FLOW CYTOMETRY CORE UNIT

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Core Unit Head

Technicians
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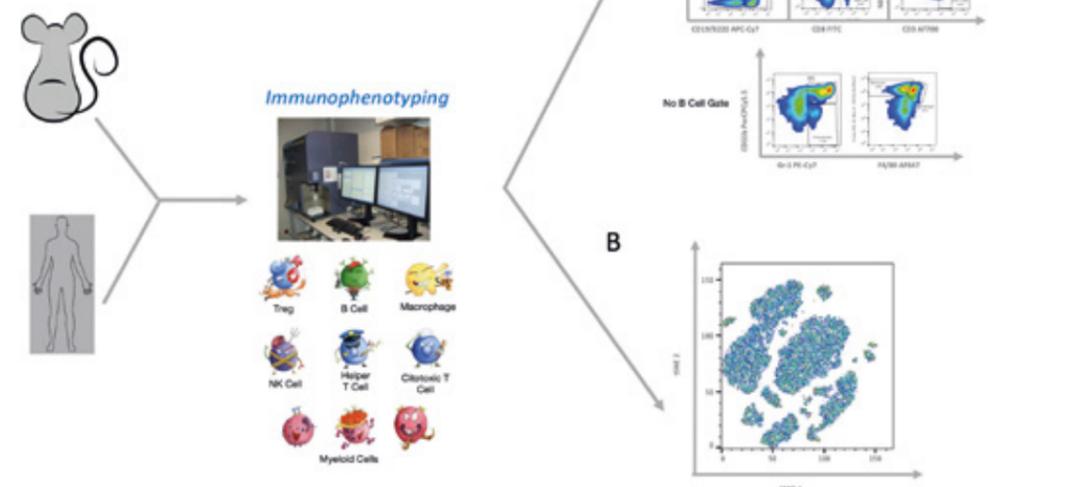*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in the setting up and optimisation of the flow cytometry techniques of their interest. Some of the applications developed and validated at our Unit are:

→ Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
→ Apoptosis studies (Annexin V, Mitochondrial Membrane Potencial, Caspase 3, etc.).
→ Multicolour Immunophenotyping panels (B and T cell development, Tregs, Inflammation, etc.).
→ Functional Assays (side population detection, Ca²⁺ flux, intracellular pH, etc.).
→ Cytometric Bead Arrays to measure several cytokines from cell extracts and plasma.
→ Platelet studies.

→ Extracellular vesicle detection (microvesicles and exosomes).
→ Single cell sorting for OMICs analysis.

We have further optimised our multicolour flow cytometry panels for the characterisation of the immune response in various samples, such as haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Single cell deposition into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We are performing 4-way sorting based on DNA content on live stained samples, and are moving forward to separate even further to isolate 6 different fractions of DNA content. Additionally, we are also pushing the power of our analytical tools by moving towards high dimensional analysis, performing 'unsupervised' clustering analysis on our multiparametric panel assays. ■

Figure Multiparametric panels for cancer immunomonitoring are commonly used in research worldwide. As the number of markers increase, so does the complexity of the analysis. Current options are conventional dot plots analysis to look at the different subsets (**A**) or the use of mathematical algorithms to look at the relation of all the markers at once (**B**).

OVERVIEW

Flow Cytometry is an indispensable tool in the oncology field. It enables multiparametric analysis for the identification, quantification and isolation of defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other.

Our aim is to provide CNIO Groups with technical and scientific advice regarding the use of cytometric technologies, collaborating with them in the design, acquisition, data analysis and interpretation.

We count with 4 analysers and 3 high-speed cell sorters with different configurations of lasers and detectors, to cater for all our users' needs. We also have an automated magnetic bead separation system (AutoMACS) and 2 automated cell counters.

"The development and optimisation of immunophenotyping panels by flow cytometry has been a key contributing factor, among others, to help us better understand the role of the immune system in the context of brain metastasis."

Analysers are user operated upon appropriate training and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6- defined populations simultaneously as well as perform single cell cloning. We can accept human samples to sort under Biosafety regulations.

▸ **PUBLICATIONS**

▸ Priego N, Zhu L, Monteiro C, Mulders M, Wasilewski D, Bindeman W, Doglio L, Martínez L, Martínez-Saez E, Ramón Y Cajal S, Megías D, Hernández-Encinas E, Blanco-Aparicio C, Martínez L, Zarzuela E, Muñoz J, Fustero-Torre C, Piñeiro-Yáñez E, Hernández-Laín A, Bertero L, Poli V, Sanchez-Martinez M, Menendez JA, Soffietti R, Bosch-Barrera J, Valiente M (2018). STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. *Nat Med* 24, 1024-1035.

▸ Hurtado B, Trakala M, Ximénez-Embún P, El-Bakkali A, Partida D, Sanz-Castillo B, Álvarez-Fernández M, Maroto M, Sánchez-Martínez R, Martínez L, Muñoz J, García de Frutos P, Malumbres M (2018). Thrombocytopenia-associated mutations in Ser/Thr kinase MASTL deregulate actin cytoskeleton dynamics in platelets. *J Clin Invest* 128, 5351-5367.

▸ Costa-Machado LF, Martín-Hernández R, Sanchez-Luengo MÁ, Hess K, Vales-Villamarin C, Barradas M, Lynch C, de la Nava D, Diaz-Ruiz A, de Cabo R, Cañamero M, Martinez L, Sanchez-Carbayo M, Herranz D, Serrano M, Fernandez-Marcos PJ. (2018). Sirt1 protects from K-Ras-driven lung carcinogenesis. *EMBO Rep* 19, e43879.

▸ Pérez-Olivares M, Trento A, Rodriguez-Acebes S, González-Acosta D, Fernández-Antorán D, Román-García S, Martínez D, López-Briones T, Torroja C, Carrasco YR, Méndez J, Moreno de Alborán I (2018). Functional interplay between c-Myc and Max in B lymphocyte differentiation. *EMBO Rep* 19, e45770.

▸ **AWARDS AND RECOGNITION**

▸ Executive Council Member, European Core Technologies for Life Science Association (CTLS).