CNIO - LILLY CELL SIGNALLING AND IMMUNOMETABOLISM SECTION

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Titulado Superior (Advanced Degree)

SCOPE OF THE ELI LILLY - CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer immunometabolism. Our Section is funded through a research contract with Eli Lilly and focuses on the identification of small molecular weight molecules that regulate the metabolism of malignant cells, with the objective of killing them either directly, acting synergistically with other antitumour agents, or activating the antitumour immune response. Exploring how to better target these mechanisms would lead to better and more efficient therapeutic options.

A combination of *in vitro* and *in vivo* approaches has been used to obtain a complete understanding of tumour metabolic reprogramming and the antitumour response. For this purpose, we have developed a series of biochemical and cell-based assays exploiting advanced techniques such as extracellular flux analysis (Seahorse technology), NMR, metabolomics and immunophenotyping. Finally, each target goes through an *in vivo* validation process using xenografts, allografts and mouse models developed at the CNIO. This includes the use of non-invasive *in vivo* imaging technologies, as well as the immunohistochemical characterisation of tumours for different metabolic, immune and tumour markers. The final step is the validation in human samples from healthy donors or patients using PBMCs or tumour tissue arrays.

SCIENTIFIC CONTEXT

Cancer can be defined as the uncontrolled growth and division of cells, leading to tumour formation, invasion, and metastases. Unlike normal cells that require growth factor signals, tumour cells often have mutations that result in constitutively active ("always on") signalling pathways that drive aberrant cell growth and division. In order to fulfil the high nutrient demand required for their continuous growth, tumour cells have reprogrammed their basal metabolism from an oxidative to a more glycolytic/anabolic one, even in the presence of oxygen, known as the Warburg effect. Both oncogenes (Ras, Myc) and tumour suppressor genes (p53, RB, LKB1) impart an altered metabolic phenotype in cancer cells through the regulation of genes involved in central metabolic pathways such as glycolysis, fatty acid metabolism, oxidative phosphorylation, nucleotide synthesis and the one carbon pool (reviewed by Gilmour & Velasco, *SLAS Discov* 2017). All these alterations lead tumours to rely heavily on specific metabolic pathways to obtain their energy, while using other pathways to grow in order to give them a growth advantage. This situation may leave tumour cells in a frail position under certain treatments, while normal cells may be able to compensate, adapt and survive. Our laboratory is searching for this metabolic weakness in order to stop tumour growth.

Furthermore, the high requirements of nutrients and other soluble factors as well as the release of metabolites with immunosuppressive properties, together with the hypoxic conditions found in tumours, create a "non-friendly" microenvironment for an anti-tumour immune surveillance, while facilitating the growth of other tumour-promoting cells such as stroma and myeloid cells (FIGURE 1A). Thus, the mechanistic understanding of cancer metabolism has led to renewed interest in developing therapeutics that target key enzymes involved in these processes. One such enzyme is Indoleamine 2,3-dioxygenase 1 (IDO1), which catalyses the initial and rate-limiting step of kynurenine synthesis from tryptophan. Tumour cells selectively upregulate IDO1 as an immune evasion mechanism through the synthesis of kynurenine, either through intrinsic expression of IDO1, or in response to IFN-γ. Our laboratory has further characterised the participation of IDO1 as an immune checkpoint by analysing two different aspects of its biology (Cerezo *et al.*, AACR Annual Meeting 2019): i) the metabolomic analysis of tryptophan metabolism using an NMR-based readout approach. This assay allowed us to detect the contribution of tryptophan catabolism to purine synthesis, suggesting further roles of tryptophan catabolism in tumours through its participation in the one carbon pool pathway (FIGURE 1B); and ii) analysing the IDO1 pattern of expression in the tumour microenvironment. It was observed that IDO1 is mostly expressed in a wide range of highly inflamed tumours, the so called "hot tumours", together with other immune checkpoint targets such as CD73 and PDL-1 (FIGURE 1C). Furthermore, the differential expression of these immune checkpoints in separate spatial compartments of the tumour adds a new level of complexity in the dynamics of the tumour/stroma/immune cell interactions. These findings justify the use of anti-immune checkpoint combination therapy and aid to a more refined patient stratification. ■

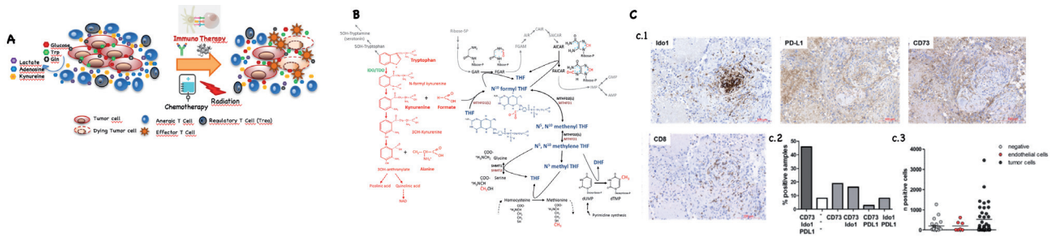

FIGURE 1 Targeting cancer metabolic immune suppression. (**A**) Tumour cells produce a battery of immunosuppressive metabolites such as lactic acid, kynurenine or adenosine that result in an anergic T cell phenotype, while consuming key metabolites such as glucose or tryptophan necessary for a proper Teff ($CD8^+$) activity. As a result, T cells are metabolically incapable of mounting an antitumour immune response. Metabolic regulation, together with immunotherapy and other classical therapies (radiation, chemotherapy), would diminish the production of immune suppressive metabolites and increase the levels of metabolites such as glucose, or the tryptophan necessary for a proper anti-tumour T cell response. (**B**)Tryptophan/one carbon pool metabolism connection. Serine (in black) and tryptophan (in red) metabolism can feed into the one-carbon metabolic pathway to support nucleotide biosynthesis. ^{13}C-labeled tryptophan and serine were used to assess the relative contribution of each amino acid to the one carbon pool by NMR. (**C**) **c.1-**Immunohistochemical analysis of the expression of the immune suppressors IDO, CD73 and PDL1, versus CD8, a marker for Teff, in a panel of 37 lung tumours. **c.2** and **c.3-** High expression of these immune checkpoints correlates with more inflamed tumours, "hot tumours". They tend to be co-expressed in different areas of the same tumour, showing a complementary pattern of expression. There are also differences in tissue distribution, while in some tumours they are homogeneously expressed in all the tissue compartments, in others they are preferentially expressed either in the tumour or in stromal or endothelial cells.