The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	Translational studies of the immune environment in newly diagnosed bladder cancer and changes induced by treatment
Short Project Title:	**Translational immuno-biology of bladder cancer**
SUPERVISORY TEAM	
Primary Supervisor(s):	Nick James
Associate Supervisor(s):	Katharine von Loga/Alan Melcher/Kevin Harrington/Anna Wilkins (in association with the Crick Institute)
Backup Supervisor: (must have IRS status)	Alan Melcher
Lead contact person for the project:	Nick James
DIVISIONAL AFFILIATION	
Primary Division:	Radiotherapy and Imaging
Primary Team:	Bladder and Prostate Cancer Research

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

There has been a growing interest in manipulation of the immune response in cancer with an increasing number of drugs being licenced, mostly in the metastatic disease setting. Bladder cancer is a promising target for approaches targeting the PD1/PDL1 axis with a number of approved agents in the clinic. The project will commence using a large tissue collection from the Bladder Cancer Prognosis Programme [1] (>1000 samples) to build a picture of the immune environment in newly diagnosed bladder cancer. Signals of interest will then be examined using a second tissue series constructed around the BC2001 trial [2] where we have mature 10 year outcomes data. The aim will be to look for correlates, not just with primary outcomes like patterns of recurrence, but also quality of life and toxicity [3]. Having built a picture based on multiplex immunohistology, the project will move to exploring detailed T cell biology using frozen tissue and free DNA from urine associated with BCPP and a more recent chemoradiotherapy study using cetuximab called TUXEDO [4]. In parallel, we will be building tissue and urine collections from the recently commenced RADIO trial comparing chemoradiation with or without durvalumab in newly diagnosed locally advanced bladder cancer in order to assess the impact of adding immunotherapy to changes detectable in the immune microenviroment and with clinical outcomes. Additional material may be available from the KEYNOTE-992 trial with a similar design based on pembrolizumab

The project will involve optimising techniques for multiplex immunohistochemistry in archive tumour samples, analysis of free DNA in urine and urine pellet samples and cross correlation with analyses based on frozen tissue

samples. Complex analysis techniques using artificial intelligence will be used to integrate the existing statistical analyses with the multi-dimensional data emerging from the project.

PROJECT AIMS

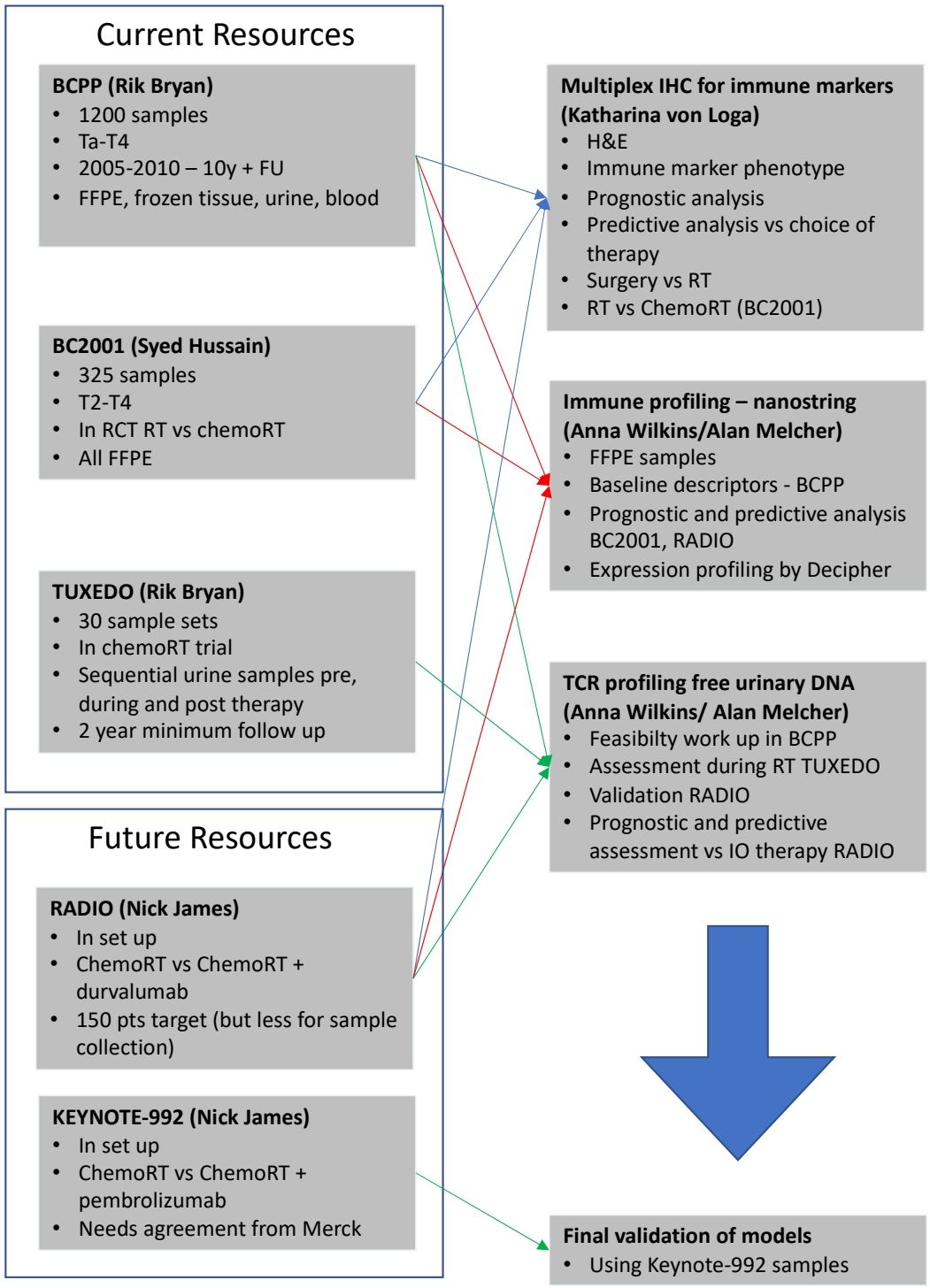
- Baseline description of immune environment in a mature, well annotated tissue collection spanning all stages of bladder cancer
- Assessment of T cell receptor changes using DNA from snap frozen material from the same tissue collection and cross-correlation with DNA from archive pathology samples and free DNA in associated urine samples
- Assessment of candidate immune profiles with clinical outcomes in the BC2001 and TUXEDO trials
- Prospective assessment of the interaction between immune phenotypes of interest and outcomes in the recently commenced RADIO trial
- Integration of analyses with pre-existing statistical analyses of these trials using artificial intelligence techniques

RESEARCH PROPOSAL

There has been a growing interest in manipulation of the immune response in cancer with an increasing number of drugs being licenced, mostly in the metastatic disease setting. Bladder cancer is a promising target for approaches targeting the PD1/PDL1 axis with a number of approved agents in the clinic. However, in the advanced disease setting, only around a quarter of patients experience worthwhile clinical responses and the reasons underlying this are poorly understood. There is both a prognostic and predictive relationship between better responses and over-expression of PD1/PDL1 either on tumour cells or the associated immune infiltrates in the tumour. However, this association is not sufficient to explain the variability in responses seen, nor to select patients for treatment in the majority of cases. Newly launched trials such as RADIO (Chief Investigator: Nick James) and Keynote-992 (Nick James part of the Trial Management Group) are exploring the first line use of immune-oncology (IO) agents as part of radical therapy for newly diagnosed patients.

The Prostate and Bladder Cancer Research Team have access to a number of mature tissue collections, summarised in the figure below. These include the Birmingham based Bladder Cancer Prognosis Programme [1], the joint Birmingham/ICR BC2001 trial [2,5] and the Birmingham based TUXEDO trial [4]. In addition, we will be prospectively collecting tissue and urine in the recently commenced RADIO trial (Chief Investigator: Nick James) comparing chemoradiation as per BC2001 [2] with chemoradiation plus neoadjuvant, synchronous and adjuvant durvalumab. This provides an ideal platform for validating any immune based signatures developed using the archive materials as well as assessing in a randomised setting the changes induced by the use of an IO agent in the first line setting. In addition, an opportunity exists via the Keynote-992 trial comparing chemoradiation with chemoradiation plus pembrolizumab to further validate any findings from the project. These resources (and key collaborators) are summarised in the figure below:

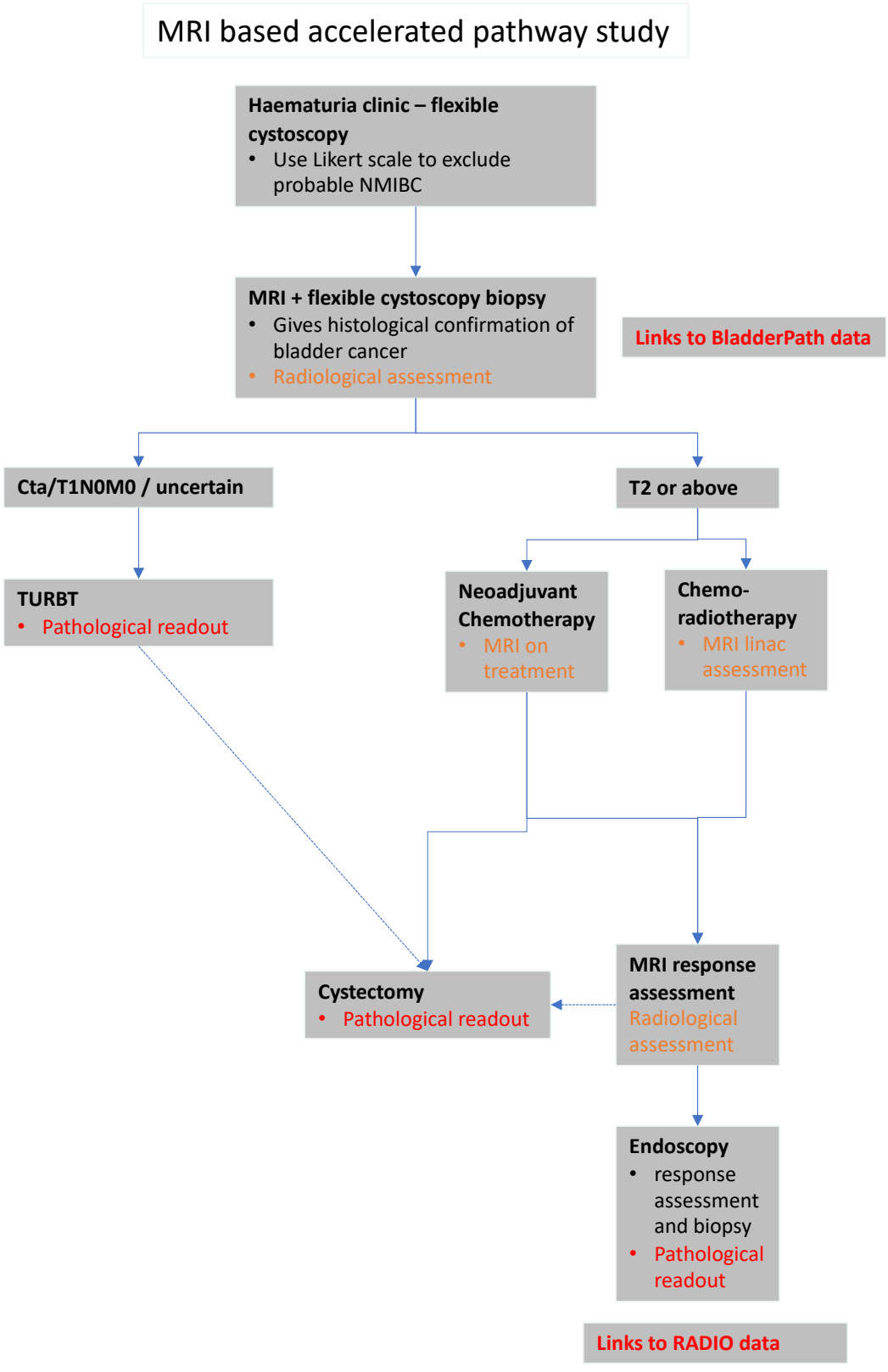
PhD project proposal outline



The BCPP study collected tissue from newly diagnosed bladder cancer between 2005-2010 across the whole spectrum from Ta to T4 and thus provides a unique platform to assess the immune landscape across the complete range of the disease at diagnosis and to relate the observed phenotypes to outcomes as well as to known risk factors such as smoking. The BC2001 trial provides another well annotated platform for the assessment of the relationship of the immune landscape to a range of clinical outcomes.

Both assessments will use multiplex immunostaining techniques already being used in the ICR (via the BRC – Katharina von Loga Group) to assess gastro-intestinal cancers but will need to be optimised for bladder cancer. Initial pilot studies will be carried out using BCPP specimens and if necessary, can be supplemented using paired fresh frozen tissue analyses from the same study. Based on pre-existing data from other tumour types, we anticipate identifying a subgroup in whom we would expect a high response rate to immune based therapies. Within BCPP we have linked snap frozen tumour samples and urine samples together with pelleted cell debris from the same samples. We have previously established that the tumour mutational spectrum is accurately represented in the urine and pellet samples (though with quantitative variations) [6,7] so we anticipate that tumour infiltrating lymphocyte DNA will be detectable in the same fashion. Urine derived lymphocytes have been shown to reflect the lymphocyte microenvironment in tumour but require specific rapid processing to isolate. High levels of urine derived lymphocytes predict a shorter recurrence free interval post cystectomy [8]. 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. Additional expression profiling will be carried out using nanostring (via Anna Wilkins Lab) and in collaboration with Decipher Biosciences [9].

Statistical analyses will be based around the existing analyses for BCPP, BC2001, TUXEDO (and in due course RADIO) but will then be linked to the multi-dimensional data emerging from this project. It is planned that these data will encompass a larger “Big Data” approach to bladder cancer encompassing magnetic resonance imaging (MRI) data from an additional study (BladderPath [10], ICR radiologist Nina Tunariou) and further planned MRI based radiotherapy approaches to bladder cancer via the ICR Radiotherapy Programme Grant (lead researchers Alison Tree and Uwe Oelfke, key collaborator Shaista Hafeez) and artificial intelligence based analyses (lead researchers: Bissan Al-Lazikani, Uwe Oelfke, Nick James). These relationships are summarised in the second figure below:


MRI based accelerated pathway study
Haematuria clinic – flexible cystoscopy
• Use Likert scale to exclude probable NMIBC
MRI + flexible cystoscopy biopsy
• Gives histological confirmation of bladder cancer
• Radiological assessment
Links to BladderPath data
Cta/T1N0M0 / uncertain
T2 or above
TURBT
• Pathological readout
Neoadjuvant Chemotherapy
• MRI on treatment
Chemo-radiotherapy
• MRI linac assessment
Cystectomy
• Pathological readout
MRI response assessment
Radiological assessment
Endoscopy
• response assessment and biopsy
• Pathological readout
Links to RADIO data

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

1. Zeegers MP, Bryan RT, Langford C, et al. The West Midlands Bladder Cancer Prognosis Programme: rationale and design. *BJU international* 2010; **105**(6): 784-8.

2. James ND, Hussain SA, Hall E, et al. Radiotherapy with or without chemotherapy in muscle-invasive bladder cancer. *The New England journal of medicine* 2012; **366**(16): 1477-88.

3. Huddart RA, Hall E, Lewis R, et al. Patient-reported Quality of Life Outcomes in Patients Treated for Muscle-invasive Bladder Cancer with Radiotherapy +/- Chemotherapy in the BC2001 Phase III Randomised Controlled Trial. *European urology* 2020; **77**(2): 260-8.

4. James N, Pirrie SJ, Liu W, et al. A phase I/II feasibility study of cetuximab with 5FU and mitomycin C with concurrent radiotherapy in muscle invasive bladder cancer. ASCO GU Symposium; 2020; San Francisco; 2020.

5. Huddart RA, Hall E, Hussain SA, et al. Randomized Noninferiority Trial of Reduced High-Dose Volume Versus Standard Volume Radiation Therapy for Muscle-Invasive Bladder Cancer: Results of the BC2001 Trial (CRUK/01/004). *Int J Radiat Oncol Biol Phys* 2013; **87**(2): 261-9.

6. Ward DG, Baxter L, Gordon NS, et al. Multiplex PCR and Next Generation Sequencing for the Non-Invasive Detection of Bladder Cancer. *PLoS One* 2016; **11**(2): e0149756.

7. Togneri FS, Ward DG, Foster JM, et al. Genomic complexity of urothelial bladder cancer revealed in urinary cfDNA. *Eur J Hum Genet* 2016; **24**(8): 1167-74.

8. Wong YNS, Joshi K, Khetrapal P, et al. Urine-derived lymphocytes as a non-invasive measure of the bladder tumor immune microenvironment. *J Exp Med* 2018; **215**(11): 2748-59.

9. de Jong JJ, Liu Y, Boorjian SA, et al. A genomic classifier for predicting clinically aggressive luminal bladder tumors with higher rates of pathological upstaging. *Journal of Urology* 2020; **In press**.

10. James N, Pirrie SJ, Liu W, et al. Replacing TURBT with mpMRI for staging MIBC: pilot data from the BladderPath trial GU Cancer Symposium; 2020; San Francisco; 2020.

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:	• Multiplex staining of archive tissue samples • Image analysis, including AI based techniques • 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 • Development of new prognostic and predictive approaches of clinical utility in bladder cancer

ADVERTISING DETAILS

Project suitable for a student with a background in: (Please tick all categories that apply – your project will be advertised under all selected categories)	x Biological Sciences ☐ Physics or Engineering ☐ Chemistry x Maths, Statistics or Epidemiology x Computer Science ☐ Other (provide details)
Keywords: Please provide 4-6 words/short phrases that potential students may type into search engines (e.g. Google) to search for PhDs similar to yours – e.g. 'cancer predisposition genes', 'physics PhD London' etc.	**1. Bladder cancer**
	2. Immunophenotyping
	3. T cell receptor repertoire
	4. Clinical outcomes
	5. Artificial intelligence
	6. PD1/PDL1 pathway biology