Hamlet.rt Collect: Use of 3D epithelioid models to improve survival from head and neck cancer

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Principal supervisor's CRUK CC theme: Thoracic Cancer Programme

Department for student registration: Department of Oncology
Department or institute where research will take place: Department of Biochemistry

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Postgraduate scheme:

- **MRes + PhD (1 + 3-year non-clinical applicants)**
- **Part-time MRes + PhD (2 + 3-year clinical applicants)**

MRes project outline:

Background
The project will address the urgent clinical need for improved therapeutic strategies targeting head and neck cancer (HNC). There are >12,000 new HNC cases each year in the UK. Radiotherapy (RT) is an essential component of primary treatment. While RT is largely effective, locoregional recurrence (LRR) in 15-60% of cases is a major factor contributing to HNC mortality with a median overall survival of 6 to 15 months. The key project objective will be to identify factors which influence LRR after RT and identify novel treatments that improve patient outcomes.

Epithelioids are a novel primary 3D culture system, similar to organotypic explant cultures, that takes advantage of the wound-healing properties of epithelia and allows establishment of trypsin-free, long-term, self-maintaining and expandable cultures of mouse and human epithelial tissues (1,2). Epithelioids derived from HNC tumours will enable patient-personalized functional studies of cell competition and mutant selection in the context of a three-dimensional epithelia and are the ideal model system for defining how cancer treatment, for instance RT, modulates competition between malignant and healthy cells.

Aims and Objectives

A) Determine how RT impacts malignant cells from primary and recurrent HNC.
i) Following on from the group's successful pilot study, Hamlet.rt Collect, the student will work with established HNC epithelioids to assess somatic mutations and copy number alterations in key genes known to be involved in HNC.
ii) Irradiation of epithelioids will be used to model RT treatment and subsequent clonal outgrowth. The student will define changes in DNA, RNA and protein expression in order to understand molecular adaptation to RT resistance.

B) Determine whether the stem cell / cancer stem cell marker LGR5 is expressed in HNC tumours and present a novel, actionable therapeutic target (3).

MRes experimental plan:

A) Phenotypic and Molecular characterisation of original biopsies and derived epithelioid cultures.
1) Epithelioids will be grown, optimized and monitored. Characterization of the original biopsies and epithelioid cultures will be conducted using tissue-specific immunofluorescence markers and confocal microscopy imaging. This analysis aims to assess the extent to which the epithelioid cultures accurately replicate levels of proliferation and differentiation as well as the structural features and cellular heterogeneity observed in primary tumours.
2) Collection of supernatant and/or basal cells to isolate tumour DNA to be sent with paired blood samples to the Sanger Institute. Germline and somatic mutational burden will be quantified using whole exome sequencing, correlating with clinical data, including HPV status. Data analyses will identify signature mutations endemic to HNC, providing a foundation for novel therapeutic strategies targeting the tumours.
3) Comparison of DNA, RNA and protein expression after irradiation of epithelioids. Is there evidence that irradiation selects for or encourages the development of resistance clones? Do mutations that arise in these irradiated epithelioids correlate with mutations that are seen in tumours that have recurred after radiotherapy?

B) Determine whether LGR5 expressing HNC cells are a therapeutic target.
1) LGR5 expression in primary HNC will be measured by immunofluorescence, co-staining with anti-beta catenin as a control in all patients recruited to the Hamlet.rt study.
2) Measurement of LGR5 expression in epithelioids before and after in vitro irradiation of primary and recurrent HNC.

PhD project outline:

Background
The PhD project builds on results from the MRes research and explores new strategies to target HNC. In HNC, immune dysregulation and hypoxia are strongly associated with resistance to RT. Resistance may be due to either selection of pre-existing radioresistant sub-clones after RT or the evolution of new radioresistant sub-clones during treatment. The design of therapeutic strategies to overcome RT resistance will require an understanding of clonal evolution of HNC after RT and the genomics of radioresistant disease.
Cancer stem cells (CSCs) are capable of self-renewal and pluripotency. HNSCC CSCs with the capacity to generate tumours represent attractive targets for novel agents; however, the challenge remains in identifying a suitable molecular biomarker of HNSCC CSCs. Antibodies for targeting the stem cell marker LGR5 have been used to distinguish high levels of LGR5 protein in colorectal and liver cancers and low to undetectable levels in healthy tissues. Preliminary data indicate that a substantial proportion of HNC patients have high LGR5 expression. The antibody drug conjugate version (α-LGR5-ADC) displays excellent efficacy targeting LGR5-expressing cancer cells in vitro and in vivo (3).
Immunotherapeutic approaches to targeting HNC are supported by significant improvements in overall survival when immune checkpoint blockade is used in the setting of recurrent and metastatic disease. This project will explore innovative new treatment strategies for treating advanced stage disease, based on newly developed therapeutic antibody targeting LGR5 expression cancer cells.

Aims and Objectives

A) As the PhD project develops, the student will focus on defining genomics, RNA and protein expression in tumours which have recurred after radical radiotherapy, and identifying specific markers of resistance to radiotherapy.

B) The efficacy of both an antibody drug conjugate (α-LGR5-ADC), anti-LGR5-CAR-Ts and MCLA-158, an EGFR-LGR5 bispecific (petosemtamab) will be tested in vitro and in vivo, with an ultimate aim of assessing suitability for phase 1 trials in HNC patients.

PhD experimental plan:

A) Molecular characterisation of locally recurrent HNC
1) The extraction and genotyping of FFPE samples will already have been performed at Informed Genomics using their Pan-Cancer Panel. For epithelioids derived from recurrent cancers, the student will compare tumour mutational burden from FFPE samples at diagnosis with mutations present at recurrent disease.
2) The Hamlet.rt Data Science Team will use the clinical imaging data to identify the site of the recurrence and correlate this with the high dose region of previous radiotherapy (4).
3) The student will analyze changes in genomics according to whether the patients recurred in the high dose RT volume (radioresistant) or outside the RT treatment volume (may be radiosensitive or radioresistant).
4) RNA-sequencing analysis to measure gene expression, including genes involved in immune function and hypoxia.

B) Determine the efficacy of α-LGR5-based immune therapeutics
1) The efficacy of the antibody drug conjugate (α-LGR5-ADC) and anti-LGR5-CAR-T cell therapy against LGR5-expressing HNC cells in vitro will be measured in vitro and treatment parameters will inform follow-on in vivo xenograft tumour targeting studies.
2) The efficacy of MCLA-158, an EGFR-LGR5 bispecific (petosemtamab) will be tested in vitro in both primary and recurrent HNC.

Main techniques:

1) Derivation and culturing of epithelioids.
2) Molecular characterisation of FFPE biopsies by IHC and immunofluorescence, and microscopy.
3) Transcriptomic and DNA sequence analysis and comparison.
4) Functional assays, primarily cell killing assays to determine drug efficacy.

Key references:

1) Fernandez-Antoran D, Piedrafita G, Murai K, Ong SH, Herms A, Frezza C, et al. Outcompeting p53-Mutant Cells in the Normal Esophagus by Redox Manipulation. Cell Stem Cell 2019;25:329-341.e6. https://doi.org/10.1016/j.stem.2019.06.011

2) Herms, Fernandez-Antoran, Alcolea., et al. Epithelioids: Self-sustaining 3D epithelial cultures to study long-term processes. BioRxiv. https://doi.org/10.1101/2023.01.03.5225893) Chen H-C, Mueller N, Stott K, Rivers E, Kapeni C, Sauer CM, et al. LGR5 targeting molecules as therapeutic agents for multiple cancer types. BioRxiv 2022:2022.09.01.506182 https://www.biorxiv.org/content/10.1101/2023.01.03.522589v1.full.pdf

3) Chen H-C, Mueller N, Stott K, Rivers E, Kapeni C, Sauer CM, et al. LGR5 targeting molecules as therapeutic agents for multiple cancer types. BioRxiv 2022:2022.09.0 https://www.biorxiv.org/content/10.1101/2022.09.01.506182v1.full.pdf

4) Ceilidh Welsh, Karl Harrison, Andrew Hoole, Sara Lightowlers, Ian Gleeson, Alfie Beard, Amy Bates, Charlotte Coles, Gillian Barnett, Rajesh Jena. Cancer agnostic methodology for classifying patterns of locoregional failure using treatment planning dose fields: application to clinical trials. To be submitted to Radiotherapy Oncology Sep 2023.(see attached poster)

5) Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Prim 2020;6. https://doi.org/10.1038/s41572-020-00224-3