Biomarker analysis beyond angiogenesis: RAS/RAF mutation status, tumour sidedness, and second-line ramucirumab efficacy in patients with metastatic colorectal carcinoma from RAISE—a global phase III study

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Background: Second-line treatment with ramucirumab+FOLFIRI improved overall survival (OS) versus placebo+FOLFIRI for patients with metastatic colorectal carcinoma (CRC) [hazard ratio (HR)=0.84, 95% CI 0.73–0.98, P = 0.022]. Post hoc analyses of RAISE patient data examined the association of RAS/RAF mutation status and the anatomical location of the primary CRC tumour (left versus right) with efficacy parameters.

Patients and methods: Patient tumour tissue was classified as BRAF mutant, KRAS/NRAS (RAS) mutant, or RAS/BRAF wild-type. Left-CRC was defined as the splenic flexure, descending and sigmoid colon, and rectum; right-CRC included transverse, ascending colon, and cecum.

Results: RAS/RAF mutation status was available for 85% of patients (912/1072) and primary tumour location was known for 94.4% of patients (1012/1072). A favourable and comparable ramucirumab treatment effect was observed for patients with RAS mutations (OS HR = 0.86, 95% CI 0.71–1.04) and patients with RAS/BRAF wild-type tumours (OS HR = 0.86, 95% CI 0.64–1.14). Among the 41 patients with BRAF-mutated tumours, the ramucirumab benefit was more notable (OS HR = 0.54, 95% CI 0.25–1.13), although, as with the other genetic sub-group analyses, differences were not statistically significant. Progression-free survival (PFS) data followed the same trend. Treatment-by-mutation status interaction tests (OS P = 0.523, PFS P = 0.655) indicated that the ramucirumab benefit was not statistically different among the mutation subgroups, although the small sample size of the BRAF group limited the analysis. Addition of ramucirumab to FOLFIRI improved left-CRC median OS by 2.5 month over placebo (HR = 0.81, 95% CI 0.68–0.97); median OS for ramucirumab-treated patients with right-CRC was
**Introduction**

The global, randomised, double-blind, placebo-controlled, RAISE phase III trial examined whether patients with metastatic colorectal carcinoma (mCRC) who had been previously treated with first-line bevacizumab, oxaliplatin, and a fluoropyrimidine would exhibit improved survival when ramucirumab was added to second-line FOLFIRI (folinic acid, 5-fluorouracil, and irinotecan) treatment [1]. The human IgG1 monoclonal antibody, ramucirumab, inhibits tumour angiogenesis by binding to vascular endothelial growth factor (VEGF) receptor-2 (VEGFR-2) and interfering with VEGF ligand binding [2]. Results from the RAISE trial indicated that the addition of ramucirumab to second-line FOLFIRI improved overall survival (OS) over placebo+FOLFIRI [median OS 13.3 versus 11.7 months; hazard ratio (HR)=0.84; 95% confidence interval (CI) 0.73–0.98; P = 0.022] [1]. Median progression-free survival (PFS) was also extended by the addition of ramucirumab (5.7 versus 4.5 months, HR = 0.79; 95% CI 0.70–0.90; P < 0.0005) [1].

Analysis of patient sub-groups and biomarkers has aimed to identify patient or tumour characteristics associated with an improved ramucirumab benefit. Using an exploratory assay, high baseline plasma VEGF-D levels (≥115 pg/ml) were associated with better survival outcomes for ramucirumab-treated patients [3]. Low baseline plasma carcinoembryonic antigen (CEA) levels (≤10 ng/ml) were also associated with an enhanced ramucirumab response [4]. The KRAS exon 2 mutation is known to affect CRC response to EGFR inhibitors, but its impact, if any, on ramucirumab is not known. A pre-specified analysis showed that both KRAS exon 2 mutant and KRAS exon 2 wild-type tumours demonstrated a consistent survival benefit in favour of the ramucirumab+FOLFIRI arm [5]. More recent data demonstrated that other RAS mutations (KRAS exons 3 and 4, NRAS) and the BRAF mutation also reduce benefit from anti-EGFR therapies [6]; therefore, the impact of these mutations on ramucirumab efficacy must be examined as well.

In addition to the possible impact of gene mutations, evidence indicates that the location of the primary CRC has prognostic implications and may be predictive of response to anti-EGFR therapy [7, 8]. This phenomenon may be explained in part by the different embryologic origin of the left and right colon and the resultant anatomical, histological, molecular, and environmental differences that impact tumours arising along its length [7].

Given evidence that additional RAS/RAF mutations and tumour sidedness impact EGFR-directed treatment, we undertook retrospective analyses of the association of these parameters and the efficacy of the VEGFR inhibitor, ramucirumab, using data from the RAISE phase III clinical trial.

**Methods**

**Study design**

The design of the RAISE phase III trial (ClinicalTrials.gov, NCT01183780) has been reported [1]. In brief, eligible patients had pathologically confirmed mCRC that had progressed during first-line treatment with bevacizumab, oxaliplatin, and a fluoropyrimidine or within 6 months of the last dose of first-line therapy. Patients were randomised (1:1) to ramucirumab or placebo, with stratification by geography (North America versus Europe versus all other regions), KRAS exon 2 status (wild-type versus mutant), and time to first-line disease progression (≥6 versus <6 months). Ramucirumab (8 mg/kg) or placebo was administered on day 1 of each 2-week cycle, followed by FOLFIRI for both treatment arms. Treatment cycles were continued until disease progression, decision by physician or patient, toxicity, or death.

Tumour tissue collection was undertaken for all study participants. In samples reported locally as KRAS wild-type, further RAS (KRAS exon 3 or 4 mutation, NRAS exon 2, 3, or 4 mutation) and BRAF mutations were assessed centrally by multiplex qPCR using the Modaplex system (Qiagen) for patients who had sufficient tumour remaining for multiple comparisons.

**Statistical analyses**

OS and PFS were evaluated by RAS/RAF and tumour sidedness subgroups using the Kaplan–Meier method. The unstratified Cox proportional hazards model was used to estimate HR and 95% CI. The study stratification factors were used as covariates in the RAS/RAF sub-group Cox models. For both OS and PFS, treatment-by-sub-group interaction was examined using the likelihood ratio test. P-values were not adjusted for multiple comparisons.

**Results**

Among the 1072 patients randomised to a treatment arm for the RAISE trial [intent-to-treat (ITT) population], RAS/RAF mutation status was available for 912 (85%), and primary tumour location was known for 1012 patients (94%). RAS mutations were found in 63% of patients (579/912); BRAF mutation in 4.5% (41/912, all...
V600E positive); 32% of patients were RAS/BRAF wild-type (292/912) (see flowchart of supplementary Figure S1 and Table S1, available at Annals of Oncology online for details). Within RAS/BRAF wild-type and RAS mutant sub-groups (Table 1), baseline characteristics were balanced between treatment arms, although the RAS/BRAF wild-type placebo arm had more males (71% versus 55%) and patients with >10 ng/ml CEA (68% versus 60%) than the ramucirumab arm. Within the 41-patient BRAF mutant sub-group, treatment arms were relatively balanced. BRAF mutations were more prevalent in right-sided tumours. Among the tumour sidedness sub-groups, left CRC predominated (69%, 699/1012) (supplementary Table S2, available at Annals of Oncology online). Within left versus right sub-groups, baseline patient and tumour characteristics were largely balanced between treatment arms. The left sub-group had a lower percentage of females (40% versus 48%) than the right.

| Table 1. Summary of patient and disease characteristics in the RAS/RAF mutation sub-groups |
|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
|                                      | Ramucirumab + FOLFIRI (N = 285)         | Placebo + FOLFIRI (N = 294)             | Ramucirumab + FOLFIRI (N = 20)          | Placebo + FOLFIRI (N = 21)             |
| Age group                              | n (%)                                   | n (%)                                   | n (%)                                   | n (%)                                   |
| ≥65 years                              | 128 (45)                                | 112 (38)                                | 6 (30)                                  | 10 (48)                                 |
| ≥70 years                              | 65 (23)                                 | 70 (24)                                 | 4 (20)                                  | 6 (29)                                  |
| Gender                                 |                                        |                                        |                                         |                                         |
| Male                                   | 150 (53)                                | 161 (55)                                | 12 (60)                                 | 12 (57)                                 |
| Female                                 | 135 (47)                                | 133 (45)                                | 8 (40)                                  | 9 (43)                                  |
| Geographical region                    |                                        |                                        |                                         |                                         |
| Japan/East Asia                        | 54 (19)                                 | 45 (15)                                 | 2 (10)                                  | 1 (5)                                   |
| Rest of world                          | 231 (81)                                | 249 (85)                                | 18 (90)                                 | 20 (95)                                 |
| Race                                    |                                        |                                        |                                         |                                         |
| Black                                  | 9 (3)                                   | 10 (3)                                  | 0                                       | 1 (5)                                   |
| Other                                  | 57 (20)                                 | 48 (16)                                 | 4 (20)                                  | 2 (10)                                  |
| White                                  | 219 (77)                                | 234 (80)                                | 16 (80)                                 | 17 (81)                                 |
| Missing                                 | 0                                       | 2 (1)                                   | 0                                       | 1 (5)                                   |
| ECOG PS                                 |                                        |                                        |                                         |                                         |
| 0                                      | 142 (50)                                | 147 (50)                                | 13 (65)                                 | 11 (52)                                 |
| 1                                      | 143 (50)                                | 146 (50)                                | 6 (30)                                  | 10 (48)                                 |
| Missing                                 | 0                                       | 1 (<1)                                  | 1 (5)                                   | 0                                       |
| Time to progression after first-line   |                                        |                                        |                                         |                                         |
| <6 months                               | 64 (22)                                 | 66 (22)                                 | 7 (35)                                  | 11 (52)                                 |
| ≥6 months                               | 221 (78)                                | 228 (78)                                | 13 (65)                                 | 10 (48)                                 |
| Colorectal tumour sidedness            |                                        |                                        |                                         |                                         |
| Left                                   | 178 (62)                                | 175 (60)                                | 7 (35)                                  | 6 (29)                                  |
| Right                                  | 95 (33)                                 | 99 (34)                                 | 11 (55)                                 | 14 (67)                                 |
| Missing                                 | 12 (4)                                  | 20 (7)                                  | 2 (10)                                  | 1 (5)                                   |
| Baseline plasma VEGF-D level\(^b\)     |                                        |                                        |                                         |                                         |
| High                                   | 143 (50)                                | 133 (45)                                | 13 (65)                                 | 14 (67)                                 |
| Low                                    | 97 (34)                                 | 100 (34)                                | 5 (25)                                  | 3 (14)                                  |
| Missing                                 | 45 (16)                                 | 61 (21)                                 | 2 (10)                                  | 4 (19)                                  |
| Baseline plasma CEA level              |                                        |                                        |                                         |                                         |
| >10 ng/ml                               | 196 (67)                                | 196 (67)                                | 13 (65)                                 | 9 (43)                                  |
| ≤10 ng/ml                               | 76 (27)                                 | 80 (27)                                 | 7 (35)                                  | 11 (52)                                 |
| ≥200 ng/ml                              | 64 (22)                                 | 82 (27)                                 | 3 (15)                                  | 2 (10)                                  |
| <200 ng/ml                              | 212 (72)                                | 17 (85)                                 | 18 (86)                                 |                                         |
| Missing                                 | 18 (6)                                  | 0                                       | 1 (5)                                   |                                         |

\(^a\) A single patient was found to have mutations in both RAS and BRAF; this patient was included only in the BRAF mutant sub-group for all summaries and analyses and in the counts listed above.

\(^b\) VEGF-D high ≥115 pg/ml; VEGF-D low <115 pg/ml.

CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; FOLFIRI, folinic acid, 5-fluorouracil and irinotecan; PS, performance status; VEGF, vascular endothelial growth factor.
A favourable ramucirumab treatment effect was found in the RAS/BRAF wild-type sub-group and the RAS mutant sub-group. Ramucirumab treatment was associated with prolonged OS (HR < 1) for the RAS/BRAF wild-type sub-group (median 16.2 months versus 15.5 months; HR = 0.86, 95% CI 0.64–1.14) and the RAS mutant sub-group (median 12.9 versus 11.5 months; HR = 0.86, 95% CI 0.71–1.04) (Figure 1A and C; Table 2). A similar trend was observed with PFS for both the RAS mutant and RAS/BRAF...
wild-type sub-group (Figure 1B and D; Table 2). Treatment–by-
mutation status interaction tests indicated that the ramucirumab
benefit was not statistically different among the three mutation
status sub-groups (OS $P = 0.523$, PFS $P = 0.655$).

Analysis of the Kaplan–Meier plots of the $BRAF$ mutant sub-
group showed that ramucirumab+FOLFIRI treatment appears
to substantially benefit patients harbouring $BRAF$-mutated
tumours. Ramucirumab-treated patients exhibited a non-
statistically significant OS and PFS benefit over placebo (median
OS 9.0 versus 4.2 months, HR $= 0.54$, 95% CI 0.25–1.13; median
PFS 5.7 versus 2.7 months, HR $= 0.55$, 95% CI 0.28–1.08) (Figure 1E and F; Table 2); although this analysis is limited by
sample size. The $RAS$/Raf sub-groups showed no substantial dif-
ference between arms in post-discontinuation treatment that
may have differentially impacted survival (supplementary Table
S3, available at Annals of Oncology online).

Since high VEGF-D levels from an exploratory assay seem to
suggest a greater benefit with ramucirumab, we examined base-
line VEGF-D expression in $RAS$/Raf mutation sub-groups and its
association with treatment effects. When treated as a con-
tinuous variable, there was no evidence suggesting different
VEGF-D expression among the $RAS$/BRAF wild-type, $RAS$ mu-
tant, and $BRAF$ mutant sub-groups ($P = 0.358$), although $BRAF$
mutant population had a slightly higher percentage of patients
classified as having high VEGF-D (Table 1). Treatment effects in
the $RAS$/Raf mutation sub-groups by baseline plasma VEGF-D
levels showed that $RAS$ mutants with high baseline VEGF-D lev-
els ($n = 276$) benefitted from ramucirumab with statistically sig-
ificantly higher OS (HR $= 0.64$, 95% CI 0.49–0.84, $P = 0.0014$)
and PFS (HR $= 0.54$, 95% CI 0.42–0.70, $P < 0.0001$) (supple-
mentary Table S4, available at Annals of Oncology online). In
contrast, patients with $RAS$ mutations with low baseline VEGF-
D ($n = 197$) exhibited no ramucirumab benefit but rather OS
and PFS favoured the placebo arm. The $RAS$/BRAF wild-type
sub-group behaved similarly to the $RAS$ mutant sub-group.
Patients with high baseline VEGF-D exhibited a significant PFS
benefit from ramucirumab (although no OS benefit was observed), and the low VEGF-D sub-group displayed no benefit
from ramucirumab (supplementary Table S4, available at
Annals of Oncology online). The small number of patients in the
$BRAF$ mutation sub-group precluded conclusions regarding ef-
fect of ramucirumab by VEGF-D level. Stem-and-leaf plots were
constructed to examine data distribution by baseline VEGF-D level
(supplementary Figure S2, available at Annals of Oncology
online). In patients with $BRAF$ mutations, there was no indica-
tion of a differential ramucirumab benefit in patients by VEGF-
D level.

The treatment effect of ramucirumab+FOLFIRI by tumour
sidedness was also evaluated. Ramucirumab–treated patients with
left-sided tumours exhibited improved OS ($HR = 0.81$, 95% CI 0.68–0.97), with median OS increasing 2.5 months for ramu-
cirumab over placebo (14.5 versus 12.0 months) (Figure 2A;
Table 2). Patients with right CRC tumours also exhibited a direc-
tional ramucirumab survival benefit on aggregate, but of smaller
magnitude, with a 1.1-month increase in median OS (12.7 versus
11.6 months, $HR = 0.97$, 95% CI 0.75–1.26) (Figure 2C; Table 2).
The interaction $P$-value was not statistically significant (0.276),
indicating that sidedness is not predictive of the efficacy of adding
ramucirumab to FOLFIRI in these analyses. A similar trend was
observed with PFS (Figure 2B and D); the interaction $P$-value was
again not significant (0.578).

There was no association between VEGF-D levels and sided-
ness (supplementary Table S5, available at Annals of Oncology
online); the ramucirumab benefit in patients with high VEGF-D
levels was seen in both right- and left-sided tumours (supple-
mentary Table S6, available at Annals of Oncology online). There
was no substantial difference among the sidedness sub-groups in
post-discontinuation treatment that likely would have impacted

| Sub-group          | Treatment arm | $n$  | Overall survival | Progression-free survival |
|--------------------|---------------|-----|-----------------|---------------------------|
|                    |               |     | Median (months) | HR (95% CI) $P$-value $^{b}$ | Interaction $P$-value $^{a}$ | Median (months) | HR (95% CI) $P$-value $^{b}$ | Interaction $P$-value $^{a}$ |
| $RAS$/BRAF wild-type | Ramucirumab   | 149 | 16.2            | 0.86 (0.64–1.14) $P = 0.2899$ | 0.523 | 5.7 | 0.78 (0.61–1.00) $P = 0.0512$ | 0.655 |
|                    | Placebo       | 143 | 15.5            | 0.86 (0.71–1.04) $P = 0.1110$ | 4.3  | 5.7 | 0.81 (0.68–0.97) $P = 0.0209$ | 0.523 |
| $RAS$ mutant       | Ramucirumab   | 285 | 12.9            | 0.86 (0.71–1.04) $P = 0.1110$ | 4.3  | 5.7 | 0.81 (0.68–0.97) $P = 0.0209$ | 0.523 |
|                    | Placebo       | 294 | 11.5            | 0.54 (0.25–1.13) $P = 0.1030$ | 4.3  | 5.7 | 0.55 (0.28–1.08) $P = 0.0826$ | 0.523 |
| $BRAF$ mutant      | Ramucirumab   | 20  | 9.0             | 0.81 (0.68–0.97) $P = 0.0188$ | 0.276| 6.0 | 0.78 (0.66–0.91) $P = 0.0014$ | 0.578 |
|                    | Placebo       | 21  | 4.2             | 0.81 (0.68–0.97) $P = 0.0188$ | 0.276| 6.0 | 0.78 (0.66–0.91) $P = 0.0014$ | 0.578 |
| Left-sided CRC     | Ramucirumab   | 353 | 14.5            | 0.97 (0.75–1.26) $P = 0.8242$ | 0.276| 6.0 | 0.86 (0.67–1.08) $P = 0.1955$ | 0.276 |
|                    | Placebo       | 346 | 12.0            | 0.97 (0.75–1.26) $P = 0.8242$ | 0.276| 6.0 | 0.86 (0.67–1.08) $P = 0.1955$ | 0.276 |

$^{a}$Both ramucirumab and placebo were given in combination with FOLFIRI.

$^{b}$Likelihood ratio.

CI, confidence interval; CRC, colorectal carcinoma; FOLFIRI, folinic acid, 5-fluorouracil and irinotecan; HR, hazard ratio.
survival results (supplementary Table S7, available at Annals of Oncology online).

Discussion

Analyses of mCRC trials have revealed that the RAS/RAF gene mutation profile and tumour sidedness are both determinants of patient prognosis and have bearing on anti-EGFR treatment efficacy in first-line trials [9, 10]. Published data on the impact of tumour sidedness and RAS/RAF mutations on the efficacy of antiangiogenic therapy is limited, especially in the second-line setting. Our exploratory retrospective analyses of the RAISE phase III trial data examined whether RAS/RAF mutation status and tumour sidedness influenced the antiangiogenic treatment efficacy of ramucirumab in patients with mCRC that progressed during or after a first-line treatment with bevacizumab, oxaliplatin, and a fluoropyrimidine. While these exploratory analyses are limited because they are retrospective and may be underpowered, they are useful indicators of areas to investigate more completely.

Analysis of patients with RAS mutations in the RAISE trial showed these mutations were associated with a worse prognosis than the RAS/BRAF wild-type. Other studies have made a similar observation [10]. Consistent with the prior RAISE analysis, this analysis showed ramucirumab added to FOLFIRI improved patient outcomes over placebo regardless of RAS mutation status. The ramucirumab benefit to patients with KRAS/NRAS mutation could not be ascribed to an imbalance between treatment arms in baseline characteristics, including any imbalance in VEGF-D and CEA baseline plasma levels. However, it was noteworthy that both RAS mutant patients and RAS/BRAF wild-type patients with high baseline VEGF-D levels displayed a more robust response to ramucirumab treatment than those with low VEGF-D levels, suggesting the predictive value of VEGF-D is independent of the RAS mutation status.

In agreement with other studies [10], the RAISE data showed that the BRAF mutation was present in a low percentage of patients with CRC (4.5%) and occurred more frequently in right-sided tumours. Patients with the BRAF mutation had worse survival than patients who were RAS/BRAF wild-type, irrespective of

Figure 2. Kaplan–Meier curves of OS and PFS in left and right CRC sub-groups. OS (A, C) and PFS (B, D) were determined using Kaplan–Meier plots of RAISE ITT patients with left (A, B) and right (C, D) CRC. HRs and 95% CI were estimated from an unstratified Cox model with treatment group as the only covariate. Tick marks represent censored events.
Tumour sidedness acted as a strong prognostic factor, but the antiangiogenic benefit was seen on both sides, with a numerically superior antiangiogenic benefit in patients with left-sided tumours. The second-line mCRC VELOUR study also found that addition of an antiangiogenic was efficacious for left- and right-sided tumours [13].

The efficacy of EGFR inhibitors appears to be limited by tumour sidedness. Studies have identified that left CRC tumours seem to be responsive to anti-EGFR therapy (cetuximab, panitumumab), but right-sided tumours are not [14, 19, 20]. Therefore, treatment guidelines currently recommend using these agents only in tumours originating from the left side of the colon [21, 22].

In conclusion, exploratory retrospective analyses of RAISE trial data have shown ramucirumab treatment is effective in a second-line setting, regardless of RAS/RAF mutation status and tumour sidedness. While the EGFR inhibitor treatments appear more circumscribed in their effective usage, ramucirumab is effective for patients with mutant RAS or BRAF tumours and patients who are RAS/BRAF wild-type. Of interest, evidence was found that patients with BRAF mutant tumours have a potentially increased benefit with ramucirumab, but the relationship was not significant in this small sub-population and requires further validation.

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