The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	Identification of combinatorial approaches to improve prostate cancer immunotherapy
Short Project Title:	**Identification of combinatorial approaches to improve prostate cancer immunotherapy**

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr. Marco Bezzi
Associate Supervisor(s):	Professor Nicola Valeri
Backup Supervisor:	Professor Andrea Sottoriva
IRS Partner :	Professor Andrea Sottoriva

DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	Tumour Functional Heterogeneity

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Cancer is a constantly evolving ecosystem composed of communities of genetically different tumoral cells that interact with each other and with the microenvironment to adopt a multitude of strategies to deceit the immune system and resist to therapies[1]. Immune checkpoint inhibitors have clearly revealed the obvious clinical relevance of this phenomenon and are revolutionizing cancer therapy. However, in many prevalent tumour types, including Prostate Cancer (PCa), significant anti-tumour activity has only been observed in subsets of patients[2,3], suggesting that both disease genetics and combination therapy could be crucial factors in determining efficacy of immunotherapies.

Tumour cells are able to evade T cell attack through a variety of strategies including expression of immune checkpoint molecules, alteration of the antigen presenting pathway, and absence of immunogenic neoantigens which could result in a "cold" tumour microenvironment (TME) devoid of any immune infiltrate[4]. PCa patients often show *de novo* resistance to immune checkpoint inhibitors characterized by either an immune "cold" TME, or by a "hot", inflamed but immunosuppressed, microenvironment infiltrated by myeloid cells that hinder cytotoxic T cells activity[2,3,5]. Notably, a high proportion of cancer types have been found to acquire detrimental HLA mutations, downregulation, and loss of heterozygosity (LOH), a mechanism that appear to be a convergent trajectory of cancer evolution in response to immunotherapy[6]. Moreover, a multitude of studies have shown how the activation of

major oncogenic pathways can have a direct effect on the cancer immune landscape and on the expression of key genes involved in the regulation of the antigen presentation pathway[7-9].

The successful candidate will mechanistically link a variety of prostate cancer genetics to specific immune responses in order to identify new combinatorial therapeutic strategies that enhance the efficacy of immune checkpoint inhibitors.

PROJECT AIMS

- **Optimization of 3D co-culture systems recreating prostate cancer microenvironment complexity**
- **Generation of *ex vivo* prostate cancer models resistant to immunotherapies**
- **Reverse genetic screenings for the identification of genes involved in tumour-immune cells interactions**
- **Uncover collateral sensitivity-based therapeutic approaches that modulate prostate cancer response to immune checkpoint inhibitors**
- **Pre-clinical combinatorial immunotherapy trials in novel syngeneic mouse models**

RESEARCH PROPOSAL (max. 1000 words) Please provide information on the approaches to be used and the expected outcomes**.**

This project focuses on tumour heterogeneity and resistance to cancer immunotherapy, two subjects that could impact the vast majority of cancer patients and that require immediate, innovative research strategies. It is becoming increasingly apparent that cancers are constantly evolving eco-systems where heterogeneous populations of malignant cells interact with a variety of normal cell. Experimental approaches that allow the modelling and functional interrogation of such complexity are lacking and a major research effort of the successful candidate will be toward the generation of both *in vivo* and *ex vivo* models that can better recapitulate the clinical scenario and therefore have higher predictive potential. PCa patients often show *de novo* resistance to immunotherapy and immune checkpoint inhibitors are not yet approved for the treatment of PCa. The successful candidate will aim to mechanistically link diverse PCa genetics to specific immune responses in order to elucidate mechanisms for the reprograming immune "cold" tumour microenvironment (TME). "Cold" TME can be categorized as either "immune excluded", a scenario in which T cells are not able to penetrate the TME, or as "immune desert", characterized by the total lack of immune response. Immune desert tumours are notoriously resistant to immune checkpoint blockade (ICB) and in this case tumour cells can escape the immune response through a variety of strategies including absence of immunogenic neoantigens and alteration of the antigen presenting pathway[4]. HLA class I molecules are critical for antigen presentation, which is required for CD8 T cells cytotoxic activity. HLA loss of heterozygosity (LOH) has been observed in a wide range of tumour samples and it has also been linked to resistance to immune checkpoint blockade (ICB), suggesting that alteration to the antigen presentation pathway may be a convergent mechanism of evolution in response to immunotherapy. While such loss allows tumours to escape recognition by functioning T cells, it potentially exposes cancer cells to attack by NK cells which specifically sense lack of HLA I[10].

The goal of this project is to identify new targets and drug treatments that synergize with ICB and enhance T cells and NK cells killing through the modulation of the antigen presentation pathway and immune modulatory molecules. To this end, the student will genetically modulate HLA expression in prostate cancer organoids. Through the combination of co-culture systems, drug screenings, genetic screenings and *in vivo* models, this project has the potential to uncover vulnerabilities and therapeutic entry points to bypass one of the most common mechanism of resistance to immunotherapy. The proposed project is subdivided in the following work packages.

<u>1. Generation of models to mimic HLA LOH and Optimization of in vitro co-culture systems</u>.

The first step of this project will be the modulation of HLA LOH in organoid murine PCa models. The student will generate prostate cancer organoid with different PCa genetics harbouring knockout alleles for MHC class I genes.

The expression of molecules of the antigen presenting pathway will be characterized and this analysis will clarify how different genetic backgrounds impact antigen presentation. HLA LOH-organoids will be functionally validated in co-culture assays in the absence or presence of antibodies that can inhibit immune checkpoint molecules. The results collected in this aim will be crucial to rationally design *in vivo* pre-clinical trials of combinatorial immunotherapies.

2. Genetic Screening of targets that modulate HLA expression.

In order to discover genes that modulate antigen presentation we will perform multi-dimensional CRISPR screening. HLA LOH-organoids of different genetic "make-ups" will be infected with pathway-specific libraries targeting epigenetic regulators, splicing regulators, and DNA repair genes. Positive hits will be identified and simultaneously characterized by mass cytometry. Targets whose knockout modulate antigen presentation will be further validated and functionalized by performing T cells and NK cell killing assays.

3. Screening for drugs that modulate HLA LOH-organoid sensitivity to immune checkpoint inhibitors.

The organoids generated in Aim 1 will be used to preform high-throughput T cells and NK killing assays in the absence or presence of antibodies that can inhibit immune checkpoint molecules, in combination with drugs currently FDA/EMA-approved or in clinical trial. Therapeutic approaches that impact immune evasion will be further evaluated in vivo pre-clinical trials.

LITERATURE REFERENCES

1. McGranahan, N. & Swanton, C. Biological and Therapeutic Impact of Intratumor Heterogeneity in Cancer Evolution. *Cancer Cell* **27**, 15–26 (2015).

2. Kwon, E. D. *et al.* Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol* **15**, 700–12 (2014).

3. Small, E. J. *et al.* A Pilot Trial of CTLA-4 Blockade with Human Anti-CTLA-4 in Patients with Hormone-Refractory Prostate Cancer. *Clin Cancer Res* **13**, 1810–1815 (2007).

4. Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer–immune set point. *Nature* **541**, 321–330 (2017).

5. Bezzi, M. *et al.* Diverse genetic-driven immune landscapes dictate tumor progression through distinct mechanisms. *Nat Med* **24**, 165–175 (2018).

6. McGranahan, N. *et al.* Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. *Cell* **171**, 1259-1271.e11 (2017).

7. Wellenstein, M. D. & Visser, K. E. de. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. *Immunity* **48**, 399–416 (2018).

8. Casey, S. C. *et al.* MYC regulates the antitumor immune response through CD47 and PD-L1. *Sci New York N Y* **352**, 227–31 (2016).

9. Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).

10. Malmberg, K.-J., Sohlberg, E., Goodridge, J. P. & Ljunggren, H.-G. Immune selection during tumor checkpoint inhibition therapy paves way for NK-cell "missing self" recognition. *Immunogenetics* **69**, 547–556 (2017).

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:	First class or upper second class honours BA or BSc Honours/MSc or equivalent in biological sciences or computational biology. - Preliminary technical experience in cell culture [essential]. - Preliminary experience in cell biology and flow cytometry [desirable].
Intended learning outcomes:	• Advanced skills in flow cytometry • Organoid-immune cells co-culture systems • Mass cytometry experimental design and data analysis • Experience in CRISPR screening approaches • Generation and characterization of prostate cancer mouse models
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. Prostate Cancer**
	2. Immunotherapy
	3. Combinatorial therapy
	4. Cancer Ecosystem
	5. Collateral sensitivity