FLOW CYTOMETRY CORE UNIT

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OVERVIEW

Flow Cytometry is a fast and multiparametric technology of great value in the study of immune responses in the context of cancer. It allows 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 at the single cell level.

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

"We hosted toxicology professor Ana Juan García from the *Universidad de Valencia* and ran a series of experiments to investigate cell death and immune responses upon treatment with mycotoxins in different cancer cell lines and primary human PBMCs."

We currently have 3 polychromatic flow cytometers and 1 spectral cytometer, plus 3 high-speed cell sorters with different optical configurations to cater our users' needs. We also have an automated magnetic bead separation system and a tissue homogeniser to standardise sample preparation. Users operate the analytical cytometers upon appropriate training, and the Unit staff operate the Unit cell sorters, which can separate up to 4- or 6- defined populations simultaneously, as well as perform single cell cloning and index sorting. We can accept human samples to sort under BSL2 regulations. ■

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 relevant to their research projects. Some applications 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.
- → Platelet studies.
- → Extracellular vesicles detection (microvesicles and exosomes).
- → CTC detection and isolation.
- → Single cell sorting for OMICs analysis.

In 2022, we further increased our multicolour flow cytometry capabilities 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, with the incorporation of an AURORA 5L. Single cell deposition using index sorting into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We also expanded our training capacities with many more workshops and small practical analysis sessions in order to provide our users with more tools to successfully perform their flow cytometry experiments. ■

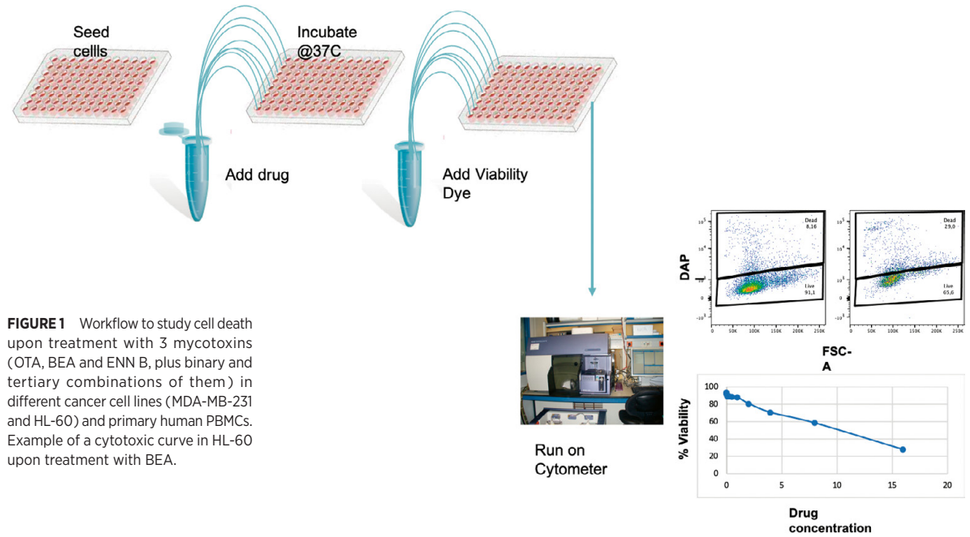

FIGURE 1 Workflow to study cell death upon treatment with 3 mycotoxins (OTA, BEA and ENN B, plus binary and tertiary combinations of them) in different cancer cell lines (MDA-MB-231 and HL-60) and primary human PBMCs. Example of a cytotoxic curve in HL-60 upon treatment with BEA.

PUBLICATIONS

- Jacobs K, Doerdelmann C, Krietsch J, González-Acosta D, Mathis N, Kushinsky S, Guarino E, Gómez-Escolar C, Martinez D, Schmid J.A, Leary P.J, Freire R, Ramiro A.R, Eischen C.M, Mendez J, Lopes M (2022). Stress-triggered hematopoietic stem cell proliferation relies on Prim-Pol-mediated repriming. *Mol Cell* 82, 41767-4188.e8.
- Back J.B, Martinez L, Nettenstrom L, Sheerar D, Thornton S (2022). Establishing a biosafety for a flow cytometry shared resource laboratory. *Cytometry A* 101, 380-386.

AWARDS AND RECOGNITION

- "Outstanding Poster Award", 37th Congress of the International Society for the Advancement of Cytometry (ISAC), CYTO 2022 Philadelphia (USA): García García S, García-Lestón J, and Martínez L. *Benefits of an open-source Similarity Score for multiparametric flow cytometry controls.*