways include chromosomal instability or less common microsatellite instability (MSI), which occurs in approximately 15% of all CRCs. MSI is a consequence of deficient DNA mismatch repair (MMR) that results in an accumulation of errors within microsatellite regions producing high mutation rates [2]. Deficient MMR (dMMR) can arise from inheritance of a germline mutation in a MMR gene (*MLH1*, *MSH2*, *MSH6*, *PMS2*) in ~2–3% of all CRCs cases [3–5] causing Lynch Syndrome [6], or more commonly results from epigenetic inactivation of *MLH1* in sporadic cases [7] in association with the CpG island methylator phenotype (CIMP) [8]. Highly concordant results have been shown for tumors evaluated by MSI testing, using a PCR-based method, or MMR protein expression by immunohistochemistry [9]. Tumors with loss of a MMR protein are considered to have dMMR and this term is often used interchangeably with MSI. Sporadic CRC with MSI are enriched with activating mutations in the *BRAF* (*B-type Raf kinase)* oncogene, which encodes a serine/threonine protein kinase and leads to stimulation of the mitogen-activated protein kinase pathway [10]. The $BRAF^{V600E}$ mutation, which consists of a valine to glutamic acid substitution, has an overall frequency of ~10% in CRCs [10, 11] and are mutually exclusive with *KRAS* (*Kirsten rat sarcoma viral oncogene homolog)* mutations [12, 13]. The *KRAS* proto-oncogene encodes a protein that is a member of the GTPase superfamily. A single amino acid substitution is responsible for abrogating the GTPase activity, resulting in a mutation that activates the RAS/RAF signalling pathway. *KRAS* mutations occur early during colorectal carcinogenesis and are found in 35% to 42% of tumors [12, 13]. *KRAS* and $BRAF^{V600E}$ mutations predict nonresponse to anti-epidermal growth factor receptor (EGFR) antibody therapy in patients with metastatic CRCs, although only *KRAS* has been validated [12, 14, 15].

Disease stage remains the strongest prognostic variable and is the key determinant of patient management. Within a given tumor stage, however, there is considerable variability in prognosis that is likely due to clinicopathological factors, molecular heterogeneity and/or tumor/host-related immunologic factors. Such variability is particularly evident in lymph node-positive cancers, i.e., stage III, and those with distant metastatic disease, i.e., stage IV. Pathway-related biomarkers hold promise for both prediction and prognosis, although most have not been studied in trials of modern combination chemotherapy regimens. Furthermore, conflicting data has been reported for the prognostic impact of $BRAF^{V600E}$ and *KRAS* mutations in non-metastatic disease. In this article, we review the current status of MMR status and mutations in *KRAS* and $BRAF^{V600E}$ as prognostic biomarkers in stage III colon cancer patients.

MMR status and clinical outcome in stage III colon cancer

Patient treated with 5-fluorouracil (5-FU)-based adjuvant therapy

Multiple studies have since shown that patients with dMMR colon cancers have more favorable survival compared to proficient MMR (pMMR) tumors [16]. This observation was confirmed in a large meta-analysis included 32 studies comprising 1,277 MSI cases among a total of 7,642 patients with stages I to IV disease [17]. The analysis included untreated patients, as well as patients treated with 5-FU-based adjuvant chemotherapy. The Hazard Ratio (HR) for overall survival (OS) associated with dMMR was 0.65 (95% CI, 0.59–0.71); benefit persisted when restricting analyses to patients with stage II or III cancers participating in clinical studies [17].

While most studies have shown a lack of benefit for 5-FU treatment in dMMR patients [18–22], early studies produced variable results with some showing a survival benefit [23–25] or even a deleterious effect [26, 27]. This discrepancy is likely due to limited sample size, inclusion of multiple tumor stages, and different 5-FU-based adjuvant regimens [16]. Sargent et al. [27] reported data on 457 stage II and III colon cancer patients who were included in five randomized trials evaluating 5-FU-based adjuvant chemotherapy. MSI was shown to be a favorable prognostic marker for the overall population of patients with stage II and III colon cancer, as well as a negative predictor of adjuvant 5-FU benefit (Table 1). These findings were maintained when data were pooled with those published in 2001 by Ribic et al. [26] to yield a total of 1,027 stage II and III colon cancer patients [27] (Table 1). In this analysis, MSI was associated with better survival in stage II and III, and was a negative predictor of adjuvant 5-FU benefit for stage II and III with a suggestion of a detrimental effect in stage II. Lack of clinical benefit for 5-FU treatment in MSI tumors is consistent with preclinical studies where human CRC cell lines with MSI display resistance to 5-FU [28].

In a study that evaluated 2,141 stages II and III colon cancers from 5-FU-based adjuvant therapy trials, patients with dMMR colon cancers were shown to have reduced rates of tumor recurrence, delayed time-to-recurrence and improved survival rates compared with pMMR colon cancers. Furthermore, a subset analysis suggested that the predictive utility of MMR for 5-FU might be different according to the molecular mechanism underlying dMMR/MSI, i.e., *MLH1* promoter methylation versus germline MMR gene mutation [29*]. A significant survival benefit was observed with 5-FU treatment in patients with suspected Lynch syndrome (disease-free-survival (DFS): HR=0.26; 95% CI, 0.09–0.77; p=0.009) but not in those with sporadic dMMR tumors (DFS: HR=0.79; 95% CI, 0.35 –1.80; p=0.58) [29*]. These data await confirmation in another patient cohort.

Patient treated with 5-FU plus oxaliplatin-based adjuvant therapy

The use of oxaliplatin in combination with adjuvant 5-FU chemotherapy is the current standard of care for stage III colon cancer patients [30–32]. Preclinical data indicate that MSI tumor cells are sensitive to oxaliplatin despite displaying resistance to 5-FU [33]. To date, however, data examining the prognostic/predictive impact of MMR on chemosensitivity to oxaliplatin-based treatment are very limited [34–37]. A preliminary

clinical study suggested that the addition of oxaliplatin may reverse the 5-FU resistance for dMMR stage III colon cancer [35]. Gavin et al reported an analysis of 2,299 stage II and III colon tumors from National Surgical Adjuvant Breast and Bowel Project (NSABP) adjuvant studies including C07 (comparing 5-FU alone or with oxaliplatin) and C08 (comparing FOLFOX with or without bevacizumab) studies [38**]. The authors showed that MSI was prognostic for recurrence in patients treated by FOLFOX (HR=0.48; 95% CI, 0.33–0.70; $p<0.0001$), but not predictive of oxaliplatin benefit [38**–40]. While these data suggest that only patients with pMMR tumors receive benefit from the addition of oxaliplatin to 5-FU and leucovorin, the interaction test between MMR status and treatment was not statistically significant and the analysis was severely underpowered due to the low number of patients and recurrences among dMMR tumors [38**–40]. Fléjou JF et al. reported the results of MMR status in 986 patients out of the 2,240 patients enrolled in the MOSAIC trial [41*]. The results of this analysis showed that the benefit of FOLFOX comparing to 5-FU alone in term of DFS was better in dMMR than in pMMR patients groups (Table 1) [41*]. While this issue is unresolved, prospective evaluation is not feasible because trials comparing fluoropyrimidines versus fluoropyrimidines plus oxaliplatin as adjuvant treatment are unethical given that the combination of a fluoropyrimidine with oxaliplatin is the current standard of care.

Patient treated with 5-FU plus irinotecan-based adjuvant therapy

Two randomized phase III studies, the CALGB 89803 and PETACC3 trials, have evaluated the benefit, if any, of adding irinotecan to 5-FU adjuvant chemotherapy in the treatment of stage III colon cancer. Unlike oxaliplatin, these studies showed that irinotecan added to 5-FU did not confer a statistically significant improvement in DFS or OS compared with 5-FU alone [42, 43]. A retrospective analyze of 702 stage III colon cancer patients included in the CALGB 89803 trial showed that dMMR patients (n=96) treated by 5-FU and irinotecan had an improved 5-year DFS as compared with pMMR patients (n=606) ($p=0.03$). This relationship was not observed among patients treated with 5-FU alone [44]. However, this finding was not confirmed by the analysis of the second study presented by Tejpar et al at the 2009 ASCO meeting [45]. In this retrospective analysis of 1,254 patients included in the PETACC-3 study, authors found that among patients with dMMR tumors, those treated with 5-FU plus irinotecan did not show significantly improved survival compared with patients treated with 5-FU alone [45].

Patient receiving targeted therapies in adjuvant setting

Given the success of biologic agents in the metastatic setting, such as directed against vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EFGR), studies were performed to investigate possible benefit of these agents in the adjuvant setting. However, these trials have shown no survival benefit for anti-EGFR or anti-VEGF antibodies combined with chemotherapy in the adjuvant setting [46–48].

The NCCTG N0147 trial tested the interest of adding cetuximab to oxaliplatin-based standard adjuvant chemotherapy in the treatment of stage III colon cancer [46]. Because of no effect from adjuvant cetuximab was reported, tumors from both study arms were pooled for the analysis of the prognostic impact of MMR status [49]. Defective MMR was detected

in 314 (12%) of 2,580 colon cancer patients. In this study, MMR status was not prognostic overall for DFS (HR=0.82; CI, 0.64–1.07; p=0.14). However, favorable DFS was observed for dMMR versus pMMR tumors in the proximal colon (HR=0.71; 95% CI, 0.53–0.94; p=0.018) but not for distal colon (HR=1.71; 95% CI, 0.99–2.95; p=0.056), adjusting for *KRAS* and $BRAF^{V600E}$ mutations [49].

In the NSABP C-08 trial that showed no benefit for the addition of bevacizumab to FOLFOX [15], a post hoc analysis showed that patients with dMMR tumors derived a statistically significant survival benefit from the addition of bevacizumab (HR=0.52; 95% CI, 0.29–0.94; p=0.02) compared to patients with pMMR tumors (HR=1.03; 95% CI, 0.84–1.27; p=0.78) [50]. The mechanism underlying this interesting finding awaits further study.

Prognostic impact of *KRAS* in patients with stage III colon cancer

The prognostic value of *KRAS* mutations has been evaluated in several studies in the literature. Most of them are small, retrospective, heterogeneous and included patients with stage III CRC but also other tumor stages. Results were conflicting, some studies reporting no prognostic value [51, 52] while some others suggested a prognostic impact of *KRAS* mutations [49, 53] or of a single specific *KRAS* mutation [54–57].

To try to clearly define the prognostic value of *KRAS* mutation in CRC, the RASCAL group developed a collaborative database that includes *KRAS* mutation data, tumor characteristics and outcomes. In the first RASCAL study of 2,721 patients, including 435 stage III CRCs, the rate of *KRAS* mutation was not different according to primary tumor site or stage. Results of multivariate analysis (including tumor stage as a covariate) suggested that the presence of a *KRAS* mutation was associated with a shorter failure-free survival (HR=1.25; CI, 1.10–1.42; p<0.001) and shorter OS (HR=1.22; 95% CI, 1.07–1.40; p=0.004) [57]. Moreover, subgroups analysis suggested that different *KRAS* mutations may not have the same prognostic value [57]. In their second publication, the RASCAL II study evaluated 4,268 patients of which 1,256 had stage III CRCs. They found that the G12V mutation had a significant worse failure-free survival (HR=1.5; 95% CI, 1.13–1.98; p=0.0076) and OS (HR=1.45; 95% CI, 1.07–1.96; p=0.02) in the subgroup of patients with stage III CRC, and in the overall population. All other *KRAS* mutations had no prognostic value [58]. The RASCAL studies were based on the collection of data from patients included in different studies with variable *KRAS* mutation assessment. Moreover, meta-analyses results reported in RASCAL study are limited by the heterogeneity of patients included and the number of analyses performed.

Prospective randomized clinical trials remain the gold standard to validate the value of putative prognostic biomarkers [59]. In the absence of such data, the alternative approach is to retrospectively analyze putative biomarkers from prospective clinical trials as has been done for the predictive value of *KRAS* mutations for anti-EGFR antibodies. The prognostic value of *KRAS* codon 12 and 13 mutations has been evaluated retrospectively in four randomized phase III adjuvant trials in patients with stage II and III colon cancer: the CKVO 90–11 trial (5FU/levamisole *vs* 5FU/levamisole/leucovorin; n=205 patients with stage III), the CALGB 89803 trial (5FU/leucovorin *vs* IFL; n=508 patients with stage III), the

PETACC-3 trial (5FU/leucovorin *vs* FOLFIRI; n=1,321 patients with stage II or III), and the NCCTG N0147 (FOLFOX *vs* FOLFOX/cetuximab; n=2,580 patients with stage III) [13, 25, 49, 52]. In three of these studies, a *KRAS* mutation had no prognostic value [13, 25, 52] with the exception of the N0147 study where *KRAS* mutations were independently associated with poorer DFS and OS after adjustment for clinicopathological features and MMR status [49, 60]. In the PETACC-3 trial, significant interactions were found between the presence of a *KRAS* mutation and tumor site, differentiation grade, age and MMR status. A *KRAS* mutation was more frequent in right tumors and well-differentiated tumors in MSS CRC [13]. In the subgroup of patients with MSS CRC, a *KRAS* mutation was associated with a slightly worse prognostic value for RFS (HR=1.29; 95% CI, 1.03–1.61; p=0.029) and OS (HR=1.33; 95% CI, 1.01–1.74; p=0.039) in patients with a stage II and III CRC. This effect seemed more important in stage II than in stage III tumors [13]. More recently, the prognosis of seven individual *KRAS* mutations in codon 12 and 13 were examined from patients included in the NCCTG N0147 trial and mutations in both codons were associated with adverse outcome [61].

Taken together, these data fail to provide consistent evidence for the prognostic impact of *KRAS* in stage III colon cancer and the explanation for discrepant results remain unclear. Most studies evaluated exon 2 *KRAS* mutations but the prognostic value of the rare mutations occurring in exons 3 or 4 of *KRAS* has not been evaluated. The predictive value of these rare *KRAS* mutations for the benefit of anti-EGFR antibodies has been recently demonstrated in metastatic CRC [62, 63]. In all cases, the absence of benefit of anti-EGFR antibodies in adjuvant setting and the unresolved queries about its prognostic value do not justify testing for *KRAS* mutation in patients with stage III CRC in routine clinical practice.

Prognostic impact of *BRAF^{V600E}* in patients with stage III colon cancer

Consistent evidence indicates that *BRAF^{V600E}* mutations are associated with poor outcome in patients with metastatic CRC as indicated by significantly shorter progression-free survival (PFS) and OS compared to *BRAF* wild-type patients [12, 64]. However, the prognostic value of *BRAF^{V600E}* status in stage II and III colon cancer patients treated with adjuvant chemotherapy remains controversial given conflicting data for RFS/DFS, whereas OS data are more consistent (Table 2) [13, 22, 38**, 49, 65, 66]. A combined data analysis of stage II and III colon cancer patients included in the Pan-European Trials in Alimentary Tract Cancers 3 (PETACC-3), the European Organisation for Research and Treatment of Cancer (EORTC 40993) and the Swiss Group for Clinical Cancer Research (SAKK) 60–00 trials, showed a *BRAF^{V600E}* mutation frequency of 7.9% (n=1,217) that was associated with reduced OS (HR=1.78; 95% CI, 1.15–2.76; p=0.010), but not RFS, in a multivariate analysis [13]. Similarly, the Cancer and Leukemia Group B (CALGB) 89803 trial showed that *BRAF^{V600E}* mutation, detected in 14.8% of cancers, was a poor prognostic factor for OS in a multivariate analysis (HR=1.66; 95% CI, 1.05–2.63; p=0.015), but not for DFS (HR=1.48; 95% CI, 0.96–1.88) in stage III colon cancer patients (n=506) [66]. The prognostic value of *BRAF^{V600E}* was also evaluated in the NSABP C-07 and C-08 adjuvant therapy trials [38**] where the frequency of *BRAF^{V600E}* mutations in 2,226 patients was 14.2%. In stage II and III colon cancer patients, *BRAF^{V600E}* was a prognostic factor for OS (HR=1.46; 95% CI, 1.20–1.79; p=0.0002) but not for RFS (HR=1.02; 95% CI, 0.82–1.28; p=0.86). The survival

after recurrence (SAR) was shortened for patients with *BRAF*^{V600E} mutations and this effects was significant by multivariable analysis (HR=2.3; 95% CI, 1.83–2.95; p<0.0001). Of note, the association of *BRAF*^{V600E} with poor SAR in this study may potentially explain why *BRAF*^{V600E} mutations were not prognostic for RFS, but were for OS and is consistent with its association with poor OS in metastatic patients [66, 67]. In contrast to other studies, *BRAF*^{V600E} mutations were found to be associated with significantly worse DFS [49] and OS [60] rates by multivariable analysis in the NCCTG N0147 trial.

As previously discussed, *BRAF*^{V600E} mutation is frequently observed in sporadic CRCs with MSI. The prognosis impact of *BRAF*^{V600E} mutation according the MMR status has been examined in some retrospective analyses of adjuvant studies [38**, 66, 68–71]. Recently, Gavin et al reported the prognostic value of *BRAF*^{V600E} and MMR status in patients with stage II and III colon cancers treated with fluoropyrimidine-based chemotherapy ± oxaliplatin ± bevacizumab [38**]. Patients whose tumors had *wild type BRAF* and dMMR had the best prognosis (HR, 0.55; p=0.0011), compared with patient tumors with wild type *BRAF* and pMMR [38**]. Patient tumors with mutated *vs* wild type *BRAF* and pMMR had the worst prognosis (HR, 1.58; p=0.0005). Of note, patients with wild type *BRAF*/pMMR or *BRAF*^{V600E} mutations/dMMR tumors had intermediate survival [38**]. In the N0147 adjuvant study, the adverse impact of *BRAF*^{V600E} mutations was limited to pMMR colon cancers [49, 60]. These data suggest that the presence of dMMR may attenuate the adverse prognostic impact of *BRAF*^{V600E} mutations that are detected in nearly 50% of sporadic dMMR tumors [10].

A combined analysis of the predictive role of *BRAF*^{V600E} mutation alone or combined with MSI status in patients treated in the Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) and in the NSAPB-C07 study is in process [31, 72]. This combined analysis aims to evaluate the predictive impact of these two biomarkers for oxaliplatin benefit.

Conclusions

Advances in the molecular characterization of CRCs have identified pathway-based biomarkers that are in current use for detection of hereditary colon cancer, prognostication, and for prediction of response to anti-EGFR antibody therapy in advanced disease. Most studies have shown an association of MSI/dMMR with more favorable patient survival in stage II and III disease. Furthermore, MSI/dMMR predicts lack of 5-FU benefit in stage II disease although data are less clear in stage III, including recent studies that included oxaliplatin. *BRAF*^{V600E} mutations appear to be an adverse prognostic marker in advanced disease and its association with adverse outcome is evident in node-positive colon cancers, especially for OS. Discrepant results exist for oncogenic mutations in *KRAS* in non-metastatic CRC patients in clinical trial cohorts and while the explanation for different results among studies are not entirely clear, relevant factors include retrospective analyses, potential interactions between biomarkers and chemotherapy agents, and the inherent limitations of cross trial comparisons. Attempts to validate findings for these biomarkers in independent patient cohorts and to examine pooled datasets that increase numbers of mutant tumors and outcome events are ongoing. Lastly, studies suggest that combinations of

biomarkers or identification of pathway-based molecular subtypes using genomic tools may be informative for prognosis and/or prediction and hold promise for advancing personalized oncology.

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Table 1

Mains studies evaluating the prognosis and predictive impact of the Mismatch Repair status in stage II and III colon cancer based on the data of randomized controlled trials.

References	Tumor Stage	Arm	Number of patients	MMR status	DFS	HR (95% CI)	p value	OS	HR (95% CI)	p value
5-fluorouracil (5FU)-based adjuvant chemotherapy										
Ribic et al, 2003 [26]	II + III	Surgery alone	245	pMMR	5 yr DFS = 58.7%	not specified	0.01	5 yr OS = 68.4%	0.72 (0.53–0.99)	0.04 *
		5FU	230		5 yr DFS = 69.8%			5 yr OS = 75.5%		
		Surgery alone	42	dMMR	5 yr DFS = 82.9%	not specified	0.11	5 yr OS = 88.0%	2.14 (0.83–5.49)	0.11 *
		5FU	53		5 yr DFS = 69.3%			5 yr OS = 70.7%		
Sargent et al, 2010 [27] (pooled data)	II + III	Surgery alone	436	pMMR	5 yr DFS = 56%	0.69 (0.55–0.86)	0.001	5 yr OS = 66%	0.73 (0.58–0.91)	0.006
		5FU	426		5 yr DFS = 67%			5 yr OS = 74%		
		Surgery alone	79	dMMR	5 yr DFS = 80%	1.61 (0.84–3.10)	0.15	5 yr OS = 85%	1.58 (0.81–3.09)	0.18
		5FU	86		5 yr DFS = 70%			5 yr OS = 73%		
Oxaliplatin-based adjuvant chemotherapy										
Gavin et al, 2013 [38, 40] (from C-07 study)	II + III	5FU	635	pMMR	not specified (TTR)	0.82 (0.67–1.00)	0.054	not specified		
		5FU + oxaliplatin	675							
		5FU	86	dMMR		1.01 (0.45–2.25)	0.98	not specified		
		5FU + oxaliplatin	85							
Gavin et al, 2013 [38, 40] (from C-07 and C-08 studies)	II + III	5FU + oxaliplatin	806	pMMR	3 yr TTR = 78.0%	0.58 (0.35–0.96)	0.03	not specified		
			102	dMMR	3 yr TTR = 87.6%					

References	Tumor Stage	Arm	Number of patients	MMR status	DFS	HR (95% CI)	p value	OS	HR (95% CI)	p value
Fléjou et al, 2013 [41] (from MOSAIC study)	II + III	5FU	50	dMMR	3 yr DFS = 78.0%	0.52 (0.24–1.14)	not specified	5 yr OS = 82.0%	0.45 (0.19–1.05)	not specified
		5FU + oxaliplatin	40		3 yr DFS = 87.5%			5 yr OS = 90.0%		
	III	5FU	28		3 yr DFS = 67.9%	0.51 (0.18–1.41)		5 yr OS = 71.3%	0.44 (0.15–1.34)	
		5FU + oxaliplatin	17		3 yr DFS = 82.4%			5 yr OS = 88.2%		

Abbreviations: MMR, Mismatch Repair; dMMR, defective Mismatch Repair; pMMR, proficient Mismatch Repair; DFS, Disease Free Survival; TTR, Time to recurrence; yr, year

*Multivariate analysis

Table 2

Retrospective analysis from randomized trials that evaluated the impact of *BRAF*^{V600E} mutation on overall survival in stage II and III colon cancer patients.

Study	Patients tested for *BRAF*^{V600E}	Tumor Stage	Frequency of *BRAF*^{V600E} mutation	HR for OS (95% CI)	Multivariate P value
PETTAC-3, EORTC 40993 and SAKK 60–00 Trial (13)	1217	II & III	7.9%	1.78 (1.15–2.76)	0.010
PETTAC-3, EORTC 40993 and SAKK 60–00 Trial (13)	829	III	8%	1.67 (1.04–2.68)	0.035
CALGB 89803 (66)	506	III	14.8%	1.66 (1.05–2.63)	0.015
NSABP C-07 and C- 08 (38**)	2226	II & III	14.2%	1.46 (1.20–1.79)	0.0002
NCCTG N0147 (60)	2831	III	12.2%	1.70 (1.31–2.20)	<0.0001

Abbreviations: PETTAC-3, Pan-European Trials in Alimentary Tract Cancers 3; EORTC 40993, European Organisation for Research and Treatment of Cancer 40993; SAKK 60-00, Swiss Group for Clinical Cancer Research SAKK 60-00; CALGB 89803, Intergroup Cancer and Leukemia Group B 89803; NSABP-C07 and -C08, National Surgical Adjuvant Breast and Bowel Project C07 and C08; NCCTTG N0147, North Central Cancer Treatment Group N0147. OS, overall survival; HR, hazard ratio; CI, confidence interval; NR, not reported