Project Details	
Project Code	MRC21IIRCa Godkin
Title	Investigating the mechanism of action of low dose cyclophosphamide in treating colorectal cancer
Research Theme	Infection, Immunity & Repair
Summary	Immunotherapy of colorectal cancer (CRC) is in its early stages, as the more commonly used checkpoint inhibitors usually do not work. This project will build on the observation that very low doses of cyclophosphamide can act as an immunotherapy controlling advanced CRC in approximately a third of cases. Mechanisms of success and failure will be explored, also employing a unique mouse model.
Description	Hypothesis: We have used low-dose cyclophosphamide (CPM) as an immunotherapy to prevent progression of advanced CRC in a 1/3 of patients. CPM may have several effects, allowing an anti-cancer immune response to develop, but also allowing the tumour stroma and microenvironment to alter facilitating tumour control. This will be tested in a new novel mouse model. Background: Cancer immunotherapy has had a resurgence in recent years due to the success of checkpoint inhibitors such as anti-PD1 antibody. However these are not effective in colorectal cancer (CRC). There is also evidence to indicate that targeting regulatory T cells represents an effective means of inducing immune responses to CRC. We recently published findings of a Phase I/II clinical study whereby CRC patients with late stage were given a course of low dose cyclophosphamide (CPM). This showed CPM to be a highly effective immunomodulator delaying disease progression in patients with advanced CRC (1), in part through depleting a subset of highly proliferative CD4+Foxp3+ regulatory T cells (Tregs). The mechanism through which CPM affects cancer progression is not fully understood. Research has been hampered by poor models not replicating the different molecular subtypes of human CRC. In collaboration (Prof Owen Sansom, Beatson Institute) we have obtained organoid cultures which have been genetically modified to replicate human molecular subtypes of CRC (2). We have recently established a superior model of colon cancer in mice, developing expertise in mouse colonoscopy to implant these organoids. This model is more physiological and allows monitoring of tumour growth/regression over time. We have already generated preliminary data demonstrating tumour regression on low dose CPM treatment of mice. We now wish to determine why CPM does not work in some cases, and whether low-dose CPM acts via immune modulation alone or includes other mechanisms such as impinging on the tumour stroma and vasculature. Aims: This project will exploit a novel mouse model of CRC to address the following aims: i) Determine how effective CPM is as treatment of CRC at different stages of development. ii) Explore the effects of CPM on tumour stroma. iii) Explore the effects of CPM on anti-tumour immune responses. Addressing these questions using this mouse model, will allow us to look at how these agents impact on mechanisms involved in tumour control, including the role of tumour stroma, the development of unique structures allowing ingress of lymphocytes (high endothelial venules [HEV] and tertiary lymphoid structures with our co-applicant in Bristol) and the infiltrate of anti-tumour lymphocytes. Understanding the mechanisms of non-response in

	tumours allows a future combination approach to improve efficacy. Methods: The methods and techniques involved in this project are established and combine the expertise of the three applicants. In particular, AG will direct the use of CPM and mouse colonoscopy, AMG is an expert on mouse models; imaging of tumours and the vasculature; and GJ has expertise in looking at HEV development and function. i) CRC organoids will be cultured in vitro. Two main types will be employed: AKPT carcinoma (imitating CRC Consensus Molecular Subtype[CMS] 2,3) and KPN carcinomas (imitating CMS4). ii). Organoid fragments are injected with a fine gauge needle into the submucosa using a mouse colonoscope. Mice used include wild type Blk/6. iii). Tumour growth kinetics monitored by repeat colonoscopy. Tumours will be treated by giving the mice CPM. Serial biopsies can be obtained via the colonoscope for proteomic analysis, resected tumours can be studied histologically to look at vascular anatomy, TILs extracted to look at T cell function in successful vs unsuccessful intervention. (1) JAMA Oncol. 2017 Oct 12;3(10):e172579 (2) Cancer Cell 2019;36, 319–336
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