Reviewer's report

Title: Beyond KRAS mutation status: influence of KRAS copy number status and microRNAs on response to cetuximab in metastatic colorectal cancer patients

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Reviewer: Peter M Wilson

Reviewer's report:

In the current manuscript the authors address an important issue which is the continuing identification of predictive biomarkers to the anti-EGFR monoclonal antibodies. While the identification of KRAS mutation and its role in resistance has improved our understanding of which patients should be candidates for these therapies, a large percentage of KRAS wild-type patients still do not benefit from the addition of these costly antibodies to standard chemotherapy. The authors address a further question regarding KRAS - the influence of gene copy number and/or amplification to clinical outcome with cetuximab. In addition, they attempt to correlate micro-RNA expression with clinical outcome to cetuximab. The concepts behind the paper and the biological rationale for analyzing these parameters is sound and the selected patient population provides a good platform with which to test these hypotheses. The manuscript overall is well constructed and well written with the exception of a few grammatical and typographical errors.

Major compulsory revisions:

1. Unfortunately, despite the sound rationale, the study suffers from a lack of patient numbers and the authors are aware of this and do acknowledge it to an extent. While some interesting statistical trends and potentially significant results are observed, the paper does not provide any robust evidence of the influence of these markers. With some of the subgroups analyzed possessing as few as 6 patients (KRAS mutant; good responders), this is not surprising. With such low patient numbers, the influence of other variables is extremely difficult to account for - although the authors did attempt to control for those that were possible. However, considering this, with appropriate acknowledgement as a pilot study and the limitations that exist with regard to the patient numbers and statistical power available, with some modifications the paper would be acceptable for publication. The authors do provide sound biological rationale and supporting references for the observed effects. The discussion is well written (if a little lengthy) but gives the distinct impression of a successful study with positive data and sound observations which may be overstating the strength of the data. I would request that the second last paragraph of the discussion expand upon the limitations of the study in more detail in a constructive manner to allow the reader to interpret the results in the context of the limitations.

2. One of my concerns with the manuscript is the authors use of the term
‘response to cetuximab’ in the manuscript title when the measure of clinical outcome used in the study was progression-free survival on cetuximab treatment. The methods state that the 34 patients were selected based on the extremes of PFS. The authors should perhaps reconsider the use of this term as a good progression-free survival is not always synonymous with a ‘good response’ and can be a measure of a cytostatic or disease stabilization scenario. The term ‘response’ in the context of clinical trials is well defined (usually by RECIST) and the authors are not comparing the influence of copy number or miRNA expression to tumour response. In the abstract the authors use the term ‘clinical outcome’ in their background - I recommend this. This also applies to other areas of the manuscript where the term ‘good responders’ and ‘poor responders’ are used.

3. What was the rationale behind selecting PFS vs response rate? This should be mentioned. Incidentally, the correlation between response rate and PFS in the 17 patients selected in each category might be useful to know - particularly in the first results section and table 1 where the PFS range for each group is specified. If there is a valid reason why PFS is more appropriate than response rate for this analysis, it should be mentioned.

4. In the introduction the authors do not adequately delineate the differences between KRAS mutations. Specifically, the recent observation that patients with the G13D KRAS mutation may actually benefit from an EGFR monoclonal antibody vs mutations in codon 12. This is particularly important since in their 34 patients, only 1 patient has a codon 13 mutation. This also applies to the discussion where the authors discuss the nature of certain KRAS mutations including mention of codon 61 mutations - the data regarding codon 13 mentioned much later in the discussion should be brought forward. De Roock et al. JAMA. 2010 Oct 27;304(16):1812-20.

5. Another point regarding the introduction - some mention of additionally tested yet unconfirmed predictive markers that have been analyzed with regard to response to anti-EGFR antibodies are mentioned in the discussion (PI3K, ligands, SNPs etc) but might benefit from being brought forward to the introduction to help support the rationale for the study...i.e. support the need for continued efforts to identify biomarkers to these therapies. This would help as the discussion is bordering on lengthy with some repetition.

6. In the discussion the authors do bring up the interesting results of the CAIRO2 trial whereby the cohort receiving chemotherapy, bevacizumab and cetuximab had decreased PFS compared to chemotherapy plus bevacizumab. An explanation as to why this limitation may not apply to this retrospective subset analysis is provided, but is not satisfactory and the potential interaction between the two biologics and the potential impact on this study should be expanded upon briefly.

Minor essential revisions

1. In the methods, the authors used a different patient population to derive their
KRAS gene copy number data. Although they provide a reference, they refer to it as ‘our previous Phase III study’. Since this population of patients contribute directly to the data in the manuscript, they should provide some brief details on this population including the nature of any therapy received and informed consent etc….simply for consistency and to inform the reader.

Minor discretionary revisions

1. In the final sentences of the introduction the authors state that there is currently no data available on the clinical relevance of miRNAs involved in KRAS activity, but I am aware of at least one study that utilized a cetuximab-treated clinical cohort and analyzed the influence of polymorphisms in a LET-7 miRNA binding site and response to cetuximab in KRAS wild-type patients. Although it is indirect evidence by analyzing SNPs vs directly measuring miRNA expression, it supports the concept nonetheless and perhaps warrants a mention. Zhang et al. Ann Oncol. 2011 Jan;22(1):104-9.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.