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Original Research

A validated prognostic classifier for ^{V600E}*BRAF*-mutated metastatic colorectal cancer: the 'BRAF BeCool' study

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Abstract ***Background:*** Despite the well-known negative prognostic value of the ^{V600E}*BRAF* mutation in patients with metastatic colorectal cancer (mCRC), its outcome is quite heterogeneous, and the basis for this prognostic heterogeneity should be better defined.
Methods: Two large retrospective series of ^{V600E}*BRAF*-mutated mCRC from 22 institutions served as an exploratory and validation set to develop a prognostic score. The model was internally and externally validated.
Results: A total of 395 ^{V600E}*BRAF*-mutated mCRCs were included in the exploratory set. Performance status, CA19.9, lactate dehydrogenase, neutrophil/lymphocyte ratio, grading and liver, lung and nodal involvement emerged as independent prognostic factors for overall survival (OS). Two different scoring systems were built: a 'complete' score (0–16) including all significant covariates and a 'simplified' score (0–9), based only on clinicopathological covariates, and excluding laboratory values. Adopting the complete score, proportions of patients with a low (0–4), intermediate (5–8) and high (9–16) score were 44.7%, 42.6% and 12.6%, respectively. The median OS was 29.6, 15.5 (hazard ratio [HR] for intermediate vs low risk: 2.16, 95% confidence interval [CI]: 1.44–3.22, $p < .001$) and 6.6 months (HR for high vs low risk: 4.72, 95% CI: 2.72–8.20, $p < .001$). Similar results were observed also after adjusting for the type of first-line treatment and adopting the simplified score. The simplified prognostic score derived from the exploratory set was then applied to the validation set for external confirmation.
Conclusions: These scoring systems are based on easy-to-collect data and defined specific subgroups with relevant differences in their life expectancy. These tools could be useful in clinical practice, would allow better stratification of patients in clinical trials and may be adopted for proper adjustments in exploratory translational analyses.

1. Background

Somatic alteration of the *BRAF* gene at the valine 600 residue (^{V600E}*BRAF*) accounts for >90% mutation of *BRAF*-mutated cancers and triggers the constitutive activation of the epidermal growth factor receptor–mediated MAPK pathway [1]. ^{V600E}*BRAF* mutation occurs in roughly 10% of metastatic colorectal cancer (mCRC) and leads to a peculiar and well-described phenotype [2]. ^{V600E}*BRAF*-mutated mCRCs are associated with female sex, advanced age, right-sided primary tumour location, peritoneal metastases and mismatch repair deficiency/microsatellite instability-high [3–6]. Moreover, the ^{V600E}*BRAF* mutation is unanimously considered a negative prognostic determinant in mCRC. In fact, the median overall survival (OS) for this molecular subgroup ranges from 10 to 20 months [7]. Nevertheless, an intragroup heterogeneity consistently emerges from all published series with about 10–20% of ^{V600E}*BRAF*-mutated cases surviving more than 2 years since the diagnosis of metastatic disease [8–12]. A pooled meta-analysis of 3 randomised clinical trials included a total of 231 patients with ^{V600E}*BRAF*-mutated mCRC and reported more than

20% of patients overcoming the survival landmark of 24 months [7]. Another real-world analysis from 503 patients showed similar results [13].

The basis of this prognostic heterogeneity is still unclear. From a clinical perspective, surgical resection of metastases with a curative intent could be a major reason for long-term survival differences in CRC series [14], but the impact of metastasectomy in ^{V600E}*BRAF*-mutated mCRC is minimal: these patients rarely have limited and exclusive liver and/or lung involvement, and at the same time, some studies reported even shorter OS and relapse-free survival after lung [15] and liver resection [16,17] for ^{V600E}*BRAF*-mutated cases. From a molecular perspective, clear differences among ^{V600E}*BRAF*-mutated CRC were recently described in gene expression [18]. Two distinct subgroups of ^{V600E}*BRAF*-mutated CRC were recently distinguished: one (named BM1) exhibiting high KRAS/mammalian target of rapamycin/AKT/4EBP1, EMK activation and immune infiltration and the other (named BM2) presenting cell cycle checkpoint dysregulation. In addition, a cell drug screen indicated that these subtypes may have different responses to specific drugs, including BRAF and MEK inhibitors.

Power and robustness of exploratory analyses in the subgroup of patients with ^{V600E}*BRAF*-mutated CRC are usually limited owing to the low incidence of the mutation. In the present multicentre study, we pooled a large number of fully annotated cases, with the aim of building reliable and simple prognostic scores, potentially useful for better informing clinicians, researchers and patients on actual disease aggressiveness and life expectancies.

2. Materials and methods

2.1. Study design and participants

Consecutive patients with ^{V600E}*BRAF*-mutated mCRC referred to 14 oncology units between January 2005 and December 2016 were gathered in an exploratory set. For details on collected data, see Appendix 1.

For building the external validation set, 8 additional centres joined the initiative, and data on consecutive eligible patients were gathered according to a predefined statistical hypothesis (see in the following section). The study was approved by local ethics committees (Oncologic Institute of Veneto, code 2017/34).

2.2. Statistical analysis

The primary end-point was to determine the presence of an independent prognostic factor for OS among patients with ^{V600E}*BRAF* mCRC. Details on formal definitions of end-points are reported in Appendix 2. An internal cross-validation procedure was applied, and the whole study population was split into a training sample (67%) and a testing one (33%); this process was repeated 10 times, obtaining 10 training samples and 10 corresponding testing samples [19]. Multivariate analysis was performed on each training sample, and covariates with an independent prognostic value were retained in the final model and included in the scoring system. The logs of median hazard ratios (HRs) obtained from the 10 Cox models were used to derive weighting factors of a prognostic index. Coefficient estimates were 'normalised' by dividing by the smallest one and rounding the resulting ratios to the nearest integer value. The sample size of the validation set was based on the following assumption: to observe the same distribution of risk categories and the same survival observed previously, 145 events were overall needed to validate prognostic differences among categories with 80% power and an alpha error of 0.05.

3. Results

3.1. Study population

A total of 395 consecutive patients with ^{V600E}*BRAF*-mutated mCRC were included in the exploratory set. Detailed patients' characteristics are listed in Table 1.

The median age was 65 years (range: 24–88), and the male/female ratio was 1:1. Of 395 patients, 65% had right-sided CRC, and 71% had a metastatic disease *ab initio*; liver metastases occurred in 56% of cases, whereas nodal and peritoneal metastases occurred in 36% and 33% of cases, respectively. The majority of patients (88%) received a first-line systemic treatment that most frequently included a fluoropyrimidine-based doublet plus bevacizumab (36%), followed by 5-fluorouracile, oxaliplatin and irinotecan triplet combination (FOLFOXIRI) plus bevacizumab (24%). One hundred eighty-six (47%) patients enrolled in at least 1 clinical study during the course of their disease.

3.2. Survival analyses in the whole population

At a median follow-up of 33.9 months, 260 patients died, and the median OS was 19.6 months.

In the univariate analysis, OS was significantly worse for an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1 or $\geq$2 (compared with an ECOG PS of 0, $p < .0001$, and $p < .0001$, respectively), not having the primary tumour resected ($p < .0001$), G3-G4 tumour grading ($p < .0001$), presence of liver metastases ($p = .014$), lung metastases ($p = .014$) or nodal metastases ($p = .002$) and the number of metastatic sites $\geq$2 ($p < .0001$).

Concerning the baseline laboratory values, OS was significantly shorter in case of CA19.9 > upper limit of normal ($p = .005$), lactate dehydrogenase (LDH) >300

Table 1
Patient characteristics.

Characteristics		Total = 395 No (%)
Sex	Female	197 (50)
	Male	198 (50)
Age	Median (range)	65 (24–88)
Age (70-year cut-off)	>70	123 (31)
	≤70	272 (69)
Baseline ECOG PS	0	212 (63)
	1	99 (30)
	≥2	25 (7)
	NA	59
Baseline CEA	Normal	111 (36)
	> ULN (5 ng/mL)	196 (64)
	NA	88
Baseline CA19.9	Normal	136 (46)
	> ULN (37 UI/L)	162 (54)
	NA	97
Baseline platelets	Normal	274 (92)
	> ULN (450 × 10^9/L)	25 (8)
	NA	96
Baseline LDH	Normal	78 (31)
	> ULN (M = 225 U/L F = 214 U/L)	175 (69)
	NA	142
Baseline ALP	Normal	185 (70)
	> ULN (M = 128 U/L F = 141 U/L)	79 (30)
	NA	131
Baseline HgB	≥11 g/dL	235 (76)
	<11 g/dL	73 (24)
	NA	87
Baseline WBC	Normal	267 (87)
	≥ ULN (11 000/ml)	39 (13)
	NA	89
NLR	≤3	167 (58)
	>3	123 (42)
	NA	105
Baseline albumin	≥4 g/dL	94 (48)
	<4 g/dl	103 (52)
	NA	198
Kohne score	Low	181 (58)
	Intermediate	94 (30)
	High	36 (12)
	NA	84
Primary tumour resected	Yes	317 (80)
	No	78 (20)
Adjuvant chemotherapy	Yes	116 (29)
	No	279 (71)
Adjuvant oxaliplatin	Yes	87 (22)
	No	29 (78)
Primary tumour location	Right	256 (65)
	Left	86 (22)
	Rectum	50 (13)
	NA	3
Mucinous histology	Yes	78 (22)
	No	280 (78)
	NA	37
Lymphovascular invasion	Yes	203 (79)
	No	55 (21)
	NA	137
Grading	G1-G2	125 (39)
	G3-G4	197 (61)
	NA	73
Presentation of metastases	Synchronous	279 (71)
	Metachronous	116 (29)
Sites of metastases at diagnosis	Liver	223 (56)
	Lung	73 (18)
	Distant nodes	143 (36)
	Peritoneum	132 (33)
	Local relapse	11 (3)
	Other	53 (1)
No. of metastatic sites	1	226 (57)
	≥2	169 (43)

ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; ULN, upper limit of normal; CEA, carcinoembryonic antigen; NLR, neutrophils to lymphocytes ratio; WBC, white blood cells; HgB, haemoglobin; ALP, alkalyne phosphatase.

mU/ml ($p < .0001$), alkalyne phosphatase (ALP) ≥300 UI ($p = .010$), haemoglobin <11 g/dL ($p = .044$), white blood cells (WBC) ≥ 11 000/ml ($p = .004$), neutrophils to lymphocytes ratio (NLR) >3 ($p < .0001$), albumin <4 g/dL ($p = .041$) and Köhne risk score intermediate ($p = .01$) or high ($p < .0001$). Detailed HR and 95% confidence interval (CI) are available in Table 2.

3.3. Multivariable analyses and the prognostic score

In the multivariable OS model, ECOG PS (1 vs 0; 2–3 vs 0), CA19.9 (high vs normal), LDH (≥300 vs low), NLR (>3 vs ≤ 3), tumour grading (3–4 vs 1–2), liver metastases (yes vs no), lung metastases (yes vs no) and nodal metastases (yes vs no) retained their prognostic impact in terms of OS. For each covariate retained in the model, median values and interquartile ranges of the 10 estimates of HR are reported. Two different scoring systems were built: a 'complete' score (0–16) including all 8 significant covariates and a 'simplified' score (0–9), selecting 5 significant covariates, excluding laboratory values (see Table 3).

In the 'complete' score, three different risk categories were defined: low (0–4), intermediate (5–8) and high (≥9). The proportion of patients assigned to the 3 categories was 44.8%, 42.6% and 12.6%, respectively (Supplementary Fig. 1). In the 10 testing samples, the median OS estimates were 26.2 months (interquartile range [IQR]: 23.0–30.9) for low the score, 15.7 months (IQR: 14.6–18.7) for the intermediate score and 6.1 months (IQR: 3.8–7.4) for the high score.

Similarly, in the 'simplified' score, three different risk categories were defined: low (0–2), intermediate (3–4) and high (≥5). The proportion of patients assigned to the 3 categories was 44.7%, 37.2% and 18.1%, respectively (Supplementary Fig. 2). In the 10 testing samples, the median OS estimates were 26.3 months (IQR: 24.7–29.8) for the low score, 15.9 months (IQR:

Table 2
Univariate analyses.

Characteristics		Median OS (months)	Overall survival		
			HR	95% CI	p
Sex	Male	19.6	1	–	–
	Female	19.3	1.00	0.79–1.29	0.972
Age	≤70	20.0	1	–	-
	>70	17.1	1.28	0.97–1.67	0.079
ECOG PS	0	23.6	1	–	–
	1	12.6	1.76	1.31–2.35	**< 0.0001**
	≥2	2.9	5.25	3.28–8.41	**< 0.0001**
Baseline CEA	Normal	19.8	1	–	–
	> ULN	17.1	1.22	0.91–1.64	0.192
Baseline CEA >200 ng/mL	No	18.8	1	–	-
	Yes	11.4	1.61	1.06–2.43	**0.025**
Baseline CA19.9	Normal	21.4	1	–	-
	> ULN	15.1	1.51	1.13–2.03	**0.005**
Baseline platelets	Normal	18.2	1	–	-
	> ULN (450 < 10^9/L)	10.8	1.25	0.76–2.06	0.386
Baseline platelets ≥400 10^9/L	No	18.8	1	–	-
	Yes	12.0	1.30	0.89–1.90	0.168
Baseline LDH	Normal	26.0	1	–	–
	> ULN (M = 225 U/L F = 214 U/L)	16.0	1.55	1.07–2.24	**0.019**
Baseline LDH ≥300 U/L	No	26.6	1	–	–
	Yes	14.2	1.93	1.42–2.64	**< 0.0001**
Baseline ALP	Normal	21.0	1	–	–
	> ULN (M = 128 U/L F = 141 U/L)	14.4	1.41	1.03–1.93	**0.031**
Baseline ALP ≥300 U/L	No	19.8	1	–	–
	Yes	11.7	1.73	1.14–2.61	**0.010**
Baseline HgB	≥11 g/dL	18.8	1	–	–
	<11 g/dl	14.5	1.38	1.01–1.89	**0.044**
Baseline WBC	Normal	19.6	1	–	–
	≥ ULN (11 000/ml)	10.8	1.78	1.20–2.63	**0.004**
NLR	≤3	25.0	1	–	-
	>3	12.7	2.01	1.50–2.68	**< 0.0001**
Baseline albumin	≥4 g/dL	22.5	1	–	–
	<4 g/dl	14.5	1.42	1.01–1.98	**0.041**
Kohne score	Low	22.8	1	–	–
	Intermediate	15.4	1.51	1.10–2.07	**0.01**
	High	5.1	3.74	2.51–5.59	**< 0.0001**
Primary tumour resection	Yes	23.0	1	–	–
	No	11.3	2.56	1.89-3.41	**< 0.0001**
Primary tumour location	Right	19.6	1	–	–
	Left	21.4	0.81	0.60-1.11	0.189
	Rectum	16.3	0.96	0.68-1.36	0.822
Primary tumour location	Right	19.6	1	–	-
	Left rectum	21.4	0.87	0.67-1.13	0.290
Mucinous histology	No	20.5	1	–	–
	Yes	19.6	0.97	0.71-1.34	0.860
Lymphovascular invasion	No	26.7	1	–	-
	Yes	18.4	1.35	0.93-1.96	0.114
Grading	G1-2	27.4	1	–	-
	G3-4	14.7	1.98	1.48-2.65	**< 0.0001**
Presentation of metastasis	Metachronous	23.8	1	–	–
	Synchronous	16.0	1.18	0.90-1.54	0.224
Liver metastasis at diagnosis	No	21.8	1	–	–
	Yes	15.9	1.37	1.07-1.76	**0.014**
Lung metastasis at diagnosis	No	22.0	1	–	–
	Yes	14.0	1.46	1.08-1.97	**0.014**
Nodal metastasis at diagnosis	No	23.0	1	–	–
	Yes	14.4	1.52	1.17-1.97	**0.002**

(*continued on next page*)

Table 2 (*continued*)

Characteristics		Median OS (months)	Overall survival		
			HR	95% CI	p
Peritoneal metastasis at diagnosis	No	21.4	1	–	–
	Yes	16.0	1.21	0.94-1.57	0.14
No. of metastatic sites	1	25.8	1	–	–
	≥2	14.0	1.95	1.52-2.50	**< 0.0001**
MSI status	MSS	22.4	1	–	–
	MSI-H	24.3	0.87	0.57-1.34	0.532

P-values below 0.05 in bold.
MSI-H, microsatellite instability-high; MSI, microsatellite instability; HR, hazard ratio; CI, confidence interval; OS, overall survival; ECOG, Eastern Cooperative Oncology Group; PS, performance status; ULN, ULN, upper limit of normal; LDH, lactate dehydrogenase; ALP, alkalyne phosphatase; WBC, white blood cells; NLR, neutrophils to lymphocytes ratio; CEA, carcinoembryonic antigen; HgB, haemoglobin; MSS, microsatellite stable.

15.1–16.0) for the intermediate score and 6.6 months (IQR: 5.5–8.4) for the high score.

3.4. Outcome for different risk score categories in the whole study population

The OS results for the 'complete' and 'simplified' prognostic classifiers in the whole study population are reported in Fig. 1.

According to the 'complete' prognostic score system, the median OS for patients included in the high-risk group was 6.6 months (95% CI: 5.1–8.0; HR high vs low risk: 4.72; 95% CI: 2.72–8.20, $p < .001$); for the intermediate-risk group, it was 15.5 months (95% CI: 10.2–20.8; HR intermediate vs low risk: 2.16; 95% CI: 1.44–3.22, $p < .001$) and for the low-risk group, it was 29.6 months (95% CI: 20.4–38.9) (Fig. 1A).

Adopting the 'simplified' prognostic score system, the 3 different risk score categories maintained their prognostic relevance with a median OS of 7.0 (95% CI: 5.1–9.0), 15.9 (95% CI: 11.0–20.7) and 26.7 (95% CI: 23.5–29.9) months for high, intermediate and low risk, respectively (HR intermediate vs low risk: 1.71; 95% CI: 1.22–2.41, $p = .002$; HR high vs low risk: 4.62; 95% CI: 3.11–6.85, $p < .001$) (Fig. 1B).

Similarly, according to either the 'complete' or 'simplified' score system, median progression-free survival (PFS) was significantly shorter for intermediate- and high-risk score subgroups than the low-risk one (Supplementary Fig. 3). Details on PFS results are available in Appendix 3.

3.5. OS adjusted for intensity of first-line systemic treatment

The impact on survival results of administered treatments and their relation to the prognostic classifiers were further explored. To that purpose, first-line therapies were

Table 3
Multivariate models and scoring systems for the 'complete score' and 'simplified score'.

Complete score						
Characteristics		Median HR	IQ range	Log (median HR)	Normalised log (HR)	Rounded score
ECOG PS	1 vs 0	2.62	2.22–3.24	0.419	2.24	2
ECOG PS	2-3 vs 0	7.26	3.68–9.36	0.861	4.61	5
CA19.9	High vs normal	1.61	1.14–2.26	0.205	1.10	1
LDH	≥300 vs low	1.86	1.66–2.33	0.270	1.44	1
NLR	>3 vs ≤ 3	1.54	1.22–1.97	0.187	1.0	1
Grading	3-4 vs 1-2	1.78	1.46–2.16	0.250	1.34	1
Liver	Yes vs no	2.22	2.08–2.50	0.347	1.86	2
Lung	Yes vs no	3.64	2.89–4.50	0.561	3.00	3
Nodes	Yes vs no	2.73	1.79–3.61	0.436	2.33	2
Simplified score						
Characteristic		Median HR	IQ range	Log (median HR)	Normalized log (HR)	Rounded score
ECOG PS	1 vs 0	2.62	2.22–3.24	0.419	1.67	2
ECOG PS	2-3 vs 0	7.26	3.68–9.36	0.861	3.44	3
Grading	3-4 vs 1-2	1.78	1.46–2.16	0.250	1.0	1
Liver	Yes vs no	2.22	2.08–2.50	0.347	1.39	1
Lung	Yes vs no	3.64	2.89–4.50	0.561	2.24	2
Nodes	Yes vs no	2.73	1.79–3.61	0.436	1.74	2

HR, hazard ratio; IQ, interquartile; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; NLR, neutrophils to lymphocytes ratio.

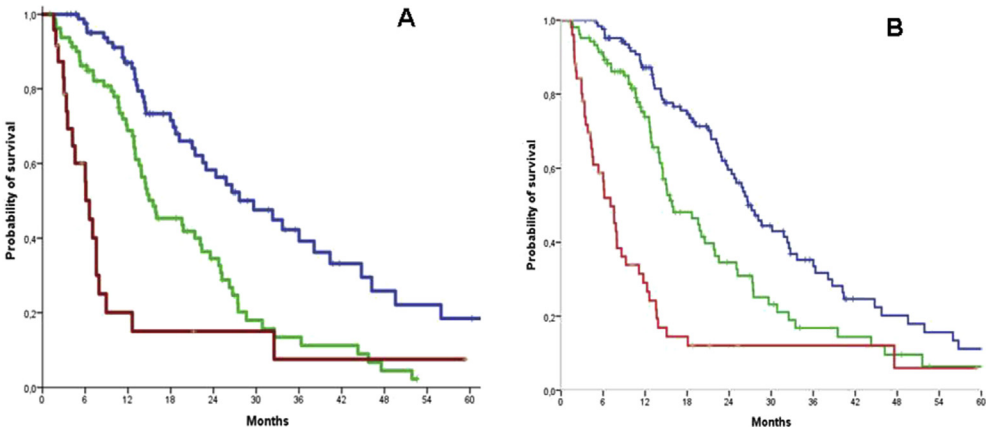

Fig. 1. Overall survival (OS) according to complete risk score categories (A) and simplified risk score categories (B). (A) Complete risk score – OS, red line: high risk (N = 24), median OS = 6.6 months (95% CI: 5.1–8.0)., green line: intermediate risk (N = 81), median OS = 15.5 months (95% CI: 10.2–20.8)., blue line: low risk (N = 85), median OS = 29.6 months (95% CI: 20.4–38.9), intermediate risk vs low risk, HR = 2.16 (95% CI: 1.44–3.22), p < .001, high risk vs low risk, HR = 4.72 (95% CI: 2.72–8.20), p < .001 (B) Simplified risk score – OS, red line: high risk (N = 51), median OS = 7.0 months (95% CI: 5.1–9.0), green line: intermediate risk (N = 105), median OS = 15.9 months (95% CI: 11.0–20.7), blue line: low risk (N = 126), median OS = 26.7 months (95% CI: 23.5–29.9), intermediate risk vs low risk, HR = 1.71 (95% CI: 1.22–2.41), p = .002, high risk vs low risk, HR = 4.62 (95% CI: 3.11–6.85), p < .001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) CI, confidence interval; HR, hazard ratio.

categorised into 3 groups according to the intensity of treatment defined on the basis of the number of concomitant chemotherapy drugs (1 vs 2- vs 3-drug regimens). Twenty-eight patients received an anti-BRAF experimental treatment (n = 7) or no treatment (n = 21). A total of 16, 85 and 61 patients were treated with single-agent chemotherapy, a doublet or a triplet as backbone chemotherapy, respectively (Supplementary Table 1).

In a multivariable analysis including the type of treatment and the risk category, OS differed significantly between patients who underwent to one- (HR: 0.27; 95% CI: 0.11–0.67, p = .005), two- (HR 0.16; 95% CI, 0.08–0.34, p < .001) or three-drug regimen (HR 0.15; 95% CI: 0.07–0.33, p < .001) compared with those untreated.

Notably, the risk category retained an independent prognostic value also in this model adjusted by type of treatment: HR for intermediate vs low risk 2.42 (95% CI: 1.59–3.70, p < .001) and HR for high vs low risk 4.69 (95% CI: 2.66–8.29, p < .001).

Similar results were observed when 'simplified score' performance was adjusted by treatment.

3.6. External validation

Data from 252 consecutive patients with ^{V600E}*BRAF*-mutated mCRC were prospectively gathered in a validation set. Adopting the simplified score, the proportion of patients assigned to low-, intermediate- and high-risk score categories was 38.5%, 31.0% and 30.5%, respectively. At the time of analyses, 150 death events occurred. The median OS for patients included in the high-risk group was 8.9 months (HR high vs low risk: 3.39; 95% CI: 3.10–7.20, p < .0001), in the intermediate-risk group was 23.7 months (HR high vs intermediate risk: 2.70; 95% CI: 2.04–4.60, p = <0.0001) and in the low-risk group was 26.1 months (HR intermediate vs low risk: 1.01; 95% CI: 0.66–1.53, p = .98). A specific web-based application (named 'BRAF BeCool') for easy calculation of the simplified score was developed and is available for download in iOS 8.0 and Android 5.0 or later.

4. Discussion

The present work is based on one of the largest series of ^{V600E}*BRAF*-mutated mCRCs. Its rationale came from the need for a specific survival classifier for these patients, generally marked as a subgroup with extremely poor prognosis. Indeed, data from previous publications suggest a high degree of heterogeneity in the outcome with a not-negligible proportion of long-term survivors [7]. However, these analyses were limited in the sample size, included different stages, and were mainly focussed on describing differences between *BRAF*-mutated and wild-type patients [7,13]. Most of the information available in the literature is derived from post hoc analyses of clinical studies [9,10,20], whose inclusion criteria clearly restrict eligibility to a selected subgroup of ^{V600E}*BRAF*-mutated patients, thus introducing a relevant bias when transferring these results to the real world. In our work, subjects enrolled in clinical trials and those who were treated outside clinical trials were balanced, thus providing a data set depicting a real-world scenario.

The major and clinically relevant finding is that patients with ^{V600E}*BRAF*-mutated mCRC might be

classified into 3 distinct prognostic subgroups (i.e. low-, intermediate- and high-risk), by means of simple and easy-to-use clinicopathological classifiers. Thus, the present scoring systems may have potential implications at 3 different levels: (i) clinical, (ii) methodological and (iii) translational.

From a clinical perspective, the prognostic classifier may affect specific crucial points of clinical decision-making. Given the retrospective and observational nature of our analysis, we cannot draw specific conclusions regarding the relative efficacy of more versus less intensive treatments. Nevertheless, the presence of extremely bad prognostic features may suggest some caution when considering specific strategies such as resection of metastases, in a careful evaluation of risk/benefit balance. To this extent, we should acknowledge that available data on secondary resection of metastases and specific outcome parameters (such as resection margins) were limited in our data sets and no specific considerations could be drawn. On the other hand, life expectancy for the good-prognosis subgroup of mutated cases is not much lower than unselected patients with wild-type tumour. Therefore, those patients should not be stigmatised simply as a group with low chance of survival based on *BRAF* mutational status because this may influence clinicians' attitude towards specific therapeutic choices. Furthermore, an informative discussion on prognosis was recently demonstrated to strengthen the therapeutic alliance between patients and oncologists, thus underlying the clinical relevance of a robust prognostic score [21].

Other than a prognostic marker, the ^{V600E}*BRAF* mutation is a potential key molecular target [22–26]. Several studies are currently testing BRAF inhibitors in mCRC. From a methodological perspective, the prognostic score could refine the criteria for the stratification of patients in those trials. This would be much more appropriate than adopting the same prognosticators derived from studies conducted in unselected mCRC. As an example, the primary tumour location has recently gained consideration a reliable prognostic factor in wild-type mCRCs [27], thus being proposed as a stratification factor for design of new trials. In our series of ^{V600E}*BRAF*-mutated patients, the primary tumour location was not prognostic. In fact, most of the data showing a significantly worse outcome for right-sided primary tumours were not powered for looking specifically at the *BRAF*-mutated subgroup [28,29]. At the same time, this observation should be considered as preliminary and hypothesis generating.

Finally, looking at the potential utility of our data from a translational perspective, it should be considered how often preliminary analyses aiming at the discovery of new useful biomarkers are hampered by limited clinical data. A better knowledge of the main specific prognostic factors in selected subgroups would allow reliable multivariate models to be built, including risk categories as covariates, in future translational studies. Similar considerations and specific proposals were made years ago for molecularly unselected patients, and those approaches improved clinical trial quality and robustness of data [30].

The retrospective part of our work has usual intrinsic limitations. First, owing to the long time frame set for retrieving such high numbers of ^{V600E}*BRAF*-mutated patients, our analysis retained an intrinsic time-lag bias. Second, we tested the microsatellite instability status in less than half of the patients; however, this will be explored in *ad hoc* translational studies. Third, despite adjusting survival analysis for the possible impact of treatment, such adjustment was limited to a simplified categorisation based on the intensity of the chemotherapy backbone (1 vs 2 vs 3 drugs); further subgroup analyses would have been impaired by very low power. Adjustments were made only according to first-line treatment data, but it should be noted that the impact on OS of second and later lines in ^{V600E}*BRAF*-mutated patients is extremely limited [7]. It should be noted that these limitations are unavoidable in retrospective analyses, which on the other hand have the advantage of describing the real-world experience.

From a methodological point of view, the prognostic model is at risk of overfitting bias [31]. We tried to reduce this bias by adopting an internal cross-validation procedure, working on training and validation samples randomly selected among the study population. However, this limitation was overcome by the prospective validation of the simplified score in an external independent series of consecutive patients. The distribution of patients in the 3 categories reproduced the initial retrospective data with a slightly higher rate of assignment to the high-risk group. It could be speculated that the prospective enrolment with absolutely no patient exclusion may have influenced that, given that the loss of bad prognosis patients could be more frequent in historical databases. Relatively short follow-up may have influenced the lack of statistical significance in differences between intermediate- and low-risk categories.

5. Conclusion

Robust clinical prognostic classifiers for patients with ^{V600E}*BRAF*-mutated mCRC are developed and validated. Both prognostic scores allow discrimination of 3 subgroups with significantly different outcomes, confirming the hypothesis of intragroup heterogeneity among ^{V600E}*BRAF*-mutated mCRC. These easy-to-collect data might be clinically useful and may guide stratification choices in future clinical trials and strengthen translational studies. The BeCool platform is currently running a translational research programme focussed on exploring how molecular factors (such as BM1/BM2 and consensus molecular subtypes (CMS)

categorisation systems) recently proposed as possible basis for ^{V600E}*BRAF*-mutated mCRC heterogeneity would fit in innovative prognostic models.

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Conflict of interest statement

Fotios Loupakis had roles as a consultant or advisor for Roche, Bayer, Amgen and Genentech.Filippo Pietrantonio received honoraria and had roles as a consultant or advisor for Amgen, Merck, Roche, Sanofi, Bayer, Servier and Lilly.Lorenza Rimassa received honoraria and had roles as a consultant or advisor for Lilly, Bayer, Sirtex Medical, Italfarmaco, Sanofi, ArQule, Baxter, Ipsen, Exelixis, Amgen, Incyte, Celgene, Eisai, AstraZeneca, AbbVie, Gilead and Roche.Sara Lonardi had roles as a consultant or advisor for Amgen, Bayer, Merck Serono and Lilly. She received research funding from Amgen and Merck Serono, and she is part of the speakers' bureau of Lilly and BMS.Vittorina Zagonel received honoraria and had roles as a consultant or advisor for Bristol-Myers Squibb, Bayer, Roche, Pfizer, Janssen, Novartis, Astellas and Servier. She had roles as a consultant or advisor for Celgene and Merck.Massimo Di Maio received honoraria, had roles as a consultant or advisor for AstraZeneca, Bristol Myers Squibb, MSD, Takeda and Janssen and received a research grant from Tesaro.All the other authors declare no conflict of interest regarding the publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.06.008.

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