PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	The role of the immune response in tumour chromosome instability, cancer evolution and sensitivity to radiotherapy

Supervisory team

Primary Supervisor:	Alan Melcher
Associate Supervisor(s):	Jyoti Choudhary, Ben O'Leary
Secondary Supervisor:	Jessica Downs

Divisional affiliation

Primary Division:	Radiotherapy and Imaging
Primary Team:	Translational Immunotherapy
Site:	Chelsea

Project background

Chromosomal instability (CIN) is a hallmark of cancer, and associated with poor prognosis, metastasis and resistance to therapy [1, 2]. One major contributor to CIN in cancer is defects in the chromatin remodelling complex SWI/SNF [3]. Whilst CIN generates genomic heterogeneity that drives natural selection and evolution of cancer, it also impacts on interactions between the tumour and the immune system, which are of major relevance in the current therapeutic era of immunotherapy (IO). In innate immunity, CIN can promote inflammatory signalling by introducing dsDNA into the cytoplasm, which engages the cGAS-STING anti-viral pathway, and potentially an anti-tumour interferon response. Alternatively, karyotypic abnormalities can drive adaptive immune evasion via reduced antigen presentation; for example, copy number loss of heterozygosity in the human-leukocyte antigen (HLA) occurs in nearly 40% of non-small cell lung cancers [4]. Conversely, CIN and genomic alterations have the potential to enhance tumour immunogenicity via the generation of neoantigens, which are key targets of the cytotoxic T cells (CTL) central to the success of clinical immunotherapy. Hence the effects of CIN on promoting or suppressing anti-tumour immunity are complex, contradictory and currently unclear.

The effects of radiotherapy (RT), which can itself induce CIN, on cancer immunology are similarly contrary. Although RT has the potential to activate an anti-tumour immune response, it also has immunosuppressive effects. Therefore, improving strategies to effectively combine RT with immunotherapy, in the context of tumours with different CIN, requires a deeper understanding of the biology of the immune response to treatment, covering the effects of RT on not only the tumour cell itself, but also the tumour immune microenvironment (TIME). In this project the candidate will use murine

models of high versus low CIN to explore the immunology of CIN and RT, with a particular focus on the dynamics of the T cell response and the antigens CTL target.

Project aims

- To evaluate the tumour immune microenvironment (TIME) in tumours with different levels of chromosome instability
- To test the sensitivity of tumours with different levels of chromosome instability to radiotherapy, and characterise RT-induced changes in the tumour microenvironment
- To investigate the CIN and TIME of tumours which recur after RT in mice, and align these findings to clinically available datasets of recurrent cancer in patients following RT
- To study the dynamics of the interaction between T cells and irradiated tumours in these systems using Tocky ('Timer of cell kinetics and activity') mice.
- To develop optimised RT/immunotherapy combination strategies for tumours with differing levels of CIN

Research proposal

This proposal specifically aligns with the Genome Stability and DNA Damage Response, and the Radiation Oncology and Biology, Themes of the MRC DTP. It is also relevant to cancer immunotherapy, as specified in this call.

We have established murine cell lines with high or low levels of CIN, determined by the presence or absence of various subunits of the SWI/SNF chromatin remodelling complex within isogenic cell lines. In complementary work ongoing in the laboratories of Profs Downs and Choudhary, a number of isogenic human and mouse models of melanoma and colorectal cancer, with SWI/SNF complex subunits including SMARCA4, BRG1 and PBRM1 knocked out, have been generated and characterised. Current and planned experiments with these cell lines are focused on their proteogenomics and immunopeptidomics, but the in-depth functional immunology of these tumours has not yet been investigated. We will initially focus on the mouse B16 melanoma model in which PBRM1 has been knocked out, which we have already shown to be less tumourigenic when seeded in vivo than the PBRM1 proficient parental cell line (Lane et al, submitted). Mechanistically, knockout of PBRM1 compromises the integrity of the centromere with profound consequences for genome integrity, so that parental and PBRM1 KO lines are ideal as models of low and high CIN respectively, for study of their interaction with the immune system.

First, the immune micronenvironment of B16 parental and PBRM1 KO tumours will be investigated as they grow in immunocompetent C57BL/6 mice. Since PBRM1 KO tumours grow more slowly, our hypothesis is that their higher CIN levels lead to a greater (though still not controlling) immune response to the growing tumour, due to mechanisms potentially including tumour mutational burden (TMB), and the recently described ability of the CIN tumour cell status to impact on the TIME [5]. The TIME of tumours of different size will be characterized using a range of techniques established in our laboratory, including multiplex immunofluorence, FACS, RNAseq (including single cell), TCRseq and digital spatial profiling. This will allow us to explore the impact of the PBRM1 subunit of the PBAF1 complex on tumour growth in the whole organism, addressing both tumour cell intrinsic factors, the immune microenvironment, and their interaction.

Next, the response of B16 +/- PBRM1 KO tumours to radiotherapy, at a range of doses and fractionations, will be tested in vitro and in vivo. We will also model recurrent tumours and their TIME, in mice given subcurative RT treatment schedules. This will allow us to address the mechanisms of tumour evolution and immune escape after radiotherapy. This is a clinically important question, and we will align

mouse findings with available comparative patient datasets, derived from ongoing clinical trials, collecting matched primary and recurrent tumours treated with RT (led by Dr Ben O'Leary).

To delve deeper into the T cell response to CIN high/low tumours +/- RT we will make use of the Tocky model [6], which elucidates the dynamics of T-cell activation, in collaboration with Dr Masahiro Ono from Imperial College. Tocky is already established in our laboratory and used, for example, to track the dynamics of the T cell response to oncolytic virotherapy [7]. In the Tocky model we will use, the promoter of Nr4a3, a gene faithfully induced by T cell receptor (TCR) stimulation, is used to demonstrate the temporal changes in effector cells undergoing T cell TCR activation. Using a fluorescent protein linked to the Nr4a3 promoter which changes colour with time from blue to red, Tocky allows us to determine the dynamic changes of Nr4a3-positive T cells (both CD8 and CD4) in tumours in vivo, between 'new' (blue), 'persistent' (blue/red) and 'arrested' (red) phases. Our recent data, which combined single cell TCRseq and RNAseq to track the dynamics of individual reactive T cell clones in response to different immunotherapies, suggests that the kinetics of T cell engagement for optimal benefit are distinct, with transient rather than persistent TCR engagement best for therapy. This project will address whether the same findings in terms of 'good' versus 'bad' TCR engagement and downstream T cell activation, apply in the context of RT for tumours (responding and recurrent), with differing levels of CIN.

Finally, and as guided by the mechanisms revealed in Aims 1-3, we will develop optimal combination RT/IO treatments for tumours with different CIN status.

Taken together, the findings from this project will explore the mechanisms underlying the interaction between cancer and the immune system in tumours with different CIN status. By performing detailed mechanistic studies, including addressing the optimal dynamics of TCR engagement with tumour antigens, in the context of effective and ineffective (tumour recurrence) RT, we aim to design novel, more effective RT/IO combination strategies for tumours of differing CIN status, informed by biological understanding, to take forward into clinical testing.

Literature references

1. Bakhoum, S.F. and L.C. Cantley, *The Multifaceted Role of Chromosomal Instability in Cancer and Its Microenvironment.* Cell, 2018. **174**(6): p. 1347-1360.
2. Kuang, X. and J. Li, *Chromosome instability and aneuploidy as context-dependent activators or inhibitors of antitumor immunity.* Front Immunol, 2022. **13**: p. 895961.
3. Nithya Krishnamurthy, S.K., Scott Lippman, Razelle Kurzrock, *Chromatin remodeling (SWI/SNF) complexes, cancer, and response to immunotherapy.* Journal for ImmunoTherapy of Cancer, 2022. **10**.
4. McGranahan, N., et al., *Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution.* Cell, 2017. **171**(6): p. 1259-1271.e11.
5. Li, J., et al., *Non-cell-autonomous cancer progression from chromosomal instability.* Nature, 2023. **620**(7976): p. 1080-1088.
6. Bending, D., et al., *A timer for analyzing temporally dynamic changes in transcription during differentiation in vivo.* J Cell Biol, 2018. **217**(8): p. 2931-2950.
7. Bozhanova, G., et al., *CD4 T cell dynamics shape the immune response to combination oncolytic herpes virus and BRAF inhibitor therapy for melanoma.* J Immunother Cancer, 2022. **10**(3).

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:	Cancer immunology Immunogenomics Bioinformatics Clinical multiomics analysis Translation 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