PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	Tracking immune cell migration in tumour and normal tissues after combined radiation-drug therapy in Kaede mice

Supervisory team

Primary Supervisor:	Kevin Harrington
Associate Supervisor(s):	Malin Pedersen
Secondary Supervisor:	TBC

Divisional affiliation

Primary Division:	Radiotherapy & Imaging
Primary Team:	Targeted Therapy
Site:	Chelsea

Project background

Previously, radiotherapy (RT) as cancer treatment was seen entirely from the perspective of the cancer cell; the goal was to inflict a burden of breaks in nuclear DNA exceeding the cancer cell's capacity for repair, without unacceptable collateral damage to adjacent normal tissues. Recently, a more nuanced view has emerged. Direct radiation-induced cancer cell killing is, of course, essential, but non-tumour cell-autonomous immune and stromal responses to radiation are increasingly appreciated as being central to successful therapy. Clinical responses to RT are inextricably linked to a functional immune system: loco-regional RT (rarely) triggers abscopal, immune-mediated responses at distant unirradiated sites, while patients with immunosuppressed states have worse outcomes from curative-intent RT.

The intrinsic biology of individual tumours and their associated tumour-immune microenvironments (TIME) shape the immunogenicity of RT and provide rich potential targets for therapeutic modulation. The TIME is not static as the tumour grows and changes with therapeutic interventions, which can exert both favourable and/or unfavourable effects. Immune cells within the TIME and adjacent lymph nodes may be depleted by RT/RT-drug combinations and RT-induced changes in chemokine/cytokine signalling and tumour vasculature may modulate immune cell migration/trafficking to and from irradiated tissues. Again, such changes may have beneficial and/or deleterious effects on tumour control outcomes. Despite the potential importance of these processes, very little is known about RT-induced dynamic changes in the TIME and their role in the therapeutic efficacy of RT or RT-drug combinations. Similarly, sparse data are available on the effects of different radiation dose-fractionation schedules and patterns of spatial dose distribution (whole tumour vs partial tumour irradiation) on the TIME.

The candidate will use syngeneic head and neck cancer models in Kaede mice to evaluate the effects of RT and RT-drug combinations on immune cell trafficking to and from the TIME and normal tissues, to define opportunities for optimised RT-immunotherapy combination regimens.

Project aims

- Evaluation of immune cell trafficking in the TIME of irradiated/unirradiated tumours and in normal tissues in tumour-bearing Kaede mice.
- Assessment of the relative effects of different radiation dose-fractionation regimens (conventional vs hypofractionated) on immune cell migration/trafficking in the TIME of irradiated/unirradiated tumours and in normal tissues in tumour-bearing Kaede mice.
- Examination of the effect of locoregional nodal irradiation on immune cell migration/trafficking in the TIME of irradiated/unirradiated tumours and in normal tissues in tumour-bearing Kaede mice.
- Evaluation of the effects of spatial dose-modulated RT of tumours on the patterns of immune cell migration/trafficking.

Research proposal

Immune checkpoint inhibitors (ICPIs) are new standard-of-care therapies across multiple tumour types. For patients with relapsed/metastatic squamous cell cancer of the head and neck (SCCHN), there have been approvals of anti-programmed death-1-inhibiting (anti-PD1) antibodies: nivolumab and pembrolizumab in second-line setting, and single-agent pembrolizumab and pembrolizumab-chemotherapy combination therapy in first-line setting [Harrington – Steering Committee Member, CheckMate-141, KEYNOTE-040, KEYNOTE-048).

Positive outcomes from CheckMate-141, KEYNOTE-040 and KEYNOTE-048 studies fed assumptions that these agents would also be effective combined with radiotherapy, including as palliative treatment of relapsed/metastatic disease and curative-intent treatment with RT/chemoradiotherapy (CRT). However, a randomised phase II study of nivolumab with or without stereotactic body radiotherapy (27 Gy/3 fractions) showed no difference in response rates, duration of response, progression-free or overall survival between arms, and no evidence of abscopal responses in irradiated patients. In curative-intent therapy, early phase studies confirmed safety and allowed us to believe that existing gold-standard CRT regimens for locally-advanced SCCHN could be combined effectively with anti-PD1 ICPI and deliver improvements in survival outcomes. The resultant JAVELIN-100 Head and Neck and KEYNOTE-412 studies [Harrington – Steering Committee member for both studies] failed to meet their primary endpoints of progression-free/event-free survival. Other studies, notably Pembro-Rad [RT-pembrolizumab vs RT-cetuximab] and REACH [RT-cetuximab-avelumab vs RT-cetuximab (platin-ineligible) or RT-platinum (platin-eligible)], have also delivered negative outcomes at primary/secondary endpoints. As a counterpoint, the PACIFIC trial of adjuvant anti-PD-L1 (durvalumab) therapy following curative-intent CRT for stage III non-small-cell lung cancer was impressively positive. Post hoc analysis showed that the greatest benefit accrued to patients who started adjuvant immunotherapy within 2 weeks of the end of CRT, raising the possibility that RT/CRT-induced conditioning or sculpting of the TIME has a significant effect on subsequent, adjuvant immunomodulatory therapy.

Therefore, in this project, the candidate will use SCCHN-bearing Kaede mice to perform studies that evaluate radiation-induced effects on immune cell trafficking within the TIME to guide rational selection of optimised combinations of RT and drug therapies.

Kaede mice are transgenic for a photoactivatable fluorescent protein derived from a stony coral, *Trachyphyllia geoffroyi*. Following in vivo ultraviolet illumination (350-400 nm), cells in the light field irreversibly photoconvert from green to red fluorescence. We will use Kaede mice to track migration and activation/suppression of immune cells in irradiated and unirradiated tissues. Specifically, we will UV-illuminate and irreversibly green-to-red photoconvert, in isolation, the following tissues: tumour, draining or non-draining lymph node, and spleen. Thereafter, we will deliver RT/CRT and, at time points between 1 and 72 hours, harvest tissues and quantify the presence of green, non-photoconverted immune cell populations in red photoconverted tissues and, vice versa, red, photoconverted immune cells in green non-photoconverted tissues. This will be achieved with flow cytometry for bulk analysis, but also with immunofluorescence microscopy to look at topography of immune cell distribution. FACS analysis will also allow us to evaluate the activation and functional status of migrated versus non-migrated cells in irradiated and unirradiated tissue zones. Well-established subcutaneous flank (MOC1, MOC2, mEER, SCC7) and orthotopic lip (mEER) tumour models (all syngeneic to Kaede mice) will be used to understand the effects of RT/CRT and drug combinations, with focus on the effects of different: (i) dose-fractionation schedules; (ii) irradiation volumes (including or excluding loco-regional draining lymph nodes); and (iii) spatial dose-modulation (exploiting an existing CRUK-funded Multidisciplinary Award [Oelfke/Harrington] on partial tumour irradiation).

This Proposal aligns specifically with the Radiation Oncology and Biology Theme of the MRC DTP.

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Specific Aims

Evaluation of radiation dose-fractionation schedules

Initial studies will focus on conventional dose-fractionation schedules, typically 10 Gy in 5 fractions, because these are universally used during curative-intent RT/CRT in SCCHN. However, we will also test hypofractionated regimens that employ dose-per-fraction levels between 5 and 8 Gy, including the widely adopted 24 Gy in 3 fraction regimen, because they are currently being assessed in certain indications in SCCHN. We have experience of using varied dose-fractionation regimens that are tailored to individual tumour models to allow detailed investigation of specific biological questions. For example, we will deliberately use relatively less effective regimens when we wish to investigate, in detail, the additional benefits of adding therapies that augment concomitant radiation-induced cell killing (e.g. DDR inhibitors).

Effect of elective nodal irradiation on immune cell migration

For decades, it has been standard-of-care practice to include clinically-uninvolved loco-regional lymph node basins (unilaterally or bilaterally) during curative-intent RT/CRT for SCCHN, with the stated goal of eradicating nodal micrometastatic disease. In the context of trying to integrate immunotherapy approaches with RT/CRT, this practice may, in fact, be detrimental rather than beneficial. Therefore, we will study the effects of treatment of adjacent nodal areas on immune cell trafficking in the TIME and normal tissues. We have defined patterns of nodal drainage for subcutaneous flank tumours, which will allow us to study the effects of combination regimens in irradiated versus unirradiated nodal tissues. Such studies can be performed relatively simply using applied fields and appropriate lead shielding on an orthovoltage X-ray source. Studies will also be extended to an orthotopic lip model (mEER) and the adjacent draining lymph nodes in the head and neck region. For studies on the effects of irradiation of tumour +/- draining lymph nodes, we will use our small animal radiation research platform (SARRP) to deliver precise image-guided radiotherapy that deliberately includes or excludes loco-regional draining cervical lymph nodes.

Effect of spatial dose-modulation (SDM)

Since its inception, the use of RT has been based on the premise that the whole of the tumour should be irradiated at each treatment fraction – an approach called broad-beam RT. Implicit in this notion is the understanding that the TIME will also be irradiated to the full therapy dose, and this may carry beneficial and/or deleterious effects. Therefore, as outlined above for nodal irradiation, it is conceivable that negative effects of RT on the TIME may, in fact, detract from the desired anti-cancer efficacy of RT/CRT. As an alternative to broad-beam RT, partial tumour irradiation using SDM offers the prospect of triggering direct RT-induced tumour cell killing (and immune activation) in irradiated areas while preserving intact elements of the TIME (and, inevitably, tumour cells) within unirradiated zones. Differential effects of broad-beam and SDM RT on immune cell trafficking and functionality will be determined in tumour-bearing Kaede mice.

Literature references

[1] McLaughlin M, Patin EC, Pedersen M, Wilkins A, Dillon MT, Melcher AA, Harrington KJ. Inflammatory microenvironment remodelling by tumour cells after radiotherapy. Nat Rev Cancer. 2020; 20(4): 203-217.

[2] Nenclares P, Rullan A, Tam K, Dunn LA, St John M, Harrington KJ. Introducing Checkpoint Inhibitors Into the Curative Setting of Head and Neck Cancers: Lessons Learned, Future Considerations. Am Soc Clin Oncol Educ Book. 2022; 42: 1-16.

[3] Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, Haddad R, Rordorf T, Kiyota N, Tahara M, Monga M, Lynch M, Geese WJ, Kopit J, Shaw JW, Gillison ML. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016; 375(19): 1856-1867.

[4] Cohen EEW, Soulières D, Le Tourneau C, Dinis J, Licitra L, Ahn MJ, Soria A, Machiels JP, Mach N, Mehra R, Burtness B, Zhang P, Cheng J, Swaby RF, Harrington KJ; KEYNOTE-040 investigators. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet 2019; 393(10167): 156-167.

[5] Burtness B, Harrington KJ, Greil R, Soulières D, Tahara M, de Castro G Jr, Psyrri A, Basté N, Neupane P, Bratland Å, Fuereder T, Hughes BGM, Mesía R, Ngamphaiboon N, Rordorf T, Wan Ishak WZ, Hong RL, González Mendoza R, Roy A, Zhang Y, Gumuscu B, Cheng JD, Jin F, Rischin D; KEYNOTE-048 Investigators. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet 2019; 394(10212): 1915-1928.

[6] Lee NY, Ferris RL, Psyrri A, Haddad RI, Tahara M, Bourhis J, Harrington K, Chang PM, Lin JC, Razaq MA, Teixeira MM, Lövey J, Chamois J, Rueda A, Hu C, Dunn LA, Dvorkin MV, De Beukelaer S, Pavlov D, Thurm H, Cohen E. 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): 450-462.

[7] Mackley EC, Houston S, Marriott CL, Halford EE, Lucas B, Cerovic V, Filbey KJ, Maizels RM, Hepworth MR, Sonnenberg GF, Milling S, Withers DR. CCR7-dependent trafficking of RORγ^{+} ILCs creates a unique microenvironment within mucosal draining lymph nodes. Nat Commun. 2015; 6: 5862.

[8] Li Z, Tuong ZK, Dean I, Willis C, Gaspal F, Fiancette R, Idris S, Kennedy B, Ferdinand JR, Peñalver A, Cabantous M, Murtuza Baker S, Fry JW, Carlesso G, Hammond SA, Dovedi SJ, Hepworth MR, Clatworthy MR, Withers DR. In vivo labeling reveals continuous trafficking of TCF-1+ T cells between tumor and lymphoid tissue. J Exp Med. 2022; 219(6): e20210749.

[9] Dewan MZ, Galloway AE, Kawashima N, Dewyngaert JK, Babb JS, Formenti SC, Demaria S. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res. 2009; 15(17): 5379-88.

[10] 10. Steel H, Brüningk SC, Box C, Oelfke U, Bartzsch SH. Quantification of Differential Response of Tumour and Normal Cells to Microbeam Radiation in the Absence of FLASH Effects. Cancers (Basel). 2021; 13(13): 3238.

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 (ideally with component of immunology)

Intended learning outcomes:	• Understanding of radiobiology of tumour/normal tissue irradiation • Understanding of radiation-drug (DNA damage response inhibitors/immunotherapy) combination therapies • Expertise in flow cytometry/immunofluorescence/immunohistochemical analysis of tumours • Expertise in RNAseq analysis (bulk/single-cell) of irradiated/unirradiated tumours and normal tissues • Understanding of biology of broad-beam versus spatial dose-modulated irradiation of tumours

Advertising details

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