

Prospective Biomarker Study in Advanced RAS Wild-Type Colorectal Cancer: POSIBA Trial (GEMCAD 10-02)

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Colorectal cancer • Biomarkers • Cetuximab • Clinical score

ABSTRACT

Background. RAS testing is used to select patients with anti-epidermal growth factor receptor (EGFR) therapies sensitivity in metastatic colorectal cancer (mCRC). However, other biomarkers such as BRAF, PIK3CA/PTEN, and p-IGF-1R+/MMP7+ (double positive [DP] phenotype) have not been prospectively assessed to predict anti-EGFR resistance.

Materials and Methods. We designed a multicenter prospective trial (NCT01276379) to evaluate whether the biomarkers BRAF mutation, PIK3CA mutation/PTEN loss, and DP phenotype can improve the prediction for 12-months progression-free survival (PFS) over the use of clinical variables exclusively in patients with RAS wild-type (WT) mCRC treated with standard chemotherapy plus biweekly cetuximab as first-line therapy. The planned sample size was 170 RAS WT patients to detect a 20% difference in 12-month PFS based on the analysis of clinical and selected biomarkers ($\alpha = .05$, $\beta = .2$). The discriminatory capacity of

the biomarkers was evaluated using receiver operating characteristic curves.

Results. We included 181 RAS WT patients. The biomarker distribution was as follows: BRAF mutant, 20 patients (11%); PIK3CA mutated/PTEN loss, 98 patients (58%); DP, 23 patients (12.7%). The clinical variables in the clinical score were progression status >0, left-sided tumor, and resectable liver metastasis as the only metastatic site. The area under the curve (AUC) of the score containing the clinical variables was 0.67 (95% confidence interval [CI], 0.60–0.75). The AUC of the score with clinical variables and BRAF mutational status was 0.68 (0.61–0.75, $p = .37$). The AUC of the score with clinical variables and PIK3CA mutation/PTEN status was 0.69 (0.61–0.76, $p = .32$). The AUC of the score with clinical variables and DP phenotype was 0.66 (0.58–0.73, $p = .09$).

Conclusion. The addition of BRAF, PIK3CA/PTEN, and DP to a clinical score does not improve the discrimination of 12-month PFS. *The Oncologist* 2019;24:e1115–e1122

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Implications for Practice: This prospective biomarker design study has important clinical implications because many prospective clinical trials are designed with the hypothesis that BRAF mutation per se and MEK and PIK3CA downstream pathways are critical for colorectal tumor survival. The results lead to the question of whether these pathways should be considered as passengers instead of drivers.

INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cause of cancer-related death in Western countries, accounting for 10% of all cancer incidence and mortality. RAS mutations are found in 50% of metastatic colorectal cancer (mCRC). BRAF mutation is found in 10% of patients with mCRC and confers poor prognosis [1]. Treatment with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (cetuximab) has shown efficacy in patients with KRAS wild-type (WT) mCRC in retrospective analyses [2, 3] and all-RAS WT both in retrospective analysis and in prospective randomized clinical trials (RCT) of first-line therapy (cetuximab and panitumumab plus chemotherapy over chemotherapy alone) [4, 5].

The evidence of other potential biomarkers to predict anti-EGFR intrinsic resistance, such as BRAF mutation, PIK3CA mutation, or PTEN loss of expression, needs further validation because available data come mainly from retrospective cohorts that constitute the lower level of evidence in biomarker studies [6–13]. Our group identified in a retrospective analysis that the coexpression of matrix metalloproteinase-7 (MMP7) and phosphorylated insulin growth factor receptor (p-IGF-R; double positive [DP] phenotype) is associated with worse prognosis in patients with WT KRAS mCRC treated with anti-EGFR [14]. BRAF, PIK3CA, and PTEN have been evaluated in RCTs, the gold standard to validate prognostic biomarkers [15], to predict the efficacy of panitumumab [16, 17] or cetuximab [17, 18], and the results were contradictory.

The objective of this prospective study was to assess whether the biomarkers BRAF, DP, and PIK3CA/PTEN improve the prediction of 12-month progression-free survival (PFS) over the use of only clinical variables in patients with mCRC RAS WT treated with standard chemotherapy, plus biweekly cetuximab, as first-line therapy

hemoglobin ≥ 9 g/dL) functions. Patients were ineligible if they were pregnant or previous recipients of anti-EGFR or chemotherapy (with the exception of adjuvant therapy more than 6 months prior) or if they had undergone surgery for metastatic disease before study entry. Patients were also excluded if they had clinically relevant coronary heart disease or myocardial infarction within the past 12 months; were at risk of uncontrolled arrhythmia, known or suspected brain metastases, or acute or subacute intestinal obstruction; or had a history of chronic inflammatory disease or chronic diarrhea, a pre-existing dihydropyrimidine dehydrogenase deficiency, a pre-existing glucuronidation defect (Gilbert syndrome), or a history of secondary malignancy within the past 5 years, except for basal cancer or carcinoma in situ of the cervix if treated with curative intent.

Patients received biweekly cetuximab (500 mg/m² every 2 weeks) with either modified mFOLFOX6 or FOLFIRI. mFOLFOX6 consists of oxaliplatin 85 mg/m², leucovorin 400mg/m², fluorouracil 400mg/m² in bolus, and fluorouracil 2,400 mg/m² in 46-hour continuous infusion. FOLFIRI consists of irinotecan 180 mg/m², leucovorin 400mg/m², fluorouracil 400mg/m² in bolus, and fluorouracil 2,400 mg/m² in 46-hour continuous infusion. Chemotherapy choice was at the discretion of the treating physician. Treatment was continued until disease progression, unacceptable toxicity, patient refusal, physician decision, or 12 cycles of mFOLFOX6 or FOLFIRI (cetuximab could be continued beyond 12 cycles in the absence of progression). Dose modifications of chemotherapy and cetuximab were permitted and specified in the protocol.

After an initial baseline assessment within 28 days of the start of study treatment, investigators assessed tumor response by computed tomography scan and/or magnetic resonance imaging every 12 \pm 2 weeks of treatment and at the end of treatment. Subsequent follow-up was done every 3 months. Adverse events were recorded from enrollment to the end of the final study visit and were classified and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. The study was approved by institutional review boards of participating centers.

RAS/BRAF and PIK3CA Mutational Analysis

Mutational analysis of genomic DNA of KRAS (exon 2) was performed by direct sequencing at each center. Extended RAS mutational analysis (including KRAS/NRAS exons 2, 3, and 4) started on October 2015 in the POSIBA trial after protocol amendments. Pyrosequencing of KRAS and NRAS codons 12, 13, 59, 61, 117, and 146 was performed using Therascreen KRAS and NRAS Pyro Kits (Qiagen, Hilden, Germany), according to manufacturer's instructions. DNA

MATERIALS AND METHODS

Patients, Treatment, and Follow-Up

The study was conceived as a prospective biomarker cohort (ClinicalTrials.gov identifier: NCT01276379). Patients were eligible if they were ≥ 18 years, with histologically confirmed WT RAS metastatic adenocarcinoma of the colon or rectum, presence of at least one radiologically measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, an Eastern Cooperative Oncology Group Performance Status (PS) of 0–1, an estimated life expectancy greater than 3 months, and adequate hepatic (serum bilirubin $\leq 1.5 \times$ upper limit of normal [ULN], alanine aminotransferase, and aspartate amino transferase $\leq 2.5 \times$ ULN or $\leq 5 \times$ ULN in the presence of liver metastases), renal (serum creatinine $\leq 1.5 \times$ ULN), and bone marrow (neutrophils $\geq 1.5 \times 10^9$ cells per L, platelets $\geq 100 \times 10^9$ per L, and

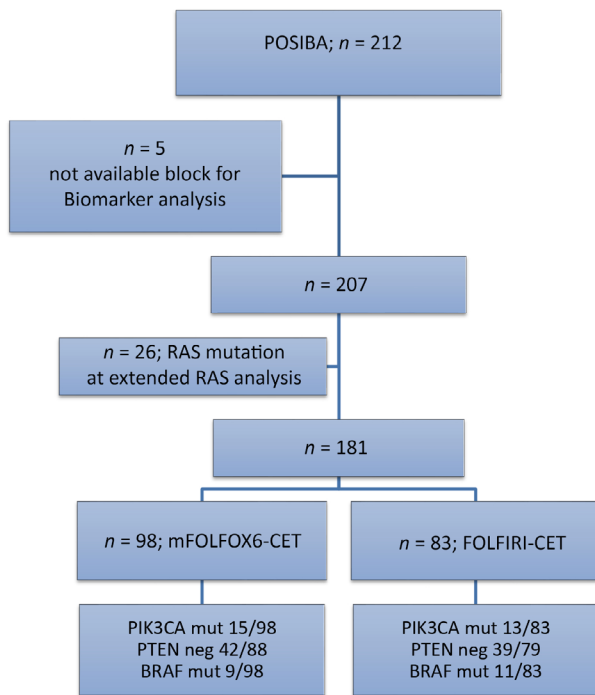


Figure 1. Patient flowchart.

from two 5- μ m tissue sections was isolated using QIAamp DNA FFPE Tissue Kit (Qiagen). Then, 10 ng/ μ l DNA templates were amplified in a SimpliAmp thermal cycler (Applied Biosystems, Foster City, CA) targeting codons of interest. Amplicons were then immobilized on Streptavidin Sepharose High Performance beads (GE Healthcare, Little Chalfont, U.K.). The thus obtained single-stranded DNA was prepared with the corresponding sequencing primers to DNA annealing. Further pyrosequencing run and analysis were carried out on the Pyromark Q24 system along with the software version 2.0 KRAS and NRAS plug-in reports (Qiagen). Mutation thresholds were identified in relation to the manufacturer's limit of detection for the specific mutations. Both wild-type control DNA and nontemplate control were included in every run for comparison and background levels screening.

The BRAF V600E mutation (exon 15) was genotyped by allelic discrimination in genomic DNA using TaqMan technology (Applied Biosystems, Foster City, CA).

Activating mutations in PIK3CA tend to cluster in hot-spots, 80% of which are accounted for by oncogenic substitution in exon 20 (H1047R) and exon 9 (E542K and E545K), which encode portions of the helical and kinase domains. Known PIK3CA mutations in these exons were evaluated using the Sequenom MALDI TOF MassARRAY system.

PTEN, p-IGF-1R, and MMP7 Immunohistochemistry Analysis

We used H&E staining to evaluate the presence and classification of the tumor specimens. Consecutive 3- μ m-thick sections were used for immunohistochemistry (IHC). Paraffin removal and heat incubation in citrate (pH = 6.0) were carried out for antigen retrieval. The primary p-IGF-1R antibody (anti-pY1316, provided by Dr. Rubini) was used at 1:100 dilution [19]. The most common pattern of expression was dot-like perinuclear

or Golgi pattern. Membrane location was unfrequent. Nuclear pattern was seen in 15%–20% of cases, and the percentage of positive nuclei was used to score the nuclear pattern of staining. MMP-7 (Monoclonal Mouse IgG_{2B} Clone no. 111433; R&D Systems, Minneapolis, MN) was used at 1:1,500 dilution, and the pattern of staining was cytoplasmatic. Detection was performed using the Dako EnVision K4011 (Agilent, Santa Clara, CA). IHC evaluation was conducted after patient inclusion. Coexpression of pIGF-1R and MMP-7 (termed DP phenotype) was defined as moderate = 2 or strong = 3 intensity and >70% expression for both MMP-7 and pIGF-1R. Normal colon mucosa expression was low for both antibodies. PTEN protein expression on tissue sections was evaluated using the mouse monoclonal anti-PTEN clone 6H2.1 (Dako; Agilent, Copenhagen, Denmark) [20], and antigen-antibody reaction was detected using Flex+ (Dako; Agilent) and developed with DAB. Quantification of PTEN expression was executed by a senior pathologist (F.R.). pIGF-1R, MMP-7, and PTEN immunostains were scored semiquantitatively using the previously described immunoreactive score (IRS) [20]: IRS = staining intensity (SI) \times percentage of positive cells (PP). SI was defined as 0 = absence of staining; 1 = weak; 2 = moderate; and 3 = strong. PP was defined as 0 < 1%; 1 = 1%–10%; 2 = 11%–50%; 3 = 51%–80%; and 4 > 80% positive cells. Ten visual fields from different areas of each tumor were evaluated for IRS. Slides without primary antibody were included as negative controls and normal epithelium of stromal cells known to express PTEN, MMP7, and p-IGF-1R were used as positive controls. IRS of 0 corresponds to PTEN-lost tumors with nondetectable level of PTEN staining, and IRS of 12 represents full PTEN expression in normal individuals. PTEN loss was defined as IRS of 3 or less.

Statistical Analysis

To quantify whether the biomarkers BRAF, DP, and PIK3CA-PTEN do improve the prediction of 12-month PFS over the use of only clinical variables in the study population, we compared the area under the curve (AUC) of a receiver operating characteristic (ROC) curve using scores composed by clinical variables with the AUC of scores with the clinical variables plus each of the biomarkers. The clinical variables were age, sex, performance status, primary tumor side (transverse and ascending colon were considered right side), number of organs affected, type of organ, surgery of primary tumor, liver-limited resectable disease (less than three nodes <5 cm), hemoglobin, leucocytes, platelets, alkaline phosphatase, and lactate dehydrogenase levels. To build the clinical score, we (a) ran univariate analyses for each variable and PFS using Cox regression models. (b) Those variables with a *p* value \leq .15 in the univariate analyses were included in a multivariate Cox model. (c) Their coefficients were added to construct the score [21].

The biomarker scores were constructed by adding each biomarker to the model of the step 2 and repeating step 3. The discriminatory capacity of each score was quantified by the AUC of a ROC curve.

Primary endpoint was progression-free survival at 12 months. PFS was defined as the time from inclusion to documented progression, death (both considered events), last follow-up, or the administrative end of follow-up (both

Table 1. Baseline characteristics by biomarker status

Characteristics	BRAF WT (n = 161) n (%)	BRAF mutant (n = 20) n (%)	p value ^a	PI3K WT and PTEN >3 (n = 69) n (%)	PI3K mutant or PTEN ≤3 (n = 98) n (%)	p value ^a	Non-DP (n = 158) n (%)	DP (n = 23) n (%)	p value ^a
Female	46 (28.6)	7 (35.0)	.60	19 (27.5)	27 (27.6)	.99	46 (29.1)	7 (30.4)	.99
Mean age (SD)	62.0 (10.5)	67 (7.4)	.031	62.5 (9.7)	63.3 (10.7)	.63	62.0 (10.5)	67 (7.4)	.031
Primary location			.0004			.50			.98
Ascending colon	23 (14.3)	9 (45.0)		11 (16.9)	21 (21.4)		28 (17.7)	4 (17.4)	
Transverse colon	12 (7.5)	2 (10.0)		5 (7.3)	7 (7.1)		13 (8.2)	1 (4.4)	
Descending colon	10 (6.2)	4 (20.0)		6 (8.7)	7 (7.1)		12 (7.6)	2 (8.7)	
Sigma	73 (45.3)	3 (15.0)		26 (37.7)	44 (44.9)		65 (41.1)	11 (47.8)	
Rectum	43 (26.7)	2 (10.0)		21 (30.4)	19 (19.4)		40 (25.3)	5 (21.7)	
Stage			.48			.056			.77
I	1 (0.6)	0		0	1 (1.0)		1 (0.6)	0	
II	13 (8.1)	0		1 (1.5)	10 (10.2)		12 (7.6)	1 (4.4)	
III	30 (18.6)	5 (25.0)		13 (18.8)	22 (22.5)		32 (20.3)	3 (13.0)	
IV	117 (72.7)	15 (75.0)		55 (79.7)	65 (66.3)		113 (71.5)	19 (82.6)	
Surgery of the primary tumor	91 (56.5)	10 (50.0)	.64	29 (42.0)	69 (70.4)	.0004	89 (56.3)	12 (52.2)	.82
Performance status			.0046			.87			.0082
0	112 (69.6)	7 (35.1)		45 (65.2)	66 (67.4)		110 (69.6)	9 (39.1)	
1	49 (30.4)	13 (65.0)		24 (34.8)	32 (32.7)		48 (30.4)	14 (60.9)	
2	0	0		0	0		0	0	
Number of metastatic organs			.30			.53			.30
0	0	0		0	0		0	0	
1	87 (54.0)	7 (35.0)		31 (44.9)	52 (53.1)		79 (50.0)	15 (65.2)	
2	58 (36.0)	11 (55.0)		29 (42.0)	37 (37.8)		61 (38.6)	8 (34.8)	
3	15 (9.3)	2 (10.0)		8 (11.6)	9 (9.2)		17 (10.8)	0	
4+	1 (0.6)	0		1 (1.5)	0		1 (0.6)	0	
Liver metastasis			.043			.89			.87
0	31 (19.3)	9 (45.0)		14 (20.3)	23 (23.5)		35 (22.2)	5 (21.7)	
Fewer than three or ≤5 cm	30 (18.6)	3 (15.0)		13 (18.8)	19 (19.4)		28 (17.7)	5 (2.8)	
More than three or >5 cm	100 (62.1)	8 (40.0)		42 (60.9)	56 (57.1)		95 (60.1)	13 (56.5)	
Node metastasis ^b	45 (28.0)	12 (60.0)	.0087	19 (27.5)	33 (33.7)	.50	50 (31.7)	7 (30.4)	.99
Lung metastasis ^b	47 (29.4)	3 (15.0)	.29	24 (34.8)	26 (26.8)	.31	48 (30.4)	2 (8.7)	.043
Peritoneal metastasis ^b	18 (11.3)	9 (45.0)	.0006	13 (18.8)	14 (14.4)	.52	23 (14.6)	4 (17.4)	.75
Patients with only resectable liver mets ^c	12 (7.5)	1 (5.0)	.99	5 (7.3)	7 (7.1)	.99	10 (6.3)	3 (13.0)	.22
Chemotherapy			.48			.76			.18
FOLFOX + cetuximab	89 (55.3)	9 (45.0)		37 (53.6)	51 (52.0)		89 (56.3)	9 (39.1)	
FOLFIRI + cetuximab	72 (44.7)	11 (55.0)		32 (46.4)	47 (48.0)		69 (43.7)	14 (60.9)	
Mean leucocytes (SD)	8.4 (3.4)	7.9 (2.9)	.49	8.3 (3.7)	8.4 (3.3)	.93	8.3 (3.3)	8.9 (3.7)	.39
Mean hemoglobin (SD)	13.6 (9.2)	12.6 (1.9)	.22	12.9 (1.8)	12.9 (1.8)	.87	13.8 (9.2)	11.9 (1.6)	.023
Mean platelets (SD)	284.4 (107.6)	283.2 (119.9)	.96	303.1 (106.4)	270.8 (108.5)	.058	282.2 (103.7)	298.3 (140.4)	.60
Mean ALP (SD)	149.7 (124.1)	170.2 (168.6)	.60	141.7 (117.7)	149.7 (117.7)	.68	148.2 (121.6)	178.9 (176.7)	.44
LDH >450	47 (29.2)	4 (20.0)	.60	23 (33.3)	24 (24.5)	.23	45 (28.5)	6 (26.1)	.99
Mean CEA (SD)	339.6 (983.5)	200.5 (398.4)	.25	269.4 (717.4)	242.9 (665.1)	.81	267.0 (731.8)	707.7 (1,771.7)	.26

^aFisher's exact test.^bMissing in one patient.^cMeaning that the patient does not have metastasis in any other location, and those in the liver are less than three in number and ≤5 cm in size. Abbreviations: ALP, alkaline phosphatase; CEA, carcinoembryonic antigen; DP, double positive; LDH, lactate dehydrogenase level.

considered censoring reasons), whichever happened first. Sample size was computed to detect an increase of 20% in 12-month PFS (i.e., from 40% in the group with worse prognostic biomarkers to 60% in those with better prognostic biomarkers) under the following assumptions: two-sided α error

of 5%, β error of 20%, percentage of patients classified in the worse prognostic biomarker group of 40%, a recruitment of 8 patients per month, a follow-up of 28 months, and 10% losses. Under these assumptions, 170 patients were required. Kaplan-Meier curves were used to plot PFS by score.

Table 2. Univariate and multivariate models for the scores

Variables	Univariate, HR (95% CI)	<i>p</i> value	Multivariate clinical, HR (95% CI)	Clinical score weight	Multivariate BRAF, HR (95% CI)	BRAF score weight	Multivariate PI3K/PTEN, HR (95% CI)	PI3K/PTEN score weight	Multivariate DP, HR (95% CI)	DP score weight
Female	0.97 (0.69–1.36)	.87								
PS >0	2.02 (1.46–2.79)	<.0001	1.80 (1.29–2.51)	0.6	1.73 (1.24–2.42)	0.5	1.72 (1.22–2.41)	0.5	1.78 (1.27–2.49)	0.6
Age >65 y	1.18 (0.87–1.61)	.30								
FOLFIRI + cetuximab ^a	1.62 (1.19–2.22)	.0024	1.48 (1.08–2.03)	NA	1.44 (1.05–1.98)	NA	1.43 (1.04–1.96)	NA	1.47 (1.07–2.02)	NA
Surgery primary	1.02 (0.74–1.39)	.92								
Left sided	0.55 (0.39–0.78)	.0008	0.59 (0.41–0.84)	−0.5	0.59 (0.42–0.84)	−0.5	0.58 (0.41–0.83)	−0.5	0.58 (0.41–0.83)	−0.5
Log CEA	1.03 (0.96–1.10)	.40								
LDH >450	1.05 (0.75–1.47)	.74								
Only resectable liver mets	0.64 (0.34–1.18)	.15	0.70 (0.37–1.32)	−0.4	0.71 (0.38–1.32)	−0.3	0.67 (0.34–1.31)	−0.4	0.70 (0.37–1.31)	−0.4
Mutant BRAF	2.33 (1.44–3.79)	.0006			2.10 (1.23–3.29)	0.7				
PI3K mutant or PTEN ≤3	0.83 (0.61–1.13)	.24					0.81 (0.59–1.11)	−0.2		
DP	1.24 (0.79–1.94)	.36							1.10 (0.68–1.76)	0.1

^aModels are adjusted for this variable, but it is not used to build the scores because it is not an intrinsic characteristic of the patients.

Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; DP, double positive; HR, hazard ratio; LDH, lactate dehydrogenase level; NA, not applicable; PS, performance status.

RESULTS

We screened 212 patients from 28 Spanish Centers between July 2011 and May 2015, 181 of whom were eligible. PI3KCA mutation/PTEN loss could be evaluated in 167 patients, and BRAF mutational status and DP expression could be evaluated in all of them. In the 212 patients included in the study, 115 patients who started treatment with mFOLFOX6 received a median number of 16.1 cycles, and the 97 patients who started treatment with FOLFIRI received a median number of 12.6 cycles. A total of 71 of the 115 (61.7%) patients treated with mFOLFOX6 received 12 or more cycles compared with 52 of 97 patients (53.6%) with FOLFIRI. See Figure 1.

Patients were followed for a median of 28.6 months (95% confidence interval [CI], 22.9–34.3), and 163 (90%) progressed during the follow-up, 103 (57%) during the first year. Baseline characteristics by biomarker are shown in Table 1. Patients with mutant BRAF tumors ($n = 20$, 11%) were older and showed a higher incidence of right-sided tumors, worse PS, and lymph node and peritoneal metastases. Patients with PI3KCA mutation/PTEN loss ($n = 98$, 58%) showed no relevant differences in baseline characteristics compared with those without it. Patients with DP phenotype ($n = 23$, 12.7%) were older and had worse PS.

Median PFS was 11.4 months in patients with WT BRAF tumors and 5.9 months in patients with mutant BRAF tumors ($p = .004$). There were no differences on prognosis according to PI3KCA mutations ($p = .43$) and PTEN loss ($p = .25$), analyzed separately. PI3KCA/PTEN pathway and DP phenotype did not discriminate PFR ($p = \text{NS}$). Baseline clinical variables with good prognosis in a multivariable model were PS = 0, left-sided tumor, and resectable liver metastases (i.e., liver-only metastases [less than three nodules <5cm]).

The clinical variables with p values $\leq .15$, thus chosen to build the clinical score, were PS >0, left-sided tumor, and resectable liver metastasis as the only metastatic site (Table 2). Chemotherapy regimen was significant in the univariate analysis. It was excluded from the scores because it is not an intrinsic characteristic of the patient, but all the score models were adjusted for it. Table 2 contains the multivariate models built with each of the biomarkers and the clinical

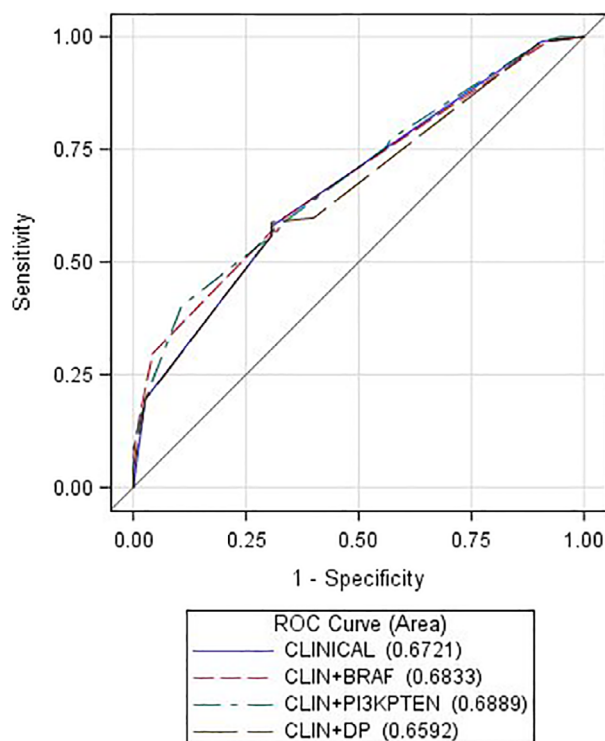


Figure 2. Area under the curve score with clinical and biomarker variables. ROC curves for comparisons.

Abbreviations: DP, double positive; ROC, receiver operating characteristic.

variables identified in the multivariate analysis, as well as the weight assigned to build each score. The AUC of the score containing the clinical variables was 0.67 (95% CI, 0.60–0.75). The AUC of the score with clinical variables and BRAF mutational status was 0.68 (0.61–0.75, $p = .37$). The AUC of the score with clinical variables and PI3KCA mutation/PTEN status was 0.69 (0.61–0.76, $p = .32$). The AUC of the score with clinical variables and DP phenotype was 0.66 (0.58–0.73, $p = .09$; Fig. 2). PFS under each score classification (dichotomized by the most discriminative cut-point) was

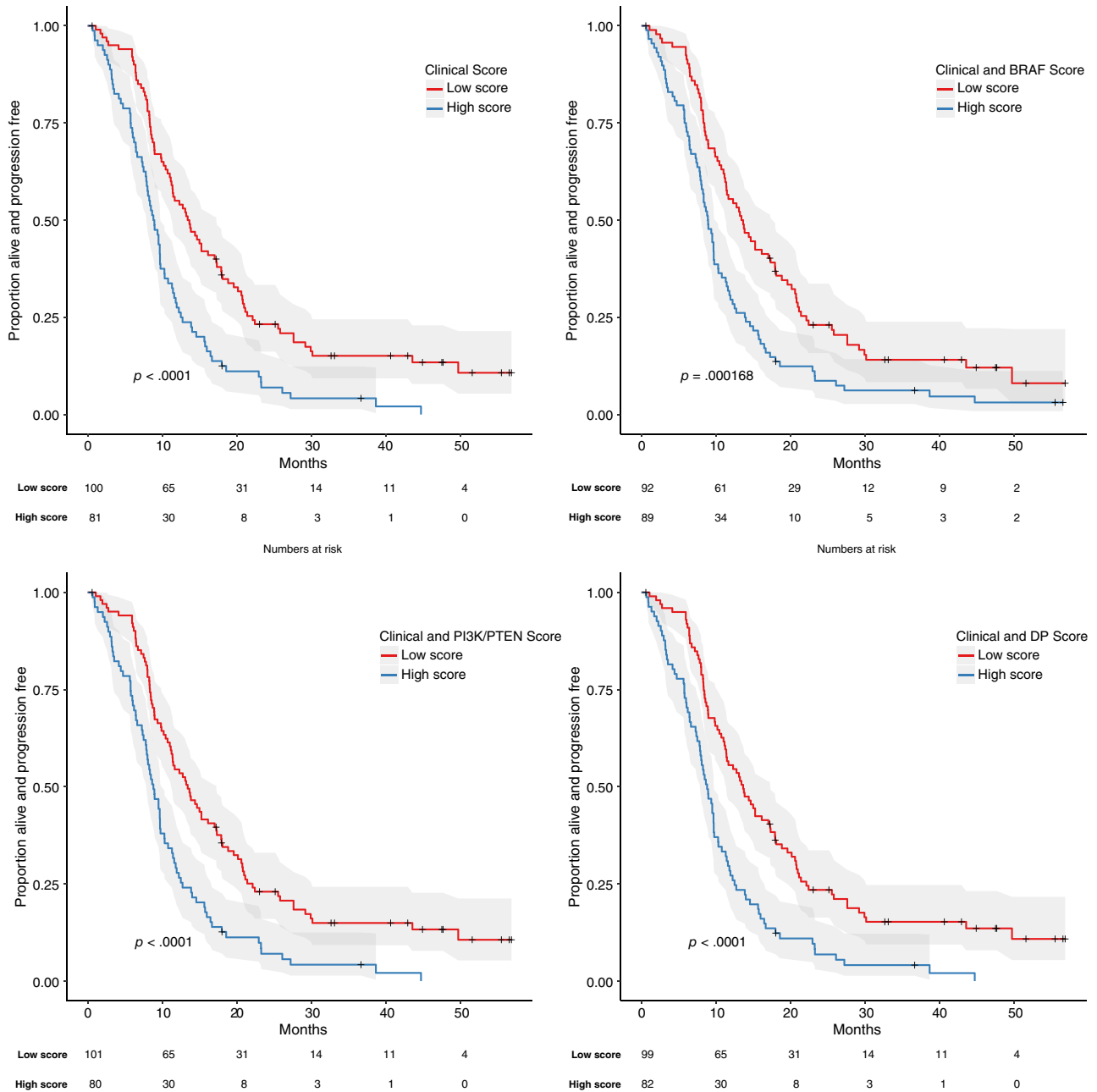


Figure 3. Progression-free survival under each score classification (dichotomized by the most discriminative cut-point) was equivalent under the four scores. Abbreviation: DP, double positive.

equivalent under the four scores (Fig. 3). The combination of the three biomarkers and the clinical variables did not improve the AUC (0.69; 95% CI, 0.62–0.77).

DISCUSSION

We present data from a prospective biomarker study to assess the prognostic value of selected biomarkers in patients with RAS WT mCRC treated in first line with a combination of standard chemotherapy plus biweekly cetuximab. We could not find proof that adding biomarker determination (BRAF, PIK3CA/PTEN, DP) to the use of only clinical variables improves patient classification into those who will progress

by the first year after diagnosis of metastatic disease and those who will not.

Several studies that have analyzed BRAF and PIK3CA/PTEN are either retrospective cohorts or retrospective biomarker analysis on RCTs (summarized in Table 3). To our knowledge, our study is the first to prospectively analyze the prognostic value of these biomarkers using an a priori-defined hypothesis and an upfront sample size.

In a retrospective analysis of refractory patients, our group found a negative prognostic value of DP phenotype for patients treated with irinotecan and cetuximab; those patients represented 25% of the sample in refractory patients, whereas in first line, only 12.7% of the patients presented DP

Table 3. Biomarker studies of anti-EGFR efficacy in metastatic colorectal cancer

Author	Biomarker	Biomarker design	Type of clinical trial design	Result	Type of BM design
Lievre, 2006 [2]	KRAS	Retrospective	Retrospective cohort	positive	NA
Benvenuti, 2007 [3]	KRAS	Retrospective	Retrospective cohort	positive	NA
Douillard, 2014 [4]	RAS	Retrospective	Prospective RCT	positive	NA
Van Cutsem, 2015 [5]	RAS	Retrospective	Prospective RCT	positive	NA
Di Nicolantonio, 2008 [6]	BRAF	Retrospective	Retrospective cohort	positive	NA
Frattini, 2007 [7]	PTEN	Retrospective	Retrospective cohort	positive	NA
Sartore-Bianchi, 2009 [8]	PIK3CA	Retrospective	Retrospective cohort	positive	NA
Laurent-Puig, 2009 [9]	PTEN, BRAF	Retrospective	Retrospective cohort	positive	NA
Perrone, 2009 [10]	PIK3CA, PTEN	Retrospective	Retrospective cohort	positive	NA
Prenen, 2009 [11]	PIK3CA	Retrospective	Retrospective cohort	negative	NA
De Roock, 2011 [12]	RAS, BRAF, PIK3CA	Retrospective	Retrospective cohort	positive	NA
Tian, 2012 [13]	KRAS, BRAF, PIK3CA	Retrospective	Retrospective cohort	positive	NA
Horndler, 2011 [14]	DP ^a	Retrospective	Retrospective cohort	positive	NA
Seymour, 2013 [16]	BRAF, PIK3CA	Retrospective	Prospective RCT	negative	NA
Peeters, 2013 [17]	BRAF, PIK3CA, PTEN ^b	Retrospective	Prospective RCT	negative	NA
Karapetis, 2014 [18]	BRAF, PIK3CA, PTEN	Retrospective	Prospective RCT	negative	NA
FOCUS4, 2017[22]	Quadruple WT ^c	Prospective	RCT	negative	Umbrella
POSIBA, 2018 [present study]	BRAF, PIK3CA, PTEN, DP	Prospective	Non RCT	negative	ROC curve score

^aDP; coexpression of metalloproteinase-7 expression and phosphorylated insulin growth factor receptor.

^bPTEN negative if mutated.

^cQuadruple WT (no mutation in RAS, BRAF and PIK3CA genes).

Abbreviations: BM, biomarker; DP, double positive; RCT, randomized clinical trial; ROC, receiver operating characteristic; WT, wild-type.

phenotype. Nevertheless, the differences between frequencies could be explained as a result of the induction of a mesenchymal phenotype in pretreated patients [22]. Activating mutations in PIK3CA and PTEN loss of expression are in the range (29%–72%) of previous published studies [6–13].

Neither DP nor PIK3CA/PTEN showed independent association with PFS in our study. Our results confirm the prognostic value of BRAF mutation in this setting [1]. However, despite the significance in multivariate analysis, it did not improve the discrimination of the clinical score, probably because of the small number of BRAF-mutant patients (11%) and collinearity with other clinical variables. Biomarkers that could actually improve the classification of these patients may be mutations other than BRAF and PIK3CA [23] or non-mutational driver pathways related to anti-EGFR resistance, located mainly in primary right-side tumors [24].

We believe that our approach has several strengths compared with enriched randomized designs [25]. First, we do not preassume that the proposed biomarkers really are drivers for targeted agent prediction and therefore allow for better evaluation of multiple biomarkers. Second, a reduced number of patients is needed to avoid patient loss because of stratification procedures. Third, all clinical variables that potentially could influence PFS are included, minimizing bias and enforcing the truly independent value of the biomarkers. The only prospective biomarker study in mCRC that uses an enriched design has recently evaluated the efficacy of AZD8931 (an EGFR, HER2, and HER3 inhibitor) in patients with quadruple WT mCRC (FOCUS4-D) [26]. As an example, in this study, only 32 out of 132 patients (24% of potentially eligible quadruple WT patients)

were finally randomized. Our study highlights the importance of clinical variables such as Eastern Cooperative Oncology Group PS, liver resectable disease, and sidedness [27]. Despite these strengths, we recognize several limitations: radiological assessment was not centralized, patients were treated with two different chemotherapy schedules, and PTEN score and DP phenotype interpretation can be subjective.

CONCLUSION

Our study has important clinical implications because many prospective clinical trials are designed with the hypothesis that BRAF mutation, in and of itself, and MEK and PIK3CA downstream pathways are critical for colorectal tumor survival. Nevertheless, the use of a triple blockage strategy (BRAF/MEK/EGFR or BRAF/PIK3CA/EGFR) in BRAF-mutant patients showed a rather modest activity (20% response rate and 4-months PFS) [28, 29]. These results support the notion that additional pathways of resistance should be evaluated in BRAF-mutant patients.

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DISCLOSURES

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