FLOW CYTOMETRY CORE UNIT

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**Titulado Superior* (Advanced Degree)
***Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)

OVERVIEW

Flow cytometry is a very useful 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 the necessary technical and scientific advice regarding the use of flow cytometric technologies, collaborating with them for the design, acquisition, data analysis and interpretation.

With our 4 analysers and 3 high-speed cell sorters, with different configurations of lasers and detectors, we can cater to all our users' needs. We also have an automated magnetic bead separation system (AutoMACS) and 2 automated cell counters. Analysers are available to users upon appropriate training and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6- defined populations at a time, as well as allow for single cell cloning. We can accept human samples to sort under Biosafety regulations.

"*In vivo* LacZ detection has always been a challenge. We have optimised a protocol for the identification and isolation of LacZ expressing cells from haematopoietic and lung tissues."

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in setting up and optimising flow cytometry techniques of their interest. Some of the applications that have been developed and validated by our Unit include:

- → 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, Ca^{2+} flux, intracellular pH, etc.).
- → Cytometric Bead Arrays to measure several cytokines from cell extracts and plasma.
- → Microvesicle detection.

We have further developed our multicolour panels for the characterisation of the immune response by incorporating the new generation of Brilliant UV dyes from samples such as haematopoietic tissues, pancreas, skin, liver, and lung. Modifications in our analytical and cell sorters have also been applied to allow for this. Moreover, these panels could still be combined with the detection of proliferation and cell death. In terms of our cell sorting capabilities we included, at the end of the year, a MoFlo ASTRIOS in our portfolio of cell sorters. This cell sorter is equipped with 4 laser lines and 15 fluorescent detectors, which enable the isolation of up to 6 different populations simultaneously. The optical configuration in the ASTRIOS will allow for the use of the new generation of Brilliant UV dyes. ■

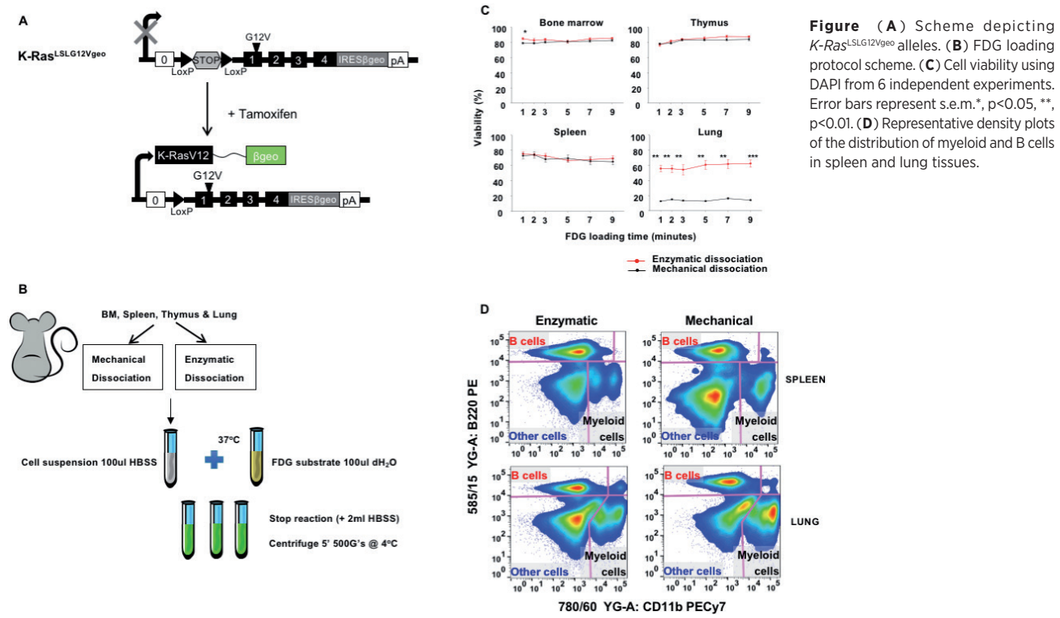

Figure (**A**) Scheme depicting *K-Ras*^{LSLG12Vgeo} alleles. (**B**) FDG loading protocol scheme. (**C**) Cell viability using DAPI from 6 independent experiments. Error bars represent s.e.m.*, p<0.05, **, p<0.01. (**D**) Representative density plots of the distribution of myeloid and B cells in spleen and lung tissues.

PUBLICATIONS

López-Guadamillas E, Fernández-Marcos PJ, Pantoja C, Muñoz-Martin M, Martínez D, Gómez-López G, Campos-Olivas R, Valverde AM, Serrano M (2016). p21Cip1 plays a critical role in the physiological adaptation to fasting through activation of PPARα. *Sci Rep* 6, 34542.