Suggested Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common form of cancer worldwide and its incidence is increasing [1]. The majority of head and neck cancers are squamous cell carcinomas that originate in the upper aerodigestive epithelium and are typically associated with exposure to carcinogens present in tobacco and alcohol [2]. The regions in which head and neck tumours typically develop are anatomically complex and play a vital physiological role in the patient; early diagnosis and selection of appropriate treatment is needed to ensure patient longevity and retention of vital organ function.

Many previous studies [3–9] have investigated the viability of a range of prognositic biomarkers for head and neck/oropharyngeal cancer (**is oropharyngeal too different?**) with varying degrees of success. The discovery of effective prognostic biomarkers is difficult, and previously has largely focused on immunohistochemistry techniques. Magnetic Resonance Imaging (MRI) has been used [10–12] to estimate various clinical biomarkers in a non-invasive manner. However the results of MRI based techniques are often in poor agreement with the results of direct measurements from the pathological staging sections [13, 14].

There is a need to establish accurate biomarkers in order to characterise the disease and optimise treatment, particularly in less aggressive disease where it is desirable to de-escalate therapy [15] and thus minimise the adverse effects of treatment and improve patient outcomes. Previous work [16–18] has hypothesized that tumours that may be responsive to novel therapeutic treatments may carry distinct molecular biomarkers. If this is correct then the identification of such biomarkers is the key to the screening of patients towards appropriate treatments.

This work explores the potential of Fourier transform infrared (FTIR) microscopy in combination with a known prognostic biomarker: α-Smooth Muscle Actin (ASMA) expression, as a method of identifying cases which may be appropriate for therapeutic treatment. Previous work [4–6] has explored the efficacy of ASMA and SERPINE1 [6] as predictive variables for extra capsular spread (ECS), which is an important prognostic biomarker for OSCC. FTIR microscopy images taken of the **same** sample set will be used in order to perform a comparison to established biomarkers.

Comment on references before drafting last section of introduction

The 1st 18 refers seem approriate but I would like to raed them which will take ,e a while.

Ref 31 is a systematic review of biomarkers for OSCC so I think it should come earlier in thereferences.

Refs 28, 30 (possibly also 29 I haven't checked) are specific to OSSC so should also come earier.

I think the refs to FTIR need to start with a review article. I am not sure of the Journal but if it is Analyst we need to use reviews from likely referees.

Mathew Bakers review (ref 1 in Barney's paper) is ideal since the author list include several people who might review this paper.

We should also consider 12, 13, 14, 15 from Barney's paper. Especially Peter Garnder's review (ref 13 since that will cheer him and he will be a co-author.

Refs 21, 22 , 23 24 are applications of FTIR to specific cancers and are probably fine.

I would also drop the excellent reference 19. The results are impressive for brain cancer but its an application to biofluids and we are studying tissue. If we keep it we should end the introduction with it but end with some statement about this paper. Its importance etc.

FTIR microscopy is a well established technique and has been utilised to investigate a range of biomedical applications in recent years [19–21]. Due to its ability to access chemical information present within the sample; analysis of FTIR microscopy data is able to lend insight into a range of label-free discrimination tasks. Zawlik et.al [22] investigated FTIR coupled with prin-cipal component analysis (PCA) to investigate the efficacy of chemotherapy in triple-negative breast cancer. They determined that it was possible to monitor changes in the biochemical composition of the tissue in order to monitor the effectiveness of received treatment.

Butler et.al [23] have undertaken development of a high-throughput ATR- FTIR based instrument for use in biofluid assays. Their work concluded that it was possible to utilise IR spectroscopy to triage brain cancer using biofluid samples with a sensitivity and specificity of 93.2% and 92.8% respectively.

Comments on rest of paper.

1) I was confused by the introduction to the Results section. I couldn't work out how many patients and what type.

Should we start with a statement that specimens were obtained from 31 patients of whom 9 where female and 24 male. Is that correct? Do we need another column in Table 1 for those that died or survived so that the numbers can be seen to add up with the first statement about how many there were?

2) Fig 2. Can we show the results of ASMA on its own?

3) I am not sure what the last statement on page 11 means?

4) Figure 4. I don't understand how this data comes from the analysis.

5) We need Janet to expand on the odd result that adding ASMA reduce the predictive capability of the analysis.

Peter Weightman 24 9 21