The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Winners versus Losers: Harnessing Cell Competition to kill tumour cells**
SUPERVISORY TEAM	
Primary Supervisor(s):	Pascal Meier
Backup Supervisor: (must have IRS status)	To be decided
Lead contact person for the project:	Pascal Meier
DIVISIONAL AFFILIATION	
Primary Division:	Breast Cancer Now
Primary Team:	Cell Death and Immunity

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Winners versus Losers: Harnessing Cell Competition to kill tumour cells.

A key problem in the treatment of cancer is the acquired resistance of the cancer cells to bypass the cell death programmes. It is now clear that cell death acts as part of a quality-control and repair mechanism that eliminates potentially harmful cells, and failure to do so is linked to cancer. Moreover, the reason for late relapse is that anti-cancer therapy for cancer is only partially effective. While tumour proliferation may be suppressed, in many cases disseminated cells survive and subsequently progress. Clearly, treatment efficiency critically depends on the ability of the anti-cancer strategy to trigger cancer cell death.

It is now clear that dying cancer cells play an active role in the initiation of an anti-cancer immune response. However, a cell can die through different cell death pathways, and not every pathway can stimulate an anti-tumour immune response. The emergence of a multitude of cell death mechanisms has shed new light on multiple molecular cross-talks between cell death pathways and innate immune pathways, and revealed new layers of complexity in the relationship between dying cells, adaptive responses and induced immunity.

The successful candidate will use interlocking cell biological, biochemical and genetic approaches to identify and understand the processes that regulate cell death, inflammation and immune homeostasis, and how this knowledge can be used to mobilise a patient's own immune system against cancer.

Cell competition is an evolutionary conserved quality control process that eliminates suboptimal, but otherwise viable cells, thereby safeguarding tissue fitness and tissue homeostasis (Johnston, 2014). How relative fitness disparities are measured across groups of cells, and how the decision is taken whether a particular cell will persist in the tissue ('winner cell') or is killed ('loser cell') is not completely understood (Claveria and Torres, 2016). This is a key issue as

competitive behaviour is exploited by cells with deregulated oncogenes or tumour suppressors, which subsequently expand at the expense of, and with the help from, their cooperating wild-type (WT) neighbours (Johnston, 2014).

Myc is a major driver of cell growth in many cancers (de la Cova et al., 2014), but direct inhibition of Myc's oncogenic activity has been challenging. Interactions between wild-type and Myc-expressing cells cause Myc cells to acquire 'supercompetitor' behaviour that increases their fitness and enables them to overtake the tissue by killing their wild-type neighbours through TNF-induced cell death during a process called cell competition.

Our preliminary data indicate that the competitive behaviour of oncogenic Myc critically depends on its ability to extract carbon fuel (Lactate) from neighbouring cells (reverse Warburg Effect). Myc cells trigger TNF-mediated activation of JNK in neighbouring cells, which in turn shuts down their mitochondrial function, making them Loser cells. Changing the ability of Myc clones to influence their neighbours prevents Loser cell elimination and erodes the supercompetitor status of oncogenic Myc clones.

Our data indicate that targeting cell competition can help to turn Myc-driven super-competitor cancer cells into super-losers, which then are killed via TNF-induced cell death.

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PROJECT AIMS

The aim of the project is as following:

1) **To identify the mechanism through which Myc Winner cells manipulate JNK signalling in Loser cells.**
2) **To use this knowledge to convert supercompetitor Winner cells (Myc cells) into superlosers.**
3) **To study how Ca2+ signalling influences the output of the TNF>JNK signalling axis.**
4) **Using mouse models of Myc-driven tumorigenesis to examine whether we can kill Myc driven tumours via cell competition.**

RESEARCH PROPOSAL

MYC is a major driver of cell growth in TNBC (Horiuchi et al., 2012), but direct inhibition of MYC's oncogenic activity has been challenging. Interactions between wild-type and MYC-expressing cells cause MYC cells to acquire 'super-competitor' behaviour that increases their fitness and enables them to overtake the tissue by killing off their wild-type neighbours through TNF-induced cell death during a process called cell competition (de la Cova et al., 2014).

We propose an interlocking cell biological, biochemical and genetic approach to delineate cell competition in mammals, and investigate how this could be exploited to kill cancerous winner cells. In particular we will aim to target MYC-overexpressing cancers, such as TNBC that is a particular hard to treat subtype of breast cancer (Braso-Maristany et al., 2016; Horiuchi et al., 2012). We will investigate whether pharmacological inhibition of cell competition in MYC-driven cancers re-defines them as unfit mutants, resulting in their elimination via cell competition by wild-type neighbours (**Objective 1 and 2**). We will also determine whether such loser cells are killed in a TNF- and RIPK1-dependent manner, and whether modulating the checkpoints of TNF-induced cell death in loser cells enhances their elimination (**Objective 3**).

This project will greatly profit from our know-how on RIPK1 signalling as well as cell competition. If successful, targeting cell competition in *Myc*-driven tumours would warrant clinical evaluation.

Objective 1

CRISPR/Cas9 knockin mice for the study of cell competition

Molecular genetic studies are continuously transforming our knowledge in biology and medicine. Forward and reverse genetics in cells and animal models is key to discovering causal mechanisms relating molecular events to phenotypes. Recently, the RNA-guided endonuclease Cas9 from microbial type II CRISPR systems has been harnessed to facilitate facile genetic manipulations in a variety of cell types and organisms. We will use a Cre-dependent Rosa26 Cas9 knock-in mouse (already available in the Meier lab) in combination with AAV-mediated delivery of Cre recombinase, guide RNA and oncogenes. Using mammary fat pad injection and Mammary Intraductal (MIND) methods we will generate clones of cells that are mutant for genes that trigger cell competition behaviour. In particular, we will generate clones of cells that express higher levels of Myc and evaluate whether these clones overpopulate the mammary epithelia and exhibit supercompetitor behaviour. Further, we will evaluate whether targeting cell competition in Myc clones reduces their oncogenic potential.

These studies will establish a new model of cell competition in the mammary epithelia.

Objective 2

Inhibition of cell competition as a targeted therapy against breast cancer with elevated MYC expression

To assess the feasibility of inhibiting cell competition as a therapy, we will treat a panel of breast cancer cell lines and PDOs with inhibitors that influence cell competitive behaviour, and study the effects on cell proliferation and cell death of TN tumour cells with elevated MYC expression. This panel of TN tumours cells has previously been characterised and will be grown as co-cultures with non-immortalized human mammary epithelial cells (HMECs) (Horiuchi et al., 2016). We will also evaluate the effect of NMDAR inhibition on the *in vivo* growth of breast cancer cell lines and the PDX series HCI-002$^{\text{high MYC}}$, HCI-004$^{\text{intermedite MYC}}$ and HCI-009$^{\text{low MYC}}$ (Horiuchi et al., 2016) that will be injected into mammary ducts of immunocompromised mice.

Furthermore, we will examine the effects of cell competitive genes in a conditional transgenic mouse model of *MYC*-driven breast cancer (TetO-MMTV;TRE-*MYC*) (D'Cruz et al., 2001). In this model, tumour growth is dependent on doxycycline-induced MYC expression in mammary tissues. TetO-MMTV;TRE-*MYC* tumours will be orthotopically transplanted into the mammary ducts of isogenic mice.

Objective 3

TNF-induced cell death of loser cells

To study the elimination process, we will examine the involvement of the TNF signalling axis, which is known to kill loser clones during cell competition from flies and mammals. To this end we will evaluate the role of TNF, TNFR1 and components of the downstream signalling axis as previously conducted (Annibaldi et al., 2018; Feltham et al., 2018; Jaco et al., 2017; Tenev et al., 2011). Furthermore, we will determine whether modulating the checkpoints of TNF-induced cell death enhances the elimination of loser cells.

Together, these experiments will address whether *MYC*-driven breast tumours require the activity of cell competition genes to maintain their supercompetitor status, and whether we can enhance their efficacies by modulating the checkpoints of cell death.

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

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Braso-Maristany, F., Filosto, S., Catchpole, S., Marlow, R., Quist, J., Francesch-Domenech, E., Plumb, D.A., Zakka, L., Gazinska, P., Liccardi, G.*, et al.* (2016). PIM1 kinase regulates cell death, tumor growth and chemotherapy response in triple-negative breast cancer. Nature medicine *22*, 1303-1313.

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de la Cova, C., Senoo-Matsuda, N., Ziosi, M., Wu, D.C., Bellosta, P., Quinzii, C.M., and Johnston, L.A. (2014). Supercompetitor status of Drosophila Myc cells requires p53 as a fitness sensor to reprogram metabolism and promote viability. Cell metabolism *19*, 470-483.

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Horiuchi, D., Kusdra, L., Huskey, N.E., Chandriani, S., Lenburg, M.E., Gonzalez-Angulo, A.M., Creasman, K.J., Bazarov, A.V., Smyth, J.W., Davis, S.E.*, et al.* (2012). MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. J Exp Med *209*, 679-696.

Jaco, I., Annibaldi, A., Lalaoui, N., Wilson, R., Tenev, T., Laurien, L., Kim, C., Jamal, K., Wicky John, S., Liccardi, G., *et al.* (2017). MK2 Phosphorylates RIPK1 to Prevent TNF-Induced Cell Death. Molecular cell *66*, 698-710 e695.

Johnston, L.A. (2014). Socializing with MYC: cell competition in development and as a model for premalignant cancer. Cold Spring Harbor perspectives in medicine *4*, a014274.

Tenev, T., Bianchi, K., Darding, M., Broemer, M., Langlais, C., Wallberg, F., Zachariou, A., Lopez, J., MacFarlane, M., Cain, K.*, et al.* (2011). The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. Molecular cell *43*, 432-448.

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)	- 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].
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 cell death regulation, which may have significant impact on the development of new therapeutic strategies for the treatment of cancer.
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. Cell Competition**
	2. Cancer
	3. Immunogenic Cell Death
	4. Immunity
	5. Targeting non-druggable oncogenes
	6. Metabolism