**Prognosis of oral squamous cell carcinoma using IR microspectroscopy**

**Background**

**Oral squamous cell carcinoma**

* Oral squamous cell carcinoma (OSCC) is the 8th most common cancer in the UK1
* OSCC is diagnosed by pathological assessment of biopsy material
* Treatment in the UK involves surgery that is usually followed by radio- or chemo radiotherapy.
* Survival beyond 5 years has remained almost static at 50% for many years, despite new treatments1. The majority of patients who died, succumbed within 2 years from diagnosis.
* Post-surgery treatment plans are dependent on an accurate prediction of the risk of recurrence (at the same site) and metastasis (at distant sites) with one of the most important predictors being cancer spread to local lymph nodes and its breaking out of the lymph node capsule (called extranodal extension [ENE] or extracapsular spread [ECS])2
* ENE can only be definitively determined after pathological assessment of the surgical resection specimen, but new treatment plans might involve pre-surgery immuno- or chemotherapy.

**Clinical need**

* An accurate prognostic indicator available prior to surgery (i.e.using biopsy tissue)
* New treatment targets

**Infrared Microspectroscopy**

* Fourier-transform infrared (FTIR) microscopy is a well-established technique that has been utilised in a range of biomedical applications in recent years3
* FTIR microscopy allows imaging of sample specimens at thousands of infra-red wavelengths simultaneously.
* Biochemical compounds typically vibrate at characteristic wavelengths in a region known as the fingerprint region (1000 cm−1-1800 cm−1)
* Differences in these absorption bands contain information that can be utilised to discriminate between samples of interest and possibly identify new therapeutic targets.

**Materials and methods**

**Biological samples & data collection**

* 1mm diameter round punches of tissue from primary OSCC tumours were mounted in an grid in paraffin wax (a tissue microarray4) to enable sectioning at 5 micrometre thickness
* Adjacent sections (5 um slices) were mounted on glass slides and calcium fluoride disks.
* The glass-mounted section was stained with haematoxylin and eosin (H&E) to visualise cell structures on microscopy
* Areas of the core containing tumour cells or underlying stroma containing fibroblasts were marked on a digital version of the H&E stained section by an oral pathologist.
* IR spectra were collected using a Varian Cary 670-FTIR spectrometer with an attached Varian Cary 620-FTIR microscope and a liquid nitrogen-cooled 128×128 pixel MCT focal plane array with an effective field of view for each pixel of 5.5 μm. Images were acquired at a resolution of 6 cm−1 over a spectral range of 990 cm−1 to 3800 cm−1 using a co-addition of 128 scans. Background scans were acquired using a blank CaF2 disk.

**Selection of data for analysis**

* 1 core from a tumour with ENE where the patient died from OSCC within 12 months of diagnosis and 1 core from a tumour with ENE where the patient survived more than 24 months were identified from clinical notes.
* The marked H&E image from each core was co-registered with the equivalent IR image at 1650 cm-1 (amide I peak) and the data from tumour areas extracted for analysis.

**Aims**

* Identification of key metrics (IR wavelength ratios) associated with poor prognosis in OSCC
* Analysis of frequently occurring IR wavelengths in these metrics and identification of biomolecules associated with poor prognosis
1. <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/head-and-neck-cancers>
2. RJ Shaw et al doi: 10.1002/hed.21244
3. D Finlayson et al doi: 10.1021/acs.analchem.9b02280
4. M Koo et al doi: 10.1007/978-1-4939-8935-5\_27.