

Metastatic colorectal cancer first-line treatment with bevacizumab: the impact of K-ras mutation

Luigi Rossi^{1,2}
 Enzo Veltri³
 Angelo Zullo⁴
 Federica Zoratto¹
 Maria Colonna⁵
 Flavia Longo⁶
 Marcella Mottolose⁷
 Diana Giannarelli⁸
 Luigi Ruco⁹
 Paolo Marchetti¹⁰
 Adriana Romiti¹⁰
 Viola Barucca¹⁰
 Giuseppe Giannini¹¹
 Loredana Bianchi¹
 Silverio Tomao¹

¹Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Rome, Italy; ²Oncology Unit, ICOT Hospital, Latina, Italy; ³Oncology Unit, SM Goretti Hospital, Latina, Italy; ⁴Gastroenterology and Digestive Endoscopy Unit, Nuovo Regina Margherita Hospital, Rome, Italy; ⁵Oncology Unit, Don Luigi di Liegro Hospital, Gaeta, Italy; ⁶Oncology Unit, Umberto I Policlinico di Roma Hospital, Sapienza University of Rome, Rome, Italy; ⁷Department of Pathology, Regina Elena National Cancer Institute, Rome, Italy; ⁸Department of Pathology, ⁹Oncology Unit, Sant'Andrea Hospital, ¹⁰Department of Pathology, ¹¹Department of Pathology, Umberto I Policlinico di Roma Hospital, Sapienza University of Rome, Rome, Italy

Correspondence: Luigi Rossi
 Oncology Unit, ICOT Hospital,
 1668 Franco Faggiana Street,
 Latina 04100, Italy
 Tel +39 349 564 3418
 Fax +39 0773 651 3342
 Email dr.rossi@ymail.com

Background: Bevacizumab plus chemotherapy prolongs progression-free survival (PFS) and overall survival (OS) in metastatic colorectal cancer (mCRC). Although there is strong evidence to suggest that the mutational status of the K-ras oncogene has a role as a predictive factor for activity in patients treated with cetuximab and panitumumab, few data have been obtained in patients treated with bevacizumab. We conducted an additional retrospective analysis to investigate the prognostic value of K-ras mutation relative to mCRC first-line treatment with bevacizumab.

Materials and methods: A total of 108 patients were retrospectively reviewed. K-ras status was assessed in the overall population by sequencing. Statistical association for PFS and OS was analyzed using the Kaplan–Meier method, and the prognostic role of K-ras was determined using the logrank test.

Results: Median PFS was 10 months both for patients with wild-type (WT) K-ras and mutated (MT) K-ras (hazard ratio [HR] 0.94, $P=0.75$); neither difference in median OS was significant (27 months WT K-ras versus 26 months MT K-ras, HR 0.92; $P=0.70$). A further analysis was carried out in the two groups according to metastatic sites. No statistically significant difference in terms of PFS and OS was demonstrated between WT K-ras and MT K-ras with liver metastases only and in those with extrahepatic disease.

Conclusion: Although further study is required, our results seem to confirm that K-ras mutation does not have a prognostic role in mCRC patients receiving first-line treatment with bevacizumab.

Keywords: K-ras, bevacizumab, prognostic factor, metastatic colorectal cancer, liver metastases, extrahepatic disease

Introduction

Predictive and prognostic tumor markers are playing an increasingly important role in personalized metastatic colorectal cancer (mCRC) care.^{1,2} These tags range from conventional single protein-, ribonucleic acid- or deoxyribonucleic acid (DNA)-based markers to molecular signatures based on multiplex assays, and they are used to guide clinical management of patients.³ Currently, biomolecular factors with ascertained predictive and prognostic value in mCRC are the K-ras and *BRAF* oncogenes, respectively.^{4–10} K-ras, the human homologue of the Kirsten rat sarcoma 2 virus oncogene, encodes a small guanosine triphosphate-binding protein that acts as a self-inactivating signal transducer by cycling from guanosine diphosphate- to guanosine triphosphate-bound states in response to stimulation of a cell-surface receptor.^{11,12} K-ras can harbor oncogenic mutation that yields a constitutively active protein. Mutations (mainly at

exons 2 and 3) occur early in the progression of colorectal adenoma to carcinoma and they are found in approximately 30%–50% of CRC.^{5,11,13,14} The identification of a biologic and a clinical role for K-ras mutation marked a turning point in the scenario of mCRC treatment. As a matter of fact, the importance of K-ras and its downstream signaling pathways in tumor development is well established. In the last few years, K-ras mutation has acquired an essential clinical significance as a negative predictive factor in mCRC patients treated with anti-epidermal growth factor receptor (EGFR) agents, such as cetuximab and panitumumab.^{1,2} In addition, the prognostic value of tumor K-ras status has been extensively evaluated, but results have been conflicting. Some studies have demonstrated a prognostic effect,^{1,5} while others have failed.⁶

The interaction of EGFR and vascular endothelial growth factor (VEGF) is well known.^{15,16} Bevacizumab, a humanized monoclonal antibody that binds to and neutralizes VEGF-A, has been widely used in various mCRC treatment lines, improving response rates, progression-free survival (PFS), and overall survival (OS).^{17–24} At the time, anti-VEGF predictive and prognostic factors were not found, and the role of K-ras-mutation status in patients undergoing treatment with bevacizumab remains of great interest. Furthermore, it has been ascertained that K-ras mutation is also associated with specific clinical signatures of tumor as an increased risk of metastasis in lung and brain, even if does not modify the risk of metastasis in the liver.²⁵

In a previous study, we examined the role of K-ras status as a predictive factor of response on antiangiogenic therapy, affirming that bevacizumab provides clinical benefit and objective response rate in mCRC patients independently of K-ras expression and metastatic sites.²⁶

Moving from such an appealing biological and clinical background, we planned an additional retrospective analysis aimed at the identification of a possible prognostic role of K-ras in mCRC patients treated with first-line chemotherapy plus bevacizumab. Correlations between K-ras status and PFS and OS were analyzed. Furthermore, we evaluated whether K-ras mutation could influence the outcome of patients with liver metastases only, in comparison to patients with extrahepatic disease.

Materials and methods

Patients and study design

The design of this study has been reported previously.²⁶ In brief, this was a retrospective study enrolling consecutive 108 unresectable mCRC patients, treated in clinical practice in

three Italian oncology centers (Rome, Latina, Gaeta) between September 2008 and March 2011. Patients with histologically confirmed mCRC, Eastern Cooperative Oncology Group performance status ≤ 2 , assessment of K-ras mutation status, no previous chemotherapy for advanced disease, treated with first-line chemotherapy with either the FOLFOX (oxaliplatin, leucovorin, and fluorouracil) or FOLFIRI (folic acid, fluorouracil, and irinotecan) regimen combined with bevacizumab, not a candidate for liver metastasis resection after chemotherapy, with adequate hepatic and renal function, and no contraindications to bevacizumab therapy were included.

The primary end point of this study was to assess the prognostic value of tumor K-ras status on PFS and OS of mCRC patients treated with first-line chemotherapy plus bevacizumab; secondary end points included evaluation of how K-ras mutation could influence the outcome of patients with liver metastases only with respect to patients with extrahepatic disease, clinical response PFS and OS of bevacizumab combined with chemotherapy in the overall population, and whether these same parameters differ between patients with hepatic metastases only and those with extrahepatic metastatic sites. Response rates to treatment with bevacizumab according to K-ras expression have been reported in our previous retrospective study.²⁶ Survival results were last updated in March 2012. PFS was defined as the time from the beginning of the treatment until the first observation of disease progression or death from any cause, while OS was defined as the time from the beginning of the treatment until death from any cause. Finally, patients with extrahepatic metastatic disease were defined as patients with metastases both in the liver and in other organs or with metastases not present in the liver but in other sites.

K-ras-mutation analysis

Evaluation of K-ras status was performed retrospectively on primary tumor samples only. K-ras analysis was performed centrally using existing platforms. DNA was extracted from paraffin-embedded CRC samples after histological control for at least 50% of tumor cells. Mutations at codons 12, 13, and 61 were assessed by means of direct sequencing with a polymerase chain reaction (PCR) method.²⁷

Statistical analysis

PFS and OS curves were calculated according to K-ras tumor-mutation status using the Kaplan–Meier method. The prognostic value of the biological marker was determined using the logrank test. A *P*-value of less than 0.05 was considered significant.

Results

A total of 108 mCRC patients were consecutively enrolled in three Italian oncology units between September 2008 and March 2011. Tumor K-ras status was assessed in the entire population. In total, 69 (64%) patients had wild-type (WT) K-ras tumors, while 39 (36%) patients had mutated (MT) K-ras. The main patient characteristics according to K-ras status are shown in Table 1. Significant differences were observed between the two groups in primary side of origin, metastatic sites, primitive tumor resection, prior adjuvant chemotherapy, and chemotherapy first-line regimen. Regarding metastatic sites, liver metastases only were present in 53 patients (41 WT K-ras patients versus twelve MT K-ras patients), extrahepatic disease affected 55 patients (28 WT K-ras patients versus 27 MT K-ras patients); among the extrahepatic sites were reported 56% lung, 31% peritoneal, 2% brain, and 11% other sites. In particular, 61.3% and 2% of patients with lung and brain metastases, respectively, had MT K-ras tumors.

PFS and OS were obtained from 106 of 108 patients, as two patients were lost to follow-up. In the overall population, median PFS (mPFS) and median OS (mOS) were 10 months (95% confidence interval [CI] 8.9–11.1) and 26 months (95% CI 23.5–28.5), respectively, with 86 (81%) deaths at last survival update.

mPFS was the same between WT K-ras and MT K-ras (10 months, hazard ratio [HR] 0.94, 95% CI

0.62–1.40, $P=0.75$) (Figure 1A). No statistically significant difference was observed in mOS either: patients with WT K-ras tumors had an mOS of 27 months versus 26 months for patients with MT K-ras tumors (HR 0.92, 95% CI 0.58–1.42; $P=0.70$), with 55 versus 31 death events in the two groups, respectively (Figure 1B).

When patients were analyzed for metastatic sites, mPFS was 10.5 months in patients with liver metastases only versus 10 months in the extrahepatic disease group (HR 0.90, 95% CI 0.59–1.32; $P=0.59$) (Figure 2A); similarly, identical mOS was observed in both groups (26 months, HR 1.28, 95% CI 0.86–1.98; $P=0.72$) (Figure 2B).

Analyzing these two subpopulations according to K-ras expression, we obtained the following results: in patients with liver metastases only, mPFS was 11 months in the WT K-ras group versus 10 months in the MT K-ras group (HR 0.88, 95% CI 0.41–1.77; $P=0.70$) (Figure 3A); mOS was greater in WT K-ras patients than in MT K-ras patients, but it was not statistically significant (27 months versus 21 months, respectively, HR 1.11, 95% CI 0.50–2.45; $P=0.78$) (Figure 3B). When metastatic disease was also extrahepatic or extrahepatic only, mPFS was 8.5 months in the WT K-ras group versus 10.5 months in the MT K-ras group (HR 1.04, 95% CI 0.61–1.80; $P=0.87$) (Figure 4A), while mOS was 25.5 versus 26.5 months, respectively (HR 0.75, 95% CI 0.39–1.27; $P=0.29$) (Figure 4B).

The administration of subsequent therapy lines after the discontinuation of first-line chemotherapy was examined. Patients treated with first-line FOLFIRI received second-line FOLFOX chemotherapy, and vice versa. All patients with WT K-ras tumors received either chemotherapy plus cetuximab in second-line therapy or one of the two anti-EGFR agents in third-line treatment (cetuximab in combination with irinotecan or monotherapy with panitumumab). However anti-EGFR agents did not induce OS difference between WT K-ras and MT K-ras tumors.

Discussion

In the overall population, the PFS and OS data regarding first-line mCRC treatment with chemotherapy and bevacizumab were modeled on literature data.^{18,21} Bevacizumab effectiveness was confirmed both in liver metastasis-only patients and in extrahepatic metastatic patients. The outcome of patients was not affected by subsequent anti-EGFR first-line chemotherapy; at the same time, several studies reported no difference between first-line chemotherapy based on the

Table 1 Baseline characteristics of patients included in the K-ras analysis according to tumor K-ras status

Characteristic	WT K-ras (n=69)	MT K-ras (n=39)	P-value
Median age years (range)	64 (41–78)	55 (39–79)	NA
Sex			
Male	44	24	0.8
Female	25	15	
ECOG PS	0	0	NA
Primary tumor			
Colon	55	29	0.5
Rectum	14	10	
Metastatic sites			
Liver only	41	12	0.004
Extrahepatic	28	27	
Primitive resection	59	30	0.3
Adjuvant treatment	14	6	0.3
First-line chemotherapy			
FOLFIRI	58	30	0.4
FOLFOX	11	9	

Abbreviations: ECOG PS, Eastern Cooperative Group performance status; WT, wild type; MT, mutant; NA, not available; FOLFIRI, folic acid, fluorouracil, and irinotecan; FOLFOX, oxaliplatin, leucovorin, and fluorouracil.

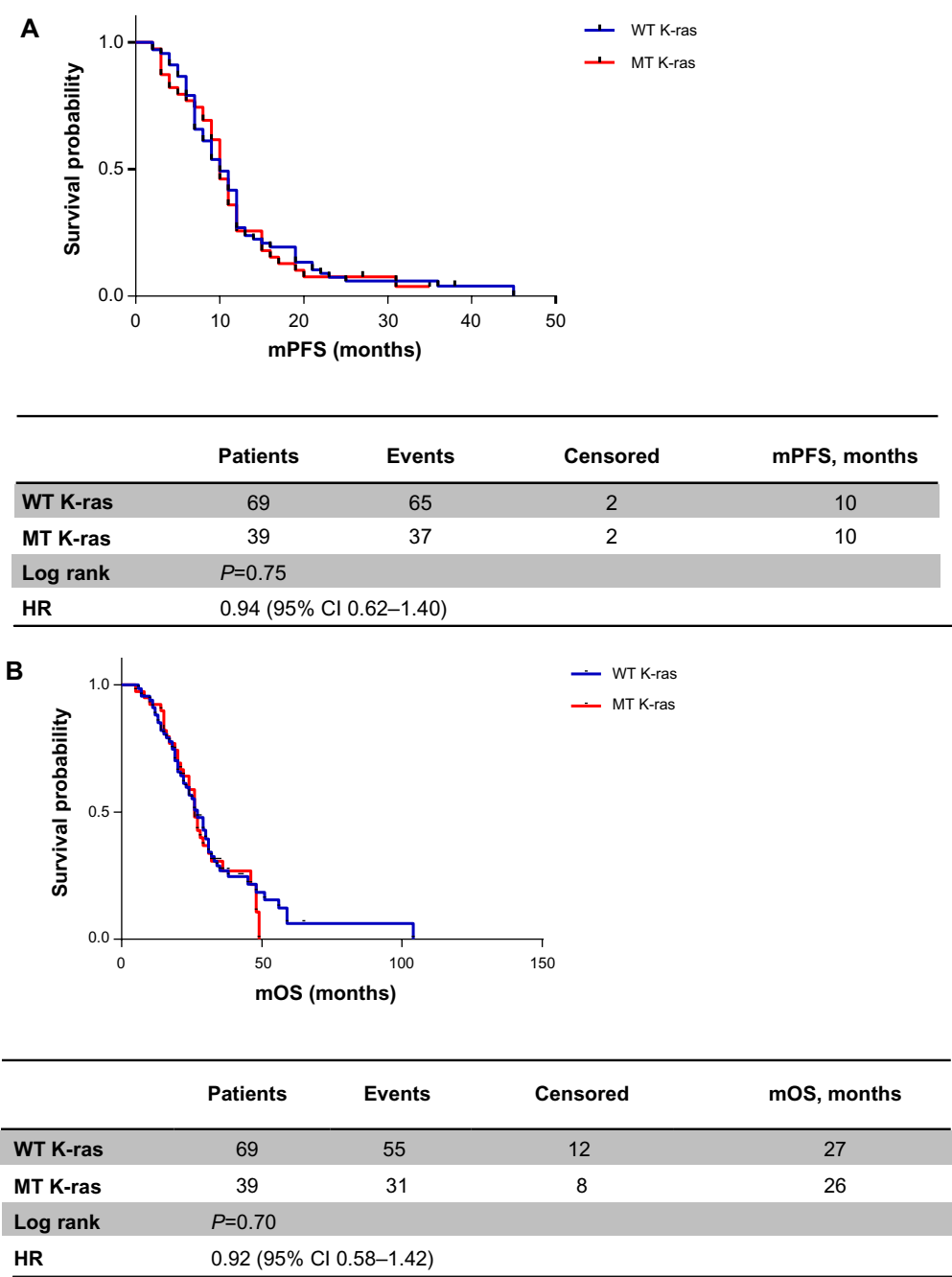


Figure 1 (A) Progression-free survival according to K-ras status; **(B)** overall survival according to K-ras status.
Abbreviations: WT, wild type; MT, mutated; mPFS, median progression-free survival; mOS, median overall survival; HR, hazard ratio; CI, confidence interval.

FOLFIRI regimen followed by second-line FOLFOX therapy, and vice versa.^{28–30}

In this study, we addressed an important question, ie, whether K-ras status is a prognostic factor in mCRC patients receiving first-line chemotherapy plus bevacizumab, given the intimate relationship between the EGFR and VEGF signaling pathways. Our retrospective experience indicates that tumor K-ras status does not influence outcomes of patients treated

with antiangiogenic therapy: patients with WT K-ras tumors had identical clinical benefit as those with MT K-ras tumors in terms of PFS (10 months) and near-identical clinical benefit in terms of OS (27 versus 26 months, respectively). A trend was observed in WT K-ras patients with liver metastases only compared with MT K-ras patients (PFS 11 versus 10 months and OS 27 versus 21 months, respectively), but this advantage was not statistically significant.

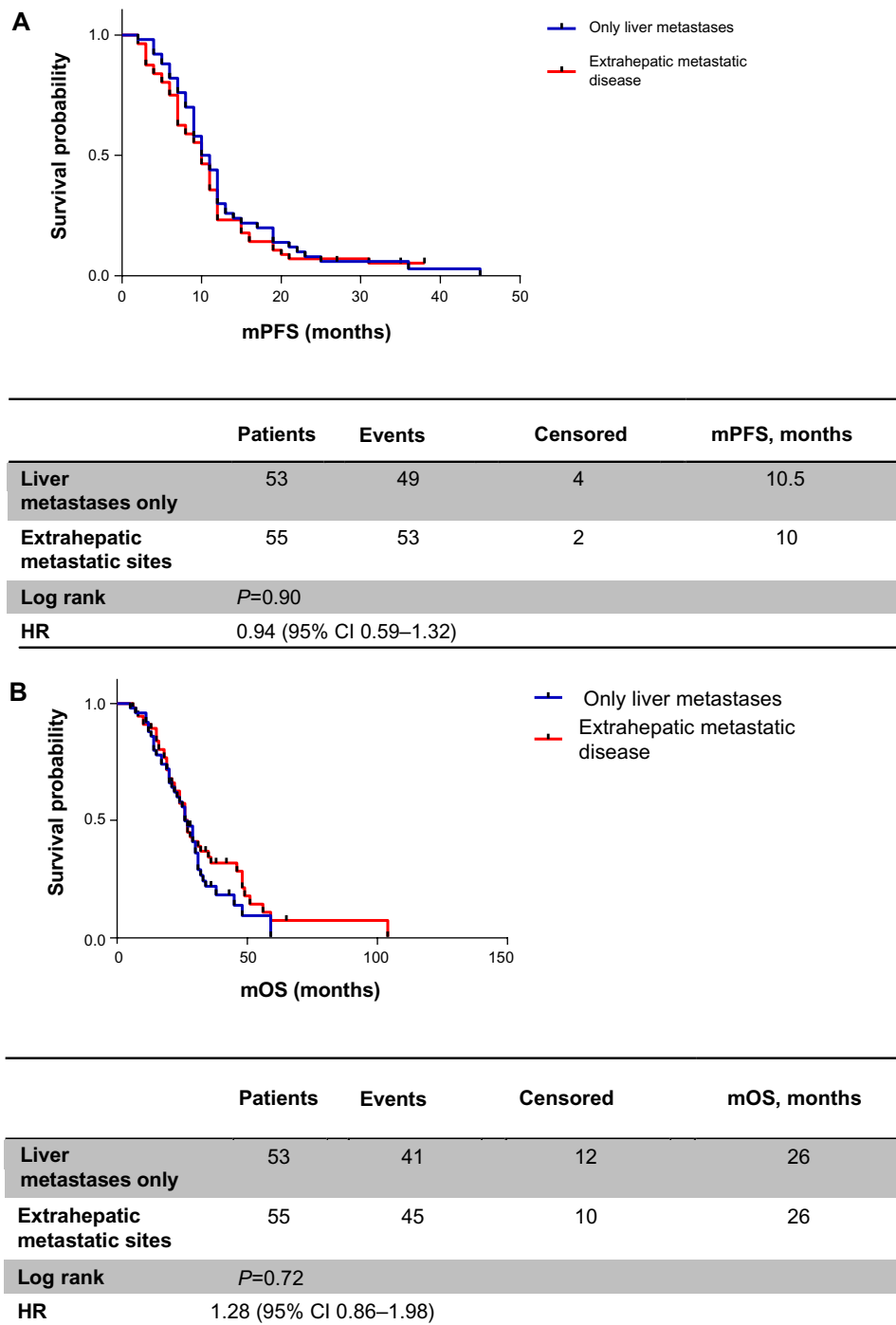


Figure 2 (A) Progression-free survival according to metastatic site; **(B)** overall survival according to metastatic site.

Abbreviations: mPFS, median progression-free survival; mOS, median overall survival; HR, hazard ratio; CI, confidence interval.

On the other hand, patients with MT K-ras tumors and extrahepatic disease appear to have a better prognosis than the WT K-ras group. Moreover, K-ras mutation is more frequently associated with extrahepatic disease, such as in the lung and brain, according to literature data.²⁵ Some studies have suggested that MT K-ras is associated with aggressive

mCRC due to the presence of extrahepatic metastases,^{31,32} though in our analysis K-ras mutation was not correlated with poor prognoses.

Several studies have evaluated the role of K-ras status in mCRC patients receiving first-line treatment with bevacizumab (Table 2). Ince et al did not establish a statistically significant

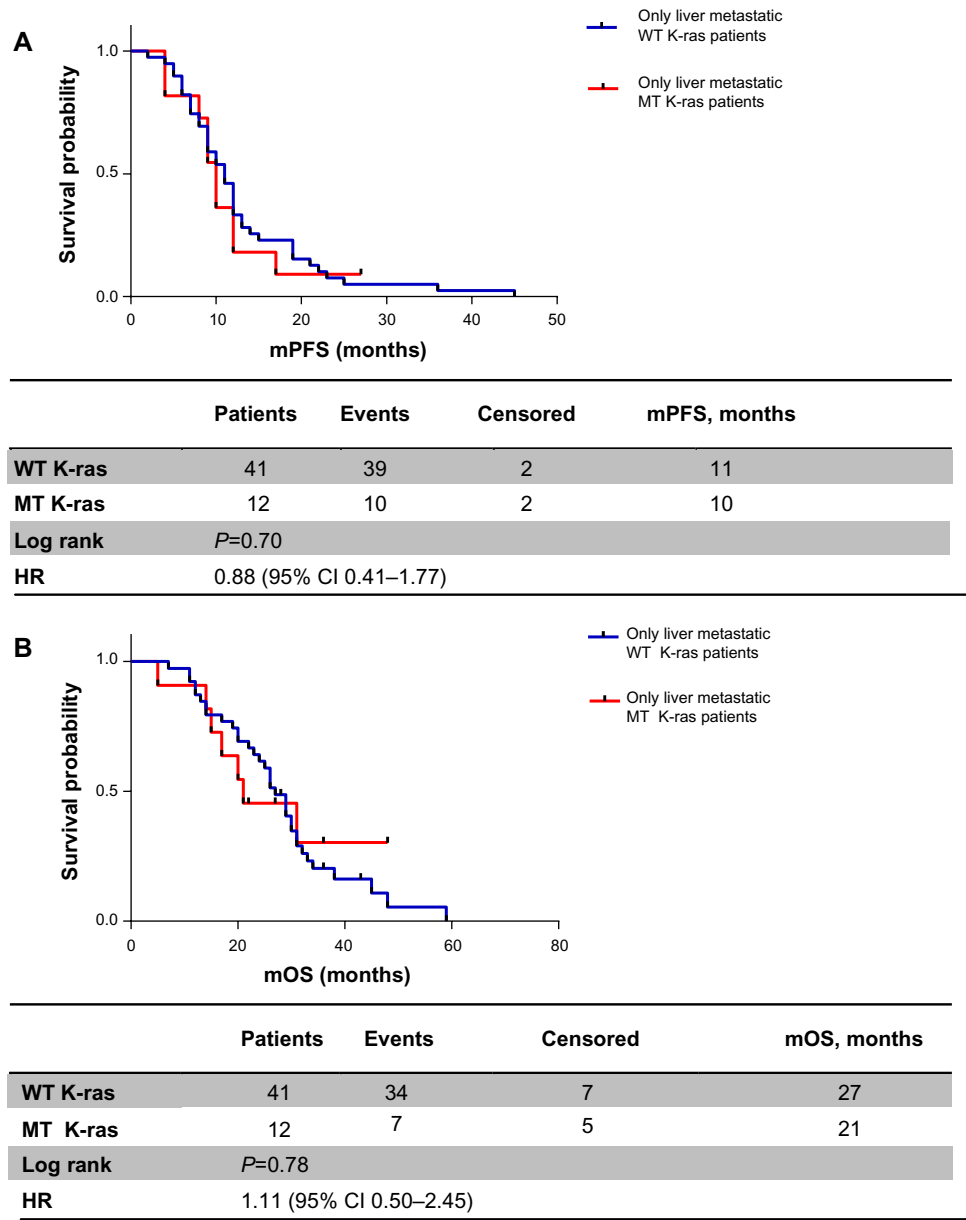


Figure 3 (A) Progression-free survival according to K-ras status in liver-only metastasis patients; **(B)** overall survival according to K-ras status in liver-only metastasis patients. **Abbreviations:** WT, wild type; MT, mutated; mPFS, median progression-free survival; mOS, median overall survival; HR, hazard ratio; CI, confidence interval.

difference in terms of OS between WT K-ras and MT K-ras in bevacizumab-treated patients.³³ In a subsequent analysis by Hurwitz et al, PFS was greater for bevacizumab-treated patients with WT versus MT K-ras tumors.³⁴ Recently, Díaz-Rubio et al suggested a prognostic role for K-ras status in mCRC patients who were enrolled in the MACRO (maintenance treatment in advanced colorectal cancer) trial.³⁵ A statistically significant difference was observed in OS and PFS between WT K-ras tumors and MT K-ras tumors, with a greater benefit of bevacizumab treatment in WT K-ras patients. On the contrary, other studies have reported that K-ras mutation is not a prognostic factor for patient outcome.^{24,36–39} Masi

et al claimed that patients with K-ras mutations derive similar clinical benefit from bevacizumab compared with patients who have WT K-ras.³⁶ This variability in results could be a result of several factors, including the number of patients included in different trials, the proportions of patients tested for K-ras mutation status and technology used, the chemotherapy regime under investigation, and subsequent second- and third-line therapies.³⁹

Our analysis seems to be in agreement with studies that did not suggest any therapeutic implication of K-ras mutation when bevacizumab was given in addition to chemotherapy,^{24,36–39} but our study has several limitations. Firstly,

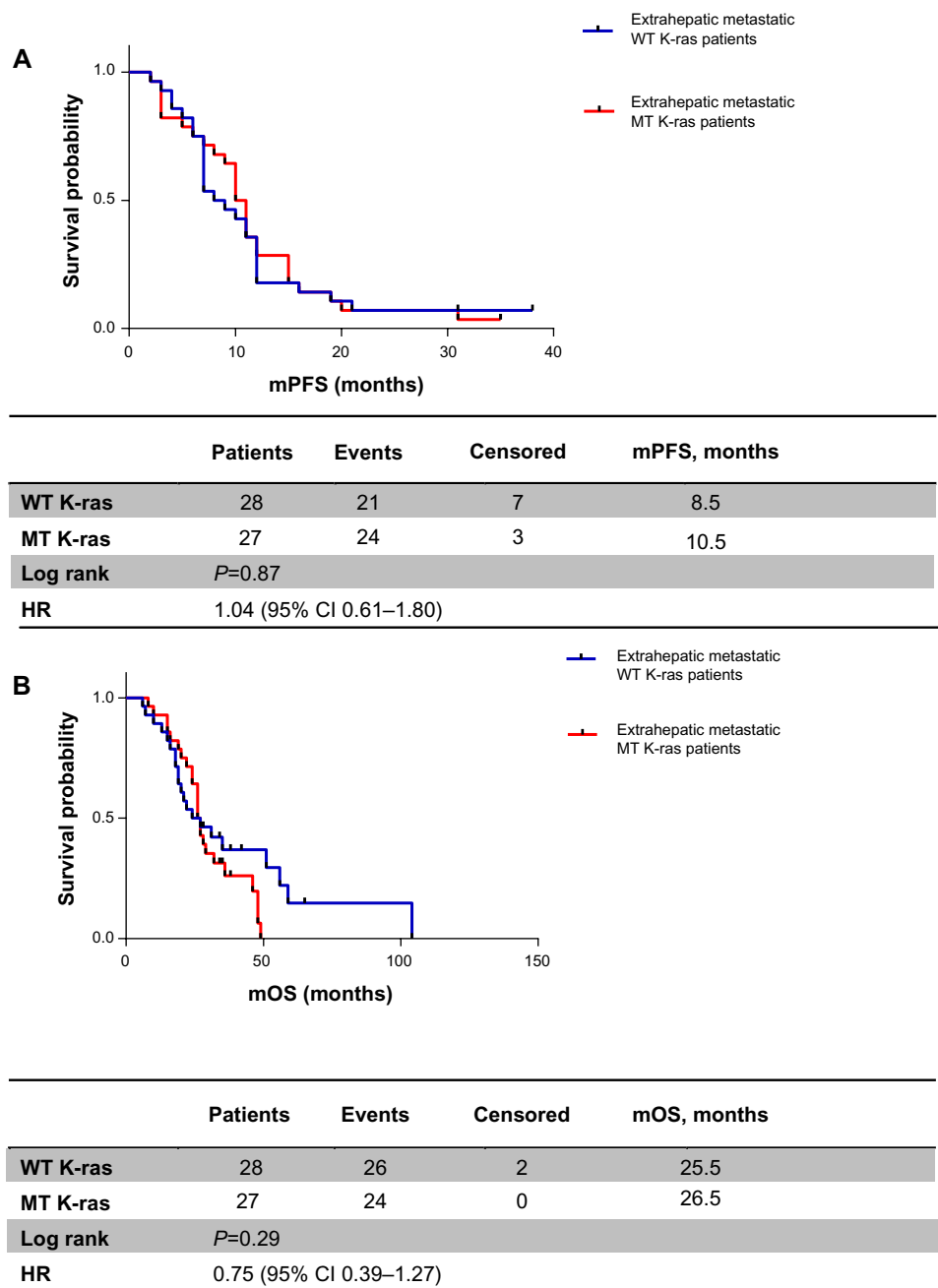


Figure 4 (A) Progression-free survival according to K-ras status in extrahepatic metastatic patients; **(B)** overall survival according to K-ras status in extrahepatic metastatic patients.

Abbreviations: WT, wild type; MT, mutated; mPFS, median progression-free survival; mOS, median overall survival; HR, hazard ratio; CI, confidence interval.

K-ras-mutation analysis was retrospective, which is common in randomized studies examining the role of biomarkers. Secondly, the number of patients in the overall population was small, resulting in imbalance parameters between WT and MT K-ras tumors. In addition, K-ras-status evaluation was performed only on primitive tumors.

In conclusion, this retrospective analysis found that chemotherapy plus bevacizumab is a reliable first-line therapeutic

approach in mCRC, regardless of whether metastases are liver only or also extrahepatic. Finally, K-ras status did not appear to affect the outcome of patients receiving antiangiogenic therapy, according to some literature reports, but not others. The reasons for the discrepancy between studies are not yet apparent or sufficiently powered. The importance of K-ras mutation status remains to be explored, and future studies will be required to answer this question.

Table 2 Summary of K-ras data from studies of bevacizumab + chemotherapy in mCRC patients

Regimen	Ince et al ³³	Hurwitz et al ³⁴	Díaz-Rubio et al ³⁵	Masi et al ³⁶	Tol et al ³⁷	Hecht et al ³⁸	Price et al ³⁹
	IFL + BV	IFL + BV	XELOX + BV→ XELOX + BV/BV alone	FOLFOXIRI + BV	CapeOx + BV	CT(Iri,OX) + BV	Cape + BV ± MitC
WT K-ras*	152/267	85/402	219/480	34/57	156/368	104/525	224/314
MT K-ras*	78/267	44/402	175/480	21/57	108/368	82/525	90/314
PFS, months							
WT K-ras	NA	13.5	10.9	13.6	10.6	11.5	8.8
MT K-ras	NA	9.3	9.4	12.6	12.5	11	8.2
P-value	NA	NA	0.0038	NA	0.80	NA	NA
OS, months							
WT K-ras	27.7	27.7	26.7	30.9	22.4	24.5	19.8
MT K-ras	19.9	19.9	18.0	NA	24.9	19.3	17.6
P-value	NA	NA	0.0002	NA	0.82	NA	NA

Note: *Number of patients WT or MT K-ras/number of patients in study.

Abbreviations: mCRC, metastatic colorectal cancer; WT wild type; MT mutant; IFL irinotecan, 5-fluorouracil, and leucovorin; BV, bevacizumab; XELOX, capecitabine and oxaliplatin; FOLFOXIRI, 5-fluorouracil, oxaliplatin, and irinotecan; CapeOx, capecitabine and oxaliplatin; Iri, irinotecan; Ox, oxaliplatin; Cape, capecitabine; MitC, mitomycin C; WT wild-type; MT, mutated; NA, not available; PFS, progression-free survival; OS, overall survival; CT, chemotherapy.

Disclosure

The authors report no conflicts of interest in this work.

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