ICR The Institute of Cancer Research

# PhD Project Proposal

## Funder details

**Studentship funded by:** The Institute of Cancer Research

## Project details

**Project title:** Investigating the Tumour Microenvironment (TME) *in vitro* and *in vivo*

## Supervisory team

**Primary Supervisor:** Dr. Ling Li

**Associate Supervisor(s):** TBD

**Secondary Supervisor:** Professor Udai Banerji

## Divisional affiliation

**Primary Division:** Cancer Therapeutics

**Primary Team:** Tumour Microenvironment and Pharmacology

**Site:** Sutton

## Project background

There have been many significant advances in our understanding of the molecular and cellular biology of cancer over the past few decades. Regardless, anti-cancer drug development is still dogged by high drug attrition rates (1). A major reason for the high failure rate is the use of suboptimal preclinical strategies due to the limitations of current *in vitro* and *in vivo* preclinical models.

Cancer involves more than just cancer cells. Many components in the surrounding microenvironment interact with cancer cells to facilitate malignant progression in a 3D architecture. Cancer cells grown in 3D culture show characteristics which better mimic *in vivo* tumours and thereby provide more rigorous models c.f. 2D culture. For example, differential zones of cancer cell proliferation have been observed in spheroids due to hypoxia and nutrition/metabolite gradients (2). Moreover, tumour cells cultured in 3D, display increased resistance to various therapies compared to cells cultured in a 2D monolayer (3). In addition, tumour organoids, established from colorectal carcinoma patients, recapitulate somatic copy number and mutation spectra in patients (4). Thus, an *in vitro* 3D (c.f. 2D) tumour culture system is likely to have distinct advantages in predicting preclinical therapeutic efficacy in the clinic.

Preclinical studies in mouse models of cancer implicate cancer-associated fibroblasts (CAFs) as well as immunosuppressive myeloid cells, including myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages (TAMs), as important for restricting the accumulation of T cells in the vicinity of cancer cells (5). Moreover, the TME regulates extravasation of T cells from the circulatory system into tumours as well as the local replication of T cells and their spatial distribution within tumours. The development of a preclinical *in vivo* model which can dynamically modulate the TME and immune response can provide unique insights into mechanisms/pathways involved in tumour development.

## Project aims

- Develop 3D scaffold-free and scaffold-based multicellular tumour spheroid models,
- Develop tumour organoids from human colorectal cancer (CRC) tissue with stromal components,
- Develop a new preclinical *in vivo* tumour, encapsulated in an air pouch mouse syngeneic model, to delineate the role of the TME and to facilitate innovative assessment of the mechanism of action of immunomodulatory agents across multiple oncological malignancies as well as evaluation of next generation therapeutics,
- Utilise 3D multicellular heterospheroids, stroma-incorporated patient-derived tumour organoids and the syngeneic air pouch tumour model to evaluate the contribution of stromal components such as CAFs and ECMs in modulating response to SOC/immune checkpoint regulators/drug combinations and to establish the *in vitro*-*in vivo* correlation.

## Research proposal

**RESEARCH PROPOSAL (max. 1000 words)** Please provide information on the approaches to be used and the expected outcomes**. For all MRC DTP proposals please include details of how this project aligns with one of the three research themes (see separate guidance) and for iCASE projects please also include the benefits to this project of industry collaboration.**

**Part 1: Generation and assembly of *in vitro* stroma-incorporated tumour models**

**Part 1a: 3D multicellular tumour spheroid models**

CRC tumour cell lines will be used to generate multicellular tumour spheroids *in vitro* with or without collagen or Matrigel as scaffold in Ultra-Low Attachment plates. The supervisor (LL) has extensive prior experience with various tumour cell line derived spheroids.

**Part 1b: Patient-derived tumour organoid model**

Patient-derived CRC tumour organoids will be generated from tumour biopsies/resections, expanded *in vitro* and cryopreserved. Several lines of banked CRC tumour organoids are also available from the second supervisor's (UB) lab.

**Part 1c: Stepwise incorporation of tumour stromal components**

Co-culture will be conducted either, (i) by co-seeding tumour cells with stromal cells or, (ii) by seeding tumour cells and stromal cells in separated microfluidic channels which allow open cell-cell communication. Microfluidic devices are a key component in the co-culture, creating a more physiological biomolecular gradient that impacts cell-cell communication and tissue formation.

***Fibroblast co-culture***: CAFs influence tumour progression. Several distinct CAF subtypes have been identified including myofibroblast-like CAFs (myCAFs), inflammatory CAFs (iCAFs) and antigen-presenting CAFs (ApCAFs). The role of CAFs in promoting tumour drug resistance, angiogenesis and invasiveness has been documented (6). Recent studies have highlighted a major role for CAFs in promoting immunotherapy resistance by excluding T cells from tumours (7). CAFs will be derived from dissociated tumour cells or obtained commercially. *In vitro* cultured CAFs will be analysed by single cell RNA-Seq or Total RNA-Seq to understand subtype heterogeneity. Incorporated fibroblasts will be analyzed by gene expression and immune staining. Condition medium will be profiled for cytokine/chemokine secretion.

***Build immune landscaping in tumour stroma:*** Immunocompetent cells (e.g. T cells, macrophages, NK cells obtained commercially or isolated from PBMCs) will be loaded to preformed tumour organoids +/- CAFs. Immune cell infiltration into tumour will be monitored by 3D imaging or immunostaining of tumour spheroids/organoids followed by quantification using advanced imaging analysis. Condition medium will be profiled for cytokines. Cancer cells and infiltrated immune cells will be immunophenotyped for expression of various markers by flow cytometry or multiplexed IHC.

**Part 1d: Evaluate the response to immune checkpoint regulators in the 3D multicellular spheroids and tumouroids**

Responses to immune checkpoint inhibitor agents, as monotherapy or in combination, targeting PD-1, PD-L1 and CTLA-4 pathways will be assessed using multicellular spheroids and patient-derived tumour organoids with various stromal subtypes and configurations employing viability assays or imaging-based cytotoxicity assays. Immune cell activation status will be profiled by flow cytometry. Level of cytokines and chemokines in the co-culture will be measured. Immunostaining or other molecular/biochemical analysis (e.g. gene and protein analysis) will also be

carried out to understand potential resistance mechanisms. Genetic mutation, MSS/MSI status and drug responses will be analysed to assess correlation.

**Part 2: Preclinical tumour air pouch model**

The *In vivo* Pharmacology group has considerable experience with syngeneic models. The supervisor (LL) is experienced with immunology models including the air-pouch model. We will develop a model in which tumours grow inside an air-pouch in the mouse (8). The air-pouch serves as the local microenvironment, modulated by pro-/anti-inflammatory stimuli, to study the impact of local cells/cytokines on tumour growth and efficacy of therapeutic agents. Fibroblasts can be introduced by co-injection with tumour cells. Spatial and temporal sampling from the TME as well as the local administration of agents can be achieved.

**Part 2a: Characterization of TME in luciferized syngeneic tumour models**

Briefly, luciferized mouse colon carcinoma syngeneic cells will be generated *in vitro* and used to assess the kinetics of tumour growth within the air-pouch c.f. standard subcutaneous models, as quantified by bioluminescence imaging. Characterization of the TME will be by MR and CT imaging, cytokine expression and cell phenotyping.

**Part 2b: Characterization of response to checkpoint inhibitor agents**

We will also evaluate checkpoint inhibitor agents, as monotherapy or in combination, targeting PD-1, PD-L1 and CTLA-4 pathways in order to evaluate their effect on tumour growth and impact on the cellular and cytokine microenvironment. Additional immune profiling will be performed to characterize cells, cytokines and gene expression in the blood, lymph nodes, air pouch and tumour pre- and post-treatment. Additional tumour models that are partially and non-responsive will be profiled in a similar manner. These data will provide information about the "TME signature" for checkpoint inhibitor agents that can be associated with full, partial and non-responsive models. The *in vitro*-*in vivo* correlation will be established.

**Part 2c: Modulation of TME to identify immunogenic components**

Modulating the TME of partially and non-responsive models to resemble the TME of checkpoint inhibitor agent-responsive models will be performed in parallel both *in vitro* and *in vivo*. Specific TME cytokines identified as more abundant in responsive models, will be applied directly to the co-culture/pouch to interrogate the contribution of local cytokines to the immune response. Alternatively, if a specific subset of cells (e.g. iCAFs) is correlated with an efficacy response, these cells will be isolated and added to the co-culture/pouch to evaluate their impact on efficacy. The direction of these studies will be largely driven by the biology uncovered in Parts 1d and 2b.

## Literature references


1. Hutchinson L. and Kirk R. (2011). High drug attrition rates--where are we going wrong? *Nat Rev Clin Oncol* 8(4), 189-190.

2. Lin R.Z. and Chang H.Y. (2008). Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnol J.* 3(9-10), 1172-1184.

3. Feder-Mengus C. et al., (2008). New dimensions in tumor immunology: what does 3D culture reveal? *Trends Mol Med.* 14(8), 333-340.

4. van de Wetering M. et al. (2015) Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 161(4), 933-945.

5. Joyce J.A. and Fearon D.T. (2015). T cell exclusion, immune privilege, and the tumour microenvironment. *Science* 348(6230), 74-80.

6. Sahai E. et al., (2020). A framework for advancing our understanding of cancer-associated fibroblasts. *Nature Reviews Cancer* 20, 174-186.

7. Hanley, C.J. and Thomas, G.J. (2020). T-cell tumour exclusion and immunotherapy resistance: a role for CAF targeting. *Br J Cancer* 123, 1353–1355.

8. Santana-Krímskaya S.E. et al., (2020) Immunepotent CRP plus doxorubicin/cyclophosphamide chemotherapy remodel the tumor microenvironment in an air pouch triple-negative breast cancer murine model. *Biomedicine & Pharmacotherapy* 126, 110062.

## 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:**

- Training and experience in modern cell biology, *in vitro* and *in vivo* model development;
- Technical skills in advanced cell biology, immunology and 3D cell model including patient derived tumour organoid generation;
- In-depth knowledge of Immuno-Oncology and tumour microenvironment biology;
- Familiarity with the principles and practice of modern drug discovery in Immuno-Oncology;
- Ability to develop and optimise new TME models;
- Facility in formulating testable scientific hypotheses and planning and executing appropriately controlled experiments to interrogate the hypotheses;
- Good scientific communication and presentation skills

## Advertising details

**Project suitable for a student with a background in:**

- [x] Biological Sciences
- [x] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science