Project Details	
Project Code	MRCIIAR25Br Shytaj
Title	Exploring the modulation of Antioxidant Metabolism to target Adult T-Cell Leukaemia Induced by HTLV-1 Infection
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Human T-lymphotropic virus 1 (HTLV-1) causes lifelong infections and can lead to severe diseases like adult T-cell leukaemia/lymphoma (ATL), with limited treatment options. Our preliminary evidence shows HTLV-1 disrupts cellular metabolism, increasing oxidative stress to support rapid cell multiplication, potentially making infected cells reliant on antioxidant responses for their survival. This project aims to study the interplay between antioxidant metabolism and cell fate in the context of ATL. The final aim of the project is identify key antioxidant pathways crucial for ATL survival and test potential therapies in cell models to selectively eliminate infected tumor cells.
Description	The human T-lymphotropic virus 1 (HTLV-1) causes chronic lifelong infections, with limited therapeutic options available to mitigate its disease manifestations(1). These include severe neurological diseases and aggressive malignancies, such as adult T-cell leukaemia/lymphoma (ATL)(1). ATL is particularly challenging due to high chemotherapy resistance and frequent recurrences. Antiretroviral drugs developed against the better-studied human immunodeficiency virus (HIV-1), which belongs to the same family of retroviruses as HTLV-1, can decrease viral replication but do not target integrated retroviral DNA(2). Similarly, cells latently infected with HTLV-1 cannot be detected and eliminated by the immune system, ensuring their survival and persistence(3). One potential approach to eliminating HTLV-1 infected cells is based on the evidence that retroviral infection can cause cellular metabolic dysregulation. Our studies show that the main cellular targets of HIV-1 infection (CD4 T cells) exhibit increased reliance on antioxidant responses compared to uninfected cells(4-6). These antioxidant responses are a consequence of the burst in reactive oxygen species (ROS) induced by the initial infection, and we have shown that they can be therapeutically exploited to eliminate latently HIV-1 infected cells ex vivo and in vivo(6,7). HTLV-1 infection also preferentially targets CD4 T cells, and oxidative stress and ROS production have been described as metabolic hallmarks of HTLV-1 infection(8). Our preliminary evidence suggests that HTLV-1 infected cells, particularly malignant ATL cells, are characterised by a hyper-energetic state, with increased transcriptional and protein expression of the oxidative phosphorylation pathway (OXPHOS). OXPHOS metabolism is a primary source of ROS generation, potentially explaining the oxidative stress induced by HTLV-1 infection. Additionally, our metabolomic data indicate that HTLV-1 infected cell lines upregulate key metabolites sustaining OXPHOS (NADH) and the antioxidant response (NADPH). NADPH in turn plays a key role in replenishing the active pool of antioxidants, including glutathione (GSH), which can detoxify ROS in mitochondria(4). In line with this, previous studies suggest that the expression of the key antioxidant GSH is increased upon retroviral infection and malignant transformation(5). This upregulation could

	favour the survival of infected/cancer cells and confer chemotherapy resistance. Overall, these data suggest that metabolic, and especially ROS-antioxidant, imbalances could serve as markers and potential therapeutic targets to eliminate persistently HTLV-1 infected cells. This project aims to study the interplay between OXPHOS, antioxidant responses, and ATL development, proliferation, and survival. The first part of the project will generate a proteomic and metabolomic comparison of ATL and non-ATL cells using primary blood cells. To improve upon our existing datasets obtained in cell lines, this aim will use primary peripheral blood mononuclear cells (PBMCs) isolated from the blood of healthy donors and infected through co-culture with an irradiated ATL cell line, in line with established protocols(9). Uninfected PBMCs will serve as genetically matched controls. The potential markers of ATL identified through these analyses will be validated using PBMCs isolated from the blood of people living with HTLV-1/ATL, provided by with collaborators at the University of São Caetano do Sul (Brazil). The second aim of the project will be the phenotypic and functional characterisation of the cells harbouring markers of metabolic dysregulation identified in the first aim. Particular attention will be given to CD4 T cells, as they are the most likely target of HTLV-1 infection and are known to be more prone to ATL transformation(3). This aim will benefit from the expertise of the co-supervisor(s) Professor Ian Humphreys and Mathew Clement who have recently characterised how chronic virus infection can lead to an alteration in metabolic properties of CD4 T cells during chronic virus infection(10) and are currently investigating how T cell metabolism impacts vaccine-induced immunogenicity. The overall goal of this aim will be to select markers that could serve as therapeutic targets. The third aim of the project will be to screen putative ATL markers for their potential to serve as drug targets, by combining literature analysis and in silico screenings, leveraging existing laboratory expertise and collaborations (e.g., our MRC project partners at the University of Perugia, Italy). Potential drug candidates will be tested on the primary PBMC-ATL model to evaluate their selective effect in blocking proliferation or inducing cytotoxicity of ATL cells compared to uninfected, genetically matched PBMCs from the same donor. Throughout the aims of the project, the student will have considerable freedom in pinpointing the most promising markers to study in more depth and to steer downstream experiments based on a combination of omics data analysis and integration, literature research, and feasibility assessment of drug candidate testing. References 1. Martin et al. 2018; 391(10133):1893-1894. 2. Siliciano et al. Cold Spring Harb Perspect Med. 2011 Sep;1(1):a007096. 3. Bangham et al. 2019; 6(1):365-385. 4. Benhar et al. 2016. 5. Shytaj et al. 2020; 39(9):e102209. 6. Shytaj et al. 2021. 7. Shytaj et al. 2015 Aug;89(15):7521-35. 8. Takahashi et al. 2013. 9. Balestrieri et al. 2008 Aug;52(8):2771-9. 10 Clements et al. Elife. 2023 Jul 13;12:e79165.

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