The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:
CRUK RadNet

Project details

Project title:
Optimising radiotherapy dose-fractionation and volume for maximal synergy with immunotherapy

Supervisory team

Primary Supervisor:
Navita Somaiah and Erik Wennerberg

Associate Supervisor(s):
Kevin Harrington, Esther Arwert

Secondary Supervisor:
TBC

Divisional affiliation

Primary Division:
Radiotherapy and Imaging

Primary Team:
Translational Breast Radiobiology and Radiation-enhanced Immunotherapy

Site: Sutton

Project background

Given the multifaceted and "double-edged sword" nature of radiation-induced immune activity, many clinical trials testing radio-immunotherapy in breast cancer – or indeed other cancer types such as head and neck squamous cell cancers – have been unsuccessful thus far, despite the clear preclinical rationale for combining the two treatment modalities [1, 2]. To bridge this translational gap, it is important to biologically optimise radiotherapy (RT) dose-fractionation and volume irradiated in the era of immune-oncology. Hypofractionated RT regimens (using >2Gy daily doses) have shown non-inferiority in tumour control and normal tissue side effects compared with conventionally fractionated RT in large, randomised trials [3]. While this change has clear benefits for patients, little is known about how hypofractionation shapes the tumour immune microenvironment, how it affects anti-tumour immunity locally and systemically, and how it could synergise with immunotherapy. Often, pre-clinical models testing RT + immunotherapy have not used clinically pertinent dose-fractionation schedules.

Further, the routine clinical practise of regional nodal irradiation has recently come into question following preclinical studies demonstrating that omitting or delaying irradiation of non-involved lymph nodes has profound positive effects on the anti-tumour immune response [4, 5]. Our data from the analysis of pre- and post-RT lymph node samples from 2 UK pre-operative breast RT trials have shown that regional lymph node RT increases tumour kill but also disrupts lymph node immune architecture and lymphocytic subpopulations, that may negatively impact the crosstalk between the primary tumour and draining lymph nodes to mount an effective immune response.

Preclinically, we have tested the immunomodulatory effects of clinically pertinent RT dose fractionation in two orthotopic breast cancer models, TSAE1 (resembling hormone-receptor-positive breast cancer (HR+BC)), and AT-3 (resembling triple-negative breast cancer (TNBC)). We assessed survival outcomes comparing conventionally fractionated RT regimen (2.67 Gy per fraction), a moderately hypofractionated RT regimen (5.2 Gy per fraction), and an ultra-hypofractionated RT regimen (8 Gy per fraction). In the second part of the study, we sought to assess immunological and metabolic responses across the different fractionation regimens. Preliminary results from the flow cytometry analysis of AT-3 tumours show clear differences in myeloid immune infiltration between the fractionation regimens. The moderately hypofractionated regimen had a reduced infiltration of inflammatory monocytes and an enrichment of neutrophils and conventional type I dendritic cells compared with the other two RT regimens, suggesting this may be the most immune-stimulatory.

Building on from the above clinical and pre-clinical data on the effects of RT alone, this PhD studentship will elucidate the optimal way in which to combine immunotherapy with RT for maximal impact in pre-clinical models and from longitudinal patient samples in upcoming/ongoing randomised trials.

Project aims

- **Identify the optimal radiotherapy (RT) dose-fractionation for maximal RT-immunotherapy synergy**
- **Interrogate the impact of lymph node sparing RT on radiation-induced immunogenicity**
- **Validate preclinical findings in patient samples from randomised clinical trials**

Research proposal

Aim 1: Identify the optimal RT dose-fractionation for maximal RT-immunotherapy synergy

This aim will build upon our data describing how RT alone shapes the tumour microenvironment. Here, clinically relevant RT fractionation regimens will be assessed for their ability to induce local and systemic anti-tumour immune responses in combination with different immunotherapies.

<u>Preclinical modelling</u>**:** In well-characterised orthotopic mouse breast cancer models (e.g. TNBC models AT3 and 4T1 as well as the HR+BC model TSAE1) and other relevant tumour models such as head and neck cancers, preclinical modelling will be performed using the small animal radiation research platform (SARRP). Following CT-guided treatment planning, RT will be delivered as clinically relevant conventionally fractionated, moderately hypofractionated, or ultra-hypofractionated regimens. The RT regimens will be tested with or without systemically administered conventional immune checkpoint blockade (anti-PD-1 and anti-CTLA-4) as well as immune agonist treatments targeting T cell co-stimulation (e.g. 41BB, OX40) and innate immunity (e.g.CD40, STING, Toll-like receptors).

<u>Immune characterisation</u>**:** Irradiated and non-irradiated tumours, tumour-infiltrated organs and secondary lymphoid organs will be analysed by spectral flow cytometry (Sony ID700 platform), multiplex immunofluorescence imaging (PhenoImager/PhenoCycler imaging platforms) to assess spatial immune and stromal contexture and single cell-RNA sequencing (10x Platform) for treatment-induced immune changes on a single-cell level. Systemic effects of combination treatments will be determined by assessing tumour growth and immune parameters in mammary fat pad tumours implanted contralaterally to the irradiated tumour. Further, to assess how selected combination treatments affect the temporal dynamics of T cell activation, the unique Timer of cell kinetics and activity (Tocky)

mouse model will be used (collaboration with Dr Masahiro Ono, Imperial College). This model allows for identification of T cells whose T cell receptor has engaged with antigen and to determine whether the interaction is sustained over time. By integrating the Tocky parameters with our multiplex flow cytometry panels assessing metabolic dependencies and cellular exhaustion, these studies will provide a unique insight into how anti-tumour T cells adapt to activation over time in response to therapy.

Aim 2: Interrogate the impact of lymph node sparing RT on radiation-induced immunogenicity

One of the promises of utilising RT as an *in-situ* tumour vaccine is its potential to promote priming of *de novo* tumour-specific immune responses, a process which largely takes part in the tumour-draining lymph nodes. Therefore, sparing lymph nodes from RT may be essential for mounting radiation-induced anti-tumour immunity. Using the RT delivery methodology and immune readouts described in Aim 1, this aim will determine whether concurrent RT abrogates therapeutic synergy with immunotherapy, and whether delayed lymph node irradiation, or sparing lymph nodes from RT altogether, is beneficial for RT-immunotherapy efficacy.

Aim 3: Validate preclinical findings in patient samples from randomised clinical trials

Following on from the hypothesis-generating single arm pre-operative breast RT trial, PRADA (NCT02771938), UK's first large, randomised trial testing pre-operative versus post-operative RT in breast cancer patients is due to open in January 2026 (PRADA-II, NIHR-HTA funded/NIHR163836, N=480 patients). In addition, MULBERRY, a trial testing pre-operative RT to the tumour alone (sparing lymph node radiation) followed by RT to the breast and lymph nodes as determined by the pathology findings at surgery, is being set up to start in the second half of 2026. A unique set of longitudinal pre- and post-RT tumour, lymph node and blood samples will be collected as part of these trials, providing the opportunity to validate findings from preclinical modelling in patients, including the impact of concurrent versus delayed lymph node radiation.

Literature references

1. Voorwerk, L., et al., *Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial.* Nat Med, 2019. **25**(6): p. 920-928.
2. Lee, N.Y., et al., *Avelumab plus standard-of-care chemoradiotherapy versus chemoradiotherapy alone in patients with locally advanced squamous cell carcinoma of the head and neck: a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial.* Lancet Oncol, 2021. **22**(4): p. 450-462.
3. Murray Brunt, A., et al., *Hypofractionated breast radiotherapy for 1 week versus 3 weeks (FAST-Forward): 5-year efficacy and late normal tissue effects results from a multicentre, non-inferiority, randomised, phase 3 trial.* Lancet, 2020. **395**(10237): p. 1613-1626.
4. Marciscano, A.E., et al., *Elective Nodal Irradiation Attenuates the Combinatorial Efficacy of Stereotactic Radiation Therapy and Immunotherapy.* Clin Cancer Res, 2018. **24**(20): p. 5058-5071.
5. Telarovic, I., et al., *Delayed tumor-draining lymph node irradiation preserves the efficacy of combined radiotherapy and immune checkpoint blockade in models of metastatic disease.* Nat Commun, 2024. **15**(1): p. 5500.

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:

First class or upper second-class BSc Honours/MSc or equivalent in biological sciences or computational biology.

Excellent communication and presentation skills

Intended learning outcomes:	• Develop an in-depth understanding of radiation-induced tumour and lymph node immune crosstalk in cancer • Understand the effects of radiation on the tumour immune microenvironment and extracellular matrix • Gain experience in pre-clinical animal modelling and use of the SAARP platform • Gain expertise in basic and advanced molecular pathology, including immunohistochemistry, multi-plex immunofluorescence, T-cell receptor sequencing and next-generation sequencing (e.g. single-cell RNA Seq) • Gain experience in spectral flow cytometry and immunometabolic analysis • Gain experience in quantitative computational pathology, bioinformatics and machine learning • Scientific writing, presenting, team working and communication skills

Advertising details

Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science