ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	The Institute of Cancer Research

Project details

Project title:	Deciphering the role of human papillomavirus (HPV) in radiotherapy resistance for HPV+ head and neck cancers.

Supervisory team

Primary Supervisor:	Ben O'Leary
Associate Supervisor(s):	Magnus Dillon
Secondary Supervisor:	Alan Melcher

Divisional affiliation

Primary Division:	Radiotherapy & Imaging
Primary Team:	Evolution & Translational Genomics
Site:	Sutton

Project background

The human papillomavirus (HPV) is responsible for approximately 5% of all human cancer, 630,000 cases worldwide each year. HPV infection in widespread, leading to squamous cell carcinomas in the cervix, head and neck, and anogenital regions and is often treated with curative intent using radiotherapy1. The incidence of head and neck squamous cell cancer (HNSCC) is rapidly increasing in the UK, where it is now the 4th most common cancer in men.

Radiotherapy treatment is toxic with a significant burden of side effects for patients, often requiring hospital admission and feeding tube insertion during treatment and resulting in long term changes in swallowing and speaking. Many HPV+ HNSCC can be cured with radiotherapy, suggesting that treatment could be de-escalated in some patients, but several large trials have failed to achieve this without compromising cure rates, highlighting that there are an important subgroup of HPV+ HNSCC who are radiotherapy resistant2,3. Identifying these is a critical step to personalising treatment and allowing safe de-escalation and focused escalation of radiotherapy in appropriately selected populations.

Our preliminary data demonstrate a wide range of HPV copy number in HPV+ HNSCC, with a group of low copy cancers with viral integration into the cancer genome, another with high viral copy number without integration, and a third that exhibits human-viral hybrid extrachromosomal DNA4,5. Further, we observe the HPV viral copy number and integration sites can evolve through radiotherapy failure in tumours from patients with recurrence after radical radiotherapy, suggesting selection for resistance. This invites the hypothesis that genome instability, and HPV copy number and integration is related to radiotherapy resistance. In this project the candidate will use a combination of

pre-clinical models, including patient-derived organoids, and clinical samples to explore the role of HPV copy number and integration in radiotherapy failure in HPV+ HNSCC.

Project aims

- Establish radiotherapy sensitivity and genomic instability in response to radiotherapy in cell line and patient-derived organoid models of HNSCC.
- Generate radiotherapy resistant pre-clinical models of HNSCC and evaluate HPV copy number and integration through evolution of radiotherapy resistance, comparing with patient datasets.
- Evaluate cell-autonomous immune profiles of radiotherapy sensitive versus resistant models and validate findings in patient samples.
- Explore targeting HPV oncoproteins as a therapeutic strategy for HPV+ HNSCC treated with radiotherapy.

Research proposal

The candidate will explore the relationship between HPV copy number and integration with genome instability and radiotherapy sensitivity in pre-clinical models, validating these in patient samples from clinical trials, in parallel testing the hypothesis that HPV oncoproteins could be used as a therapeutic target for combination with radiotherapy.

1) Establish radiotherapy sensitivity and genomic instability in response to radiotherapy in cell line and patient-derived organoid models of HNSCC.

The available clinical evidence suggests integration of the HPV viral genome into the cancer genome is associated with a poor prognosis6. Using a panel of 11 HNSCC cell lines that have been characterised at the DNA, RNA, proteomic and phosphoproteomic levels, the candidate will characterise the comparative radiotherapy sensitivity of different HPV+ genomic profiles, with HPV- HNSCC as controls, using clinically relevant radiotherapy dose fractionation and assessment of cell survival, proliferation, and DNA damage and repair. This work will be supported by collaboration with the Dillon lab, with their established expertise in radiotherapy and the DNA damage response. The candidate will further examine radiotherapy sensitivity in patient-derived HNSCC organoid models generated in the ORIGINS study at The Royal Marsden Hospital, in collaboration with the CRUK Convergence Science Organoid Centre. The workflow for organoid generation is well-established, with ongoing enrolment of patients in the clinical study.

2) Generate radiotherapy resistant pre-clinical models of HNSCC and evaluate HPV copy number and integration through evolution of radiotherapy resistance, comparing with patient datasets.

Fractionated radiotherapy treatment at clinically relevant doses will be used to deliver sub-lethal treatment to the panel of pre-clinical models to select populations for radiotherapy resistance. Cancer genome copy number will be assessed with whole genome sequencing, with HPV copy number and integration assessed with a custom in-house HPV sequencing and digital PCR assays, Lineage tracing will be used to assess the cancer clonal dynamics during radiotherapy treatment and relapse – answering the question as to whether sub clonal selection drives radiotherapy failure, or whether multiple sub clones populate the recurrence or plasticity within the clonally dominant population present prior to treatment, and the role HPV plays in this selection. The available data suggests that cancers treated with cytotoxic chemotherapies can demonstrate convergence towards stereotyped patterns of aneuploidy, but whether this occurs with radiotherapy is unknown7,8. Single cell sequencing of DNA, RNAseq, and ATACseq will be used to assess the tumour population diversity in terms of genome instability and aneuploidy, with concurrent assessment of epigenetic and transcriptional response to radiotherapy in tumours. Profiles will be compared to patient datasets characterised within the lab. The INOVATE trial, a study of patients with HPV+ HNSCC treated with

radiotherapy, will be used to test whether features identified in the pre-clinical models with radiotherapy resistance are associated with poorer clinical outcomes.

3) Evaluate cell-autonomous immune profiles of radiotherapy sensitive versus resistant models and validate findings in patient samples.

HPV+ HNSCC has a better prognosis than HPV- HNSCC when treated with curative intent using radiotherapy, for reasons that are not understood. As HPV+ HNSCC is the direct result of viral infection, it must evolve with some degree of tolerance to the presence of viral DNA and the expression of viral oncoproteins. Recent work demonstrates that in addition to its direct action through DNA damage, radiotherapy also activates evolutionarily conserved antiviral pathways - cytosolic DNA released through radiotherapy damage provoking a type I interferon response through cGAS/STING9. In tumours this can prime an immune response against the neoantigens from the cancer and is potentially important for tumour response to radiotherapy – tumour control is dependent on type I interferon response in some murine models, and T cell checkpoint inhibition such as anti-CTLA-4 and PD-1 can enhance radiotherapy response in cancers9. In HPV+ HNSCC the viral genome presents an obvious point of tumour-immune tension, with the integration and expression of the HPV viral genome potentially related to radiotherapy sensitivity through the immune stimulatory action of radiotherapy. The candidate will explore this hypothesis by examining cGAS/STING-related pathways and upregulation of neoantigen expression in different radiotherapy-treated pre-clinical models, validating these observations within patient cohorts with intact immune microenvironments, and collaborating with the Melcher group to validate in immune competent mouse models.

4) Explore targeting HPV oncoproteins as a therapeutic strategy for HPV+ HNSCC treated with radiotherapy.

Recent advances in proteolysis targeting chimera (PROTAC) technology presents a novel approach for cancer precision medicine by inducing selective degradation of a target protein. The HPV-related oncoproteins E6 and E7 potentially offer ideal targets for the treatment of HPV+ HNSCC in being exclusively expressed in cancer cells in these patients, offering in theory a wide therapeutic window with less off target-related toxicity10. In this complementary workstream, the candidate will assess the ongoing functional importance of HPV oncoproteins in HPV+ HNSCC models, and their importance for radiotherapy resistance and sensitivity, through exploring knock down and knock out of E6 and E7 in the cell line and 3D patient-derived organoid models. This will allow assessment of these as appropriate targets for PROTAC development in collaboration with the ICR's Centre for Target Validation, from whose expertise the candidate will benefit from.

Literature references

[1] de Martel, C., Plummer, M., Vignat, J. & Franceschi, S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer 141, 664-670 (2017).
[2] Mehanna, H., et al. 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, 51-60 (2019).
[3] Gillison, M.L., et al. Radiotherapy plus cetuximab or cisplatin in human papillomavirus-positive oropharyngeal cancer (NRG Oncology RTOG 1016): a randomised, multicentre, non-inferiority trial. The Lancet 393, 40-50 (2019).
[4] Gillison, M.L., et al. Human papillomavirus and the landscape of secondary genetic alterations in oral cancers. Genome research 29, 1-17 (2019).
[5] Akagi, K., et al. Intratumoral heterogeneity and clonal evolution induced by HPV integration. Cancer Discovery (2023).
[6] Koneva, L.A., et al. HPV Integration in HNSCC Correlates with Survival Outcomes, Immune Response Signatures, and Candidate Drivers. Molecular Cancer Research 16, 90-102 (2018).
[7] Ippolito, M.R., et al. Gene copy-number changes and chromosomal instability induced by aneuploidy confer resistance to chemotherapy. Developmental Cell 56, 2440-2454.e2446 (2021).
[8] Santaguida, S. & Amon, A. Short- and long-term effects of chromosome mis-segregation and aneuploidy. Nat Rev Mol Cell Biol 16, 473-485 (2015).
[9] Twyman-Saint Victor, C., et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 520, 373-377 (2015).

[10] Mangano, K., et al. VIPER-TACs leverage viral E3 ligases for disease-specific targeted protein degradation. bioRxiv, 2024.2008.2013.607762 (2024).

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:

Intended learning outcomes:

- Radiobiology
- 3D organoid culture
- Bioinformatics
- Clinical multi-omics analysis
- Translational cancer biology

Advertising details

Project suitable for a student with a background in:

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