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FOLFOXIRI plus bevacizumab versus FOLFOX plus bevacizumab for patients with metastatic colorectal cancer and ≥3 circulating tumour cells: the randomised phase III VISNÚ-1 trial

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ABSTRACT

Purpose 5-Fluorouracil/leucovorin, oxaliplatin, irinotecan (FOLFOXIRI) plus bevacizumab is more effective than doublets plus bevacizumab as first-line therapy for metastatic colorectal cancer, but is not widely used because of concerns about toxicity and lack of predictive biomarkers. This study was designed to explore the role of circulating tumour cell (CTC) count as a biomarker to select patients for therapy with FOLFOXIRI-bevacizumab.

Patients and methods VISNÚ-1 was a multicentre, open-label, randomised, phase III study in patients with previously untreated, unresectable, metastatic colorectal carcinoma and ≥3 CTC/7.5 mL blood. Patients received bevacizumab 5 mg/kg plus FOLFOXIRI (irinotecan 165 mg/m^2, oxaliplatin 85 mg/m^2, leucovorin 400 mg/m^2 and 5-fluorouracil 3200 mg/m^2) or FOLFOX (oxaliplatin 85 mg/m^2, leucovorin 400 mg/m^2, 5-fluorouracil 400 mg/m^2 then 2400 mg/m^2) by intravenous administration every 2 weeks. The primary outcome was progression-free survival (PFS).

Results The intention-to-treat population comprised 349 patients (FOLFOXIRI-bevacizumab, n=172; FOLFOX-bevacizumab, n=177). Median PFS was 12.4 months (95% CI 11.2 to 14.0) with FOLFOXIRI bevacizumab and 9.3 months (95% CI 8.5 to 10.7) with FOLFOX-bevacizumab (stratified HR, 0.64; 95% CI 0.49 to 0.82; p=0.0006). Grade≥3 adverse events were more common with FOLFOXIRI-bevacizumab 85.3% vs 75.1% with FOLFOX-bevacizumab (p=0.0178). Treatment-related deaths occurred in 8 (4.7%) and 6 (3.4%) patients, respectively.

Conclusions First-line FOLFOXIRI-bevacizumab significantly improved PFS compared with FOLFOX-bevacizumab in patients with metastatic colorectal cancer and ≥3 CTCs at baseline, which indicate a poor prognosis. CTC count may be a useful non-invasive biomarker to assist with the selection of patients for intensive first-line therapy.

Summary

What is already known about this subject?

► 5-Fluorouracil/leucovorin, oxaliplatin, irinotecan (FOLFOXIRI) plus bevacizumab is more effective than doublets plus bevacizumab as first-line therapy for metastatic colorectal cancer, but is not widely used because of concerns about toxicity and lack of predictive biomarkers. Circulating tumour cell (CTC) count is an independent prognostic marker in patients with metastatic colorectal cancer that offers the possibility of selecting high-risk patients suitable for intensive first-line therapy.

What does this study add?

► The phase III VISNÚ-1 study showed that first-line FOLFOXIRI plus bevacizumab significantly improved progression-free survival compared with 5-fluorouracil/leucovorin and oxaliplatin plus bevacizumab in patients with metastatic colorectal cancer and ≥3 CTCs at baseline, which indicate a poor prognosis.

How might this impact on clinical practice?

► CTC count may be a useful non-invasive biomarker to assist with the selection of patients suitable for intensive first-line therapy in metastatic colorectal cancer.

INTRODUCTION

In fit patients with metastatic colorectal cancer, the cytotoxic triplet of 5-fluorouracil/leucovorin, oxaliplatin plus irinotecan (FOLFOXIRI) ± bevacizumab is more effective as first-line therapy than a doublet ± bevacizumab,[1–7] but is not widely used in routine

clinical practice because of concerns about toxicity and because no specific predictive biomarkers are available.

Circulating tumour cells (CTCs) are released from primary tumours or metastases into the bloodstream, and are detectable in the blood of many patients with advanced primary carcinomas, including colorectal cancer.[8–10] In patients with metastatic colorectal cancer, CTC count is a strong independent prognostic marker,[11 12] and patients with ≥3 CTCs/7.5 mL of peripheral blood prior to chemotherapy have a significantly shorter progression-free survival (PFS) and overall survival (OS) when compared with patients with <3 CTCs/7.5 mL blood.[11 13 14] Furthermore, CTC count at baseline is an important prognostic indicator within patient subgroups defined by line of therapy, type of chemotherapy, age or performance status.[12]

In 2012, the Spanish Cooperative Group for the Treatment of Digestive Tumours designed the VISNÚ project in which patients with metastatic colorectal cancer were eligible for one of two studies based on their CTC counts enumerated using the CellSearch System. We report findings from the phase III VISNÚ-1 trial, which was designed to explore the role of CTC count as a biomarker to select patients for therapy with FOLFOXIRI plus bevacizumab. In this study, FOLFOXIRI plus bevacizumab was compared with 5-fluorouracil/leucovorin and oxaliplatin (FOLFOX) plus bevacizumab in patients with metastatic colorectal cancer and an unfavourable prognosis defined by CTC count (ie, ≥3 CTCs/7.5 mL blood), irrespective of *RAS* or *BRAF* status.

METHODS

Study design

VISNÚ-1 was a multicentre, open-label, randomised, phase III study conducted in 51 university or community hospitals in Spain (ClinicalTrials.gov identifier: NCT01640405).

Patients

Adult patients 18–70 years of age with an Eastern Cooperative Oncology Group (ECOG) performance status of ≤1, and adequate bone marrow, liver and renal function were eligible. Patients had histologically confirmed metastatic colorectal adenocarcinoma that was deemed unresectable with curative intent and ≥3 CTCs/7.5 mL of blood at baseline. Prior chemotherapy for advanced disease was not permitted, although radiotherapy was allowed if completed within 4 weeks before randomisation. Written informed consent was obtained from all patients before enrolment.

The main exclusion criteria were uncontrolled hypertension, relevant cardiovascular disease, significant bleeding in the last month, major surgery, open surgical biopsy or significant traumatic injury within 4 weeks before randomisation, serious non-healing wound, ulcer or bone fracture, abdominal fistula or perforation in the last 6 months, a history of pulmonary fibrosis, acute lung disease or interstitial pneumonia, proteinuria >1 g/24 hours, or a history of peripheral neuropathy.

Randomisation and masking

Patients were randomly assigned (1:1 ratio) to receive FOLFOXIRI plus bevacizumab or FOLFOX plus bevacizumab. The randomisation sequence was generated using permuted blocks and stratified according to site, *KRAS* status (exon 2 and 3 mutations vs wildtype), and number of organs affected (1 vs >1). Stratification and randomisation were performed centrally at the study data centre, and the randomisation number and treatment group for each patient made available to the investigator (either electronically or by facsimile). Treatment allocation was not masked.

Procedures

Bevacizumab 5 mg/kg was delivered as a 30–90 min intravenous infusion on day 1, followed by FOLFOXIRI or modified FOLFOX. FOLFOXIRI consisted of irinotecan 165 mg/m^2 administered as a 30–90 min intravenous infusion, followed by oxaliplatin 85 mg/m^2 administered as a 2-hour intravenous infusion given concurrently with or followed by (according to the practice of each institution) leucovorin 400 mg/m^2 administered as a 2-hour intravenous infusion, and 5-fluorouracil 3200 mg/m^2 by continuous intravenous infusion over 46 hours repeated every 2 weeks. Modified FOLFOX consisted of oxaliplatin 85 mg/m^2 administered as a 2-hour intravenous infusion given concurrently with or followed by (according to the practice of each institution) leucovorin 400 mg/m^2 administered as a 2-hour intravenous infusion, 5-fluorouracil 400 mg/m^2 as an intravenous bolus then 2400 mg/m^2 by continuous infusion over 46 hours repeated every 2 weeks. Treatment was continued until disease progression, unacceptable toxicity or withdrawal of consent. Treatment was suspended and surgery scheduled in patients with metastases that became resectable during the study. Protocol-specified treatment modifications were recommended when predefined toxicities occurred. The protocol was amended after enrolling 63 patients to recommend the use of prophylactic granulocyte-colony stimulating factor (G-CSF) in the FOLFOXIRI–bevacizumab group due to a high rate of neutropenia.

CTCs were determined at baseline by central testing (San Carlos Hospital, Madrid, Spain). Peripheral blood (10 mL) was collected in CellSave Preservative Tubes, and CTCs were enumerated using the CellSearch Tumor Circulating Cell Kit (Veridex LLC, Raritan, New Jersey, USA). Mutational analyses of *KRAS, NRAS, BRAF* and *PIK3CA* genes were done on primary tumour or metastatic tissue samples at baseline 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, and mutations in *KRAS* exon 4 and *NRAS* exons 2, 3 and 4 were analysed by pyrosequencing (Qiagen NRAS kit, RAS Extension Pyro Kit or Therascreen RAS Extension Pyro Kit). Microsatellite instability analysis in tumour

tissue was performed using a Promega Kit containing five monomorphic mononucleotide repeats (BAT-21, BAT-26, Mono-27, NR-21 and NR-24) and two polymorphic pentanucleotide repeats (Penta C and D).

Tumour assessments using CT of the chest, abdomen and pelvic region and measurement of carcinoembryonic antigen levels were performed at baseline and then every 12 weeks until disease progression. After discontinuing treatment, patients were followed every 3 months for 24 months after inclusion of the last patient for any additional anticancer therapies and for survival.

Outcomes

The primary endpoint was PFS, defined as the time from randomisation to investigator-assessed disease progression according to Response Evaluation Criteria in Solid Tumours (RECIST), V.1.1,[15] or death from any cause. Secondary endpoints were overall response rate (ORR; complete or partial response using RECIST, V.1.1), OS (defined as the time from randomisation until death from any cause), rate of radical R0 resection (ie, surgical margins free of tumour cells on histological examination), safety and tolerability. Safety assessments were graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events, V.4.0.

Statistical analysis

The addition of irinotecan to FOLFOX plus bevacizumab was anticipated to increase the median PFS from 8 to 11.2 months, corresponding to an HR of 0.71. Assuming that 10% of patients would be lost to follow-up prior to progression, inclusion of 350 patients (280 progression events) was required to detect a difference of 3.2 months with 80% power at a two-sided significance level of 0.05. Assuming that approximately 47% of patients with metastatic colorectal cancer have ≥3 CTC/7.5 mL blood,[14] it was estimated that a total of 750 patients would need to be screened for the study.

Efficacy analyses were performed on an intention-to-treat basis, and safety analyses were performed in all randomised patients who received ≥1 dose of any study drug. Event-free survival rates were estimated using the Kaplan-Meier method and compared using a stratified two-sided log-rank test. A stratified Cox proportional hazard model was used to estimate HR with 95% CIs for FOLFOXIRI-bevacizumab (experimental group) versus FOLFOX-bevacizumab (control group). Subgroup analyses of PFS were performed to determine treatment effect according to key baseline factors. A Cox proportional hazard model was used to evaluate the effects of predefined prognostic factors on PFS in univariate and multivariate analyses, including effect size of treatment. Categorical variables were compared by χ^2 test or Fisher's exact test, and continuous variables by t-test or Wilcoxon test. P values ≤0.05 were considered to indicate statistical significance. SAS V.9.4 or later was used for statistical analyses.

RESULTS

From 8 October 2012 to 30 November 2016, 487 of 1208 patients (40.3%) who were screened for CTCs and molecular markers had ≥3 CTCs/7.5 mL blood (figure 1). Overall, 349 patients were eligible and comprised the intention-to-treat population (FOLFOXIRI-bevacizumab, n=172; FOLFOX-bevacizumab, n=177). Two patients in the FOLFOXIRI-bevacizumab group did not receive any study treatment and were not included in the safety population, which comprised a total of 347 patients (FOLFOXIRI-bevacizumab; n=170; FOLFOX-bevacizumab, n=177).

Demographic and baseline characteristics of patients were similar between treatment groups (table 1). The median age of the study population was 60 years (IQR, 53–65), 166 (47.6%) patients had an ECOG performance status of 0, 327 (93.7%) presented with synchronous metastases, and 216 (61.9%) had disease at multiple sites. A total of 114 (32.7%) patients had had surgical resection of their primary tumour, and 16 (4.6%) patients had received (neo)adjuvant chemotherapy. *RAS* (*KRAS* or *NRAS*) mutations were documented in 169 patients (48.4%), *BRAF* mutations in 33 patients (9.5%), and *PI3K* mutations in 43 patients (12.3%).

Treatment

The median number of treatment cycles administered was 12 (IQR 6–21) in the FOLFOXIRI-bevacizumab group and 14 (IQR 8–20) in the FOLFOX-bevacizumab group (online supplemental table S1). The median duration of treatment was 32.1 weeks (IQR 13.9–50.6) in the FOLFOXIRI-bevacizumab group and 32.0 weeks (IQR 18.0–47.7) in the FOLFOX-bevacizumab group. Delays and dose reductions were required in 150 (88.2%) and 107 (62.9%) patients in the FOLFOXIRI-bevacizumab group, respectively, and in 147 (83.1%) and 91 (51.4%) patients in the FOLFOX-bevacizumab group, respectively.

Efficacy

At the cut-off date (November 2018), the median duration of follow-up was 52.1 months (95% CI 45.3 to 54.5). An efficacy summary is presented in table 2.

In the intention-to-treat population, PFS events occurred in 112 patients (65.1%) in the FOLFOXIRI-bevacizumab group and 129 patients (72.9%) in the FOLFOX-bevacizumab group (online supplemental table S2). Median PFS was 12.4 months (95% CI 11.1 to 14.0) in the FOLFOXIRI-bevacizumab group and 9.3 months (95% CI 8.5 to 10.7) in the FOLFOX-bevacizumab group (stratified HR 0.64; 95% CI 0.49 to 0.82; p=0.0006) (figure 2A). PFS rates at 12 and 24 months were 52.4% (95% CI 43.2% to 60.8%) and 15.2% (95% CI 9.1% to 22.8%), respectively, in the FOLFOXIRI-bevacizumab group, and 29.0% (95% CI 21.4% to 37.0%) and 3.5% (95% CI 1.1% to 8.5%), respectively, in the FOLFOX-bevacizumab group (online supplemental table S2). The treatment effect of FOLFOXIRI-bevacizumab was consistent across analysed subgroups (figure 3). *RAS* mutations, *BRAF* mutations,

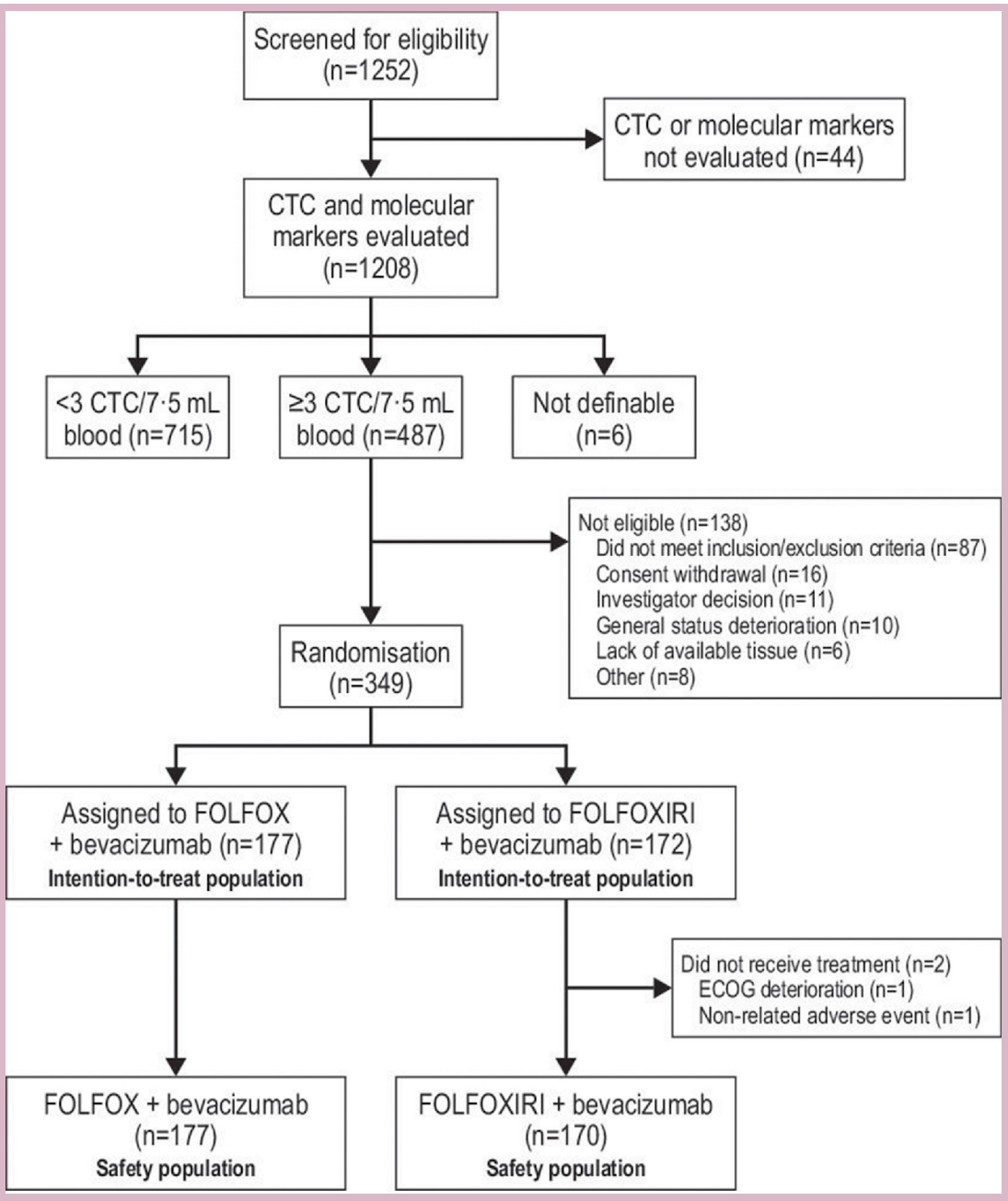

Figure 1 CONSORT flow chart. CONSORT, Consolidated Standards of Reporting Trials; CTC, circulating tumour cells; ECOG, Eastern Cooperative Oncology Group; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; FOLFOXIRI, 5-fluorouracil, leucovorin, oxaliplatin and irinotecan.

>20 CTC/7.5 mL blood, male sex, ECOG score of 1, right-sided tumours and tumours at multiple sites were identified as adverse prognostic factors for PFS in the univariate analysis (online supplemental table S3). *RAS* mutations, *BRAF* mutations, >20 CTCs/7.5 mL blood and an ECOG score of 1 were identified as independent adverse prognostic factors in the multivariate analysis (online supplemental table S4). The effect of FOLFOXIRI-bevacizumab versus FOLFOX-bevacizumab on PFS remained statistically significant after adjusting for these factors (adjusted HR 0.66; 95% CI 0.51 to 0.87; p=0.003) (online supplemental table S4). As *RAS* and *BRAF* status were related, the model was rerun after excluding *BRAF* status and yielded a similar result (adjusted HR 0.64; 95% CI, 0.49 to 0.83; p=0.001).

ORR in the intention-to-treat population was 59.3% (102/172 patients) in the FOLFOXIRI-bevacizumab group and 52.0% (92/177 patients) in the FOLFOX-bevacizumab group (OR 0.74; 95% CI 0.49 to 1.14; p=0.1685) (table 2). ORR in the response-evaluable population was 68.9% (102/148 patients) in the FOLFOXIRI-bevacizumab group and 57.5% (92/160 patients) in the FOLFOX-bevacizumab group (OR 0.61, 95% CI 0.38 to 0.97; p=0.0381). The median duration of response in the intention-to-treat population was 9.9 months in the FOLFOXIRI-bevacizumab group and 8.1 months in the

Table 1 Baseline characteristics in the intention-to-treat population

Variable	FOLFOX plus bevacizumab (n=177)	FOLFOXIRI plus bevacizumab (n=172)
Age (years)	59 (53–65)	61 (54–66)
Sex		
Male	119 (67.2%)	118 (68.6%)
Female	58 (32.8%)	54 (31.4%)
ECOG performance status		
0	85 (48.0%)	81 (47.1%)
1	92 (52.0%)	91 (52.9%)
Tumour localisation		
Colon	108 (61.0%)	113 (65.7%)
Rectum	48 (27.1%)	38 (22.1%)
Colorectal	21 (11.9%)	21 (12.2%)
Site of primary tumour		
Left colon	137 (77.4%)	119 (69.2%)
Right colon	39 (22.0%)	48 (28.0%)
Both	1 (0.6%)	5 (2.9%)
Site of metastases		
Liver only	62 (35.0%)	62 (36.1%)
Multiple sites	115 (65.0%)	110 (64.0%)
Metastatic sites		
≤1	65 (36.7%)	68 (39.5%)
>1	112 (63.3%)	104 (60.5%)
Presentation		
Synchronous	167 (94.4%)	160 (93.0%)
Metachronous	10 (5.7%)	12 (7.0%)
Prior treatment		
Surgery	55 (31.0%)	59 (34.3%)
Radiotherapy	4 (2.3%)	5 (2.9%)
Chemotherapy*	7 (4.0%)	9 (5.2%)
CEA levels		
≤5 ng/mL	8 (4.5%)	16 (9.3%)
>5 ng/mL	169 (95.5%)	156 (90.7%)
RAS status		
Mutated†	84 (47.5%)	85 (49.4%)
Wildtype	88 (49.7%)	85 (49.4%)
Data not available	5 (2.8%)	2 (1.2%)
PI3K status		
Mutated	17 (9.6%)	26 (15.1%)
Wildtype	159 (89.8%)	146 (84.9%)
Data not available	1 (0.6%)	0 (0%)
BRAF status		
Mutated	17 (9.6%)	16 (9.3%)
Wildtype	160 (90.4%)	156 (90.7%)
MSI		
MSI high	1 (0.6%)	2 (1.2%)
MSI low	7 (4.0%)	8 (4.7%)
MSS	156 (88.1%)	156 (90.7%)
Data not available	13 (7.3%)	6 (3.5%)

Data are no (%) or median (IQR).
*Adjuvant or neoadjuvant setting.
†Mutated in *KRAS* (exon 2, 3 or 4) or *NRAS* (exon 2, 3, or 4).
CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; FOLFOXIRI, 5-fluorouracil, leucovorin, oxaliplatin and irinotecan; MSI, microsatellite instability; MSS, microsatellite stable.

FOLFOX-bevacizumab group (HR 0.56; 95% CI 0.40 to 0.79; p=0.0010). The rate of R0 resection was 7.0% (95% CI 3.7% to 11.9%) and 7.9% (95% CI 4.4% to 12.9%) in the FOLFOXIRI-bevacizumab and FOLFOX-bevacizumab groups, respectively (p=0.7400).

In the intention-to-treat population, a total of 285 patients died of whom 136 (79.1%) were in the FOLFOXIRI-bevacizumab group and 149 (84.2%) were in the FOLFOX-bevacizumab group. Median OS was 22.3 months (95% CI 17.8 to 26.4) in the FOLFOXIRI-bevacizumab group and 17.6 months (95% CI 15.1 to 21.2) in the FOLFOX-bevacizumab group (stratified HR 0.84; 95% CI 0.66 to 1.06; p=0.1411) (figure 2B). OS rates at 60 months were 15.8% (95% CI 10.3% to 22.5%) and 6.9% (95% CI 2.2% to 15.4%) in the FOLFOXIRI-bevacizumab and FOLFOX-bevacizumab groups, respectively (online supplemental table S5).

Safety

A safety summary is provided in online supplemental table S6, and the most common treatment-emergent adverse events are presented in online supplemental table S7. Grade 3 or greater treatment-emergent adverse events were significantly more common in the FOLFOXIRI-bevacizumab group (145/170 patients, 85.3%) than in the FOLFOX-bevacizumab group (133/177 patients, 75.1%) (p=0.0178). Grade 3/4 treatment-emergent adverse events that occurred significantly more frequently in the FOLFOXIRI-bevacizumab group were diarrhoea (n=39, 22.9% vs n=12, 6.8% in the FOLFOX-bevacizumab group; p<0.0001), asthenia (n=29, 17.1% vs n=15, 8.5%; p=0.0163), and febrile neutropenia (n=14, 8.2% vs n=4, 2.3%; p=0.0121).

Serious treatment-emergent adverse events were also significantly more common in the FOLFOXIRI-bevacizumab group (n=83, 48.8%) than in the FOLFOX-bevacizumab group (n=64, 36.2%; p=0.0170). The most

Table 2 Efficacy in the intention-to-treat population

Variable	FOLFOX plus bevacizumab (n=177)	FOLFOXIRI plus bevacizumab (n=172)	HR or OR (95% CI)	P value
Progression-free survival				
Events	129 (72.9%)	112 (65.1%)	0.64 (0.49 to 0.82)	0.0006
Median (95% CI), months	9.3 (8.5 to 10.7)	12.4 (11.1 to 14.0)		
Response				
Complete response	1 (0.6%)	4 (2.3%)		
Partial response	91 (51.4%)	98 (57.0%)		
Stable disease	64 (36.2%)	40 (23.3%)		
Progressive disease	4 (2.3%)	6 (3.5%)		
Not evaluable	17 (9.6%)	24 (14.0%)		
Overall response rate	92 (52.0%)	102 (59.3%)	0.74 (0.49 to 1.13)	0.1685
95% CI	44.4 to 59.5	51.6 to 66.7		
Duration of response				
Median, months	8.1	9.9	0.56 (0.40 to 0.79)	0.0010
R0 resection	14 (7.9%)	12 (7.0%)	–	0.7400
95% CI	4.4 to 12.9	3.7 to 11.9		
Overall survival				
Events	149 (84.2%)	136 (79.1%)	0.84 (0.66 to 1.06)	0.1411
Median (95% CI), months	17.6 (15.1 to 21.2)	22.3 (17.8 to 26.4)		

Data are n (%) unless otherwise stated.
Ratios listed are HRs, except for overall response rate for which an OR is presented.
FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; FOLFOXIRI, 5-fluorouracil, leucovorin, oxaliplatin and irinotecan.

common serious adverse events in the FOLFOXIRI-bevacizumab group were febrile neutropenia (n=15, 8.8% vs n=4, 2.3% in the FOLFOX-bevacizumab group), diarrhoea (n=14, 8.2% vs n=5, 2.8%), neutropenia (n=9, 5.3% vs n=1, 0.6%) and vomiting (n=8, 4.7% vs n=4, 2.3%).

Treatment-related deaths occurred in eight patients (4.7%) in the FOLFOXIRI-bevacizumab group and six patients (3.4%) in the FOLFOX-bevacizumab group: sepsis (n=5) and intestinal perforation (n=3) in the FOLFOXIRI-bevacizumab group; and intestinal perforation (n=2), sepsis (n=2), interstitial lung disease (n=1) and pneumonitis (n=1) in the FOLFOX-bevacizumab group.

Subsequent anticancer treatments

The distribution of second-line and later-line anticancer treatments was similar in both groups, except that more patients in the FOLFOX-bevacizumab group received irinotecan (64.4% vs 48.8% with FOLFOXIRI-bevacizumab), and more patients in the FOLFOXIRI-bevacizumab group received regorafenib (19.2% vs 9.6% with FOLFOX-bevacizumab) and trifluridine/tipiracil (12.8% vs 6.8%) (online supplemental table S8).

DISCUSSION

Our findings suggest that patients with metastatic colorectal cancer and an unfavourable prognosis defined by CTC count at baseline have improved survival outcomes with intensive upfront treatment with the cytotoxic triplet of FOLFOXIRI plus bevacizumab compared with a doublet plus bevacizumab. In this study, FOLFOXIRI-bevacizumab significantly improved median PFS by 3.1 months compared with FOLFOX-bevacizumab, with clear separation of the PFS curves over time indicative of long-term benefit. There was also a clinically relevant improvement of 4.7 months in median OS with FOLFOXIRI-bevacizumab versus FOLFOX-bevacizumab, although the study was not powered for OS and the finding did not attain statistical significance. Further long-term follow-up of patients is ongoing. We did note an initial crossing of both PFS and OS curves, which is likely due to a higher occurrence of drop-outs and early deaths attributable to adverse events with FOLFOXIRI-bevacizumab. It is notable that a similar pattern of survival events was recently reported with FOLFOXIRI-bevacizumab in the TRIBE-2 study.[6] The R0 resection rate in our study was low but similar in both study groups, although we did not look specifically at those with disease confined to the liver, a patient group that has previously been shown to benefit from FOLFOXIRI-bevacizumab.[5 16]

In terms of safety, the profile of adverse events reported with FOLFOXIRI-bevacizumab in the present study was consistent with other phase III trials of this regimen.[3 6] We observed high rates of neutropenia with FOLFOXIRI-bevacizumab among the first patients enrolled, which was subsequently corrected

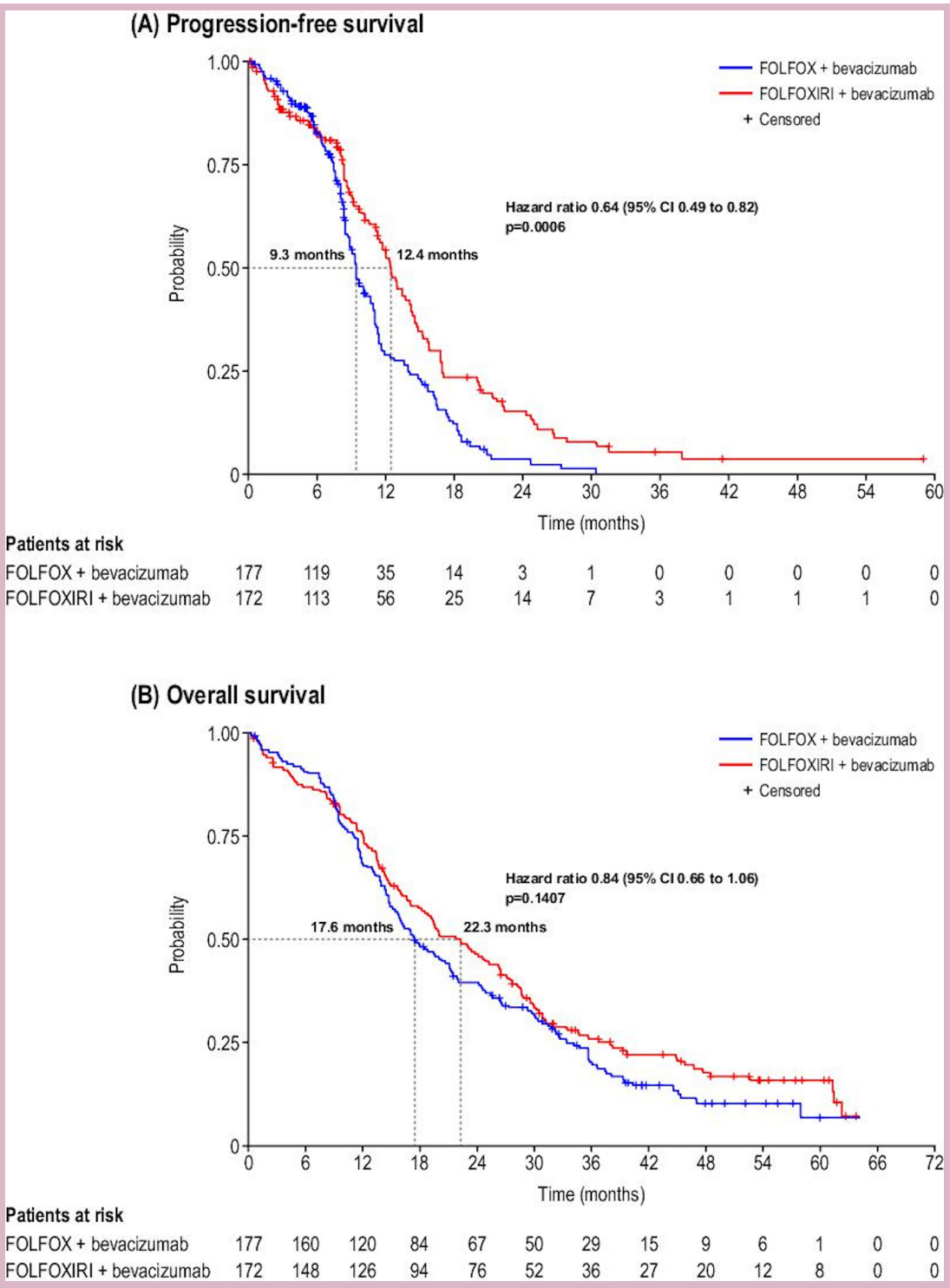

Figure 2 Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival in the intention-to-treat population. FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; FOLFOXIRI, 5-fluorouracil, leucovorin, oxaliplatin and irinotecan.

by the addition of G-CSF prophylaxis. The overall rate of grade 3/4 neutropenia observed in our study with FOLFOXIRI-bevacizumab (35%) compares favourably with rates reported in phase III trials that did not include G-CSF prophylaxis (50%).[3 6] Importantly, the rates of treatment-related deaths in both treatment groups were similar at study end.

The subgroup analysis of PFS from our study showed that FOLFOXIRI-bevacizumab was superior in all patient subgroups, with the exception of those with *PI3K*-mutated tumours (n=43) or right-sided tumours (n=93), thus confirming the benefit of intensified therapy across a range of prognostic factors among patients with ≥3 CTC/7.5 mL blood. These subgroups included patients

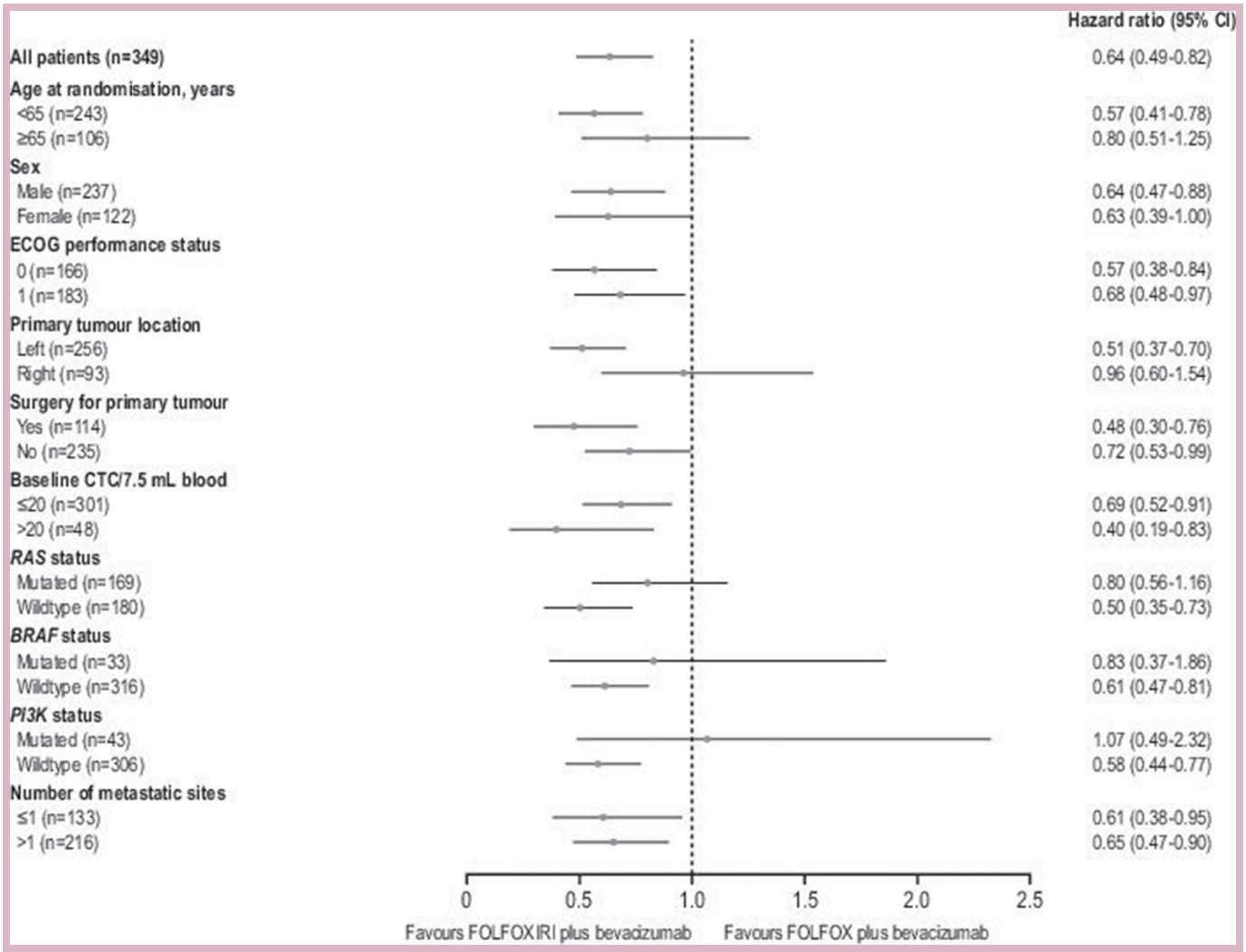

Figure 3 Progression-free survival: subgroup analysis. The vertical dashed line indicates a HR of 1.00—the null hypothesis value. Error bars represent 95% CIs. CTC, circulating tumour cell; ECOG, Eastern Cooperative Oncology Group; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; FOLFOXIRI, 5-fluorouracil, leucovorin, oxaliplatin and irinotecan.

with *RAS*-mutated tumours (HR 0.80; 95% CI 0.56 to 1.16), and are consistent with a recent meta-analysis[17] and phase II study[18] supporting the first-line use of FOLFOXIRI-bevacizumab in *RAS*-mutated tumours. A positive effect of FOLFOXIRI-bevacizumab was also evident in patients with *BRAF* mutations (HR 0.83; 95% CI 0.37 to 1.86), although the effect size for this subgroup was smaller than reported in the TRIBE study (HR=0.55).[3] Possible explanations for the difference between studies may be small patient numbers, or that there was a higher proportion of patients with a poorer performance status in the present study (ECOG score 1, 52% vs ECOG score 1/2, 10% in TRIBE).[3]

In VISNÚ-1, study treatment was recommended until disease progression or unacceptable toxicity, with protocol-specified dose modifications to manage adverse events and cumulative toxicities, consistent with what was known at the time the study was designed. Subsequently, the concept of induction therapy followed by maintenance therapy was developed to manage cumulative toxicities, an approach that has been used in other trials investigating FOLFOXIRI-bevacizumab, that is initial treatment limited to 4–6 months (induction therapy) followed by treatment with a de-escalated regimen (maintenance therapy).[3 5–7 19] We acknowledge these differences and that the duration of treatment in VISNÚ-1 was longer than other related studies, but it should be noted that dose modifications were used frequently in VISNÚ-1 to manage toxicities.

To our knowledge, VISNÚ-1 is the first prospective study to use CTC as a method to guide treatment choices in patients with metastatic colorectal cancer. Previous clinical research on CTCs has focused primarily on their role as either prognostic or predictive biomarkers. These results are particularly relevant as more treatments for colorectal cancer become available and the delineation between traditional lines of therapy becomes less distinct. It is hoped that our findings will encourage future studies using CTC count to guide treatment choices, as well as comparisons between

CTC-based measures with other markers of tumour burden (eg, circulating tumour DNA (ctDNA)[20]) or prognostic methods based on clinicopathological factors (eg, ARCAD nomogram[21]).

VISNÚ-1 was conducted in a carefully selected patient population appropriate for intensive first-line therapy and should not be generalised to broader patient populations. CellSearch, which isolates and enumerates CTC of epithelial origin, was used in the present study. The relevance of our findings to patients selected using other methods of CTC detection (ie, expression of other proteins or physical properties) or according to ctDNA is unknown. Our study included an assessment of CTC count at baseline only. Enumeration of CTCs during therapy is predictive of outcomes in patients with metastatic colorectal cancer,[11 13] and studies investigating escalation or de-escalation of therapy according to CTC count after initiation of therapy would be of interest. Future trials will also consider CTC mutations and gene expression, as well as CTC quantification, to provide a real-time genetic profile of the disease and to guide therapy. The present study did not include any patient-reported outcomes, and the effects of FOLFOXIRI–bevacizumab on patient quality of life and whether or not the benefits of treatment outweighed the increased risk of toxicity is unknown. Cost-effectiveness data that capture the use of CTC as a screening method would also be of interest.

In conclusion, first-line therapy with FOLFOXIRI-bevacizumab significantly improved PFS compared with FOLFOX-bevacizumab in patients with metastatic colorectal cancer and ≥3 CTCs at baseline, which indicate a poor prognosis, although with a higher rate of serious toxicities. These findings suggest CTC count may be a useful non-invasive biomarker to assist with the selection of patients suitable for intensive first-line therapy.

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Competing interests EA has done consulting or advisory roles for Amgen, Bayer, Celgene, Merck, Roche and Sanofi. JMV has done consulting or advisory roles for Amgen, Roche, Servier, received research funding from Amgen and Roche, done expert testimonies for Servier, and received travel, accommodation, and expenses from Amgen, Bristol-Myers Squibb, Roche, and Servier. AG-E has received travel, accommodation, and expenses from Amgen, Merck, Roche and Servier. BG has done consulting or advisory roles for Celgene and Sanofi, received research funding from Amgen, Celgene, Erytech Pharma, Gilead Sciences, Lilly, Parexel, and Sanofi, and travel, accommodation, and expenses from Amgen, Celgene, Roche, Sanofi and Servier. JJR-Z has done consulting or advisory roles for Amgen, Archimedes, Bayer, Ipsen, Merck, Novartis, Pfizer, Prostrakan, Roche, Sanofi, and Servier, and received travel, accommodation, and expenses from Amgen, Bayer, Ipsen, Merck, Novartis, Prostrakan, Roche, Sanofi, and Servier. CG-P has received travel, accommodation, and expenses from Sanofi/Aventis. RG-C declares having provided scientific advice and/or received honoraria from AAA, Amgen, Bayer, Bristol-Myers Squibb, Ipsen, Lilly, Merck, MSD, Novartis, PharmaMar, Pfizer, Roche and Sanofi, and has received research support from Bristol-Myers Squibb and Pfizer. MJS has done consulting or advisory roles for Amgen, Merck, Roche, Sanofi and Servier, and received travel, accommodation, and expenses from Amgen, Merck and Roche. ALCM has received travel, accommodation and expenses from Amgen. RLL has stock and other ownership interests in MTrap and Nasasbiotech SL, has done consulting or advisory roles for Bayer, Bristol-Myers Squibb, Janssen, Lilly, MSD, and Roche, done speakers' bureaus for Novartis and Roche, received institutional research funding from Lilly, Merck, and Roche, and received travel, accommodation, and expenses from Pierre Fabre, Roche and Tesaro. JS has done speaking honoraria for Ipsen, Lilly, Merck, MSD, Pfizer, Roche, Shire, and Servier, advisory roles for Amgen, Bayer, Bristol-Myers Squibb, Celgene, Ipsen, Merck, Roche, Sanofi, and Servier, and received travelling and accommodation support from Ipsen and Merck. ED-R has done consulting or advisory roles for Amgen, Bayer, Genomica, Merck Serono and Servier, speakers' bureaus for MSD and Servier, and received research funding from Amgen, AstraZeneca, Merck Serono, Roche and Sysmex. SGC, ASS, PG-A, FR, GAQ-A, EGF, MSF and BG declare no potential conflicts of interest.

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