Author's response to reviews

Title: Prognostic significance of multiple kallikreins in high-grade astrocytoma

Authors:

Kristen L Drucker (drucker.kristen@mayo.edu)
Caterina Gianinni (giannini.caterina@mayo.edu)
Paul A Decker (decker.paul@mayo.edu)
Eleftherios P Diamandis (ediamandis@mtsinai.on.ca)
Isobel A Scarisbrick (scarisbrick.isobel@mayo.edu)

Version: 3
Date: 13 June 2015

Author's response to reviews: see over
Dr. Dafne Solera  
Executive Editor  
BMC Cancer

June 12, 2015

Dear Dr. Solera,

We very much appreciate the careful reviews provided to manuscript 2074495428156407 “Prognostic significance of multiple kallikreins in high-grade astrocytoma”. We were pleased that across both Reviews that the findings presented were found to be “novel and interesting”, the Results to be “detailed” and of “high quality”, and our overall findings to be of “importance to the field”.

We have addressed each of the Reviewer comments point by point and highlight our changes in the revised manuscript. All of the recommended changes were considered minor, were readily addressed and improved the overall quality of the data presented. It was also suggested (but not required) that we perform additional studies to determine the mechanism by which KLK7 and KLK9 might contribute to reduced GBM patient survival. This is an important suggestion and an avenue that we, or others, will need to pursue in the future. The extensive additional experiments that would be needed to establish mechanism for each kallikrein studied however is beyond the scope of this initial effort in which we rigorously studied the potential clinical significance of six unique kallikreins to GBM patient survival. We discuss potential mechanisms of action based on our own prior studies regarding KLK6 and the studies of others. We note that the mechanism(s) involved for KLK7 and KLK9 may be very different from that of KLK6 since each kallikreins has unique substrate specificities and enzymatic activities. We anticipate that the publication of the current manuscript will facilitate our efforts and those of others to obtain new sources of funding to pursue the mechanistic studies that Reviewer 1 suggested.

Thank you again for the careful and thoughtful review and we look forward to publishing this research in BMC Cancer where we believe it will receive wide readership.

Sincerely,

Isobel A. Scarisbrick Ph.D.  
Associate Professor of Physiology  
Neurobiology of Disease Program  
Department of Physical Medicine & Rehabilitation  
642B Guggenheim  
Mayo Clinic and Foundation  
200 First St. SW, Rochester MN, 55905  
Work Tel: 507-284-0124 (538-3348)
Title: Prognostic significance of multiple kallikreins in high-grade astrocytoma

Version: 2 Date: 13 April 2015

Reviewer: Krishna P. Bhat

Reviewer's report:
In the manuscript titled “Prognostic significance of multiple kallikreins (KLK) in high-grade astrocytoma” by Drucker et al., the authors evaluate the expression of multiple isoforms of KLK in high grade astrocytomas using immunohistochemical approaches. Using Kaplan-Meier and multi-variate analyses for association, the authors conclude that of the various isoforms, KLK6, KLK7 and KLK9 have utility as prognostic markers of patient survival in these brain tumors. The authors have extensive experience studying KLKs and data presented is very detailed statistical analyses. The figures and the IHC images are all high quality. The main concern moving forward with this manuscript is a lack of sufficient data. The “Results” section alone is described in two pages. Previous studies by the same authors have shown prognostic as well as mechanistic studies on KLK6 in glioma (Drucker et al., Neuro-onc, 2013). Similar approaches could be taken for characterizing KLK7 and KLK9 in glioma, which undoubtedly will enhance the quality of the manuscript.

Reviewer 1 Response:
We would like to thank the reviewer for a careful and thoughtful review of our manuscript. With the establishment of the potential significance of both KLK7 and KLK9 to GBM patient survival we are also very interested in the potential mechanism(s) of action as well as contributions to therapy resistance. We have discussed potential mechanisms of action based on our own prior studies and those of others, which may include direct and/or indirect effects (see Discussion p. 14 to 15). The intended scope of the current paper however was to make a comprehensive examination of six kallikreins in grade III and grade IV astrocytoma and to determine any impact of their levels on patient survival. Since this study has revealed that both KLK7 and KLK9, like KLK6, are indicators of poor GBM patient survival, additional mechanistic studies are now warranted. However, we feel that these additional studies will require effort to a level that is outside of the intended scope of the current manuscript. We anticipate that publication of the current findings in a timely manner will facilitate our efforts, and those of others, to obtain additional funding to perform the mechanistic studies suggested. The mechanism underlying the decreased survival in each case may be different among the different kallikreins as they are each unique family members with different cleavage specificities and enzymatic activities. The current manuscript demonstrates the importance of these proteins to GBM patient survival. We hope these findings will generate wide attention and spur additional efforts to delineate the mechanisms of action of multiple kallikreins in GBM and other cancers (see Conclusion, p. 15).

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

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: None
Reviewer's report  Reviewer 2

Title: Prognostic significance of multiple kallikreins in high-grade astrocytoma

Version: 2  Date: 7 June 2015

Reviewer: Michael Karsy

Reviewer's report:
The authors present a laboratory analysis of various kallikreins in high-grade glioma as they relate to patient survival. The expressions of six different kallikreins (KLK1, KLK6, KLK7, KLK8, KLK9 and KLK10) were quantified in a tissue microarray of 60 grade IV and 8 grade III astrocytomas. Analysis of patient survival by Kaplan-Meier survival analysis and Cox proportional hazards modeling was performed. Overall, the authors performed a very reasonable analysis, with good use of methodology as well as explanation of their findings in the context about what is known regarding kallikreins. Their findings of poorer prognosis with greater expression of individual kallikreins are novel and interesting in high-grade gliomas. New findings regarding the role of KLK9 may also be of interest for further study.

Major compulsory revisions
1. None

Minor essential revisions
1. Include cities and states for manufacturers of various reagents discussed in methods section

Reviewer 2 Comment 1 Response:
The city and state for each manufacturer has been added to the Methods section (p. 6 to 7).

2. X axis labels for figure 3A and B are cutoff

Reviewer 2 Comment 2 Response:
Thank you for your feedback. We have added X axis labels to figure 3A and 3B for clarity.

Discretionary revisions
1. One comment is in regards to the combination of grade III and IV gliomas. It may be interesting to factor glioma grade into some of the survival and multivariate analysis in order to control for this variable. There are “some” survival differences between these types of pathologies.

Reviewer 2 Comment 3 Response:
In this study, the survival analysis was only completed for the grade IV samples (n=60). The grade III samples were excluded from the analysis due to low sample number (n=8). These groups were not combined due to the inherent differences in the tumor populations.

Level of interest: An article of importance in its field

Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.

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