Author's response to reviews

Title: FCGR2A and FCGR3A polymorphisms and clinical outcome in metastatic colorectal cancer patients treated with 1st line 5-fluorouracil/folinic acid and oxaliplatin +/- cetuximab

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

Thank you for giving us the opportunity to revise and resubmit the manuscript. Below is our response to the reviewers’ comments.

Reviewer: Marco BL MBL Rocchi

Major compulsory revisions

In detail I wonder if the authors performed the necessary check of parametric assumptions before using both Anova and Cox's model (normality and omoschedasticity, and proportional hazard checks, respectively). So, I suggest to state these checks (if performed) or to perform them.

We did perform the necessary checks and have added the following sentences to the Result section, under Statistical analyses, lines: 158.-159; “Homoscedasticity was ascertained and the non-parametric Kruskal-Wallis test was applied as a sensitivity analysis”, and lines 164.-165; “The assumption of proportional hazards was checked by inspection of log minus log plots.”

Minor essential revisions

Table 1. Please, use X instead of x to indicate "chi" inside the symbol of chi square.

We have changed x to X in Table 1.

Tables 2,3,4,5. I suggest to add, in captions, the statistical tests performed.
Reviewer: Patricia de Cremoux

Major compulsory revisions

1. This paper analyses FCGR2A & FCGR3A polymorphisms in the context of metastatic colon cancer with and without KRAS exon 2 mutations. These results are interesting since the paper showed that these KRAS mutation status in the context of NORDIC-VII trial is not predictive of response to cetuximab whatever will be the arm of chemotherapy regimen. This remains either in accordance and also contradictory of some recent published trials with similar or different chemotherapy regimen. The subject remains to be elucidated. In fact, even if the datas on KRAS status are not in front line, they represents in addition to BRAF status a major element of the analyse of this paper and it has to be completed.

In the NORDIC-VII trial (published in 2012), the authors analysed for KRAS gene only hot spots mutations of codons 12 and 13 (7 mutations analysed). These alterations represent the majority of KRAS gene mutations, however, recent papers demonstrated that “wt type RAS “colon cancer are defined by the absence of KRAS exons 2, 3 and 4 mutations and the absence of NRAS exons 2, 3 and 4 mutations. This represents 17% additional RAS mutations that are not negligible. The predictive value of non-response to EGFR antibodies targeted therapy is increased by these determinations as recently published in the PRIME study (with another chemotherapy regimen and panitumumab).

The authors could precise in the material and methods section the KRAS exons (or codons) analysed in NORDIC-VII trial (they were indicated in the referred paper, however of major importance in this context).

We have added the following sentences in the Material and methods section, (lines 125 -131): “DNA from primary tumors was screened for the presence of seven KRAS mutations (codons 12 (G12D, G12A, G12V, G12S, G12C, G12R) and 13 (G13D)) and one BRAF (BRAF V600) mutation as previously described (Tveit et al). KRAS and BRAF mutation analyses were obtained in 498 (88%) and 457 patients (81%), respectively. KRAS mutations in codons 12 and 13 were found in 39% of the tumors. BRAF mutations (V600E) were present in 12% of the tumors. The mutational frequencies of the 195 KRAS (codons 12 and 13) mutations in the NORDIC VII cohort were; G12A (9.7%), G12R (1.5%), G12D (35.4%), G12C (9.7%), G12S (6.2%), G12V (15.4%), and G13D (22.1%)”. (lines 136-138); KRAS status was available from 442 and 437 patients with FCGR2A and FCGR3A status, respectively. BRAF status was available from 405 patients with FCGR2A and FCGR3A status.”
If it is possible for the authors, could they obtain KRAS exons 3 and 4 and NRAS exons 2, 3, and 4 data’s in their series of patients and analyse them in their study?

A follow up study of the NORDIC VII cohort will include additional analyses of KRAS (exons 3 and 4) and NRAS (exons 2, 3, and 4). We may expect to find additionally 17% RAS mutations when we screen for selected codons in these exons [1]. Taking into account that there was no effect of cetuximab in the KRAS wild-type (exon 2) population in the published NORDIC VII study, we find it unlikely that the additional mutations in the KRAS exon 2 wild-type population will affect the outcome (ORR, PFS, OS) significantly, or have an impact on the subgroups of the FCGR polymorphisms. Moreover, it is possible that the reported outcome of the KRAS exon 2 subgroup, in the NORDIC VII trial per se or in the different subgroups of the FCGR polymorphisms will be more pronounced, however not principally different, with a larger RAS mutation subgroup including cases with NRAS and additional KRAS (exons 3,4) mutations. We therefore suggest the current KRAS mutation data as sufficient for the conclusions in the present publication. We hope this is acceptable to the referees.

If not possible, could the authors clearly mention it in the material and method section, and largely discuss this point in the discussion section?

We have added the following paragraph in the Material and methods section, lines 143 - 147: “Primary tumors in the NORDIC VII study were screened for KRAS exon 2 (codons 12 and 13) mutations. However, recent studies have demonstrated that wild-type RAS should be defined by the absence of KRAS exons 2,3, and 4 mutations and the absence of NRAS exons 2, 3, and 4 mutations [1-3]. A follow-up study of the NORDIC VII cohort will include these additional mutational analyses.”

We have added the following paragraph in the Discussion section, lines 270 - 276: “Primary tumors in the NORDIC VII study were screened for KRAS exon 2 (codons 12 and 13) mutations. Recent studies have though demonstrated that the selection of patients for anti-EGFR therapy may improve by considering RAS mutations other than KRAS exon 2 mutations (NRAS exons 2, 3, and 4 and KRAS exons 3 and 4)[1-3]. It is expected to find up to 17% mutations in the KRAS exon 2 wild-type population in the NORDIC VII cohort. We do not expect that the contribution of the additional mutations will considerably alter the outcome of the FCGR polymorphisms. Lack of this data is however a limitation of the present study.”

2. In the results section the authors showed that they observed a higher response rate in patients with FGFR2A R/R polymorphism when cetuximab was added either regardless to KRAS mutational status, and in the group of KRAS mutated tumours. However, as showed in table 3, we observed that the response rate of this group of patients is similar to those of all other groups with the exception of the group of patients with FGFR2A R/R polymorphism that is clearly lower. One conclusion could be that patients with FGFR2A R/R polymorphism have a significant decreased response rate in the group of patients treated without cetuximab. All other genotypes gave similar response rate with or without
cetuximab, and with or without KRAS mutated tumour. Could the authors discuss these results?

The addition of cetuximab to Nordic FLOX lead to an increased response rate in patients with the FCGR2A R/R genotype. This difference was even larger in a subgroup analysis of KRAS mutated patients.

We have revised the conclusion in the abstract to emphasize our main finding more clearly in the manuscript., lines 82-84: “Patients with KRAS mutated tumors and the FCGR2A R/R polymorphism responded poorly when treated with chemotherapy only, and experienced the most benefit of the addition of cetuximab in terms of response rate.”.

We revised the first paragraph in the Discussion section and added the following sentence (lines 206-207); “Subgroup analysis of patients with KRAS mutated tumors and the FCGR2A R/R genotype showed an even larger increase in response after the addition of cetuximab”.

We amended a paragraph in the in the Discussion section in the previous manuscript to the following (lines 248-254); “Our study show that patients with KRAS mutated tumors and the FCGR2A R/R genotype responded poorly when treated with chemotherapy only and experienced the most benefit of the addition of cetuximab in terms of response rate. In line with this, Correale et al. demonstrated that activating KRAS mutations in colon cancer cell lines may correlate with a higher susceptibility to cetuximab-mediated ADCC [4]. Another study by Schlaeth et al. found that KRAS mutated tumor cells could be effectively killed by ADCC, indicating that mutated KRAS is not enough to confer resistance to antibody-mediated cell killing [5].

The first paragraph in the Conclusion was revised (lines 278-280): Patients with KRAS mutated tumors and the FCGR2A R/R genotype responded poorly when treated with chemotherapy only and experienced the most benefit of the addition of cetuximab in terms of response rate.

We have added one figure (Figure 2 in the revised version) to the manuscript which illustrates the response rates in patients with KRAS wild-type tumors according to the FCGR2A subgroups. We added this figure to better illustrate the difference in response rates between patients with KRAS wild-type vs KRAS mutated tumors according to the FCGR2A polymorphisms. The following sentence has therefore been added in the Result section (lines 186-188); “There was no significant difference in response rates in the FCGR2A subgroups in patients with KRAS wild-type tumors after the addition of cetuximab, Table 4 and Figure 2.”

Minor Essential Revisions

3. The authors could also include the recent references including the data of RAS (KRAS and NRAS) determination and response to EGFR antibodies

We added the following references;
Reference List


Please find enclosed our revised manuscript which we hope will be acceptable for publication.

Kind regards,

Janne Beathe Kjersem on behalf of Elin Kure (corresponding author)

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