Author’s response to reviews

Title: Microdissection: A method developed to investigate mechanisms involved in TSE pathogenesis

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PDF covering letter
Dear Dr Gadd,

Please find the revised manuscript, taking into consideration the reviewers’ comments. I accept that the manuscript should be considered as a technical advance rather than a research article and have re-assigned accordingly.

We would like to thank the reviewers for their comments and appreciate the time and effort they have given to the review process. We have taken their points into consideration and feel that the article has been enhanced by this process.

Please find below the authors responses to reviewers comments:

**Reviewer 1. Theodoros Sklaviadis**

**Discretionary Revisions**

To address the concerns about magnetic resonance spectroscopy and proteomics the ‘Conclusions’ section has been re-written. We are currently completing a four-year study on the use of magnetic resonance imaging and spectroscopy in transmissible spongiform encephalopathies in collaboration with Dr J. Bell and Dr Y-L Chung, MRC Clinical Science Centre, Imperial College School of Medicine, Hammersmith Hospital, London. Results from this study will hopefully be published in the near future. A proteomics study is also currently in progress.

*page 3 third paragraph use the word unglycosylated instead of aglycosyl- Changed*

*Proper use of units ie page 3 & page 8 use molecular mass 25 kDa instead of Mr=25k*

The authors have changed all molecular weights to kDa however we consider Mr = as scientifically acceptable

*Proper use of terms ie page 3 use 1 dimentional SDS electrophoresis instead of 1D SDS-PAGE and give all abbrevations analytically.*

We have changed the wording of this sentence both to read more clearly and to include 1 dimentional however SDS- PAGE is a well documented method and it was thought that the abbreviated form was sufficient.

*page 4 methods 2.1 give the amounts of the inoculum.*

20µl has been inserted
page 6

2.3.1 centrifugation in g forces instead of rpm - changed

Table 1 use wet weights
A fuller explanation of these weights has now been added to section 2.2 Microdissection.

Table 3 should refer to figure 2 A-G instead of fig1 - corrected

Table 3 & 4 explain scoring - removed

Figure 4 EP=endpoint?NB=normal brain? give numbers for images on the third column as well – Figure 4 has been modified to take in both reviewers points and make it easier to understand

Major Compulsory Revisions
Densitometry must be done in order to corelate microdissection and western blotting.
Statistics of the performed experiments to prove reproducibility.

Statistics
Three brain samples at each time point were microdissected and immunoblotted. We have added sentences to Section 3.2 detailing the scoring seen in the tables basically a plus denotes where PrPSc is visualised in at least two out of the three brains.

Reviewer 2 Catherine Botteron

Minor Essential Revisions
Explain with more precisions what happens to the mice brains (frozen vs formalin-fixed).
Section 2.1 has had a paragraph added making clear that brain samples for histology were fixed in 10% formol saline.

Figure 4: Figure 4 has been re-designed hopefully making it easier to understand.

The conclusions should be rephrased to better reflect the data presented in this paper. The Conclusions section has been re-written and some amendments to the Discussion have been added.

All abbreviations and typos have been corrected.
In general all the figures and tables have been edited to conform with BMC format. The only changes made in the 20th October manuscript was authors added however in this manuscript only one more author, Yuen-Li Chung differs from the original manuscript and has been included in the Title and Authors Contributions sections.

Yours sincerely

Janice Barr