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ORIGINAL ARTICLE

The Prognostic Influence of BRAF Mutation and other Molecular, Clinical and Laboratory Parameters in Stage IV Colorectal Cancer

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Abstract Our aim was to evaluate the predictive and prognostic influence of *BRAF* mutation and other molecular, clinical and laboratory parameters in stage IV colorectal cancer (CRC). 60 patients were included in this retrospective analysis, and 17 variables were examined for their relation with treatment response and survival. *KRAS* mutation was identified in 40.3 % of cases, *BRAF* and *PIK3CA* in 8.8 % and 10.5 % respectively. 29.8 % of patients responded to treatment. Median survival time was 14.3 months. Weight loss, fever, abdominal metastases, blood transfusion, hypoalbuminaimia, *BRAF* and *PIK3CA* mutations, CRP and DNA Index were associated with survival. In multivariate analysis, male patients had 3.8 times higher probability of response, increased DNA Index was inversely correlated with response and one unit raise of DNA Index augmented 6 times the probability of death. Our findings potentiate the prognostic role of *BRAF*, *PIK3CA* mutations and ploidy in advanced CRC.

Keywords Colorectal cancer · BRAF · KRAS · PIK3CA · DNA ploidy · Prognosis

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Introduction

Colorectal cancer is one of the most frequently diagnosed cancers in western world and one of the leading causes of death. In United States, it is estimated that about 136, 830 were diagnosed with colorectal cancer during 2014, and 50,310 persons had been died of the disease. Progress in prevention and early detection by screening tests as well as the molecular understanding of the disease and the breakthrough of targeted therapies are the cornerstones that allow us to ameliorate the response to treatment and the prognosis of patients with CRC.

TNM staging at time of diagnosis has been largely applied as a useful prognostic tool. Nonetheless, it cannot provide us all the information required to recognize patients with high risk of relapse nor predict response to targeted treatment. So a panel of biomarkers is necessary to be added to the current staging system.

KRAS codon 12 and 13 wild type state has already been used as a biomarker of response to treatment with cetuximab and panitumumab. *KRAS* gene product is a G protein which is a downstream effector of EGFR signal pathway and motivates the activation of MAPK and promoting cell growth and survival. It is well documented that *KRAS* mutation is an early event in CRC occurring in about 40 % of cases conferring resistance to anti-EGFR regimens [1]. As not all *KRAS* wild type tumors will respond to anti-EGFR treatment, there are studies supporting the potential predictive and prognostic role of *BRAF*, *PIK3CA* and *PTEN* mutations which are the main effectors of EGFR downstream signal pathway [2].

BRAF gene encodes a serine-theonine protein kinase that is a component of the RAS/MEK/ERK pathway. Mutations in *BRAF*, found in approximately 10 % of patients with metastatic CRC, constitute an early event in colon tumorigenesis. In

95 % of cases, *BRAF* mutations occur in V600E and activate the signaling pathway [3]. Furthermore, they are strongly associated with microsatellite instability (MSI) and CpG methylator phenotypes (CIMP) [4]. More specifically, *hMLH1* promoter hypermethylation in sporadic CRC causes the defective function of DNA mismatch repair (dMMR) system leading to MSI. *BRAF* and *KRAS* mutations are almost exclusive in patients with CRC and prior studies support their role as negative predictors of response to anti-EGFR regimens [5]. Although further studies have not confirmed a predictive role [6, 7], *BRAF* mutations have been implicated with poor prognosis in metastatic CRC patients [8]. Vemurafenib is a selective inhibitor of BRAF V600E demonstrating high response rates in melanoma tumors, but notably poorer responses in CRC patients with *BRAF* mutation. Based on recent studies, resistance to *BRAF* inhibition in these patients can be overcome by inhibiting PI3K or with demethylating agents [9].

Mutations in PI3K pathway genes occur in about 40 % of patients with CRC and most frequently in the p110a catalytic subunit phosphatidynlinositol-4, 5-biphosphate 3-kinase catalytic subunit alpha (gene *PIK3CA*) or in phosphatase and tensin homolog (*PTEN* gene) that suppresses PI3K signaling [10]. EGFR via KRAS activation modulates PI3K signaling and there are indications that mutations in PI3K may have a potent role as predictors of response to anti-EGFR treatment as well as anti-PI3K therapies [2]. Nevertheless recent data propose *PIK3CA* mutations as predictive markers of response to aspirin [11]. *PIK3CA* mutations are not mutually exclusive neither with *KRAS* nor with *BRAF* mutations leading to unresponsiveness to anti-EGFR treatment.

Chromosomal instability is referred to the presence of numerical or structural alterations of chromosomes and represents the commonest form of genomic instability detected to up to 85 % of CRCs patients [12]. CIN status can be assessed by DNA flow cytometry or image flow cytometry. There are indications that CIN increases clonal diversity leading to tumor progression and constitutes a marker of poor prognosis [12]. Furthermore, it was suggested that low DNA ploidy score might derive greatest benefit from palliative chemotherapy [13].

The aim of the present study was to examine the correlation between molecular, clinical and laboratory markers with response to treatment and overall survival in patients with advanced CRC in an effort to form a multivariate system which help us to optimize individual therapy regimen and estimate survival rates. For that reason we searched for mutations in *BRAF*, *KRAS*, *PIK3CA* genes and correlated the results with various clinical and laboratory parameters.

Material and Methods

Patients and Data Sources

The medical records of 60 patients with histologically proven CRC (UICC stage IV) between 1998 and 2009 were retrospectively reviewed. All were consecutive non selected cases from a single centre and all patients were treated outside of clinical trials. No patients were candidates for surgical treatment (either curative or palliative); however, all received palliative chemotherapy according to established protocols. Chemotherapy regimens were based on single agent leucovorin modulated 5-FU (Mayo clinic or AIO regimens) or combination treatments of 5-FU (DeGrammont or simple infusion and leucovorin) with either oxaliplatin or irinotecan, or capecitabine with or without bevacizumab or cetuximab. Records with complete data (for the parameters used as prognostic factors) were included in the analysis. Follow-up was continued until death from CRC or from any other cause, and patients who remained alive were censored as of January 1, 2010. Overall survival was the primary endpoint. This protocol has been approved by the National and Kapodistrian University of Athens ethics committee.

Prognostic Variables

Seventeen potential prognostic variables were selected. These included patient-related variables such as age (≤60 years or >60 years), gender, performance status (PS) according to the Karnofsky Performance Status Scale Index, Body Surface Area (BSA) (≤1.8m^2 or >1.8m^2), symptoms of fever and pain, weight loss or gain, the location of distant metastases (lymph nodes, liver, lung, abdominal, pelvic, locoregional disease, bone), need of administration of erythropoietin and red blood cells transfusion and the detection of mutations in *BRAF*, *KRAS* or *PIK3CA* genes. For the evaluation of continuous laboratory parameters, we used group categorizations: for carcinoembryonic antigen (CEA): normal ≤5 mg/dL and elevated >5 mg/dL; for cancer antigen 19–9 (CA 19–9): normal ≤30 U/L and elevated >30 U/L; for C-reactive protein (CRP): normal <5 mg/dL, moderately elevated 5–15 mg/dL, and highly elevated >15 mg/dL; and for albumin: normal >5 g/dL and low ≤5 g/dL. For ploidy score (DNA index), group categorization was also applied for analytical purposes: <2.2, 2.2–2.6, >3.6.

DNA Extraction from Paraffin Embedded Tissues (FFPE)

Sections 10 μm thick were cut from paraffin-embedded tissue blocks after tumor enrichment under the Light Microscope by an experienced pathologist. DNA was extracted from the selected tissue areas following a standard DNA extraction kit protocol (NucleoSpin tissue, Macherey-Nagel, Duren,

Germany). The extracted DNA was quantitated on a Picodrop Microliter spectrophotometer.

Real-Time PCR High Resolution Melting Analysis

Samples were screened in duplicate for mutations in exon 15 of *BRAF*, exon 2 of *KRAS* and exons 9 and 20 of *PIK3CA* gene using a Real-Time polymerase chain reaction approach followed by High Resolution melting analysis (HRM) on a Light Cycler 480 (Roche Diagnostics, GmbH, Germany) using primers previously described [14, 15].

Each reaction mixture contained ~20 ng of genomic DNA, 200 nmol/μl of each primer, 10 μl of Light Cycler LC480 High Resolution Melting Master (Roche Diagnostics, GmbH, Germany), 3.5 mM $MgCl_2$ and PCR-grade water adjusted to a total volume of 20 μl. An initial cycle at 95 °C for 10 min, followed by 50 cycles of 95 °C for 10s, 60 °C for 15 s, 72 °C for 12 s was performed. Using the Gene Scanning software (Roche Diagnostics, GmbH, Germany) melting curves were normalized and temperature-adjusted; then a difference plot was generated for HRM analysis. DNA extracted from human colon cancer cell lines, HT29, harboring a mutation (p.V600E) in exon 15 of *BRAF* gene and HCT116 with p.H1047R mutation in exon 20 of *PIK3CA* gene, were used as positive controls. For *KRAS* genes, DNA samples displaying mutations previously identified (colon cancer tumors) were used as positive controls [14, 15]. PCR products positive by HRM analysis were purified and subjected to Sanger sequencing and/or pyrosequencing.

DNA Measurements (Ploidy)

For DNA measurements, the Feulgen staining technique was applied as previously described [16]. The nuclei of Feulgen-stained cells were evaluated for DNA ploidy using a Nikon eclipse microscope (Nikon, Japan) connected with a Nikon CCD videocamera and an IBM Pentium 4/PC cell measurement software (Image Pro Plus v. 5.1, Media Cybernetics Inc., Silver Springs, MD, USA). Areas of the Feulgen-stained sections containing pathological lesions, identified in adjacent H&E stained slides, were selected for DNA content analysis. A total of 200–300 nuclei with clear boundaries appearing to have no loss of membrane integrity were analyzed in each tissue sample. Cytometry measurements were performed with a magnification of ×200 and calculated automatically according to the algorithms described previously by measuring the nuclear integrated optical density (IOD), representing the cytometrical equivalent of DNA content [17]. The procedure was performed for all nuclei, and the overall mean represents DNA content or DNA index (DI). Mean IOD of human lymphocytes (control cells) was used as the diploid standard (2c) and reference for DI calculation for targeted cells. DNA histograms were generated and a tumor was classified as diploid if the DI ranged from 0.9 to 1.1 and the relevant DNA histogram revealed only 1 peak at 2c and aneuploid if any from the previous 2 criteria was absent.

Statistical Analysis

Descriptive statistics were calculated with the measures of means, medians, and standard deviation for quantitative parameters. Frequency tables were used to present discrete variables.

Comparisons of quantitative measures between discrete factors were made with the use of student t-test. Associations between discrete variables were studied by using Chi square test or Fisher Exact tests (for 2 × 2 tables). Overall survival was studied by Kaplan-Meier method. Survival differences between groups were studied with the use of log-rank test.

Multivariate logistic regression was performed in order to study the simultaneous effect of prognostic factors on overall response. A cox regression model was also set to estimate the simultaneous effect of prognostic factors on overall survival.

All analyses were performed with SPSS 12.0 statistical package. An initial significance level of 10 % was applied to identify potential prognostic factors. In order to detect the strongly significant associations through the multivariate models, a significance level of a = 5 % was used.

Results

Patients

Patients' characteristics are presented in Table 1. Among 57 patients included in the study, 50.9 % were male and 49.1 % female with a mean age of 63.1 years (median: 63.0 years, s.d.: 8.0). 47.4 % of the patients exhibited an initial PS value of 80/100, 21.1 % a PS of 70/100, 14.0 % a PS of 90/100 and the rest 17.5 % a PS of 100/100. Performance status was revaluated after the treatment and it remained stable in 19.3 % of cases, it was improved in 40.4 % and it deteriorated in 40.4 %. The mean CRP value was 9.68 mg/dl (median: 1.0 mg/dl, s.d.: 16.07). The mean BSA value was estimated at 1.80 (median: 1.81, s.d.: 0.19). 29.8 % of patients presented fever, 64.9 % (49.1 % up to 5 %) weight loss and 26.3 % weight gain. 50.9 % of patients referred pain. Mean CEA value was estimated at 64.3 (median: 11.0, s.d.: 159.8) and mean CA19.9 value at 124.9 (median: 37.3, s.d.: 320.8). Metastases' existence was identified as follows: LN (57.9 %), Liver (70.2 %), Lung (26.3 %), ABD (50.9 %), PELV (36.8 %), LOC (40.4 %) and BON (5.3 %). EPO was administrated in 14.0 % of all cases and red blood cells transfusion in 17.5 %. Hypoalbuminemia was detected in 15.8 %

Table 1 Patients characteristics and clinical, molecular and laboratory parameters

Characteristic	N	%
Sex		
Male	29	50.9
Female	28	49.1
PS value		
70/100	12	21.1
80/100	27	47.4
90/100	8	14.0
100/100	10	17.5
PS after treatment		
Stable	11	19.3
Improvement	23	40.4
Deterioration	23	40.4
Symptoms		
Fever	17	29.8
Weight loss	37	64.9
Weight gain	15	26.3
Pain existence	29	50.9
Metastases		
LN	33	57.9
Liver	40	70.2
Lung	15	26.3
ABD	29	50.9
PELV	21	36.8
LOC	23	40.4
BON	3	5.3
Treatments		
EPO	8	14.0
Transfusion	10	17.5
Albumin detection	9	15.8
Mutations		
KRAS	23	40.3
BRAF	5	8.8
PIK3CA exon 9	2	3.5
PIK3CA exon 20	4	7.0
Age, years		
Mean	63.1	
Median	63	
BSA		
Mean	1.81	
Median	1.80	
CRP		
Mean	9.68	
Median	1.0	
CEA value		
Mean	64.3	
Median	11.0	
CA19–9 value		
Mean	124.9	
Median	37.3	
Ploidy value		
Mean	2.92	
Median	2.84	

PS Performance Status, LN Lymph Nodes, ABD Abdominal, PELV Pelvic, LOC Locoregional, BON Bone, EPO Erythropoietin, BSA Body Surface Area, CRP C-Reactive protein, CEA Carcinoembryonic antigen, CA 19–9 Cancer antigen 19–9

of cases. The average Ploidy value was estimated at 2.92 (median: 2.84, s.d.: 0.72).

Mutational Analysis

From the total of 60 patients included initially in the study, three were excluded due to poor quality DNA extracted from FFPE specimens. The rest 57 patients were included in the statistical analysis.

KRAS status of 57 patients with adequate quality DNA was determined by HRM analysis followed by pyrosequencing. 23 of patients (40.3 %) presented *KRAS* mutation, 18 in codon 12 (78.2 %) and the rest 5 in codon 13 (21.7 %). *KRAS* mutations appeared more frequently in patients aged over 60 years. The most frequent *KRAS* alteration was p.G12D observed in 11 cases (47.8 %), followed by p.G12 V (3 cases), p.G12C (2 cases) and p.G12 A (2 cases) while p.G13D was the only mutation detected in codon 13 (5 cases). Five specimens carried a mutation in *BRAF* gene (8.8 %) that was identified as p.V600E in 40 % of cases. *BRAF* mutations were found in three male and two female patients with mean age of 62 years. In detail, mutations were as follows: p.V600E (2 cases), p.Q609* (1case), p.H608Y (1 case) and p.L597R (1 case). One *BRAF* mutant case was found to display high microsatellite instability (MSI-H). In our study, *BRAF* and *KRAS* mutations were mutually exclusive as none of KRAS mutant tumors harbored a *BRAF* mutation. In total, MAPK pathway was found activated by mutations in 49 % of the cases in our cohort.

PIK3CA exon 9 mutations were found in 3.5 % of the cases whereas *PIK3CA* exon 20 mutations in 7.0 %. In total 10.5 % of the examined cases displayed a *PIK3CA* gene mutation. *BRAF* and *PIK3CA* mutations were not mutually excluded as a *BRAF* (p.V600E) and a *PIK3CA* exon 9 mutation (p.D527V) coexisted in one case. In addition, concurrent mutations were detected in *KRAS* (p.G13D) and *PIK3CA* exon 9 (p.R537R) in one patient and *KRAS* (p.G12 V or p.G12D) along with *PIK3CA* exon 20 (p.H1047R) in two patients. As there were only two patients with mutations in *PIK3CA* exon

9, their association with prognosis could not be evaluated. Mutation in *PIK3CA* exon 20 was not found to be importantly related with treatment response (p-value = 0.08) but it was associated with overall survival (p-value = 0.04).

Ten of the examined cases (five *BRAF*-mutants and 5 *BRAF*-normal) were further analyzed for microsatellite instability (MSI) and promoter methylation for *MLH1*, *MGMT*, *DAPK1*, *RASSF1A* and *APC* gene. Only one *BRAF*-mutant case, harboring p.V600E, displayed MSI-H based on the analysis of BAT-25 and BAT-26 mononucleotide markers. This patient, a female 60 years old, who exhibited a twelve months overall survival, also displayed *hMLH1*, *MGMT*, *DAPK1* and *RASSF1A* promoter methylation. Due to the limited number of cases examined, the effect of MSI to response to treatment and prognosis could not be introduced in the statistical analysis. Descriptive statistics are summarized in Table 1.

Response to Treatment

29.8 % of all patients presented a total or partial response to treatment (5.3 % total response and 24.6 % partial response). Bivariate relationships between overall response and prognostic factors under study were evaluated at a significance level of 10 % in order to detect possible associations with response.

Potential prognostic factors of response were gender (p-value: 0.08), weight loss (p-value: 0.08), transfusion (p-value = 0.03), hypoalbuminemia (p-value: 0.05), CEA (p-value: 0.06), ploidy (p-value: 0.05) and *PIK3CA* exon 20 mutation (p-value 0.08).

Neither *BRAF* nor *KRAS* mutation were found to be significantly associated with response to treatment (p-value: 1 and p-value: 0.56, respectively) as 30.8 % of patients with wild-type *BRAF* responded to treatment versus 20 % of those carrying *BRAF* mutation. Moreover, 24 % of *KRAS* mutant patients vs 34 % of *KRAS* wild type patients responded to treatment. The effect of *PI3KCA* mutation to response to treatment could not be studied due to limited number of mutant cases (only six).

In order to estimate the simultaneous effect of the factors above on overall response a multivariate logistic regression model was set. Due to limited number of cases, transfusion and albumin were excluded from the model. Out of the 10 patients going through red blood cells transfusion, there had not been any responding to the treatment vs 36.2 % of those being transfused. Additionally, out of the 9 patients with hypoalbuminemia, none responded to treatment vs 35.4 % of those with normal albumin values.

From the remaining parameters, gender and ploidy were entered in the model (Table 2). Male patients had 3.8 times higher probability to respond than females (95 % C.I.: 1.05–13.72), while as ploidy values were increased the probability of response to treatment was reduced (p-value: 0.04).

Overall Survival

The estimated mean survival was 25.6 months (95 % C.I.: 19.3–31.9) and the median survival was 14.3 months (95 % C.I.: 6.0–22.6). Survival plot is shown below (Fig. 1).

Indications of association between the prognostic factors under study and overall survival have been detected with regards to the following parameters: weigh loss (p-value: <0.001), fever (p-value:<0.001), abdominal metastases (p-value:0.08), blood transfusion (p-value: <0.001), hypoalbuminemia (p-value:<0.001), *BRAF* mutation (p-value: 0.01) (Table 3), *PIK3CA* mutation, either exon 9 or exon 20 (p-value: 0.04), CRP value (p-value:0.01) and ploidy (p-value: <0.001). All above parameters had a significant association with overall survival at a significance level of a = 10 %.

In order to study the simultaneous effect of the above factors on overall survival, a Cox regression model was set. All parameters were entered into the model so as to study the effect of one after taking into account the effect of others on overall survival. The final model included the parameter ploidy (Table 4). Based on the results of the Cox regression model, one unit raise of ploidy, leads to the increase of the risk of death by 6 times (95 % C.I.: 3.9–12.0).

Discussion

Mutations in RAS/MAPK and PI3K/AKT signaling networks frequently occur in colorectal cancer promoting uncontrolled

Table 2 Multivariate logistic regression on overall response to treatment (parameters checked: gender, weight loss, Performance Status, CEA and ploidy, best model selected by stepwise forward method)

Variable	B	Standard error	Wald	*P*-value	Exp(B)	95,0 % C.I.for EXP(B)	
						Lower	Upper
SEX (Males vs Females)	1.33	0.66	4.11	0.043	3.79	1.05	13.72
PLOIDY	−1.02	0.51	4.07	0.044	0.36	0.13	0.97
Constant	1.28	1.41	0.82	0.365	3.58		

CI Confidence interval

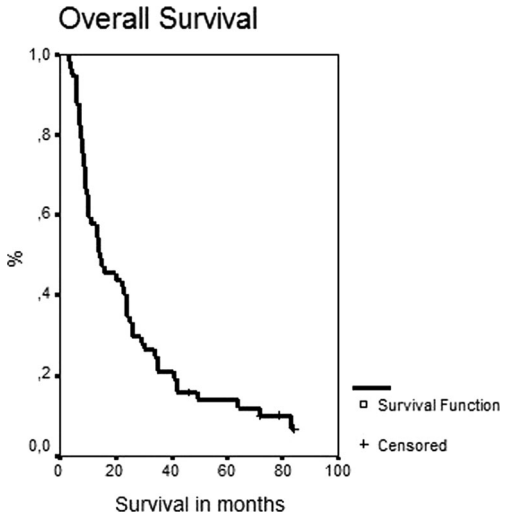

Fig. 1 Overall survival

activation of EGFR downstream cascades. As a result tumor cells undergo deregulated proliferation and exhibit unresponsiveness to anti-EGFR agents. Hence, lack of mutations in codon 12 and 13 of *KRAS* gene has been established as predictive biomarker for treatment with cetuximab and panitumimab [18]. Even though, as previous data support, only 40–60 % of exon 2 *KRAS* wild type tumors will respond to anti-EGFR treatment [19]. This effect potentiates the need for the introduction of additional molecular predictive markers.

In our study we tried to evaluate the prognostic importance *of KRAS*, *BRAF* and *PIK3CA* mutations which constitute important nodes of both RAS/RAF/MEK/MAPK and PI3K/AKT signaling networks in relation with clinical and laboratory parameters in a multivariate model.

In this cohort *KRAS* mutation was found in 40.3 % of cases, *BRAF* in 8.8 % and *PIK3CA* in 10.5 % in accordance with results of large population series [20, 21]. *KRAS* exon 2 mutations which are the most common *KRAS* alterations in colorectal cancer, were evaluated by HRM followed by sequencing and were detected predominately in older patients aged over 60 years. The most frequent mutation was p. G12D, followed by p.G12 V, in accordance with previous studies. *KRAS* mutation was not found to be importantly related with prediction of response to treatment as literature suggests [3, 18, 22] nor to prognosis, possibly due to the retrospective

Table 3 Mean Survival and BRAF mutation

	Mean (95 % C.I.)	Median (95 % C.I.)
BRAF-mutant	9.5 (3.8–15.2)	8.5 (3.1–13.9)
BRAF-normal	27.2 (20.4–33.9)	15.0 (5.4–24.6)
p-value = 0.01		

CI Confidence interval

Table 4 Cox regression on overall survival (parameters checked: weight loss, fever, metabd, transfusion, albumin, BRAF-mutant, PIK3CA-mutant, crp and ploidy, best model selected by stepwise forward method)

Variable	B	Standard error	Wald	*P*-value	Exp(B)	95,0 % C.I.for EXP(B)	
						Lower	Upper
PLOIDY	1.92	0.29	45.12	0.000	6.84	3.90	11.99

CI Confidence interval

method of the study and therefore the small number of patients who attended to anti-EGFR treatment.

BRAF mutation frequency (8.8 %) in the present study was within the range of previous results, with p.V600E mutation identified as well as other rare *BRAF* mutations [23]. In accordance with literature, *BRAF* and *KRAS* mutations in our cohort were mutually excluded in the analyzed samples [5]. *BRAF* mutant samples were predominately localized in proximal colon (60 % of cases) as it has been demonstrated previously [20]. The presence of *BRAF* mutation was strongly associated with poor prognosis with mean overall survival of 9.5 months [8, 22]. Although there are studies suggesting the negative predictive role of *BRAF* mutation for response to anti-EGFR agents when used as second or subsequent line therapy [5, 20], the present study did not identify any relation with response to treatment probably due to the small number of patients.

As far as *PIK3CA* gene mutations are concerned, hot spot exons 9 and 20 have been investigated in the present study. *PIK3CA* and *KRAS* mutations as well as *PIK3CA* and *BRAF* were not mutually exclusive as supported by previous studies [23]. Due to the small number of mutant cases in exon 9 of *PIK3CA* gene, we could not support any association with prognosis or treatment response. Interestingly, *PIK3CA* exon 20 mutations were associated with overall survival, although not related with treatment response. This is in line with recent data, supporting relation of *PIK3CA* exon 20 mutations with poorer PFS, prognosis and objective response to anti-EGFR treatment.

In the present study DNA ploidy was found to be strongly related to treatment response and overall survival as other studies had indicated [13]. More specifically, based on the results of the Cox regression model, one unit raise of ploidy increases six times the risk of death.

This analysis also confirms the prognostic significance of previously identified factors, such as CRP, albumin, anemia, fever and weight loss. CRP consist an acute-phase protein which is produced in liver as response to proinflammatory cytokines such as interleukine-6 (IL-6), IL-8 and tumor necrosis factor alpha (TNF-a). Previous studies indicated that CRP levels correlates with weight loss, anorexia-cachexia syndrome, disease extent and recurrence in many cancers, including CRC [24]. Serum albumin levels reflect the nutritional

status of patients and therefore their general condition having a documented predictive role in metastatic CRC [25]. The general status of patient is also negatively influenced by the presence of anemia, fever and weight loss which also deteriorate patient performance status and survival [25].

Conclusion

In conclusion, this study represents a comprehensive analysis of molecular, clinical and laboratory parameters with possible predictive and prognostic role in patients with stage IV colorectal cancer receiving palliative chemotherapy. Our results are in favor of the potential role of *BRAF* mutations as a prognostic biomarker for stage IV colon cancer patients. Our analysis demonstrated that common molecular tests, such as mutational status and DNA ploidy along with simple laboratory parameters such as CRP and clinical status can give us important information for the assessment of prediction and prognosis in CRC patients. Randomized controlled population studies in a perspective setting should be performed in order to accurately introduce a panel of markers which will allow us to better assess personalized medicine for colorectal cancer patients.

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Compliance with Ethical Standards

Conflict of Interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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