

## Magnetic nanotechnology for sentinel lymph node mapping in oral cancer

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### Introduction:

Oral Squamous Cell Carcinoma (OSCC) can metastasise at an early stage to cervical lymph nodes, and this has an associated 50% decrease in survival. Currently these patients undergo neck dissection even if they have a clinically and radiologically negative neck, as even our most sensitive investigative options will miss microscopic cancer deposits in the cervical lymph nodes.

Sentinel lymph node biopsy (SLNB) is a technique to identify microscopic metastatic deposits in the first draining lymph nodes, which obviates the need for complete surgical clearance of the regional nodal basin in patients with cancer who test negative and is used commonly in breast cancer and melanoma treatment. It is an option for OSCC patients but current SLNB technology has several limitations for use in the head and neck, which has hampered its widespread adoption.

Our study evaluates the accuracy of a novel nano-magnetic approach for sentinel lymph node mapping as an innovative diagnostic option. The project is broken down into two parts in order to achieve this overarching objective. The first part is to establish a rabbit model of oral cancer, and the second part is to use this pre-clinical model to trial the new SLNB technology as part of a proof-of-concept study. A Phase I Clinical trial will follow to see the full translation of this technology.

This report covers our progress with the first part of this study.

### Methodology

To establish a rabbit model of OSCC an immortalized VX2 cell-line was obtained from the National Cancer Institute (NCI) in the USA and cultured *in vitro* in Dulbecco's modified Eagle medium. Cells were harvested with 0.25% trypsin, counted and collected by centrifugation prior to re-suspension in phosphate buffered solution (PBS).

An injection of 0.3mL containing various amounts of VX2 cells in suspension was administered orthotopically into New Zealand White rabbits. Different cell dosages were trialed ranging from  $10 - 50 \times 10^6$  vital cells. Cells were injected either extra orally into the buccinator muscle or into the anterior lateral tongue in order to cover two subsites of the oral cavity.

The cell line was also propagated *in vivo* in order to investigate a different method of culture. In this method, the rabbit's hind limb was inoculated with cells, which were allowed to grow for 1-2 weeks at which point the developed tumour was resected, and cryopreserved. When needed, frozen tumor stocks were thawed in a water bath, pulverized through a 70microlitre cell strainer with PBS and then counted, centrifuged and re-suspended in PBS prior to orthotopic inoculation in a rabbit.

The primary oral tumour and regional lymph nodes were examined by palpation, photography, and MRI at weekly intervals. Cohorts of rabbits were humanely killed 2-weeks post tumour injection, at which point excision of the primary tumour and bilateral neck dissections were performed in order to examine tumour growth and regional spread by histology, which was correlated with imaging.

## **ANZHNCs Research Grant Progress Report:**

### **Preliminary results**

So far we have been successful in culturing a rabbit squamous cell carcinoma (SCC) cell-line both *in vitro* and *in vivo*. The *in vitro* cultured cancer cells have been analysed with cytopathology and immunohistochemistry and demonstrate a poorly differentiated SCC of epithelial origin.

When we inoculate rabbits with the cancer cells in their oral cavity we are now consistently able to produce tumours. These are typically palpable between 1-2 weeks and we are able to visualise them on MRI with contrast at 1 week. Tumours average 2cm in maximal width at 1 week and growth tends to plateau after this presumably related to the aggressive tumour outgrowing its blood supply and resulting in central autonecrosis. MRI scans and correlated histopathology from dissected lymph nodes in the necks of the rabbits demonstrate cancer spread to lymph nodes at 2 weeks.

### **Discussion**

As part of optimising this model we have compared two methods of culturing cancer cells prior to inoculation in the oral cavity. One method involves a standard cell-culture laboratory technique and the other method involves creating a tumour cell suspension from tumour pieces propagated in rabbit hind-limbs. We have found that both methods are able to produce tumours and neck node spread with no significant difference in rate of growth or spread.

The establishment and optimisation of our pre-clinical oral cancer model has been invaluable and places us on track to trial our new magnetic nano-technology as part of phase 2 of this study in the coming month.

### **Academic Output**

#### **Oral Presentations:**

1. Establishing a Rabbit Model of Oral Cancer as a tool for Preclinical Investigation in Head and Neck Surgery - Poster Presentation at the Australian Society of Otorhinolaryngology, Head and Neck Surgeons - Perth, Australia, March 2018
2. Magnetic Nanotechnology for Sentinel Lymph Node Mapping in the Head and Neck - Oral Presentation at the 8th international Symposium on sentinel lymph node biopsy in the Head and Neck - London, UK, May 2018
3. Establishing a Rabbit Model of Oral Cancer as a tool for Preclinical Investigation in Head and Neck Surgery - Oral Presentation at the American Academy of Otorhinolaryngology, Head and Neck Surgery Foundation Conference - Atlanta, USA, October 2018