ICR The Institute of Cancer Research

CANCER RESEARCH UK | RadNet The Institute of Cancer Research The Royal Marsden Hospital

PhD Project Proposal

Funder details

Studentship funded by:	CRUK RadNet

Project details

Project title:	HPV- and genome instability-driven innate immune responses in head and neck cancer

Supervisory team

Primary Supervisor:	Ben O'Leary; Christian Zierhut
Associate Supervisor(s):	Kevin Harrington (IRS partner)
Secondary Supervisor:	Jessica Downs

Divisional affiliation

Primary Division:	Radiotherapy and Imaging/Cancer Biology
Primary Team:	Evolution & Translational Genomics/Genome Stability and Innate Immunity
Site:	Chelsea

Project background

Head and neck cancer is rising in incidence, driven largely by oropharyngeal cancer related to human papillomavirus (HPV+ HNSCC). The principal curative treatment for HPV+ HNSCC is radiotherapy, which is often highly toxic. Intrituingly, HPV+ HNSCC can be cured with much lower radiotherapy doses than conventionally administered, but the mechanisms of radiotherapy sensitivity and how to select patients for reduced dose is unknown (Mehanna *et al.,* 2019; Lee *et al.,* 2024). For HPV to persist and drive HNSCC it must subvert the immune system. How this is achieved is not known, but could be relevant to radiotherapy, which works in part through immune responses. Radiotherapy also causes increased chromosomal instability, often already baseline present at lower rates in HNSCC. Whilst chromosomal instability can potentially act oncogenic by generating genotypic variability, it is also thought to represent a potential Achilles heel by promoting innate immune signalling, which may ultimately generate long-term anti-tumour immunity (Zierhut and Funabiki, 2020). This is because immunogenic responses to pathogen DNA can also be activated by by-products of chromosomal instability. Foremost amongst these pathways are the cGAS-STING and the AIM2 inflammasome pathways, but how these impact head and neck

cancer is poorly understood (Karki and Kanneganti, 2019; Zierhut and Funabiki, 2020). Intriguingly, although the HPV genome could potentially act as a stimulant for these pathways, HPV has rather been suggested to act inhibitory on innate immune responses (Lau *et al.*, 2015). However, the cross-talk between chromosomal instability, HPV and innate immunity is very poorly understood and recent studies combining radiotherapy with T cell-targeting immunotherapy have been unsuccessful (Machiels *et al.*, 2024). We propose to explore the impact of radiotherapy on this cross-talk, and investigate the hypothesis that the intersection between the innate immune response and HPV directly impacts radiotherapy efficacy and chance of cure in the clinic.

Project aims

- Identify the innate immune status of human head and neck cancers within the context of chromosomal instability and HPV.
- Functionally validate the relationship between the identified pathways in laboratory models of head and neck cancers, including using novel 3D co-cultures of patient-derived organoid models and endogenous matched immune cells, and explore manipulation of these pathways as potential novel therapeutic approaches.
- Evaluate how immune responses and therapy outcome correlate with molecular phenotypes defined by CIN, HPV and innate immune signalling
- Explore potential co-treatments modulating the innate immune responses to support head and neck cancer treatment.

Research proposal

This interdisciplinary project combines state-of-the art analysis of human tumour samples with cutting-edge cell biology and pre-clinical models, and has the potential to inform the design of future clinical trials. The project will benefit from the combination of the fundamental science expertise of Christian Zierhut, a cell biologist who recently developed several new approaches to monitor innate immune signalling in cultured cells at unprecedented spatial and temporal resolution, and the co-supervision from Ben O'Leary, a clinician scientist leading multiple translational studies in patients being treated for head and neck cancer at The Royal Marsden Hospital.

First, we will examine in HPV+ HNSCC the interplay between the innate immune system and existing and ongoing chromosomal instability (CIN) within the human and HPV viral genome. Focusing initially on human cancer samples, we will use a combination of multi-omic approaches including spatially conserved assessment of genomics, transcriptomics and protein to unpick the relationship between chromosomal instability, HPV, and the nature of the innate immune response.

Next, using laboratory models of HNSCC we will explore the mechanistic relationships underlying these observations these pathways will be manipulated in *in vitro* models of head and neck cancer. This work will be carried out in cell line models of head and neck cancer and will involve both mono-culture as well as co-culture with cells from the tumour microenvironment such as cancer-associated fibroblasts and immune cells. This part of the project will also incorporate expertise in 3D culture from both the O'Leary and the Zierhut lab, and the CRUK Convergence Science Organoids Centre. Leveraging clinical samples being collected in ORIGINS (Organoid Generation in Cancer Study), we will develop 3D co-culture assays combining patient-derived tumour organoids with matched fibroblasts and immune cells collected contemporaneously at the time of surgery, providing a unique platform for investigating the innate immune response to chromosomal instability in HPV+ HNSCC. A particular emphasis will be put on the question how HPV modulates innate immune responses, and how HPV co-signals with other intracellular

signalling in order to re-wire cellular functions. At this point, we will also integrate key new findings from the O'Leary and Zierhut groups on pathways that regulate cancer cell behaviour in response to HPV and in the context of cGAS and AIM2 signalling. Finally, these findings will be integrated with animal models of head and neck cancer and its treatment, to test the impact of the identified pathways, through genetic and/or pharmacological manipulation. These experiments will be facilitated by the extensive catalogue of murine head and neck models available to us through collaboration with the groups of Professors Alan Melcher and Kevin Harrington, as well as the extensive expertise available at the ICR in their analysis using state-of-the-art technologies.

Having dissected the dynamic relationship between CIN and the innate immune response in HPV+ HNSCC, we will correlate the identified molecular phenotypes with the pattern of responses to radiotherapy in clinical cohorts of HNSCC, linking our pre-clinical mechanistic work to real-world clinical data. To this end, we will analyse impacts on the tumour microenvironment (TME), immune cell infiltration specific to the innate immune response, and overall treatment response to radiotherapy. This part of the project will be based on extensive data that is available through Ben O'Leary's involvement in multiple clinical studies on the treatment of head and neck cancer.

Overall, this project will reveal important principles for how head and neck cancer modulates immune responses to re-wire cell fate and the microenvironment, and how this impacts radiotherapy sensitivity and the chance of cure. Ultimately, this work could lead to the identification of potential biomarkers and co-treatments to improve personalisation of radiotherapy treatments for patients with HPV+ HNSCC, allowing clinical trials of reduced radiotherapy dosing in rationally selected populations and targeted combination treatment in high-risk cases.

Literature references

Karki, R. and Kanneganti, T.-D. (2019) "Diverging inflammasome signals in tumorigenesis and potential targeting," *Nature Reviews Cancer*, 19(4), pp. 197–214. Available at: https://doi.org/10.1038/s41568-019-0123-y.

Lau, L. *et al.* (2015) "DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway.," *Science (New York, N.Y.)*, 350(6260), pp. 568–571. Available at: https://doi.org/10.1126/science.aab3291.

Lee, N.Y. *et al.* (2024) "Hypoxia-Directed Treatment of Human Papillomavirus–Related Oropharyngeal Carcinoma," *Journal of Clinical Oncology*, 42(8), pp. 940–950. Available at: https://doi.org/10.1200/jco.23.01308.

Machiels, J.-P. *et al.* (2024) "Pembrolizumab plus concurrent chemoradiotherapy versus placebo plus concurrent chemoradiotherapy in patients with locally advanced squamous cell carcinoma of the head and neck (KEYNOTE-412): a randomised, double-blind, phase 3 trial," *The Lancet Oncology*, 25(5), pp. 572–587. Available at: https://doi.org/10.1016/s1470-2045(24)00100-1.

Mehanna, H. *et al.* (2019) "Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus-positive oropharyngeal cancer (De-ESCALaTE HPV): an open-label randomised controlled phase 3 trial," *The Lancet*, 393(10166), pp. 51–60. Available at: https://doi.org/10.1016/s0140-6736(18)32752-1.

Zierhut, C. and Funabiki, H. (2020) "Regulation and Consequences of cGAS Activation by Self-DNA.," *Trends in cell biology*, 30(8), pp. 594–605. Available at: https://doi.org/10.1016/j.tcb.2020.05.006.

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants:	BSc in Biology or similar, ideally an MSc or MRes degree
Intended learning outcomes:	• The ability to design, and interpret experiments • The ability to evaluate and interpret published literature • The ability to present and discuss research data and ideas • Understand and be able to use state-of-the-art molecular biology, cell biology and tumour analysis techniques • Become an expert in innate immunity, chromosomal instability and the biology of HPV with respect to cancer • Become an expert in the cell biology and tumour context of HNSCC

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☐ Physics or Engineering
- ☐ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science