Project Details	
Project Code	MRC22IIARBa Jungwirth
Title	The regulation of tumour cell cytolysis by cancer associated fibroblasts
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Cytotoxic T cells can kill tumour target cells. However, this ability is widely suppressed in the tumour microenvironment. Using the in vitro reconstruction of this environment with three-dimensional tissue cultures, you will investigate how tumour associated fibroblasts in various differentiation states regulate cytolytic tumour cell killing per se and, in collaboration with our industrial partner AstraZeneca, upon therapeutic intervention.
Description	Tumours need and co-evolve with a permissive microenvironment. Cancer-associated fibroblasts (CAFs) are one of the most abundant cell types in the tumour microenvironment, supporting tumour progression (Jungwirth et al., Nat Coms, 2021). CAFs have heterogeneous phenotypes with divergent functions such as enhanced matrix remodelling and immune regulation. CAFs can secrete cytokines and chemokines to regulate immune cell differentiation and inhibit cytotoxic lymphocytes (CTL). CAFs thereby mediate an immune-suppressive microenvironment which impacts the efficacy of immunotherapy. CAFs and their interaction with CTL are of therapeutic interest in the immunotherapy of cancer for two reasons: The numbers and subtypes of CAFs associated with a tumour may allow the efficient stratification of patients in deciding which immunotherapy to use and inhibiting the immune-suppressive CAF phenotype may enhance the ability of CTL to kill tumour cells. For the effective characterisation and manipulation of different CAF populations in the context of tumour cell cytolysis, a robust in vitro dual/triple co-culture system of tumour cells, CTL with or without addition of CAFs would be invaluable. This project will establish such system using non-small lung cell carcinoma (NSCLC) tumour cells expressing NY-ESO as a tumour antigen, primary human T cells expressing the 1G4 T cell receptor that recognises a NY-ESO-derived peptide and human CAFs. This human co-culture system will be validated in our laboratories using data from already completed and future parallel work on a similar, murine tumour cell/ CTL/CAF co-culture system. We propose to address two questions. (1) The regulation of CTL function by distinct CAF subtypes. Adding CAF subtypes to a human dual co-culture system of NSCLC tumour cell spheroids and CTL expressing the 1G4 T cell receptor will allow ex vivo studies of the immune suppressive function of fibroblasts/CAFs. Distinct CAF populations and their markers have been identified in NSCLC. To recapitulate and investigate such subtypes, we will compare normal human lung fibroblasts and CAF cultures. We will test a range of in vitro activation strategies to generate distinct CAF characteristics (including activation through TGFβ, IL-1, IL-6 and direct NSCLC co-culture). A particular focus will be on podoplanin (PDPN)high and PDPNlow -expressing CAFs, which have been associated with an immunosuppressive CAF phenotype in NSCLC. 24h incubation of CTL with CAF-conditioned medium (including assessment of distinct cytokines and chemokines) and on CAF-derived matrices will allow a delineation of direct CAF influences on the cytolytic ability of CTL as determined in a subsequent tumour cell killing assay.

	(2) The mechanism of action of bi-specific T cell engagers as regulated by CAFs. The identification of tumour antigen-specific T cell receptors (TCR) is still a significant clinical challenge. Reagents to activate T cells independently of their endogenous TCR specificity, such as bi-specific T cell engagers, are, therefore, of therapeutic interest. However, substantial questions remain about how similar T cell function is in response to such engagers when compared to TCR engagement by peptide MHC. Using a dual human NSCLC tumour cell spheroid/ 1G4 TCR CTL co-culture system, we will determine T cell infiltration and tumour cell killing in spheroids as well as CTL calcium signalling and cytoskeletal polarisation after 16h spheroid co-culture to compare T cell activation through the 1G4 TCR versus bi-specific T cell engagers. Methods are established as described in Ambler et al. (Sci. Signal. 2020) and Edmunds et al. (BioRxiv 2021). Using findings from the first part of the project to determine most relevant CAF subtypes, we will finally determine how CAFs regulate the killing of tumour cell spheroids using T cell activation with bi-specific T cell engagers.
Supervisory Team	
Lead Supervisor	
Name	Dr Ute Jungwirth
Affiliation	Bath
College/Faculty	Faculty of Science
Department/School	Department of Pharmacy & Pharmacology
Email Address	uj217@bath.ac.uk
Co-Supervisor 1	
Name	Professor Christoph Wuelfing
Affiliation	Bristol
College/Faculty	Faculty of Life Sciences
Department/School	School of Cellular and Molecular Medicine
Co-Supervisor 2	
Name	Dr Simon Dovedi
Affiliation	Other
College/Faculty	
Department/School	
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	
Co-Supervisor 4	
Name	
Affiliation	
College/Faculty	
Department/School	