Author's response to reviews

Title: Prognostic value of KRAS genotype in metastatic colorectal cancer (MCRC) patients treated with intensive triplet chemotherapy plus bevacizumab (FIr-B/FOX) according to extension of metastatic disease

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Author's response to reviews: see over
Enclosed you’ll find the manuscript 1158812950717649

"Clinical outcome of metastatic colorectal cancer (MCRC) patients treated with intensive triplet chemotherapy plus bevacizumab (FIR-B/FOx) according to KRAS genotype and disease extension", by Gemma Bruera et al., whose title was modified into "Prognostic value of KRAS genotype in metastatic colorectal cancer (MCRC) patients treated with intensive triplet chemotherapy plus bevacizumab (FIR-B/FOx) according to extension of metastatic disease", revised in light of the reviewer’s comments.

A point-by-point response to the comments is described in this cover letter and has been provided in the revised manuscript.

Our manuscript benefited from improved organization and some copy editing for language.
Referee 1:

“Overall evaluation: the authors should stress the retrospective and exploratory value of this analysis and they should better explain the limits of the observed data. Indeed the small sample of patients analysed do not allow to draw any conclusion about the question (different outcome achieved from the treatment by KRAS WT patients with liver limited disease).”

We revised the manuscript taking in account this criticism and we stressed the followings.

Focus of the title, page 1: “Prognostic value of KRAS genotype in metastatic colorectal cancer (MCRC) patients treated with intensive triplet chemotherapy plus bevacizumab (FIr-B/FOx) according to extension of metastatic disease”;

The retrospective and exploratory value and the limits of the observed data in this analysis were introduced in: Abstract, page 3 “Prognostic value of KRAS genotype in L-L and other or multiple metastatic (O/MM) MCRC pts treated with FIr-B/FOx regimen was retrospectively evaluated.”;

Background, page 7: “We report a retrospective exploratory analysis evaluating prognostic value of KRAS genotype in MCRC pts enrolled in a previously reported phase II study [4] and in an expanded clinical program proposing FIr-B/FOx intensive regimen as first line treatment, also verifying recently reported significantly better effectiveness in L-L compared to O/MM pts [6].”;

Methods, Study design, page 9: “A retrospective analysis has been planned to evaluate prognostic relevance of KRAS genotype on clinical outcome of MCRC pts treated with FIr-B/FOx as first line. More, pts were classified according to involved metastatic sites, L-L and O/MM [6], to evaluate the relevance of metastatic extension in KRAS wild-type and mutant MCRC pts.”;

Discussion, page 16: “A significantly favourable prognosis was demonstrated in KRAS wild-type L-L compared to O/MM patients, even if it represents a retrospective, exploratory analysis in a small cohort of MCRC patients.”.

“Abstract: Methods should report main patients’ selection criteria and treatment schedule. Results: this section is almost unreadable because it is too full of numbers, authors should focalize the main message to do to readers. Conclusions: the sentence “ FIr-B/FOX regimen can increase activity and efficacy of KRAS WT and mutant MCRC patients” should be removed. Indeed this is not a randomized, comparative trial and therefore there is not a reference arm with which results can be compared. The present analysis can only suggest that KRAS WT patients with liver limited disease could achieve a greater benefit from the treatment with respect to KRAS MUT patients.”
We refined abstract as it follows:

Methods, addiction of main patients’ selection criteria and treatment schedule (Abstract, page 3):
“Fit MCRC pts <75 years have been consecutively treated with FIr-B/FOx regimen: weekly 12 h-timed-flat-infusion/5-fluorouracil 900 mg/m², days 1-2, 8-9, 15-16, 22-23; irinotecan 160 mg/m² plus bevacizumab 5 mg/kg, days 1,15; oxaliplatin 80 mg/m², days 8, 22; every 4 weeks.”;

Results, (Abstract, page 3), main message was focalized: “At 21.5 months median follow-up, objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) were, respectively: KRAS wild-type 90%, 14 months, 38 months; KRAS mutant 67%, 11 months, 20 months. PFS and OS were not significantly different. PFS and OS were significantly different in L-L compared to O/MM evaluable pts. In KRAS wild-type pts, clinical outcome of 12 L-L compared to 18 O/MM was significantly different: PFS 21 versus 12 months and OS 47 versus 28 months, respectively. In KRAS mutant pts, clinical outcome of 13 L-L compared to 14 O/MM was not significantly different: PFS 11 months equivalently and OS 39 versus 19 months, respectively.”

Conclusions were clarified, Abstract, page 3. “KRAS genotype wild-type and mutant does not significantly affects different clinical outcome of MCRC pts treated with first line FIr-B/FOx intensive regimen. KRAS wild-type pts with L-L disease may achieve significantly prolonged clinical outcome due to integration with secondary liver surgery, with respect to KRAS mutant patients.”

“Background: the authors should better explain the possible prognostic role of KRAS status, independently from its predictive role.”

The relationship between prognostic role of KRAS status and predictive value of bevacizumab addition in KRAS wild-type and mutant genotype was better explained (Background, page 6): “Clinical outcome (PFS, OS) according to wild-type and mutant genotype assesses prognostic relevance of a specific biomarker, potentially including predictive role of effectiveness of treatment strategies. In randomized studies, predictive relevance of wild-type or mutant genotype can be also specifically assessed by comparing experimental and control arms. Reported median OS of KRAS wild-type and mutant MCRC pts treated with IFL plus BEV were 27.7 and 19.9 months, respectively [18,21]. Prognostic relevance of KRAS or BRAF wild-type compared to KRAS or BRAF
mutant genotype was not significantly different, even if hazard ratio (HR) was 0.64 and 0.38, respectively. Significantly better prognosis was reported only when KRAS/BRAF wild-type pts were compared with pts harbouring mutations in KRAS or BRAF gene (HR 0.51) [18]. KRAS wild-type genotype significantly predicts favourable clinical outcome of anti-EGFR or anti-VEGF drugs added to doublet chemotherapy [18,21-23]. In KRAS mutant genotype, BEV addition to irinotecan, 5-fluorouracil and leucovorin (IFL) significantly prolonged PFS up to 9.3 months, without increasing OS and activity, compared to IFL [18,21].

“Methods: Study design: this section do not clarify the design of the reported analysis (hypothesis? statistics?).”

Study design was reorganized to clarify the plan of reported analysis and statistics (Methods, Study design, pages 9-10): “A retrospective analysis has been planned to evaluate prognostic relevance of KRAS genotype on clinical outcome of MCRC pts treated with FIr-B/FOx as first line. More, pts were classified according to involved metastatic sites, L-L and O/MM [6], to evaluate the relevance of metastatic extension in KRAS wild-type and mutant MCRC pts. Patients with L-L metastases were evaluated at baseline and every 3 cycles of treatment by a multidisciplinary team, consisting of a medical oncologist, liver surgeon, radiologist, to dynamically evaluate resectability defined according to resectability categories previously reported [6]. Resection rate was evaluated in intent-to-treat population enrolled. Liver metastasectomies were defined as: R0, if radical surgery; R1, if radiofrequency was added. Surgery was recommended > 4 weeks after BEV discontinuation. Clinical evaluation of response was made by CT-scan; PET was added based on investigators’ assessment.

Clinical criteria of activity and efficacy were: ORR, PFS, OS. ORR was evaluated according to RECIST criteria [28]; pathologic complete response was defined as absence of residual cancer cells in surgically resected specimens. Overall activity of integrated medical treatment and secondary liver surgery, consisting of the sum of clinical complete responses (cCR) and liver metastasectomies was also evaluated, as previously reported [6]. PFS and OS were evaluated using Kaplan and Meier method [29]. PFS and PFS from surgery were defined respectively, as the length of time from the beginning of treatment or the date of liver metastasectomy and disease progression or death (resulting from any cause) or to the last contact; OS as length of time between beginning of treatment and death or to last contact. Log-rank test was used to compare PFS and OS in KRAS
wild-type versus mutant, L-L versus O/MM, and KRAS wild-type L-L versus O/MM, and KRAS mutant L-L versus O/MM MCRC pts [30].”

“Results: Patients’ demographics: authors should better clarify why there are 31 KRAS WT patients, among the 32 patients analyzed for BRAF there are not mutated patients and finally only 18 patients are KRAS and BRAF WT (?).”

As clarified in Results, page 11: “Thirty-two tumoral samples (54%) were also analysed for BRAF and no BRAF mutation was detected; 18 out of 31 KRAS wild-type MCRC pts were KRAS and BRAF wild-type; 14 out of 28 KRAS mutant MCRC pts were BRAF wild-type.”

“Discussion: the sentence “a significant interaction was demonstrated between ...” should be removed because this assertion imply that a formal comparison and an interaction test was performed, but this is not applicable to the reported analysis.”

We changed this sentence as it follows (Discussion, page 16): “A significantly favourable prognosis was demonstrated in KRAS wild-type L-L compared to O/MM patients,...”

“Conclusion: the sentence “FIR-B/FOX regimen can increase activity and efficacy of KRAS WT and mutant MCRC patients” should be removed. Indeed this is not a randomized, comparative trial and therefore there is not a reference arm with which results can be compared. The present analysis can only suggests that KRAS WT patients with liver limited disease could achieve a greater benefit from the treatment with respect to KRAS MUT patients.”

Conclusions were changed as it follows (Conclusions, pages 16-17): “KRAS genotype wild-type and mutant does not significantly affects different clinical outcome of MCRC pts treated with first line FIR-B/FOX intensive regimen. KRAS wild-type pts with L-L disease may achieve significantly greater benefit from integration with liver metastasectomies compared to O/MM metastatic extension, with respect to KRAS mutant patients. Present findings would be verified in prospective trials of multidisciplinary strategies comparing clinical outcome according to KRAS genotype in patients with L-L and O/MM disease.”
“Figures: the figures that I could download are of poor quality”

We maximized the quality of our figures.
Referee 2:

“In the abstract the result section is very confounding as it describe at some timed a large amount of results which include in the same time mix LL /MCRC, kras, Braf etc and the reader is lost”

In Abstract, Results, page 3, we better focused main data of the paper as it follows: “Fifty-nine pts were evaluated: 31 KRAS wild-type, 53%; 28 KRAS mutant, 47%. At 21.5 months median follow-up, objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) were, respectively: KRAS wild-type 90%, 14 months, 38 months; KRAS mutant 67%, 11 months, 20 months. PFS and OS were not significantly different. PFS and OS were significantly different in L-L compared to O/MM evaluable pts. In KRAS wild-type pts, clinical outcome of 12 L-L compared to 18 O/MM was significantly different: PFS 21 versus 12 months and OS 47 versus 28 months, respectively. In KRAS mutant pts, clinical outcome of 13 L-L compared to 14 O/MM was not significantly different: PFS 11 months equivalently and OS 39 versus 19 months, respectively.”

Introduction

“The authors should describe much more clearly from which kind of study they have extrapolated their analysis (It was a Phase II, III, retrospective analysis or what?) and their objectives.

“Activity and efficacy ... according to… on a retrospective study is too ambitious for this kind of study”

“May be that they evaluated the predictive and prognostic value of several parameters in patients undergone to Flr-B/Fox biochemotherapy”

As suggested, we better described and signified the prognostic and predictive objectives of the present analysis, the phase II clinical trial and expanded clinical program from which we evaluated MCRC patients (Background, page 7): “We report a retrospective exploratory analysis evaluating prognostic value of KRAS genotype in MCRC pts enrolled in a previously reported phase II study [4] and in an expanded clinical program proposing Flr-B/FOX intensive regimen as first line treatment, also verifying recently reported significantly better effectiveness in L-L compared to O/MM pts [6].”
“Authors should describe what is bevacizumab and cetuximab etc and in a synthetic way they chose to evaluate kras, braf and disease extension as predictive markers or response to their treatment containing bevacizumab.”

We briefly described what is bevacizumab and cetuximab in Background, page 5: “...BEV (anti-vascular endothelial growth factor monoclonal antibody) or cetuximab (anti-epithelial growth factor receptor monoclonal antibody) in EGFR-overexpressing and KRAS wild-type MCRC,…”

The background of our evaluation of KRAS (and BRAF) genotype, and extension of metastatic disease is described in Background, pages 6-7: “Clinical outcome (PFS, OS) according to wild-type and mutant genotype assesses prognostic relevance of a specific biomarker, potentially including predictive role of effectiveness of treatment strategies. In randomized studies, predictive relevance of wild-type or mutant genotype can be also specifically assessed by comparing experimental and control arms. Reported median OS of KRAS wild-type and mutant MCRC pts treated with IFL plus BEV were 27.7 and 19.9 months, respectively [18,21]. Prognostic relevance of KRAS or BRAF wild-type compared to KRAS or BRAF mutant genotype was not significantly different, even if hazard ratio (HR) was 0.64 and 0.38, respectively. Significantly better prognosis was reported only when KRAS/BRAF wild-type pts were compared with pts harbouring mutations in KRAS or BRAF gene (HR 0.51) [18]. KRAS wild-type genotype significantly predicts favourable clinical outcome of anti-EGFR or anti-VEGF drugs added to doublet chemotherapy [18,21-23]. In KRAS mutant genotype, BEV addition to irinotecan, 5-fluorouracil and leucovorin (IFL) significantly prolonged PFS up to 9.3 months, without increasing OS and activity, compared to IFL [18,21].

We report a retrospective exploratory analysis evaluating prognostic value of KRAS genotype in MCRC pts enrolled in a previously reported phase II study [4] and in an expanded clinical program proposing FIr-B/FOx intensive regimen as first line treatment, also verifying recently reported significantly better effectiveness in L-L compared to O/MM pts [6].”

“They should explain why Kras BRAF genotype status was taken in consideration in cancer patients undergone bevacizumab based treatment. It should not be considered as a statement that activating Kras mutation is a negative prognostic/predictive factor in this patient setting.”
As answered in previous question, prognostic role of KRAS status and predictive value of bevacizumab addition in KRAS wild-type and mutant genotype was better explained in Background, page 6.

We underline that in a previous retrospective analysis, KRAS status did not affect significantly different prognosis in MCRC patients treated with doublet chemotherapy (IFL) ± bevacizumab. We took in consideration KRAS (BRAF) genotype status as well, in order to retrospectively verify its prognostic value using a regimen consisting of bevacizumab addition to triplet chemotherapy.

“The sentence increased survival over doublet requires reference”

We added references to this sentence (Background, page 5): “...significantly increased survival over doublet regimens [1,2].”

“Role of Liver metastasectomy subset and description should be included in the discussion concerning statistical analysis of that subgroup of patients who could receive surgery”

The emerging role of liver metastasectomies to increase effectiveness of integrated medical and surgical treatment in liver-limited MCRC patients was better described (Background, page 5): “Thus, MCRC pts with L-L disease, integrating Flr-B/FOx intensive regimen and secondary liver surgery significantly improved clinical outcome compared to MCRC pts with multiple metastatic disease, up to median PFS 17 months and median OS 44 months [6].”

Methods

“Authors should describe treatment protocol, ethical procedures and clinical study design not just the treatment”

Treatment protocol, ethical procedures and clinical study design were described, as it follows in Methods:
Patient Eligibility, page 7: “MCRC pts were enrolled in previously reported phase II study [4] and in the expanded clinical program proposing Flr-B/FOx association as first line treatment. Pts were eligible if they had histologically confirmed diagnosis of measurable MCRC; age 18-75 years;
World Health Organization (WHO) performance status \( \leq 2 \); adequate hematological, renal and hepatic functions; life expectancy more than 3 months. The study was approved by the Local Ethical Committee (Comitato Etico, Azienda Sanitaria Locale n.4 L’Aquila, Regione Abruzzo, Italia) and conducted in accordance with the Declaration of Helsinki. All patients provided written, informed consent.”

Study design, pages 9-10: “A retrospective analysis has been planned to evaluate prognostic relevance of \( \text{KRAS} \) genotype on clinical outcome of MCRC pts treated with Flr-B/FOx as first line. More, pts were classified according to involved metastatic sites, L-L and O/MM [6], to evaluate the relevance of metastatic extension in \( \text{KRAS} \) wild-type and mutant MCRC pts. Patients with L-L metastases were evaluated at baseline and every 3 cycles of treatment by a multidisciplinary team, consisting of a medical oncologist, liver surgeon, radiologist, to dynamically evaluate resectability defined according to resectability categories previously reported [6]. Resection rate was evaluated in intent-to-treat population enrolled. Liver metastasectomies were defined as: R0, if radical surgery; R1, if radiofrequency was added. Surgery was recommended > 4 weeks after BEV discontinuation. Clinical evaluation of response was made by CT-scan; PET was added based on investigators’ assessment.

Clinical criteria of activity and efficacy were: ORR, PFS, OS. ORR was evaluated according to RECIST criteria [28]; pathologic complete response was defined as absence of residual cancer cells in surgically resected specimens. Overall activity of integrated medical treatment and secondary liver surgery, consisting of the sum of clinical complete responses (cCR) and liver metastasectomies was also evaluated, as previously reported [6]. PFS and OS were evaluated using Kaplan and Meier method [29]. PFS and PFS from surgery were defined respectively, as the length of time from the beginning of treatment or the date of liver metastasectomy and disease progression or death (resulting from any cause) or to the last contact; OS as length of time between beginning of treatment and death or to last contact. Log-rank test was used to compare PFS and OS in \( \text{KRAS} \) wild-type versus mutant, L-L versus O/MM, and \( \text{KRAS} \) wild-type L-L versus O/MM, and \( \text{KRAS} \) mutant L-L versus O/MM MCRC pts [30].”

“\( \text{Kras} \) and \( \text{BRAF} \) genotypic analysis should be shortened”

\( \text{KRAS} \) and \( \text{BRAF} \) mutational analysis section was shortened, Methods, Mutational analysis, pages 8-9: “\( \text{KRAS} \) and \( \text{BRAF} \) genetic analyses were performed on paraffin-embedded tissue blocks from primary tumor and/or metastatic site. Genotype status was assessed for \( \text{KRAS} \) codon 12-13
mutations and $BRAF$ c.1799 T $>$ A (V600E) mutation by SNaPshot® multiplex screening for $KRAS$ mutations and $KRAS/BRAF$ mutations in 36 and 32 samples, respectively [26,27]; direct sequencing was performed for detection of $KRAS$ mutations in 23 samples and to confirm detected mutations. After treatment with xylene thyocyanate and selection of tumoral cell clusters, DNA was isolated using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE Tissues (Applied Biosystems, Courtaboeuf, France) according to manufacturer’s instructions. Considering the contamination of tumoral samples by non malignant cells, a $KRAS$ mutation in the tumour was defined as appearance of a mutant peak with an height of at least one-third compared to the wild type.

**SNaPshot® and Direct Sequencing assays**

SNaPshot® multiplex assay was performed as elsewhere reported [26,27]. Briefly, $KRAS$ exon 2 and $BRAF$ exon 15 were simultaneously PCR-amplified using specific primers and purified using NucleoSpin® Extract II kit (Macherey-Nagel EURL, Hoerdt, France). PCR-amplified DNA was analyzed using the ABI PRISM SNaPshot® Multiplex kit (Applied Biosystems, Foster City, CA, USA) and five primers including at their 5’-end an additional tail allowing their simultaneous detection. Sense primers allowing the extension at nucleotides $KRAS$ c.34G, c.35G, c.37G, c.38G and $BRAF$ c.1799T were used and multiplex SNaPshot® reaction was performed as reported [26]. $KRAS$ exon 2 sequence was performed from PCR-amplified tumour DNA using the Big Dye V3.1 Terminator Kit (Applied Biosystems, Foster City, CA, USA). Labelled products were separated in ABI Prism 3130xl Genetic Analyzer Applied Biosystems. Data were analysed using the GeneMapper Analysis Software version 4.0 (Applied Biosystems).”

“Statistical analysis should be described and referenced”

Statistical analysis has been described and referenced in Methods, Study design, pages 9-10, as it follows: “A retrospective analysis has been planned to evaluate prognostic relevance of $KRAS$ genotype on clinical outcome of MCRC pts treated with FIr-B/FOx as first line. More, pts were classified according to involved metastatic sites, L-L and O/MM [6], to evaluate the relevance of metastatic extension in $KRAS$ wild-type and mutant MCRC pts. Patients with L-L metastases were evaluated at baseline and every 3 cycles of treatment by a multidisciplinary team, consisting of a medical oncologist, liver surgeon, radiologist, to dynamically evaluate resectability defined according to resectability categories previously reported [6]. Resection rate was evaluated in intent-to-treat population enrolled. Liver metastasectomies were defined as: R0, if radical surgery; R1, if
radiofrequency was added. Surgery was recommended > 4 weeks after BEV discontinuation. Clinical evaluation of response was made by CT-scan; PET was added based on investigators’ assessment.

Clinical criteria of activity and efficacy were: ORR, PFS, OS. ORR was evaluated according to RECIST criteria [28]; pathologic complete response was defined as absence of residual cancer cells in surgically resected specimens. Overall activity of integrated medical treatment and secondary liver surgery, consisting of the sum of clinical complete responses (cCR) and liver metastasectomies was also evaluated, as previously reported [6]. PFS and OS were evaluated using Kaplan and Meier method [29]. PFS and PFS from surgery were defined respectively, as the length of time from the beginning of treatment or the date of liver metastasectomy and disease progression or death (resulting from any cause) or to the last contact; OS as length of time between beginning of treatment and death or to last contact. Log-rank test was used to compare PFS and OS in KRAS wild-type versus mutant, L-L versus O/MM, and KRAS wild-type L-L versus O/MM, and KRAS mutant L-L versus O/MM MCRC pts [30].”

Results

“These interesting results are presented in inconsistent manner and should be reorganized”

We revised Results section in order to better describe retrospective evaluation of clinical relevance of KRAS genotype and extension of metastatic disease:
First, we evaluated and compared prognostic relevance of KRAS genotype in overall MCRC patients treated with triplet chemotherapy plus bevacizumab (FIr-B/FOx). Then, we verified the relevance of extension of metastatic disease (liver-limited compared with other/multiple metastatic sites) in KRAS wild-type and mutant patients: a significantly better prognosis was shown in liver-limited KRAS wild-type pts, while not in KRAS mutant patients. It suggests that integrated Fir-B/FOx regimen and secondary liver surgery may significantly predict better prognosis of KRAS wild-type liver limited pts, while not of KRAS mutant liver-limited pts.

“All of the patients should be considered in the analysis further subgroup analysis should be performed for liver limited patients undergone surgery after treatment.”
As previously described, evaluation of prognostic role of KRAS genotype and metastatic extension was firstly performed in overall MCRC pts (Table 3, Table 4). Further subgroup analysis “for liver limited patients undergone surgery after treatment” was added in Results, Activity and efficacy, page 13, as it follows: “Among the 17 L-L pts who underwent liver metastasectomies, median PFS was 18 months (8-35+ months); median OS was 47 months (10+-56+ months).”

“I suggest a better use of table and use of COX analysis for subgroups LL or LO should be included as parameters and not as separate setting of patients”

Indeed, in the above mentioned plan of results evaluation, L-L (and O/MM) subgroups represent clinical parameters potentially driving different treatment strategies and not a different patients’ setting.

Clinical outcome in terms of progression-free survival and overall survival was compared using log-rank test, as described in Methods, Study design, page 10: “Log-rank test was used to compare PFS and OS in KRAS wild-type versus mutant, L-L versus O/MM, and KRAS wild-type L-L versus O/MM, and KRAS mutant L-L versus O/MM MCRC pts [30].”

“In the survival curves there is a trend to difference this should be commented. Even though PFS and OS are not significant within their patient group the results should be expressed as RISC RATIO and P value showed. In this kind of study a trend may be stimulation for further studies with a greater patient sample”

Effectively, OS curves show a trend toward favourable prognosis of KRAS wild-type compared to mutant patients, even if not significantly. We integrated it in Results, page 12: “KRAS wild-type compared with mutant pts did not show significantly different PFS nor OS, even if OS seems to be favourable in KRAS wild-type pts (Figure 1).”

It was commented in Discussion, page 15: “Median PFS and OS of MCRC pts treated with FIr-B/Fox were different in KRAS wild-type and mutant pts, even if not significantly, while they were equivalent in the FOLFOXIRI plus BEV study [3]. BEV addition to doublet IFL chemotherapy reported median PFS 13.5 and 9.3, median OS 27.7 and 19.9 months, in KRAS wild-type and mutant pts, respectively [18,21]. Significantly better prognosis was reported in KRAS/BRAF wild-
type pts compared with pts harbouring mutations in KRAS or BRAF gene (HR 0.51) [18]. Direct comparison of OS between KRAS wild-type and mutant MCRC pts treated with BEV-containing chemotherapy failed to significantly differentiate prognosis, as in present study. Thus, intensive regimens adding BEV to triplet chemotherapy can further increase activity and efficacy in KRAS wild-type and mutant pts. Randomized studies would properly evaluate it.”

Discussion

“Is too long with conclusions which are not clear.”

“The manuscript should be shortened reorganized. The authors should make a much better effort to clarify the importance of this study.”

Manuscript and conclusions were shortened and reorganized.

We refined discussion and conclusions and we clarified the importance of the study, Discussion, Conclusions, pages 14-17:

“Discussion

In KRAS wild-type pts, BEV addition to doublet chemotherapy significantly increased ORR, PFS and OS up to 60-61%, 10.5-13.5 months and 21.8-27.7 months, respectively [18,21,31,32]. Randomized studies of anti-EGFR added to doublets, in EGFR-overexpressing pts, reported ORR 50-61%, PFS 7.7-10.6 months, OS 22.4-24.9 months [22,23,31-33]. First line cetuximab plus FOLFOX4, significantly improved ORR and PFS in KRAS/BRAF wild-type population, similarly to KRAS wild-type pts [34]. In KRAS mutant pts, BEV addition to doublet chemotherapy (IFL) significantly increased median PFS up to 9.3 months, while ORR was equivalent to doublet arm (43.2% and 41.2%, respectively), and median OS increased up to 19.9 months, even if not significantly [35,21].

In KRAS wild-type and mutant MCRC pts, BEV addition to triplet chemotherapy, according to FIr-B/FOx schedule, reported high activity and efficacy: ORR 90% and 67%, median PFS 14 and 11 months, median OS 38 and 20 months, respectively. Similar clinical outcome was also obtained in KRAS/BRAF wild-type pts. Equivalent efficacy was reported with FOLFOXIRI/BEV regimen; ORR 82% and 71%, median PFS 13.6 and 12.6 months, respectively [3]. In unresectable colorectal liver metastases, ORR 79%, median PFS 14 months, median OS 37 months were reported with chrono-IFLO/cetuximab [5].
Median PFS and OS of MCRC pts treated with Flr-B/FOx were different in KRAS wild-type and mutant pts, even if not significantly, while they were equivalent in the FOLFOXIRI plus BEV study [3]. BEV addition to doublet IFL chemotherapy reported median PFS 13.5 and 9.3, median OS 27.7 and 19.9 months in KRAS wild-type and mutant pts, respectively [18,21]. Significantly better prognosis was reported in KRAS/BRAF wild-type pts compared with pts harbouring mutations in KRAS or BRAF gene (HR 0.51) [18]. Direct comparison of OS between KRAS wild-type and mutant MCRC pts treated with BEV-containing chemotherapy failed to significantly differentiate prognosis, as in present study. Thus, intensive regimens adding BEV to triplet chemotherapy can further increase activity and efficacy in KRAS wild-type and mutant pts. Randomized studies would properly evaluate it.

High activity of triplet chemotherapy plus BEV regimens correlated with increased resection rate of liver metastases and pathologic CR, particularly in L-L MCRC pts [1,3-4,6]. We recently reported that clinical outcome of L-L compared to multiple metastatic disease was significantly improved up to median PFS 17 months and median OS 44 months [6] due to effectiveness of integrated Flr-B/FOx intensive treatment and secondary liver surgery. Present analysis confirm significantly favourable prognosis of L-L compared to MM pts and show that KRAS wild-type L-L pts, accounting for 20% fit MCRC pts, could gain 100% overall activity with integrated medical and surgical approach, due performed liver metastasectomies and long-lasting cCRs, median PFS 21 months and OS 47 months. A significantly favourable prognosis was demonstrated in KRAS wild-type L-L compared to O/MM patients, even if it represents a retrospective, exploratory analysis in a small cohort of MCRC patients. Using neoadjuvant cetuximab with either FOLFOX6 or FOLFIRI for unresectable colorectal liver metastases, metastasectomies were performed in 38% and 30% pts, respectively [36]. Chrono-IFLO/cetuximab reported 60% R0 resection rate in unresectable colorectal liver metastases, with ORR 79%, median PFS 14 months and median OS 37 months [5]. Further prospective studies will properly address if intensive medical treatments, such as Flr-B/FOx, and secondary liver surgery could represent the standard multidisciplinary strategy for KRAS wild-type L-L MCRC pts. In KRAS mutant pts, prevalently harbouring c.35 G > A transversion (53.5%), integrated medical and surgical treatment failed to significantly increase PFS and OS in L-L compared to O/MM pts: median PFS was equivalent (11 months), in spite of 54% performed liver metastasectomies in L-L pts. Proper multidisciplinary treatment strategy of KRAS mutant pts, showing different aggressiveness [37], sensitivity to medical treatment, and worse clinical behaviour, represents an unmet need.

Conclusions
KRAS genotype wild-type and mutant does not significantly affects different clinical outcome of MCRC pts treated with first line FIr-B/FOx intensive regimen. KRAS wild-type pts with L-L disease may achieve significantly greater benefit from integration with liver metastasectomies compared to O/MM metastatic extension, with respect to KRAS mutant patients. Present findings would be verified in prospective trials of multidisciplinary strategies comparing clinical outcome according to KRAS genotype in patients with L-L and O/MM disease.”

We thank very much for the contribution to further increase the relevance of our article by the above criticisms and we hope it will be considered for publication in BMC Medicine.

Sincerely,

Enrico Ricevuto

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