PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	Imaging to characterise the biomechanical phenotype of cancer and its association with the immune landscape and radiation response.

Supervisory team

Primary Supervisor:	Emma Harris
Associate Supervisor(s):	Anna Wilkins; John Civale
Secondary Supervisor:	Simon Robinson

Divisional affiliation

Primary Division:	Radiotherapy and Imaging
Primary Group:	Imaging for therapy adaptation
Site:	Sutton

Project background

This PhD will develop novel elastographic imaging techniques and exploit them to test the hypothesis that non-invasive mapping of the biomechanical properties of tumours pre- and post-radiation can inform on the distribution of immune infiltrates in stromal-rich cancers.

Stromal-rich tumours can be difficult to treat. They are typically characterised by greater collagen density and cross-linking that increases tumour stiffness, which impedes the transport of drugs and oxygen[1] reducing the effectiveness of cancer therapies. Cancer cells promote stiffness via cells that manufacture extracellular matrix (ECM), including cancer-associated fibroblasts (CAFs). This is now believed to be an immune system escape mechanism, which effectively excludes immune cells from the tumour, hindering response to therapies that activate the immune system, for example immunotherapy and radiation therapy[2,3]. We have strong evidence that CAFs are associated with poor outcomes in rectal cancer and that the stromal-rich bladder cancer subtypes exhibit an association of CAFs and greater radiation and chemoradiation resistance. In models of melanoma, immune cell (CD8+) infiltration was restricted to the tumour boundaries post-radiation. Intriguingly, these models showed marked peritumoural increase in fibrillar collagen in a pattern which mimicked immune cell infiltration, suggestive of CAF-induced fibrotic response, only one week after irradiation.

Interventions that modulate the tumour microenvironment (TME) to reduce tumour stiffness and increase the penetration of drugs are under intense investigation but little is known about how they could complement radiotherapy by preventing tumour escape from a radiation-induced immune response. Neither do we understand the dynamic interaction between immune cell infiltration and radiation-induced fibrosis, and radiotherapy dose fractionation/scheduling. We need to understand the interplay between the biomechanical properties of tumours, immune cell infiltration and radiation-induced fibrosis if we are to deliver optimal TME modulation plus radiotherapy approaches to patients.

Preclinical elastographic imaging techniques, deployed using ultrasound or magnetic resonance imaging (MRI) and developed at the ICR show great promise for accurately mapping and quantifying tumour biomechanics. They offer non-invasive interrogation of the tumour, allowing longitudinal study of preclinical tumours before and after therapy. Further, using these non-invasive methods which are employed clinically, will help to translate our findings directly into the clinic to optimise therapy for patients by informing on baseline tumour stiffness and therapy response, allowing clinicians to adapt administration of TME modulators and radiotherapy scheduling.

Project aims

1. Establish spatial correlations between the biomechanical properties of bladder cancer models measured by ultrasound and MR elastography, and collagen density and structure, CAF distribution, and the infiltration of immune cells, using digital pathology.
2. To characterise the changes in the distribution and number of immune infiltrates over a course of radiotherapy, and the onset of radiation-induced fibrosis.
3. To exploit the different strengths of MRE and VSWE and how they might provide complementary information to improve the accuracy and speed with which we can map the elastic and viscous moduli using both techniques.
4. To devise novel methods of mapping the viscoelastic properties of orthotopic bladder cancer models using both VSWE and MRE.

Research proposal

The biomechanical properties of tissues can be determined via the detection and characterisation of shear waves as they propagate through tissue. Elastic or storage modulus, G', (which quantifies tissue stiffness) is directly related to shear-wave speed (SWS), and viscous or loss modulus, G'', is related to the variation in SWS with shear wave frequency (SW phase velocity dispersion). The Harris lab has developed preclinical Vibrational SWE (VSWE) which uses tuneable external mechanical sources for SW generation[5]. Our novel system mapped tumour $G^{'}$ which was spatially correlated to tumour integrity and was sensitive to TME modulation in subcutaneous models of breast and brain cancer[6]. Magnetic resonance elastography (MRE) is an emerging technique that uses MR imaging of shear waves also generated using an external mechanical source to map G' and G''. The Robinson lab validated MRE generated maps of G' and $G^{''}$ against histopathology in a range of subcutaneous, orthotopic and GEM models and shown that the contribution of collagen and collagen structure is a primary determinant of the biomechanical phenotype[7]. Further, Robinson demonstrated both increased G' and collagen density in models of triple-negative breast cancer post radiotherapy[8].

Both VSWE and MRE are emerging techniques with neither being considered superior and current preclinical implementations have strengths and weaknesses. VSWE is faster and greater access to the tumour allows optimisation of the frequency and placement of mechanical sources, one drawback is that measures of G'' require multiple frequencies. MRE has superior spatial resolution and G'' can be measured using a single vibrational frequency, although low signal to noise ratio increases scan times, which limits preclinical acquisition to a small central volume of the tumour (~ 3mm in thickness).

<u>Technical challenges we will address in this project:</u>

- We will develop methods of using multifrequency vibrations to acquire measures of $G^{''}$ for VSWE and investigate their benefit for increased SNR and therefore reduced scan time in MRE.
- Neither technique is optimised for orthotopic models of bladder cancer which poses challenges in terms of shear wave transmission through bladder (fluid). Evaluation of mechanical source position and frequencies will be initially performed using VSWE and the results used to optimise mechanical source/animal positioning for MRE.
- Accurate spatial registration of *in vivo* images with histology remains a challenge. Referencing to high contrast anatomical T_2-weighted MR images and registration of ultrasound images will help mitigate this problem. For all endpoint images an intermediate step is carried out in which the *ex vivo* tumour is set in gelatine and imaged immediately after in vivo imaging and tumour extraction. Using this approach tumour orientation and scan planes can be more accurately maintained across modalities.

Clinical relevance:

Increasing evidence indicates that tumour stiffness and increased fibroblast proportion in tumours is associated with an aggressive tumour phenotype and poor response to radiotherapy and systemic agents[9]. We lack the ability to identify "stiffer" fibroblast-enriched tumours which are likely to require treatment intensification and there is no detailed understanding of how longitudinal biomechanical changes in the tumour microenvironment following radiation and/or systemic agents impact the immune response and ultimate treatment efficacy. Non-invasive imaging has the potential to provide solutions to both challenges and can be readily translated to the clinic. Further, novel non-invasive imaging readouts could assist in the optimal design of clinical trials of stromal-targeting agents alongside radiation.

Proposed project/training plan:

Months 1 – 6: Taught courses in Ultrasound, MRI, Bladder Cancer, Radiotherapy, Radiobiology, Image Processing; Development of expertise in preclinical SWE and MRE; Training in animal handling (and certification); Small animal irradiation training; multicolour immunofluorescence training; Literature review.

Months 7 – 12: Develop novel methods and protocols to implement multifrequency VSWE, test multifrequency MRE and acquire sequential VSWE and MRE *in vivo* data.

Months 13 – 22*: In vivo* and *ex vivo* VSWE and MRE to characterise the spatial heterogeneity of elastic and viscous moduli and establish spatial correspondence of moduli with the distribution of collagen, CAFs and other immune cells.

Months 23 – 30: Establish spatial and temporal correlations between changes in moduli on *in vivo* images, immune cell & CAF population/distribution, the structure and density of fibrillar collagen in response to radiation +/- TME modulation in subcutaneous models of bladder cancer.

Months 31 - 33: Novel methods and optimisation for imaging orthotopic bladder models.

Months 34 - 43: Establish spatial and temporal correlations between changes in moduli on *in vivo* images, immune cell population/distribution, the structure and density of fibrillar collagen in response to radiation +/- TME modulation in orthotopic models of bladder cancer.

Months: 44 - 48: Thesis writing.

Literature references

[1] Dewhirst, M. W., & Secomb, T. W. (2017). Transport of drugs from blood vessels to tumour tissue. Nature Reviews Cancer, 17(12), 738-750.

[2] Nicolas-Boluda, A., Vaquero, J., Vimeux, L., Guilbert, T., Barrin, S., Kantari-Mimoun, C., ... & Donnadieu, E. (2021). Tumor stiffening reversion through collagen crosslinking inhibition improves T cell migration and anti-PD-1 treatment. Elife, 10, e58688.

[3] Mariathasan, S., Turley, S. J., Nickles, D., Castiglioni, A., Yuen, K., Wang, Y., ... & Powles, T. (2018). TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature, 554(7693), 544-548.

[4] Mohammadi, H., & Sahai, E. (2018). Mechanisms and impact of altered tumour mechanics. Nature cell biology, 20(7), 766-774.

[5] Civale, J., Parasaram, V., Bamber, J. C., & Harris, E. J. (2022). High frequency ultrasound vibrational shear wave elastography for preclinical research. Physics in Medicine & Biology, 67(24), 245005.

[6] Parasaram, V., Civale, J., Bamber, J. C., Robinson, S. P., Jamin, Y., & Harris, E. (2022). Preclinical Three-Dimensional Vibrational Shear Wave Elastography for Mapping of Tumour Biomechanical Properties In Vivo. Cancers, 14(19), 4832.

[7] Li, J., Zormpas-Petridis, K., Boult, J. K., Reeves, E. L., Heindl, A., Vinci, M., ... & Robinson, S. P. (2019). Investigating the contribution of collagen to the tumor biomechanical phenotype with noninvasive magnetic resonance elastography. Cancer Research, 79(22), 5874-5883.

[8] Lesbats, C., Roy, U., Reeves, E. L., Boult, J. K., Jamin, Y., Cummings, C., ... & Robinson, S. P. Early tumour response to radiotherapy assessed by magnetic resonance elastography.

[9] Wang, L., Saci, A., Szabo, P. M., Chasalow, S. D., Castillo-Martin, M., Domingo-Domenech, J., ... & Galsky, M. D. (2018). EMT-and stroma-related gene expression and resistance to PD-1 blockade in urothelial cancer. Nature communications, 9(1), 3503.

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:

- Secure a Home Office licence, become a responsible licensee and become proficient in the propagation of subcutaneous and orthotopic bladder tumour models in vivo.
- Development and application of non-invasive, clinically translatable US and MRI modalities for the preclinical assessment of tumours and therapeutic response in vivo.
- Establish and implement targeted irradiation protocols using the SARRP for the treatment of subcutaneous and orthotopic bladder cancer models in vivo.
- Gain an appreciation of clinical imaging approaches for the assessment of bladder cancer.
- Develop strong and confident communication skills through regular presentations of their work at lab meetings, departmental seminars and report writing.
- Competency in the maintenance of tissue cultures of bladder cancer cell lines
- Training will be provided within a stimulating research environment in which many projects are of a multi-disciplinary or collaborative nature, providing an insight into a wide range of imaging techniques and expertise.

Advertising details

Project suitable for a student with a background in:

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