Reviewer's report

Title: Epidermal Growth Factor Receptor (EGFR) mutation analysis, gene expression profiling and EGFR protein expression in androgen-dependent prostate cancer

Version: 1 Date: 1 October 2010

Reviewer: Samantha Larkin

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Minor Essential Revisions

1) Sample size quite low, particularly in the gene profiling analysis
   a. Some confusion over sample size in the gene profiling actually. It is mentioned that 13 EGFR mutant samples were analysed using microarrays but in the figure (Fig 2) there are only 8 samples shown. What happened to the other 5?
   b. Again, qRT-PCR is based on 8 samples – what happened to the n=13 mentioned earlier?
   c. Discussion completely lacks any mention of the low sample size in the mutational analysis of EGFR+ and EGFR- tumours – this was based on a very small number of samples and there needs to be clear critique of this in the discussion and how that might affect the power of the work

2) Biochemical relapse definition is not very clear.

3) No mention is made of how many histopathologists scored the Gleason of each radical specimen. This is important as the Gleason score can vary hugely between histopathologists. Was the same histo used for all samples? Was the Gleason verified by another histo?
   a. Why were the Gleason groupings 4-6 and 7-9 chosen? No justification given for this division.
   b. Discussion of this should be included to show the limitations of any Gleason based analysis...

4) It’s a shame that there is such limited follow up on the T3 staged tumours – only 9/26 have follow up – it would have been interesting to see how tumour stage correlated with some of your findings. Why did so many patients not complete follow up? It would be worth expanding on the reason behind the lack of follow up in those 32 patients

5) Summary of clinical pathological characteristics needs amending – 26 were T3.

6) All figure legends need expanding to detail the experiments more precisely.
   a. Figure 1 should have some annotations – point out normal glandular staining and over expression in the tumour areas.

7) On the immunostaining, how was the 1% of tumour staining (was this the
basal level?) quantified? And by whom?

9) Mutational analysis – why is the association between EGFR protein expression and mutation status given a p-value of >0.05 whereas the other p-values in that sentence have a specific p-value? Be consistent.

10) How were the Ct values analysed in the qRT-PCR gene profiling experiment. I feel that a brief summary of how the Ct values were manipulated and analysed should be included rather than there just being a column in the table stating ‘Log10Ratio’ and a reference.

11) Some references missing (‘a PSA follow-up of 4 years is considered sufficient to declare a patient disease free’ should be referenced)

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests