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:	Enhancing immune responses to radiation with spatiotemporal dose modulation and drug combinations

Supervisory team

Primary Supervisor:	Magnus Dillon
Associate Supervisor(s):	
Secondary Supervisor:	Uwe Oelfke

Divisional affiliation

Primary Division:	Radiotherapy and Imaging
Primary Team:	Biological enhancement of radiotherapy
Site:	Sutton

Project background

Radiation is a critical treatment component for the cure of around half of cancer patients. The predominant effect of radiotherapy is through death of tumour cells, however it is increasingly recognised that the immune response is critical for the effect of radiation. Radiation can result in tumour inflammation, immunogenic cell death and increased antigen presentation, but it can also cause the death of sensitive effector immune cells and infiltration of suppressor cells (Lynch et al., 2024). This balance may be affected by physical factors such as dose, dose-rate, field size and dose-per-fraction (Arnold et al., 2018) as well as biological factors including the mode of cell death, presence of radiosensitising drugs (Dillon et al., 2019) and the initial immune landscape of the tumour. Clinical trials of immune checkpoint blockade with radiotherapy have also given mixed results, with phase III studies of immunotherapy given after chemoradiotherapy showing a benefit (Antonia et al., 2018), but not when given concomitantly (Lee et al., 2021), reinforcing the need for better understanding of these effects.

Radiation with the intent of immune activation presents a new paradigm for radiotherapy. Both preclinical and clinical data suggest that, in combination with immune checkpoint blockade, partial tumour irradiation provides comparable tumour control to total irradiation (Korpics et al., 2023, Markovsky et al., 2019). Microbeam

radiotherapy is a method of systematic partial tumour irradiation, early data suggest that it may lead to a more favourable immune microenvironment (Takashima et al., 2024).

FLASH radiation is another modality which seems to spare normal tissues and is entering clinical practice. In animal models, the same degree of tumour control is possible with reduced normal tissue toxicities (Vozenin et al., 2022). The immune and microenvironmental effects of FLASH radiation have not been well described, and this knowledge is critical in the era of immunotherapy.

Project aims

- Characterise differences in immune responses to FLASH versus broad beam (standard) radiation
- Examine changes in immune biology of irradiated tumours with spatially fractionated radiation, combined with DNA damage response inhibitors
- Assess whether addition of immune-targeted drugs is more beneficial in spatiotemporally modulated radiation dosing (FLASH and microbeam), compared with broad beam irradiation
- Assess translatability of findings using human tumour explants and patient-derived organoids

Research proposal

The candidate will use preclinical models to explore the microenvironmental changes after FLASH radiation versus broad beam, and the potential for adding drugs targeting the DNA damage and immune response to microbeam and FLASH radiation.

1. **Characterise differences in immune responses to FLASH and microbeam-FLASH versus broad beam (standard) radiation**: FLASH irradiation appears to have a greater therapeutic window between tumour and normal cells compared with standard radiation. Using immunocompetent mouse models of gastrointestinal (pancreatic and colorectal) cancers, single or dual fraction irradiations will be performed on the FLASH-SARRP (small animal radiation research platform), which delivers ultra-high dose-rate radiation. These will be compared with equivalent doses of broad beam radiation. The changes in tumour microenvironmental cells (immune cells and fibroblasts) and their activation/exhaustion status will be assessed, using multicolour flow cytometry and transcriptomics. Additional methods will be used to determine (i) whether there are differences in antigen-specific responses (using tetramer staining of CD8 cells); (ii) whether there are differences in infiltrating vs resident immune cells (using the kaede mouse, whose cells change colour when they are exposed to UV light and thus can track whether cells were present at the time of irradiation or have infiltrated since); and (iii) whether there is a difference in the spatial organisation of the tumour (using multiplex immunofluorescence, IHC and/or spatial transcriptomics). This will confirm whether there are microenvironmental differences after treatment with the novel radiation modalities.
2. **Examine changes in immune biology of irradiated tumours with spatially fractionated radiation, combined with DNA damage response inhibitors:** Using the SARRP with microbeam collimator, tiny stripes of radiation can be delivered. In theory, high-dose regions will experience more tumour cell death, which will lead to more immune activation of the immune cells in low-dose regions that are not killed by irradiation. The close proximity of these regions within the tumour may lead to enhanced antigen presentation and activation of immune cells, with less radiation-induced immune cell death. Adding radiosensitizers may further enhance this effect, increasing cell death in the irradiated regions and making the modality of death more immunogenic. We have access to clinical compounds of PARP1-selective inhibitors, ATR inhibitors and ATM inhibitors to test whether these could increase immunogenic cell death in the high-dose regions. As well as the techniques outlined above, live cell imaging of tumour slices will be used to assess spatial modulation of cell death with various combination treatments. Ovalbumin-transduced tumour cells will be used to check for differences in the level of tumour antigen presentation. This work will confirm whether microbeam effects can be enhanced with DNA repair inhibition and will run in parallel with immunotherapy combination experiments.
3. **Assess whether addition of immune-targeted drugs is more beneficial in spatiotemporally modulated radiation dosing (FLASH and microbeam), compared with broad beam irradiation:** Initially, responses with FLASH vs. microbeam vs. broad beam in combination with anti-PD-1 antibodies will be tested to determine if anti-tumour immunity can be enhanced. Ongoing work is looking at immune response to microbeam irradiation. This will generate potential novel immune-related targets for microbeam-immunotherapy combinations. Assessing differential efficacy of these targets in microbeam and broad beam-treated animals in terms of tumour control, survival and antigen-specific responses will clarify the role

of microbeam radiotherapy as an immune-sparing or immune-stimulating treatment and pave the way for future clinical trials of microbeam radiation.

4. **Assess translatability of findings using human tumour explants and patient-derived organoids**: Ongoing work has set up collaboration with RMH surgeons, Kings College Hospital surgeons and biobank in order to access fresh tissue from pancreatectomy specimens, both irradiated and unirradiated. Organoid-generation protocols are being optimised in the lab in order to bank patient-derived organoids for future analysis. Organoids will be used to assess differences in cell-intrinsic reaction to FLASH and broad-beam radiation (secretome, proteome, radiation response). Autologous fibroblasts and lymphocytes will also be collected for co-culture experiments to ascertain similarities between the pre-clinical model findings. Live tumour explants (precision-cut tumour slices) will be used as a proof-of-concept to investigate the short-term effects of microbeam and FLASH irradiation on the tumour microenvironment. Slices can be used to test multiple conditions on the same tumour, and a variety of techniques will be used to characterise the secretome (ELISA), cell infiltration, survival and activation (flow cytometry) and spatial localisation of microenvironmental cells (Phenocycler, Visium HD). This provides a critical step to clinical translation.

Overall, this project will contribute essential knowledge as to whether these novel radiation modalities can have a role in immunotherapy combinations through immune sparing or immune activation.

Literature references

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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 biological sciences or equivalent
Intended learning outcomes:	
Potential Publications from this project:	2 original research articles and 1 review article.

Advertising details

Project suitable for a student with a background in:

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