Ann Surg Oncol (2019) 26:3561–3567
https://doi.org/10.1245/s10434-019-07513-6

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – COLORECTAL CANCER

Influence of Molecular Status on Recurrence Site in Patients Treated for a Stage III Colon Cancer: a Post Hoc Analysis of the PETACC-8 Trial

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ABSTRACT

Background. Recurrence patterns in stage III colon cancer (CC) patients according to molecular markers remain unclear. The objective of the study was to assess recurrence patterns according to microsatellite instability (MSI), *RAS* and $BRAF^{V600E}$ status in stage III CC patients.

Methods. All stage III CC patients from the PETACC-8 randomized trial tested for MSI, *RAS* and $BRAF^{V600E}$ status were included. The site and characteristics of recurrence were analyzed according to molecular status. Survival after recurrence (SAR) was analyzed.

Results. A total of 1650 patients were included. Recurrence occurred in 434 patients (26.3%). Microsatellite stable (MSS) patients had a significantly higher recurrence rate (27.2% vs. 18.7%, $P = 0.02$) with a trend to more pulmonary recurrence (28.8% vs. 12.9%, $P = 0.06$) when compared to MSI patients. MSI patients experienced more regional lymph nodes compared to MSS (12.9% vs. 4%, $P = 0.046$). In the MSS population, the recurrence rate was significantly higher in *RAS* (32.2%) or *BRAF* (32.3%) patients when compared to double wild-type patients (19.9%) ($p < 0.001$); no preferential site of recurrence was observed according to *RAS* and $BRAF^{V600E}$ mutations.

Electronic supplementary material The online version of this article (https://doi.org/10.1245/s10434-019-07513-6) contains supplementary material, which is available to authorized users.

First Received: 2 March 2019;
Published Online: 17 June 2019

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Finally, decreased SAR was observed in the case of peritoneal recurrence or more than two recurrence sites.
Conclusions. Microsatellite, *RAS* and $BRAF^{V600E}$ status influences recurrence rates in stage III CC patients. However, only microsatellite status seems to be associated with specific recurrence patterns. More than two recurrence sites and recurrence in the peritoneum were associated with poorer SAR.

Colon cancer (CC) is the third most common cancer worldwide.[1] When diagnosed at a localized stage, curative management including colectomy with complete mesocolic excision is required and fluoropyrimidine-based adjuvant chemotherapy is proposed when nodal involvement is observed (stage III disease).[2–4] In 2004, adjuvant oxaliplatin-fluoropyrimidine combination therapy became the worldwide standard treatment for stage III CC patients able to receive.[3] It increases the 3-year, disease-free survival (DFS) rate and the long-term overall survival (OS) rate compared with fluoropyrimidine alone.[3]

However, 50% of stage III patients are cured by surgery alone, 20% with the addition of adjuvant chemotherapy, and 30% will develop recurrence, which is generally fatal within 2–3 years.[2] Disease stage remains the most important prognostic variable, but there is considerable stage-independent prognostic variability, likely due to tumor characteristics and biological heterogeneity.[5–8] Colorectal cancer is biologically heterogeneous and two major pathways of colorectal carcinogenesis have been described: chromosomal instability and, less commonly, microsatellite instability (MSI), which is reported in approximately 15% of sporadic cases.[5] Recent publications also show that $BRAF^{V600E}$ and *RAS* mutations are prognostic factors and are significantly associated with shorter DFS and OS in stage III CC patients with microsatellite-stable (MSS) tumors, but not in MSI tumors.[6,8–10] With regard to recurrence patterns of stage III CC patients, data analyzing the association between the recurrence site and these molecular markers remain poor and unclear.

The PETACC-8 trial was an open randomized, controlled, multinational/multicenter European phase III study that evaluated the efficacy of cetuximab in addition to FOLFOX-4 for 6 months in patients with fully resected stage III colon cancer.[11] The purpose of this post hoc analysis was to assess, in stage III colon cancer patients derived from the PETACC-8 trial, recurrence patterns according to MSI, *RAS,* and $BRAF^{V600E}$ status.[11] We also examined survival after recurrence (SAR) according to the site, the number of recurring sites, and biomolecular markers.

METHODS

Population of the Study

This study included all stage III CC patients (any T, N1 or N2, M0) from the PETACC-8 trial, (NCT00265811) promoted by the FFCD (Fédération Francophone de Cancérologie Digestive), with a signed informed consent for biological sample collection and tested for MSI, *RAS,* and *BRAF* status.[11] The trial failed to demonstrate the benefit in DFS of the addition of cetuximab to the FOLFOX (folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin) regimen in stage III CC.[11] The PETACC-8 trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the appropriate Ethics Committees.

Follow-up

For patient follow-up, a standard radiological evaluation was made at randomization and during adjuvant treatment and then every 6 months during the first 5 years of follow-up and then annually, including at least chest X-ray and abdominal US, but more generally a chest-abdomen-pelvis CT scan. Neurological explorations were guided by the clinical exam. Diagnosis of recurrence was established either histologically or by imaging. The PETACC-8 database used in this study was locked on October 27, 2016.

Data Extraction

Demographics, cancer history, pathological, clinical, and biological parameters were prospectively collected at the time of randomization, as were efficacy outcomes (DFS, OS, and SAR).

Mismatch Repair Status

Mismatch repair (MMR) tumor status was determined by immunohistochemical analysis (IHC) or by MSI testing when IHC was indeterminate. MSI phenotype tumors were defined as exhibiting the loss of expression of one or more MMR proteins by IHC or exhibiting high-level tumor DNA MSI on MSI testing. MSS phenotype tumors were defined by normal MMR protein expression in IHC or MSS or low-level MSI status on MSI testing. Mismatch repair protein (MLH1, MSH2, MSH6, PMS2) expression was analyzed by IHC on tissue microarrays. Concerning MSI testing, DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissues for MSI analysis using five monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, NR-27). Specimens with at least three unstable markers were scored as highly unstable, and

specimens with fewer than three unstable markers were scored as stable.

RAS and BRAF^{V600E} Status

RAS hotspot mutations (c.34G > A/p.G12S, c.34G > C/p.G12R, c.34G > T/p.G12C, c.35G > A/p.G12D, c.35G > C/p.G12A, c.35G > T/p.G12 V and c.38G > A p.G13D) and the *BRAF^{V600E}* mutation (c.1799T > A/p.V600E) were detected by real-time PCR with TaqMan® probes (Applied Biosystems, Paris, France). Exons 2, 3, and 4 of KRAS and NRAS, as well as BRAF exons 11 and 15, were sequenced with the Ampliseq colon-lung cancer panel version 2 in the PETACC8 trial participants who consented to translational research.

Statistical Analysis

Median (interquartile range) values and proportions (percentage) were provided for the description of continuous and categorical variables, respectively. Median and proportions were compared using the Wilcoxon-Mann–Whitney test and χ^2-test (or Fisher's exact test, if appropriate), respectively.

Survival after recurrence (SAR) was defined as time between recurrence and death from any cause and was estimated using the Kaplan–Meier method and described using median or rate at specific time points with their 95% confidence intervals (95% CI). Differences between groups of patients were analyzed with unstratified log-rank tests.

All analyses were performed using R software version 2.15.2 (R Development Core Team, Vienna, Austria; http://www.r-project. org). P values < 0.05 were considered statistically significant, and all tests were two-sided.

RESULTS

Patients

Among the 2559 patients enrolled in the PETACC-8 phase III trial, between December 2005 and November 2009, 1650 patients with signed informed consent for biological sample collection and tested for MMR, *RAS* and *BRAF^{V600E}* status were included (Fig. 1). In the present study population, MSI tumors and *RAS* and *BRAF^{V600E}* mutations were found in 10% (166 of 1650), 45% (748 of 1650), and 7.7% (127 of 1650) of patients, respectively. The overall median follow-up was 7.82 (95% CI, 7.69–7.91) years.

Recurrence

Recurrence occurred in 26.3% of patients of the stage III CC patients included (434/1650). The mean time to recurrence was 1.7 ± 1.4 years. Of the 434 patients who experienced recurrence, 31 were MSI (7%) and 403 MSS (93%). MSS patients (403 of 1484) had a significantly higher recurrence rate compared with MSI patients (31 of 166) (27.2% vs. 18.7%, $P = 0.02$), with a trend to more frequent pulmonary recurrence in MSS patients as compared to MSI patients (28.8% vs. 12.9%, $P = 0.06$) (Fig. 2; Supplementary Table 1). Conversely, the preferential site of recurrence in the MSI group was regional lymph nodes compared with MSS patients (12.9% vs. 4%, $P = 0.046$) (Fig. 2; Supplementary Table 1).

In the MSS population, *RAS* and *BRAF^{V600E}* mutations were found in 50.4% (748 of 1484) and 8.6% (127 of 1484) of patients, respectively. The recurrence rate was significantly higher in *RAS* (241/748, 32.2%) and *BRAF^{V600E}* (41/127, 32.3%) patients compared with double wild-type patients (121/609, 19.9%; $P < 0.001$) (Fig. 3; Supplementary Table 2). However, no preferential site of recurrence was observed in this MSS population according to *RAS* and *BRAF^{V600E}* mutations (Fig. 3, Supplementary Table 2). Concerning the RAS alterations, no significant differences were found between KRAS and NRAS mutant cases concerning the number and the site of recurrences.

Survival after Recurrence

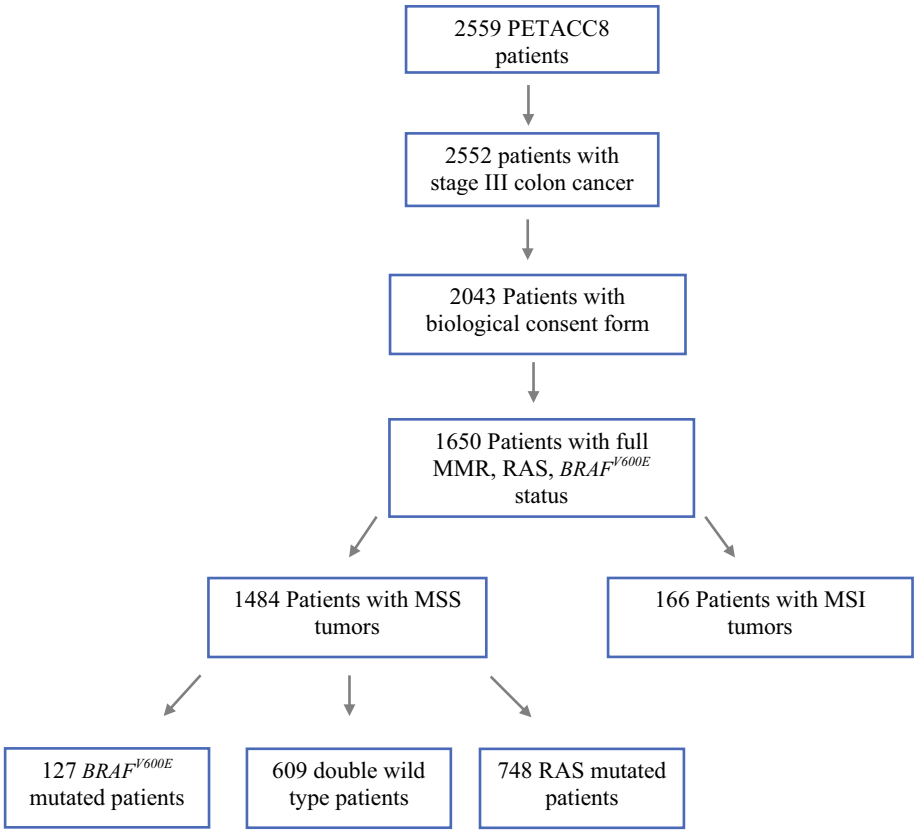
In the 434 patients who experienced recurrence, the median survival after recurrence (SAR) was 24 months (95% CI 21.8–26.5). No significant differences in SAR were found between MSI and MSS patients (19.4 vs. 24.2 months, HR 1.05, 95% CI 0.66–1.67, P =0.83).

In the entire recurrence population, *BRAF^{V600E}* and *RAS* mutations were associated with significantly poorer SAR compared with wild-type patients (median SAR for double wild-type: 29 months, *RAS*mut 24 months and *BRAF^{V600E}*mut 15.2 months; HR for *BRAF^{V600E}*mut = 2.46; 95% CI 1.72–3.53 and HR for *RAS*mut = 1.23; 95% CI 0.96–1.59, $P < 0.0001$, respectively) (Fig. 4a).

In all patients who experienced recurrence, peritoneal recurrence was significantly associated with poorer SAR (median SAR: 19.7 vs. 24.3 months, HR 1.79, 95% CI 1.26–2.54, $P = 0.001$) (Fig. 4b). A trend to a better prognosis was found for lung recurrence (27.8 vs. 22.6 months, HR 0.80, 95% CI 0.62–1.03, $P = 0.08$) (Fig. 4c). SAR according to liver recurrence was not modified (HR 1.12, 95% CI 0.92–1.44, $P = 0.22$) (Fig. 4d).

Interestingly, the number of recurrence sites was prognostic. More than two recurrence sites was associated with poorer SAR compared with one or two sites (median SAR:

FIG. 1 Flow chart



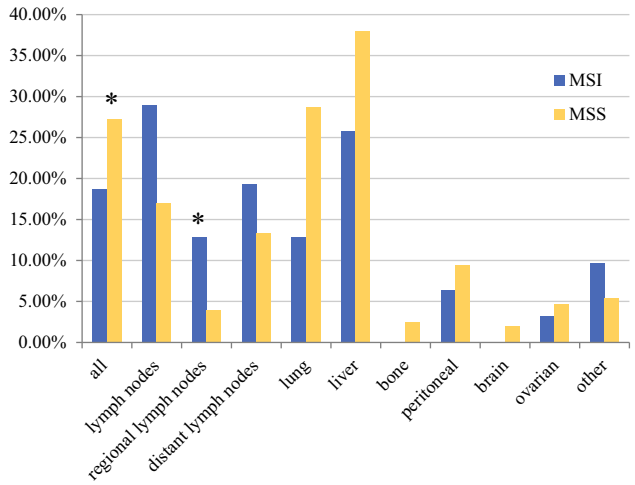


FIG. 2 Percentage of patients with disease recurrence according to metastatic site and MSS status. *$p < 0.05$

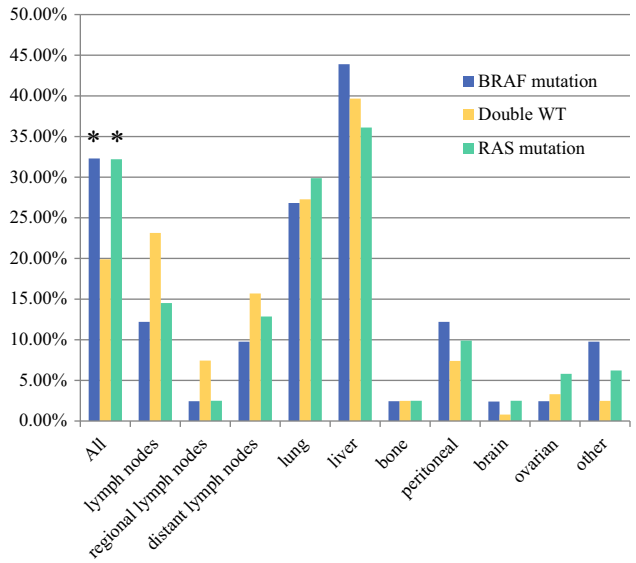


FIG. 3 Percentage of patients with disease recurrence according to metastatic site, *BRAF*^{V600E} and RAS status in the MSS population. *$p < 0.05$. BRAF mutation = BRAF^{V600E} mutation

15.2 vs. 25.9 months, HR 2.7, 95% CI 1.71–4.25, $P < 0.0001$) (Fig. 4e).

DISCUSSION

Biological molecular status in metastatic colorectal cancer is becoming increasingly relevant; *RAS*, *BRAF*^{V600E}, and MSI assessments are nowadays part of our routine practice and are recommended upfront to drive patients' treatment in the metastatic setting.[12] However, little is known about their correlation with site-specific patterns of recurrence in patients initially nonmetastatic.

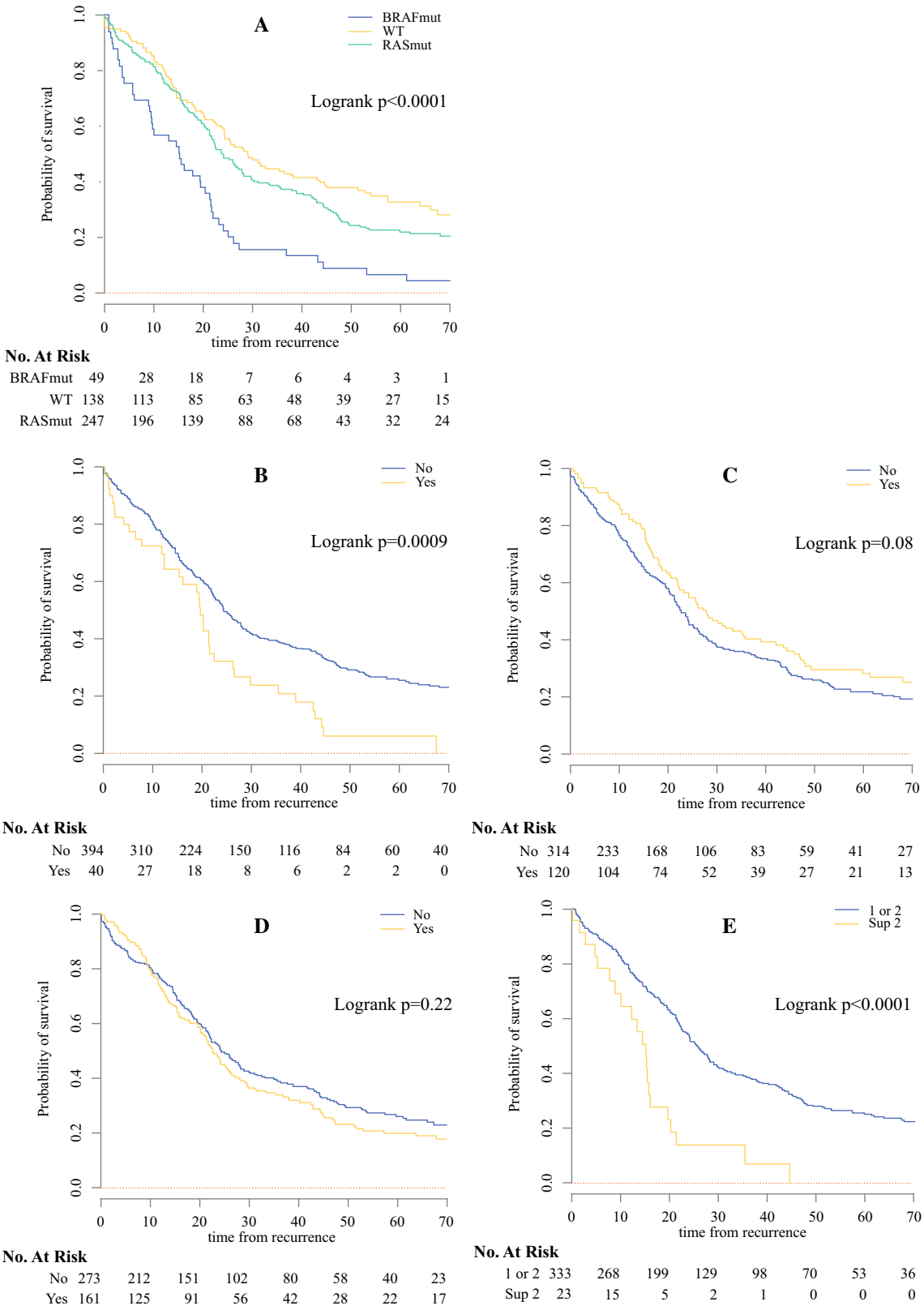


FIG. 4 Survival after recurrence according to molecular profile and recurrence site. **a** Survival after recurrence according to *RAS* and *BRAF*^{V600E} status. BRAFmut = BRAF^{V600E} mutation. **b** Survival after recurrence according to peritoneal recurrence. **c** Survival after recurrence according to lung recurrence. **d** Survival after recurrence according to liver recurrence. **e** Survival after recurrence according to number of recurrence sites

The present study analyzed recurrence rate and site of recurrence in 1650 stage III CC patients of the PETACC-8 trial tested for biological molecular markers. The main findings were a higher recurrence rate in the MSS population with the "lung" as a preferential site of recurrence, as MSI patients experienced fewer recurrences with "regional lymph nodes" as a preferential site of recurrence. Survival after recurrence was not significantly different between MSI and MSS patients. In the MSS population, *RAS* and $BRAF^{V600E}$ mutations were associated with significantly higher recurrence rates, but no preferential sites of recurrence according to these mutations were observed. Finally, peritoneal recurrence and more than two recurrence sites were associated with poorer SAR.

Concerning the impact of MMR status on the site of recurrence, Prasanna et al.[13] recently published a retrospective study of two Australian databases including more than 5000 stage IV CC patients and analyzed the association between tumor biological characteristics (including MSI status) and the site of metastatic lesions. They found that liver-only metastases were less frequent in MSI patients (relative risk [RR] = 0.7, $P = 0.01$) but that MSI status was not associated with other specific sites of metastatic disease. In our study, MSI tumors were not associated with a lower rate of liver recurrence but with a higher rate of regional node metastases, a finding that has been already reported by others.[14,15] We also found a strong trend to more lung recurrences in MSS patients, which has, to our knowledge, not been described before. The prognostic significance of MSI phenotype in metastatic disease is still controversial; some studies reported poor outcome in metastatic MSI tumors and other studies not.[7,14,16–18] Here, SAR was not significantly different between the MSS and MSI patients after a median follow-up exceeding 7 years (median SAR: 19.4 vs. 24.2 months, $P = 0.82$). However, our study focused on recurrence patterns and SAR of stage III patients and not on initially nonresectable metastatic patients, unlike previous studies of that topic.[17]

Concerning *RAS* status, we found no preferential site of metastatic relapse in our population, whereas a major part of the available literature in patients with resected stage II and III colon cancer reports that the presence of a *RAS* mutation significantly increases the rate of lung recurrence, with HR ranging from 1.4 to 2.1.[13,19–21] Interestingly, Yaeger et al.[22] described that at the time of metastasis diagnosis, *RAS* mutated tumors are more likely to be associated with lung metastasis compared with *RAS* wild-type (22% vs. 13%, $P < 0.01$). In our study, the rate of lung recurrence in *RAS* mutant patients was slightly but not significantly increased compared with *RAS*/$BRAF^{V600E}$ wild-type and $BRAF^{V600E}$ mutant patients (29.9%, 27.3%, 26.7%, respectively, NS).

$BRAF^{V600E}$ mutated tumors were not statistically associated with a specific site of recurrence in the present work. However, we found a trend for a higher rate of peritoneal recurrence in $BRAF^{V600E}$ mutant patients as compared to *RAS* mutant and double wild-type patients (12.2% vs. 7.44% vs. 9.96%, respectively) as previously described in the literature.[13,14,21,23–26] These patterns of metastatic spread have even been suggested to be responsible, at least in part, for the poor outcomes of $BRAF^{V600E}$ mutant mCRC. Here, although no significant difference was found for peritoneal recurrence in $BRAF^{V600E}$ mutants, the poor prognosis of these patients was clearly observed.

We also observed a trend to more regional node recurrence in double wild-type MSS patients when compared to $BRAF^{V600E}$ or *RAS* mutated patients, although this recurrence pattern occurs in less than 10% of patients in all molecular subgroups. $BRAF^{V600E}$ and *RAS* mutations were associated with significantly poorer SAR in the entire series compared with wild-type patients. Moreover, peritoneal recurrence and number of recurrence sites (more than two) were significantly associated with poorer SAR. These results are in line with the available literature and confirm that a peritoneal recurrence pattern is a poor prognostic indicator.[5–9,16,21,25–27]

The strengths of the present study include the analysis of biological markers in a large, prospective collection of tumors, from a homogenous population of stage III colon cancer patients, treated in a randomized trial with the standard adjuvant chemotherapy. Limitations include the retrospective analysis of data from subgroups with limited numbers of patients in some cases, which may preclude the drawing of any definitive conclusions. Moreover, the absence of data on the treatment of recurrence may impact the SAR results.

To conclude, this large analysis of stage III colon cancer patients tested for MSI, $BRAF^{V600E}$ and *RAS* shows that MSS patients had more recurrence and that recurrence is more frequent in MSS patients with *RAS* and $BRAF^{V600E}$ mutations. Recurrence pattern analysis showed more lung recurrence for MSS patients and more regional node recurrence for MSI patients, but no preferential site for disease recurrence for *BRAF* or *RAS* patients in this series.

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