The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	**Deciphering and targeting of novel immune evasion mechanisms in patient derived gastrointestinal cancer models**

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Marco Gerlinger
Other supervisory team members:	Prof Alan Melcher
Lead contact person for the project:	Dr Marco Gerlinger

DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	Translational Oncogenomics

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Checkpoint-inhibiting immunotherapies have revolutionized the treatment of melanomas and lung cancers but they had only modest impact to date on other common malignancies such as colorectal (CRC) and oesophago-gastric (OGC) cancers. Most CRCs and OGCs have lower mutation loads than the average melanoma or lung cancer and this likely to contribute to the limited activity of single agent checkpoint inhibitors[1]. Additional cancer cell intrinsic factors, such as the activation of oncogenic signalling pathways, the disruption of antigen-presentation pathways or the expression of immune inhibitory cytokines also influence cancer immunogenicity[2-4]. Overall, it is thought that the balance of pro-immunogenic factors (in particular the mutation/neoantigen load) vs. the immune evasion mechanisms that are active in a cancer determine whether a tumour responds to immunotherapy or not.

The identification of immune evasion mechanisms and of therapeutic strategies to reverse these provides the opportunity to increase immunotherapy success in tumours with moderate mutation loads. This has for example been shown in a study that increased immune checkpoint-inhibitor activity in CRCs through co-targeting of the immune suppressive MAP-kinase pathway[5].

However, the identification and validation of immune evasion mechanism in OGC and CRC has been significantly hindered by the lack of immunogenic laboratory models. We have established patient derived CRC and OGC cancer cell cultures and methods for large scale T cell expansion and have combined these into co-culture systems that enable us to study how tumour targeted T cells interact with cancer cells. The aim of this PhD is to apply genetic perturbations and high throughput transcriptional and genetic analyses to these model systems in order to

reveal the immune evasion mechanisms commonly employed by CRCs and OGCs to escape T cell recognition and killing. This will define novel therapeutic targets and inform combination therapies to increase the potency of immunotherapy in these highly prevalent and aggressive cancers.

PROJECT AIMS

-utilize existing immunogenic CRC and OGC models for the development of a versatile screening platform for CRISPR/CAS9 and cDNA screens

-perform reverse genetic (CRISPR/CAS9- and cDNA-) screens to identify OGC and CRC driver genes that confer resistance to killing by cancer targeted T cells

-prioritize the identified immune evasion drivers for mechanistic dissection and therapeutic targeting

-robustly validate therapeutic approaches to reverse such immune evasion mechanisms in our CRCs and OGC model systems

RESEARCH PROPOSAL

1. Existing immunogenic CRC and OGC model systems:

The laboratory has established and genetically characterized (by exome sequencing) over 15 patient-derived *in vitro* cancer models from OGCs and CRCs. They stably express a nuclear green fluorescent protein (GFP) for cell tracking and can be cultured long term. In addition, we developed protocols to expand cytotoxic CD8 T cells from healthy donor blood samples. Using novel bispecific immunotherapy antibodies and immunological model antigens, we have shown that these T cells and the patient derived tumour cells can be combined in vitro into immunogenic model systems that enable the study of immune recognition and killing under controlled conditions. This permits us to study how cancers can escape from T cell killing when endogenous antigens presented on the Class I MHC are recognized. In addition, we can study immune evasion mechanisms to bispecific T cell engaging antibodies. This is important as bispecific antibodies may be the most effective means to generate clinically relevant immune responses against tumours with very low mutation loads.

2. Reverse genetic screen identification of immune evasion mechanisms:

Culture and reverse transfection conditions will be optimized for 96 well plate formats to enable screening of several dozen target genes and relevant controls per patient-derived cancer cell line. We will then use cDNA overexpression (to model amplifications or introduce gain of function mutations) or CRISPR/CAS9 (to model deletions or loss of function mutations) to genetically perturb:

1) the most frequently altered CRC driver genes, in each of 5 CRC lines
2) the most frequently altered OGC driver genes, in each of 5 OGC lines

Perturbed GOA and CRC cell lines will be combined at various effector to target (E:T) ratios in established killing assays and imaged high throughput microscopy to quantify cancer cell survival over time. The genetically perturbed cancer cell lines will be killed by the T cells unless the perturbation impairs immune recognition or killing. Well

characterized genes regulating immune recognition will be perturbed as positive controls that rescues target cells from T cell mediated killing. The surviving cancer cells will be normalized against the controls and gene perturbations showing the strongest inhibition of T cell killing will be validated in standard chromium killing assays.

3. Mechanistic dissection and targeting of screen-identified immune evasion drivers:

Subsequent experiments will dissect the mechanisms of immune evasion induced by the perturbed genes. For example, we will assess if immune evasion is mediated by defective antigen processing or MHC loading or other mechanisms, such as upregulation of immune-inhibitory checkpoints. RNA-Seq will be applied to wild type and genetically perturbed cell lines prior to and during T cell challenge to further characterize the impact of immune evasion drivers on gene expression and to identify dynamic immune checkpoint activation during T cell targeting. Validation in additional patient derived models and in independent datasets (eg TCGA CRC or OGC data and internal clinical trial databases) will be performed.

Validated immune evasion drivers may be targetable with existing drugs and novel drug targets will be discussed with the ICR Cancer Therapeutics team to evaluate opportunities for drug development. We will use our immunogenic *in vitro* models to assess whether small molecule inhibitors of targetable drivers restore T cell killing, or if these agents are ineffective, for example if the drug inhibits signalling pathways which are critical for T cell activation. In addition, we will assess if inhibition of novel immune evasion mechanisms in combination with anti-PDL1-antbodies further augments immunotherapy responses in these model systems. This should reveal novel therapeutic strategies that counter immune evasion mechanisms in CRCs and OGCs as single agent or in combination with immune checkpoint inhibitors and increase immunotherapy efficacy in these tumours.

LITERATURE REFERENCES

1. Rizvi, Hellmann, Snyder, Kvistborg, Makarov et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124-8.
2. Spranger, Bao, Gajewski. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 2015;523:231-5.
3. Davies, Barber, Spain, Gerlinger. Evolution and Immune Evasion in gastro-oesophageal cancer. PLOS Biology, under review
4. Yoshihama, Roszik, Downs, Meissner, Vijayan et al. NLRC5/MHC class I transactivator is a target for immune evasion in cancer. PNAS, 2016; 113:5999-4
5. Bendell, Kim, Goh, Wallin, Oh et al. Clinical activity and safety of cobimetinib (cobi) and atezolizumab in colorectal cancer (CRC). J Clin Oncol; 2016; Abstract 3502

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)	BSc or equivalent in relevant areas such as cancer biology, immunology or molecular biology

Intended learning outcomes:

Acquire detailed knowledge of cancer immunity and immunotherapy

Culture, optimisation and genetic manipulation of immunogenic in vitro cancer models

CRISPR/CAS9 and cDNA-overexpression screens

Immunological assays such as killing and cytokine assays and genetic introduction of model antigens

Application of the latest solid tumour immunotherapy technologies including engineered bispecific antibodies

Functional dissection of immune evasion mechanisms through a broad range of molecular biology and immunological techniques

Development and validation of therapeutic approaches to reverse immune evasion mechanisms in cancer