The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL : iCASE SCHEME

GUIDANCE	
PROJECT DETAILS	
Project Title:	Evaluation of cancer-associated fibroblasts (CAFs) to combat therapy resistance in bladder cancer.
SUPERVISORY TEAM	
Primary Supervisor(s):	Anna Wilkins
Associate Supervisor(s):	Nick James, Katharina von Loga, Alan Melcher, Andrew Furness Nick James has extensive experience of industrial collaborations. This is firstly as Chief Investigator of a number of clinical trials sponsored by pharmaceutical companies. Secondly, he has collaborated extensively with industry for translational science embedded in the STAMPEDE trial (http://www.stampedetrial.org/)
Industry supervisor:	To be identified
Secondary Supervisor:	Alan Melcher
Lead contact person for the project:	Anna Wilkins
DIVISIONAL AFFILIATION	
Primary Division:	Radiotherapy and Imaging
Primary Team:	Prostate and Bladder Cancer Research
PROJECT PROPOSAL	

SHORT ABSTRACT

Approximately 50% of locally-advanced and metastatic bladder tumours show enrichment of cancer-associated fibroblasts (CAFs) with increased Transforming Growth Factor–beta (TGFβ) signalling and extracellular matrix (ECM) remodelling. Survival outcomes and therapy responses are particularly poor in these “immune-excluded” tumours. The role of CAFs in radiotherapy resistance is poorly understood and likely to be clinically important. In this proposal, immunogenomic and immune phenotyping approaches will characterise CAF-enrichment, the ECM and the wider immune microenvironment in large retrospective tissue collections and a prospective randomised trial using

immune checkpoint blockade with standard chemo-radiation. Novel non-invasive biomarkers including urinary TCR-Seq will also be evaluated.

BACKGROUND TO THE PROJECT

Radical radiotherapy is commonly used in the curative treatment of bladder cancer but long term disease control rates remain sub-optimal; in muscle-invasive bladder cancer overall survival rates five years after radical chemoradiotherapy are less than 50% (1). There is therefore an urgent need to identify and subsequently target aspects of bladder cancer biology that drive radioresistance to improve outcomes. Approximately 50% of locally-advanced and metastatic bladder tumours show enrichment of cancer-associated fibroblasts (CAFs), with increased Transforming Growth Factor–beta (TGFβ) signalling and extracellular matrix (ECM) remodelling, which result in an "immune-excluded" tumour phenotype (2). In both surgically-treated cohorts, and in metastatic disease, CAF-enriched tumours are associated with inferior overall survival outcomes (2, 3). However, the impact of CAF-enrichment on survival outcomes following radical radiotherapy is not known.

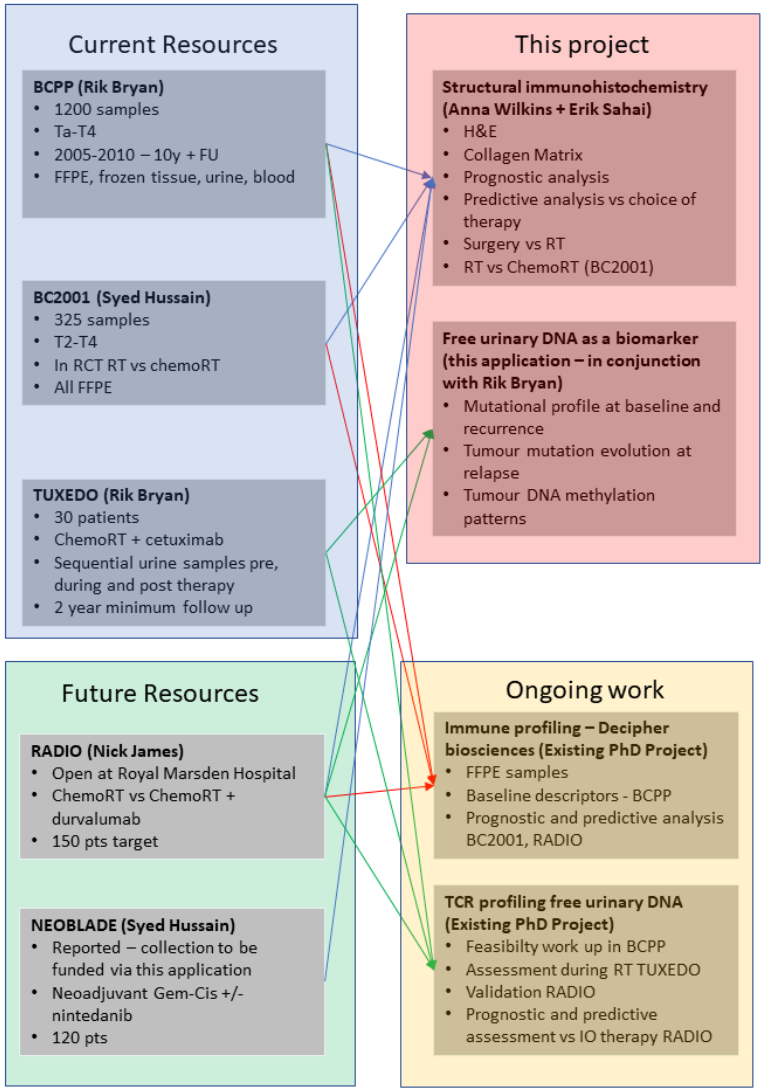
The central hypothesis for this project is that CAF enrichment of bladder tumours is associated with inferior survival outcomes following radiotherapy. In a range of pre-clinical models, CAFs contribute to radioresistance via both immunological and non-immunological mechanisms. However, the contribution of CAFs to radioresistance in a human context is very poorly understood. Both early stage and metastatic bladder cancer are treated with immunomodulatory agents (BCG and inhibition of the PD-1/PD-L1 axis, respectively), which suggests that immune mechanisms of radioresistance may be important in bladder cancer. Targeting of CAFs, alongside radical radiotherapy, has the potential to reverse CAF-induced immune suppression and improve survival outcomes. This project will also address the hypothesis that the impact of CAFs on the immune response to radiotherapy can be monitored using novel non-invasive biomarkers including urinary TCR-Seq and urinary free tumour DNA (4).

PROJECT AIMS

RESEARCH PROPOSAL

The overriding hypothesis for this project is that CAF enrichment is associated with inferior survival outcomes following radiotherapy for bladder cancer. A summary outline of the PhD proposal, including relevant patient cohorts, is provided in figure 1.

Figure 1: PhD project proposal outline



Analysis of retrospective cohorts:

To address the above hypothesis, initially large retrospective tissue collections with mature survival outcomes from the Bladder Cancer Prognosis Programme (BCCP) (n=1200) (5), will be studied in collaboration with **Richard Bryan, Douglas Ward (both University of Birmingham), Syed Hussain (Sheffield) and Emma Hall (Institute for Cancer Research)**. The successful candidate will receive training in immune phenotyping with image analysis, transcriptional profiling and quantitative analysis of the ECM, including the use of TWOMBLI (The Workflow of Matrix Biology Informatics) software (6). This will enable evaluation of how CAF-enrichment changes with increasing tumour stage and correlative analyses between CAF-enrichment, features of the ECM, immune cells in the tumour microenvironment and TCR-Seq in the urinary DNA. The BCPP sample collection includes linked snap frozen tumour samples and urine samples together with pelleted cell debris from the same samples. The group has established that the tumour mutational spectrum is accurately represented in the urine and pellet samples (though

with quantitative variations) (4, 7). We hypothesise that the urine-derived lymphocyte DNA will be detectable in stored urine and urine pellet and that changes in the TCR will be reflected in this DNA.

The immune phenotyping and immunogenomic approaches described above will also be applied to the BC2001 tissue collection (n=325) to answer the question of whether CAF-enrichment in tumours leads to inferior survival outcomes after radiotherapy alone and chemo-radiation. BC2001 was run by the ICR-CTSU and this practice-changing randomised trial has excellent clinical annotation and mature survival outcomes. In addition, profiling of tissue collected from NEOBLADE (n=120) will enable specific study of the impact of the CAF-targeting agent nintedanib on the bladder immune microenvironment in association with survival outcomes. The NEOBLADE trial demonstrated improved failure free and overall survival in the nintedanib arm (8).

Analysis of prospective cohorts

Translational profiling of the prospective RADIO study (Chief Investigator: Nick James) of chemoradiation +/- neo-adjuvant, concomitant and adjuvant use of the PD-L1 inhibitor Durvalumab will build on the above findings using the same collaborative approach. RADIO aims to recruit 150 patients. Here, comprehensive molecular profiling will include longitudinal evaluation of the evolving immune landscape using non-invasive TCR-Seq and ctDNA biomarkers in blood and urinary TCR-Seq and urinary free tumour DNA, which will be integrated with baseline tumour biology, including CAF and ECM patterns. In collaboration with **Shaista Hafeez**'s team, functional MRI imaging will be used to derive imaging biomarkers of CAF-enrichment or desmoplasia. Such imaging biomarkers have the potential to aid treatment stratification based on CAF infiltration and collaboration with industry will provide expertise and insight into how to maximise the clinical potential of such imaging biomarkers. The emerging multidimensional datasets will be combined in a "Big Data" approach using Artificial Intelligence approaches (in collaboration with **Bissan Al-Lazikani**) enabling a detailed insight to how CAFs contribute to inferior radiotherapy outcomes in bladder cancer.

The work in this proposal will be carried out in close collaboration with a second recently-appointed PhD student who is due to start in October 2020 (primary supervisor Nick James). Their complementary project addresses separate immunological hypotheses using the same clinical cohorts and trials. This second PhD will help maximise scientific productivity and biological insights from these extensive retrospective and prospective clinical datasets. In addition, ongoing work with our Birmingham collaborators is evaluating the use of free urinary tumour DNA as a diagnostic tool and as a method of assessing tumour prognosis and response to treatment. Again this work will be proceeding in parallel and is complementary to the proposed project.

Benefits of industrial collaboration

A particular benefit to this project is the expertise and insight that industry will provide to enable development of urinary TCR-Seq as a non-invasive biomarker for clinical use. Industrial collaboration is also likely to provide valuable insights to non-invasive MRI biomarkers of tumour desmoplasia. In addition, we anticipate that industry collaboration will enable greater access to cutting edge immunogenomic technology and bioinformatics approaches so as to maximise the scientific impact of this project. This wider exposure to cutting edge immunogenomic technology and industry approaches to the development of novel immune targets will be enhanced by a 3-6-month placement with the relevant industrial partner. In addition, the RADIO trial is an investigator initiated study funded by AstraZeneca, hence the proposed work complements the study.

Training

The successful candidate will receive training in a range of immunogenomic techniques described above. The large patient cohorts described means there is excellent statistical power for this work and attendance on formal courses in statistics and bioinformatics will be prioritised. The PhD student will then work closely with, and learn from, bioinformaticians embedded within the newly-formed Centre for Translational Immunology at the ICR and

statisticians in the ICR-CTSU. In the second half of this project, they will build on this training by learning Artificial Intelligence techniques working with the Al-Lazikani team. Alongside this project, Dr Anna Wilkins will be running a pre-clinical work program between the ICR and the Sahai lab at the Francis Crick Institute. This program is evaluating novel drug candidates to target CAFs in bladder cancer in combination with radiotherapy. The student will therefore gain an understanding of the interplay between pre-clinical and human translational science, including the importance of reverse translation.

The PhD experience will include attending bladder cancer clinics and radiotherapy planning with Dr Anna Wilkins and Prof Nick James thus enabling the candidate to learn about the management of different stages of bladder cancer. We will also arrange opportunities to observe bladder cancer surgery. The candidate will attend a Good Clinical Practice Course and will work with the bladder trials team in the running of clinical trials, including sample processing. We believe this clinical context is key to understanding how to carry out effective translational research. Finally, the opportunity to collaborate with industry will provide educational insights as to how academic and industrial partners can identify new therapeutic targets and maximise the scientific impact of human and pre-clinical translational research.

LITERATURE REFERENCES (Please use the Harvard system of referencing and provide up to 10 key references)

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4. TOGNERI, F. S., WARD, D. G., FOSTER, J. M., DEVALL, A. J., WOJTOWICZ, P., ALYAS, S., VASQUES, F. R., OUMIE, A., JAMES, N. D., CHENG, K. K., ZEEGERS, M. P., DESHMUKH, N., O'SULLIVAN, B., TANIERE, P., SPINK, K. G., MCMULLAN, D. J., GRIFFITHS, M. & BRYAN, R. T. 2016. Genomic complexity of urothelial bladder cancer revealed in urinary cfDNA. *Eur J Hum Genet,* 24**,** 1167-74.
5. ZEEGERS, M. P., BRYAN, R. T., LANGFORD, C., BILLINGHAM, L., MURRAY, P., DESHMUKH, N. S., HUSSAIN, S., JAMES, N., WALLACE, D. M. & CHENG, K. K. 2010. The West Midlands Bladder Cancer Prognosis Programme: rationale and design. *BJU Int,* 105**,** 784-8
6. WERSHOF E, BARRY D, JENKINS R, RULLAN A, WILKINS A, ROXANIS I, ANDERSON K, PARK D, BATES P, SAHAI E. A FIJI Macro for quantifying pattern in extracellular matrix http://dxdoiorg/101101/867507 2019.
7. WARD, D. G., BAXTER, L., GORDON, N. S., OTT, S., SAVAGE, R. S., BEGGS, A. D., JAMES, J. D., LICKISS, J., GREEN, S., WALLIS, Y., WEI, W., JAMES, N. D., ZEEGERS, M. P., CHENG, K. K., MATHEWS, G. M., PATEL, P.,

GRIFFITHS, M. & BRYAN, R. T. 2016. Multiplex PCR and Next Generation Sequencing for the Non-Invasive Detection of Bladder Cancer. *PLoS One,* 11**,** e0149756.

8. HUSSAIN SA, LESTER JF, JACKSON R, GORNALL M, ELLIOTT A, CRABB SJ, HUDDART RA, VASUDEV N, BIRTLE AJ, WORLDING J, JAMES ND, PARIKH O, VARELA MV, ALONZI R, LINCH MD, POWLES T, JONES RJ. 2020. Phase II randomized placebo-controlled neoadjuvant trial of nintedanib or placebo with gemcitabine and cisplatin in locally advanced muscle invasive bladder cancer (NEO-BLADE). Journal of Clinical Oncology 38:438-438.

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: e.g. BSc or equivalent in specific subject area(s)	B.Sc. in relevant biological or mathematical discipline
Intended learning outcomes (including those arising from the industry collaboration):	• Extensive knowledge of immunobiology of human bladder cancer, cancer-associated fibroblasts and the extracellular matrix • Multiplex staining of archive tissue samples • Quantitative image analysis, including AI based techniques • Gene expression profiling • T cell receptor repertoire analysis using DNA from tumour infiltrating lymphocytes, optimisation of detection methods for detection in free urine DNA • Integration of biological and clinical trials data with statistical and "big data" approaches • Overview of all stages of the "bench to bedside" translational research process
Potential publications arising from project:	1. Integrated transcriptional and immunophenotyping report of how CAF enrichment changes as bladder tumours progress in the context of the wider bladder tumour microenvironment and according to survival following radiotherapy 2. Technical report of feasibility of TCR-Seq from urinary DNA as a non-invasive biomarker (working with newly-appointed PhD student) 3. Prospective characterisation of immunological and molecular changes occurring in a prospective trial of chemo-radiation +/-PD-L1 inhibition, focusing on cancer-associated fibroblasts and the extracellular matrix
Estimated amount and distribution of time spent with industrial partner:	3-6 months

MRC requires a cumulative period of no less than three months spent working in the facilities of the industrial collaborator.	
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☒ Maths, Statistics or Epidemiology ☒ Computer Science ☐ Other (provide details)
Keywords:	**1. Cancer-associated fibroblasts**
	2. Bladder cancer
	3. Extracellular matrix
	4. Radiotherapy
	5. Immunomodulation
	6. T cell receptor sequencing