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ORIGINAL RESEARCH

Influence of *BRAF* and *PIK3CA* mutations on the efficacy of FOLFIRI plus bevacizumab or cetuximab as first-line therapy in patients with *RAS* wild-type metastatic colorectal carcinoma and <3 baseline circulating tumour cells: the randomised phase II VISNÚ-2 study

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Background: We explored the influence of *BRAF* and *PIK3CA* mutational status on the efficacy of bevacizumab or cetuximab plus 5-fluorouracil/leucovorin and irinotecan (FOLFIRI) as first-line therapy in patients with *RAS* wild-type metastatic colorectal cancer (mCRC).

Patients and methods: VISNÚ-2 was a multicentre, randomised, phase II study. Patients with *RAS* wild-type mCRC and <3 circulating tumour cells/7.5 ml blood were stratified by *BRAF*/*PIK3CA* status (wild-type versus mutated) and number of affected organs (1 versus >1), and allocated to bevacizumab (5 mg/kg every 2 weeks) or cetuximab (400 mg/m^2 then 250 mg/m^2 weekly) plus FOLFIRI [irinotecan 180 mg/m^2, leucovorin 400 mg/m^2, 5-fluorouracil 400 mg/m^2 (bolus) then 2400 mg/m^2 (46-h continuous infusion) every 2 weeks]. The primary endpoint was progression-free survival (PFS). All analyses were exploratory.

Results: Two hundred and forty patients with *BRAF*/*PIK3CA* wild-type ($n = 196$) or *BRAF*- and/or *PIK3CA*-mutated tumours ($n = 44$) were enrolled. Median PFS was 12.7 and 8.8 months in patients with *BRAF*/*PIK3CA* wild-type and *BRAF*/*PIK3CA*-mutated tumours, respectively [hazard ratio (HR) = 1.22; 95% confidence interval (CI) 0.80-1.85; $P = 0.3602$]. In the *BRAF*- and/or *PIK3CA*-mutated cohort, median PFS was 2.8, 8.8 and 15.0 months in patients with *BRAF*/*PI3KCA*-mutated ($n = 8$), *BRAF*-mutated/*PI3KCA* wild-type ($n = 16$) and *BRAF* wild-type/*PI3KCA*-mutated ($n = 20$) tumours, respectively ($P = 0.0002$). PFS was similar with bevacizumab plus FOLFIRI versus cetuximab plus FOLFIRI in *BRAF*/*PIK3CA* wild-type (HR = 0.99; 95% CI 0.67-1.45; $P = 0.9486$) and *BRAF*/*PIK3CA*-mutated tumours (HR = 1.11; 95% CI 0.53-2.35; $P = 0.7820$). The most common grade 3/4 treatment-related adverse events were neutropenia, diarrhoea and asthenia in both treatment groups.

Conclusions: *BRAF*/*PIK3CA* status influences outcomes in patients with *RAS* wild-type mCRC but does not appear to assist with the selection of first-line targeted therapy.

Key words: BRAF, colorectal cancer, PIK3CA, RAS, targeted therapy

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INTRODUCTION

Over the past two decades, the survival of patients with metastatic colorectal cancer (mCRC) has improved through the use of combination chemotherapy (fluoropyrimidines, irinotecan and oxaliplatin) and monoclonal antibodies targeting vascular endothelial growth factor (VEGF) (bevacizumab) and the epidermal growth factor receptor (EGFR) (cetuximab, panitumumab). The discovery that activating *RAS* mutations (*KRAS* exons 2, 3 and 4 and *NRAS* exons 2, 3 and 4) were predictive of a lack of response to EGFR inhibitors[1,2] provided the first molecular markers for this disease. Although *RAS* testing has helped with the selection of patients likely to respond to EGFR-directed monoclonal antibodies, testing of other tumour-specific genetic markers encoding for molecular transducers of EGFR activation, such as *BRAF* and *PIK3CA* mutations, may further inform clinical decision-making.

The presence of circulating tumour cells (CTCs) in the peripheral blood is a strong independent prognostic marker for progression-free survival (PFS) and overall survival in patients with mCRC.[3-5] A threshold value of 3 CTCs/7.5 ml blood has been shown to distinguish between patients with favourable and unfavourable risk profiles,[3-5] and may help to guide treatment choices in patients with mCRC independently of *RAS* status.

In 2012, the Spanish Cooperative Group for the Treatment of Digestive Tumours (TTD) designed the VISNÚ project in which patients with mCRC were enrolled into one of two studies based on their CTC count. We report the findings from VISNÚ-2, a randomised phase II study that explored the influence of *BRAF* and *PIK3CA* mutational status on the efficacy of 5-fluorouracil/leucovorin and irinotecan (FOLFIRI) plus bevacizumab or FOLFIRI plus cetuximab as first-line therapy in patients with *RAS* wild-type mCRC and <3 CTCs/7.5 ml blood at baseline. VISNÚ-1, which investigated bevacizumab-based regimens as first-line therapy in patients with ≥ 3 CTCs/7.5 ml blood irrespective of *RAS* or *BRAF* status, will be reported separately.[6]

METHODS

Study design

VISNÚ-2 was an exploratory, multicentre, open-label, randomised phase II study conducted at 43 centres in Spain involving chemotherapy-naive patients with *RAS* wild-type mCRC and <3 CTCs/7.5 ml blood. Randomisation was carried out centrally at the study data centre using permuted blocks, and the treatment group made available to the investigator (either electronically or by facsimile). Patients were stratified according to *BRAF* and *PIK3CA* status [wild-type (defined as *BRAF* and *PIK3CA* wild-type) versus mutated (defined as *BRAF*-mutated, *PIK3CA*-mutated or both)] and number of affected organs (1 versus >1), and then randomised (1 : 1 ratio) to receive FOLFIRI plus bevacizumab or FOLFIRI plus cetuximab (Figure 1).

The study was conducted in accordance with Good Clinical Practice guidelines, the Royal Decree 223/2004 (Spain), and the latest version of the Declaration of Helsinki. The study protocol was approved by local ethics committees at each participating centre. All patients provided written informed consent before study entry. The trial is registered on ClinicalTrials.gov (NCT01640444).

Patients

Adults aged 18-70 years with histologically confirmed metastatic colorectal adenocarcinoma without *KRAS* exon 2 and 3 mutations and <3 CTCs/7.5 ml peripheral blood were eligible. Owing to evidence emerging on the negative predictive value of additional *RAS* mutations during the study, the protocol was amended in March 2014 to further exclude patients with mutations in *KRAS* exon 4 and *NRAS* exons 2, 3 and 4. Previous chemotherapy for metastatic disease and prior treatment with bevacizumab or an EGFR inhibitor was not allowed. Adjuvant therapy for colorectal cancer must have been completed within 6 months before randomisation. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate bone marrow, liver and renal function.

Key exclusion criteria were uncontrolled hypertension, history of hypertensive encephalopathy or hypertensive crises, significant cardiovascular disease, major surgery, history of haemoptysis, bleeding diathesis or coagulopathy, need for anticoagulation therapy or thrombolytics, previous abdominal fistula or gastrointestinal perforation, serious non-healing wound, ulcer or bone fracture, intestinal occlusion or intestinal inflammatory disease, pulmonary fibrosis, acute lung disease or interstitial pneumonia, or proteinuria.

Treatment

Patients received bevacizumab 5 mg/kg as a 30- to 90-min intravenous (i.v.) infusion on day 1 every 2 weeks, or cetuximab 400 mg/m^2 as a 120-min i.v. infusion on day 1 (cycle 1 only) then 250 mg/m^2 as a 60-min infusion once weekly. Patients in both groups received FOLFIRI, which comprised irinotecan 180 mg/m^2 as a 30- to 90-min i.v. infusion on day 1, followed by leucovorin 400 mg/m^2 as a 2-h i.v. infusion, then 5-fluorouracil 400 mg/m^2 as an i.v. bolus then 2400 mg/m^2 as a 46-h continuous infusion every 2 weeks (one cycle). Treatment was continued until disease progression, toxicity or withdrawal of consent. For patients with unresectable metastases that became resectable during the study, treatment was suspended and surgery was scheduled. Protocol-specified treatment modifications were recommended when treatment-emergent toxicities occurred.

Assessments

During screening, CTCs were determined by central testing (San Carlos Hospital, Madrid, Spain). Peripheral blood (10 ml) was collected in CellSave® Preservative Tubes (Veridex LLC, Raritan, NJ), and CTCs were enumerated using the

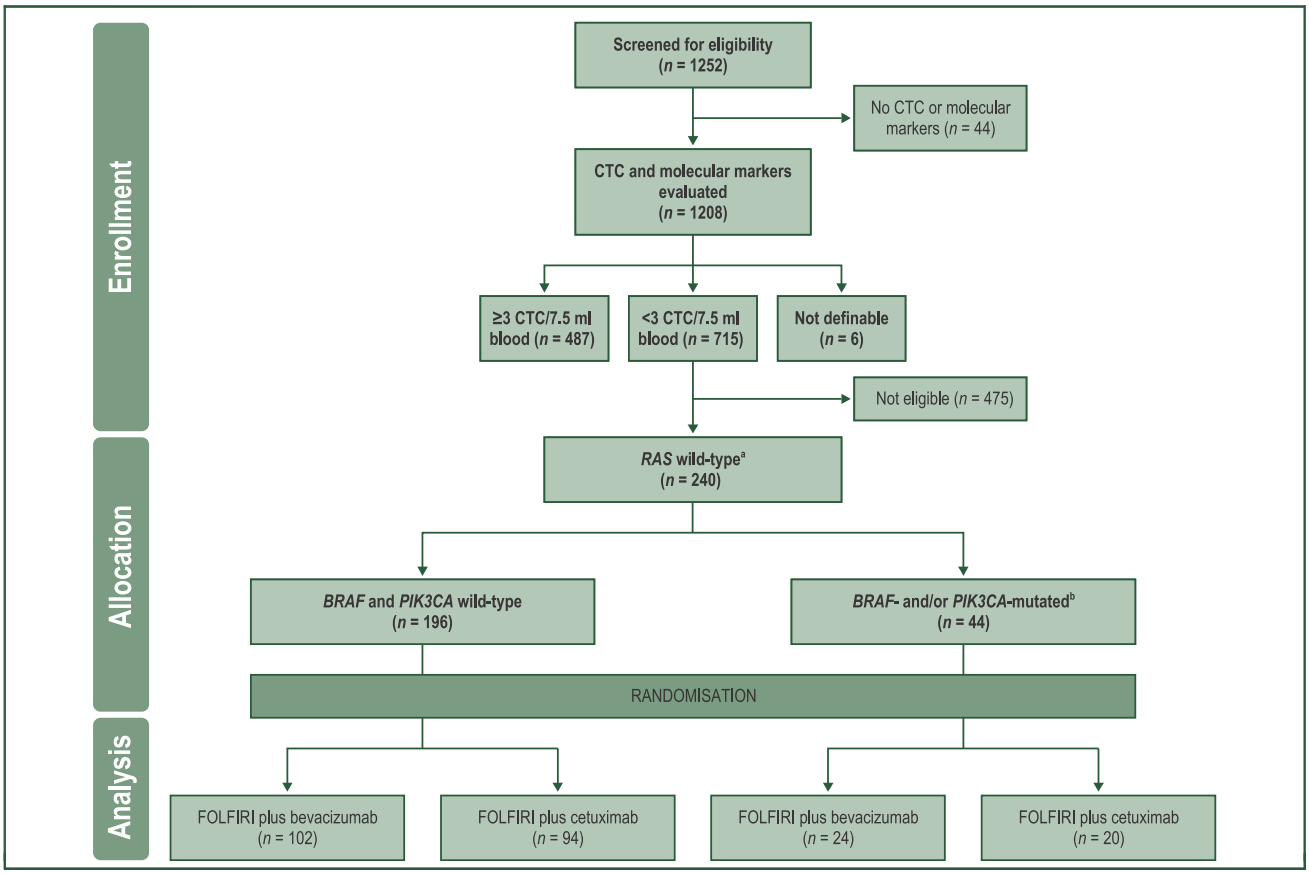

Figure 1. VISNÚ-2: CONSORT flowchart.
CTC, circulating tumour cell; FOLFIRI, 5-fluorouracil, leucovorin and irinotecan.
[a] Sixteen patients had extended *RAS* mutations (*KRAS* exon 4 mutations, $n = 3$; *NRAS* exon 2, 3 or 4 mutations, $n = 9$) or their *RAS* status was unknown ($n = 4$).
[b] *BRAF*-mutated/*PI3KCA*-mutated ($n = 8$), *BRAF*-mutated/*PI3KCA*-wild-type ($n = 16$) and *BRAF*-wild-type/*PI3KCA*-mutated ($n = 20$).

CellSearch® Tumor Circulating Cell kit (Veridex LLC, Raritan, NJ). Mutational analyses of *KRAS*, *NRAS*, *BRAF* and *PIK3CA* genes were done on primary tumour or metastatic tissue samples at six reference laboratories. Mutations in *KRAS* exons 2 and 3, $BRAF^{V600}$ and *PIK3CA* exons 9 and 20 were determined by the Cobas® test (Roche Diagnostics, Indianapolis, IN) and mutations in *KRAS* exon 4 and *NRAS* exons 2, 3 and 4 were analysed by pyrosequencing [Therascreen® NRAS Pyro Kit or Therascreen® RAS Extension Pyro Kit (Qiagen, Venlo, Netherlands)].

Tumour assessments were carried out using computed tomography of the chest, abdomen and pelvic region at baseline and then every 12 weeks until disease progression, and evaluated according to RECIST, version 1.1. After discontinuing treatment, patients were followed every 3 months for 2 years for any tumour assessments and survival. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0.

Outcomes

The primary endpoint was PFS, defined as time from randomisation to disease progression or death from any cause, whichever occurred first. Secondary endpoints were overall survival (defined as time from randomisation until death from any cause), overall response rate (defined as the proportion of patients with complete or partial responses), R0 resection rate (defined as the proportion of patients with surgical margins free of tumour cells on histological examination following rescue surgery), safety and tolerability.

Statistical analysis

The sample size for each treatment group was estimated using the Brookmeyer-Crowley method. Assuming an unilateral alpha error of 0.1, 90% power, 36-month recruitment period and 24-month follow-up period, it was necessary to enrol a total of 240 patients (*BRAF*/*PIK3CA* wild-type, $n = 194$; *BRAF*- and/or *PIK3CA*-mutated, $n = 46$) (see Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2021.100062, for details).

All analyses were exploratory. Efficacy analyses were carried out on an intention-to-treat basis, and safety analyses were carried out in all randomised patients who received at least one dose of any study drug. Overall response rate was evaluated in response-assessable patients, defined as those having at least one evaluation by RECIST.

Efficacy findings were presented according to *BRAF* and *PIK3CA* status and by study treatment. Time-to-event endpoints were analysed using the Kaplan—Meier method. A Cox proportional-hazards model was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs), and between-group comparisons made using the log-rank test. A multivariate analysis of PFS was carried out. Categorical variables were compared by chi-square or Fisher's exact test, and continuous variables by *t*-test or Wilcoxon's test. Statistical analyses were generated using SAS version 9.4 or greater and SAS Enterprise Guide version 7.1 or greater (SAS Institute Inc., Cary, NC).

RESULTS

From October 2012 to November 2016, 240 patients were enrolled and included in the intention-to-treat population. Overall, 196 patients had *BRAF/PIK3CA* wild-type tumours (FOLFIRI plus bevacizumab, $n = 102$; FOLFIRI plus cetuximab, $n = 94$), and 44 patients had *BRAF*- and/or *PIK3CA*-mutated tumours (FOLFIRI plus bevacizumab, $n = 24$; FOLFIRI plus cetuximab, $n = 20$) (Figure 1). A total of 16 patients in the intention-to-treat population had extended *RAS* mutations (*KRAS* exon 4 mutations, $n = 3$; *NRAS* exon 2, 3 or 4 mutations, $n = 9$) or their *RAS* status was unknown ($n = 4$).

Demographic and baseline characteristics were similar between patients with *BRAF/PIK3CA* wild-type and *BRAF*- and/or *PIK3CA*-mutated tumours (Table 1), except that *BRAF*- and/or *PIK3CA*-mutated tumours were significantly more likely to be right-sided (43% versus 16% for *BRAF/PIK3CA* wild-type tumours; $P = 0.0003$).

Table 1. Baseline characteristics in patients with *RAS* wild-type metastatic colorectal cancer and <3 CTC/7.5 ml blood (intention-to-treat population)

Variable	*BRAF/PIK3CA* wild-type ($n = 196$)	*BRAF*- and/or *PIK3CA*-mutated ($n = 44$)	Total ($n = 240$)
Median age (range), years	60 (32-70)	62 (47-70)	60 (32-70)
Sex, *n* (%)			
Male	133 (68)	31 (71)	164 (68)
Female	63 (32)	13 (30)	76 (32)
ECOG performance status, *n* (%)			
0	111 (56)	29 (66)	140 (58)
1	85 (43)	15 (34)	100 (42)
Tumour localisation, *n* (%)			
Colon	117 (60)	32 (73)	149 (62)
Rectum	59 (30)	6 (14)	65 (27)
Colorectal	20 (10)	6 (14)	26 (11)
Site of primary tumour, *n* (%)			
Left colon	162 (83)	24 (55)	186 (78)
Right colon	32 (16)	19 (43)	51 (21)
Both	2 (1)	1 (2)	3 (1)
Site of metastases, *n* (%)			
Liver only	74 (38)	14 (37)	88 (37)
Multiple sites	122 (62)	30 (68)	152 (63)
Number of organs affected			
≤1	97 (50)	18 (41)	115 (48)
>1	99 (50)	26 (59)	125 (52)
Presentation, *n* (%)			
Synchronous	172 (88)	39 (87)	211 (88)
Metachronous	24 (12)	5 (11)	29 (12)
Prior treatment, *n* (%)			
Surgery	96 (49)	28 (63)	124 (52)
Radiotherapy	10 (5)	2 (5)	12 (5)
Chemotherapy[a]	23 (12)	4 (9)	27 (11)
CEA levels, *n* (%)			
≤5 μg/l	40 (20)	13 (30)	53 (22)
>5 μg/l	156 (80)	31 (71)	187 (78)
RAS status, *n* (%)			
Mutated	11 (6)	1 (2)	12 (5)
KRAS exon 4	3 (2)	0 (0)	3 (1)
NRAS exons 2, 3 or 4	8 (4)	1 (2)	9 (4)
Wild-type	181 (92)	43 (98)	224 (93)
Not definable	4 (2)	0 (0)	4 (2)
PIK3CA status, *n* (%)			
Mutated	0 (0)	28 (64)	28 (12)
Wild-type	196 (100)	16 (36)	212 (88)
BRAF status, *n* (%)			
Mutated	0 (0)	24 (55)	24 (10)
Wild-type	196 (100)	20 (46)	216 (90)

CEA, carcinoembryonic antigen; CTC, circulating tumour cell; ECOG, Eastern Cooperative Oncology Group.
[a] Adjuvant or neoadjuvant setting.

Treatment

The median duration of treatment was 28.0 weeks (range, 2.0-166.6) in the FOLFIRI plus bevacizumab group and 26.1 weeks (range, 2.0-143.4) in the FOLFIRI plus cetuximab group. Median relative dose intensities were at least 80% for all agents in both treatment groups (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2021.100062).

Efficacy

Efficacy outcomes by *BRAF/PIK3CA* status and by treatment group are presented in Table 2. At the cut-off date (November 2018), the median duration of follow-up was 44.1 months (95% CI, 39.7-48.0).

By *BRAF/PIK3CA* status. Median PFS in the intention-to-treat population was 12.7 months in patients with *BRAF/PIK3CA* wild-type tumours and 8.8 months in patients with *BRAF*- and/or *PIK3CA*-mutated tumours (HR 1.22; 95% CI 0.80-1.85; $P = 0.3602$) (Figure 2A). The results were unchanged after excluding 16 patients with extended *RAS* mutations or whose *RAS* status was unknown (HR 1.18; 95% CI 0.77-1.80; $P = 0.4608$) (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2021.100062). A similar trend of improved PFS in patients with *BRAF/PIK3CA* wild-type versus *BRAF*- and/or *PIK3CA*-mutated tumours was evident in both treatment groups (FOLFIRI plus bevacizumab: HR 1.07; 95% CI 0.61-1.88; $P = 0.8251$; FOLFIRI plus cetuximab: HR 1.55; 95% CI 0.83-2.89; $P = 0.1669$) (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2021.100062). Right-sided tumours and an ECOG performance status of 1 were identified as independent adverse prognostic factors in the multivariate analysis (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2021.100062). After adjusting for these factors, the influence of *BRAF/PIK3CA* status on

Table 2. Efficacy in patients with *RAS* wild-type metastatic colorectal cancer and <3 CTCs/7.5 ml blood at baseline (intention-to-treat population)

Variable	*BRAF/PIK3CA* wild-type (n = 196)		*BRAF*- and/or *PIK3CA*-mutated (n = 44)		Total (n = 240)	
	FOLFIRI plus bevacizumab (n = 102)	FOLFIRI plus cetuximab (n = 94)	FOLFIRI plus bevacizumab (n = 24)	FOLFIRI plus cetuximab (n = 20)	*BRAF/PIK3CA* wild-type (n = 196)	*BRAF*- and/or *PIK3CA*-mutated (n = 44)
Primary endpoint						
Progression-free survival						
Median (95% CI), months	12.9 (10.3-14.9)	12.5 (10.5-16.1)	9.3 (3.7-15.0)	8.5 (5.3-12.4)	12.7 (11.3-14.9)	8.8 (8.2-12.4)
HR (95% CI)	0.99 (0.67-1.45)		1.11 (0.53-2.35)		1.22 (0.80-1.85)[a]	
P value	0.9486		0.7820		0.3602	
Secondary endpoints						
Overall survival						
Median (95% CI), months	36.0 (28.3-43.7)	34.1 (29.9-42.1)	18.6 (13.7-28.4)	23.7 (14.3-34.6)	34.7 (30.1-41.6)	20.7 (15.6-28.4)
HR (95% CI)	0.91 (0.63-1.33)		0.79 (0.39-1.59)		1.87 (1.27-2.77)[b]	
P value	0.6294		0.5061		0.0017	
Overall response rate,[c] n (%)	62/93 (67)	68/87 (78)	13/22 (59)	11/17 (65)	130/180 (72)	24/39 (62)
P value					0.1855	
Complete response	2 (2)	8 (9)	1 (5)	2 (12)	10 (6)	3 (8)
Partial response	60 (65)	60 (69)	12 (55)	9 (53)	120 (67)	21 (54)
Stable disease	22 (24)	13 (15)	5 (23)	4 (24)	35 (19)	9 (23)
Progressive disease	9 (10)	6 (7)	4 (18)	2 (12)	15 (8)	6 (15)
R0 resection,[d] n (%)	—	—	—	—	36 (18)	4 (9)
P value	—	—	—	—	0.1357	

CI, confidence interval; CTC, circulating tumour cell; FOLFIRI, 5-fluorouracil, leucovorin and irinotecan; HR, hazard ratio.
[a] *BRAF*- and/or *PIK3CA*-mutated versus *BRAF/PIK3CA* wild-type in the FOLFIRI plus bevacizumab group (HR = 1.07; 95% CI 0.61-1.88; P = 0.8251), and in the FOLFIRI plus cetuximab group (HR = 1.55; 95% CI 0.83-2.89; P = 0.1669).
[b] *BRAF*- and/or *PIK3CA*-mutated versus *BRAF/PIK3CA* wild-type in the FOLFIRI plus bevacizumab group (HR = 2.01; 95% CI 1.19-3.40; P = 0.0094), and in the FOLFIRI plus cetuximab group (HR = 1.71; 95% CI 0.95-3.10; P = 0.0748).
[c] Analysis in patients assessable for response (n = 219) defined as those having at least one evaluation by RECIST.
[d] For surgeries registered during the treatment period until the start of second-line therapy.

PFS was diminished (adjusted HR 0.94; 95% CI 0.65-1.36; P = 0.760).

Median overall survival was 34.7 months in patients with *BRAF/PIK3CA* wild-type tumours and 20.7 months in patients with *BRAF*- and/or *PIK3CA*-mutated tumours (HR 1.87; 95% CI 1.27-2.77; P = 0.0017) (Figure 2B). In the response-assessable population (n = 219), the overall response rate in patients with *BRAF/PIK3CA* wild-type tumours was 72%, and in patients with *BRAF*- and/or *PIK3CA*-mutated tumours was 62% (P = 0.1855). R0 resection rates were 18% and 9%, respectively (P = 0.1357).

Among the 44 patients with *BRAF*- and/or *PIK3CA*-mutated tumours, median PFS and overall survival appeared to differ in patients with *BRAF* mutations (n = 16), *PI3KCA* mutations (n = 20) or both (n = 8) (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2021.100062). Median PFS was 2.8 months in patients with *BRAF*- and *PI3KCA*-mutant tumours versus 8.8 months for *BRAF*-mutant/*PI3KCA* wild-type, and 15.0 months for *BRAF* wild-type/*PI3KCA*-mutant tumours (P = 0.0002). Median overall survival was 11.7 months for patients with *BRAF*- and *PI3KCA*-mutant tumours versus 18.7 months for *BRAF*-mutant/*PI3KCA* wild-type, and 38.4 months for *BRAF* wild-type/*PI3KCA*-mutant tumours (P = 0.0001).

In this subgroup of 44 patients with *BRAF*- and/or *PIK3CA*-mutated tumours, the impact of primary tumour location was also analysed (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2021.100062). Median PFS for left-sided versus right-sided tumours was 9.3 versus 8.2 months (P = 0.0504), and median overall survival was 24.2 versus 18.4 months, respectively (P = 0.2811).

By treatment group. In patients with *BRAF/PIK3CA* wild-type tumours, median PFS was 12.9 months with FOLFIRI plus bevacizumab and 12.5 months with FOLFIRI plus cetuximab (HR 0.99; 95% CI 0.67-1.45; P = 0.9486) (Figure 3A), and median overall survival was 36.0 months with FOLFIRI plus bevacizumab and 34.1 months with FOLFIRI plus cetuximab (HR 0.91; 95% CI 0.62-1.33; P = 0.6294). In the response-assessable population with *BRAF/PIK3CA* wild-type tumours, the overall response rate was 67% with FOLFIRI plus bevacizumab and 78% with FOLFIRI plus cetuximab.

Among patients with *BRAF/PIK3CA* wild-type tumours, tumours were located on the left side of the colon in 162 patients (82.7%). In this subgroup, median PFS was 12.5 months with FOLFIRI plus bevacizumab and 12.7 months with FOLFIRI plus cetuximab (HR 0.94; 95% CI 0.61-1.44; P = 0.7682) (Supplementary Figure S5A, available at https://doi.org/10.1016/j.esmoop.2021.100062), and median overall survival was 41.6 months with FOLFIRI plus bevacizumab and 34.6 months with FOLFIRI plus cetuximab (HR 0.87; 95% CI 0.56-1.33; P = 0.5060) (Supplementary Figure S5B, available at https://doi.org/10.1016/j.esmoop.2021.100062).

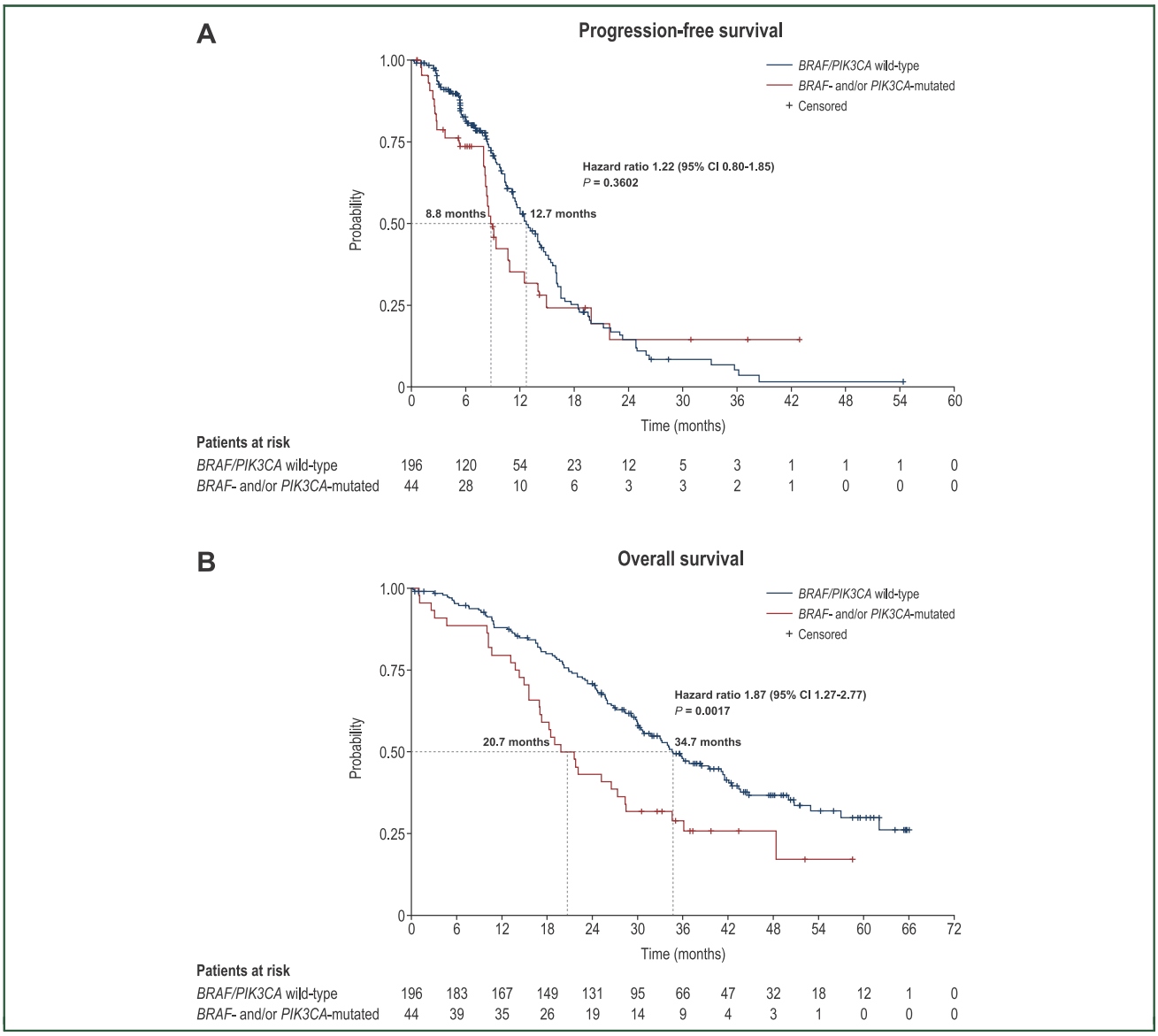

Figure 2. Kaplan—Meier estimates of progression-free survival (A) and overall survival (B) by *BRAF* and *PIK3CA* status (intention-to-treat population).
CI, confidence interval.

In patients with *BRAF*- and/or *PIK3CA*-mutated tumours, median PFS was 9.3 months with FOLFIRI plus bevacizumab and 8.5 months with FOLFIRI plus cetuximab (HR 1.11; 95% CI 0.53-2.35; $P = 0.7820$) (Figure 3B), and median overall survival was 18.6 months with FOLFIRI plus bevacizumab and 23.7 months with FOLFIRI plus cetuximab (HR 0.51; 95% CI 0.39-1.59; $P = 0.5061$). In the response-assessable population with *BRAF*- and/or *PIK3CA*-mutated tumours, the overall response rate was 59% with FOLFIRI plus bevacizumab and 65% with FOLFIRI plus cetuximab.

Safety

A total of 239 patients received at least one dose of study treatment and were included in the safety population (*BRAF/PIK3CA*-wild-type, $n = 195$; *BRAF*- and/or *PIK3CA*-mutated, $n = 44$). A safety summary is provided in Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2021.100062 and the most common treatment-related adverse events by *BRAF/PIK3CA* status and by treatment group are presented in Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2021.100062. The safety profiles in both treatment groups were consistent with the known side-effects of the individual drugs. The most common treatment-related grade 3/4 adverse events in both treatment groups were neutropenia, diarrhoea and asthenia (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2021.100062).

Serious adverse events judged to be treatment-related occurred in 20 patients with FOLFIRI plus bevacizumab and 19 patients with FOLFIRI plus cetuximab (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2021.100062). The most common serious events with FOLFIRI plus bevacizumab were diarrhoea

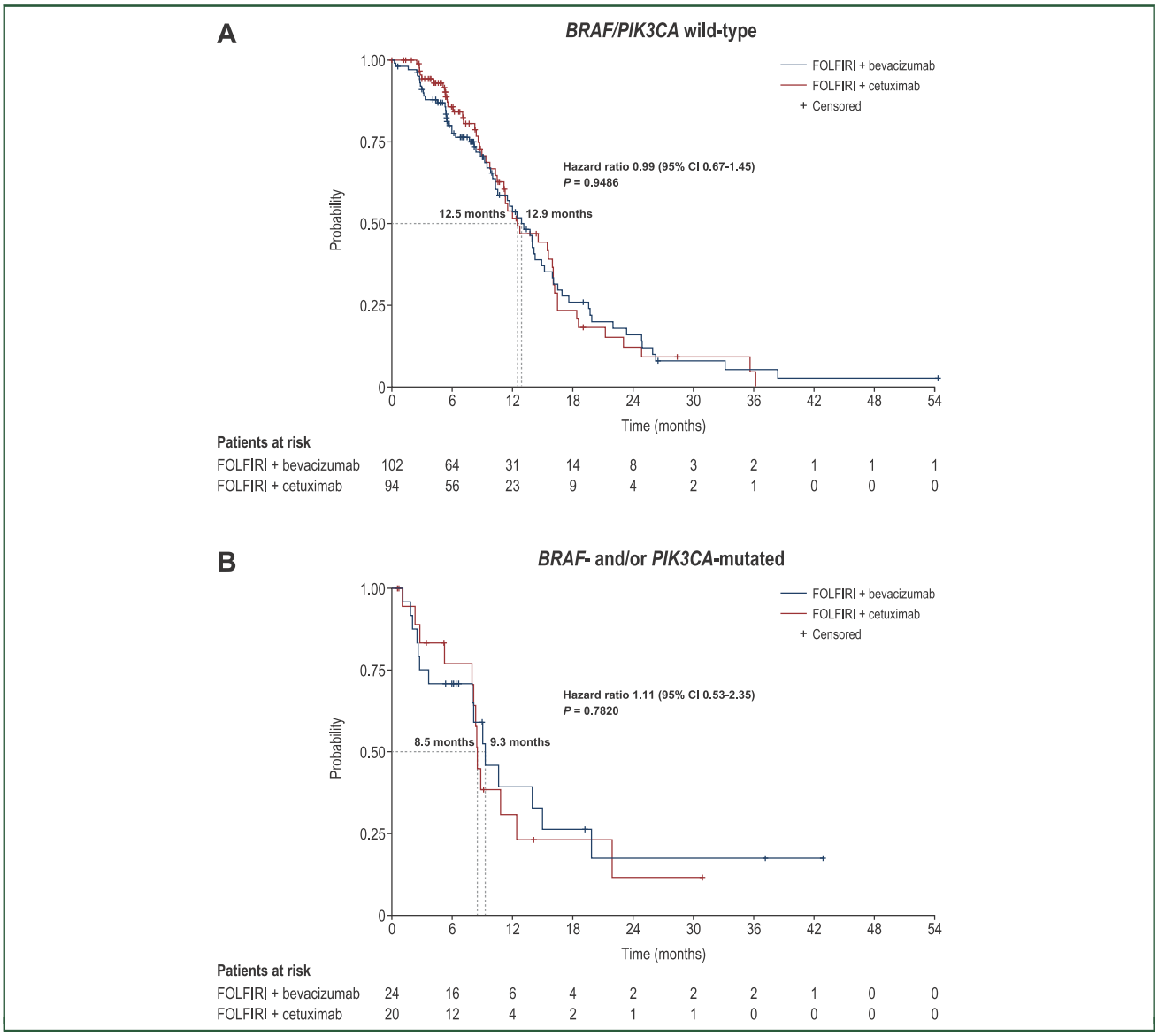

Figure 3. Kaplan—Meier estimates of progression-free survival for FOLFIRI plus bevacizumab versus FOLFIRI plus cetuximab by *BRAF*/*PIK3CA* status: (A) *BRAF*/*PIK3CA* wild-type and (B) *BRAF*- and/or *PIK3CA*-mutated tumours (intention-to-treat population).
CI, confidence interval; FOLFIRI, 5-fluorouracil, leucovorin and irinotecan.

($n = 6$) and intestinal perforation ($n = 4$), and with FOLFIRI plus cetuximab were febrile neutropenia ($n = 5$) and diarrhoea ($n = 3$).

Six patients (5%) in the FOLFIRI plus bevacizumab group and two patients (2%) in the FOLFIRI plus cetuximab group died as a result of adverse events. These events were considered treatment-related in 3 patients (2%) in the FOLFIRI plus bevacizumab group (intestinal perforation, $n = 1$; dehydration, $n = 1$; pulmonary embolism, $n = 1$).

Subsequent anticancer treatments

Second-line treatment was given in similar proportions of patients in the FOLFIRI plus bevacizumab group ($n = 95$, 75%) and in the FOLFIRI plus cetuximab group ($n = 87$, 76%). The proportions of patients receiving third-line and fourth-line or later treatments were also similar in the FOLFIRI plus bevacizumab group ($n = 61$, 48% and $n = 37$, 29%, respectively) and in the FOLFIRI plus cetuximab group ($n = 61$, 54% and $n = 36$, 32%, respectively). The distribution of anticancer agents was similar in both groups, except that more patients in the FOLFIRI plus bevacizumab group received EGFR-directed agents ($n = 67$, 53% versus $n = 39$, 34% in the FOLFIRI plus cetuximab group), and more patients in the FOLFIRI plus cetuximab group received anti-angiogenic agents ($n = 63$, 55% versus $n = 45$, 36% in the FOLFIRI plus bevacizumab group) (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2021.100062).

DISCUSSION

The findings from our study suggest that the presence of *BRAF* and/or *PIK3CA* mutations is associated with poorer outcomes versus the absence of these mutations in patients

with *RAS* wild-type mCRC and a favourable prognosis defined by CTC count. Median PFS and overall survival were 4 and 14 months shorter, respectively, in the *BRAF*- and/or *PIK3CA*-mutated cohort compared with the *BRAF*/*PIK3CA* wild-type cohort, the difference in overall survival being statistically significant ($P = 0.0017$). Although the number of patients with *BRAF* and/or *PI3KCA* mutations was low, the impact of these mutations appeared to be different. *BRAF* mutations had a marked impact on PFS and overall survival, which seemed to be further potentiated by the addition of *PI3KCA* mutations. However, patients with only *PI3KCA* mutations seemed to achieve similar PFS and overall survival times as patients with *BRAF*/*PI3KCA* wild-type tumours. Similar results were reported in a subgroup analysis of the CALGB/SWOG 80405 study, in which *PI3KCA* status did not have any impact on survival outcomes in patients with *KRAS*/*NRAS*/*BRAF* wild-type tumours.[7] It is also notable that *BRAF* and *PIK3CA* mutations occurred significantly more frequently in right-sided tumours, an independent adverse prognostic indicator in our study population, raising questions about the relative contribution of mutational status and tumour location on outcomes. In our multivariate analysis, the prognostic influence of *BRAF* and *PIK3CA* mutations on outcomes was minimised after adjusting for tumour sidedness. Furthermore, even in the reduced subgroup of patients with *BRAF*- and/or *PI3KCA*-mutant tumours, a tendency to worse PFS and overall survival was observed in right-sided tumours, indicating that more factors beyond *BRAF* and *PI3KCA* mutations may be implicated. The *V600E BRAF* mutation is a well-recognised poor prognostic factor for PFS and overall survival in patients with mCRC treated with chemotherapy, and could be related to the early development of mechanisms of resistance.[8] The role of *BRAF* mutations as a predictive factor for anti-EGFR therapy is still controversial, since several studies and meta-analyses suggest resistance to anti-EGFR therapy,[9,10] but others do not completely exclude a potential benefit from anti-EGFR therapy.[11] In fact, a gene expression profile study in *BRAF*-mutated patients suggests a heterogeneous population subclassified in BM1 and BM2 subgroups according to activation of different pathways.[12]

Three other randomised studies (FIRE-3, CALGB/SWOG 80405, PEAK) have directly compared a VEGF inhibitor (bevacizumab) plus chemotherapy versus an EGFR inhibitor (cetuximab or panitumumab) plus chemotherapy as first-line therapy in patients with *KRAS* wild-type mCRC.[13-15] To our knowledge, VISNÚ-2 is the first study to compare these treatments in a *RAS* wild-type population with further consideration given to other mutations (*BRAF* and *PIK3CA*). In our study, neither treatment was clearly more effective in the *BRAF*/*PIK3CA* wild-type or *BRAF*/*PIK3CA*-mutated cohorts, and poorer outcomes were observed with both regimens in patients with *BRAF/PIK3CA*-mutated tumours. Our findings suggest that *BRAF*/*PIK3CA* status does not appear to provide any additional information over *RAS* status for selecting bevacizumab or cetuximab as first-line therapy, although a larger study is needed to confirm these observations. Consistent with our study, a subgroup analysis from the CALGB/SWOG 80405 study in patients harbouring *BRAF* mutations showed no difference in overall survival between bevacizumab or cetuximab plus chemotherapy.[7]

A subgroup analysis in left-sided, quadruple-negative (*KRAS*/*NRAS*/*BRAF*/*PI3KCA*) tumours comparing cetuximab- versus bevacizumab-based treatment, while of interest, has not been carried out. Nevertheless, in the left-sided colon VISNÚ-2 population, 93% of tumours were quadruple-negative, and no differences between treatments were found according to sidedness in an exploratory analysis. It should be noted that eight patients in this patient subgroup had *RAS*-mutant tumours and three patients had unknown *RAS* status, although we suggest that this number would not have influenced the results.

More than 1200 patients with mCRC were screened for the VISNÚ project and enrolled into VISNÚ-1 (high-risk population with $\geq$3 CTCs/7.5 ml blood) or VISNÚ-2 (low-risk population with $<$3 CTCs/7.5 ml blood) based on the CTC threshold defined by Cohen et al.[3] The outcomes in our low-risk study population were generally better than those reported in other studies involving *RAS* wild-type populations,[13-15] and provide further support for a CTC count of $<$3/7.5 ml blood as an indicator of favourable prognosis in mCRC. For example, the median PFS times in VISNÚ-2 (bevacizumab plus FOLFIRI: 12.5 months; cetuximab plus FOLFIRI: 11.5 months) were longer than in the FIRE-3 *RAS* wild-type study population which was selected without regard for baseline CTC count (bevacizumab plus FOLFIRI: 10.2 months; cetuximab plus FOLFIRI: 10.4 months).[13]

Our study was exploratory and designed with the intention of informing future research efforts. We acknowledge that the number of patients, particularly in the *BRAF*- and/or *PIK3CA*-mutated cohort, was small. During the course of the study, the inclusion criteria were updated to exclude patients with extended *RAS* mutations as evidence confirming their predictive value emerged. For this reason, the study population included 12 patients with *KRAS/NRAS* mutations, and was not solely *RAS* wild-type.

In conclusion, this exploratory study suggests that *BRAF* mutations had a negative effect on PFS in patients with *RAS* wild-type mCRC, which was further potentiated by the addition of *PI3KCA* mutations. The association between *BRAF*/*PI3KCA* mutations and right-sided tumours, and their relative contribution to patient outcomes requires further study. Neither bevacizumab nor cetuximab given in combination with FOLFIRI was superior as first-line therapy in the *RAS*/*BRAF*/*PIK3CA* wild-type or *RAS* wild-type/*BRAF*/*PIK3CA*-mutant subpopulations suggesting that *BRAF* and *PI3KCA* mutations have a role as prognostic but not predictive factors.

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DATA SHARING

The study data (i.e. de-identified participant data and datasets analysed for the present manuscript) are available from the corresponding author, Dr J. Sastre (e-mail: jsastrev@salud.madrid.org), on reasonable request and subject to approval from the lead investigators. Additional, related documents will also be available (study protocol, statistical analysis plan, informed consent form).

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