Reviewer's report

Title: Testing of human papillomavirus in lung cancer and non-tumor lung tissue

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Reviewer: Jill Koshiol

Reviewer's report:

The purpose of this study was to test for the presence of HPV DNA in lung tumor and adjacent non-tumor tissue in order to clarify whether HPV was present and may therefore have been involved in the development of cancer. This study was well designed, and the paper was well and clearly written. Although not a large study, 100 cases is a sufficient number to establish an HPV prevalence of about 5% or more. The inclusion of adjacent non-tumor samples is a nice addition.

The authors were careful to address both major concerns in any study of the prevalence of HPV outside of the cervix: 1) the potential for false positives, and 2) the potential for false negatives. They addressed the first concern by describing their pre-post amplification procedures to avoid contamination. They also included a pair of primers for the amplification of a modified plasmid as a positive control to ensure that negative results were not due to PCR inhibition. To address the potential for false negatives, they amplified a fragment of the human CFTR gene as a genomic DNA control to ensure that negative HPV results were not due to inadequate DNA. Although the 450-bp amplicon is large, the amplification should still be sufficiently sensitive since the investigators used frozen tissue samples rather than paraffin-embedded formalin-fixed samples. Finally, as described above, 100 cases should be sufficient to identify a meaningful proportion of HPV-related cases.

Discretionary Revisions:

1. It would be useful to have more information about how the frozen tissue was collected. Were the tissues immediately snap frozen in LN at the time of removal? How long on average was the time from removal from the vasculature to snap freezing?

2. A little more description of the “strict procedures…developed to avoid specimen contamination” might be useful for readers to wish to perform similar experiments in the future.

3. The findings that repeat testing with a new assay kit lot failed to confirm initial weakly positive results seem particularly useful. It may be worth noting that other studies reporting on HPV in controversial areas could to use this approach as an additional way to help assess their findings.

4. While I tend to agree that these results argue against any pathogenic role of HPV in lung cancer, it should be pointed out that this study was conducted in a Western population and the majority of cancer cases (81%) were smokers. It
may therefore be prudent to add the qualification “in this population” to the end of
the Conclusion at the end of the paper, just as the authors did in the Conclusions
section of the Abstract.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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