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Functional characterisation of pre S1/pre S2 deletion mutants of hepatitis B virus isolated from Southern Africans

Wolhuter, Suzanne

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Suzanne Wolhuter – Masters Corrections List and Comments

Dissertation Title:

Functional Characterisation of pre-S1/preS2 Deletion Mutants of Subgenotype A1 Hepatitis B Virus Isolated from southern African HIV-positive Adults

Candidate Details:

Suzanne Wolhuter – 0402869J

Suzanne.wolhuter@gmail.com

0832874441

List of Corrections and Comments:

1. Corrected: In the abstract, Viral Load indicated by VL is now explained when used for the first time, thank you this was an oversight.
2. Corrected: the resolution of image for Figure 1.1 on page 7 has been corrected as much as possible, the original image is unfortunately not a high resolution image.
3. Comment: The examiner felt unsure whether HBeAg is the abbreviation for Hepatitis B enigmatic antigen on page 8. The Hepatitis B e antigen was named for its enigmatic appearance in serum, HBeAg status can change as the natural history of HBV changes or the immune status of the infected person changes. However, the examiner is correct, it turns out the HBeAg stands for Hepatitis B e antigen. This is mentioned on page 8 in the introduction. The reference to the 'enigmatic core protein on page 15 refers to why it was named as such, and not what HBeAg stands for.
4. Corrected: Figures 1.2 and 1.3 on pages 9 and 10, as well as Figure 1.13 on page 43 all have the *Eco*RI site listed as position 3221, which is correct. Thank you, this was a mistake I did not pick up in proof reading.
5. Corrected: Instead of 399 amino acids in the LHBs, it was corrected to 400 amino acids in Table 1.4 on page 12, thank you, I was confused about this.
6. Corrected: The examiner is correct in saying that vaccine escape is not cause by mutation in the T cell epitope of the HBV virus, immune escape mutants however are caused by these mutations. I have corrected that the sentence on page 13 to: "Mutations in this region are often responsible for immune escape and viral breakthrough by reducing T cell reactivity". Thank you to the examiner for pointing this out.
7. Correction: The section explaining the length of the core protein in amino acids was corrected as per the suggestion on page 16. New suitable references were included. It

now reads: 'On the other hand, the 181 aa core protein is translated from the pgRNA gene transcript, starting at the core ATG initiation (position 1901 from the *Eco*RI site) and ending at the gene termination site (2458 from *Eco*RI) [54, 55]. It should be noted that in some HBV genotypes, including subgenotype A1, the core protein is 183 aa long. This is due to a 6-nt insertion and is the reason for which the subgenotype A1 genome is 3221 bp in length [54, 55]. This core protein is composed of an assembly domain as well as a nucleic acid binding domain, from aa's 1-149 and 150-183 respectively [55]."

8. Corrected: On page 26 the examiner suggests a change in the sentence about sequence divergence and genotypes. The change suggested certainly would make it less confusing, thus the sentence now reads: "Genotypes are identified where there is 7.5% or more intergenomic sequence divergence between strains, and subgenotypes where there is more than 4% intergenomic sequence divergence [33, 71, 107, 108]".
9. Comment: The examiner questions the use of the definition of occult HBV where HBV DNA is detectable but under 200 IU/ml on page 31. While the question of the restricted copy number as being necessary for the definition may be a valid one, I know certainly many scientists agree with this view, I used the definition of occult HBV as outlined in the publication: Raimondo, G., et al., Statements from the Taormina expert meeting on occult hepatitis B virus infection. Journal of Hepatology, 2008. 49(4): p. 652-657." This publication was supposed to be a meeting of an expert panel to agree on the definition of occult HBV, and thus I decided to use this definition in my dissertation.
10. Corrected: On page 36, in Table 1.9 the examiner made a valid suggestion that pre-core mutants should be included in Table 1.9. This has been done and a relevant reference was added as well.
11. Comment: It was requested that a section outlining the statistics be included, this is already present in the Material and Methods Chapter on page 54, this is also shown in the Index. The examiner mentions standard deviations, which were not one of the statistical methods employed during this study, however the statistics section outlines how the p-values (significance values) were calculated.
12. Correction: On page 69, the examiner suggests a change of the heading of section 3.1.1, the suggestion does make it clearer that the deletions are found only in preS1 and preS2 and not in the S region of the envelope gene, thus the new heading reads: "PCR Amplification of the Envelope (preS1/pre2/S) Region and Sequencing".
13. Comment: The examiner made some interesting suggestions and comments on the discussion chapter (Chapter 4 pages 111 – 122). I am unsure whether they need to be corrected, however I do feel they are valid commentary. The comment on decreased sequence heterogeneity in HBV-HIV coinfection. It is true that it was hard to find consensus in the literature as to how co-infection should influence heterogeneity, and it seems my study is one of precious few that compared so many clones from a groups of patients, and also one of very few to carry out Bayesian phylogenetic analysis. Overall it was challenging to find many yardsticks to compare my results to. I guess I wanted to simply highlight that I had thought about points such as this, and had a difficult time putting that into my discussion. We (my supervisor and I) agree that we should have added Southern Blot analysis to the study to confirm virion formation and replication *in vitro*, however, we ran out of time and funding. A student who has taken over this project is about to embark on this goal, and hopefully that will shed more light on virion

formation and whether the mutants produce all replication intermediates *in vitro*. We should however, have made this clearer in the discussion. I was struggling with how to put all the information together in a succinct way, I feel I have a better understanding now of what my results did and did not indicate. I thank you for this comment, it has brought more clarity to my thinking on what the results mean in terms of measuring the life cycle and protein production of the virus. Finally the comment as to PCR error, we did use a high fidelity Polymerase (please see Materials and Methods pages 46 and 56 , the polymerase used was Taq/Tgo Polymerase mix in the High Fidelity Expand Kit). Thus we were referring rather to the HBV polymerase being error prone, and not the PCR method. My apologies for the lack of clarity there.

14. Comment: There was a strong recommendation that the entire dissertation be printed one-sided throughout, with no pages turned so that the printed side is facing one another. Currently the entire dissertation is printed one sided, but my supervisor and I decided to turn some pages to face each other as the text and tables or text and figures on those pages were related to each other. Thus we felt that for the ease of reading them together, it was important to face the print towards each other by turning the pages. While we appreciated the examiners comment, we still feel that leaving these pages facing each other increases the ease of understanding of the content of those pages, and therefore we will be leaving them to face each other.