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Cetuximab continuation after first progression in metastatic colorectal cancer (CAPRI-GOIM): a randomized phase II trial of FOLFOX plus cetuximab versus FOLFOX

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Background: Cetuximab plus chemotherapy is a first-line treatment option in metastatic KRAS and NRAS wild-type colorectal cancer (CRC) patients. No data are currently available on continuing anti-epidermal growth factor receptor (EGFR) therapy beyond progression.

Patients and methods: We did this open-label, 1:1 randomized phase II trial at 25 hospitals in Italy to evaluate the efficacy of cetuximab plus 5-fluorouracil, folinic acid and oxaliplatin (FOLFOX) as second-line treatment of KRAS exon 2 wild-type metastatic CRC patients treated in first line with 5-fluorouracil, folinic acid and irinotecan (FOLFIRI) plus cetuximab. Patients received FOLFOX plus cetuximab (arm A) or FOLFOX (arm B). Primary end point was progression-free survival (PFS). Tumour tissues were assessed by next-generation sequencing (NGS). This report is the final analysis.

Results: Between 1 February 2010 and 28 September 2014, 153 patients were randomized (74 in arm A and 79 in arm B). Median PFS was 6.4 [95% confidence interval (CI) 4.7–8.0] versus 4.5 months (95% CI 3.3–5.7); [hazard ratio (HR), 0.81; 95% CI 0.58–1.12; $P = 0.19$], respectively. NGS was performed in 117/153 (76.5%) cases; 66/117 patients (34 in arm A and 32 in arm B) had KRAS, NRAS, BRAF and PIK3CA wild-type tumours. For these patients, PFS was longer in the FOLFOX plus cetuximab arm [median 6.9 (95% CI 5.5–8.2) versus 5.3 months (95% CI 3.7–6.9); HR, 0.56 (95% CI 0.33–0.94); $P = 0.025$]. There was a trend in better overall survival: median 23.7 [(95% CI 19.4–28.0) versus 19.8 months (95% CI 14.9–24.7); HR, 0.57 (95% CI 0.32–1.02); $P = 0.056$].

Conclusions: Continuing cetuximab treatment in combination with chemotherapy is of potential therapeutic efficacy in molecularly selected patients and should be validated in randomized phase III trials.

Key words: colorectal cancer, cetuximab, FOLFOX, NGS

introduction

Epidermal growth factor receptor (EGFR) is a key cell growth regulator activated in several human malignancies [1]. A subset of colorectal cancer (CRC) depends on EGFR activation and, therefore, treatment with blocking anti-EGFR monoclonal antibodies

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(moAbs), such as cetuximab or panitumumab, in combination with 5-fluorouracil, folinic acid and irinotecan (FOLFIRI) or 5-fluorouracil, folinic acid and oxaliplatin (FOLFOX) chemotherapies, represents a valuable therapeutic approach in these patients [2, 3]. Activating mutations in KRAS exon 2 (codon 12 or 13) and less frequently in exons 3 and 4, or NRAS mutations, are predictive biomarkers of resistance to anti-EGFR moAbs in a metastatic CRC (mCRC) [4–7]. In addition, mutations occurring in BRAF, PIK3CA and PTEN genes have been associated with resistance to cetuximab and panitumumab [6, 7].

We recently reported that mCRC patients with KRAS/NRAS/BRAF/PIK3CA wild-type (WT) tumours treated in first line with FOLFIRI plus cetuximab had better response rate (RR) and progression-free survival (PFS) compared with patients harbouring a mutation in any of these genes [6, 7].

In the continuum of care of mCRC patients treated in first line with chemotherapy plus the anti-vascular endothelial growth factor moAb bevacizumab, it has been shown that a therapeutic option is a second-line chemotherapy in combination with any anti-angiogenic drug such as bevacizumab, aflibercept and ramucirumab, suggesting that anti-angiogenic therapy beyond first progression could be effective [8–10]. There is clinical evidence of therapeutic efficacy of blocking a growth factor receptor in subsequent lines of therapy, as in breast cancer patients overexpressing HER2 gene. In fact, in breast cancer patients, whose tumours have an amplified and overexpressed HER2 gene, continuum blockade of HER2-signalling has been established as an effective therapeutic strategy from adjuvant therapy to sequential lines of treatment for metastatic disease. It is conceivable that continuum inhibition of EGFR signalling could have therapeutic efficacy also in mCRC patients with and EGFR-dependent tumour [11].

The Cetuximab After Progression in KRAS wIld type colorectal cancer patients (CAPRI)–Gruppo Oncologico dell'Italia Meridionale (GOIM) (CAPRI-GOIM) study was designed to evaluate the role of EGFR inhibition in the second-line treatment of KRAS exon 2 WT mCRC patients after progression from first-line treatment with cetuximab. Here, we report the final results of the CAPRI-GOIM study.

methods

study design and participants

We did an academic, open-label, 1:1 randomized phase II trial at 25 hospitals in Italy. We enrolled patients aged 18 years or older with WT KRAS exon 2 histologically confirmed adenocarcinoma of colon or rectum (assessed by local pathology), with measurable metastatic disease, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, previously treated in first line with FOLFIRI plus cetuximab, as previously reported [10], until disease progression or unacceptable toxicity and that achieved complete response (CR), partial response (PR) or stable disease (SD) (supplementary Figure S1, available at *Annals of Oncology* online). The trial was approved by the Ethics Committees at each participating institution and carried out in accordance with the Declaration of Helsinki. All patients provided written informed consent. From November 2013, when the European Medicine Agency (EMA) reviewed the selection criteria for treatment of mCRC patients with anti-EGFR moAbs, only patients whose tumours were RAS WT (KRAS exons 2, 3, 4 and NRAS exons 2, 3, 4) were eligible and enrolled in the trial. EudraCT number 2009-014041-81.

randomization and treatment

For prospective KRAS exon 2 screening before patient treatment in first line with FOLFIRI plus cetuximab, tumour samples were analysed by local pathology laboratory [6]. Archival formalin-fixed, paraffin-embedded tissue samples were collected for 117/153 (76.5%) patients and centrally assessed by next-generation sequencing (NGS), as previously reported [6, 7].

After progression from first-line therapy, the GOIM Clinical Trials Unit randomized the patients centrally, using the method of minimization with a random element. Patients were stratified according to performance status and to BRAF mutation. Patients were randomized (1:1) to FOLFOX plus cetuximab (arm A) or FOLFOX (arm B). Treatment allocation was not masked.

Patients in arm A received the FOLFOX regimen: i.v. infusion of 200 mg L-folinic acid, 85 mg/m^2 oxaliplatin over 2 h, 400 mg/m^2 i.v. bolus of 5-fluorouracil, followed by 2400 mg/m^2 5-fluorouracil i.v. over 46 h; cetuximab 500 mg/m^2 i.v. over 120 min for the first dose, over 90 min for the second and over 60 min for the subsequent doses. Treatment was repeated every 2 weeks. Patients in arm B received only FOLFOX every 2 weeks. Patients were treated until disease progression (PD), unacceptable toxicity or patient refusal.

Toxicity was graded using the National Cancer Institute-Common Toxicity Criteria for adverse events (AEs), version 3.0. Tumour assessments were performed according to Response Evaluation Criteria in Solid Tumours (RECIST, version 1.1) using spiral or conventional CT scan, or MRI, if required; tumour measurements were assessed at baseline, at week 6 and, thereafter, every 8 weeks.

Detailed protocol provided in supplementary Materials, available at *Annals of Oncology* online.

statistical analysis

The study initiated in July 2009 screened ~600 patients for KRAS exon 2 mutations to identify at least 320 patients eligible for first-line treatment with FOLFIRI plus cetuximab. For the second line, 211 events of progression were necessary to detect a difference in the superiority of FOLFOX plus cetuximab versus FOLFOX alone with a power at 80% for the hazard ratio (HR) of 0.68. Actually, 340 patients were recruited in first-line treatment with FOLFIRI plus cetuximab [6] (supplementary Figure S1, available at *Annals of Oncology* online). In the second-line treatment, 153 patients [intention-to-treat (ITT) population] were enrolled: 74 in arm A and 79 in arm B, respectively (Figure 1 for patients' distribution). We used the Kaplan–Meier method to assess PFS and overall survival (OS) and compared them with log-rank tests at a significance level of 5%. Cox regression models were used to generate HRs and corresponding 95% confidence intervals (CIs). We used IBM-SPSS statistics version 22.0 for all statistical analyses.

results

From 1 July 2009 to 30 June 2013, 340 WT KRAS exon 2 mCRC patients were treated in first line with FOLFIRI plus cetuximab until progression or unacceptable toxicity (supplementary Figure S1, available at *Annals of Oncology* online). Results of antitumor activity have been recently reported [6]. From 1 February 2010 to 28 September 2014, among 307/340 patients, that received in first-line FOLFIRI plus cetuximab, and obtained a clinical benefit (CR, PR or SD as best response), 153 patients with progression were randomized to receive FOLFOX plus cetuximab (arm A, $n = 74$) or FOLFOX (arm B, $n = 79$) as second-line treatment, respectively (Figure 1 and supplementary Figure S1, available at *Annals of Oncology* online). Patient demographic and clinical characteristics were balanced between the

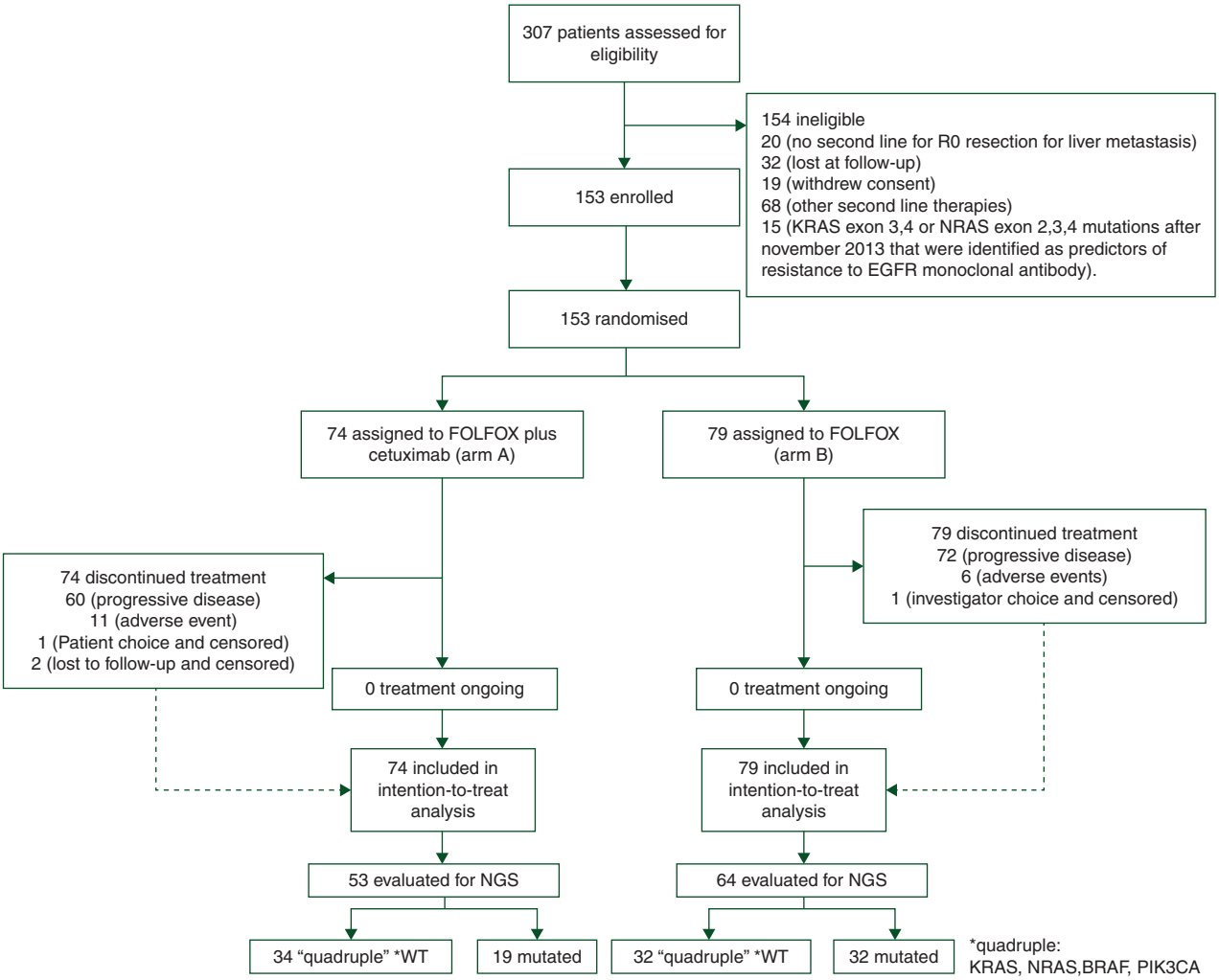

Figure 1. Patient enrolment and disposition CONSORT diagram.

two arms (supplementary Table S1, available at *Annals of Oncology* online).

At the time of the final analysis on 31 July 2015, PFS events were 149 and occurred over a median follow-up of 35.3 months. We recorded 71 PFS events with a median follow-up of 35.3 months in arm A and 78 events with a median follow-up of 34.1 months in arm B. In the ITT population (153 patients), median PFS was 6.4 months (95% CI 4.7–8.0) in arm A and 4.5 months (95% CI 3.3–5.7) in arm B (HR, 0.81; 95% CI 0.58–1.12; $P = 0.19$) (Figure 2A).

We recorded 128 deaths: 63 occurred in arm A and 65 in arm B. The median OS was 17.6 months (95% CI 14.1–21.1) in the FOLFOX plus cetuximab arm and 14.0 months (95% CI 12.9–15.1) in the FOLFOX arm (HR, 0.86; 95% CI 0.61–1.20; $P = 0.41$) (Figure 2B). Tumour response was evaluable in 144/153 patients (94.1%): 70/74 (94.6%) in arm A and 74/79 (93.7%) in arm B. Response rate in the ITT population (CR+PR) was 21.6% (95% CI 11.0% to 32.2%) in arm A and 12.7% (95% CI 5.4% to 20.0%) in arm B, respectively (supplementary Table S2, available at *Annals of Oncology* online).

To assess the potential effect of mutations in KRAS, NRAS, BRAF or PIK3CA genes on the second-line efficacy of FOLFOX plus cetuximab when compared with FOLFOX, a retrospective NGS analysis on 22 genes involved in EGFR-pathway was done in 117/153 (76.5%) cases. At least one mutation in either KRAS/NRAS/BRAF or PIK3CA genes was found in 51/117 (43.6%) cases (supplementary Table S3, available at *Annals of Oncology* online). In 32 patients, 27.4% of KRAS mutations were found: 19 in exon 2 (16.2%), and 13 in exons 3 or 4 (11.1%). The higher sensitivity of NGS (2%) compared with standard genotyping methods might in part explain the high rate of KRAS exon 2 mutations identified.

In 10 patients, 8.5% of NRAS mutations at exons 2 or 3 were found. BRAF mutations were detected in 7 cases (6.0%): 6 were codon V600E mutations (exon 15). Mutations in exon 9 and 20 of PIK3CA gene occurred in most cases together with KRAS, NRAS or BRAF mutations (data not shown) [6, 7]. PIK3CA gene was mutated in only two cases (1.8%) in which KRAS, NRAS and BRAF genes were WT [6].

Sixty-six out of 117 patients had KRAS/NRAS/BRAF/PIK3CA WT tumours (34 patients in arm A, 32 patients in arm B) whereas, in 51/177 patients (19 in arm A and 32 in arm B), a mutation in one of these genes was found. Median PFS of the KRAS/NRAS/BRAF/PIK3CA WT patients was 6.9 months (95% CI 5.5–8.2) for arm A when compared with 5.3 months

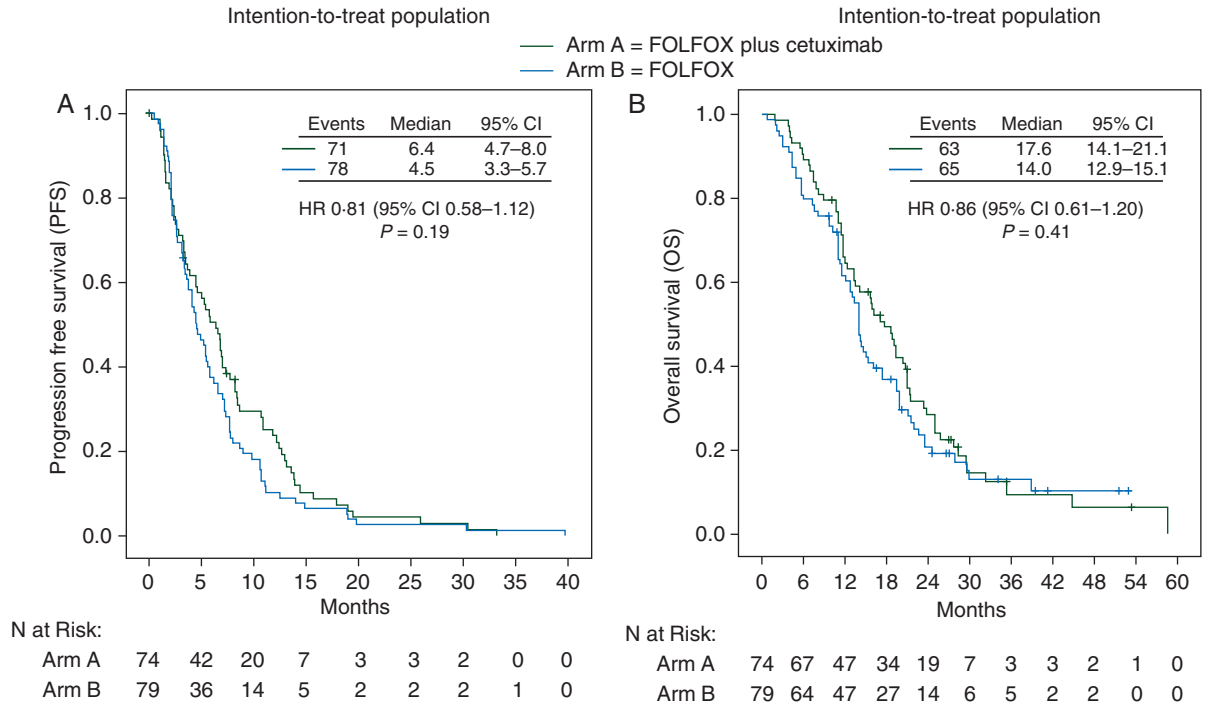

Figure 2. Kaplan–Meier analysis of progression-free survival and overall survival in the intention-to-treat population.

(95% CI 3.7–6.9) for arm B (HR, 0.56; 95% CI 0.33–0.94; $P = 0.025$) (Figure 3A). On the contrary, the median PFS of mutated (any mutation in KRAS/NRAS/BRAF/PIK3CA genes) patients was 2.7 months (95% CI 1.1–4.3) for arm A versus 4.4 months (95% CI 3.3–5.5) for arm B (HR, 1.70; 95% CI 0.94–3.05; $P = 0.07$) (Figure 3B). The median OS for the KRAS/NRAS/BRAF/PIK3CA WT patients was 23.7 months (95% CI 19.4–28.0) in arm A compared with 19.8 months (95% CI 14.9–24.7) in arm B (HR, 0.57; 95% CI 0.32–1.02; $P = 0.056$) (Figure 4A). However, median OS for KRAS/NRAS/BRAF and/or PIK3CA mutated population was 11.6 months (95% CI 11.2–12.0) in arm A versus 14.0 months (95% CI 11.9–16.0) in arm B (HR, 1.60; 95% CI 0.89–2.96; $P = 0.10$) (Figure 4B). The RR also differed according to mutation status. In particular, the overall responses (CR+PR) were 10/34 (29.4%) (95% CI 14.1–43.5%) in arm A when compared with 3/32 (9.4%) (95% CI 0% to 41.3%) in arm B for patients whose tumours were WT for the four genes. RAS WT population data are described in supplementary Results, available at *Annals of Oncology* online.

Analysis of safety and tolerability was performed in all 153 patients. No unexpected AEs or toxic deaths were recorded (supplementary Table S4, available at *Annals of Oncology* online). The most frequently reported grade 3–4 AEs were neutropenia, diarrhoea, nausea, asthenia, peripheral neuropathy and cutaneous reactions. Grade 3–4 AEs were more common in the arm A than in the arm B. In particular, febrile neutropenia was reported in 1 patient (1.4%) in arm A and in none in arm B, grade 3 diarrhoea in 5 patients (6.8%) in arm A and 2 patients (2.6%) in arm B. As expected, cutaneous reactions occurred only in patients treated with FOLFOX plus cetuximab (G3 in 20 patients, 27.0%).

Treatment was discontinued for AEs in 11 patients (15%) in arm A and in 6 patients (7.6%) in arm B. In arm A, 10 patients (13.5%) discontinued chemotherapy or both chemotherapy and cetuximab, whereas 1 patient (1.3%) discontinued only cetuximab. The median number of cycles in arm A was 7, whereas it was 8 (inter-quartile range 4–12) in arm B. Dose modifications occurred in each arms similarly. For cetuximab dose modifications were necessary in 57 patients (77.0%); oxaliplatin dose was modified for 36 patients (48.6%) in arm A and for 41 patients (51.9%) in arm B; 5-fluorouracil dosing was modified in 43 (58.1%) in arm A and in 42 patients (53.2%) in arm B.

discussion

To our knowledge, this is the first clinical trial evaluating the potential role of continuing EGFR inhibition by treating KRAS exon2 WT mCRC patients in second line with cetuximab plus chemotherapy after treatment in first line with cetuximab-based therapy. In the 153 ITT population, no significant difference in PFS, in RR and in OS was observed among the two arms. However, a trend in favour of the FOLFOX plus cetuximab combination was recorded in all end points.

More importantly, in 66 patients, WT for KRAS/NRAS/BRAF/PIK3CA genes, we report a significant prolonged PFS for the treatment with FOLFOX plus cetuximab when compared with FOLFOX alone with an HR of 0.56 and a P value of 0.025. OS was also increased in these patients with an HR of 0.57, approaching statistical significance ($P = 0.056$). Similarly, mCRC patients WT for KRAS/NRAS/BRAF/PIK3CA genes had the best clinical benefit by FOLFIRI plus cetuximab, in first line, compared with patients with a mutation in any of these gene (RR, 64.4%; median PFS, 11.3 months versus RR, 47.4%; median PFS, 7.7 months) [6]. This is the first clinical evidence that, in patients whose tumours are WT for KRAS/NRAS/BRAF and PIK3CA

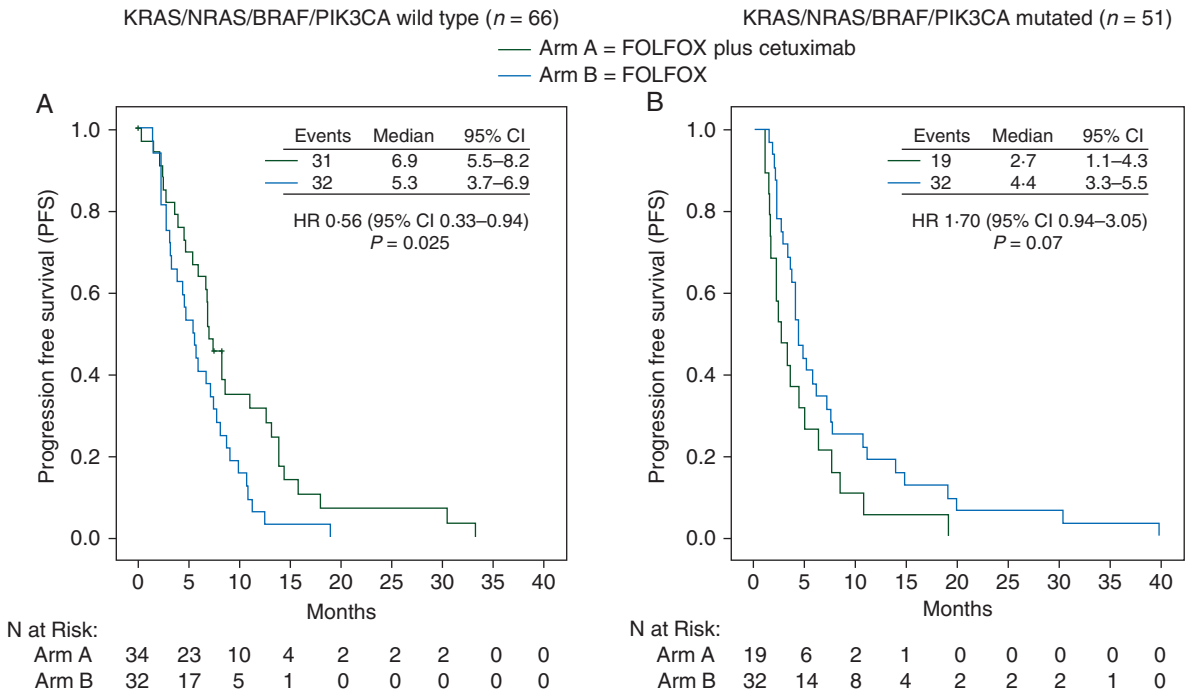

Figure 3. Kaplan–Meier analysis of progression-free survival according to KRAS, NRAS, BRAF and PIK3CA status.

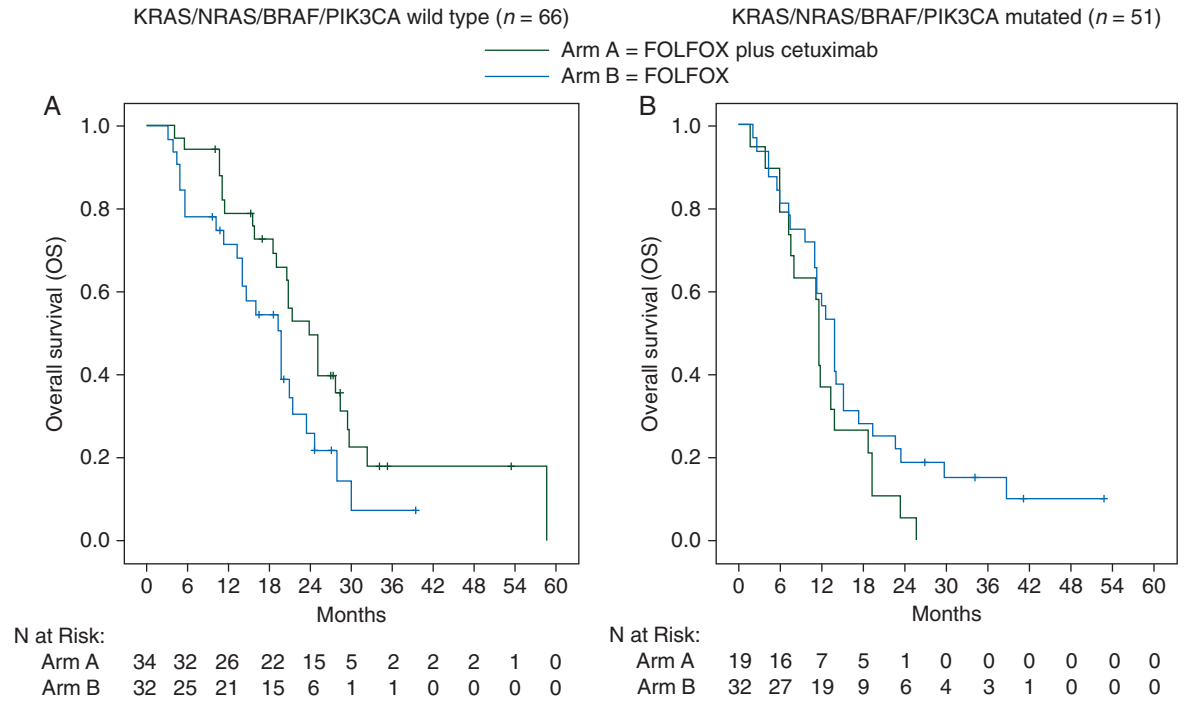

Figure 4. Kaplan–Meier analysis of overall survival according to KRAS, NRAS, BRAF and PIK3CA status.

genes, cetuximab treatment in first and second line in combination with two sequential chemotherapy regimens (FOLFIRI followed by FOLFOX) could be a potential effective therapeutic option, achieving a median OS of ~35 versus 31.1 months in WT patients treated in second line with FOLFOX alone [6]. Overall, within the limitation of a retrospective subgroup analysis, these data suggest that a better molecular classification for genes, acting downstream to EGFR and whose mutation and functional activation could determine escape from EGFR inhibition, might identify tumours that are highly dependent on EGFR signalling for their growth and that, therefore, might respond to anti-EGFR treatment beyond progression.

In contrast, a detrimental effect of cetuximab plus FOLFOX on both PFS and OS was observed in patients with tumours having any mutation in KRAS/NRAS/BRAF or PIK3CA genes. However, this phenomenon is likely driven by the negative interaction of KRAS/NRAS mutations with EGFR monoclonal antibodies in mCRC patients treated with oxaliplatin-based chemotherapies, as previously shown [12].

A series of experimental studies have identified different potential mechanisms of cancer cell resistance to anti-EGFR drugs [13]. In particular, intrinsic resistance may be due to the activation of other growth factor receptors such as MET and HER2 [13–17]. In the present study, tumours WT for KRAS/NRAS/BRAF/PIK3CA genes did not present any ERBB2 or MET gene mutations according to NGS testing (data not shown). Moreover, it has been shown that extracellular domain EGFR gene mutation could be responsible of acquired resistance to anti-EGFR therapies in mCRC [18]. We did not find any EGFR mutation in pre-treatment biopsies of WT cases for KRAS/NRAS/BRAF/PIK3CA genes (data not shown), confirming that these mutations are generally acquired [18, 19].

Several studies have suggested that RAS WT patients, becoming resistant to anti-EGFR moAbs, develop RAS mutations. This phenomenon should have led to a negative interaction of cetuximab and FOLFOX in patients progressing after first-line cetuximab. However, the frequency of RAS WT tumours switching to an RAS mutant phenotype ranges between 37% and 96% in different studies, employing liquid biopsy and mainly enrolling patients treated with EGFR moAbs as monotherapy or in combination with irinotecan [20, 21]. Moreover, it has been shown that plasma levels of RAS mutations rapidly decline in patients after suspension of therapy, suggesting that even a short treatment interruption might reduce RAS mutant clones in the resistant tumour. Finally, molecular alterations in different genes have been shown to co-exist with RAS mutations. In this regard, negative interaction of cetuximab and FOLFOX in cells carrying mechanisms of resistance to anti-EGFR moAbs other than RAS mutations have not been shown.

In conclusion, these findings suggest that continuing cetuximab treatment while switching to a non-cross-resistant chemotherapy combination after first progression is a promising therapeutic approach in molecularly selected mCRC patients with an EGFR-dependent cancer. This strategy deserves further clinical evaluation in randomized phase III trials. Furthermore, since it has been shown that EGFR-resistant cancer cell clones could be identified by the detection of mutations in plasma circulating free tumour-DNA during treatment with anti-EGFR drugs [20, 21], monitoring the presence of such mutations by liquid biopsy could be an additional strategy to optimize continuous EGFR blockade in these patients.

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funding

This was an academic non-profit study, which was sponsored by GOIM. Cetuximab was provided by Merck Serono, Italy. Merck Serono provided also an unrestricted research grant to partially cover administrative costs of the study. Merck Serono had no role in study design, data collection, analysis, interpretation of the results and writing of the manuscript. FC and NN received research grants from Associazione Italiana per la Ricerca sul Cancro (AIRC) (IG2013-14800) for the gene mutation analysis in the tumour tissues by NGS. All the authors had full access to the data. FC had the final responsibility to submit the manuscript for publication.

disclosure

FC has participated to Advisory Boards for Merck Serono, Roche, Bayer, Lilly, Sanofi, AstraZeneca. NN has participated to Advisory Boards for Merck Serono, Roche, Lilly. EM has participated to Advisory Boards for Bayer, Roche, Lilly, Sanofi. TT has participated to Advisory Boards for Bayer, Sanofi. All remaining authors have declared no conflicts of interests.

references

1. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008; 358: 1160–1174.
2. Van Cutsem E, Lenz HJ, Köhne CH et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in cancer. J Clin Oncol 2015; 33: 692–700.
3. Douillard JY, Oliner KS, Siena S et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013; 369: 1023–1034.
4. Heinemann V, von Weikersthal LF, Decker T et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. Lancet Oncol 2014; 15: 1065–1075.
5. De Roock W, De Vriendt V, Normanno N et al. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 2011; 12: 594–603.
6. Ciardiello F, Normanno N, Maiello E et al. Clinical activity of FOLFIRI plus cetuximab according to extended gene mutation status by next-generation sequencing: findings from the CAPRI-GOIM trial. Ann Oncol 2014; 25: 1756–1761.
7. Normanno N, Rachiglio AM, Lambiase M et al. Heterogeneity of KRAS, NRAS, BRAF and PIK3CA mutations in metastatic colorectal cancer and potential effects on therapy in the CAPRI GOIM trial. Ann Oncol 2015; 26: 1710–1714.
8. Bennouna J, Sastre J, Arnold D et al. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. Lancet Oncol 2013; 14: 29–37.
9. Van Cutsem E, Tabernero J, Lakomy R et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. J Clin Oncol 2012; 30: 3499–3506.
10. Tabernero J, Yoshino T, Cohn AL et al. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. Lancet Oncol 2015; 16: 499–508.
11. Baselga J. Treatment of HER2-overexpressing breast cancer. Ann Oncol 2010; 21 (Suppl 7): vii36-vii40.
12. Bokemeyer C, Köhne CH, Ciardiello F et al. FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. Eur J Cancer 2015; 51: 1243–1252.
13. Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. Cancer Discov 2014; 4: 1269–1280.

14. Bertotti A, Migliardi G, Galimi F et al. A molecularly annotated platform of patient-derived xenografts ('xenopatients') identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov 2011; 1: 508–523.
15. Bardelli A, Corso S, Bertotti A et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 2013; 3: 658–673.
16. Troiani T, Martinelli E, Napolitano S et al. Increased TGF-α as a mechanism of acquired resistance to the anti-EGFR inhibitor cetuximab through EGFR-MET interaction and activation of MET signaling in colon cancer cells. Clin Cancer Res 2013; 19: 6751–6765.
17. Ciardiello F, Normanno N. HER2 signaling and resistance to the anti-EGFR monoclonal antibody cetuximab: a further step toward personalized medicine for patients with colorectal cancer. Cancer Discov 2011; 6: 472–474.
18. Montagut C, Dalmases A, Bellosillo B et al. Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer. Nat Med 2012; 22: 221–223.
19. Esposito C, Rachiglio AM, La Porta ML et al. The S492R EGFR ectodomani mutation is never detected in KRAS wild-type colorectal carcinoma before exposure to EGFR monoclonal antibodies. Cancer Biol Ther. 2013; 14(12): 1143–1146.
20. Bettegowda C, Sausen M, Leary RJ et al. Detection of circulating tumor DNA in early- and late-stage malignancies. Sci Transl Med 2014; 6(224): 224–248.
21. Morelli MP, Overman MJ, Dasari A et al. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. Ann Oncol 2015; 26(4): 731–736.

appendix 1

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