Project Details	
Project Code	MRCIIAR24Br Itasaki
Title	How do tumour cells respond to infiltrated T cells and what factors are involved in the response?
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Tumour infiltrating lymphocytes (TILs) are lymphocytes that can be found within solid tumours. Some TILs are cancer-specific and if expanded can be used for immunotherapy. However, not all patients respond to TIL therapy. We aim to elucidate the impact that cancer cells might have on TIL function. Using 3D culture imaging, we reveal the interaction between cancer cells and TILs and examine possible factors that impact the way in which cancer cells respond to TILs.
Description	Tumour infiltrating lymphocytes (TILs) are lymphocytes that enter and reside in solid tumours as a part of the immune response. TILs have the potential to be able to kill cancer cells and as such are used as a promising immunotherapy. Here, TILs are collected from a patient's tumour, grown in vitro to a large number and then infused back into the patient to mediate immune-mediated cancer cell death. It is particularly effective in metastatic melanoma, however, only in some cases, not in all cases. It is yet to be understood what conditions and factors might impact the ability of immune cells to attack and kill cancer cells. Current studies mostly focus on the function of TILs, especially on CD8+ T cells that are capable of directly killing cancer cells. Little is known about how tumour cells respond to TILs. In this project, which will involve collaboration between experts in immunology and cancer cell biology, we will investigate the cancer cell response to TILs by (1) establishing an in vitro 3D culture imaging system and (2) identifying possible factors that affect the effective killing of cancer cells by TILs. We have studied the interaction of cancer cells and non-cancerous epithelial cells by 3D imaging analysis and found active interaction between them (Ivers et al., 2014). Cancer cells attack non-cancerous spheroids by extending a process toward the non-cancerous cells and attaching them. Other nearby cancer cells then gather at the site and eventually engulf the non-cancerous spheroids when the cancer cells dominate the population. This suggests that the cancer cells communicate with each other by secreting cytokines and cooperating. We have extensive experience in cancer 3D cultures both with and without the extracellular matrix (ECM) (Abe-Fukasawa et al., 2018; 2021). We also found the crucial role of ECM on TILs in exerting its function in attacking tumour cells (Pires et al., 2020). In this project, we will examine the way in which cancer cells respond to autologous TILs extracted from the same tumour. In the first objective, we will establish a 3D culture system that allows monitoring of the interaction between cancer cells and T-cells. Initially, we will use melanoma cell lines that are stably labelled with GFP, and melanoma-specific T cells that are immortalised and labelled. Once this system is technically established, we will use primary tumour cells and autologous TILs derived from melanoma patients. In the second objective, we will identify and test factors/conditions that impact cell-cell interaction and cancer cell survival. They will include the populational ratio of cancer cells and T cells, the concentration of immunoregulatory factors such as IL-2, the culture matrix and the use of

	CD8+ T cells with or without high-affinity CD8 mutants previously identified by our group which enhanced antigen sensitivity (Knezevic et al., 2023). We will also investigate the expression of immunosuppressive proteins that block T cells from attacking cancer cells, such as PD-1 on T cells and the ligand PD-L1 on cancer cells, thus, aiming to find out the mechanism of immunosuppression of tumours that hinders the immunotherapy efficacy. We will further employ transcriptome analysis to identify genes that are expressed in cancer cells and specifically involved in the response to T cells. Cancer cells cultured in different conditions will be collected to extract mRNA, which will be subject to RNAseq analysis. Such-obtained candidate genes will be validated by gain- and loss-of-function analyses in vitro in the 3D culture. Thus, we aim to identify factors that would facilitate effective immune therapy for cancer patients. Abe-Fukasawa et al., doi:10.1038/s41598-018-21950-5 (NI lab) Abe-Fukasawa et al., doi:10.1111/febs.15867 (NI lab) Ivers et al., doi:10.1186/s12935-014-0108-6 (NI lab) Knezevic et al., doi:10.1016/j.jbc.2023.104981 (LW lab) Pires et al., doi:10.1158/2326-6066.CIR-20-0070 (MS lab)
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