The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title	Biology of Breast Cancer Metastasis
Short Project Title	Biology of Breast Cancer Metastasis

SUPERVISORY TEAM	
Primary Supervisor	Clare Isacke
Associate Supervisor(s)	Andrew Tutt

DIVISIONAL AFFILIATION	
Primary Division	Breast Cancer Research
Primary Team	Molecular Cell Biology
Site	Chelsea

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

This studentship is part of an Innovative Training Network (ITN) Marie Skłodowska-Curie Actions of the European Commission (https://h2020.org.tr/en/h2020/marie-sklodowska-curie-actions/itn). 15 PhD students will be recruited across 13 European institutes to participate in the 'EVOMET' training network 'Deconstructing the Evolution of Metastasis' (https://cordis.europa.eu/project/id/955951), tackling different aspects of the metastatic process. In addition to undertaking a dedicated research project at the ICR, the student will participate in EVOMET meetings, workshops and two 2-month secondments abroad.

This project will focus on invasive lobular breast cancer (ILC). ILC is the 2nd most common form of breast cancer representing 10 - 15% of all newly diagnosed breast cancers. Biologically, ILC is distinguished by the loss of functional cell-cell adhesions, usually through genetic deletion of the E-cadherin (*CDH1*) gene or functional E-cadherin inactivation of E-cadherin. At present, the treatment of ILC patients is complicated by a number of factors. First, due to the loss of E-cadherin, lobular breast cancer cells invade into in a diffuse 'single file' pattern which limits early detection. Second ILCs have a different metastatic spectrum with a greater propensity to develop ovarian, gastrointestinal and leptomeningeal metastases, with the tumour cells typically spreading over the organ surfaces. Finally, ILCs respond poorly to chemotherapy limiting the therapeutic options for ILC patients that have become insensitive to hormonal therapies (Thomas et al., 2019; Reed et al., 2015).

In this project, the student will interrogate the biology of ILCs, specifically the mechanisms which govern the unique pattern of ILC dissemination, to identify potential therapeutic opportunities for targeting ILCs in the clinic.

PROJECT AIMS

The overall goal of this project is to identify new strategies for the treatment of ILC patients.

Using a combination of biochemical, in vitro, ex vivo and in vivo approaches the student will

(a) establish assays to study the interaction of ILC cells with the metastatic environment

(b) develop screens to identify key pathways that could be targeted to limit ILC metastatic spread

(c) perform ultra-low pass whole genome and whole exome sequencing of primary tumour samples and liquid biopsies (plasma, peritoneal or pleural fluid, cerebrospinal fluid) from ILC patients to (i) identify potential genetic drives of ILC metastatic spread, (ii) determine the clonal evolution of ILC metastases, and (iii) for the development of diagnostic and disease monitoring biomarkers.

RESEARCH PROPOSAL

The overall goal of this project is to identify new strategies for the treatment of ILC patients in the clinic.

Our laboratory has a wealth of experience in studying the tumour microenvironment and mechanism of metastatic spread, as well as in performing genetic screens (Avgustinova et al., 2016; Jungwirth et al., 2020; Perkins et al, 2020; Murugaesu et al., 2014; van Weverwijk et al 2019). We have developed a number of reagents and protocols for this project. These include (i) the generation of ILC patient-derived organoids, (ii) the generation of mouse models of ILC metastasis, (iii) the collaborations and necessary ethical approval for the collection of ILC patient samples from the clinic. In addition to being part of the EVOMET training programme (https://cordis.europa.eu/project/id/955951), our laboratory is a member of the European Lobular Breast Cancer Consortium (ELBCC, https://www.elbcc.org/). These European networks will provide the student with a wealth of expertise to draw up and a highly collaborative community to interact with.

Aim 1: Establish assays to study the interaction of ILC cells with the metastatic environment
The student will focus on understanding how loss of E-cadherin junctions in ILC alters tumour cell drives an altered biology at the metastatic sites. In particular the student will address why ILCs tend to spread over the surface of the ovaries, intestinal organs and meninges, rather than invading into the tissue parenchyma. To achieve this the student will develop co-culture assays in vitro and ex vivo, using PDO models and cell lines generated in our laboratory and provided by our collaborators. Using these assays, the student will address the role of E-cadherin loss in driving this unique biology

Aim 2. Develop screens to identify key pathways that could be targeted to limit ILC metastatic spread
Using the assays developed in Aim 1, the student will devise unbiased shRNA and/or CRISPR-Cas9 based screens to identify synthetic lethal interactions that operate in this biologically relevant environment. These genetic screens will be complemented with small molecule inhibitor screens. In conjunction with these screens, the student will extend their findings from Aim 1 to determine how disrupting unique features of the ILC biology modulates metastatic potential. Validation studies will be performed using the clinically relevant ILC mouse models developed in our laboratory. This part of the project will benefit from the extensive screening expertise in the neighbouring laboratory led by Professor Chris Lord (Bajrami et al., 2018). In addition, advice on the identification of potential therapeutics will be provided by the Division of Cancer Therapeutics at the ICR.

Aim 3: Perform ultra-low pass whole genome and whole exome sequencing of primary tumour samples and liquid biopsies from ILC patients.
In conjunction with our colleagues at the Royal Marsden and King's College London, the student will collect liquid biopsy samples (plasma, peritoneal or pleural fluid, cerebrospinal fluid) from ILC patients in the clinic. Circulating tumour DNA (ctDNA) will be isolated using well established protocols used in our laboratory and, together with DNA extracted from archival patient primary tumour samples subject to ultra-low pass whole genome and whole exome sequencing to (i) identify potential genetic drives of ILC metastatic spread, (ii) determine the clonal evolution of ILC metastases, and (iii) for the development of diagnostic an disease monitoring biomarkers. These studies will be

supported by the expertise in ctDNA analysis in Nick Turner's laboratory at the ICR (O'Leary et al., 2020). Data analysis will be performed by the in-house Bioinformatics Facility with appropriate training provided for the student.

All of these studies will also be supported by members of the EVOMET Training Network.

Conclusion

This is an exciting project which addresses a clinically important question in breast cancer. The student will be provided with a wealth of training opportunities and benefit strongly from our world-class local environment, the specific expertise provided by the European Lobular Breast Cancer Consortium (ELBCC) and the broader and extensive expertise of the EVOMET training network.

LITERATURE REFERENCES

Avgustinova, A., Iravani, M., Robertson, D., Fearns, A., Gao, Q., Klingbeil, P., Hanby, A., Speirs, V., Sahai, E., Calvo, F. and Isacke, C. M. (2016) Tumour cell-derived Wnt7a recruits and activates fibroblasts to promote tumour aggressiveness. *Nature Communications* 7:10305

Bajrami I, Marlow R, van de Ven M, Brough R, Pemberton HN, Frankum J, Song F, Rafiq R, Konde A, Krastev DB, Menon M, Campbell J, Gulati A, Kumar R, Pettitt SJ, Gurden MD, Cardenosa ML, Chong I, Gazinska P, Wallberg F, Sawyer EJ, Martin LA, Dowsett M, Linardopoulos S, Natrajan R, Ryan CJ, Derksen PWB, Jonkers J, Tutt ANJ, Ashworth A, Lord CJ. (2018) E-Cadherin/ROS1 Inhibitor Synthetic Lethality in Breast Cancer. *Cancer Discov*. 2018:498-515.

Jungwirth, U., van Weverwijk, A., Jenkins, L., Alexander, J. Vicente, D., Gao, Q., Haider, S., Iravani, M. and Isacke, C.M. (2020) Impairment of a distinct cancer-associated fibroblast population limits tumour growth and metastasis (*in revision*): *bioRxiv* 2020.05.17.100412; doi: https://doi.org/10.1101/2020.05.17.100412

Murugaesu, N., Iravani, M., van Weverwijk, A., Ivetic, A., Johnson, D.A., Antonopoulos, A., Fearns, A., Jamal-Hanjani, M., Sims, D., Fenwick, K., Mitsopoulos, C., Gao, Q., Orr, N., Zvelebil, M., Haslam, S.M. Dell A., Yarwood, H., Lord, C.J., Ashworth, A. and Isacke, C.M. (2014) An *in vivo* functional screen identifies ST6GalNAc2 sialyltransferase as a breast cancer metastasis suppressor *Cancer Discovery* 4:204-317

O'Leary B, Cutts RJ, Huang X, Hrebien S, Liu Y, André F, Loibl S, Loi S, Garcia-Murillas I, Cristofanilli M, Bartlett CH, Turner NC.(2020) Circulating Tumor DNA Markers for Early Progression on Fulvestrant With or Without Palbociclib in ER+ Advanced Breast Cancer. *J. Natl Cancer Inst.* 2020 doi: 10.1093/jnci/djaa087. Online ahead of print.PMID: 32940689

Perkins, D.W., Haider, S., Robertson, D., Buus, R., O'Leary, L. and Isacke, C.M. (2020) Therapy-induced senescence in normal tissue promotes breast cancer metastasis. *BioRxiv* doi: https://doi.org/10.1101/2020.10.17.343590

Reed, A., Kutasovic, J., Lakhani, S.R. and Simpson, P.T. (2015) Invasive lobular carcinoma of the breast:morphology, biomarkers and 'omics. Breast Cancer Research 17:12

Thomas, M., Kelly, R.D., Abraham, J. and Kruse, M. (2019) Invasive lobular breast cancer: A review of pathogenesis, diagnosis, management, and future directions of early stage disease *Seminars in Oncology.* 46:121-132

van Weverwijk, A., Koundouros, N., Iravani, M., Ashenden, M., Gao, Q., Poulogiannis, G., Jungwirth, U. and Isacke, C.M. (2018) Metabolic adaptability in metastatic breast cancer by AKR1B10-dependent

balancing of glycolysis and fatty acid oxidation. *Nature Communications* 10(1):2698. doi: 10.1038/s41467-019-10592-

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)	SPECIAL REQUIREMENTS • Applicants can be of any nationality • If the applicant's first language is not English: They must be able to demonstrate a proficiency in English to the equivalent of an IELTS score of 7.0, with a minimum of 6 in any one component; -or- within the last two years in a majority English speaking country, have either education experience in English for a minimum of 1 year; or work experience in English for a minimum of 18 months and be able to satisfy Home Office visa criteria where necessary. • Applicants must be eligible to enroll in a PhD programme at the ICR. • Being a PhD student in an ITN includes a considerable amount of travelling including participation in meetings and workshops and will complete two 2-month secondments at Fundació Institut d'Investigació Biomèdica de Bellvitge (IDIBELL, Spain) and at Intomics A/S (IS, Denmark). **Candidates will be required to meet the Marie Skłodowska-Curie Early Stage Researcher eligibility criteria:** • Mobility Rule: researchers must not have resided or carried out their main activity (work, studies, etc.) in the United Kingdom for more than 12 months in the 3 years immediately before the recruitment date. Compulsory national service, short stays such as holidays, and time spent as part of a procedure for obtaining refugee status are not taken into account. The earliest possible recruitment date is 1 March 2021, with a student expected to be in place by August 2021. • Early Stage Researcher (ESR) criteria: ESRs must, at the date of recruitment by the host organisation, be in the first four years (full-time equivalent research experience) of their research careers and have not been awarded a doctoral degree. Full-Time Equivalent Research Experience is measured from the date when the researcher obtained the degree entitling him/her to embark on a doctorate (either in the country in which the degree was obtained or in the country in which the researcher is recruited, even if a doctorate was never started or envisaged).

	• **You will only be considered for the position if you fulfil (and can prove so) the above eligibility criteria.** The project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 955951"
Intended learning outcomes	
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. Breast cancer**
	2. Invasive lobular breast cancer
	3. Metastasis
	4. Microenvironment
	5. Screen
	6.