# **Queen’s Doctoral Training Programme - Multi-dimensional approaches to understanding microbe/host interactions in the context of disease, therapeutics and community resilience**

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| **Title of studentship** | Investigating the clinical and molecular significance of the microbiome in breast cancer |
| **Project summary (max 250 words – this will be used to advertise the project if selected).** | Breast Cancer (BC) is the most common cancer in the UK, accounting for 15% of new cancer cases. There is a significant unmet clinical need to understand why some cancers do not respond to treatment and what drives metastasis.  With the advent of next-generation sequencing, we have an unprecedented ability to study tumour and host genomes as well as those of the vast array of microorganisms that exist within living organisms. Evidence now suggests that these microbes may confer susceptibility to certain cancers and may also influence response to therapeutics and metastatic potential. Recently, a unique microbiome has been identified in the breast – a site previously thought to be sterile. Furthermore, a number of studies have shown significant differences in the bacteria present in BC and normal breast suggesting that microbial dysbiosis may contribute to BC development and progression.  We hypothesise that the microbiome, and specifically bacteria, associated with BC can have a significant impact the cancer cell and tumour microenvironment thereby impacting key phenotypes such as chemotherapy response and metastasis. By better understanding the molecular consequences of the presence of the bacteria, we can develop prognostic/predictive biomarkers and/or novel therapeutic strategies to improve BC outcome.  This project will investigate the clinical and molecular consequences of bacterial colonisation integrating molecular pathology, *in silico*, *in vitro* and *in vivo* approaches using both patient samples and cell lines providing the student with a range of molecular biology and bioinformatic skills to tackle a highly translational research question with real potential for impact. |
| **Supervisor(s)** | Dr Niamh Buckley, Prof. Michael Tunney and Dr Mark Tagney (UCC) |
| **What types of new collaborative relationships would this studentship support (e.g. development of national and international collaborations or industrial involvement/financial support) (100 words max)** | This project will develop a new collaborative link between Dr Buckley and Prof Tunney bringing together expertise in cancer biology and microbiome research. The supervisory team will also seek guidance from Prof Dan Longley (PGJCCR) who has experience in investigating the impact of *F. nucleatum* in colorectal cancer using both clinical and *in vitro* samples. A new collaborative link will also be established with the Cork Cancer Research Centre and APC Microbiome Centre at University College Cork though links with Dr Mark Tagney who has expertise in investigating the microbiome in breast cancer, harnessing knowledge for diagnostic and therapeutic applications. |
| **Research centre / School** | School of Pharmacy |
| **Subject area** | Breast Cancer |
| **Candidate requirements / Key skills required for the post.** Please note for the QUB-DTP awards applicants must have a 1st or 2.1 Honours degree (or equivalent) in a relevant subject. | Applicants should have a 1st or high 2.1 honours degree (or equivalent) in a relevant subject. Relevant subjects include Pharmacy, Molecular Biology, Pharmaceutical Sciences, Biochemistry, Biological/Biomedical Sciences, Microbiology or a closely related discipline. Students who have a 2.2 honours degree and a Master’s degree may also be considered, but the School reserves the right to shortlist for interview only those applicants who have demonstrated high academic attainment to date |
| **Relevant links for project advertisement/ more information** | <https://www.qub.ac.uk/schools/SchoolofPharmacy/Research/find-a-phd-supervisor/dr-niamh-buckley.html>  <https://www.qub.ac.uk/schools/SchoolofPharmacy/Research/find-a-phd-supervisor/professor-michael-tunney.html>  <https://www.qub.ac.uk/schools/SchoolofPharmacy/> |
| **Keywords for search filters** | Breast cancer, personalised medicine, microbiome, biomarkers, novel therapies |
| **Training provided through the research project** | This project will investigate the clinical and molecular consequences of bacterial colonisation integrating molecular pathology, *in silico*, *in vitro* and *in vivo* approaches using both patient samples and cell lines providing the student with a range of molecular biology and bioinformatic skills to tackle a highly translational research question with real potential for impact. |
| **Expected impact activities** | This project has significant potential for impact on the management of breast cancer and specifically secondary breast cancer.  Research will be shared at national and international conferences as well as outreach activities including the annual patient information event organised by Dr Buckley in collaboration with Action Cancer and BRCALinkNI  Research findings will be published in high impact journals and relevant resources/data made available to the wider research community as appropriate. |

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**Project details including expertise in area and workplan (max 1200 words)**

Breast Cancer (BC) is the most common cancer in the UK, accounting for 15% of new cancer cases. While survival rates from primary/localised BC have significantly improved, there is no cure for secondary BC with an estimated 1000 women dying each month and 35,000 people living with the disease in the UK1. There is therefore a significant unmet clinical need to understand why some cancers do not respond to treatment and what drives cancer metastasis.

With the advent of next-generation sequencing, we have an unprecedented ability to study tumour and host genomes as well as those of the vast array of microorganisms that exist within living organisms. Evidence now suggests that these microbes may confer susceptibility to certain cancers and may also influence response to therapeutics (including immunotherapy) and metastatic potential2. This is best described in the context of the gut microbiome and colorectal cancer (CRC), however, recently, a unique microbiome has been identified in the breast – a site previously thought to be sterile3,4. Furthermore, a number of studies have shown significant differences in the bacteria present in BC and normal breast suggesting that microbial dysbiosis may contribute to both BC development and progression3,4.

This includes overabundance of *Fusobacterium nucleatum*, an obligate Gram-negative anaerobic bacterium which is the most abundant bacterial species in the oral cavity. *F. nucleatum* is prevalent in CRC where it has been shown to promote tumourigenesis, inhibit anti-tumour immune cells and induce resistance to chemotherapy, all leading to poor outcome5,6. It is thought to spread through the hematogenous rather than gastrointestinal route where it colonises the CRC cells which display high levels of Gal-GalNAc. More recently, high levels of both *F. nucleatum* and Gal-GalNAc have also been detected in BC with *in vivo* studies showing accelerated tumour growth and metastatic progression associated when mice are inoculated with the bacteria7. This is thought to immune mediated with decreased CD4+ and CD8+ T cells observed in the tumours while increased metastasis may be linked to increased expression of MMP97.

Other BC-associated bacteria, *E.coli* and *L. welshimeri,* have also been shown to modulate the efficacy of chemotherapeutic drugs including decreasing the efficacy of gemcitabine8, which is often used for recurrent BC treatment. This was shown to through biotransformation of the drug however other studies have also shown that the microbiota can enhance chemotherapy response by activating innate immune cells as well as local/systemic inflammation9. Bacteria have also been shown to modulate pathways within the host cell associated with chemotherapy response and oncogenesis including p53, DNA Damage Response, Wnt-β-catenin and MAPK/AKT.

*We hypothesise that the microbiome, and specifically bacteria, associated with BC can have a significant impact the cancer cell and tumour microenvironment thereby impacting key oncogenic phenotypes such as chemotherapy response and metastasis. By better understanding the molecular consequences of the presence of the bacteria, we can develop prognostic/predictive biomarkers and/or novel therapeutic strategies to improve BC outcome.*

**Aim1: Investigate the prevalence of cancer associated bacteria in a BC cohort**

The prevalence of key BC-associated bacteria will be assessed in a well described cohort of 300 BC samples with clinicopathological data and >5yr follow up10,11. Given the links to BC, *F. nucleatum* will be prioritisedand quantified using validated qPCR primers using DNA already extracted available through the Northern Ireland Biobank. The abundance (copy number) will be correlated with outcome (RFS/OS), molecular subtype12,13 and other clinical data such as age, grade and stage. If required further bacteria with known links to BC, such as *E.coli* and *L. welshimeri,* can be investigated although the number will been limited to facilitate the downstream studies.

*This will provide the student with key skills in qPCR from clinical samples as well as analysis using relevant statistical approaches.*

**Aim2: Investigate the molecular significance of the presence of bacteria in cancer samples**

Extensive molecular profiling is also available for this cohort including transcriptomics and IHC-based profiling of the tumour microenvironment (TME) including markers of T and B cells, macrophages and immune checkpoints. Gene expression data has also been deconvoluted to further profile the TME11. Bacterial abundance will be correlated with TME markers as a continuous and also stratified into high/low levels based on median or as guided by the clinical significance determined in Aim1. The gene expression profiles of tumours with high vs low expression will be compared using differential gene expression as well as Gene Set Enrichment Analysis to identify key genes/pathways associated with bacteria colonisation. This will be compared to findings from CRC studies to gain insight into any breast cancer specific signalling. These may serve as biomarkers and/or represent therapeutic targets.

*This will provide the student with key skills in bioinformatics using both online tools and R-based packages.*

**Aim3: Assess impact of bacteria on key cancer phenotypes *in vitro* and *in vivo* to identify biomarkers and/or actionable pathways modulated by bacterial infection.**

The impact of co-culture of BC-associated bacteria on BC cell lines will be assessed in the context of key cancer phenotypes (proliferation/viability, migration/invasion, response to chemotherapy (especially Standard of Care e.g. FEC) using a range of MOI. Cell lines representing the molecular heterogeneity will be used (e.g. intrinsic subtypes, p53wt/mut) and choice may be guided by the clinical significance determined in Aim1. Bacterial colonisation will be assessed (e.g. biofilm formation) and correlated with Gal-GalNac levels of the cell lines (measured using FITC labelled PNA, a Gal-GalNac specific lectin) to give further insight into whether different subtypes of cancer are more susceptible to colonisation.

While the immunosuppressive role of bacteria such as *F. nucleatum* has been well described, it is not clear whether this is only due to direct effects of the bacteria on the immune cells or whether the bacteria can modulate the cancer cell itself which in turn influences the TME. Therefore, key immune-related signalling pathways within the cancer cells +/- co-culture will be investigated using luciferase-based reporter assays (NFκB, Jak/STAT) and qPCR. *F. nucleatum* has also been shown to modulate intracellular signalling (EMT, Wnt-β-catenin pathway and autophagy) within cancer cells so these pathways will also be investigated using established reagents in the lab. Results from Aim2 will also guide additional pathways for investigation. The *in vitro* and *in silico* findings will be integrated to identify clinically relevant, actionable pathways modulated by bacteria with findings validated *in vitro* using pharmacological and/or RNAi-based pathways inhibitors.

Key findings will be validated *in vivo* using syngeneic murine models (e.g.4T1,AT3) which have previously been shown to be colonised by *F. nucleatum7*. The 4T1 model provides an excellent *in vivo* model as both primary tumour and spontaneous metastasis are easily established. Cells will be implanted orthotopically followed by tail vein delivery of bacteria plus relevant treatments with tumour progression and metastasis as key endpoints with ex-vivo analysis if required.

*This will provide the student with a broad range of molecular biology and in vivo skills and allow integration of in silico and in vitro approaches.*

Dr Buckley is an expert in breast cancer research with extensive experience in the integration of molecular pathology, in silico and in vitro approaches. She will provide training in molecular and bioinformatics skills and ensure the translational relevance of the project. Prof Tunney is an expert in microbiome research and will provide expertise in culture and molecular microbiology methods. Dr Tagney is an expert in studying and exploiting the microbiome for therapeutic gain and has experience in identifying breast cancer specific bacteria.

1 *Secondary Breast Cancer Information: breastcancernow.org*.

2 Helmink, B. A., Khan, M. A. W., Hermann, A., Gopalakrishnan, V. & Wargo, J. A. The microbiome, cancer, and cancer therapy. *Nature medicine* **25**, 377-388, (2019).

3 Chen, J. *et al.* The microbiome and breast cancer: a review. *Breast Cancer Res Treat* **178**, 493-496, (2019).

4 O'Connor, H. *et al.* Resident bacteria in breast cancer tissue: pathogenic agents or harmless commensals? *Discov Med* **26**, 93-102, (2018).

5 Castellarin, M. *et al.* Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res* **22**, 299-306, (2012).

6 Kostic, A. D. *et al.* Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. *Genome Res* **22**, 292-298, (2012).

7 Parhi, L. *et al.* Breast cancer colonization by Fusobacterium nucleatum accelerates tumor growth and metastatic progression. *Nature communications* **11**, 3259, (2020).

8 Lehouritis, P. *et al.* Local bacteria affect the efficacy of chemotherapeutic drugs. *Sci Rep* **5**, 14554, (2015).

9 Roy, S. & Trinchieri, G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer* **17**, 271-285, (2017).

10 Boyle, D. P. *et al.* The prognostic significance of the aberrant extremes of p53 immunophenotypes in breast cancer. *Histopathology* **65**, 340-352, (2014).

11 Parkes, E. *et al.* The clinical and molecular significance associated with STING signaling in estrogen receptor-positive early breast cancer. *bioRxiv*, (2020).

12 Perou, C. M. *et al.* Molecular portraits of human breast tumours. *Nature* **406**, 747-752, (2000).

13 Goldhirsch, A. *et al.* Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* **22**, 1736-1747, (2011).