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ORIGINAL ARTICLE

Analysis of angiogenesis biomarkers for ramucirumab efficacy in patients with metastatic colorectal cancer from RAISE, a global, randomized, double-blind, phase III study

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Background: The phase III RAISE trial (NCT01183780) demonstrated that the vascular endothelial growth factor (VEGF) receptor (VEGFR)-2 binding monoclonal antibody ramucirumab plus 5-fluororuracil, leucovorin, and irinotecan (FOLFIRI) significantly improved overall survival (OS) and progression-free survival (PFS) compared with placebo + FOLFIRI as second-line metastatic colorectal cancer (mCRC) treatment. To identify patients who benefit the most from VEGFR-2 blockade, the RAISE trial design included a prospective and comprehensive biomarker program that assessed the association of biomarkers with ramucirumab efficacy outcomes.

Patients and methods: Plasma and tumor tissue collection was mandatory. Overall, 1072 patients were randomized 1 : 1 to the addition of ramucirumab or placebo to FOLFIRI chemotherapy. Patients were then randomized 1 : 2, for the biomarker program, to marker exploratory (ME) and marker confirmatory (MC) groups. Analyses were carried out using exploratory assays to assess the correlations of baseline marker levels [VEGF-C, VEGF-D, sVEGFR-1, sVEGFR-2, sVEGFR-3 (plasma), and VEGFR-2 (tumor tissue)] with clinical outcomes. Cox regression analyses were carried out for each candidate biomarker with stratification factor adjustment.

Results: Biomarker results were available from >80% ($n = 894$) of patients. Analysis of the ME subset determined a VEGF-D level of 115 pg/ml was appropriate for high/low subgroup analyses. Evaluation of the combined ME + MC populations found that the median OS in the ramucirumab + FOLFIRI arm compared with placebo + FOLFIRI showed an improvement of 2.4 months in the high VEGF-D subgroup [13.9 months (95% CI 12.5–15.6) versus 11.5 months (95% CI 10.1–12.4), respectively], and a decrease of 0.5 month in the low VEGF-D subgroup [12.6 months (95% CI 10.7–14.0) versus 13.1 months (95% CI 11.8–17.0), respectively]. PFS results were consistent with OS. No trends were evident with the other antiangiogenic candidate biomarkers.

Conclusions: The RAISE biomarker program identified VEGF-D as a potential predictive biomarker for ramucirumab efficacy in second-line mCRC. Development of an assay appropriate for testing in clinical practice is currently ongoing.

Clinical trials registration: NCT01183780.

Key words: ramucirumab, colorectal cancer, VEGF-D, biomarkers, predictive

Introduction

Colorectal carcinoma (CRC) is the third leading type of cancer and cause of cancer deaths worldwide [1, 2]. Metastatic CRC (mCRC) develops in approximately half of the patients diagnosed with CRC [3]. The poor prognosis of mCRC drives ongoing efforts to find treatments that improve patients' outcomes [4]. A principal goal of translational research (TR) is the identification of biomarkers to better select treatment options for patients. The discovery of predictive biomarkers for drug efficacy, particularly for antiangiogenic treatments, has been disappointing despite huge efforts and investments. Therefore, selection of patients more likely to benefit from antiangiogenic therapy has not yet been possible, and, to date, no biomarker has been identified and validated in mCRC to predict antiangiogenic treatment efficacy.

Ramucirumab is a human IgG1 monoclonal antibody that specifically binds to the extracellular domain of vascular endothelial growth factor (VEGF) receptor (VEGFR)-2 with high affinity, preventing binding of the agonist ligands VEGF-A, VEGF-C, and VEGF-D and, consequently, VEGFR-2 activation [5].

The safety and efficacy of ramucirumab in combination with 5-fluororuracil, leucovorin, and irinotecan (FOLFIRI) as second-line therapy for patients with mCRC that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine were evaluated in a randomized, double-blind, placebo-controlled, phase III trial (RAISE; NCT01183780) [6]. The RAISE trial demonstrated a statistically significant survival benefit for patients treated with ramucirumab + FOLFIRI versus placebo + FOLFIRI with a median overall survival (OS) of 13.3 months for the ramucirumab group versus 11.7 months for the placebo group [hazard ratio (HR) 0.84; 95% confidence interval (CI) 0.73–0.98; log-rank $P = 0.0219$]. The prespecified subgroup analyses did not identify any factors that predicted ramucirumab efficacy, showing a consistent treatment effect for the analyzed variables [6, 7].

One of the planned study end points was to identify predictive biomarkers for ramucirumab efficacy in second-line mCRC. Investigations focused on angiogenesis-related mediators such as VEGF family members and their receptors.

Methods

Study design

Details of the RAISE trial, including patient eligibility, trial design, randomization, dose administration, clinical outcome definitions, and statistical analyses, were published [6]. Briefly, eligible patients included those with pathologically confirmed mCRC, known *KRAS* exon 2 mutation status (mutant or wild-type); an Eastern Cooperative Oncology Group performance status of 0 or 1; and disease progression during or within 6 months of the last dose of first-line combination therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine for metastatic disease. Patients were randomized 1: 1 to receive on day 1 of each 2-week cycle either 8 mg/kg ramucirumab or placebo as a 60-min intravenous infusion, followed by the FOLFIRI regimen (180 mg/m^2 irinotecan, 400 mg/m^2 leucovorin, and 400 mg/m^2 fluorouracil as a bolus, then 2400 mg/m^2 as a 48-h infusion).

Sample collection and VEGF ligand and receptor analysis

Plasma and tumor tissue collection was mandatory. Analyses were carried out to assess the correlations of the baseline individual marker levels with clinical outcomes. Plasma samples were collected from whole blood before cycle 1. VEGF-C, VEGF-D, soluble VEGFR-1 (sVEGFR-1), sVEGFR-2, and sVEGFR-3 were assessed by exploratory, individual, proprietary Eli Lilly and Company—developed dual-monoclonal sandwich immunoassays (Version 1 for each) [8]. Additional method details for the VEGF-D assay are provided as supplementary material, available at *Annals of Oncology* online. VEGF-A was not assessed because blood samples were collected in heparin tubes, and it has been determined that heparin interferes with the bioanalytical assay for VEGF-A, such that reliable results cannot be obtained. Archived tumor samples were submitted to the central laboratory for VEGFR-2 immunohistochemistry assay [9]. A semi-quantitative VEGFR-2 vascular H-score (range 0–300) was determined, based on the immunostaining intensity and the proportion of vessel cells stained within the invasive tumor stroma. All assays were carried out and scored while blinded to the study treatment and outcomes.

Statistical analysis

An adaptive analysis design [10] was used in which the population for the study was randomly and prospectively split, after initial randomization, into a marker exploratory (ME) set and a marker confirmatory (MC) set in a 1 : 2 ratio. This approach allowed the broad exploration of markers in the ME set of patients and then pre-specification for any noteworthy findings to be confirmed independently in the MC set of patients. Stratification was applied to balance the ME and MC sets according to treatment assignment and the three study stratification factors: geographic region, *KRAS* exon 2 mutation status, and time to disease progression on the first-line treatment. Kaplan–Meier estimates and 95% CIs were used to analyze OS and progression-free survival (PFS). Cox regression analyses were carried out for each marker with stratification factors adjusted. A subpopulation treatment effect pattern plot (STEPP) was used to evaluate the relationship of each marker's levels with efficacy outcomes. In generating the STEPP, the treatment effect was assessed in subsets of patients who had similar biomarker levels, with the subsets together spanning the full range of that marker's values (known as a sliding window approach).

Results

Biomarker results were available from >80% ($n = 894$) of patients. Median levels for markers are shown in supplementary Table S1, available at *Annals of Oncology* online.

At the initial plasma ME subset analyses (ramucirumab, $n = 153$; placebo, $n = 146$), VEGF-D showed a strong signal associating higher levels with greater improvement in OS and PFS in the ramucirumab arm (supplementary Figure S1, available at *Annals of Oncology* online). To test the relationship of VEGF-D levels with efficacy outcomes seen in the dataset from the ME patients, a VEGF-D level of 115 pg/ml was pre-specified (based on multiple analyses from ME set) as the cut-off value for high and low subgroup analysis of the independent MC population and the full TR population (ME + MC populations). The flow of VEGF-D patients is diagrammed in supplementary Figure S2, available at *Annals of Oncology* online.

As shown in supplementary Table S2, available at *Annals of Oncology* online, the results in the MC population independently confirmed the ability of the pre-specified cutoff from the ME dataset to predict ramucirumab efficacy (interaction $P = 0.0107$ and 0.0013 for OS and PFS, respectively). Here we present the full TR population (ME + MC) analysis to provide the overall results of the candidate biomarkers from the RAISE trial. The TR population had similar demographics and baseline disease characteristics compared with the intent-to-treat (ITT) population

(supplementary Table S3, available at *Annals of Oncology* online). Additionally, the OS stratified HR for the plasma TR population was 0.89 (supplementary Figure S3A, available at *Annals of Oncology* online), and the PFS stratified HR for the plasma TR population was 0.80 (supplementary Figure S3B, available at *Annals of Oncology* online). Altogether, these results indicate that the overall plasma TR population is representative of the ITT population.

The TR population was divided into subgroups of patients with high VEGF-D plasma levels (≥115 pg/ml; $n = 536$), representing 61% of the TR population, and patients with low VEGF-D levels (<115 pg/ml; $n = 348$), representing 39% of the TR population. The demographics and baseline characteristics for patients in the high and low VEGF-D groups are shown in Table 1.

In the high VEGF-D group, ramucirumab + FOLFIRI patients ($n = 270$) had a median OS of 13.9 months (95% CI 12.5–15.6), and placebo + FOLFIRI patients ($n = 266$) had a median OS of 11.5 months (95% CI 10.1–12.4), with a stratified HR of 0.73 (95% CI 0.60–0.89; $P = 0.0022$; Figure 1A). Ramucirumab + FOLFIRI patients had a median PFS of 6.0 months (95% CI 5.6–7.0), and placebo + FOLFIRI patients had a median PFS of 4.2 months (95% CI 4.1–4.5), with a stratified HR of 0.62 (95% CI 0.52–0.74; $P < 0.0001$; Figure 2A). In the low VEGF-D group, ramucirumab + FOLFIRI patients ($n = 176$) had a median OS of 12.6 months (95% CI 10.7–14.0), and placebo + FOLFIRI patients ($n = 172$) had a median OS of 13.1 months (95% CI 11.8–17.0), with a stratified HR of 1.32 (95% CI 1.02–1.70; $P = 0.0344$; Figure 1B). Ramucirumab + FOLFIRI patients had a median PFS of 5.4 months (95% CI 4.2–5.8), and placebo + FOLFIRI patients had a median PFS of 5.6 months (95% CI 5.3–6.9), with a stratified HR of 1.16 (95% CI 0.93–1.45; $P = 0.1930$; Figure 2B). Interaction analyses using the 115 pg/ml cut-off were statistically significant for both OS and PFS ($P = 0.0005$ and $P < 0.0001$, respectively).

Table 1. Baseline patient characteristics by VEGF-D levels

	VEGF-D High		VEGF-D Low	
	Ramucirumab + FOLFIRI (*N* = 270) *n* (%)	**Placebo + FOLFIRI (*N* = 266) *n* (%)**	**Ramucirumab + FOLFIRI (*N* = 176) *n* (%)**	**Placebo + FOLFIRI (*N* = 172) *n* (%)**
Age group				
≥65 years	111 (41)	109 (41)	64 (36)	70 (41)
≥70 years	59 (22)	53 (20)	31 (18)	42 (24)
Gender				
Male	137 (51)	162 (61)	100 (57)	110 (64)
Female	133 (49)	104 (39)	76 (43)	62 (36)
Geographic region				
Japan/East Asia	67 (25)	61 (23)	33 (19)	28 (16)
Rest of world	203 (75)	205 (77)	143 (81)	144 (84)
Race				
Black	8 (3)	9 (3)	6 (3)	5 (3)
Other	72 (27)	70 (26)	38 (22)	30 (17)
White	189 (70)	185 (70)	132 (75)	134 (78)
Missing	1 (<1)	2 (1)	–	3 (2)
ECOG PS				
0	143 (53)	126 (47)	88 (50)	93 (54)
1	127 (47)	140 (53)	87 (49)	79 (46)
Missing	–	–	1 (1)	–
Time to progression after first-line				
<6 months	63 (23)	71 (27)	43 (24)	36 (21)
≥6 months	207 (77)	195 (73)	133 (76)	136 (79)
***KRAS* status**				
Mutant	137 (51)	119 (45)	89 (51)	86 (50)
Wild type	133 (49)	147 (55)	87 (49)	86 (50)
CEA				
>10 μg/l	179 (66)	178 (67)	126 (72)	111 (65)
≤10 μg/l	75 (28)	74 (28)	43 (24)	49 (28)
≥200 μg/l	51 (19)	59 (22)	43 (24)	28 (16)
<200 μg/l	203 (75)	193 (73)	126 (72)	132 (77)
Missing	16 (6)	14 (5)	7 (4)	12 (7)

CEA, carcinoembryonic antigen; ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFIRI, 5-fluororuracil, leucovorin, and irinotecan; VEGF-D, vascular endothelial growth factor D.

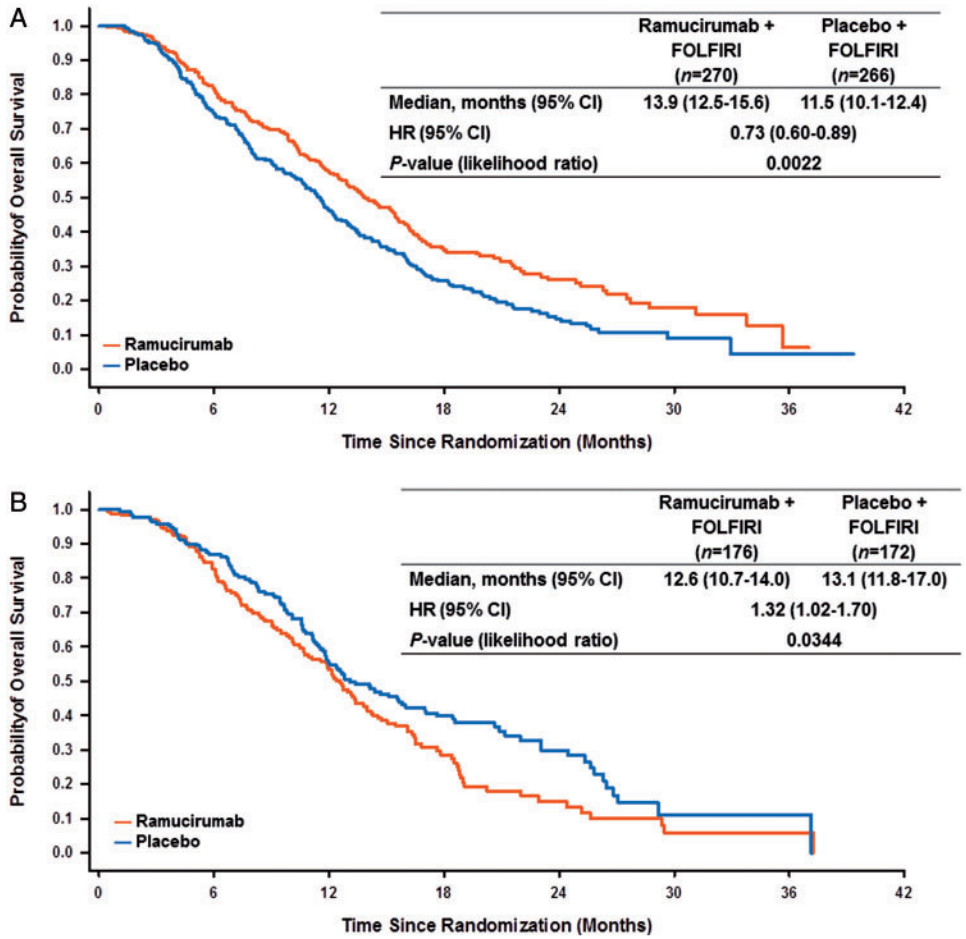

Figure 1. Overall survival in patients receiving ramucirumab + FOLFIRI compared with that in patients receiving placebo + FOLFIRI in patients with (A) high VEGF-D expression levels (≥115 pg/ml) and (B) low VEGF-D expression levels (<115 pg/ml). CI, confidence interval; FOLFIRI, 5-fluororuracil, leucovorin, and irinotecan; HR, hazard ratio; VEGF-D, vascular endothelial growth factor D.

The response rate for both VEGF-D groups (supplementary Table S4, available at *Annals of Oncology* online) was roughly similar to the ITT population [6].

In addition to the predictive relationship, VEGF-D was also found to be prognostic, as indicated by the worse outcomes within the placebo + FOLFIRI group for patients with high VEGF-D [median OS 11.5 months (95% CI 10.1–12.4)] compared with patients with low VEGF-D [median OS 13.1 months (95% CI 11.8–17.0); marker prognostic HR 1.42 (95% CI 1.1–1.8); $P = 0.0025$].

To examine the VEGF-D predictive relationship more granularly, STEPP figures were created. These figures show the point estimate for the treatment HR across a range of VEGF-D levels. For both OS and PFS, a consistent relationship was observed between HR and VEGF-D values (Figure 3A and B, respectively). These figures also demonstrate that the 115 pg/ml cut-off identified, based on the results from the ME dataset, is well suited to the data from the TR population. Examination of VEGF-D plasma levels by tumor side revealed a similar distribution for VEGF-D levels among patients in the left versus right subgroups (supplementary Table S5, available at *Annals of Oncology* online). Additionally, no correlation was found between VEGF-D and carcinoembryonic antigen (CEA) levels (supplementary Table S6, available at *Annals of Oncology* online). These results suggest that the greater ramucirumab efficacy shown in patients with high VEGF-D levels is independent of primary CRC tumor side and CEA baseline values.

The relationships of OS and PFS results to levels of sVEGFR-1, sVEGFR-2, sVEGFR-3, VEGF-C in plasma, and vascular VEGFR-2 by immunohistochemistry in tumor are summarized in supplementary Figures S4–S8, respectively. As with the results from the ME set alone, no clear trends were evident for these markers in the TR population.

Treatment-emergent adverse events (TEAEs) and adverse events of special interest were analyzed by treatment arm and VEGF-D level (Table 2). Most TEAEs that occurred more frequently among ITT patients treated with ramucirumab + FOLFIRI (hypertension, thrombocytopenia, diarrhea, and fatigue) [6] were elevated to a similar extent in both VEGF-D groups. Grade ≥3 neutropenia was higher in the high VEGF-D ramucirumab group (42%) than in the low VEGF-D ramucirumab group (32%; Table 2); however, febrile neutropenia events were infrequent and had similar incidences in the high and low VEGF-D groups. Adverse events of special interest (those associated with anti-VEGF therapies) showed a similar incidence across the TR population (Table 2).

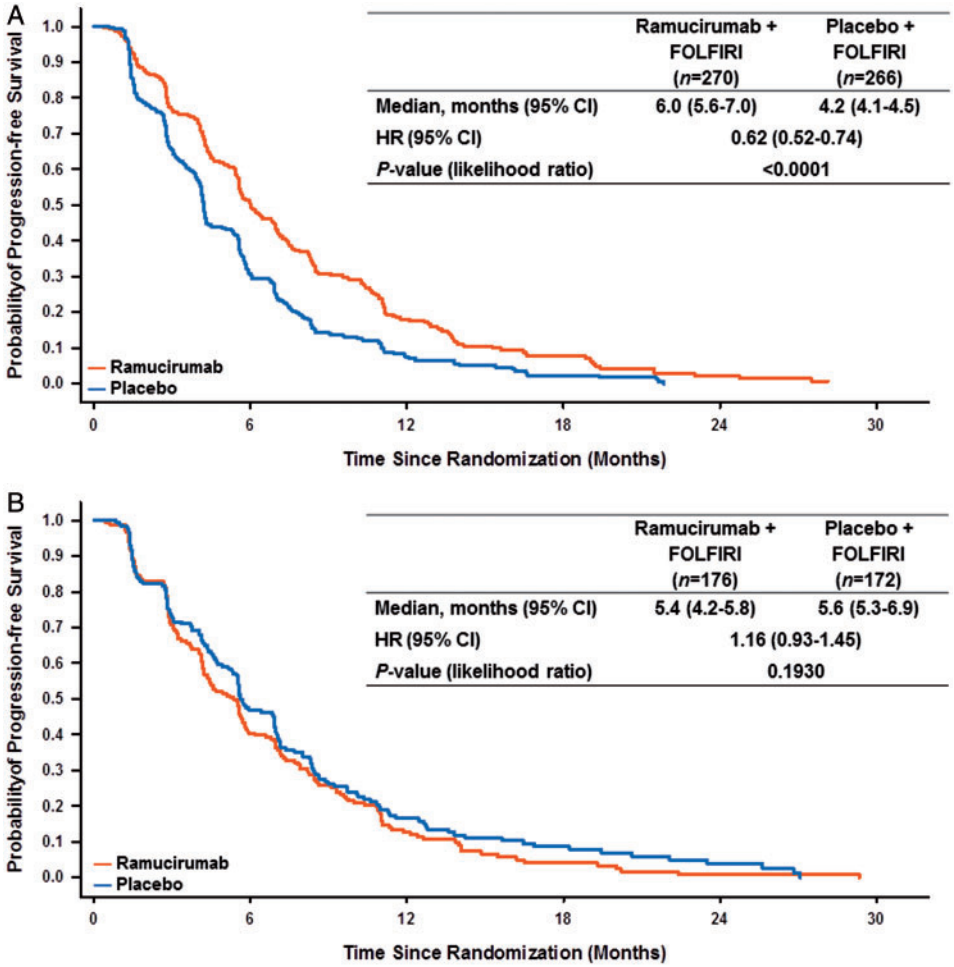

Figure 2. Progression-free survival in patients receiving ramucirumab + FOLFIRI compared with that in patients receiving placebo + FOLFIRI in patients with (A) high VEGF-D expression levels (≥115 pg/ml) and (B) low VEGF-D expression levels (<115 pg/ml). CI, confidence interval; FOLFIRI, 5-fluororuracil, leucovorin, and irinotecan; HR, hazard ratio; VEGF-D, vascular endothelial growth factor D.

Discussion

In the RAISE study, second-line treatment with ramucirumab in combination with FOLFIRI demonstrated a statistically significant survival benefit for mCRC patients when compared with placebo + FOLFIRI [6]. This report presents an evaluation of VEGF ligands and receptors as predictive or prognostic markers for ramucirumab efficacy. A consistent relationship was observed for greater ramucirumab efficacy (both OS and PFS) in mCRC patients with higher baseline plasma VEGF-D levels, with a median OS benefit from ramucirumab of 2.4 months. On the other hand, in patients with low VEGF-D, the difference in median OS was 15 days, favoring placebo. Similar differences were observed for PFS. The other markers evaluated—sVEGFR-1, sVEGFR-2, sVEGFR-3, VEGF-C, and vascular VEGFR-2 in tumor tissue—did not demonstrate any clear trends.

Angiogenic factors play different roles in the angiogenesis pathways. VEGF-A binding to VEGFR-2 is believed to be the key signaling for the activation of the VEGF angiogenesis pathway. However, other factors and receptors are also involved in the activation of the VEGF pathway, such as VEGF-C and VEGF-D binding to VEGFR-2 and VEGFR-3. Redundancy among family members of angiogenic regulators has been established [11], and it was hypothesized that related VEGF family members such as VEGF-C and VEGF-D may continue to stimulate angiogenesis despite inhibition by VEGF-A antibodies such as bevacizumab [12, 13]. Published data associating the efficacy of antiangiogenic therapy with VEGF-D levels in mCRC are scarce, but two publications analyzed that relationship in trials containing bevacizumab. The first one, from the AGITG MAX trial, assessed the relationship of angiogenic biomarker levels, assayed by immunohistochemistry in tumor tissue, with patients' outcomes [14]. It was a three-arm phase III study evaluating the effect on PFS of adding bevacizumab with or without mitomycin to capecitabine chemotherapy as first-line therapy for mCRC [14]. The AGITG MAX analysis evaluated the predictive correlation between expression of angiogenesis-related factors (VEGF-A to VEGF-D, VEGFR-1, and VEGFR-2 in tumor tissue) and outcomes of treatments with or without bevacizumab [15]. Tumor specimens were available for examination of VEGF and VEGFR expression from 57% of the study population ($n = 268$). Of the six biomarkers analyzed, only VEGF-D served as a predictor of bevacizumab efficacy on survival (OS and PFS). High expression of VEGF-D predicted resistance to bevacizumab plus chemotherapy, whereas

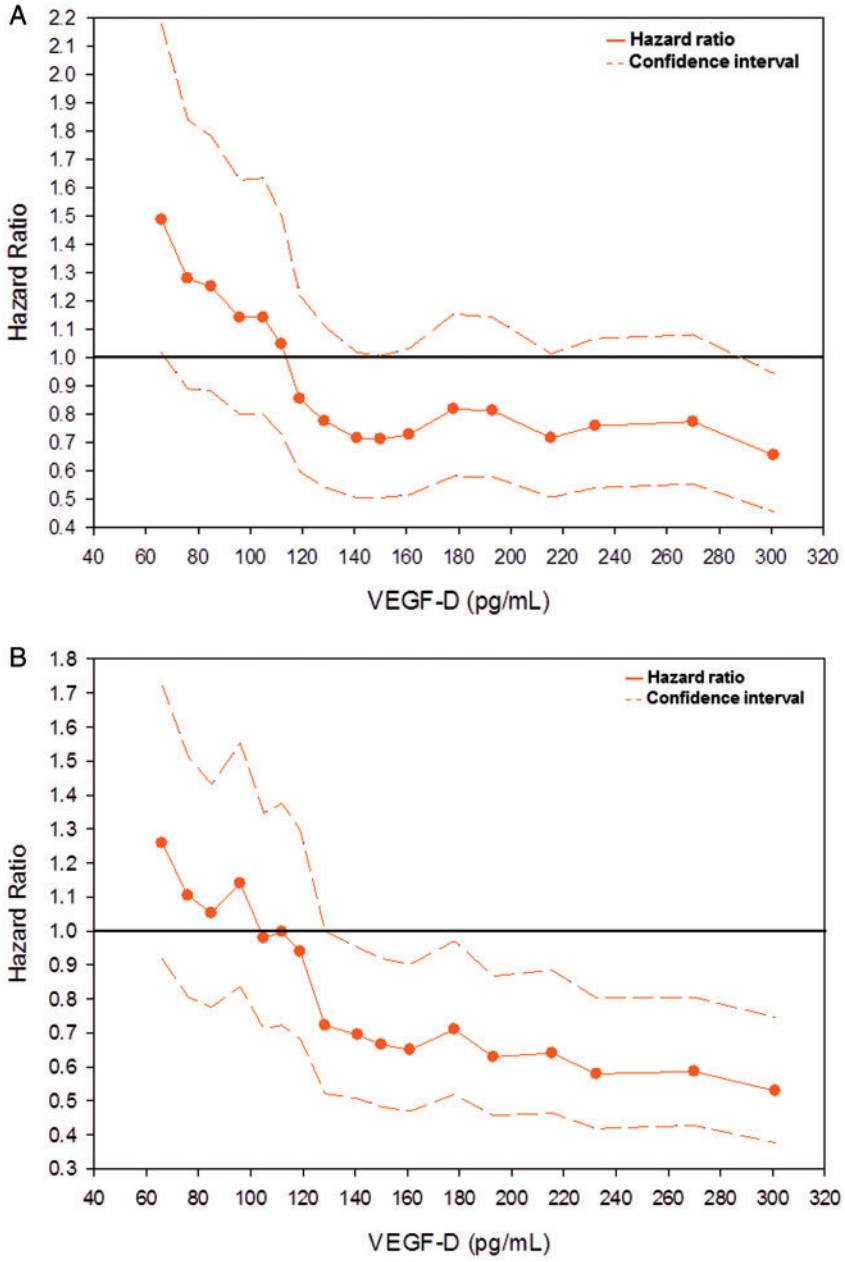

Figure 3. VEGF-D ($N = 884$) subpopulation treatment effect pattern plot (STEPP) with sliding windows of size 200 with the largest overlap between windows of size 160 for (A) overall survival and (B) progression-free survival. VEGF-D, vascular endothelial growth factor D.

low expression of VEGF-D was associated with longer OS (HR 0.35; 95% CI 0.13–0.90) and PFS (HR 0.22; 95% CI 0.08–0.55) (P interaction <0.05). The apparent higher benefit from bevacizumab in patients with low VEGF-D is in contrast to the findings in the RAISE trial, in which patients with higher levels of VEGF-D derived more benefit with ramucirumab.

The second related publication was the biomarker analysis of the Cancer and Leukemia Group B (CALGB)/Southwest Oncology Group Trial 80405. The phase III CALGB study ($N = 1137$) evaluated the efficacy and safety of adding bevacizumab, cetuximab, or both to either FOLFOX or FOLFIRI as first-line treatment of advanced CRC [11, 16]. In the biomarker analysis, seven previously identified candidate biomarkers associated with outcomes for either bevacizumab or cetuximab were assessed via multiplex enzyme-linked immunosorbent assay (ELISA) systems [12]. Once again, VEGF-D associated with a signal. Lower plasma VEGF-D levels, analyzed by quartiles, were associated with greater OS benefit from bevacizumab [HR 0.62 (95% CI 0.41–0.92) for the first quartile]. For the higher three quartiles, no benefit from bevacizumab could be demonstrated [HRs ranging from 1.02 (95% CI 0.71–1.47) to 1.34 (95% CI 0.91–1.97)]. A similar relationship was identified for PFS [11]. This analysis and that of the AGITG MAX trial [14] suggest that bevacizumab seems to provide the greatest benefit to patients with low circulating VEGF-D levels. One noteworthy comparison between trials is that the baseline plasma levels of VEGF-D measured in the CALGB trial were 10 times higher than those measured in RAISE (median = 1.1 ng/ml for CALGB versus

Table 2. Treatment-emergent adverse events by VEGF-D cut point (115 pg/ml)

	Any grade				Grade ≥3			
	RAM+FOLFIRI		PBO+FOLFIRI		RAM+FOLFIRI		PBO+FOLFIRI	
	VEGF-D Low (*N* =176) *n* (%)	VEGF-D High (*N* =271) *n* (%)	VEGF-D Low (*N* =172) *n* (%)	VEGF-D High (*N* =265) *n* (%)	VEGF-D Low (*N* =176) *n* (%)	VEGF-D High (*N* =271) *n* (%)	VEGF-D Low (*N* =172) *n* (%)	VEGF-D High (*N* =265) *n* (%)
Any TEAE[a]	173 (98)	268 (99)	168 (98)	263 (99)	133 (76)	219 (81)	110 (64)	160 (60)
Diarrhea	106 (60)	163 (60)	100 (58)	130 (49)	18 (10)	33 (12)	21 (12)	22 (8)
Neutropenia	98 (56)	171 (63)	79 (46)	127 (48)	56 (32)	115 (42)	36 (21)	73 (28)
Fatigue	96 (55)	158 (58)	95 (55)	128 (48)	15 (9)	33 (12)	17 (10)	15 (6)
Nausea	91 (52)	138 (51)	90 (52)	136 (51)	5 (3)	6 (2)	3 (2)	9 (3)
Decreased appetite	65 (37)	114 (42)	47 (27)	73 (28)	3 (2)	9 (3)	3 (2)	2 (1)
Stomatitis	58 (33)	89 (33)	41 (24)	53 (20)	10 (6)	8 (3)	5 (3)	6 (2)
Constipation	57 (32)	72 (27)	44 (26)	56 (21)	0	4 (1)	4 (2)	3 (1)
Alopecia	55 (31)	78 (29)	67 (39)	79 (30)	0	0	0	0
Vomiting	54 (31)	80 (30)	52 (30)	70 (26)	5 (3)	8 (3)	3 (2)	9 (3)
Hypertension	49 (28)	71 (26)	19 (11)	20 (8)	22 (13)	28 (10)	8 (5)	4 (2)
Abdominal pain	49 (28)	69 (25)	38 (22)	72 (27)	7 (4)	9 (3)	7 (4)	8 (3)
Epistaxis	48 (27)	101 (37)	27 (16)	42 (16)	0	0	0	0
Thrombocytopenia	38 (22)	83 (31)	25 (15)	37 (14)	4 (2)	9 (3)	0	3 (1)
Peripheral edema	27 (15)	63 (23)	15 (9)	26 (10)	1 (<1)	0	0	0
Anemia	29 (16)	49 (18)	43 (25)	51 (19)	2 (1)	6 (2)	6 (3)	10 (4)
Adverse events of special interest								
Hypertension	50 (28)	72 (27)	19 (11)	20 (8)	23 (13)	29 (11)	8 (5)	4 (2)
Venous thromboembolic events	16 (9)	21 (8)	14 (8)	12 (5)	10 (6)	10 (4)	4 (2)	3 (1)
Proteinuria	32 (18)	47 (17)	3 (2)	16 (6)	6 (3)	8 (3)	0	1 (<1)
Bleeding/hemorrhage event	65 (37)	130 (48)	38 (22)	60 (23)	3 (2)	9 (3)	4 (2)	3 (1)
GI perforation	3 (2)	5 (2)	0	3 (1)	3 (2)	5 (2)	0	3 (1)
Congestive heart failure	3 (2)	0	0	1 (<1)	3 (2)	0	0	1 (<1)
GI hemorrhage events	13 (7)	40 (15)	12 (7)	13 (5)	2 (1)	7 (3)	2 (1)	2 (1)
Infusion-related reaction	11 (6)	17 (6)	7 (4)	8 (3)	2 (1)	2 (1)	2 (1)	0
Renal failure	4 (2)	10 (4)	9 (5)	6 (2)	1 (1)	4 (1)	3 (2)	2 (1)
Arterial thromboembolic events	1 (1)	6 (2)	1 (1)	9 (3)	0	3 (1)	1 (1)	4 (2)
Healing complication	3 (2)	2 (<1)	1 (<1)	0	1 (<1)	0	0	0
Congestive heart failure	3 (2)	0	0	1 (<1)	3 (2)	0	0	1 (<1)
Fistula	2 (1)	1 (<1)	1 (<1)	0	0	0	0	0
Pulmonary hemorrhage events	1 (<1)	5 (2)	2 (1)	1 (<1)	0	0	0	0
Reversible posterior leukoencephalopathy syndrome	0	1 (<1)	0	1 (<1)	0	0	0	0
Hepatic hemorrhage events	0	0	1 (<1)	0	0	0	1 (<1)	0

Terms in italics are consolidated terms. TEAEs graded by NCI-CTCAE v4.0.

[a]All grades 20% or higher or grade ≥3 5% or higher in either treatment arm.

FOLFIRI, 5-fluororuracil, leucovorin, and irinotecan; GI, gastrointestinal; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; PBO, placebo; RAM, ramucirumab; TEAE, treatment-emergent adverse event; VEGF-D, vascular endothelial growth factor D.

0.135 ng/ml for RAISE), which raises a question regarding potential differences in assays used.

In patients with high levels of VEGF-D, the angiogenesis pathway may be mainly activated by VEGF-D, which is not blocked by VEGF-A binding antibodies such as bevacizumab. The high VEGF-D levels could exist as an initial disease state or potentially could arise as a mechanism of antiangiogenic resistance to therapeutic strategies that selectively block VEGF-A but not VEGF-D. An increase in VEGF-D could thus serve as an angiogenic switch from VEGF-A driving tumors to VEGF-D. Therefore, patients with high VEGF-D levels who progressed after first-line therapy containing bevacizumab, as in the RAISE study population, could derive greater benefit from VEGFR-2 inhibition with ramucirumab. The RAISE results suggest that ramucirumab-containing treatment may compensate for the negative prognostic value associated with high VEGF-D levels, yielding a difference in median OS of 2.4 months and similar outcomes to those seen with low VEGF-D and a better prognosis.

In an unselected population, second-line ramucirumab treatment could provide a remarkable benefit for those with high VEGF-D levels, whereas the 15-day difference observed between treatment arms for those with low VEGF-D is of questionable clinical relevance. Overall, the risk-benefit ratio from the unselected ITT population continues to favor the use of ramucirumab.

In conclusion, higher levels of VEGF-D expression are a potential predictive biomarker for ramucirumab efficacy (OS and PFS). Despite the biologic plausibility of the association between VEGF-D levels and patients' outcomes in the RAISE trial, these findings were obtained with an assay that was developed and validated for exploratory research purposes only. Development and validation of an assay appropriate for clinical testing and decision making are currently underway. If successful, this assay will be used to confirm the relationship observed in the RAISE samples.

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