

# CNIO - LILLY CELL SIGNALLING THERAPIES SECTION

Susana Velasco  
Section Head

Staff Scientists  
Ana Cerezo, Eva P. Lospitao, Gloria Martínez del Hoyo, Sonia Hernández

Tiedra (since July), Juan Manuel Funes (until May)

Technicians  
Laura Díezma, Tamara Mondejar (TS)\*, Sandra Peregrina (TS)\*

\*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 anti-tumour agents, or activating the anti-tumour immune response. Exploring how to better target these mechanisms would lead to better and more efficient therapeutic options.

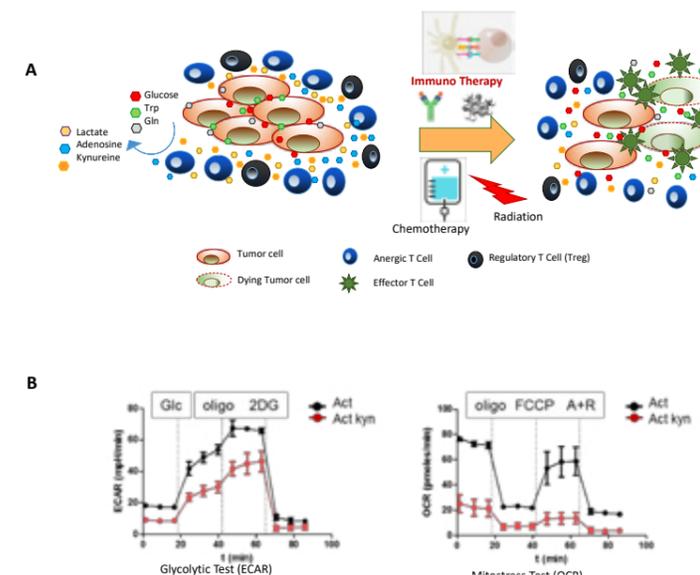
A combination of *in vitro* and *in vivo* approaches is being utilised to obtain a complete understanding of tumour metabolic reprogramming. For this purpose, we have developed a series of biochemical and cell-based assays exploiting advanced

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 that includes the use of non-invasive *in vivo* imaging technologies, and 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. Otto Warburg proposed in the early XX century that, 'this altered metabolic state was the underlying cause for cancer' (Warburg 1956). The past decade has been a period of very active research in the area of tumour metabolic reprogramming, and major molecular mechanisms involved in the process have been identified and characterised. It was found that 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, 2017). All these alterations have led tumours to rely heavily on specific metabolic pathways to obtain their energy, while using other pathways to grow in order to give tumour cells a growth advantage. This situation may leave tumour cells in a frail position under certain treatments or circumstances, 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 A, B). Thus, the mechanistic understanding of cancer metabolism has led to renew interest in developing therapeutics that target key enzymes involved in this process. Checkpoint-blockade immunotherapy has been one of the most exciting advances made in cancer treatment in recent years. Metabolic interplay in the local microenvironment can mediate T cell differentiation and function. 'Checkpoint-blockade' antibodies can also influence cellular metabolism. Finally, recent clinical trials have shown that combination immunotherapy, based on immune checkpoints blockade and targeted and non-targeted therapies, provides even higher response rates than either approach alone. Several clinical trials are currently using this approach, however, not all patients respond to immunotherapy and it is, therefore, necessary to determine which patients would be good candidates for the treatment. It has been found that an inflammatory tumour microenvironment – 'hot' tumours – greatly increases patient survival. One of the objectives of our laboratory has been to identify, and characterise the expression of novel and known tumour markers that may enable a better patient stratification for future therapies. This approach has shown that, in addition to the levels of expression of an immunotherapy target, the type of cells that express the marker may also be a feature to consider. ■



**Figure** 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 T cell response. (B) Extracellular flux analysis for the acidification rate (glycolytic test) and O<sub>2</sub> consumption OCR (mitochondrial test). Fully active effector T cells require an activated glycolytic and an oxidative metabolism in order to synthesize cytokines and other molecules necessary for their cytotoxic activity. Immune suppressive metabolites, like kynurenine, suppress the metabolic activity of effector T cells inhibiting their cytotoxic activity.