Reviewer's report

Title: Expression profiling of blood samples from an SU5416 Phase III metastatic colorectal cancer clinical trial: a novel strategy for biomarker identification.

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Level of interest: A paper of considerable general medical or scientific interest

Advice on publication: Accept without revision

In this study, the authors sought to identify gene transcripts (ie, 'surrogate biomarkers') in peripheral blood mononuclear cells (from cancer patients in a Phase III clinical trial) that predict for patient treatment with the VEGF RTK inhibitor, SU5416. By microarray analysis, 13 transcripts were detected that met certain significance and consistency criteria, and a subset of these was investigated further by RT-PCR. Four confirmed transcripts were then independently validated by RT-PCR on a larger patient sample population, and found to have fairly high class prediction accuracy.

In general, I feel the authors covered their bases well. The study design, though apparently not originally conceived with microarray analysis in mind, was adequate (in terms of technical considerations and sample population size) for addressing the scientific question via DNA microarrays. I felt the analytical and validation strategies were appropriate and the interpretation of the results sound. Overall, I found the study to be interesting (as surrogate biomarkers could have particular clinical value, and microarrays have the potential to facilitate their discovery) and the manuscript to be well written. As such, I have no compulsory revisions to recommend. I do, however, have several minor discretionary suggestions and comments.

Discretionary Revisions:

1. The data is convincing that the identified transcripts do indeed predict for the SU5416 treatment arm. This fact alone is of singular importance and is rightly the major focus of the study. However, knowing the factor(s) responsible for these transcripts is important for their clinical utility, and from this study it is not clear what, exactly, these transcripts are biomarkers of. The authors address this issue in their discussion and acknowledge that follow-up studies in vitro suggest that the transcripts may not be induced directly by SU5416 or the premedication, dexamethasone. Other possibilities that the authors point out include the administration vehicle, Cremophor (which is itself highly cytotoxic), and other SU5416 trial arm-specific premeds such as H1- and H2 blocker antihistamines (used to minimize the side effects of Cremophor). It is quite plausible that one or more of these
agents could be directly responsible for the transcript profiles. Why then were these possibilities not tested on the in vitro model together with SU5416 and dexamethasone? Since the authors investigated the effects of SU5416 and dexamethasone on the transcripts and reported the preliminary observations, I think they should have simultaneously tested and reported on the other obvious possibilities as well.

2. It is interesting to speculate that in the validation studies perhaps the proteins corresponding to the transcripts might serve as more reliable biomarkers than the transcripts themselves thus augmenting class prediction accuracy. For example, in the case of Defensin alpha 3 (which had the lowest p-value (0.0002) of all candidates identified in the initial Affy analysis), its failure to recapitulate the same level of significance in the RT-PCR study could be the result of a suboptimal oligo primer for RT-PCR resulting in reduced sensitivity of transcript detection or cross-hybridization. Also, RNA levels do not consistently reflect protein levels, and given the paucity of RNA sample available in this study, protein levels might be more reliably detected relative to transcript levels by RT-PCR with limited template. This approach might be considered further in follow-up investigations.

3. In the discussion, the authors briefly mention that they found no correlation between gene expression patterns and clinical response (which, interestingly enough, could be divided into only two categories in the 23 patients: partial response (PR) or progressive disease (PD), with the exception of a single complete response (CR)). It is not said what type of analysis was done. It would be interesting to see the result of a supervised analysis that disregarded the SU5416 and control arm designations and only took into account partial and complete responders (12 total) versus those with progressive disease (11 total).

**Competing interests:**

None declared.