The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

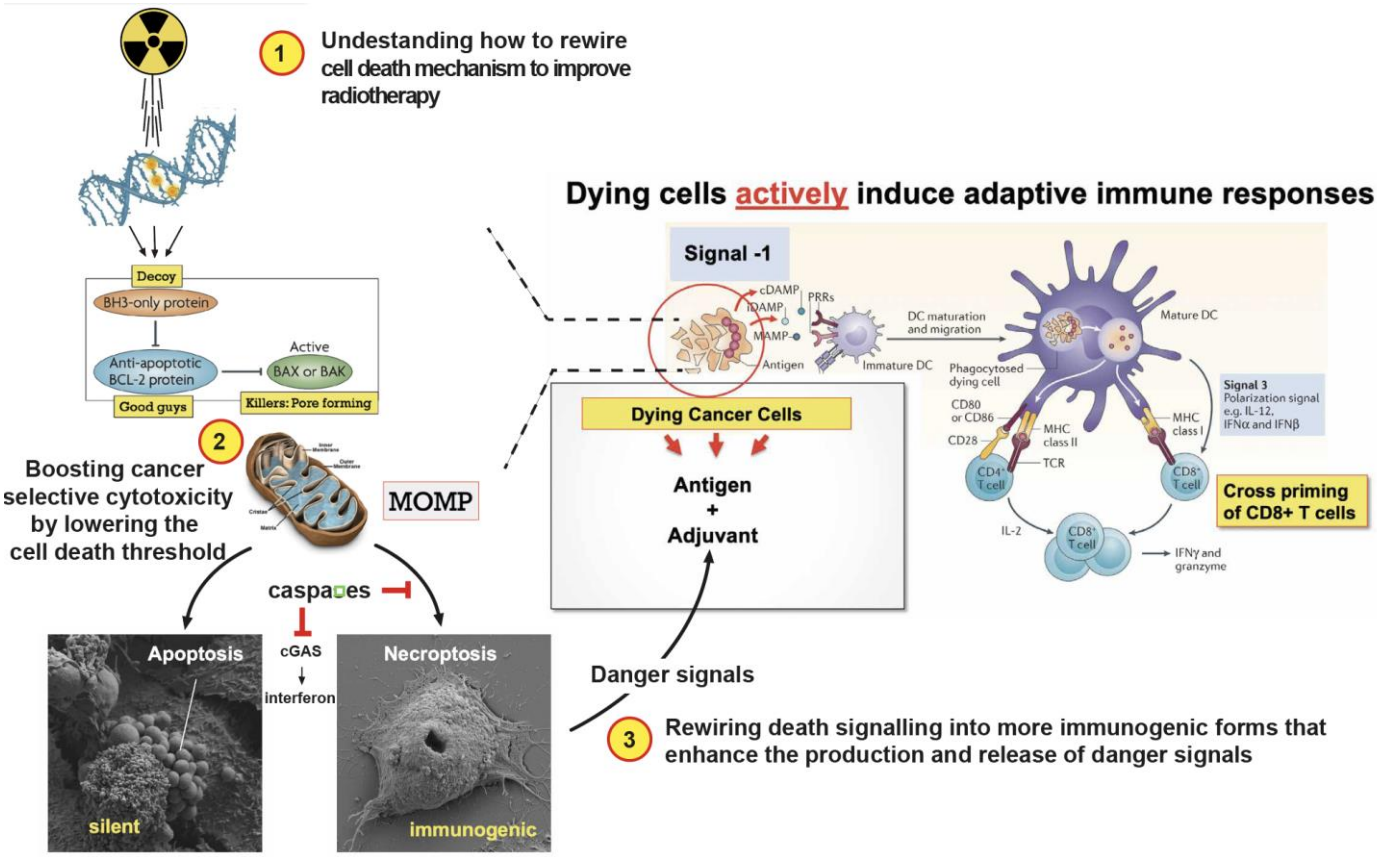
FUNDER DETAILS	
Studentship funded by:	Medical Research Council - Doctoral Training Partnership (MRC DTP)
Funder specific requirements:	All MRC DTP students will attend taught courses one day a week for the first nine months of the PhD. This training will cover computational and thematic science training as well as core and transferable skills. Students will spend the remainder of the four years on their PhD project full time with monthly cohort activities.
PROJECT DETAILS	
Project Title:	**Driving immunogenic cell death to trigger anti-tumour immunity**
Short Project Title:	Immunogenic Cell Death in Cancer
SUPERVISORY TEAM	
Primary Supervisor(s):	Professor Pascal Meier
Associate Supervisor(s):	Professor Alan Melcher
Secondary Supervisor:	Professor Kevin Harrington
DIVISIONAL AFFILIATION	
Primary Division:	Division of Breast Cancer
Primary Team:	Cell Death and Immunity
Other Division (if applicable):	Division of Radiotherapy and Imaging
Other Team (if applicable):	RadNet

SHORT ABSTRACT

Death is not an endpoint, but the beginning of a novel communication axis between dying cells and cells of the immune system[1]. The way in which a cell dies dramatically alters the danger signals that get released. While apoptosis tilts towards an immunologic silent outcome, non-apoptotic forms of cell death, such as caspase-independent necroptosis, can be highly immunogenic.

The successful PhD candidate will investigate how non-apoptotic forms of cell death can be used to open a new line of communication with immune cells. Specifically, he/she will use novel ICR-developed **PROTAC degraders** in combination with **radiation therapy** to rewire cell death signalling pathways into caspase-independent forms of cell death. PROTACs or Proteolysis Targeting Chimeric Molecules are heterobifunctional nanomolecules that target a protein-of-interest for ubiquitylation and degradation. We will use such compounds to test whether killing cancer cells by non-apoptotic forms of cell death more effectively drives an immune response against tumours, hence enhancing the effectiveness of radiotherapy.

Schematic abstract of proposed research:



BACKGROUND TO THE PROJECT

Radiotherapy typically kills tumour cells by apoptosis. However, apoptosis is an immunologically silent form of cell death that does not stimulate the immune system against the tumour. Moreover, resistance to radiation therapy (RT) in cancer patients is common and can arise as tumour cells evolve to become resilient to undergo apoptosis.

While it is clear that dying cells play an active role in inducing anti-tumour immunity, cells can die through **very different modalities**, with **apoptosis** being more immunologically silent whereas **caspase-independent cell death (CICD)** alerts the immune system of danger[2]. This is because apoptotic caspases cleave and block the production of 'danger' signals, causing a more immunologically silent form of death. In contrast, cells that die by CICD release molecules that function as adjuvants or danger signals for immune cells. Such damage- or stress-associated molecular patterns (DAMPs and SAMPs) can engage innate pattern recognition receptors (PRR), resulting in the activation of tumour-specific immune responses.

Therefore, rewiring cell death signalling pathways into caspase-independent forms of cell death is a promising strategy to engage the immune system against the tumour. These types of cell death, such as necroptosis, are based on the strategy of 'pathogen mimicry'. They simulate the presence of an imaginary pathogen, while collaterally triggering an attack on the tumour and an immunization against tumour antigens. While cancer cells have grown to manipulate the immune system into tolerating the tumour, there is an enemy the immune system will never ignore: **pathogens**. Therefore, engaging pathogen-sensing pathways while rewiring their death signalling outputs into **CICD might be a better way to kill cancer cells.**

Recent data demonstrate that radiotherapy-mediated anti-tumor efficacy critically depends on activation of innate immunity pathways[3-8]. Thus, radiation triggers an immune response by causing cytosolic DNA, which activates the cGAS/STING pathway. Additionally, the effects of radiation can also induce expression of **endogenous retroviruses (ERVs)**[9] that in turn are detected by dsRNA sensors, such as ZBP1 and RIG-I. Both, STING and ZBP1 can drive interferon and necroptosis signalling, which potently alerts the immune system of danger.

PROJECT AIMS

The **overarching goal** is to gain a better understanding how to boost the immunogenicity of radiotherapy.

We aim to achieve this by manipulating the innate immunity pathways triggered by radiation so that they **maximally** drive interferon signalling, NF-kB activation and lytic forms of cell death. Importantly, we will investigate cancer cell autonomous as well as non-autonomous effects, such as those mediated by cancer associated fibroblasts (CAFs) and macrophages.

Specifically, we will pursue the following aims:

- identify strategies to enhance the tumour-selective cytotoxicity of radiation
- understanding how to rewire radiation-induced apoptosis into caspase-independent forms of cell death
- gain a better understanding how radiation drives cGAS/STING and ZBP1 activation
- characterise how we can manipulate regulators of the cGAS/STING and ZBP1 pathways to enhance their immunogenic signalling outputs (interferon signalling and cell death)
- evaluate the immunogenicity of novel radiation-based treatment protocols using state-of-the-art syngeneic mouse tumour models for breast cancer

Manipulating radiation-induced cytotoxicity and immunogenicity may ultimately translate into novel therapeutic developments to induce effective anti-tumour immunity in nearly all cancer patients. It will also allow the identification of radiosensitivity biomarkers and promote immunogenic approaches to cancer treatment.

RESEARCH PROPOSAL

We aim to elucidate how we can unleash the full potential of cGAS/STING and ZBP1-mediated signalling in response to radiation so that we can trigger an effective immune response against tumours.

Objective 1: ***Manipulating cell death priming to enhance the efficacy of radiation***

Many mechanisms of resistance to radiotherapy have been observed, including resistance to apoptosis. Accordingly, our preliminary data indicate that Navitoclax, a BCL2 inhibitor, sensitises Patient-derived tumour organoids and murine tumour organoids to radiation. Therefore, we will evaluate whether manipulating the cell death threshold improves radiation-induced cell death. Particularly, we will combine radiation with BH3 mimetics, which antagonise BCL-2 family members and increase the extent of mitochondrial cell death priming. We will use: ***Navitoclax*** *(targeting BCL-2, BCL-XL, BCL-W),* ***Venetoclax*** *(BCL-2), and* ***S6845*** *(MCL-1). Using* murine breast cancer organoids and human mutant PDOs, we will test whether antagonism of BCL-2 family members enhances the sensitivity to radiation. Increasing the readiness to undergo MOMP implies that radiation causes upstream activation of cell death signalling via BH3-only protein induction (e.g. PUMA or NOXA).

Together, it will allow us to determine which anti-apoptotic BCL-2 family member sustains resistance to radiation-induced cell death, and to design an efficient treatment protocol (see **Objective 3**).

Objective 2. Caspase-independent cell death (CICD) triggered by radiation

2.1 MOMP-induced caspase-independent cell death

While mitochondrial outer membrane permeabilization (MOMP) is a key event for intrinsic apoptosis, caspases are not required for cell death as cells die post-MOMP even in the absence of caspase activity[2]. While caspases are dispensable for MOMP-induced cell death, they function primarily to accelerate cell death, which serves important roles during development and keeps apoptosis immunologically silent. For example, apoptotic caspases cleave and inactivate cGAS and IRF3 to suppress type I interferon (IFN) response. Caspases also prevent the release of mitochondrial DNA (mtDNA). Thus engaging MOMP, while blocking caspases (CICD) strongly provokes immunogenic cell death through the activation of NFkB and the induction of mtDNA-mediated IFN response. Accordingly, caspase inhibition has been shown to induce anti-tumour activities accompanied by tumour regression[2].

To provide a proof-of-concept and profile the innate immune signalling outputs of this approach in breast cancer (PDOs and murine tumour organoids), we will trigger radiation-induced MOMP while inhibiting caspase activity with clinical-grade pan-caspase inhibitors. We will assess mtDNA release and cGAS/STING-dependent interferon production as well as NF-kB signalling. Particularly, we evaluate whether caspase blockade boosts activation of cGAS/STING signalling, thereby altering the communication between dying cancer cells and cells of the immune system.

To study the communication between dying cells and immune cells, we will study the cell signalling events triggered by heterotypic co-culture assays, and what activates/polarises the immune cells. This will identify the danger signals that mediate cancer/immune communication.

Ultimately, we will evaluate whether radiation-induced and MOMP-mediated CICD confers therapeutic benefit in cancer treatment. To this end we will perform tumour allograft and treatment experiments using murine tumour model (**see Objective 3**).

Objective 3: Heating up tumours via immunogenic cell death

3.1 Studying radiation-mediated activation of the immune system

We will use transplantation-based syngeneic mouse tumour models of TNBC to study the ability of dying tumour cells, killed by radiation-induced apoptosis or CICD (Objective 2), to stimulate the adaptive immune system. The effect of CICD on anti-tumour immunogenicity will be conducted in collaboration with **Alan Melcher and Kevin Harrington**, as previously described[10-12]. These assay systems have already been set up in my lab. Changes in the immune landscape and immune activation will be evaluated *as previously described*[13]. Furthermore, we will measure the cross-priming, proliferation, and cytokine (INFg and TNF) release of cytotoxic T cells ($CD8a^+$) isolated from these locations. We will also test the *in vivo* cytolytic activity of the primed $CD8^+$ T cells in these systems.

Together, these approaches will allow us to further understand the language used by dying cells and tailor our combinations to achieve optimal anti-tumour immune responses by radiation.

LITERATURE REFERENCES

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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:	- Candidates must have a first class or upper second class honours degree or an MSc in an appropriate scientific discipline [essential]. - Preliminary technical experience [essential]. - Experience in cell biology, biochemistry or molecular biology [essential]. - Ability to design and implement experiments using state-of-the-art techniques [essential]. - Good communication and presentation skills [essential]. - Team player [essential]
Intended learning outcomes:	- To work independently on a defined project and to consult when appropriate. - To take an interest in the relevant scientific literature. - To present work at conferences and participate regularly in group meetings. - To publish work in the scientific press. - To generate insight and leads to further our understanding of the complex relationship between cell death and immunity, and how this could be exploited to improve tumour kill and drive anti-tumour immunity
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. Radiation
	2. PROTAC (ICR-developed Advanced Therapeutics)

	3. Epigenetic modulation
	4. Immunogenic Cell Death
	5. Innate and adaptive immunity