Author's response to reviews

Title: Prognostic impact of three immunohistochemically detected markers of neuroendocrine differentiation in prostate cancer

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Author's response to reviews: see over
Dear Robin Cassady-Cain,

Many thanks for the opportunity to modify the manuscript in the light of recommendation made by the reviewers. I have addressed point all changes recommended by the reviewer and the editorial board and these are detailed below:

- We recommend that you ask a native English speaking colleague to help you copyedit the paper.
  - Authors’ response: reviewed and necessary corrections made in the manuscript.

- Please re-write the background of your abstract to include some context for your study; the objective can appear as the last statement of the background section.
  - Authors’ response: changes made as recommended

- Please include a statement of ethics and consent in your methods as outlined below:
  - Authors’ response: Done, see first 2 line in para 1 of the methods sections.

- Informed consent must also be documented. Manuscripts may be rejected if the editorial office considers that the research has not been carried out within an ethical framework, e.g. if the severity of the experimental procedure is not justified by the value of the knowledge gained.
  - Authors’ response: in view of the nature of study; ERC waived the requirement for informed consent. (see methods section line 2-3 para 1.

- Please include Competing Interests and Authors’ Contribution statements as outlined below:
  - Authors’ response: Added, following conclusions and competing interest stataemnt..

- Competing interests
  - Competing interests - Please include a 'Competing interests' section between the Conclusions and Authors' contributions.
    - Authors’ response: ‘The authors declare that they have no competing interests

- The questions that are asked of authors are:
  - Financial competing interests
    - In the past five years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify.
Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.

Authors’ response: None

Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.

Authors’ response: None

Do you have any other financial competing interests? If so, please specify.

Authors’ response: None

- Non-financial competing interests
- Are there any non-financial competing interests (political, personal, religious, academic, ideological, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.

Authors’ response: None

- Authors' contributions

Authors’ response: Added

- Acknowledgements?

Authors’ response: Added following authors’ contribution

Please list the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section. Authors must describe the role of the funding body, if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Authors’ response: Added following acknowledgements.

Reviewer: Jiaoti Huang

My only suggestion is that the authors have the clinical follow-up data and should be used, including survival and metastasis.

Authors’ response: Table 2 added to address this issue.
An attempt should be made to correlate these with the expression of NE markers in the original tumor, which is a more objective criterion of the tumors' biologic behavior.

Authors’ response: The IHC staining was performed in the original tumor only and not in the nodes or metastatic deposits.

Reviewer: Roberto Mario Scarpa  
Reviewer’s report:  
Major compulsory revisions:  
1) Detailed characteristics of tumors and patients should be provided in a table.  
95 specimens from 84 patients (83 in the abstract… clarify the discordance) were included in the study. Therefore 12 patients underwent two surgical procedures. Information about these patients is needed. Did they undergo 2 TURs or a radical prostatectomy followed by a TUR?  
Authors’ response: Table 1 added to address the issue. It is a typographical error, there were 84 patients and 95 specimen, some of the patients had TURP followed by a radical prostatectomy.  
2) The Authors classify the patients according to the TNM stage, but most of the specimens are TURP specimens and a correct pathological stage can be assigned only after a radical prostatectomy.  
Authors’ response: the TNM classification was clinical, local and loco regional staging on DRE, TRUS and CT or MRI for patients who only had TURP and metastatic work using, CT scan and bone scan.  
3) In the tables percentages should be used rather than absolute numbers.  
Authors’ response: changed

Reviewer: Ruoqian Shen  
Reviewer’s report:  
1. In “Introduction”, the authors used the statistic data in 2006 (page 3). As it is 2008 now, it is better to cite the statistic data in 2007.  
Authors’ response: Changed  
2. Some sentences are unclear, such as in “Results” “As a median follow up --- patients 21---”(page 7). It should be corrected as 21 patients.  
Authors’ response: Corrected  
3. Some sentences are redundant, such as “for about 33% of incident cases” (page 3) and “about 33%of all new cases” (page 8).  
Authors’ response: Corrected  
4. The authors indicated that “--- current evidence suggests that it has an influence on prognosis related to hormone resistant tumors or a role in the conversion to a hormone resistant phenotype”. However, the authors did not show any detailed analysis on the expression of CgA, NSE and SYN in patients with or without anti-androgen (or androgen withdrawal) therapies.  
Authors’ response: sentence deleted.  
5. There are numerous reports demonstrated that elevated levels of chromogranin A and NSE were detected in blood samples of prostate cancer patients. Nevertheless, the authors did not show any corresponding data of the levels of CgA, NSE and SYN in the blood samples of these patients in supporting
their results from tissue sections.
Authors’ response: I agree with the reviewer concerning significant evidence in favour of serum CgA levels in D2 prostate cancer; however we have only analyzed IHC detection of NE markers in the current work.
6. These tissue staining pictures are poor. Also in these representative pictures, there are no arrows showing the positive staining regions. Figure legends are too simple.
Authors’ response: Deleted

Reviewer: Pierre-Jean Lamy
Reviewer's report:
Major Compulsory Revisions:
1) Prognosis value of biomarkers cannot be determined without a follow-up study
Authors’ response: we have a median follow up of 8.4 + 3.5 years, table 2 added to better demonstrate the relation between NE marker expression and development of metastases and overall and cause specific survival.
2) Sample sizes are too small (particularly for the group I)
Authors’ response: I agree and that’s why recommendations and conclusions are modest.
3) Providing a table presenting patient’s characteristics makes it easier for readers to grasp majority of information.
Authors’ response: Table 1 added
4) Statistical analysis is inappropriate to draw meaningful conclusions. Univariate and multivariate analysis should be done and Kaplan Meier curves should be added.
Authors’ response:
5) It should underlined  that a p value = 0.059 is not statistically significant but could be analyzed as a trend.
Authors’ response: I agree and amends made in the manuscript (results section, para3 page 8)
Minor Essential Revision:
1) “CGA appears to be the best overall tissue and serum marker of neuroendocrine differentiation”. Could you corroborate this affirmation with recent references?
Authors’ response: reference 9 added
Discretionary Revisions:
1) Figures are dispensable
Authors’ response: removed

Kind regards,

Dr M H Ather