FULL PAPER

BJC

British Journal of Cancer (2016) 114, 30–36 | doi: 10.1038/bjc.2015.399

Keywords: BRAF; nomogram; metastatic colorectal cancer

Clinico-pathological nomogram for predicting *BRAF* mutational status of metastatic colorectal cancer

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Background: In metastatic colorectal cancer (mCRC), *BRAFV600E* mutation has been variously associated to specific clinico-pathological features.

Methods: Two large retrospective series of mCRC patients from two Italian Institutions were used as training-set (TS) and validation-set (VS) for developing a nomogram predictive of *BRAFV600E* status. The model was internally and externally validated.

Results: In the TS, data from 596 mCRC patients were gathered (*RAS* wild-type (wt) 281 (47.1%); *BRAFV600E* mutated 54 (9.1%)); *RAS* and *BRAFV600E* mutations were mutually exclusive. In the *RAS*-wt population, right-sided primary (odds ratio (OR): 7.80, 95% confidence interval (CI) 3.05–19.92), female gender (OR: 2.90, 95% CI 1.14–7.37) and mucinous histology (OR: 4.95, 95% CI 1.90–12.90) were independent predictors of *BRAFV600E* mutation, with high replication at internal validation (100%, 93% and 98%, respectively). A predictive nomogram was calculated: patients with the highest score (right-sided primary, female and mucinous) had a 81% chance to bear a *BRAFV600E*-mutant tumour; accuracy measures: AUC = 0.812, SE:0.034, sensitivity:81.2%; specificity:72.1%. In the VS (508 pts, *RAS* wt: 262 (51.6%), *BRAFV600E* mutated: 49 (9.6%)), right-sided primary, female gender and mucinous histology were confirmed as independent predictors of *BRAFV600E* mutation with high accuracy.

Conclusions: Three simple and easy-to-collect characteristics define a useful nomogram for predicting *BRAF* status in mCRC with high specificity and sensitivity.

In the last years, significant improvements in the treatment of metastatic colorectal cancer (mCRC) progressively increased the survival expectancy of the overall patients' population to over 2 years (Heinemann *et al*, 2014; Lenz *et al*, 2014; Loupakis *et al*, 2014). A major contribution to these achievements was given by the introduction of *RAS* testing and the opportunity of treating

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Received 26 July 2015; revised 15 September 2015; accepted 20 October 2015; published online 17 November 2015

wild-type (wt) patients with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (Atreya *et al*, 2015). Although *RAS* status ascertainment is recommended by all major guidelines (Van Cutsem *et al*, 2014; Clinical Practice Guidelines in Oncology (NCCN Guidelines), 2015), the predictive role towards anti-EGFRs of V600E activating mutation of *BRAF* is still debated (Di Nicolantonio *et al*, 2008; Laurent-Puig *et al*, 2009; Loupakis *et al*, 2009; Souglakos *et al*, 2009; De Roock *et al*, 2010) To this extent, studies have not been conclusive maybe due to the low incidence of *BRAFV600E* mutation ($<10\%$ of mCRC; Davies *et al*, 2002) and to the intrinsic limitations of retrospective subgroup analyses. Nevertheless, all the published series recognised that *BRAFV600E* mutation is a strong negative prognostic determinant in mCRC and *BRAF*-mutated metastatic patients have an extremely poor life-expectancy of around 12 months (Richman *et al*, 2009; Souglakos *et al*, 2009; Saridaki *et al*, 2010; Tie *et al*, 2011; Tran *et al*, 2011; Yokota *et al*, 2011; Saridaki *et al*, 2013; Yaeger *et al*, 2014).

BRAF-mutated CRCs constitute a distinct subgroup with specific characteristics as underlined by their peculiar gene expression signature (Popovici *et al*, 2012). The presence of a *BRAF* mutation has also been associated to specific clinico-pathological features (Samowitz *et al*, 2006; Roth *et al*, 2010; Tie *et al*, 2011; Tran *et al*, 2011; Yokota *et al*, 2011; Clancy *et al*, 2013; Saridaki *et al*, 2013; Gonsalves *et al*, 2014; Yaeger *et al*, 2014). In some published series, *BRAF* mutation occurred more frequently in older patients and in females, and showed a higher rate of nodal and peritoneal metastases and a lower rate of lung involvement. *BRAF*-mutant CRCs were also more frequently right-sided, poorly differentiated, mucinous, microsatellite instable and T4-staged. In addition, patients bearing a *BRAF*-mutant tumour often had a poor performance status (PS) and multiple metastatic sites at diagnosis. However, the association between these features and *BRAF* mutation was only preliminarily described and up today, no clear and definitive comprehensive data are available, especially in terms of multivariate modelling.

Moving from such considerations, we tested the specific contribution of each clinico-pathological feature for predicting *BRAF* mutational status in *RAS*-wt mCRC in a large training-set (TS) population. On those basis, we built a nomogram to predict the likelihood of *BRAF* mutation occurrence and validated it in a confirmatory external data set (Iasonos *et al*, 2008).

MATERIALS AND METHODS

A specific database including the variables previously associated to the presence of *BRAF* mutation in CRC patients was built (Samowitz *et al*, 2006; Roth *et al*, 2010; Tie *et al*, 2011; Tran *et al*, 2011; Yokota *et al*, 2011; Clancy *et al*, 2013; Saridaki *et al*, 2013; Gonsalves *et al*, 2014; Yaeger *et al*, 2014). The following characteristics were selected: age, ECOG-PS, time to metastatic presentation (i.e. synchronous *vs* metachronous), primary tumour site (i.e. right-sided, from caecum up to transverse colon included *vs* left-sided, from splenic flexure to rectum), resection of the primary tumour, mucinous histology (as indicated in pathological report), number of metastatic sites, peritoneal, lung, distant lymph nodes as metastatic sites, tumour grading, *RAS* and *BRAF* mutational status.

The analysis was conducted as follows: (1) to determine (and confirm) the independent prognostic role for survival of *BRAF* mutation in our series of mCRC patients; (2) to identity the clinico-pathological predictive factors of the presence of *BRAF* mutation (predictive nomogram) in RAS-wt patients; (3) to measure the predictive accuracy of the generated nomogram; (4) to internally and externally validate the predictive nomogram. Thus, a step-by-step protocol was followed according to the methodological approach for building a nomogram according to Iasonos *et al* (2008), with respect to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria for the conduction of a retrospective study in the context of an unselected population (Simon *et al*, 2009).

Patients' population. Consecutive mCRC patients with available clinical and pathological data (including *RAS*, *BRAF* mutational status) referred to the Unit of Oncology, Azienda Ospedaliero-Universitaria Pisana (Pisa, Italy) from February 2000 to October 2014, were retrospectively gathered (TS). Using the same database, data of patients with overlapping entry criteria, referred to the Department of Oncology, Azienda Ospedaliero-Universitaria Santa Maria della Misericordia (Udine, Italy) in the same time frame were gathered for the validation set (VS).

End point. The aim was to generate a predictive nomogram according to clinical and pathological factors for the identification of *RAS*-wt patients more likely to carry the *BRAF* mutation.

Mutational analyses. DNA was extracted from a single formalin-fixed-paraffin-embedded block. Haematoxylin-eosin slides were revised by expert pathologists who macrodissected proper representative areas, to obtain an amount of neoplastic cells of at least 50%. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) with overnight proteinase K digestion and DNA concentration was determined by NanoDrop 2000c spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE, USA). *KRAS* (exons 2, 3 and 4), *NRAS* (exons 2, 3 and 4) and *BRAFV600E* mutational status was tested by means of Pyrosequencing on the PyroMarkQ96 ID instrument (Qiagen) with commercially available kits (Diatech Pharmacogenetics, Italy). Sensitivity (detectable percentage of mutant alleles) of the Pyrosequencing technique is around 5%.

Statistics. Descriptive statistics was used to summarise pertinent study information. Follow-up was analysed and reported according to Shuster (1991). The correlation between variables was analysed according to χ^2. A multivariate Cox proportional hazard model was developed using stepwise regression (forward selection, enter/remove limits $P=0.10$ and $P=0.15$) to identify independent predictors of the presence of *BRAF* mutation; the odds ratio (OR) and the 95% confidence intervals (95% CI) were estimated for each variable. The assessment of interactions between significant investigation variables was taken into account when developing the multivariate model. Overall survival (OS) was calculated by the Kaplan–Meier product limit method from the date of diagnosis of metastatic disease until death due to cancer or death for any cause. The hazard ratio (HR) and 95% CI were estimated for each variable using the Cox multivariate model. The log-rank test was used to assess differences between subgroups. Significance was defined at the $P<0.05$ level. The SPSS (21.0), and MedCalc (14.12.0) licensed statistical programs were used for all analyses.

Internal validation. To address the over-fitting of multivariate model and to validate the results, a cross-validation technique that evaluates the replication stability of the Cox multivariate model in predicting the presence of *BRAF* mutation was investigated, using a resampling procedure considering those variables independent at the multivariate analysis (Iasonos *et al*, 2008). This technique generates a number of simulation data sets (at least 100, each $\sim$80% of the original size), by randomly selecting patients from the original sample, to establish the consistency of the model across less-powered patient samples (Iasonos *et al*, 2008).

Predictive score assessment. The log-ORs obtained from the Cox model were used to derive weighting factors of a predictive index, aimed at identifying differential probability of the presence of *BRAF* mutation. Coefficients estimates were 'normalised' dividing by the smallest one and rounding the resulting ratios to the nearest integer value.

External validation. The predictive accuracy of the derived nomogram predictive of *BRAF* mutation was evaluated in the context of the VS. The sample size of the VS was calculated based on the predictive performance of the model estimated in the TS, to have a similar predictive performance between the two populations with a null hypothesis of 0.65, a power of 95% and an alpha-error of 5%. To assess the prognostic value of *BRAF* mutation, a multivariate model for OS was derived in the VS as well. A χ^2 comparison between the predictive performances at the ROC analyses of the nomogram in the TS and in the VS was thereafter carried out.

RESULTS

Data for overall 1104 advanced CRC patients were gathered (TS: 596, VS: 508 patients, respectively). Patients' characteristics are reported in Table 1. Overall, the two populations were similar, although the VS cohort included more patients with ECOG-PS $\geqslant$ 2, on site primary tumour, higher histological grade and number of metastatic sites. Median age was 65 (range 25–92) and 67 years (range 32–85) in the TS and VS, respectively. Median follow-up was 24 (range 0–163) and 20 months (range 1–165) in the TS and VS, respectively.

In the TS, data from 596 advanced CRC patients were gathered (*RAS* wt: 281 (47.1%); *BRAF* mutant: 54 (9.1%)). *BRAF* mutation was more frequent in female (13.1% *vs* 6.4%, $P = 0.005$), right-sided primary (16.3% *vs* 8.0%, $P < 0.0001$), mucinous tumours (19.1% *vs* 6.8%, $P < 0.0001$), poorly differentiated tumours (16.4% *vs* 4.7%, $P < 0.0001$), patients with peritoneal metastases (14.6% *vs* 7.5%, $P = 0.01$) and distant lymph-node metastases (15.4% *vs* 7.5%, $P = 0.008$; Supplementary Table 1).

In the overall sample, age $\geqslant$65-years-old, ECOG-PS $\geqslant$ 2, unresected primary tumour, multiple metastatic sites and *BRAF* mutation were independent prognostic factors for poorer OS with a trend towards significance for mucinous histology (Table 2). *BRAF* mutation had the higher prognostic power at the multivariate analysis (HR: 2.98, 95% CI 1.96–4.52, $P < 0.0001$). Thus, the identification of clinical and pathological predictors of the presence of *BRAF* mutation in the context of *RAS*-wt patients was justified.

Patients' characteristics of the *RAS*0-wt population for the TS are reported in Supplementary Table 2. Female gender (OR: 2.90, 95% CI 1.14–7.37, $P = 0.025$), right-sided primary site (OR: 7.80, 95% CI 3.05–19.92, $P < 0.0001$) and mucinous histology (OR: 4.95, 95% CI 1.90–12.90, $P < 0.0001$), resulted to be significant independent predictors of the presence of *BRAF* mutation in the TS (Table 3). These factors replicated at the internal cross-validation with a high rate, as follows: gender (93%), primary site (100%) and histology (98%). Figure 1 shows the probability of harbouring *BRAF* mutation according to the scoring index assigned to each patient combining the three independent variables. At the ROC analysis, the predictive accuracy of such nomogram was high (AUC: 0.812, standard error: 0.034), with a sensitivity of 81.2% and a specificity of 72.1% (Figure 2, panel A).

Patients' characteristics of the VS are reported in Table 1. In the VS, data from 508 mCRC patients were gathered (*RAS* wt: 262 (51.6%); *BRAF* mutant: 49 (9.6%)). Patients' characteristics of the *RAS*-wt population for the VS are reported in Supplementary Table 2. Right-sided primary site (OR: 8.68, 95% CI 4.18–18.02, $P < 0.0001$) and mucinous histology (OR: 3.23, 95% CI 1.49–7.02, $P = 0.003$) were confirmed as independent predictors of *BRAF* mutation in the VS, with a trend towards significance for female gender (OR: 1.92, 95% CI 0.92–3.97, $P = 0.081$; Table 3). The predictive nomogram derived in the TS was then applied to the VS; at the ROC analysis, the predictive accuracy was high (AUC: 0.811, standard error: 0.041), with a sensitivity of 73.5% and a specificity of 80.3% (Figure 2, panel B). No significant difference between the predictive performance of the model in both patients' cohorts was found ($P = 1.0$).

Table 1. Patients' characteristics in the TS and VS (overall population, *N* = 1104)

	TS *N* (%)	VS *N* (%)	*P*-value
Number of patients	596 (100)	508 (100)	–
Gender			
Male	359 (60.2)	318 (62.6)	0.46
Female	237 (39.8)	190 (37.4)	
Age (years)			
<65	287 (48.2)	224 (44.1)	0.20
⩾65	309 (51.8)	284 (55.9)	
ECOG-PS			
0–1	468 (78.5)	433 (85.2)	<0.0001
⩾2	10 (1.7)	75 (14.8)	
Missing	118 (19.8)	0 (0)	
Driver mutation			
Wt	227 (38.1)	213 (41.8)	0.96
BRAF mutant (V600E)	54 (9.1)	49 (9.6)	
RAS mutant	315 (52.8)	246 (48.6)	
Number of metastatic sites			
1	378 (63.4)	216 (42.5)	<0.0001
>1	218 (36.6)	292 (57.5)	
Peritoneal metastases			
Yes	130 (21.8)	107 (21.1)	0.82
No	466 (78.2)	401 (78.9)	
Lung metastases			
Yes	147 (24.7)	152 (29.9)	0.06
No	449 (75.3)	356 (70.1)	
Synchronous metastases			
Yes	412 (69.1)	367 (72.2)	0.29
No	184 (30.9)	141 (27.8)	
Distant lymph-node metastases			
Yes	117 (19.6)	110 (21.7)	0.45
No	479 (80.4)	398 (78.3)	
Mucinous histology			
Yes	110 (18.5)	76 (15.0)	<0.0001
No	395 (66.3)	416 (81.9)	
Missing	91 (15.3)	16 (3.1)	
Primary tumour site			
Right	202 (33.9)	162 (31.9)	0.52
Left	394 (66.1)	346 (68.1)	
Primary tumour resected			
Yes	466 (78.2)	344 (67.8)	<0.0001
No	130 (21.8)	164 (32.2)	
Tumour grading			
G1–2	248 (41.6)	143 (28.1)	<0.0001
G3–4	195 (32.7)	221 (43.5)	
Missing	153 (25.7)	144 (28.3)	

Abbreviations: N = number; PS = performance status; TS = training set; VS = validation set; wt = wild type. %: rate; *P*-value: χ^2 test.

The Kaplan–Meier survival curves of patients in the TS and VS according to *RAS* and *BRAF* are shown in Figure 3. As expected, *BRAF*-mutant mCRC patients had a worse prognosis compared with *RAS* mutant, and *RAS* and *BRAF*-wt patients with an OS rate at 3 years of 19.8%, 37.3% and 45.5%, respectively ($P < 0.0001$).

DISCUSSION

BRAFV600E mutation occurs in 8–10% of mCRC and is associated with an extremely poor prognosis (Richman *et al*, 2009; Souglakos

Table 2. Uni- and multivariate analyses for OS

	TS (*N* = 596)				VS (*N* = 508)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*
Gender								
F *vs* M	1.14 (0.92–1.42)	0.222	–	–	1.17 (0.95–1.46)	0.15	–	–
Age (cut-off: 65 years)								
⩾65 *vs* <65	1.67 (1.35–2.07)	0.0001	1.86 (1.38–2.49)	<0.0001	1.21 (0.98–1.49)	0.08	–	–
ECOG-PS								
⩾2 *vs* <2	2.12 (1.70–2.63)	0.0001	1.54 (1.14–2.07)	0.004	1.11 (0.83–1.47)	0.49	–	–
Synchronous metastases								
yes *vs* no	1.31 (1.03–1.66)	0.026	–	–	1.05 (0.85–1.30)	0.66	–	–
Primary site								
right *vs* left	1.52 (1.22–1.88)	0.0001	–	–	1.48 (1.18–1.86)	0.001	1.33 (1.05–1.69)	0.02
Primary resected								
no *vs* yes	1.97 (1.53–2.53)	0.0001	2.27 (1.52–3.39)	<0.0001	1.11 (0.95–1.38)	0.23	–	–
Mucinous histology								
yes *vs* no	1.38 (1.05–1.82)	0.018	1.43 (0.97–2.08)	0.065	1.26 (0.97–1.63)	0.09	–	–
Number of metastatic sites								
>1 *vs* 1	2.08 (1.67–2.59)	0.0001	1.83 (1.38–2.43)	<0.0001	1.94 (1.56–2.42)	<0.0001	1.76 (1.38–2.25)	<0.0001
Peritoneal metastases								
yes *vs* no	1.69 (1.31–2.18)	0.0001	–	–	1.37 (1.06–1.76)	0.01	–	–
Lung metastases								
yes *vs* no	1.05 (0.82–1.35)	0.646	–	–	0.99 (0.74–1.21)	0.53	–	–
Distant lymph-node metastases								
yes *vs* no	1.54 (1.18–2.02)	0.002	–	–	1.92 (1.50–2.47)	<0.0001	1.31 (0.99–1.74)	0.06
Tumour grading								
3–4 *vs* 1–2	2.34 (1.83–3.01)	0.0001	–	–	1.23 (0.98–1.56)	0.08	–	–
RAS								
mut *vs* wt	1.20 (0.97–1.49)	0.088	–	–	1.01 (0.82–1.25)	0.91	–	–
BRAF								
mut *vs* wt	2.56 (1.84–3.56)	0.0001	2.98 (1.96–4.52)	<0.0001	2.72 (1.92–3.85)	<0.0001	2.33 (1.61–3.35)	<0.0001

Abbreviations: CI = confidence intervals; HR = Hazard Ratio; N = number; OS = Overall Survival; PS = performance status; TS = training set; VS = validation set; wt = wild type.

et al, 2009; Saridaki *et al*, 2010; Tie *et al*, 2011; Tran *et al*, 2011; Yokota *et al*, 2011; Saridaki *et al*, 2013; Yaeger *et al*, 2014). Despite this, the need of a routine assessment of *BRAF* mutational status for clinical practice is a matter of debate, because of the limited therapeutic implications outside of clinical trials (Van Cutsem *et al*, 2014; Clinical Practice Guidelines in Oncology (NCCN Guidelines), 2015). Conversely, the analysis of *RAS* mutational status is essential for defining resistance to anti-EGFR monoclonal antibodies (Atreya *et al*, 2015). Among *RAS*-wt patients the incidence of *BRAF* mutation is relatively higher (around 20%), because of the mutual exclusivity between *RAS* and *BRAF* mutations (Peeters *et al*, 2013). Recently, many retrospective series preliminary described some clinical features specifically associated with *BRAF* mutation (Samowitz *et al*, 2006; Roth *et al*, 2010; Tie *et al*, 2011; Tran *et al*, 2011; Yokota *et al*, 2011; Clancy *et al*, 2013; Saridaki *et al*, 2013; Gonsalves *et al*, 2014; Yaeger *et al*, 2014). Nevertheless, these studies were exploratory and included different stages (i.e. from I to IV), different settings, (i.e. first *vs* later lines of treatment), had an incomplete molecular assessment (in most of the cases only *KRAS* exon 2 was tested) and lacked of VSs. Hence, oncologists need to clarify and to properly measure the association between *BRAFV600E* mutational status and specific patients' and disease's characteristics in the context of the *RAS*-wt subgroup.

The data reported herein female sex, age ⩾65 years, worse ECOG-PS, right-sided primary tumour, mucinous histology, presence of nodal and peritoneal metastases and higher tumour grading, were associated to *BRAF*V600E mutation in the TS and these findings are consistent with most previous studies (Samowitz *et al*, 2006; Roth *et al*, 2010; Tie *et al*, 2011; Tran *et al*, 2011; Yokota *et al*, 2011; Clancy *et al*, 2013; Saridaki *et al*, 2013; Gonsalves *et al*, 2014; Yaeger *et al*, 2014). At the multivariate analysis, only female gender, right-sided primary and mucinous histology retained their significance as predictors of *BRAF*V600E mutation (OR: 2.90, 4.95 and 7.80 respectively). At internal cross-validation these three features were replicated with high rates (93%, 100% and 98%, respectively). These robust data allowed to build a nomogram for predicting the presence of *BRAF* mutation by combining the three independent variables. The probability to carry a *BRAF*-mutated tumour ranged from 4% to 81% with a predictive accuracy >80% and with high sensitivity and specificity (81.2% and 72.1%, respectively). In particular, a *RAS*-wt mCRC, not mucinous and originated from a left-sided primary occurring in male patients have an extremely poor likelihood to be *BRAF* mutant (4%). Conversely, female patients with mucinous histology and a right-sided primary *RAS*-wt tumour have a high probability to carry a *BRAF*-mutated cancer (81%). Finally, these data were replicated and validated in an external independent population. The predictive performance of the derived model in the context of the VS was impressively superimposable.

In a previous experience, Tie *et al* (Tie *et al*, 2011) reported a 50% incidence of *BRAF* mutation in *KRAS*-wt females aged ⩾70 years at diagnosis affected by a right-sided colon cancer.

Table 3. Uni- and multivariate analyses for the presence of *BRAF* mutation in the population of *RAS*-wt patients

	TS (*N* = 281)				VS (*N* = 262)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
Gender								
F *vs* M	2.78 (1.51–5.11)	0.001	2.90 (1.14–7.37)	0.025	2.31 (1.23–4.33)	0.009	1.92 (0.92–3.97)	0.08
Age (cut-off: 65 years)								
⩾65 *vs* <65	1.03 (1.00–1.06)	0.030	–	0.11	1.42 (0.76–2.68)	0.27	–	–
ECOG-PS								
⩾2 *vs* <2	2.09 (1.02–4.28)	0.044	–	0.87	1.30 (0.54–3.12)	0.55	–	–
Synchronous metastases								
yes *vs* no	1.17 (0.63–2.19)	0.606	–	–	1.34 (0.72–2.50)	0.35	–	-
Primary site								
right *vs* left	7.12 (3.74–13.56)	0.0001	7.80 (3.05–19.92)	<0.0001	11.14 (5.50–22.56)	<0.0001	8.68 (4.18–18.02)	<0.0001
Primary resected								
no *vs* yes	1.43 (0.68–3.02)	0.339	–	–	1.29 (0.78–2.86)	0.229	–	-
Mucinous histology								
yes *vs* no	4.69 (2.33–9.46)	0.0001	4.95 (1.90–12.90)	<0.0001	4.61 (2.34–9.07)	<0.0001	3.23 (1.49–7.02)	0.003
Number of metastatic site								
>1 *vs* 1	1.21 (0.68–2.14)	0.506	–	–	1.92 (1.03–3.60)	0.04	–	0.34
Peritoneal metastases								
yes *vs* no	2.19 (1.15–4.19)	0.017	–	0.17	2.53 (1.27–5.02)	0.01	–	0.15
Lung metastases								
yes *vs* no	1.22 (0.59–2.51)	0.586	–	–	1.12 (0.65–1.91)	0.69	–	–
Distant lymp-node metastases								
yes *vs* no	2.02 (1.05–3.88)	0.009	–	0.64	3.30 (1.72–6.33)	<0.0001	–	0.19
Tumour grading								
3–4 *vs* 1–2	4.54 (2.18–9.48)	0.0001	–	0.46	1.63 (0.85–3.13)	0.14	–	–

Abbreviations: CI = confidence intervals; N = number; OR = odds ratio; PS = performance status; TS = training set; VS = validation set; wt = wild type.

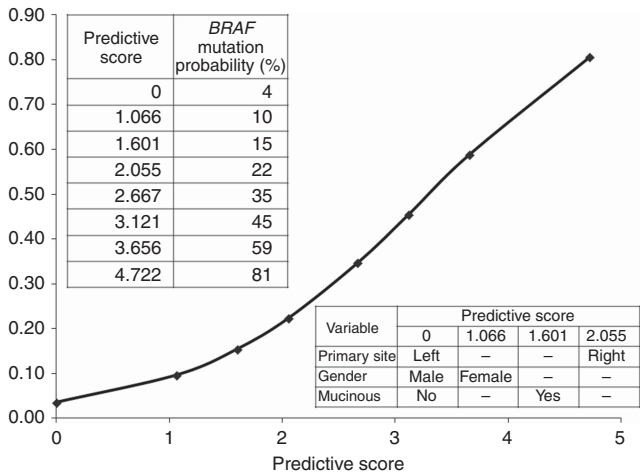

Figure 1. Clinico-pathological nomogram for predicting *BRAF* mutational status. A predictive score is assigned to each variable and the sum of scores is converted to the probability of *BRAF* mutation occurrence.

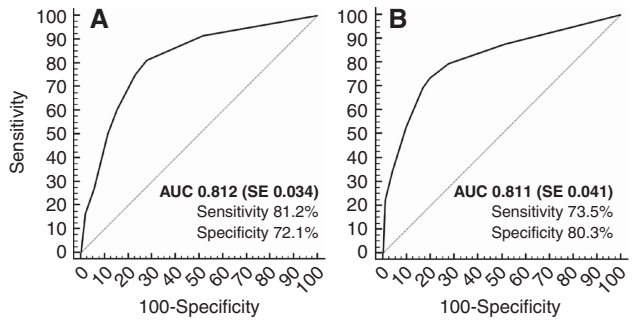

Figure 2. ROC curves in TS (panel **A**) and in VS (panel **B**) populations. Abbreviations: AUC = area under the curve; SE = standard error.

Nevertheless, the inclusion of stage I–IV patients, *RAS* testing limited to *KRAS* exon 2 mutations and the exclusion of mucinous histology from the model, attenuated the value of the determined correlations.

A weakness point of our study is the lack of data on microsatellite instability (MSI). Although the association between MSI-high status and *BRAF* mutation is well-established (Tran *et al*, 2011), the positive prognostic effect of MSI-high makes its occurrence in metastatic patients very uncommon (2–6%; Richman *et al*, 2009; Goldstein *et al*, 2014). Given the above considerations and taking into account that MSI is not routinely tested in the metastatic setting (Van Cutsem *et al*, 2014), we could speculate that the inclusion of such variable in our nomogram would not have significantly affected the performance of the model. Also, we did not consider rare *BRAF* mutations other than V600E. In addition, we did not assess the status of other pathological characteristics potentially associated with *BRAF*

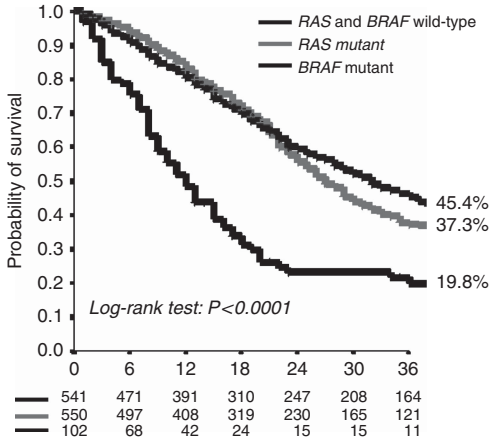

Figure 3. Kaplan–Meier curves of the probability of OS in *RAS* and *BRAF* wt (blue), *RAS*-mutant (red) and *BRAF*-mutant (black) patients. A full colour version of this figure is available at the *British Journal of Cancer* journal online.

mutational status (Schirripa *et al*, 2015; i.e. T and N pathological stage, vascular invasion, tumour budding, lymphocytic infiltrate and number of lymph nodes resected), because this information were not always available and were beyond the purpose of present study.

In the era of molecular characterisation, the present nomogram should not be considered a tool to replace the mutational analysis of CRC, but it could allow physicians to better estimate patients' prognosis where *BRAF* testing is not available or reimbursed because of regulatory restrictions.

Moreover, several studies are undergoing worldwide for exploring the efficacy of *BRAF*-targeting agents. We believe that by applying the proposed nomogram the molecular screening and therefore the overall accrual of those studies could be better implemented in terms of both costs and enrolment performance. Furthermore, the poor prognosis of *BRAF*-mutant patients makes their earliest identification essential to enable enrolment in clinical trials.

In addition, our nomogram may also potentially guide for prospective stratification of future randomized trials thus avoiding costly and time-consuming upfront testing procedures.

In addition, the identification of subgroups where *BRAF* mutation is very likely to occur would theoretically help to decrease the attrition bias of retrospective studies when tumour blocks are no longer available or difficult to retrieve. In the context of CRC, a clear example is given by the Analysis and Research in CAncers of the Digestive system (ARCAD) database: a large international effort for pooling data from major randomized trials, that nowadays includes $>20\,000$ patients from >20 first-line mCRC trials (de Gramont *et al*, 2010; Lieu *et al*, 2014) and our nomogram may represent a valuable tool for guiding and interpreting the results of subgroup analyses.

From a broader perspective, what does a nomogram add to a multivariate model? We believe that while a simple multivariate model allows physicians to identify which are the independent predictors for the occurrence of a specific event, a nomogram, as the one herein proposed, may translate the statistical output of the identified predictors into a single numerical estimate of the probability of an event (Iasonos *et al*, 2008), which is, in this case, the chance to have a *BRAF* mutation. Given the nature of the required information (gender, primary site and histology) and the ease of the graphical interface (Figure 1) this nomogram is extremely valuable and ready-to-use.

ACKNOWLEDGEMENTS

SP, LC, IS and EB are supported by a grant of the Italian Association for Cancer Research (AIRC My First AIRC Grant No. 14282). This work was partially supported by ARCO No-profit Foundation and by a donation from Raddi family in loving memory of Bianca Lucarelli. FL and EB were responsible for study concept and design. MM, LS, MS, DR, LC, SP, LC, DI, MC and LF were responsible for data collection. FL, RM, CC, IS, GA, AF, GT and EB were responsible for analysis and interpretation of data. FL, RM and EB were responsible for drafting the manuscript. IS and EB were responsible for statistical analysis. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on *British Journal of Cancer* website (http://www.nature.com/bjc).