Reviewer's report

Title: Prediction of response to anti-EGFR antibody-based therapies by multigene sequencing in colorectal cancer patients

Version: 2

Date: 18 June 2015

Reviewer: cecily vaughn

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This paper tests colorectal cancer patient samples wildtype for KRAS mutations for mutations in additional genes, and correlates the occurrence of mutations with therapy response. Clinical correlation with these lesser occurring mutations is often lacking. Although the sample size of mutations in specific genes is small, this paper adds to this body of knowledge, and is thus an important contribution.

Minor Essential Revisions:

1. On pg. 4, line 27, “equipments” should be either “equipment” or “instruments.”

2. On pg. 6, line 1, I believe the comma after insertions is not intended.

3. In the Methods describing Sanger sequencing, the manner in which the primer sequences are presented is a little confusing. Are these sequencing primers used with amplicons generated using the same primers as in the NGS assay? Clarification here would be appreciated. (However, I don’t think the inclusion of all of the Sanger methods and data is strictly needed, as noted in my comments below.)

4. The statistical significance of KRAS mutations detected alone and in addition to other mutations is noted several times. In this paper, it is referring to the additional KRAS mutations not previously detected by the TheraScreen assay, rather than all KRAS mutations. The paper would benefit from clarifying that the statistics refer to the additional KRAS mutations. For example, on pg. 7, line 27, “Mutations in KRAS…” could be amended to “Previously undetected mutations in KRAS…” Another is example is on pg. 10, line 27. There may be additional places where this clarification would be beneficial.

Discretionary Revisions:

1. The feasibility of this testing in clinical practice is referenced in both the Background and in the Conclusion portion of the Abstract. This is an important point; thus, it may be useful to include information about the number of samples that can be multiplexed on a single chip (in the Methods section) and/or estimated costs of performing a panel of this size on the Ion Torrent PGM.

2. In the Methods section, consider adding whether the tissue samples were enriched for tumor content, e.g., by microdissection, prior to DNA extraction.
3. It is stated that only mutations at greater than 15% were considered. Could you comment on whether mutations below this limit were present in any of the samples?

4. The Sanger confirmations displayed in Figures 2 and S3 look fine. However, now that NGS technologies are well established, I think the Sanger confirmations (methods, results, or figures) could be removed from the paper if desired – with the exception of keeping the results (figures not necessarily needed) regarding the confirmation of the KRAS mutations G12C, G12V, and G13D missed by TheraScreen in five patients.

**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.