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:	Investigating how myeloid populations may modify radiotherapy/immunotherapy responses in muscle-invasive bladder cancer.

Supervisory team

Primary Supervisor:	Anna Wilkins
Associate Supervisor(s):	Anguraj Sadandam, Nick James (IRS Partner), Maggie Cheang
Secondary Supervisor:	Alan Melcher

Divisional affiliation

Primary Division:	Radiotherapy and Imaging
Primary Team:	Stromal Radiobiology Group (Prostate and Bladder Cancer Research Team)
Site:	Sutton

Project background

Muscle-invasive bladder cancer patients undergoing treatment with curative intent have 5-year survival rates of approximately 50%, (Hall et al., 2022) meaning there is a crucial unmet need for novel therapeutic combinations. Radiotherapy is an important bladder-sparing treatment in this context and the addition of immunotherapy has considerable potential to improve outcomes (Nagumo et al., 2022). The majority of patients with bladder cancer fail to respond to immunotherapy (Lopez-Beltran et al., 2021). Different components of the bladder tumour microenvironment (TME) are known to limit therapeutic responses; both myeloid cell populations (Wang et al., 2021) and cancer-associated fibroblasts (Mariathasan et al., 2018, Wang et al., 2018) can suppress effector T cell responses in this context. Myeloid populations are also known to drive inferior responses to radiotherapy across tumour types, (Liang et al., 2017) yet we lack a specific understanding of this in bladder cancer (Yu et al., 2022). We hypothesize that multimodal evaluation of myeloid populations, alongside wider components of the bladder TME, could elucidate how these populations contribute to radiotherapy/immunotherapy

responses, thus identifying novel therapeutic targets and prognostic and predictive biomarkers to utilize in the clinic.

We have a substantial number of formalin-fixed paraffin-embedded (FFPE) tissue samples from various clinical trials in bladder cancer. These include the 1000 patient Bladder Cancer Prognosis programme and the landmark BC2001 trial led by the ICR Clinical Trials and Statistics Unit (ICR-CTSU)(both with mature survival outcomes),(Zeegers et al., 2010, James et al., 2012) as well as the ongoing radio-immunotherapy RADIO (*ISRCTN: 43698103)* and PLUMMB trials (the latter recently completed accrual). A number of these samples have undergone detailed "multi-omic" profiling including multiplex immunofluorescence (mIF) to profile elements of the TME, incorporating lymphocyte, myeloid and cancer-associated fibroblast populations. Using flow cytometry panels from longitudinal blood and urine samples from patients in these collections, we have investigated lymphocytes and now plan to assess myeloid involvement. We have established an immunogenomics pipeline from tissue, blood and urine samples to obtain transcriptomic and whole exome sequencing data on these patients. The project will build on the established pipelines and offer opportunities for new analyses based around myeloid cell populations in this well-annotated sample set. There is scope to validate findings in the RAIDER trial of bladder radiotherapy, also led by the ICR-CTSU.

Project aims

- Assess existing multicolour immunofluorescence (mIF) data relating to myeloid populations and their association with bladder cancer outcomes (in clinical trials outlined above)
- Extend mIF evaluation to include myeloid derived suppressor cells (MDSCs) of bladder tumours in clinical trials and integrate findings with (1)
- Integrate findings from 1. and 2. with other constituents of the tumour microenvironment including cancer-associated fibroblasts, natural killer cells and different lymphocyte populations
- Integrate findings from 1, 2 and 3 with transcriptomic/exomic data including spatial transcriptomic approaches.
- Evaluate myeloid populations in longitudinal blood and urine samples to evaluate changes before, during and after radiotherapy +/- immunotherapy.

Research proposal

The overarching hypothesis to address in this project is that myeloid populations in the bladder TME contribute to radiotherapy/immunotherapy responses. A comprehensive understanding of this, especially in the context of the wider tumour microenvironment, including different CAF subtypes, will enable identification of novel therapeutic targets and prognostic and predictive biomarkers to use in the clinic. Radiotherapy and immunotherapy are increasingly being combined in a trial setting to treat muscle-invasive bladder cancer, as outlined above. A robust understanding of how the bladder cancer TME impacts radiotherapy response is important for the success of these clinical trials.

In order to address the above hypothesis, multimodal evaluation of tissues samples in retrospective and prospective clinical trials will be conducted followed by integration of the emerging biological datasets. The key approaches to be used include:

1. Multicolour immunofluorescence (IF): the group has established a pipeline of three IF panels using the VECTRA Polaris system, in collaboration with the Integrated Pathology Unit (IPU) based at the Royal Marsden Hospital/ICR. The panels focus on 1. Lymphocytes, 2. Innate immune cell populations including a limited number of myeloid populations and 3. Cancer-associated fibroblasts. Analysis to date has focussed on cancer-associated fibroblasts and lymphocytes meaning there is exciting potential to explore myeloid cell biology and integrate this with other cell populations. The IPU and our team have established computational pipelines that can harmonise data from serial sections into a single dataset meaning that all 3 panels can be evaluated in combination. This enables a detailed interrogation of wider spatial relationships between myeloid populations and other components of the tumour microenvironment and assessment of how such

relationships may impact therapy responses. The VECTRA Polaris system permits digital image acquisition of large tumour areas enabling detailed evaluation of intra-tumoural heterogeneity on each slide. To further develop this, the PhD student will develop a panel focussed on myeloid-derived suppressor cells (MDSCs) which are known to contribute to poor radiotherapy outcomes, potentially via interaction with cancer-associated fibroblasts.

2. Bulk transcriptomics: we have collated bulk transcriptomic data for over 300 tumours from patients with MIBC recruited to trials of radical radiotherapy and are currently expanding this dataset. This dataset will enable deconvolution of cell populations which can be combined with the IF data obtained above.

3. Spatial transcriptomics: in a selected cohort of patients, spatial transcriptomics centred on NanoString Digital Spatial Profiling and other FFPE-compatible approaches will be used to interrogate spatial relationships and signalling between myeloid populations and other TME constituents in greater detail.

4. Flow cytometry: We have collected longitudinal blood and urine samples from patients recruited to the RADIO and PLUMMB trials (which combine radiotherapy and immunotherapy) before, during and after radiotherapy. TCR-Seq and flow cytometry using these samples has focussed in lymphocyte populations. Intriguingly, early findings show the emergence of new TCR clones in the blood prior to macroscopic disease recurrence. A better understanding of longitudinal changes in innate immune cells, including myeloid populations will provide further insight to how different radiation-induced immune responses associate with complete response versus disease recurrence.

5. Multimodal modelling: the PhD student will work with computational biology and bioinformatics colleagues from the Integrated Pathology Unit, the Centre for Translational Immunotherapy and the Centre for Evolution and Cancer to construct multimodal models using the datasets obtained above plus exome sequencing data. This will maximise the opportunity to establish how signalling between myeloid populations, cancer-associated fibroblasts and other cells in the TME impact the response to radiotherapy +/- immunotherapy in bladder tumours with different mutational profiles.

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, computational biology or bioinformatics

Intended learning outcomes:

- Optimising, running and analysing outputs from multiplex immunofluorescence
- Analysis of bulk transcriptomic data
- Spatial transcriptomics methodology, including analysis of such data
- Performance and analysis of flow cytometry using blood and urine samples
- Multimodal modelling strategies

Potential Publications from this project:

1. Manuscript of multimodal models describing the association between specific TME networks centred on myeloid cell signalling and linked with outcomes following radiotherapy +/- immunotherapy (these outputs may form more than 1 manuscript).

2. Methodology manuscript of multimodal modelling

Advertising details

Project suitable for a student with a background in:

☒ Biological Sciences

☐ Physics or Engineering

☐ Chemistry

☒ Maths, Statistics or Epidemiology

☒ Computer Science