Author’s response to reviews

Title: Molecular Risk Assessment of BIG 1-98 Participants by Expression Profiling using RNA from Archival Tissue

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Author’s response to reviews: see over
Dear Dr. Le Good

Thank you for your reply and the reviews regarding our manuscript 1967450280304825 "Molecular Risk Assessment of BIG 1-98 Participants by Expression Profiling using RNA from Archival Tissue" by Antonov et al.

We included a statement about the ethical approval of our study and we refer to the reference Viale et al. J Clin Oncol 2008; 26: 5569-5575 [ref. 23] who have previously used the same tumor material for their analyses (page 5).

We also addressed the comments raised by Reviewer #3 and we give a point by point answer to all of them:

- We added a sentence in Materials about prospective, in silico selection of genes for molecular scores (page 7)
- We also include a link to the requested data that was previously published by Wirapati et al. in Breast Cancer Research: http://breast-cancer-research.com/content/10/4/R65/table/T1 (please inform me know if you prefer a separate Table added as suppl. information to this manuscript)
- We included a statement about tumor cell content in the samples used in this manuscript ("Methods" section, page 6)
- We present RIN numbers (RNA integrity numbers) for RNA from FF and RNA from FFPE material (Methods, page 6)
- A statement is included that all patients were treated by mastectomy or breast conserving surgery (page 6)
- A sentence was added which says that RNA was isolated from representative tumor regions (page 6)
- Following the suggestion of the reviewer we modified the sentence in the Discussion which said "Tumor cells contain considerably more RNA than tumor-surrounding fat cells and therefore, molecular parameters are not or only marginally affected by contaminating fat cells". It now reads: "For comparison, RNA was also isolated from tumor-surrounding cells which led to rather poor RNA recoveries from comparable tissue areas (data not shown). This does not exclude that tumor-surrounding cells may have a limited impact on molecular scores in such analyses. (page 13)
We agree with the reviewer that macrodissection of tumors may reduce non-tumor cell contamination. We will test this more carefully in future. We extended the discussion by including a new sentence: "Contamination by non-tumor cells may be reduced by macrodissecting tumors before RNA isolation and molecular assessment. The same procedure would also make tumors accessible to molecular analysis when sections contain less than 30% tumor cells." (page 12)

We have also checked the formatting of the manuscript according to the guidelines of the Journal. We hope very much that our manuscript is now acceptable for publication in BMC Cancer.

Kind regards

Rolf Jaggi