Report for the Australian and New Zealand Head and Neck Cancer Society Foundation Grant 2024 (Lauren Barrett Fund)

Principal Investigator: Professor Ruta Gupta (MBBS; MD; FRCPA; DMedSc)[1,2]

Associate Investigators: Professor Jeremy Crook[2]; Dr Robin Hill; Dr Mun Hui[4]; Professor Carsten Palme[3], A/Professor Chris Milross[3].

1. *NSW Health Pathology: Department of Tissue Pathology and Diagnostic Oncology at Royal Prince Alfred Hospital*
2. *Chris O'Brien Lifehouse: Biomedical Innovation Hub*
3. *Chris O'Brien Lifehouse: Head and Neck Department*
4. *Chris O'Brien Lifehouse: Medical Oncology Department*

Title: Tailoring Adjuvant Therapy for Oral Cancer in Young People Through BioPrinting

Introduction/Description: Oral cancer is a mutilating and lethal disease. The current 'one size fits all' treatment approach not only carries highly debilitating side effects, but nearly 60% patients do not respond, develop recurrence, or die. There is an urgent need to tailor adjuvant therapy to tumour biology.

In this proposal, we combine bioprinting of oral cancers from young patients with ex vivo testing of sensitivity to adjuvant chemotherapy and radiotherapy to provide clinically actionable results within 14 days. We have established scalable production of bioprinted patient tumour replicates from dissociated primary tumours obtained from surgery. We are now ready to fully characterise the 3D replicates and correlate cellular and molecular information with clinical response. This will be achieved by leveraging our current phase II clinical trial where we have collected tumour dissociates from young oral cancer patients with known treatment responses. We will bioprint replicates of these tumours and refine the ex vivo testing protocols until they reliably simulate the clinical trial results.

Aims of the project: The aims of this project were to:

1. To establish the clinical reproducibility of ex-vivo adjuvant therapy testing using bioprinted patient derived cancer models.
2. To implement a real time bioprinted oral cancer treatment screening pipeline.
3. To validate the biomarkers of response to therapy using the head and neck cancer biobank.

Results: Bioprinting tumour dissociates was optimised by systematically modulating nozzle size and type (Fig 1a), with printed droplet consistency evaluated across a range of droplet volumes (Fig 1b). Optimal printing parameters included 6 µL droplet volume, 4 µL/s flow rate, 5 µL retraction volume, 30°C printhead temperature, 15°C printbed temperature, 0.05 mm syringe height, using a 22G tapered conical nozzle.

Chemotherapy dose-response analysis based on metabolic activity supported the use of 8 concentrations for robust dose-response curve fitting. Furthermore, assessment 3-days after treatment was consistent with published standards (Fig 1c)[1-3]. Radiation dose-response was similarly evaluated using metabolic assay, in addition to live/dead cell staining (Fig 1d). Unlike chemoresponse, day 3 assessment was insufficient for detecting single-dose regimen treatment effects consistently across all patient samples tested using these assays. Extended observation up to Day 21 demonstrated that the pattern of cellular response was conserved beyond Day 7, indicating that longer timeframes were not necessary for response characterisation (data not shown). Fractionated radiation treatment was also investigated (data not shown) and induced differential metabolic responses compared to single-dose exposure, warranting further investigation to

determine whether observed fractionation patterns are relevant to clinical outcomes. Future analysis involving normalisation of growth rate across different patient tumours will be essential for comparative analysis of both chemotherapy and radiotherapy modalities.

Comparative analysis of individual patient-derived cell dose-response profiles revealed substantial heterogeneity in key pharmacodynamic parameters, including hill slope, maximum effect magnitude, and time to response onset. As patient-derived cell datasets expand, these *ex vivo* dose-response characteristics will be correlated with corresponding clinical treatment responses to establish potential predictive biomarkers for personalised treatment selection.

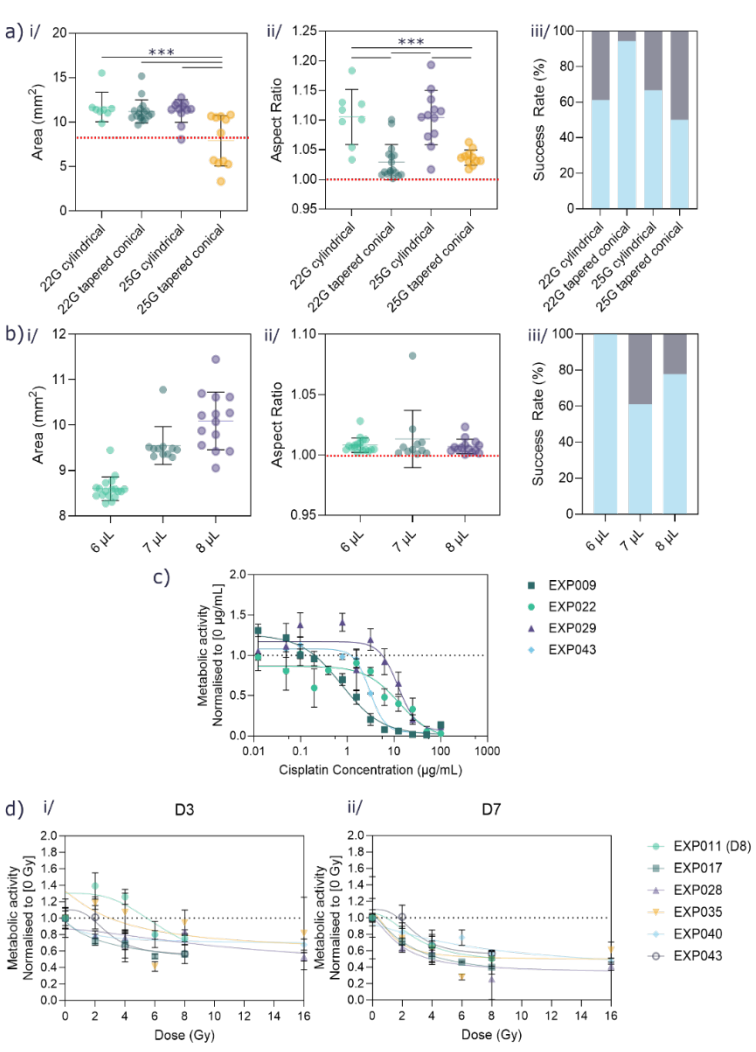

Fig 1. a) *and* ***b)*** *Droplet consistency analysis of i/ area, ii/ aspect ratio and iii/ success rate, as a function of a) nozzle type and size, and b) droplet volume. n=18. Data presented as individual measurements, mean ± standard deviation.* ***c)*** *Overlayed dose response curves of different patient-derived bioprinted droplets 3-days post cisplatin treatment. n=3. Data presented as mean ± standard deviation, and line of best fit (non-linear regression – variable slope).* ***d)*** *Overlayed dose response curves of different patient-derived bioprinted droplets i/ 3-days and ii/ 7-days post irradiation using a linear accelerator. n=6. Data presented as mean ± standard deviation, and line of best fit (non-linear regression – variable slope).*

Conclusions: Progress to date supports bio-printability of patient tumour dissociates and proof-of-concept treatment and analysis of printed tumour constructs. Future work will expand the assay range to better define treatment–response nuances, including senescence, cell cycle arrest, and DNA damage preceding cell death.

References:

1. Camacho-Alonso, F., et al., In Vitro Study of Synergic Effect of Cisplatin and Low Molecular Weight Heparin on Oral Squamous Cell Carcinoma. Front Oncol, 2020. **10**: p. 549412.
2. Wahbi, W., et al., Evaluation of in vitro and in vivo personalized cancer treatment assays for oral squamous cell carcinoma. Translational Oncology, 2023. **33**.
3. Miyamoto, S., et al., Three-Dimensional Spheroid Configurations and Cellular Metabolic Properties of Oral Squamous Carcinomas Are Possible Pharmacological and Pathological Indicators. Cancers (Basel), 2023. **15**(10).