Author's response to reviews

**Title:** Retrospective exploratory analysis of VEGF polymorphisms in the prediction of benefit from first-line FOLFIRI plus bevacizumab in metastatic colorectal cancer

**Authors:**

Fotios Loupakis (fotiosloupakis@gmail.com)  
Annamaria Ruzzo (annamaria.ruzzo@uniurb.it)  
Lisa Salvatore (salvatorelisa@libero.it)  
Chiara Cremolini (c.cremolini@sssup.it)  
Gianluca Masi (gl.masi@tin.it)  
Paolo Frumento (dyno@sssup.it)  
Marta Schirripa (martaschirripa@gmail.com)  
Vincenzo Catalano (catalano_v@yahoo.it)  
Nadia Galluccio (nadiagalluccio@virgilio.it)  
Emanuele Canestrari (e.canestrari@uniurb.it)  
Bruno Vincenzi (b.vincenzi@unicampus.it)  
Daniele Santini (d.santini@unicampus.it)  
Katia Bencardino (katia.bencardino@ospedaleniguarda.it)  
Vincenzo Ricci (ricci.vincenzo@hsr.it)  
Mariangela Manzoni (mariangelamanzoni@libero.it)  
Marco Danova (m.danova@smatteo.pv.it)  
Giuseppe Tonini (g.tonini@unicampus.it)  
Mauro Magnani (magnani@uniurb.it)  
Alfredo Falcone (a.falcone@med.unipi.it)  
Francesco Graziano (frada@tin.it)

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

we are pleased to re-submit a corrected version of our manuscript titled “Retrospective exploratory analysis of VEGF polymorphisms in the prediction of benefit from first-line FOLFIRI plus bevacizumab in metastatic colorectal cancer” to BMC Cancer.

We read carefully the considerations done by the reviewers and we revised the manuscript following their suggestion.

POINT-BY-POINT RESPONSE

Referee 1

This is a relatively straight forward retrospective tissue evaluation of prognostic putative markers of bevacizumab sensitivity in patients with metastatic colorectal cancer. The authors clearly acknowledge the limitations of such a study and furthermore state that plans are ongoing to validate their preliminary findings in a prospective fashion. The conclusions adequately address the limitations of this particular data set. This data is of interest particularly since the field of biomarker development is becoming increasingly important in directing the clinical care of cancer patients.

The manuscript is well written but could do with editorial rewrite to make the English in the more common vernacular and therefore easier to read.

Minor Essential Revisions:

Authors reply: corrected as suggested.

2. “While it has been proven that cetuximab is active only in patients bearing KRAS wild-type tumors, up today there are no predictive biomarkers of cetuximab efficacy.” I would revise this to incorporate new evidence that some patients with KRAS mutations may possibly benefit from anti-EGFR therapy, and that it is not entirely clear that benefit is limited to KRAS wild-type patients [De Roock W et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA. 2010 Oct 27;304(16):1812-20].

Authors reply: we have included a comment about De Roock’s study in the background section.
3. Conclusions: remove the word “utmost”

*Authors reply*: removed as suggested.

4. Background: “anti-VEGF MoAb therapy” instead of anti-VEGF’s

*Authors reply*: corrected as suggested.

5. “These events do not compromise the overall efficacy of VEGF inhibition, but especially when life-threatening, have a deep impact on the outcome of a single patient.” I would omit this sentence; although I understand what the authors are trying to imply, any life-threatening intervention would be expected to have an impact on a patient.

*Authors reply*: removed.

6. I think it would be easier to read if the mutations were listed as “VEGF-2578 C/A” instead of “-2578 C/A” throughout the manuscript.

*Authors reply*: we added “VEGF” to each polymorphism mentioned.

7. Page 6: not sure why the abbreviation VEGF is variably italicized throughout the manuscript.

*Authors reply*: “VEGF” is italicized when it refers to the gene and it is not when it indicates the protein. This is in accordance with major guidelines. It could be corrected if there is any specific editorial need.

8. Page 6: tumoral should read tumor

*Authors reply*: changed.

9. Page 6: ECOG 2100 and throughout the manuscript.

*Authors reply*: we changed ECOG2100 in ECOG 2100.

10. Page 6: treated with the anti-VEGF should read anti-VEGF MoAb.

*Authors reply*: changed.

Authors reply: we included a comment about the Koutras’ study in the discussion, even if congress reports are always difficult to be judged in term of quality since only a few data are provided. We did not include a comment about Chae’s study because it refers to an heterogeneous population which included stage I-IV patients, regardless of treatment.

12. Page 8: which version of RECIST was used?
Authors reply: we used RECIST criteria version 1.0. We specified it in the text (Study population, page 8).

Authors reply: corrected.

Authors reply: corrected.

15. Page 9: omit apostrophe “patients”
Authors reply: corrected.

Authors reply: corrected.

17. Page 11: I would not include the doses of FOLFIRI but just state given in the standard fashion with a reference. The manuscript should state that the bevacizumab was given biweekly with the FOFIRI (the way it is written now suggests that it was only given day 1).
Authors reply: the doses of FOLFIRI have been removed and it has been specified that bevacizumab is administered on day 1 every two weeks.

18. Results, page 11: all of the patient information data collected is present in Tables 2 and 3 do not have to be repeated in the narrative (redundant).
Authors reply: removed from the text as suggested.

19. Page 12: mPFS should read median PFS
Authors reply: corrected.

20. Discussion: instead of “blockade efficacy” I would say “clinical efficacy”.
Authors reply: changed.
Referee 2

1) This paper needs a review with a keen eye to grammar and style. There are several areas that are poorly worded, missing words, and at times have grammatical errors. For example, in the first sentence in the background section, "has notably changed in last years," is not appropriately worded. Multiple other examples exist throughout the paper and must be fixed.

Authors reply: The text was extensively revised so that grammar and style were improved as suggested.

2) The background section is too long and needs to be shortened. The background should not include an extensive literature review; this is reserved for the discussion section. The background should be more focused, concentrating on reasons why you took on your study and a concluding sentence clearly indicating your hypothesis.

Authors reply: Background section was shortened and focused as proposed.

3) In the background, the SNP 1154 A/G is discussed in regard to the ECOG trial, yet not studied in this paper. It is important to discuss why you chose the particular SNP's you looked at, and if citing data on a SNP evaluated, discuss why you didn't use a particular SNP from that data cited.

Authors reply: As indicated by the referee the citation was removed from the background section. With respect to which VEGF polymorphisms were chosen it should be considered that data from the ECOG trial were still not available when our study was planned. This latter consideration makes it even more correct to remove the reference to the ECOG trial from the background while citing it in the discussion.

4) Include citations for the ECOG performance status, Kohne prognostic score, RECIST criteria, and the NCI-CTCAE criteria.

Authors reply: Done, except for the ECOG PS that is worldwide known, recognized and routinely used in the clinical practice.

5) Is there any data that could be cited that confirms VEGF SNP's in peripheral blood are the same as that in tumor samples? If so, please cite in your methods section under genotyping. If not, look into this and write another paper.

Authors reply: Schneider and colleagues in the following paper "Analysis of angiogenesis genes from paraffin-embedded breast tumor and lymph nodes. Breast Cancer Res Treat 96:209-215, 2006", demonstrated that the host angiogenic genotype imprints the tumor genotype. We referred to this paper in the background.

6) Add some subheadings to the Results section, such as, "toxicity," and, "treatment response," etc.

Authors reply: We modified the results section according to referee’s 1 comments and therefore this suggestion is no longer applicable.

7) If a p value is not <.05, report it only as a "trend."
Authors reply: corrected when applicable.

8) In the results section, break up major findings with appropriate paragraphs.
Authors reply: considering that, as reported above, we radically shortened the results section following referee’s indication there was no longer need for adding new subheadings.

9) The discussion needs better organization. Focus on your results, what you think they mean, compare with others’ results, discuss the limitations of your study, and finish with a conclusion paragraph. The conclusion paragraph should include conclusions on what your data means ONLY. Your conclusion contains a bunch of info unrelated to your study.
Authors reply: Discussion section and conclusion paragraph were organized as proposed.

10) In the discussion, focus on what you think your data means. Certainly admit that prospective validation is necessary, but absolutely include a discussion of the possible importance of this data, and what might be driving it at the patient level. Do you think Bev only helps in 1498 C/C, or does it hurt patients with 1498 T/T, etc. Make a statement of your thoughts on the meaning of your data, attempt to support it with other literature, and comment on limitations. This is what a discussion section is for.
Authors reply: Our interpretation of the results is that patients with VEGF -1498 T/T genotype seem to benefit less than others from antiangiogenic treatment comparing their outcome with that of other studies with FOLFIRI plus bevacizumab in the same setting. In fact, VEGF -1498 T/T patients achieved a mPFS of 7.5 months that is quite inferior to the about 11 months of the subjects included, for example, in the AVIRI trial. Our results could not immediately influence the clinical practice thus excluding -1498 T/T patients from beva-containing treatments, but need a new prospective validation for verifying this specific assumption. We added a comment about this in the discussion and in the conclusion paragraphs as suggested.

11) Did ECOG performance status maintain significance in the various analyses when grouped by, “0-1,” and “ 2 and higher?” This may be a better break point and if this alters the data, it should be included.
Authors reply: from a theoretical point of view subgrouping the patients in 0-1 vs 2 on the basis of their PS would be more correct, nevertheless only 9 patients in the Beva-group had a PS of 1 or 2 therefore a different way of subgrouping has no value in the present study.
Please note in your Methods section whether ethical approval was sought for this study, and if that approval was not required please state why.

Authors reply: Local EC has approved the study. A specific statement has been included in the Methods section.

Best regards,
on behalf of the authors,

Fotios Loupakis, MD

Polo Oncologico, Azienda Ospedaliero-Universitaria Pisana
Department of Oncology, Transplants and New Technologies in Medicine,
University of Pisa
Via Roma, 67
56126 PISA, Italy
Phone: +39 050 992451
Fax: +39 050 992467
e-mail: fotiosloupakis@gmail.com