Reviewer's report

Title: Thymidylate Synthase, Dihydropyrimidine Dehydrogenase, ERCC1, and Thymidine Phosphorylase Gene Expression in Primary and Metastatic Gastrointestinal Adenocarcinoma Tissue in Patients Treated on a Phase I Trial of Oxaliplatin and Capecitabine

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Reviewer: Elisa Giovannetti

Reviewer's report:

The present work describes the gene expression analysis of selected molecular markers in paired microdissected metastatic and primary tumors (97% colorectal cancers).

Furthermore, gene expression was correlated with clinical outcome (response, TTF and survival) in patients enrolled in a Phase I clinical trial examining the feasibility of administering a fixed dose of oxaliplatin with escalating doses of capecitabine.

Overall, the manuscript deals with an issue of topical interest, which has been the focus of a large series of studies in colorectal cancer patients treated with various schedules of 5-FU and leucovorin.

However the experimental results add some novel information, including the analysis of paired metastatic and primary tumors and the evaluation of several clinical parameters to understand the prognostic and/or the predictive value of critical molecular markers in patients treated with the combination of oxaliplatin and capecitabine, which is commonly used in the second- and third-line treatment of colorectal cancer.

The research is well presented, however there are some essential revisions that might clarify some points to the reader:

1. The abstract and text of the manuscript should clearly identify which was the aim and the primary endpoint of the pharmacogenetic study

2. The authors stated that they enrolled in the study a heterogenous population, with a median of two prior chemotherapy regimens. However the Authors should add a table with the main clinical characteristics of the patients.

3. It is not clear, from the methods and the results section, why the Authors analyzed disease control of 60 (see Table 2) out of the 65 and 64 patients with TS and ERCC1 gene expression values, respectively (see Table 1). In order to assist the reader, the Authors should add a flowchart showing the number of the tissues (primary and metastatic) as well as of the clinical and pharmacogenetics data available from the subjects enrolled in the study

4. The discussion should be more careful. The number (N) of patients in this study is small, especially when stratified by gene expression. This may explain
that some of the differences observed in this population versus others in previous studies may simply be attributable to chance. Furthermore, the Authors should add in the legend the N of the patients in the groups presented in the figure 3 and 4.

5. Although considering the small N it does not appear appropriate to discuss non-statistically significant findings in detail, the Authors should explain which possible combinations of molecular marker expression (See last statement in the Results section) were analyzed.

6. The samples from metastatic tumors were frozen tissue sections, while the archival primary tumor tissue was available as formalin fixed paraffin embedded (PFFE) tissue blocks. Several previous studies have shown that, compared with formalin fixation, cryofixation and sectioning result in samples with significantly more preserved RNA (Goldsworthy et al. Effects of fixation on RNA extraction and amplification from laser capture microdissected tissue. Mol Carcinog 1999; 25(2):86-91). Indeed, formalin fixation results in chemical modification of cellular nucleic acids, effectively limiting the size, quantity and quality of cDNAs that can be produced by reverse transcription. The need to pick up more material is time-consuming for the operator, increases the costs for the materials, and implies a significant large period of time for samples harvesting, which is likely to start the process of RNA degradation. Do the Authors have any data about the amount of cells they obtained in the different cases and whether their procedure from PFFE samples might affect the quality of the RNA and the following analysis of gene expression? I would really appreciate to have more data about quality of RNA and the results on possible control tissues that are essential for the analysis in paired microdissected metastatic and primary tumors.

7. The Authors should better clarify why they think that “some subjects received potentially lower than optimal therapeutic doses” and whether this could affect not only the TTF, but also disease control (CR+PR+SD) and OS.

8. It would be interesting to have further information about comparison between ERCC1, DPD, TS and TP expression measurement and data obtained from the usual pathologic exam (i.e. differentiation grade).

Minor revision:
1. Regarding the quantitative PCR, could the Authors give more details, including the reproducibility (C.V. etc.)?

2. The Authors stated that there was a “moderately strong correlation between DPD and TP expression”. Since the p value is below 0.0005. I suggest to delete “moderately”.

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests