Author’s response to reviews

Title: PDGFRalpha/beta AND VEGFR2 polymorphisms in colorectal cancer: Incidence and implications in clinical outcome

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Author’s response to reviews: see over
Sevilla, October 5th 2012

**Manuscript Title:** “PDGFRα/β and VEGFR2 polymorphisms in colorectal cancer: incidence and implications in clinical outcome”  
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Dear Editor,

On behalf of all co-authors, please find enclosed the revised version of the above-referenced manuscript in accordance with your suggestions and concerns, as detailed in the following point-by-point reply. The manuscript has been reviewed by our institutional editing service for English quality. We hope that we have satisfactorily addressed the issues raised by the reviewers and look forward to hearing from you soon.

Thank you again for your assistance in this process.

Sincerely yours,

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REFEREE #1

Major Compulsory Revisions

1. Author should clarify differences of the oncological therapies between WT and SNP in PDGFR B19 and PDGFR B19. If author are unable to clarify differences of the oncological therapies between WT and SNP in PDGFR B19 and PDGFR B19, author should not describe the relationship between PDGFR/#/PDGFR# genetic variants and colon cancer survival.

As requested by the reviewer, oncological therapies (surgery and chemotherapy) by PDGFR A13 and B19 genotypes have been described in Table 2. As illustrated in the new table, no major disbalances were observed by study group.

2. P17, l: 17-19

“In this study, response rates were significantly higher in patients homozygous for the A-allele (AA) than in patients with the C-allele genotype (CC or CA) (56% vs 39%, p=0.015).”

Author should clarify the evidences mentioned above in Methods and Result sections.

These results regarding the association between VEGFR-1 genotype and response rate are not derived from our own results but rather on the study by Hansen et al that we are commenting upon in the discussion section. Therefore, it does not apply to our own Methods or Results sections.

In order to avoid misunderstanding in this regard, the sentence has been now modified and the full paragraph now reads as follows:

“Very recently, the VEGFR-1 319 C/A SNP, located in the promoter region of the gene, has been reported to be associated with response to therapy in a cohort of 218 CRC patients treated with different bevacizumab-containing regimens. In this study by Hansen et al, response rates were significantly higher in patients homozygous for the A-allele (AA) than in patients with the C-allele genotype (CC or CA) (56% vs 39%, p=0.015).”

Minor Essential Revisions

1. p2 (abstract)
   l: 12
   c.2439+58C>A) => c.2439+58C>A
   l:13

2. p14 (discussion)
   l:9
   a significant => a substantial

Editorial and style corrections have been made in page 2 and page 14 following the reviewers suggestions.
REFEREE #2

First of all, the authors should be congratulated with a fine study. I especially acknowledge two parts of the design. The originality of focusing at a specific location of the genes of interest and afterwards testing the possible functional influence of their results based on CRC cell lines.

Having said that there are also a few smaller issues that should be clarified:

Discretionary Revisions

1. The introduction (p. 3 l.14): It could be specified, that bevacizumab target VEGF-A instead of writing just VEGF especially since the authors later in the introduction decide to differentiate between the ligand subtypes.

As suggested by the referee, the introduction (p.3 l.14) has been modified to specify that bevacizumab targets VEGF-A rather than VEGF.

2. Material and Methods. The source of DNA for the present study is not entirely clear. The author’s state that DNA was isolated from tissue sections from the tumours and by that must represent tumour-derived genomic DNA which is not necessarily the same as “normal” genomic DNA. Please clarify this.

The source of DNA for the present study was tumor DNA as we describe in the Material and Methods section (third paragraph of page 6): “DNA isolation from human tumor samples and culture cells. …….Three tissue sections of each tumor were first deparaffinized and rehydrated…. Then, DNA isolation from both human tissue samples and culture cells was performed…….”

However, this paragraph has been modified to clarify this issue and now reads as follows: “DNA isolation from human tumor samples and culture cells. …….Three tissue sections of each tumor were first deparaffinized and rehydrated ……….Then, DNA isolation from both human tumor tissue samples and culture cells was performed…….”

3. Table 2: According to the results section, the rare allele of the PDGFR# 19 SNP exists in heterozygous as well as homozygous genotypes. Why is this not shown? The 45 patients are grouped together under “SNP”. This could easily influence the presented relationship and especially when near significant results are obtained (the correlation with tumour location) it becomes even more important for the reader to have access to the “raw data” showing the relationship between all three genotypes and tumour location.

PDGFR B19 SNP was present in homozygosis only in 7 cases. The presence of the SNP in homozygosis or heterozygosis did not significantly impact the association with the different clinical and pathological features nor its impact in clinical outcome (survival). The low numbers of the homozigotic variant per subgroup preclude, however, any firm conclusions in this regard and certainly makes the table more hazardous to read. For these reasons we decided to group the SNP variants together.

Minor Essential Revisions

4. Table 1: Why is tumour differentiation only available for 80 patients? – and in the same context this could very well have an influence on the near significant results
presented in Table 2 regarding the correlation between “SNP 13” and tumour differentiation.

Tumour differentiation was “only” available in 80 of 92 cases as this histological feature is usually not provided for mucinous or colloid variants (12 cases in our cohort). Therefore, no further conclusions may be achieved in this regard.

5. Table 3: The presented results regarding PDGFR-A13 are a bit surprising. After 37 months (approximately 3 years) 50% of the WT (AA) patients have died and after two more years (5-year survival) 14% is still alive. This seems plausible. On the other hand when one takes a look at data for the SNP (AG) group results are a bit surprising. 50% have died after less than 2 years (21.7 months) and for the following 3 years only an additional 9% dies? I know that this group only consists of 13 patients but it still surprises be – are these numbers correct?

We have indeed checked the figures regarding this very pertinent observation, which as the reviewer points out may likely be due to the low figures at the “tail” of the curves, and have verified that the numbers are correct.

6. Figure 2. Please specify the reasons for censoring survival data.

Censored data in Kaplan-Meier survival curves refer to all those patients in whom the event (death) has not occurred, that is, patients that were still alive at last follow-up visit.