Author’s response to reviews

Title: Evaluation of FTIR Spectroscopy as a diagnostic tool for lung cancer using sputum

Authors:

Paul D Lewis (p.d.lewis@swansea.ac.uk)
Keir E Lewis (keir_lewis@hotmail.com)
Robin Ghosal (ghosalr@hotmail.com)
Sion Bayliss (474488@swansea.ac.uk)
Amanda J Lloyd (abl@aber.ac.uk)
John Wills (438626@swansea.ac.uk)
Ruth Godfrey (a.r.godfrey@swansea.ac.uk)
Philip Kloer (Philip.Kloer@carmarthen.wales.nhs.uk)
Luis Mur (lum@aber.ac.uk)

Version: 2 Date: 28 June 2010

Author’s response to reviews:

Dear Dr Bruzzi,

Please find below details of the revisions requested by each reviewer for our manuscript. We are extremely grateful to each of the reviewers for their comments and suggestions, many of which have strengthened the interpretation and discussion of our results. We are also grateful for your further consideration of our manuscript.

Best wishes,

Paul Lewis

Revision Details

------------------

Editorial Revision

1. Statement concerning informed consent added on p.7

Reviewer 1 - Donna Maziak

Major Revision:

1. Amendments have been made to patient details on p.7 to include information pertaining to tumour location. We have also stated in our Conclusion on p.23 that we are currently evaluating the ability of FTIR to detect peripheral lung tumours.

Reviewer 2 - Luca Quaroni
Major Revisions:

1. To address the issue raised concerning band assignment we chose the second option as recommended by the reviewer. We fully agree that in describing a diagnostic methodology of this kind we should not concern ourselves with attempting to interpret each wavenumber without absolute knowledge of the structural changes. Changes have been made throughout the manuscript accordingly.

Given these changes we felt that the additional DNA spectra work did not fit so well and has been removed. We now think that an attempt to indicate DNA changes between cancer and normal sputa cells be best presented as a separate publication at a later stage.

Major section changes are made on p. 8-9, p.14-15, p.18-19.

2. We have addressed the issue of potential cellular contamination as suggested by the addition of a paragraph on p.20

Minor Revisions:

1. Details of the baseline correction method have been added on p.10
2. FTIR instrumentation details have been added on p.9
3. Minus sign has now been inserted on p.10

Reviewer 3 - Nick Stone

Major Revisions:

1. Annotation of patient histological subtype now amended and details added on p.8 to state why subtypes were not obtained.

Minor Revisions:

1. Reference to Raman work added on p.6
2. This has now been corrected on p.12

3. It is unusual to see median spectra reported. For a robust statistical analysis we felt it pertinent to assess the data distribution of each wavenumber and found that all followed a non-normal distribution. Thus, we felt the correct procedure would be to use a non-parametric inferential statistical test (Mann Whitney U test) to establish any significant differences at each wavenumber between cancer and non cancer cases. Interestingly, Whiteman et al. (2008) used the same approach for their statistical analysis on data from sputum in COPD samples for the same reason. We have amended text on p.12 to attempt to make clearer that median
absorbance values are calculated at each individual wavenumber.

4. We state in the Results (highlighted in yellow on p.15) that we selected 6 highly significant wavenumbers that had prominent second derivative peaks and were interpretable (in terms of prior annotation in the literature). These 6 wavenumbers were used for multivariate analysis. After applying PCA, we had to retain 3 components according to the rule of only using those principal components (in order of decreasing variation explained) that collectively explain at least 95% of the variation. Thus, interpretation of patterns of loadings can only be be based on these 3 components and we would be unwise to look for other patterns of how cases group using further components that explain very small amounts of variation. We feel that use of the 6 significant wavenumbers and the retention of the first 3 components in PCA is the most robust approach prior to interpretation of patterns in how cases group according to similarity.

Discretionary Revisions:

1. Details of the baseline correction method have been added on p.10

2. 'P value' changed to 'p-value' throughout manuscript

3. This is how spectra have been presented in most of the relevant FTIR publications we have read.