Project Details	
Project Code	MRC23IIARBr Koh
Title	Immune and therapeutic implications of intratumour bacteria
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Intratumour microbiota (i.e. bacteria living inside tumour cells) is an emerging hallmark that has been clinically validated in many tumour types. You will study the role of intratumour microbiota in promoting chemoresistance and characterise the associated tumour immune microenvironment. Once validated, this project will be the first demonstration of how intratumour microbes alter tumour behaviours, paving the way to more mechanism-directed clinical interventions.
Description	Tumour-resident intracellular microbiota is an emerging tumour feature that has been clinically established in a number of tumour types including breast cancer. Recently, a seminal paper shows that intratumour microbiota reorganises actin cytoskeleton in breast cancer cells, leading to increased tolerance towards mechanical stress and hence metastatic potential [1]. Based on our initial discovery that aberrant expression of RASAL2 (an endogenous RAS regulator implicated in cytoskeletal organisation and metastasis) leads to breast cancer chemoresistance [2], we hypothesise that intratumour microbiota represents an independent but potentially cooperative mechanism through which tumour cells gain tolerance towards chemotherapy insult. The student will work towards the following Objectives. Objective 1: Establish the role of intratumour microbiota in breast cancer chemoresistance (Year 1-2) The student will test the hypothesis that intratumour microbiota confers chemoresistance. To this end, breast cancer cells will be co-cultured with Staphylococcus aureus at clinically relevant ratios (up to ~0.2 million bacteria per gram of tumour cells). Staphylococcus aureus is chosen as the representative microbial model because it is one of the facultative intracellular bacteria most commonly found in breast tumour patient specimens. Cancer cells harbouring the bacteria will be exposed to conventional chemotherapy, such as doxorubicin and carboplatin. Their viability will be assessed by comparing it with the results from bacteria-free cells and RASAL2 cells (which are chemoresistant, thus serving as positive controls). Further validation will be done using independent breast cancer cell lines, 3D cultures (spheroids and organoids) and primary ex vivo human cultures. Objective 2: Establish the impact of intratumour microbiota on the tumour immune microenvironment (Year 3-4) The student will test the hypothesis that intratumour microbiota alters the tumour immune microenvironment. To this end, murine 4T1 breast cancer cells harbouring Staphylococcus aureus will be implanted orthotopically into the mammary fat pads of the syngeneic Balb/c mouse model. The immune microenvironment (specifically the composition of immune cells) will be profiled at predefined timepoints using multiplex flow cytometry and immunohistochemistry. Further immunohistochemical validation will be done on clinical specimens of breast tumour, which will be obtained through collaboration with Bristol-based breast clinicians. While this project has defined, achievable and hypothesis-driven Objectives, it has been designed to be open-ended. Thus, the student will have the flexibility to steer the project in numerous directions that

	can lead to high-impact outcomes. For instance, in Objective 1, the student may choose to compare the implications by other types of common microbes found in breast tumour (Lactobacillus, Streptococcus and Enterococcus). In addition to chemoresistance, a few other implications worthy of investigation include tumour growth, tumour cell cycle and tumour secretome. In Objective 2, depending on their interest and initial findings, the student may focus their effort on a subset of immune cells, or on testing out immunotherapy/antibiotic-based treatment regimens relevant to the context they are studying. For example, T lymphocyte infiltration is highly prognostic in triple-negative breast cancer (of which 4T1 is a preclinical model). The student will be able to exploit our established co-culture tumour-T lymphocyte model to investigate underlying mechanisms related to intratumour microbes. References: [1] Fu et al. 2022. Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. Cell, 185(8):1356-1372. [2] Koh et al. 2021. RASAL2 Confers Collateral MEK/EGFR Dependency in Chemoresistant Triple-Negative Breast Cancer. Clin Cancer Res, 27(17):4883-4897.
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