Author's response to reviews

Title: Efficacy of bevacizumab and chemotherapy in the first-line treatment of metastatic colorectal cancer: broadening KRAS-focused clinical view

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Author's response to reviews: see over
Dear Editor,

We resubmit the revised version of the manuscript entitled “Efficacy of bevacizumab and chemotherapy in the first-line treatment of metastatic colorectal cancer: broadening KRAS-focused clinical view”.

Here is the response to reviewers’ comments:

Reviewer 1

General comments:
1. Previous studies have looked at the same question in the past (Rossi L 2013 and Hurwitz HI 2009) as well as clinical trials (as mentioned in Line 234-237); which makes the current study important only to those with closely related research interests and less interesting clinically.
AR: The comment was accepted and no further action is required.

2. This study is still interesting because of its large sample size; nevertheless the role of some other mutations (BRAF, NRAS, PIK3CA, other-KRAS mutations) have not been explored (although this has been alluded to by the authors as a limitation of the study)
AR: The comment was accepted and no further action is required.

Major Compulsory Revisions
1. Line 202-205: “similarly to PFS…” authors have mentioned that the risk was higher whilst the confidence intervals overlap: the p value should be provided, and if not significant it would be better to avoid the statement: “this risk was higher in patients with KRAS mutation…” (Line 204).
AR: The comment was accepted and the text revised. The statement “this risk was higher in patients with KRAS mutation (HR = 2.00; 95% CI 1.52 - 2.63) compared to patients with wtKRAS (HR = 1.43; 95% CI 1.15 - 1.77).” was omitted from the text.

2. Line 263-265: “…patients who received in the first line…” is the difference is statistically significant?
AR: The comments was accepted and further explanation has been provided. Here we discussed the trend. The overall difference between four subgroups was not statistically significant. However, presence of the trend is justified by non-existent [WT KRAS + OX-based CHT OS=31.0 months (28.7; 33.4) vs KRAS mutation + OX-based CHT OS=29.1 months (26.2; 32.1)] or minimal [KRAS mutation + IRI-based CHT OS=24.2 months (21.9; 26.4) vs. KRAS mutation + OX-based CHT OS=29.1 months (26.2; 32.1)] overlap between confidence intervals between certain subgroup.

3. Line 280-281: “this hazardous effect was stronger…” again this is not based on a statistically significant difference
AR: The comment was accepted and the text revised. The statement “and this hazardous effect was stronger in patients with mutKRAS tumors.” was omitted from the text.
4. This study has looked at patients who have received chemotherapy and therefore patients with poor performance and/or with co-morbidities who were not chemotherapy candidates have all been excluded: this is a source of bias (and is worth mentioning in the discussions) for this reason, any conclusions regarding prognostic value of the variables should be made cautiously. The authors need to clarify this in the discussion section.

AR: The comment was accepted and the text revised. In the “adverse event” paragraph in the discussion section, we clarify performance status of the patients in the study “Here it is of note, that according to the national therapeutic indication criteria for bevacizumab, only patients with good performance status (ECOG of 0 or 1) were included into the study. Patients with co-morbidities controlled by medication have not been excluded from the study and standard contraindications for bevacizumab therapy have been applied into all treatment subgroups.” To address the issue of performance status and overall survival in chemotherapy subgroup, we provided additional information in the third paragraph of the discussion section: “As we did not observe a difference in performance status (ECOG of 0 or 1) between patients who started treatment with bev/OX versus bev/IRI-based therapy in the first-line setting (data not shown), the trend toward better OS in bev/OX subgroup is unlikely to be attributed to better performance status in these patients.”

Minor Essential Revisions
1. Page 6 lines 101-109: Naming all different ethics committees is unnecessary
AR: The comment was accepted and the text revised. Omitted from the text.

2. Line 140: RECIST: which version?
AR: The comment was accepted and the text revised. The text now reads as follows “RECIST version 1.0”.

2. Line 211-214: (“thus, the trend …”) it would be better to moved this to discussion
AR: The comment was accepted and the text revised. The statement “Thus, the trend toward relatively improved overall survival in patients who started with bev plus XELOX or FOLFOX may be explained by shorter OS in KRAS mutant patients who received anti-angiogenic therapy on an irinotecan backbone.” was omitted from the text as it was discussed in the original version of the manuscript.

3. Line 287: (“…KRAS mutation in colorectal cancer…”) it would be interesting if the authors discussed any potential molecular explanations
AR: The comment was accepted and the text revised. We discussed kras mutation and metastatic spread to lungs as follows: “Considering the decreased proportion of lymph node metastasis in mutated KRAS patients compared to wtKRAS subgroup (Table 1, [23, 24]), it seems that carcinoma cells with activating mutation in KRAS may exhibit a more hematogenous metastatic spread rather than along a lymphogenous path. Survival of tumor cells within the bloodstream and adhesion in the vasculature at the metastatic sites depend on tumor cell – platelet interactions [25]. We hypothesize that
activating mutation of KRAS inducing expression of molecules responsible for interaction with platelets, such as tissue factor [26], cyclooxygenase and metalloproteinase-9 [27], or cathepsin B [28] might contribute to increased protection of these carcinoma cells against shear stress as well as to enhanced adhesion properties which in turn leads to onset of pulmonary metastasis of mutated KRAS carcinoma cells and higher metastatic activity in general [29].“

Reviewer 2

Discretionary Revisions

1. Previous evidence has shown that there is no association between KRAS status and efficacy of bevacizumab in mCRC, but most of them were based on subgroup analysis of phase III trials. The present manuscript is a large-scale retrospective study that evaluated the prognostic and predictive value of KRAS status in mCRC patients treated with bevacizumab plus chemotherapy which is widely used in clinical practice.
AR: The comment was accepted and no further action is required.

2. In this paper, the investigators defined KRAS mutation only in mutations on exon 2, more evidence suggested RAS status including KRAS (both exon 2 and non-exon 2) and NRAS may have stronger predictive and prognostic value in treatment of mCRC using anti-EGFR therapy, but its value in treatment using anti-VEGF therapy is still unknown. The results would be more valuable if RAS status being used in the analysis.
AR: The comments was accepted and further explanation has been provided.
We are aware of this limitation saying that “An existing limitation of the present study is that we had no data specifying the type of KRAS mutation, NRAS mutation or data on BRAF mutation.”, followed by further discussion.

3. The subsequent treatment after first-line therapy would have significant influence on OS. The manuscript gave information of subsequent anti-EGFR therapy but failed to give information on second-line or third-line intensity of chemotherapy in both groups.
AR: The comment was accepted and the text revised. “The regimen with anti-EGFR antibodies were following: FOLFOX4+ panitumumab, FOLFIRI+panitumumab, FOLFOX4+cetuximab, FOLFIRI+cetuximab, irinotecan (250mg/m² IV every 2 weeks)+cetuximab, panitumumab or cetuximab as single agents. Schedules and dosage of chemotherapy was identical to those applied in the first-line treatment. Panitumumab was administered 6mg/kg IV every 2 weeks, cetuximab was administered 500mg/m² IV every 2 weeks or 400mg/m² IV first infusion, then 250mg/m² IV weekly. The registry did not provide reliable data on number of patients treated subsequently with chemotherapy alone. The chemotherapeutic regimen applied without addition of anti-EGFR antibodies were FOLFOX, FOLFIRI, XELOX or XELIRI as defined in Material and Method section.” This information was included into the legend to figure 1. Despite the fact that the data from the
CORECT registry did not provide information on number of patients treated with chemotherapy alone, patients from both groups (wt-KRAS and mutKRAS) had the same access to subsequent treatment with chemotherapy.

Moreover, additional revisions of the manuscript have been made:
- few typing and formal errors have been corrected;
- title page has been edited according the instruction for authors and affiliations have been updated;
- the text has been updated (the first line in the third paragraph of introduction section);
- formal changes in table 1 and “treatment” chapter in the Material and Method section have been made.

We resubmit
- Revised manuscript with highlighted changes
- Revised manuscript – clean version
- Revised table 1

Figure 1-4 and supplementary Table have not been modified from the original submission.

Kind regards, Lenka Zdrazilova Dubska