Author's response to reviews

Title: Increased tartrate-resistant acid phosphatase (Trap) expression in several malign tumors, patients sera and cell lines, an investigational study.

Authors:

Arnd Honig (arnd_hoenig@hotmail.com)
Lorenz Rieger (rieger@gmx.de)
Michaela Kapp (M.Kapp@mail.uni-wuerzburg.de)
Mathias Krockenberger (matze.krock@web.de)
Matthias Eck (Matth.Eck@gmx.de)
Johannes Dietl (frauenklinik@mail.uni-wuerzburg.de)
Ulrike Kammerer (FRAK057@mail.UNI-wuerzburg.de)

Version: 5 Date: 29 June 2006

Author's response to reviews: see over
Dear Sir or Madam:

Thank you very much for your comments on our manuscript “Increased tartrate-resistant acid phosphatases…” . We have revised the manuscript in accordance with the reviewers’ comments as shown below.

Response to reviewer No. 1 (Wolfgang Janni)

- The manuscript has now been subjected to language editing by a native speaker. Changes such as malign to malignant, progress to progression have now been made.

- *(Material and Methods)* The reviewer points out that there is a lack of a Methods section in the Abstract, which has now been added.

- *(General design)* We have tried to improve the manuscript by making it clearer and more concise. We have deleted the results pertaining to TRAP expression in placenta and serum TRAP levels in pregnant women in order to focus on the major subject of the paper.

- *(General design)* We have made the tables clearer and more concise by abolishing unnecessary information. They now clearly reflect our main statements.

- *(Methods)* The reviewer is right that some of the information given in the Methods section belongs in the Results. We have now removed the tables with the results from
the Methods section and put them into the Results section. No statistical analysis or quantification was performed due to the descriptive nature of the manuscript.

- **(RT-PCR: cut off):** RT-PCR was considered positive when a clear band of the expected size was visible on an ethidium bromide-stained gel. This is now mentioned in the Material and Methods section.

- **(Immunohistochemistry: cut off):** For evaluation, the cells and tissues were compared with control sections incubated with an IgG control antibody. These evaluation criteria are now given at the end of the Immunohistochemistry paragraph in the Material and Methods section.

- **(Results)** We describe a relatively new finding and therefore performed various different assays, evaluating several cell types, in order to be sure that cancer cells express TRAP. To some extent the manuscript is therefore descriptive.

- **(Discussion)** The reviewer was right in pointing out that malignant melanoma results are not an integral part of the study. The central aim of the study was to prove that cancer cells express TRAP. The question was whether only breast cancer cells express TRAP or whether TRAP expression is a common feature of malignant cells. We therefore evaluated various different malignant tumors, including malignant melanoma. In order to make the results concerning malignant melanoma more relevant to the study we have now included RT-PCR results for TRAP mRNA detection in malignant melanoma cells. We also evaluated cultured malignant melanoma cells for TRAP expression by immunocytology and have included these results in the revised version of the manuscript.
• *(Discussion)* The reviewer asked why healthy people like pregnant women express TRAP. It is a well-known fact that the placenta expresses almost every protein that has been linked to malignant transformation of cells at some stage of gestation. Placental tissue is used as a positive control in many fields of basic cancer research. The point in describing this finding was to illustrate the fact that TRAP expression is not restricted to cells of the monocytic lineage like osteoclasts and dendritic cells, but is also found in placental tissue. Therefore, TRAP expression is not confined to a particular type of cell or lineage. Such widespread expression of the protein makes expression in tumors seem possible. However, since we have now omitted the information on TRAP expression in the placenta according to the reviewer’s suggestion, this information is not given in the text.

Response to reviewer No. 2 (Alison Hayman)

1. We agree with the reviewer about the high TRAP values determined by ELISA. The method for determining these concentrations was not correctly described in the previous version of the manuscript. We now describe the method of dilution and calculation of the TRAP concentration in the ELISA paragraph of our Material and Methods section.

2. Page 11, last sentence: We are grateful for this comment and now show RT-PCR results for the detection of TRAP mRNA in stimulated dendritic cells (Figure 4).
3. Page 9 paragraph 3: We have omitted the section on gestational tissues and the results dealing with serum TRAP concentrations in pregnant women. Almost all proteins that seem to play a role in malignant transformation are expressed in the placenta at some stage during gestation. This is not surprising, because the placenta shows invasive growth at least until week 20 of gestation. Furthermore, this adds to the point that TRAP expression is not restricted to osteoclasts but can be detected in placental tissue, as well as a variety of different cancer cells.

4. The question as to whether concentration or activity was determined is, of course an important one. We have clarified this issue in the Material and methods section and made it clear that we did not measure TRAP activity but concentration of TRAP protein. In addition, we have replaced the expression TRAP “level” with TRAP “concentration” wherever serum concentrations are referred to.

5. Table 1: Units/l have now been added in the column.

6. We have subjected the manuscript to language editing by a native speaker and corrected the points mentioned by the reviewer. a) The word “malign” has been replaced by “malignant” throughout the text. b) “Bone affection” has been replaced by “bone disease” or “bone involvement”. c) The word “cultivated” is replaced by “cultured”. d) The reviewer asked for the correction of minor grammatical errors. We asked for the help of a native speaker to correct these.

7. We have mentioned in the introduction that—although we detected marked TRAP protein expression in cancer cells—we cannot comment on TRAP activity, because we
did not attempt to measure the enzyme’s activity in the various tissues and cells investigated.

Yours sincerely

Arnd Honig