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BRAF and *RAS* mutations as prognostic factors in metastatic colorectal cancer patients undergoing liver resection

M Schirripa[1,9], F Bergamo[2,9], C Cremolini[1], M Casagrande[3], S Lonardi[2], G Aprile[3], D Yang[4], F Marmorino[1], G Pasquini[1], E Sensi[5], C Lupi[5], G De Maglio[6], N Borrelli[5], S Pizzolitto[6], G Fasola[3], R Bertorelle[7], M Rugge[8], G Fontanini[5], V Zagonel[2], F Loupakis*[,1,10] and A Falcone[1,10]

[1]*Unit of Medical Oncology 2, Department of Translational Research and New Technologies in Medicine and Surgery, Azienda Ospedaliero-Universitaria Pisana and University of Pisa, Via Roma 67, 56126 Pisa, Italy;* [2]*Unit of Medical Oncology 1, Department of Medical Oncology, Oncology Institute of Veneto—IRCCS, via Gattamelata 64, 35128 Padova, Italy;* [3]*Department of Oncology, Azienda Ospedaliero-Universitaria 'Santa Maria della Misericordia', Piazzale Santa Maria della Misericordia 15, 33100 Udine, Italy;* [4]*Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School Of Medicine, University of Southern California, 1441 Eastlake Avenue, 90033 Los Angeles, California, USA;* [5]*Department of Surgery, Division of Pathology, University of Pisa, Via Roma 57, 56126 Pisa, Italy;* [6]*Department of Laboratory Medicine, Division of Pathology, Azienda Ospedaliero-Universitaria 'Santa Maria della Misericordia', Piazzale Santa Maria della Misericordia 15, 33100 Udine, Italy;* [7]*Unit of Immunology and of Oncological Molecular Diagnostics, Department of Oncological Diagnostics, Oncology Institute of Veneto—IRCCS, via Gattamelata 64, 35128 Padova, Italy and* [8]*Unit of Pathology, University Hospital of Padova, Via Gabelli 61, 35128 Padova, Italy*

Background: Despite major advances in the management of metastatic colorectal cancer (mCRC) with liver-only involvement, relapse rates are high and reliable prognostic markers are needed.

Methods: To assess the prognostic impact of *BRAF* and *RAS* mutations in a large series of liver-resected patients, medical records of 3024 mCRC patients were reviewed. Eligible cases undergoing potentially curative liver resection were selected. *BRAF* and *RAS* mutational status was tested on primary and/or metastases by means of pyrosequencing and mass spectrometry genotyping assay. Primary endpoint was relapse-free survival (RFS).

Results: In the final study population ($N = 309$) *BRAF* mutant, *RAS* mutant and all wild-type (wt) patients were 12(4%), 160(52%) and 137(44%), respectively. Median RFS was 5.7, 11.0 and 14.4 months respectively and differed significantly (Log-rank, $P = 0.043$). At multivariate analyses, *BRAF* mutant had a higher risk of relapse in comparison to all wt (multivariate hazard ratio (HR) = 2.31; 95% CI, 1.09–4.87; $P = 0.029$) and to *RAS* mutant (multivariate HR = 2.06; 95% CI, 1.02–4.14; $P = 0.044$). Similar results were obtained in terms of overall survival. Compared with all wt patients, *RAS* mutant showed a higher risk of death (HR = 1.47; 95% CI, 1.05–2.07; $P = 0.025$), but such effect was lost at multivariate analyses.

Conclusions: *BRAF* mutation is associated with an extremely poor median RFS after liver resection and with higher probability of relapse and death. Knowledge of *BRAF* mutational status may optimise clinical decision making in mCRC patients potentially candidate to hepatic surgery. *RAS* status as useful marker in this setting might require further studies.

*Correspondence: Dr F Loupakis; E-mail: fotiosloupakis@gmail.com
[9]These authors contributed equally as first authors.
[10]These authors contributed equally as senior authors.

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Resection of liver metastases (CLM) represents a possibility of cure in metastatic colorectal cancer (mCRC) (Tomlinson *et al*, 2007). Currently, the number of patients candidate to hepatic resection has dramatically increased thanks to the integration of new surgical techniques with more effective therapies (Kopetz *et al*, 2009; Primrose, 2010). Consequently, overall survival (OS) rates progressively increased, exceeding 50% at 5 years in resected patients (Hayashi *et al*, 2010).

However, liver resection is a complex and costly procedure and tumour relapse occurs in almost two-thirds of patients after a potentially curative resection (de Jong *et al*, 2009). Thus, it is evident that the need for prompt identification of patients at higher risk of recurrence. Several studies examined prognostic markers for recurrence after CLM resection but only common clinico-pathological characteristics are included in risk estimating scoring systems (Nordlinger *et al*, 1996; Fong *et al*, 1999; Rees *et al*, 2008; Primrose, 2010). Available scores are not sensitive enough to definitely exclude patients from a potentially useless surgery, which, at the same time, may stand as the only chance for cure.

During the last decade, the assessment of *RAS* and *BRAF* mutational status gained increasing importance for an optimal management of CRC (Schmoll *et al*, 2012; Douillard *et al*, 2013). *BRAF* V600E mutation, occurring in 6–10% of mCRC, defines a subgroup with low probability of long-term survival, specific clinico-biological features with high rate of nodal and peritoneal metastases (Richman *et al*, 2009; Saridaki *et al*, 2010; Tran *et al*, 2011; Yokota *et al*, 2011). *RAS* mutations, occurring in about 50% of mCRC(Peeters *et al*, 2013; Morris *et al*, 2014; Schirripa *et al*, 2014), are determinants of resistance to anti-EGFR monoclonal antibodies (Lievre *et al*, 2006; Amado *et al*, 2008; Karapetis *et al*, 2008; Van Cutsem *et al*, 2011; Douillard *et al*, 2013) and are linked to higher incidence of lung and brain metastases (Cejas *et al*, 2009; Tie *et al*, 2011; Kim *et al*, 2012). The prognostic role of *RAS* mutations is controversial and a mild negative effect is reported both in the adjuvant and in the metastatic setting (Andreyev *et al*, 2001; Richman *et al*, 2009; Van Cutsem *et al*, 2011).

In this complex scenario, *BRAF* and *RAS* mutations might increase the chance of selecting appropriate candidates for liver resection. Some authors suggested a possible negative prognostic role for *RAS* mutations in patients undergoing CLM resection, while the extremely small number of *BRAF* mutant patients identified in the published series did not allow to draw definitive conclusions (Teng *et al*, 2012; Karagkounis *et al*, 2013; Umeda *et al*, 2013; Vauthey *et al*, 2013).

Moving from the above-mentioned considerations, we carried out the present work to investigate *BRAF* and *RAS* mutations as prognostic biomarkers in a wide population of patients who underwent liver resection with curative intent.

MATERIALS AND METHODS

Patients' selection and clinical data collection. Clinical records from three Italian Oncology Units with a high volume of mCRC patients were reviewed. Data from consecutive mCRC patients referred to the Units of Pisa (2005–2012), Padova (1995–2012) and Udine (2000–2012) were evaluated for inclusion.

Patients with histological diagnosis of colorectal adenocarcinoma who underwent liver resection with curative intent defined by a multidisciplinary team were selected according to the following eligibility criteria:

(1) Availability of tumour tissue for mutational status evaluation;
(2) Adequate follow-up defined as 'clinical visits, including evaluation of CEA level and a chest/abdomen CT scan performed within 3 months from liver resection and then repeated at least once every 4 months for 3 years after resection'.

Baseline characteristics collected are reported in Table 1. Data concerning systemic therapies before and/or after liver resection and sites of first relapse were also collected.

Patients who met these selection criteria were included in the 'eligible patients' population'.

Molecular analyses. Primary and/or corresponding liver metastasis were retrieved from the archives of Pathology Departments of the three collaborating Institutions.

A screening genotyping for *KRAS* (exon 2) and *BRAF* V600E mutation was run by means of Pyrosequencing on the PyroMark

Table 1. Final study population characteristics according to mutational status

Characteristics	*BRAF* mut (*N* = 12)	*RAS* mut (*N* = 160)	All wt (*N* = 137)	*P*
	N (%)	*N* (%)	*N* (%)	
Sex				
Male	7 (58)	94 (59)	91 (66)	0.38
Female	5 (42)	66 (41)	46 (34)	—
Age				
<65 years	8 (67)	89 (56)	80 (58)	0.71
⩾65 years	4 (33)	71 (44)	57 (42)	—
ECOG PS				
0	8 (67)	139 (87)	120 (88)	0.12
1–2	4 (33)	21 (13)	17 (12)	—
Primary tumour site				
Right colon	7 (58)	61 (38)	19 (14)	**<0.0001**
Left colon	3 (25)	55 (35)	80 (59)	—
Rectum	2 (17)	43 (27)	37 (27)	—
Liver only				
Yes	10 (83)	142 (89)	129 (94)	0.17
No	2 (17)	18 (11)	8 (6)	—
Unilobar mts				
Yes	9 (75)	102 (64)	87 (64)	0.72
No	3 (25)	58 (36)	50 (36)	—
Time to mts				
Synchronous	10 (83)	110 (69)	88 (64)	0.34
Metachronous	2 (17)	50 (31)	49 (36)	—
Resection outcome				
R0	10 (83)	129 (81)	119 (87)	0.34
R1/R2 Expl. Lapar.	2 (17)	31 (19)	18 (13)	—
Primary lymph nodes				
No	2 (17)	45 (29)	49 (36)	0.21
Yes	10 (83)	111 (71)	86 (64)	—
NA	0	4	2	—
DFI <12 months				
Yes	11 (92)	128 (80)	103 (75)	0.31
No	1 (8)	32 (20)	34 (25)	—
>1 liver mts				
No	5 (42)	63 (40)	64 (48)	0.37
Yes	7 (58)	96 (60)	70 (52)	—
NA	0	1	3	—
Mts diameter >5 cm				
No	8 (67)	127 (82)	96 (76)	0.29
Yes	4 (33)	28 (18)	30 (24)	—
NA	0	5	11	—
CEA >200 ng ml^{-1}				
No	12 (100)	117 (98)	84 (93)	0.24
Yes	0 (0)	3 (2)	6 (7)	—
NA	0	40	47	—
Clinical risk score[a]				
Low	5 (42)	67 (47)	64 (54)	0.42
High	7 (58)	76 (53)	54 (46)	—
NA	0	17	19	

Abbreviations: CEA = carcinoembryonic antigen; DFI = disease-free interval; ECOG = Eastern Cooperative Oncology Group; Expl. Lapar. = exploratory laparotomy; mts = metastasis; mut = mutant; *N* = number; NA = not available; PS = performance status; wt = wild-type.
[a]Clinical risk score is defined as previously described by Fong *et al* (1999): patients with 0 to 2 risk features were categorised as 'low risk', while those with 3 to 5 features as 'high risk'.
Bold entries indicate significant results.

Q96 ID instrument (Qiagen, Hilden, Germany) with commercially available kits (Diatech Pharmacogenetics, Ancona, Italy).

KRAS (exon 2) wild-type (wt), *BRAF* wt and patients with discordant results on primary and corresponding liver metastasis were centrally re-evaluated by means of MassARRAY (Sequenom Inc., San Diego, CA, USA) with the CE-IVD marked kit Myriapod Colon Status (Diatech Pharmacogenetics) on primary or corresponding liver metastasis. The assay allows simultaneous analyses of *KRAS*, *BRAF* and *NRAS*, tested mutations are listed in Supplementary Table 1.

Patients with informative mutational status results were defined as 'final study population', and based on their mutational status were categorised as: *BRAF* mut, *RAS* mut and all wt (*BRAF* and *RAS* wt).

Methods for microsatellite instability determination are described in the Supplementary Appendix 1.

Statistical considerations. Results of *BRAF* and *RAS* mutational analyses were used as categorical variables. Fisher's exact test or χ^2-test was used to compare clinical and biological features according to mutational status.

The primary endpoint was relapse-free survival (RFS) according to *BRAF* and *RAS* status in the final study population. All other analyses were exploratory and aimed to assess secondary endpoints. RFS was defined as the time from liver resection to first disease recurrence or death due to any cause; OS was defined as the time from liver resection to death due to any cause. Overall survival and RFS analyses were determined according to the Kaplan–Meier method and survival curves were compared using the log-rank test. Statistical significance was set at $P<0.05$ for a bilateral test.

The correlation of mutational status and clinico-pathological characteristics with survival was firstly assessed in the univariate analyses. Cox proportional hazard model was adopted in the multivariate analysis, including as covariates variables correlated with survival in the univariate analyses ($P<0.1$). An exploratory recursive partitioning analysis was performed.

RESULTS

Patient populations and mutational analyses. A total of 3024 mCRC patients were referred to the three institutions during the specified time frame. Case-by-case revision of medical records allowed to identify 494 subjects who underwent liver resection. Among them, 360 patients met eligibility criteria and were included in the 'eligible patients' population' (Figure 1). Baseline characteristics are reported in Supplementary Table 2.

Pyrosequencing analyses. BRAF V600E mutational status was performed in the primary tumour, in a liver metastasis or both in 63 (17.5%), 59 (16.5%) and 238 (66%) cases, respectively. Eleven cases (3%) resulted *BRAF* mut. No discordance between primaries and related metastases was observed.

KRAS exon 2 mutational status was performed in the primary tumour, in a liver metastasis or both in 63 (17.5%), 61 (17%) and 234 (65%) cases, respectively; 2 (0.5%) samples were not evaluable. One-hundred-fourteen cases (32%) resulted *KRAS* mut. A discordant result between the primary tumour and related liver metastasis was found in 18 cases (8%), 12 primaries were mut with wt metastases and 6 metastases were mut with wt primaries. *BRAF* and *KRAS* exon 2 wt patients were 215 (60%).

Sequenom analyses. About 233 cases (from 215 *BRAF* and *KRAS* exon 2 wt patients and from 18 patients showing discordant *KRAS* mutational status results) were tested. Forty-three cases were excluded due to tumour tissue and/or DNA insufficient and/or inadequate. Six out of 18 primary-metastases couples discordant at pyrosequencing had the previous results confirmed at MassARRAY (Sequenom Inc.) testing. Ten out of 18 discordant couples were found mut both on the primary tumour and on the corresponding liver metastasis. A *BRAF*, *NRAS* or *KRAS* mutation was found in 1, 17 and 29 cases, respectively, out of 190 cases.

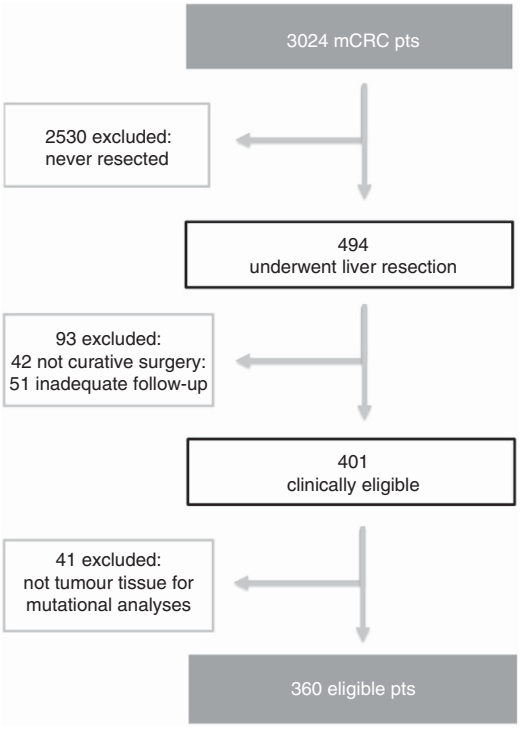

Figure 1. Diagram of eligible patients population selection.

The final study population included 309 patients with informative results: 12 (4%) *BRAF* mut, 160 (52%) *RAS* mut and 137 (44%) all wt patients. A diagram showing the selection process is shown in Figure 2. A detailed mutational status description of the final study population is shown in Supplementary Table 3.

Clinical characteristics and their association with mutational status. No differences were observed between the eligible patients population and the final study population (Supplementary Table 2).

Among patients included in the final study population: 67% had synchronous disease, 91% had liver limited disease, 57% had more than one liver metastasis and 36% had bilobar liver involvement. With regard to medical treatments administered: 50% of patients received a systemic treatment before and after liver resection (including bevacizumab or anti-EGFR monoclonal antibodies in 31% and 5% of cases, respectively), 36% received a systemic treatment only after liver resection; 14% were not treated neither before nor after liver resection; 26% received an anti-EGFR in subsequent lines; 21% received an adjuvant treatment after primary tumour resection.

Only 51 patients (17%) underwent R1/R2-exploratory laparotomy instead of curative surgery due to unexpected metastatic spread observed during surgery.

No differences in clinical or pathological characteristics were observed according to mutational status, except for primary tumour location: all wt tumours were right-, left-sided or rectal in 14%, 59% and 27% of cases, respectively. All wt tumours showed different primary tumour location in comparison to *BRAF* mut tumours (right-, left-sided or rectal in 58%, 25% and 17% of cases, respectively, $P<0.0001$) and to *RAS* mut tumours (right-, left-sided or rectal in 38%, 35% and 27% of cases, respectively, $P<0.0001$) (Table 1).

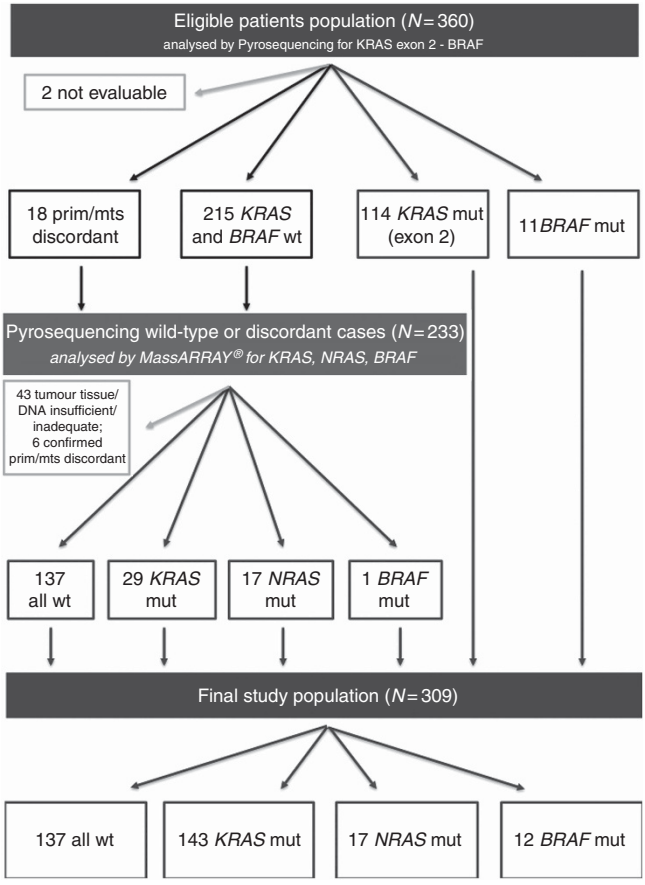

Figure 2. Diagram of final study population selection. *N* = number; prim = primary; mts = metastasis; wt = wild-type; mut = mutant.

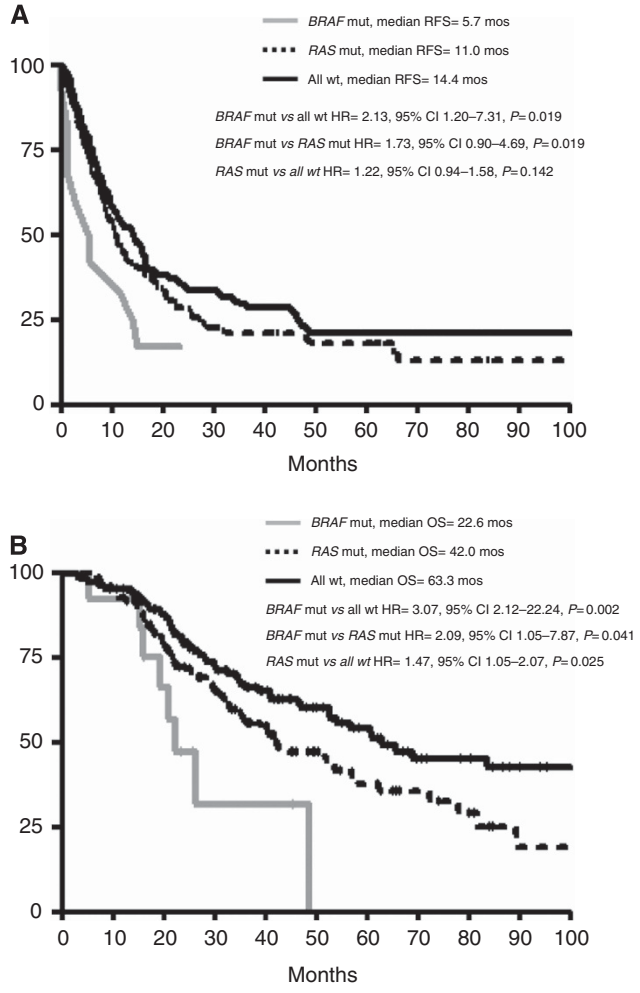

Figure 3. Relapse-free survival and overall survival according to mutational status. (**A**) Relapse-free survival; (**B**) overall survival. Mut = mutant; wt = wild-type; HR = hazard ratio; CI = confidence interval.

Survival analyses. At a median follow-up of 45.6 months, 236 (76%) patients showed disease recurrence and 144 (47%) patients had died.

Relapse-free survival outcomes differed significantly according to mutational status ($P = 0.043$) and median RFS were 5.7 months, 11.0 months and 14.4 months in *BRAF* mut, *RAS* mut and all wt patients, respectively. *BRAF* mut patients showed a significantly higher risk of relapse in comparison to all wt patients (hazard ratio (HR), 2.13; 95% CI, 1.20–7.31; $P = 0.019$). *RAS* mut compared with all wt patients showed no difference in terms of RFS (HR, 1.22; 95% CI, 0.94–1.58; $P = 0.142$) (Figure 3A). Other clinical and pathological covariates that significantly associated with inferior RFS were: presence of extra-hepatic disease (HR, 1.92; 95% CI, 1.41–4.20; $P = 0.001$); bilobar liver involvement (HR, 1.55; 95% CI, 1.22–2.13; $P = 0.0009$), synchronous disease (HR, 1.48; 95% CI, 1.11–1.89; $P = 0.006$) and not R0 liver resection (HR, 2.39; 95% CI, 2.27–5.44; $P < 0.0001$). Patients with high clinical risk score (CRS) had shorter RFS in comparison to low CRS (HR, 1.75; 95% CI, 1.35–2.35; $P < 0.0001$) (Table 2).

At the RFS multivariate models, *BRAF* mutation retained its prognostic impact in terms of RFS compared with all wt patients (HR, 2.31; 95% CI, 1.09–4.87; $P = 0.029$) and to *RAS* mut patients (HR, 2.06; 95% CI, 1.02–4.14; $P = 0.044$) (Table 3).

Overall survival outcomes differed significantly according to mutational status ($P = 0.003$), and median OS were 22.6, 42.0 and 63.3 months in *BRAF* mut, *RAS* mut and all wt patients, respectively. *BRAF* mut patients showed a significantly higher risk of death in comparison to all wt patients (HR, 3.07; 95% CI, 2.12–22.94; $P = 0.002$) and to *RAS* mut patients (HR, 2.09; 95% CI, 1.05–7.87; $P = 0.041$). A significant difference was also observed comparing *RAS* mut and all wt patients (HR, 1.47; 95% CI, 1.05–2.07; $P = 0.025$) (Figure 3B). Other covariates associated with inferior OS were ECOG PS > 0 (HR, 1.95; 95% CI, 1.42–3.95; $P = 0.001$); extra-hepatic disease (HR, 2.22; 95% CI, 1.57–6.43; $P = 0.001$); bilobar liver metastases (HR, 1.59; 95% CI, 1.16–2.33; $P = 0.006$), right-sided primary tumour (HR, 1.59; 95% CI, 1.10–2.45; $P = 0.017$) and not R0 liver resection (HR, 3.21; 95% CI, 3.66–10.85; $P < 0.0001$). Patients with high CRS showed worse OS compared with low CRS (HR, 1.65; 95% CI, 1.16–2.35; $P = 0.005$) (Table 2).

At the OS multivariate model, *BRAF* mutation was independently associated with worse outcome compared with all wt patients (HR, 2.76; 95% CI, 1.12–6.81; $P = 0.029$) and with *RAS* mut patients (HR, 2.73; 95% CI, 1.25–5.92; $P = 0.012$). *RAS* mutation lost its association with worse OS (HR, 1.08; 95% CI, 0.73–1.59; $P = 0.712$) (Table 3).

Recursive partitioning analyses showed that not R0 liver resection was the most important factor in the prediction of RFS and OS. Other characteristics affecting RFS and OS were time from date of metastatic disease diagnosis to liver resection, age, bilobar liver metastases (for RFS only) and primary nodal involvement (for OS only) (Supplementary Figure 1).

Sites of first relapse. At the time of analyses, relapsed *BRAF* mut, *RAS* mut and all wt patients were 10 (83%), 127 (79%) and 99 (72%), respectively. Liver-only relapse was not associated with mutational status and was observed in 60, 43 and 49% of patients

Table 2. Univariate analyses for relapse-free survival and overall survival

		Relapse-free survival				Overall survival			
Characteristics	***N***	**Median (months)**	**HR**	**95% CI**	***P***	**Median (months)**	**HR**	**95% CI**	***P***
Mutational status									
All wt	137	14.4	1	—	—	63.3	1	—	—
BRAF mut	12	5.7	2.13	1.20–7.31	**0.019**	22.6	3.07	2.12–22.94	**0.002**
RAS mut	160	11.0	1.22	0.94–1.58	0.142	42.0	1.47	1.05–2.07	**0.025**
Sex									
Male	192	12.2	1	—	—	53.8	1	—	—
Female	117	10.7	0.98	0.75–1.28	0.878	40.7	1.19	0.85–1.68	0.313
Age									
<65 years	177	11.7	1	—	—	52.8	1	—	—
⩾65 years	132	11.4	1.09	0.84–1.41	0.526	46.6	1.17	0.84–1.64	0.349
ECOG PS									
0	267	12.2	1	—	—	54.0	1	—	—
1–2	42	9.4	1.38	0.96–2.16	0.076	26.5	1.95	1.42–3.95	**0.001**
Primary tumour site									
Left colon	138	12.0	1	—	—	57.3	1	—	—
Right colon	87	10.7	1.23	0.91–1.69	0.179	35.5	1.59	1.10–2.45	**0.017**
Rectum	82	12.6	1.04	0.76–1.43	0.794	61.1	0.95	0.63–1.43	0.804
Liver only									
Yes	281	12.6	1	—	—	53.8	1	—	—
No	28	7.5	1.92	1.41–4.20	**0.001**	25.5	2.22	1.57–6.43	**0.001**
Unilobar mts									
Yes	198	15.1	1	—	—	61.1	1	—	—
No	111	7.9	1.55	1.22–2.13	**0.0009**	34.8	1.59	1.16–2.33	**0.006**
Time to mts									
Metachronous	101	15.1	1	—	—	56.8	1	—	—
Synchronous	208	10.4	1.48	1.11–1.89	**0.006**	48.7	1.30	0.91–1.82	0.158
Resection outcome									
R0	258	14.2	1	—	—	61.1	1	—	—
R1/R2-Expl. Lapar.	51	6.3	2.39	2.27–5.44	<**0.0001**	21.6	3.21	3.66–10.85	<**0.0001**
Primary lymph nodes									
No	96	17.7	1	—	—	56.8	1	—	—
Yes	206	10.4	1.58	1.17–2.00	**0.002**	51.9	1.35	0.94–1.89	0.107
DFI <12 months									
Yes	242	10.7	1	—	—	47.0	1	—	—
No	67	17.1	0.67	0.52–0.93	**0.015**	61.1	0.80	0.54–1.21	0.312
>1 liver mts									
No	132	16.8	1	—	—	69.3	1	—	—
Yes	173	8.8	1.76	1.36–2.27	<**0.0001**	36.0	1.84	1.30–2.49	**0.0005**
Mts diameter >5 cm									
No	231	11.7	1	—	—	56.8	1	—	—
Yes	62	11.2	1.13	0.82–1.58	0.437	52.7	1.19	0.79–1.85	0.396
CEA>200 ng ml^{-1}									
No	212	11.0	1	—	—	46.6	1	—	—
Yes	9	9.0	1.21	0.54–2.82	0.620	17.5	2.40	1.24–12.11	**0.021**
Clinical risk score[a]									
Low	138	16.6	1	—	—	58.6	1	—	—
High	137	8.6	1.75	1.35–2.35	<**0.0001**	35.5	1.65	1.16–2.35	**0.005**

Abbreviations: CI = confidence interval; DFI = disease-free interval; Expl. Lapar. = explorative laparotomy; HR = hazard ratio; mts = metastasis; mut = mutant; N = number; PS = performance status; wt = wild-type.
[a]Clinical risk score is defined as previously described by Fong *et al* (1999): patients with 0 to 2 risk features were categorised as 'low risk', while those with 3 to 5 features as 'high risk'. Bold entries indicate significant results.

in the three groups ($P = 0.45$). No differences were observed in terms of peritoneal ($P = 0.89$), nodal ($P = 0.10$) and liver relapse ($P = 0.61$). Lung relapse was more frequently observed in *RAS* mut (35%) patients in comparison to all wt (21%) and *BRAF* mut (0%) patients ($P = 0.008$; all wt *vs* *RAS* mut $P = 0.027$; *RAS* mut *vs* *BRAF* mut $P = 0.030$) (Supplementary Table 4).

MSI status and *BRAF* mutation. All *BRAF* mut cases were analysed for MSI status. Two cases resulted MSI-H and 1 MSI-L. Interestingly, of the 2 *BRAF* mut patients free of relapse at the time of the analyses 1 had a MSI-H tumour and the other a MSI-L tumour; on the other hand, 9 out of 10 relapsed patients had a MSS tumour ($P = 0.046$).

Table 3. Multivariate analyses for relapse-free survival and overall survival

Characteristics	HR	95% CI	*P*
Relapse-free survival			
BRAF mut vs all wt			
Mutational status (*BRAF* mut *vs* all wt)	2.31	1.09–4.87	**0.029**
ECOG PS (1–2 *vs* 0)	1.89	0.97–3.33	0.063
Liver-only metastases (No *vs* Yes)	0.78	0.35–1.70	0.528
Unilobar mts (No *vs* Yes)	2.11	1.32–3.37	**0.002**
Time to mts (synchronous *vs* metachronous)	1.03	0.56–1.88	0.930
Resection outcome (R1/R2-Expl. Lapar. *vs* R0)	2.28	1.27–4.07	**0.006**
Clinical risk score (High *vs* Low)	1.51	0.87–2.62	0.149
BRAF mut vs RAS mut			
Mutational status (*BRAF* mut *vs* *RAS* mut)	2.06	1.02–4.14	**0.044**
ECOG PS (1–2 *vs* 0)	0.95	0.58–1.55	0.833
Liver-only metastases (No *vs* Yes)	1.08	0.62–1.89	0.789
Unilobar mts (No *vs* Yes)	0.97	0.65–1.43	0.864
Time to mts (synchronous *vs* metachronous)	1.15	0.74–1.79	0.548
Resection outcome (R1/R2-Expl. Lapar. *vs* R0)	3.22	2.05–5.06	**<0.0001**
Clinical risk score (High *vs* Low)	1.53	1.01–2.33	**0.046**
Overall survival			
BRAF mut vs all wt			
Mutational status (*BRAF* mut *vs* all wt)	2.76	1.12–6.81	**0.029**
ECOG PS (1–2 *vs* 0)	2.81	1.37–5.78	**0.005**
Tumour site (Right *vs* Left and Rectum)	1.25	0.60–2.59	0.549
Liver-only metastases (No *vs* Yes)	1.40	0.60–3.28	0.437
Unilobar mts (No *vs* Yes)	2.19	1.17–4.08	**0.014**
Resection outcome (R1/R2-Expl. Lapar. *vs* R0)	4.54	2.32–8.89	**<0.0001**
Clinical risk score (High *vs* Low)	0.98	0.55–1.57	0.952
BRAF mut vs RAS mut			
Mutational status (*BRAF* mut *vs* *RAS* mut)	2.73	1.25–5.92	**0.012**
ECOG PS (1–2 *vs* 0)	1.07	0.59–1.93	0.833
Tumour site (right *vs* left and rectum)	1.22	0.76–1.95	0.415
Liver-only metastases (No *vs* Yes)	0.95	0.45–2.03	0.903
Unilobar mts (No *vs* Yes)	1.13	0.69–1.85	0.618
Resection outcome (R1/R2-Expl. Lapar. *vs* R0)	3.98	2.34–6.76	**<0.0001**
Clinical risk score (High *vs* Low)	2.17	1..33–3.54	**0.002**
RAS mut vs all wt			
Mutational status (*RAS* mut *vs* all wt)	1.08	0.73–1.59	0.712
ECOG PS (1–2 *vs* 0)	1.79	1.12–2.84	**0.015**
Tumour site (right *vs* left and rectum)	1.34	0.89–2.02	0.165
Liver-only metastases (No *vs* Yes)	1.68	0.95–2.98	0.078
Unilobar mts (No *vs* Yes)	1.37	0.93–2.04	0.115
Resection outcome (R1/R2-Expl. Lapar. *vs* R0)	3.24	2.07–5.08	**<0.0001**
Clinical risk score (High *vs* Low)	1.50	0.10–2.24	0.053

Abbreviations: HR = hazard ratio; CI = confidence interval; mut = mutant; wt = wild-type; PS = performance status; mts = metastasis; Expl. Lapar. = explorative laparotomy. Bold entries indicate significant results.

DISCUSSION

Extensive molecular characterisation of CRC has gained more and more importance both with predictive and prognostic intent. In the present work, starting from the revision of 3024 medical records of mCRC patients, after a careful clinical selection, we identified 360 eligible patients and collected as much data as possible on markers potentially affecting prognosis after liver resection. Finally, we performed a comprehensive *RAS* and *BRAF* molecular characterisation that lead us to identify a final study population of 309 cases.

The major and clinically relevant finding is that *BRAF* mutation emerges as an independent and strong negative prognostic factor also in this specific setting. *BRAF* mut patients had an extremely poor median RFS of 5.7 months and a significantly higher risk of relapse, as compared with both *RAS* mut (HR, 2.06; $P = 0.044$) and all wt patients (HR, 2.31; $P = 0.029$).

Other studies tried to address the same issue, but as admitted by their authors, were limited in sample size to catch the independent prognostic effect of *BRAF* status (Stremitzer *et al*, 2012; Karagkounis *et al*, 2013; Kemeny *et al*, 2013; Umeda *et al*, 2013; Vauthey and Kopetz, 2013; Vauthey *et al*, 2013). A previous study showed a significantly shorter OS for *BRAF* mut patients undergoing liver resection, but no data on RFS were available, while two out of six *BRAF* mut patients had a mutation different from the V600E, thus limiting possible conclusions (Teng *et al*, 2012). A recent retrospective analysis conducted at Memorial Sloan Kettering Cancer Centre on the prognostic impact of *BRAF* mutation in mCRC, confirmed a shorter OS for *BRAF* mutant patients in the subgroup undergoing resection of metastases with radical intent (Yaeger *et al*, 2014).

All previous experiences, as well as ours, are in line in reporting a very low incidence of *BRAF* mutation in patients undergoing liver resection, ranging from 2 to 4%. These data find a possible explanation in the specific clinical features and the peculiar metastatic spread usually observed in *BRAF* mut patients, rarely presenting with liver limited metastatic disease and just in a few cases achieve favourable clinical conditions leading to consider a radical liver resection. The low mutation rate of *BRAF* in this setting dilutes the clinical impact of its prognostic value possibly raising some concerns about the cost-effectiveness of its routinary use, but the implications and consequences at the 'single-patient' level could be extremely relevant.

The high rate of nodal relapse observed in our series, although not reaching the statistical significance, possibly due to the small number of *BRAF* mut patients, might allow to assume that *BRAF* mut patients could more frequently recur in a shorter time and in extra-hepatic locations due to the presence of occult micro-metastatic disease. As a consequence, an intensive preoperative work-up in *BRAF* mut patients, potentially candidates for liver resection, could be proposed. In particular, MRI with liver-specific contrast, ultrasound scans with contrast medium and PET-CT have recently been shown to have a higher sensitivity in comparison to CT scan (Schmidt *et al*, 2009).

Whether the prognostic impact of *BRAF* mutation is independent or not from MSI status, a condition to which it is significantly associated, is still a matter of debate, also because of the extremely low frequency of the concomitant presence of these features in the metastatic setting (Goldstein *et al*, 2014). An interesting finding coming out from our experience is that both the 2 *BRAF* mutant patients free of relapse at the time of the analyses (16.3 months and 23.6 months after resection, respectively) were not MSS.

The prognostic role of *RAS* mutations is not confirmed in our multivariate models and this is apparently inconsistent with results by other groups. However, some explanations can be hypothesised: first of all, different inclusion criteria were adopted and this is reflected also by the relatively higher incidence of *RAS* mutations in our patients; second, available data come from major surgical referral centres, while our patients' selection moved from oncologic units, thus leading to a slightly different study population; third, different covariates were included in the multivariable models as a result of different selection rules for these variables. As compared with the experience by Karagkounis *et al* (2013) our results at the univariate analyses are very similar and not statistically significant in terms of RFS. Our pre-specified analytical criteria did not allow variables with statistical significance $\geqslant 0.1$ to enter the multivariate model. As a consequence, the models differed and this may have affected the results. Similar constraints apply to the comparison with data by Vauthey *et al* (2013) that were reported as 3-year survival rates and again included different variables in the multivariate models.

Interestingly, our results confirm the association of *RAS* mutations with an higher risk of lung relapse, as previously reported by Kemeny *et al* (2013) These data enforce the role of an adequate thoracic staging in preoperative work-up as well as the mandatory inclusion of intensive chest follow-up of *RAS* mut patients after liver resection (Maithel *et al*, 2010).

No specific analyses were carried out on the basis of received treatment due to the wide heterogeneity of received treatments and to the specific objective of the present study.

At recursive partitioning analyses, traditional clinico-pathological prognostic factors (such as resection margins, time to resection, extension of liver involvement, age and primary tumour nodal involvement) emerged as primary determinants (Supplementary Figure 1). This underlines the importance of coupling old and new

markers to optimise future prognostication skills and clinical decision making.

Taken together, all the available data support the implementation of molecular testing in defining the risk of relapse of candidates to curative liver resection. Although data on *BRAF* refer to a rather small group of patients, their significance is relevant to balance pros and contra of the indication for a major surgery, with a non-negligible risk of post-operative morbidity and mortality, high costs and a great clinical commitment for patients and for health care facilities. Ultimately, many innovative therapeutic strategies are under investigation for targeting *RAS* and *BRAF* mutant CRCs. The extensive knowledge of their clinical behaviour might be crucial for the development of new therapeutic approaches in specific settings, such as the perioperative and adjuvant treatment of liver limited disease.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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