Reviewer’s report

Title: Downstream Mutational Status and Association to Clinical Outcome of Third-line Cetuximab-Irinotecan in Metastatic Colorectal Cancer

Version: 1 Date: 20 September 2010

Reviewer: Annamaria Ruzzo

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This is a potentially interesting manuscript with the aim to assesses the association between mutational status of the most relevant EGFR regulators (KRAS, BRAF, PIK3CA, PTEN) and EGFR comprised encompass and clinical outcome of metastatic colorectal cancer (mCRC) patients treated in third line with cetuximab-irinotecan. The basic scientific methods used to analyze the mutational status of the EGFR regulators appear to be appropriate.

Major Compulsory Revisions

1- The title of this manuscript is: Downstream Mutational Status and Association to Clinical Outcome of Third-line Cetuximab-Irinotecan in Metastatic Colorectal Cancer.
Questions: “Downstream mutational status” of what? Please clarify

2- In the section “abstract”, under “background” the Authors report: “The aim of the present study was to investigate the clinical value of these mutations in a homogeneous material of patients with mCRC treated with third-line cetuximab/irinotecan”. Please clarify this sentence in the manuscript: if you mean that patients were homogenous relatively to the treatment because the histological specimens were not, as reported in “material methods” (see also point 6)

3- In the section “abstract”, under “methods” the Authors report that 107 patients were prospectively included in the study, but the mutational status analysis performed was assessed only on 94 patients as reported at the section “results” under “mutational status”, for EGFR expression 68 tumors only. The mutational status is the aim of the study so I think it should be better report also in the abstract the exact number of patients analyzed for the mutational status.

4- In the section “abstract”, under “results” the sentence “Supplementary BRAF and PIK3CA analysis indentified an additional 11% of non-responders”. It is not clear if this 11% of not-responder was found in addition to KRAS mutated non-responders or other. Please clarify

5- In the section “introduction” the Authors hypothesize that the relatively low frequency of BRAF mutation found in the study, may be due to poor sensitivity assay similar to previously published data on KRAS analysis. But in their study
the Authors report for KRAS the same percentage reported in literature. Why didn’t the Authors chance the kit or the method (sequencing?) to detect BRAF mutations? In addition, I’m surprised that a BRAF kit is not sensitive. Did the Authors make questions to the manufacturer? The formalin-fixed paraffin-embedded tissues were of good quality for the analyses?

6- In the section “material and method” under “patient material” the Authors report that primary tumor AND/OR corresponding metastasis were collected for this mutational study; this means that mutational analysis was performed in primary tumor and/or metastasis depending on the tissue available, is this interpretation correct? Please clarify in the section. Moreover, it has been reported that there is a high concordance of KRAS status between primary and related metastatic sites in literature but not for BRAF and PIK3CA status. In particular Santini et al (2010) report that the level of concordance in primary and related metastatic sites is lower when the primary tumor harbors the BRAF mutation. The Authors should report how many primary tumors and how many metastases they analyzed when the primary tumors wasn’t available.

7- for the section “discussion” I have the same questions reported at point 4. Moreover, if the percentage of BRAF mutation as reported in literature reach 8-10% and in this work submitted is 3%, this means that a number of patients were no detected and this could influence heavily the triple mutation analysis, but not only that. I’d suggest to change the BRAF kit and do over again the BRAF mutation analysis, also on metastatic tissues.

Minor Essential Revisions

1. Please correct wildtype to wild-type or wild type
2. Please correct Di Nicolantonio and check the reference (5)

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

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Very minor compulsory revisions

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I declare that I have no competing interests