

PhD Project Proposal

Funder details

Studentship funded by:

ICR

Project details

Project title:

Investigating the cytoprotective role of the ectonucleotidase CD38 in immune responses to cancer

Supervisory team

Primary Supervisor:

Dr Erik Wennerberg

Associate Supervisor(s):

Dr Esther Arwert

Secondary Supervisor:

Prof Pascal Meier

Divisional affiliation

Primary Division:

Radiotherapy and Imaging

Primary Team:

Radiation-enhanced
Immunotherapy

Site: Sutton

Project background

Immunotherapy in the form of immune checkpoint blockade (ICB) has transformed cancer treatment, becoming the standard of care in many settings. However, most cancer patients either do not respond to ICB, or do not experience long-term benefit [1]. To develop immunotherapeutic strategies that work for these patients, we need a better understanding of how tumours dysregulate tissue immune homeostasis, how they create immune checkpoints of their own, and ultimately how they escape immune attack.

We have recently identified the mono-ADP-ribosyltransferase ART1 as a novel tumour-expressed immune checkpoint [2]. Expression of ART1 allows cancer cells to use extracellular NAD⁺ (which is abundant in the tumour microenvironment) to ADP-ribosylate the purinergic P2X7 receptor (P2X7R) on tumour-infiltrating T cells. ART1-

mediated ADP-ribosylation of T cells can trigger an apoptotic cascade resulting in NAD-induced cell death (NICD), and subsequently tumour immune escape [2].

The ectonucleotidase CD38, is expressed on activated immune cells[3]. Through its function as a NAD⁺ glycohydrolase, CD38 catabolises extracellular NAD⁺ into ADPR, which serves two roles in maintaining tissue immune homeostasis in inflamed tissues: firstly by modulating local NAD⁺ levels to regulate ART-mediated NICD, and secondly by generating precursors for production of immunosuppressive adenosine [4-7]. The literature describing the CD38 regulation of NICD originate from pre-clinical models of tissue damage and infection where extracellular NAD⁺ concentrations are elevated. Although a similar inflammatory microenvironment is observed in solid tumours, the impact of CD38-mediated NICD regulation on the anti-tumour response remains entirely unexplored.

Pre-clinical lung cancer models have shown that CD38 expression promotes resistance to ICB, and attributes this to CD38-mediated adenosine generation [8]. However, none of the clinical trials which have combined anti-CD38 antibodies (daratumumab, isatuximab) with ICB (anti-PD-1/PD-L1) in patients with lung cancer (or other solid tumours) have generated therapeutic benefit compared with ICB alone [9, 10].

Hypothesis/Aims

We hypothesise that T cell expression of CD38 has a cytoprotective function in cancer, by safeguarding T cells and other lymphocytes against NICD in ART1-expressing solid tumours. Depending on the tumour context, this protective role of CD38 may be a critical determinant for the success or failure of anti-tumour immune responses, particularly in the context of ICB.

In this project, the student will elucidate the role of CD38 in maintaining viability and cell activation of T cells, as well as other adaptive and innate immune cells, using in vitro tumour-immune cell co-culture models. Further, the student will use cutting-edge techniques, including multi-parameter immunofluorescence and flow cytometry, as well as single cell RNA-sequencing and digital spatial profiling to characterize the tumour immune landscape and determine the immune-stimulatory and suppressive effects of anti-CD38/ICB combination treatments in different mouse models of lung cancer, as well as in unique patient samples.

The lab

Our lab is dedicated to developing immunotherapeutic strategies that can effectively treat advanced and poorly immunogenic cancers. We are a recently established lab building a supportive team which is based in a dynamic and diverse environment in the Division of Radiotherapy and Imaging. We are part of the wider Centre for Translational Immunotherapy, which brings together multidisciplinary immunology researchers across the ICR and the Royal Marsden. The student will have access to a wide network of national and international collaborators, including our close collaborators at Albert Einstein School of Medicine, New York. Our lab has an active public engagement programme that is central to our research approach and creates opportunities for development. The ICR provides an inspiring training environment for PhD students and early career researchers with opportunities and tailored courses to support scientific, personal, and wider career development. This fully funded 4-year ICR studentship will be supervised by Dr Erik Wennerberg, with co-supervision from Prof Pascal Meier and Dr Esther Arwert, who are experts in immunogenic cell death mechanisms and functional tumour immunology respectively.

Project aims

- **Aim 1:** Elucidate the role of CD38 in maintaining viability and cell activation of innate and adaptive immune cell subsets in tumour co-culture models.
- **Aim 2:** Characterize the tumour immune landscape in mouse models of lung cancer following treatment with anti-CD38 monoclonal antibodies with or without immunotherapy.
- **Aim 3:** Investigate therapy-induced changes in purinergic signalling molecules in clinical samples from cancer patients.

Research proposal

Aim 1:

Elucidate the role of CD38 in maintaining viability and cell activation of innate and adaptive immune cell subsets in tumour co-culture models.

Research question

Does expression of CD38 on different immune cell subsets convey protection against NICD and how does it impact cell activation, proliferation, and cytotoxic function?

Methodology

Murine lymphocytes (T cells, B cells and NK cells) as well as myeloid cells, (DCs, macrophages and neutrophils) will be isolated from spleen, bone-marrow, tumour, and tumour-draining lymph nodes of mice with proficient or deficient expression of CD38 and P2X7R, using magnetic bead- or flow-sorting. These will be co-cultured with recombinant NAD⁺ (including etheno-NAD), recombinant ART1, or with tumour cells. We have already generated lung cancer cells (KP1) with ART1 overexpression and conditional ART1 knockdown cells of ART1 (KP1-ART1OE, KP1-shART1). This aim will also determine whether pre-treatment of tumours with chemo/radiotherapy, and/or immunotherapy (anti-PD-1, anti-CTLA-4, anti-ART1 antibodies) modulates the extent and selectivity of NICD.

To accurately measure NICD, we have adapted a flow cytometry-based etheno-NAD assay to measure mono-ADP-ribosylation of immune cells[6]. Together with viability staining by annexin V/Propidium iodide we will determine cell death by apoptosis. This method will be complemented with live cell imaging (Phoenix Opera System) to determine the kinetics and sensitivity to MARYlation-induced calcium flux (Fura-2) as well as viability determined by nuclear dyes (NucBlue). Additionally, cell activation and function will be determined including assessment of cytokine production (intracellular staining, ELISA/ELISPOT) and in vitro killing assays.

The P2X7R exists in several splice variants with varying susceptibility to NICD following P2X7R MARYlation, which constitutes an immune escape strategy employed by tumour cells[11, 12]. qPCR analysis will be performed to determine expression of P2X7R variants by immune cells and tumour cells included in co-culture assays.

Expected outcomes

This aim will determine the susceptibility of different immune cells to NICD induced by tumour-derived NAD⁺ as well as the extent to which they rely on CD38 expression to counteract this process in modelled tumour conditions. Results from this aim will inform the focus of Aim 2 in terms of selection of immune markers for in vivo analysis.

Aim 2:

Characterize the tumour immune landscape in mouse models of solid cancer following treatment with anti-CD38 monoclonal antibodies in the context of immunotherapy and radiotherapy.

Research question

How does anti-CD38 treatment shape the immune landscape of solid tumours in the context of immunotherapy and radiotherapy, and does tumour ART1 expression predict treatment response?

Methodology

Initial studies will make use of ectopic flank and systemic lung tumour models including KP1-ART1OE, where modulation of ART1 expression by the tumour will be induced by doxycycline-mediated ART1 knockdown or with ART1-blocking antibodies (22C12)[2]. The treatment design will mimic the failed clinical trials in lung cancer patients where anti-CD38 antibodies were combined with anti-PD-1/anti-PD-L1 inhibitors. In vivo administration of murine equivalents of anti-CD38 (NIMR-5) and anti-PD-1 (RMP1-14) antibodies has already been established in the lab. Tumour-bearing lungs from treated mice will be analyzed for tumour burden by QuPath-based analysis of H&E-stained lung tissue sections and immune characterization will be performed using the following methods.

We have designed two standardized flow cytometry panels (20 parameters each) for immune characterization of murine tumours using the FACSymphony A5 platform. The two panels are customized for this project to quantify expression of P2X7R, CD38 and CD39 on lymphocytes and myeloid cells respectively. The lymphocyte panel captures memory T cell subsets, NK cell subsets and B cells while the myeloid panel captures conventional DC subsets, tumour-associated macrophages (TAMs), neutrophils, myeloid-derived suppressor cells (MDSCs) and cancer-associated fibroblasts (CAFs). This methodology will guide the use of bulk- and single cell-RNA sequencing on sorted leukocytes to determine therapy-induced changes in transcriptional patterns of each immune subset. Finally, our findings will be validated using multiparameter immunofluorescence as well as spatial transcriptomics analysis (GeoMX DSP platform) of mouse tumour tissues. This will further provide spatial information on expression patterns of purinergic signalling molecules (including CD38/P2X7R/ART1) and how these relate to the overall immune contexture.

An important focus of our team is to develop treatment strategies against advanced and immunologically cold tumours using radiotherapy/immunotherapy combinations. Guided by findings from Aim 1 and Aim 2, we will employ the immunotherapy-resistant Lewis Lung Carcinoma (LLC1) and immune cold MOC2 head and neck squamous cell carcinoma (HNSCC) model delivering immunogenic radiotherapy using the small animal radiation research platform (SARRP) in conjunction with ICB, ART1 blockade and evaluate the immunomodulatory role of CD38 inhibitors and agonists.

Expected outcomes

This aim will determine whether CD38 blockade has a detrimental or beneficial effect on anti-tumour immune responses in lung cancer and whether ART1 tumour expression stratifies the responses to CD38 therapy.

Aim 3:

Investigate therapy-induced changes in purinergic signalling molecules in clinical samples from cancer patients.

Research Question

Does radiotherapy and/or immunotherapy modulate the expression of purinergic signalling molecules in patients with solid tumours.

Methodology

In collaboration with clinical investigators at the Royal Marsden Hospital, we will analyse tumour biopsies collected pre-, during-, and post-treatment with radiotherapy and/or immunotherapy combinations. Several trials providing such samples are already completed/ongoing including the CHIMERA platform trial (Principal Investigator Anna Wilkins) which includes samples from lung cancer, HNSCC, bladder cancer, cervix cancer and rectal cancer. Whole exome, RNA and TCR sequencing of tumour biopsies and on PBMC from longitudinal blood samples will allow for differential gene expression analysis and identification of neo-antigens associated with therapy, as well as changes in clonality of the TCR repertoire over time, and correlation with treatment response. Guided by transcriptomic pathway analysis, we will, in selected samples, perform multiparameter immunofluorescence staining using the VECTRA system, and digital spatial profiling using the GeoMX DSP platform.

Expected outcomes

This aim will determine whether therapy-induced alterations of purinergic enzyme expression correlate with tumour immune infiltration. Studies of samples from radiotherapy-treated patients from the CHIMERA trial, will provide spatial and temporal data on CD38/P2X7R/ART1 expression and provide correlative data on how immune contexture and immunometabolic gene signatures correlate with changes in TCR repertoire and with treatment response.

Literature references

1. Karasarides, M., et al., *Hallmarks of Resistance to Immune-Checkpoint Inhibitors*. *Cancer Immunol Res*, 2022. **10**(4): p. 372-383.
2. Wennerberg, E., et al., *Expression of the mono-ADP-ribosyltransferase ART1 by tumor cells mediates immune resistance in non-small cell lung cancer*. *Sci Transl Med*, 2022. **14**(636): p. eabe8195.

3. Sandoval-Montes, C. and L. Santos-Argumedo, *CD38 is expressed selectively during the activation of a subset of mature T cells with reduced proliferation but improved potential to produce cytokines*. J Leukoc Biol, 2005. **77**(4): p. 513-21.
4. Krebs, C., et al., *CD38 controls ADP-ribosyltransferase-2-catalyzed ADP-ribosylation of T cell surface proteins*. J Immunol, 2005. **174**(6): p. 3298-305.
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6. Adriouch, S., et al., *NAD⁺ released during inflammation participates in T cell homeostasis by inducing ART2-mediated death of naive T cells in vivo*. J Immunol, 2007. **179**(1): p. 186-94.
7. Stark, R., et al., *T RM maintenance is regulated by tissue damage via P2RX7*. Sci Immunol, 2018. **3**(30).
8. Chen, L., et al., *CD38-Mediated Immunosuppression as a Mechanism of Tumor Cell Escape from PD-1/PD-L1 Blockade*. Cancer Discov, 2018. **8**(9): p. 1156-1175.
9. Pillai, R.N., et al., *Daratumumab Plus Atezolizumab in Previously Treated Advanced or Metastatic NSCLC: Brief Report on a Randomized, Open-Label, Phase 1b/2 Study (LUC2001 JNJ-54767414)*. JTO Clin Res Rep, 2021. **2**(2): p. 100104.
10. Simonelli, M., et al., *Isatuximab plus atezolizumab in patients with advanced solid tumors: results from a phase I/II, open-label, multicenter study*. ESMO Open, 2022. **7**(5): p. 100562.
11. Schwarz, N., et al., *Alternative splicing of the N-terminal cytosolic and transmembrane domains of P2X7 controls gating of the ion channel by ADP-ribosylation*. PLoS One, 2012. **7**(7): p. e41269.
12. Sainz, R.M., et al., *Tumour immune escape via P2X7 receptor signalling*. Front Immunol, 2023. **14**: p. 1287310.

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants:

Master's Degree in Biological Science

Intended learning outcomes:

- Develop a deep understanding of tumour immunology and cancer immunotherapy.
- Develop skills required to work with syngeneic immunocompetent mouse models of lung cancer, including acquiring a personal licence (PIL) from the Home Office.
- Proficiency and development of several lab techniques including isolation and culture of immune cells from mouse tissues, cell culture of tumour cell lines, flow cytometry acquisition and analysis, immunofluorescence microscopy and image analysis, RNA-extraction, and RNA-sequencing analysis.
- Develop critical thinking skills and being able to assess collected data in light of emerging literature.
- Presentation of data in lab meetings, workshops, as well as national and international conferences. Writing manuscript for peer-review publication in recognized journals.

Advertising details

Project suitable for a student with a background in:

Biological Sciences

Physics or Engineering

Chemistry

Maths, Statistics or Epidemiology

Computer Science