**The Institute of Cancer Research**

## PHD STUDENTSHIP PROJECT PROPOSAL

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| **FUNDER DETAILS** | |
| **Studentship funded by:** | The Institute of Cancer Research (ICR) |
| **PROJECT DETAILS** | |
| **Project Title:** | **Deciphering the metabolic determinants of bone metastasis in breast cancer** |
| **SUPERVISORY TEAM** | |
| **Primary Supervisor:** | Dr George Poulogiannis |
| **Secondary Supervisor:** | Professor Clare Isacke |
| **DIVISIONAL AFFILIATION** | |
| **Primary Division:** | Cancer Biology |
| **Primary Team:** | Signalling & Cancer Metabolism |
| **Other Division (if applicable):** | Breast Cancer Research |
| **Other Team (if applicable):** | Molecular Cell Biology |

**SHORT ABSTRACT**

Rewiring of metabolic programmes is crucial for cancer cells to shed from a primary tumour, overcome the nutrient and energy deficit, and eventually survive and form metastases. However, the role of specific metabolic enzymes and pathways conferring the aggressive properties of malignant cancers remains unclear. This proposal aims to identify (i) distinct metabolic hallmarks underlying metastatic potential and spread to different organ sites and in particular to the bone, and (ii) metabolic targets that could prevent or suppress the aforementioned processes. Exploiting this information could help identify a role for metabolic therapies and/or dietary restriction control in optimal therapy response.

**BACKGROUND TO THE PROJECT**

Metastasis is the dissemination and formation of secondary tumours at distal sites that causes most of the morbidity and mortality associated with breast cancer and most other cancer types [1, 2]. Key characteristics of metastatic cells include their ability to migrate, invade surrounding tissue, survive in the periphery, and extravasate and colonize to distal sites of the body. Some of the advantageous traits that equip them with the capacity to metastasize may originate in the primary tumour, while others continue to evolve during the multistep cascade of the metastatic process [3]. One of these traits is metabolic reprogramming, which could be of relevance to additional selective fitness characteristics for metastatic cells, including their ability to evade host immune responses and co-opt with other tumour and stromal cells to promote their growth and invasion. The hostile microenvironment (i.e., hypoxia, low pH,

and low glucose) and the metabolic requirements of a fast-growing tumour sustain the Epithelial-to-Mesenchymal Transition (EMT) allowing cancer cells to bypass the nutrients and oxygen supply limitation caused by the rapid primary cancer growth, and colonize secondary sites to secure the adequate support of energy and nutrients.

Regarding metabolic reprogramming involved in breast cancer metastasis, recent evidence suggests that breast cancer cells with a high capacity to utilize or synthesize aspartic acid are more likely to survive in the blood circulation and promote the formation of lung metastases [4]. In addition, it has been reported that breast cancer cells utilize pyruvate to modify collagen in the microenvironment to promote the formation of lung metastases [5] and that proline catabolism promotes breast cancer metastasis to the lung [6]. However, the mechanistic basis of metabolic reprogramming events associated with breast cancer metastasis to the bone is still obscure.

**PROJECT AIMS**

This project focuses on gaining a mechanistic understanding of the metabolic reprogramming events associated with breast cancer metastasis to the bone. We propose to address the following Aims:

**Aim 1. Identification of metabolic enzymes and signaling pathways that promote bone metastasis**

a. Comprehensive analysis of the signaling and metabolic alterations underpinning tissue-specific metastasis

b. Tracking the metabolic determinants of bone-specific metastasis

c. Clinical validation of metabolic determinants of bone-specific metastasis

**Aim 2. Systematic interrogation of metabolic vulnerabilities for effective anti-metastatic therapeutic strategies**

a. Targeting cancer metabolism to combat bone metastasis of breast cancer

b. Elucidating the role of dietary interventions in therapy response

**RESEARCH PROPOSAL**

In the context of this Proposal, and building on our preliminary results, two main aims will be pursued:

**1. Identification of metabolic enzymes and signaling pathways that promote bone metastasis**

<u>a. Comprehensive analysis of the signaling and metabolic alterations underpinning tissue-specific metastasis</u>

In the preliminary experiments, MDA-MB-231 cells derived from the primary site (breast) following orthotopic injection, or brain and lung metastases following intravenous injection, or bone metastases following intracardial inoculation and consecutive rounds of *in vivo* selection in BALB/c nude mice were established, and showed distinct metabolic signatures. We will establish another set of metastatic clones using the same approach following injection of the 4T1 mouse breast cancer cells in Balb/c mice. Using these lines, we will conduct RNA-sequence analysis alongside, multiplex proteome and phosphoproteome analysis using tandem mass tags (TMTs) labelling to identify the metabolic genes/enzymes and signalling pathways that are differentially regulated in these cells. Stable isotope labelling studies using media supplemented with either 13C-Glucose, 13C-Glutamine or 15N-Glutamine will be performed to trace key metabolic pathways across the different metastatic clones.

<u>b. Tracking the metabolic determinants of bone-specific metastasis</u>

A pooled *in vivo* loss-of-function genetic screening approach using CRISPR–Cas9 genome editing in xenograft tumors will be used with two different purposes: (i) identifying metabolic genes required for cell migration and invasion, and (ii) identifying metabolic genes associated with metastatic tropism to specific secondary sites. After transduction and *in vitro* passage to allow gene editing to take place, tumor cells will be orthotopically injected into NSG mice. Inspection of the list of genes targeted by sgRNAs enriched in different metastatic sites will lead to the identification of genes conferring either (i) high metastatic potential or (ii) specific metastatic site phenotype. The effect of gene silencing will be evaluated on the formation of bone metastasis following intracardial inoculation of the bone metastatic subclones. Finally, a number of different metabolic tracking tools (bioenergetic profiling, central carbon metabolomics, lipidomics, stable-isotope labelling experiments) will be used to examine whether the metabolic enzymes identified in the aforementioned screen contribute to the characteristic metabolic features of the bone metastatic BC subclones.

c. Clinical validation of metabolic determinants of bone-specific metastasis

We will examine the expression of metabolic enzymes or signaling pathway proteins that have been shown to promote bone metastasis in the series of experiments described in section 1a and 1b using publicly available datasets e.g. TCGA or INSERM, Provisional. Our lab has also established a biobank of >100 primary and metastatic breast tumors that have been extensively characterized for their central carbon and lipid metabolism. For tissue specimens where both primary tumors and bone metastases are available from the same patient, RNA sequence analysis and immunohistochemical staining will be used to identify biomarkers driving preferential metastasis to the bone.

**2. Systematic interrogation of metabolic vulnerabilities for effective anti-metastatic therapeutic strategies**

a. Targeting cancer metabolism to combat bone metastasis of breast cancer

Taking advantage of the disease models generated in the previous stages of this project, *in vivo* experiments will be performed to explore the therapeutic relevance of the genes identified as potential therapeutic targets. Experimental approaches similar to those already developed by our group will be followed to perform these experiments. Therefore, a specific search for small molecule inhibitors targeting the metabolic enzymes of interest will be performed. Priority will be given to the evaluation of these metabolic inhibitors that are already in the clinic or under clinical trials. Moreover, to investigate whether the metabolic inhibitors can be used to prevent or slow down the formation of bone metastases, we will conduct another experiment in which the breast cancer cells and the inhibitors will be administered simultaneously. If there are no existing inhibitors for the metabolic enzymes or signaling pathway, we will conduct in silico and in vitro drug screening to identify drugs that inhibit these metabolic enzymes or signaling pathway components, and conduct *in vivo* treatment experiments as described previously.

b. Elucidating the role of dietary interventions in therapy response

The metabolites and metabolic pathways identified in the series of experiments described in Aim 1 are considered to promote bone metastasis of breast cancer cells. Therefore, we will examine whether bone metastasis can be inhibited by feeding mice specific diets that suppress these metabolites. In particular, it is expected that bone metastasis may be inhibited by the combination of a metabolic inhibitor and dietary intervention. We will first assess the synergistic effect *in vitro* and then examine whether the formation of bone metastases and/or the growth of tumors in the bone are inhibited through a “drug and diet” combination.

The present study is original and is likely to have wider implications both in terms of preventing breast cancer disease progression to the bone and/or effectively treating bone metastasis based on the metabolic dependencies of the breast cancer cells with higher propensity to metastasize to the bone. More generally, this study could demonstrate

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that cancer cells with specific metabolic traits can metastasize to specific organs, providing a new concept for the seed and soil theory, which could be mediated by changes in metabolism.

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


1. Chaffer, C.L. and R.A. Weinberg, *A perspective on cancer cell metastasis.* Science, 2011. **331**(6024): p. 1559-64.
2. Perez, E.A. and J.P. Spano, *Current and emerging targeted therapies for metastatic breast cancer.* Cancer, 2012. **118**(12): p. 3014-25.
3. Celia-Terrassa, T. and Y. Kang, *Distinctive properties of metastasis-initiating cells.* Genes Dev, 2016. **30**(8): p. 892-908.
4. Knott, S.R.V., et al., *Asparagine bioavailability governs metastasis in a model of breast cancer.* Nature, 2018. **554**(7692): p. 378-381.
5. Elia, I., et al., *Breast cancer cells rely on environmental pyruvate to shape the metastatic niche.* Nature, 2019. **568**(7750): p. 117-121.
6. Elia, I., et al., *Proline metabolism supports metastasis formation and could be inhibited to selectively target metastasizing cancer cells.* Nat Commun, 2017. **8**: p. 15267.


**CANDIDATE PROFILE**

**Note:** the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

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| **Pre-requisite qualifications of applicants:** | BSc in Biochemistry, Cell Biology or equivalent |
| **Intended learning outcomes:** | • Attain a thorough knowledge of the literature and a comprehensive understanding in the area of metastasis and cancer metabolism.<br>• Be able to demonstrate originality in the application of targeting tumour metabolism.<br>• Learn how to perform label free and isotope-assisted metabolomics and acquire expertise in *in vivo* CRISPR knockout screening.<br>• Gain skills in bioinformatics and data analysis.<br>• Acquire proficiency in acting autonomously in the planning and implementation of research, as well as working as part of a team.<br>• Gain oral presentation and scientific writing skills. |

**ADVERTISING DETAILS**

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| **Project suitable for a student with a background in:** | ☒ Biological Sciences<br>☐ Physics or Engineering<br>☐ Chemistry<br>☐ Maths, Statistics or Epidemiology |

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| | ☐ Computer Science<br>☐ Other (provide details) |
| **Keywords:** | 1. Cancer metabolism |
| | 2. Breast cancer |
| | 3. Bone metastasis |
| | 4. Metabolomics |
| | 5. Metabolic vulnerabilities |
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