# BIOTECHNOLOGY PROGRAMME

**FERNANDO PELÁEZ** Programme Director



The main mission of the Biotechnology Programme Core Units is to provide expert technical and scientific support to CNIO Research Groups in a number of disciplines and technologies widely used in biomedical research, as well as to implement and develop state-of-the-art biotechnological tools and protocols. The Programme consists of 9 Core Units covering major areas in Biotechnology, namely Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Mouse Genome Editing, as well as an Animal Facility. Although the Core Units are mainly focused on providing support and collaborating with the CNIO Research Groups, they also collaborate with groups from other research institutions as well as with private companies.

**"The excellence of the Core Units of the Biotechnology Programme is one of the critical factors behind the outstanding scientific productivity of the CNIO in research and innovation."**

In 2022 the Programme incorporated 2 new Unit Heads to replace former Unit leaders who left the CNIO in 2021. Thus, Marta Isasa joined the CNIO as Head of the Proteomics Unit in October 2022. She brings more than 10 years' experience in this field, including a postdoctoral stay in one of the top proteomics laboratories in the world, with Prof. Steven Gygi at Harvard Medical School (Boston, USA). Until then, Marta was Associate Director of the Proteomics and Chemical Biology Group at Odyssey Therapeutics (Boston, USA). In addition, in December 2022, we incorporated Isabel Peset as new Head of the Confocal Microscopy Unit. She comes with over 10 years of postdoctoral experience in the UK, in several laboratories working in advanced microscopy. Before joining the CNIO, Isabel was Lead Scientist in Advanced Imaging at Medicines Discovery Catapult (Cambridge, UK). We wish them both great success in this new step in their professional careers.

Regarding the projects led by the Units, it is worth mentioning the grant awarded to the Histopathology Unit through the call *Ayudas a Proyectos de Colaboración Público-Privada* from the Ministry of Science and Innovation (*MCI*), for a project in collaboration with the company MedLumics and the *Universitat Pompeu Fabra*. The project focuses on the development of a system to treat auricular fibrillation using irreversible electroporation, and the role of the Unit will focus on the analysis of the pathological features and the mechanisms mediating cell death in cardiac tissue upon auricular fibrillation ablation.

On the other hand, our technological capabilities continued to be upgraded during 2022. Some examples include the acquisition of an optical imaging IVIS Lumina III system in the Molecular Imaging Unit, used for imaging studies in animal models; several automated platforms for histochemical and immunohistochemical staining (Agilent, Ventana-Roche) for the Histopathology Unit; and a new Chromium iX system for single cell RNA sequencing analysis.

As usual, the Core Units were active in attracting funding from external sources through innovation related activities, including contracts and agreements with private companies and public institutions based on the technologies mastered by several of our Core Units. The royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit continue representing a significant funding source for the CNIO. This year the total income derived from these licenses exceeded €1.5 million, an impressive achievement that represents an increase of more than 40% over the figure from 2021, positioning the CNIO as a true worldwide reference in this field.

Last but not least, 2022 was again a very productive year scientifically for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in the more than 30 publications co-authored by members of the Units, many of them in top journals. ■

# GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Laura Conde, José Luis Espadas (until April) (PEJ)*, Guadalupe Luengo, Ruth Micha (since October), Jorge Monsech, Ángeles Rubio

*_Plan de Empleo Joven_ (Youth Employment Plan)

## OVERVIEW

The Genomics Unit provides centralised research services as well as expert consultation in the fields of genomics and genetics. Contributing to uncover biological mechanisms, therapeutic targets, or prognostic biomarkers, these services encompass a broad range of applications, from traditional to cutting-edge technologies. These technologies, with their capacity to interrogate whole genomes and their activities, can reveal the entire package of structural features (mutation landscapes, chromosomal protein location, or chromatin structure) and molecular programmes (transcriptomic RNA profiles), even at the single-cell level. So-called next-generation sequencing (NGS) is a staple among them. More traditional methodologies, like Sanger capillary DNA sequencing, are also provided. As a side activity, we manage a genetically engineered mouse genotyping service.

**"Our service portfolio is shaped by the requirements of CNIO's scientists in genomics and genetic technologies. It represents a flexible response to both generic and boutique services, from basic housekeeping activities to advanced explorations of biological complexity."**





## RESEARCH HIGHLIGHTS

Every cancerous tumour, even those of the same type and with a similar outcome, is different at the chromosomal level, has distinct molecular origins, and will likely differ in its most suitable therapeutic intervention. This variability can be comprehended through the use of powerful genomic technologies. These tools, with their capacity to analyse even whole genomes in a single assay, permit decoding structural changes and functional molecular programmes.

The Genomics Unit, with its array of molecular services, contributes to the dissection of molecular processes of biological complexity in research projects conducted by CNIO Research Groups. The genomic-wide level is addressed by NGS-based technologies. NGS constitutes the final readout for a variety of different applications at either the structural or functional levels: on the one hand, genome or exome tumour characterisations, mutation repertoires, location of relevant DNA-bound protein factors, variations in chromatin folding, or on/off functional states; on the other hand, transcriptional profiles reflecting functional choreographies, useful to decipher tumour compositions, uncover therapeutic targets, or predict disease course. Tissue composition, heterogeneity, and fate can be further explored with single cell resolution, by capturing individual cells in microdroplet emulsions and studying them by the tens of thousands through analysis in the NGS platform.

At the single locus level other services are provided. A traditional DNA capillary sequencing service is being used to find and confirm mutations in candidate genes, or to verify cloned genes or inserts. A cell authentication service, based on individual STR marker profiles, provides confidence in the identity of the samples used for experimentation. The Unit also manages a transgenic mouse genotyping service with custom allele-specific, real-time PCR test assays for a quick and efficient turnaround time. ■

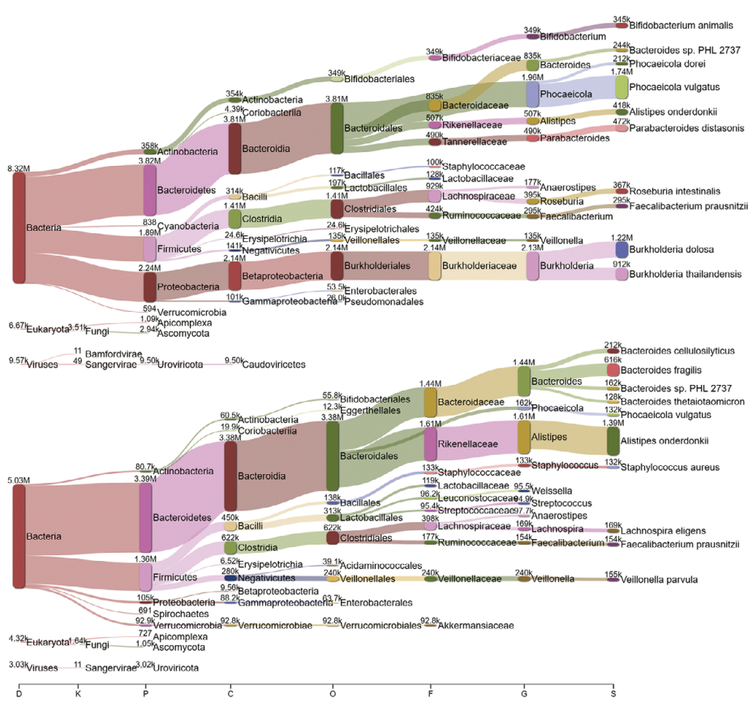


**FIGURE 1** In order to ascertain variability factors that might influence each person's oncological process, gut microbiota diversity was explored. The Figure shows metagenomics classifications for 2 samples obtained at different time points from the same patient. Faecal DNA was sequenced (NGS), analysed with Kraken taxonomic classification software, and diagrams obtained from the Pavian web tool. Data kindly shared by M.A. Quintela and M.J. Bueno, from the CNIO Breast Cancer Clinical Research Unit.

# MOUSE GENOME EDITING CORE UNIT

Sagrario Ortega
Core Unit Head

Gradutate Student
Aleida Pujol (until April)

Technicians
Estefania Ayala (until July), Marina Cabrerizo (until March) (TS)* (PEJ)**,
Beatriz Escobar (since July) (TS)*, Carmen Gómez, Melani Margullón (since December), Jaime Muñoz (TS)*, Patricia Prieto (TS)*, Pierfrancesco Vargiu (TS)*

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

## OVERVIEW

Cancer encompasses a wide spectrum of extremely complex diseases. Genetic and epigenetic modifications in tumour cells lead to the acquisition of "malignant" phenotypes that enable them to escape normal physiological control. Genome editing and transgenesis technologies are used to accurately reproduce these modifications in the mouse, creating animal models that are crucial to understand and better treat cancer. Tumour cells interact at different levels with other systems in the body such as the immune, cardiovascular or lymphatic systems, which in turn modulate tumour growth, invasion, and expansion. Behavioural factors such as diet also have an impact on cancer development. The study of such complexity demands reliable *in vivo* models that reproduce the features of cancer in a "whole body" context. The precise, targeted, and controlled modification of the mouse genome, using the most advanced genome editing tools, sustains the generation of genetic mouse models of cancer that are crucial for understanding the molecular basis of tumour development and the preclinical validation of new and more efficient cancer therapies.

**"The Unit has more than 20 years of experience in the design, generation, and validation of genetically modified mouse models using state-of-the-art genome editing techniques. It also maintains a cryoarchive of the hundreds of genetically modified mouse lines created at the CNIO."**





## RESEARCH HIGHLIGHTS

Since the outbreak of the SARS-CoV-2 pandemic in 2020, the Unit has dedicated extra effort to generating and characterising mouse models for COVID disease. For this purpose, and supported by a dedicated grant from the Spanish Institute of Health *Carlos III* and a *SINERGIAS*-grant from the Madrid Local Government (*CAM*), the Unit has created "humanized" mouse models for COVID19 research, in collaboration with the company Gen-H Genetic Engineering, Heidelberg (Germany).

The laboratory mouse is the most widely used animal model in biomedicine, but it is not a permissive species for SARS-CoV-2 infection. Structural differences between the human angiotensin converting enzyme-2 (ACE2) protein, the cellular receptor for SARS-CoV-2, and its murine ortholog are the cause, at least in part, of the different sensitivity to viral infection in humans and mice.

Using the latest gene editing technologies, based on the CRISPR/Cas9 system, we created knockin mice in which the human ACE2 protein is expressed under the transcriptional control of the endogenous mouse *Ace2* promoter, interrupting simultaneously the *Ace2* coding sequence and resulting in the knockout of the mouse *Ace2* gene (FIGURE 1). We generated two knockin mouse models, co-expressing the human ACE2 protein together with a fluorescent reporter or with the human TMPRSS2 serine protease that plays a critical role, together with ACE2, in the virus entry into cells. These humanized mice provide a more physiological platform than the currently available models for studying the long term effects of SARS-CoV-2 infection in the mouse.

We are presently collaborating with Dr Luis Enjuanes (Coronavirus Laboratory) at the National Centre for Biotechnology (*CNB/CSIC*, Madrid), and with Dr Maria A. Blasco at the CNIO (Telomeres and Telomerase Group-*Fundación Humanismo y Ciencia*), to characterise these mouse models and their application to study the effect of aging by telomere shortening in COVID19. ■

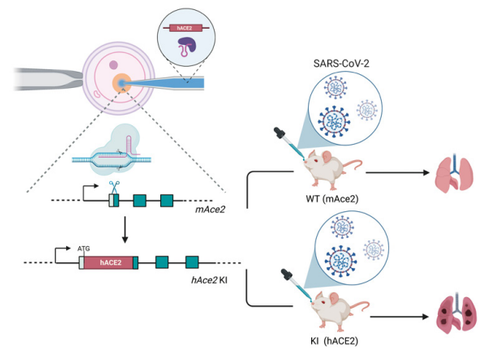


**FIGURE 1** Gene editing strategy used to create humanized knockin mouse models to study COVID19. Using CRISPR/Cas9 in embryos, we replaced the mouse *Ace2* gene with its human ACE2 ortholog. The human receptor is expressed under the transcriptional control of the mouse *Ace2* promoter in the knockin and, simultaneously, the mouse *Ace2* gene is knocked out. *Created with BioRender.*

### PUBLICATIONS

- Beucher A, Miguel-Escalada I, Balboa D, De Vas MG, Maestro MA, Garcia-Hurtado J, Bernal A, Gonzalez-Franco R, Vargiu P, Heyn H, Ravassard P, Ortega S, Ferrer J (2022). The HASTER lncRNA promoter is a cis-acting transcriptional stabilizer of HNF1A. *Nat Cell Biol* 24, 1528-1540.
- Fernández LP, Deleyto-Seldas N, Colmenarejo G, Sanz A, Wagner S, Plata-Gómez AB, Gómez-Patiño M, Molina S, Espinosa-Salinas I, Aguilar-Aguilar E, Ortega S, Graña-Castro O, Loria-Kohen V, Fernández-Marcos PJ, Efeyan A, Ramírez de Molina A (2022). Folliculin-interacting protein FNIP2 impacts on overweight and obesity through a polymorphism in a conserved 3' untranslated region. *Genome Biol* 23, 230.

# MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
Álvaro García (until August), Scherezade Jiménez-Villa, Lorena Maestre (TS)*, Ana I. Reyes

**Titulado Superior* (Advanced Degree)

## OVERVIEW

The Monoclonal Antibodies Unit provides CNIO and other national and international research groups with the capability to generate "à la carte" monoclonal antibodies (mAbs) that are used as research tools to isolate, identify, and characterise new pathways relevant to cancer diagnosis, prevention, and treatment.

Our mAbs are useful tools to understand cancer biology and to diagnose neoplastic diseases, since they allow the identification of molecular markers that are selectively expressed by specific tumour subtypes.

We are particularly specialised in the production and validation of mAbs for immunohistochemistry (IHC), a technique that allows the localisation and study of proteins in tissue sections. This type of reagent allows for a more accurate diagnosis, resulting in a better classification of cancer and the selection of the most adequate cancer treatment.

**"The Monoclonal Antibodies Unit is highly specialised in mAbs production and characterisation, providing CNIO researchers with reliable and well-validated reagents that give added value to their research projects."**

The Unit also offers mAb characterisation and validation, medium-scale mAb production, as well as a service of *Mycoplasma* testing for the cell culture facility.





## RESEARCH HIGHLIGHTS

During the last 22 years, the Monoclonal Antibodies Unit has generated a large number of mAbs, directed against more than 180 different antigens, mostly targeting molecules for which mAbs are not commercially available. Many of those mAbs (63) have been licensed to external companies, generating royalties that represent an important source of revenues for the CNIO.

Each year we prepare and update a detailed CNIO mAbs Catalogue, which contains the datasheets of more than 100 thoroughly validated, high-quality mAbs (accessible at http://www.cnio.es/ing/servicios/anticuerpos/default.aspx). This catalogue is offered to specialised companies looking for licensing opportunities.

**Research activities:**

**National and international collaboration**. In addition to our collaboration with the CNIO's Research Groups, during the last 22 years we have also developed many joint projects with groups from other national and international research institutions. In these collaborations, the scientists provide their extensive and profound knowledge of cancer research, generating fresh perspectives, diverse viewpoints, and innovative methodologies, which allow the targeting of proteins that play an important role in tumour transformation. We provide them with access to the generation of reliable tools (mAbs), useful both to confirm the results obtained, as well as to further investigate in their research field. In addition, we can develop and set up novel products that can lead to the generation of diagnostic tools for the prevention and diagnosis of cancer. Some of our most recent (last 2 years) and successful collaborations have been with the Spanish National Centre for Cardiovascular Research, *CNIC* (anti-ALDH14 mAb), the *Hospital Universitario Fundación Jiménez Diaz* (anti-hPIGR mAb), and the Centre for Cooperative Research in Biosciences, *CICbioGUNE* (anti-IL4I1 mAbs).

**TACI (CD267) in lymphomas.** In 2022, we produced and characterised a novel mAb against TACI protein (encoded by *TNFRSF13B* gene) that belongs to the tumour necrosis factor receptor superfamily. TACI, also known as CD267, promotes T-independent antibody production, in part by facilitating plasma cell differentiation. Since the distribution of CD267 in reactive and neoplastic lymphoid tissues has not been investigated, we are currently evaluating its expression using a novel rat monoclonal antibody (CLOE240B) against the CD267 intracellular domain, which recognises its target in paraffin-embedded tissue sections. Large series of normal tissues and B and T-cell lymphomas are being studied using whole sections and tissue microarrays. The aim is to determine the pathological diagnostic roles and clinical significance of the CD267 receptor in B-cell neoplasms.

**EuroMAbNet.** In 2008, in collaboration with Oxford University, we founded EuroMAbNet (www.euromabnet.com), a non-profit organisation that currently spans 13 European countries. EuroMAbNet's primary goal is to provide an arena for people working in the field of monoclonal antibody production and technology to exchange knowledge and updated methodologies, and to create common strategies to improve and standardise the production of properly validated antibodies. ■

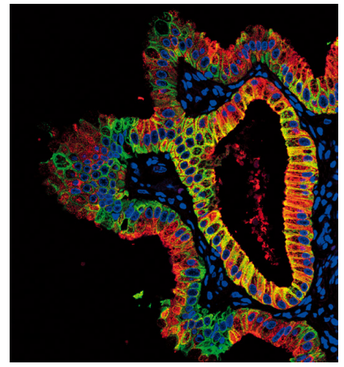

**FIGURE 1** Double immunofluorescence staining of PIGR mAb (red) and cytokeratin (green) in paraffin section of human epithelium.

**PUBLICATIONS**


- Yamamoto Y *et al.* (incl. Roncador G) (2022). Anti-HBV drug entecavir ameliorates DSS-induced colitis through PD-L1 induction. *Pharmacol Res* 179, 105918.
- Roncador G, Puñet-Ortiz J, Maestre L, Rodríguez-Lobato LG, Jiménez S, Reyes-García AI, García-González Á, García JF, Piris MÁ, Montes-Moreno S, Rodríguez-Justo M, Mena MP, Fernández de Larrea C, Engel P (2022). CD229 (Ly9) a novel biomarker for B-cell malignancies and multiple myeloma. *Cancers (Basel)* 14, 2154.
- Carreras J, Roncador G, Hamoudi R (2022). Artificial intelligence predicted overall survival and classified mature B-cell neoplasms based on immuno-oncology and immune checkpoint panels. *Cancers (Basel)* 14, 5318.
- Peña-Cardelles JF *et al.* (incl. Roncador G) (2022). Prognosis value of immunoregulatory molecules in oral cancer microenvironment: an immunohistochemical study. *Biomedicines* 10, 710.
- Pileri A, Cavicchi M, Bertuzzi C, Righi S, Zengarini C, Sabattini E, Roncador G, Agostinelli C (2022). TOX expression in mycosis fungoides and Sezary Syndrome. *Diagnostics (Basel)* 12, 1582.
- Righi S *et al.* (incl. Roncador G) (2022). Myeloid nuclear differentiation antigen: an aid in differentiating lymphoplasmacytic lymphoma and splenic marginal zone lymphoma in bone marrow biopsies at presentation. *Hum Pathol* 124, 67-75.

# MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Tatiana Álvarez, Guillermo Garaulet (TS)˚, Guillermo Medrano (TS)˚, Jorge Rodríguez (since March), Judit Rey (since November) (TS)˚, David Sabador (TS)˚ (PEJ) ˚˚, Gloria Visdomine

˚*Titulado Superior* (Advanced Degree)
˚˚*Plan de Empleo Joven* (Youth Employment Plan, until February)


## OVERVIEW

Molecular imaging techniques aim to characterise and quantify biological processes at the molecular and cellular levels, facilitating a repetitive, non-invasive, uniform, and relatively automated study of the same living subject using identical or alternative biological imaging assays at different time points. The statistical power of longitudinal studies is therefore harnessed, and the number of animals required and costs incurred are reduced. Combining techniques using multimodality (PET-CT, optical imaging-CT, and ultrasound) allows pathophysiological changes in early disease phases to be detected with high structural resolution. Other advantages include the ability to interrogate the whole body and to visualise the molecular target of interest in 3D space.

**"Specific imaging of targets will allow a more fundamental understanding of the disease process."**





## RESEARCH HIGHLIGHTS

The services offered to CNIO researchers by the Molecular Imaging Unit cover different technologies to non-invasively and repetitively image targeted macromolecules in living organisms. We enjoy state-of-the-art technical equipment:

- → A micro-PET-CT system (eXplore Vista) from GE to detect early tumour development was acquired, and it is now fully operational. We changed the flat panel to increase the resolution with less radiation.
- → A CT system (CompaCT) from Sedecal for the follow-up of tumours and to phenotype different genetically modified mouse strains. Upgraded with the Advanced Bone Analysis Tool.
- → Two ultrasound systems (Vevo 3100) from Fujifilm VisualSonics to obtain high-resolution abdominal and soft tissue tumour images.
- → A densitometer system (Lunar PixiMus) from GE to perform bone and fat analysis.
- → Two optical imaging devices (IVIS Lumina III) from PerkinElmer to acquire fluorescence and bioluminescence. One of them was installed in September 2022.

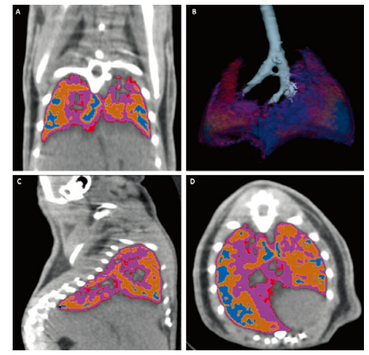


**FIGURE 1** Computed Tomography of a mouse with fibrosis in the lungs. Density changes could be segmented and represented in different colours: normal lung (blue), infiltrated fibrotic tissue (orange), and collapsed lung (pink). (**A**) Coronal projection. (**B**) 3D rendering. (**C**) Sagittal projection. (**D**) Axial projection.

We continued our work on theranostic applications of radiolabelled antibodies, looking for the best-matched isotope pair for imaging and therapy, and employing the pre-targeting approach, in a project supported by a grant from the BBVA foundation. We also renewed our grant project with the *Red Madrileña de Nanomedicina en Imagen Molecular* (*RENIM* 2), which focuses on developing and optimising molecular imaging probes and tools for oncology research.

During 2022, and as a result of our increasing expertise in ImmunoPET techniques, we published a special edition entitled "ImmunoPET Imaging in Disease Diagnosis and Therapy Assessment" in the Nuclear Medicine section of *Frontiers in Medicine*. We also obtained a Next Generation EU infrastructures grant to buy an MRI (Magnetic Resonance Imaging) machine. With this system, we will have a complete set-up for imaging, including all the current techniques available. ■

### PUBLICATIONS

- González-Martínez J, Cwetsch AW, Gilabert-Juan J, Gómez J, Garaulet G, Schneider P, de Cárcer G, Mulero F, Caleiras E, Megías D, Porlan E, Malumbres M (2022). Genetic interaction between PLK1 and downstream MCPH proteins in the control of centrosome asymmetry and cell fate during neural progenitor division. *Cell Death Differ* 29, 1474-1485.
- Gerke C, Zabala Gutierrez I, Méndez-González D, Cruz MCI, Mulero F, Jaque D, Rubio-Retama J (2022). Clickable albumin nanoparticles for pretargeted drug delivery toward PD-L1 overexpressing tumors in combination immunotherapy. *Bioconjug Chem* 33, 821-828.
- Mulero F, Oteo M, Garaulet G, Magro N, Rebollo L, Medrano G, Santiveri C, Romero E, Sellek RE, Margolles Y, Campos-Olivas R, Arroyo AG, Fernández LA, Morcillo MA, Martínez-Torrecuadrada JL (2022). Development of anti-membrane type 1-matrix metalloproteinase nanobodies as immunoPET probes for triple negative breast cancer imaging. *Front Med* 9, 1058455.
- Francisca Mulero (2022). ImmunoPET in oncology. *Rev Esp Med Nucl Imagen Mol* (Eng Ed) 41, 332-339.

### AWARDS & RECOGNITION

- Faculty and Mentor of *IDEA2* 2022 MIT linQ, Massachusetts Institute of Technology, USA.

# FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Irene Fernández-Delgado (TS)* (since October), Julia García-Lestón (TS)*, Sara García García (until May), Ana M. Elizabeth Ilie (since July)

*Titulado Superior* (Advanced Degree)

Visiting Scientist
Ana Juan García (June-September) (*Universidad de Valencia*, Spain)

## 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. ■

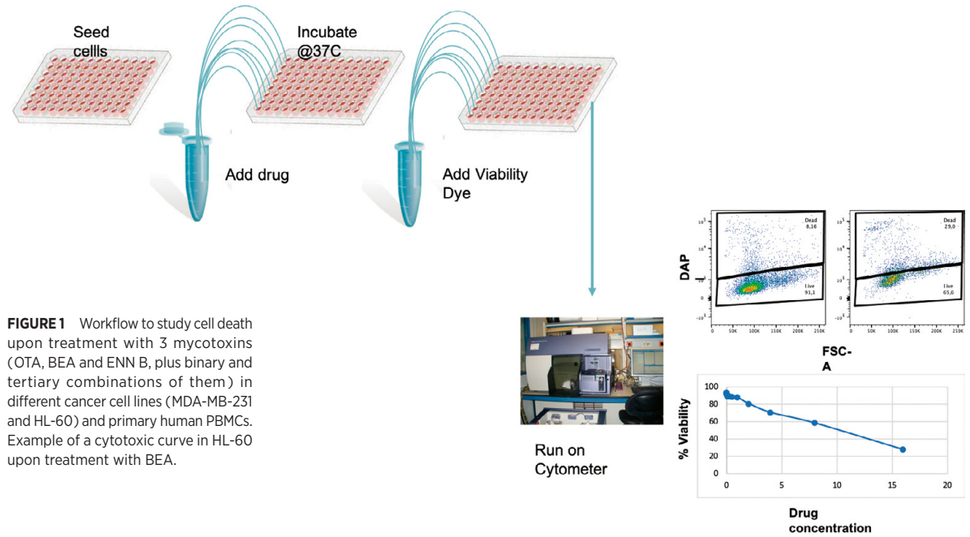


**FIGURE 1** Workflow to study cell death upon treatment with 3 mycotoxins (OTA, BEA and ENN B, plus binary and tertiary combinations of them) in different cancer cell lines (MDA-MB-231 and HL-60) and primary human PBMCs. Example of a cytotoxic curve in HL-60 upon treatment with BEA.

### 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.*

# CONFOCAL MICROSCOPY CORE UNIT

Isabel Peset (since December)
Core Unit Head

Technicians
Jesús Gómez (since February) (TS)*,
Manuel Pérez (TS) *

*Titulado Superior* (Advanced Degree)

## OVERVIEW

One of the main challenges in oncology research is the study of specific markers, expression patterns or individual cells in the tumour environment. Optical microscopy has traditionally been an indispensable tool in cell biology studies and has become essential for understanding cancer biology.

The Confocal Microscopy Unit (CMU) provides the CNIO research groups with the latest advances in optical microscopy, offering access to state-of-the-art equipment and image analysis software, including scientific advice and technical support. The Unit is also actively involved in developing and implementing new advanced imaging methods that could have an impact on the work of CNIO research groups. Advanced microscopy training and science disseminating activities are also an essential component of our mission. We organise courses, talks and visits, always with the aim of increasing our understanding of the cellular and molecular disorders that lead to cancer and the study of potential treatments.

**"The CMU is committed to applying advanced microscopy methods to visualise at subcellular level different cancer markers simultaneously, providing a deep understanding of tumour progression and treatment responses."**





## RESEARCH HIGHLIGHTS

The CMU has continued developing automated imaging technologies applied to confocal and widefield microscopy to improve the high-throughput (HT) of highly resolved visualisation and analysis of different samples.

In 2022, the Unit has focused its efforts on implementing High Content Screening (HCS) methods using the new Opera Phenix Plus HCS microscope installed last year. This instrument is a high-end HCS system equipped with a robotic plate handler and an analysis software, which enables the monitoring of cells processes in multi-well plates of fixed and live samples. Together with CNIO Research Groups, the Unit has developed multi-well plate-based methods to analyse cell cycle profiles, cell viability and mitosis phenotyping studies at high-resolution using the PreciScan feature (object-of-interest-driven acquisition) provided by the system (FIGURE 1A). The platform will also allow 3D HT analysis of organoids or spheroids campaigns and live-cell imaging assays, boosting thereby the screening capacity at the CNIO.

In addition, the Unit implemented a sample navigation application integrated into the SP8 and SP5 confocal systems and Thunder imaging widefield system. This enables fast and semi-automated HT feeding of the instrument, both in multi-well plates (FIGURE 1B) and in tissue sections, including Tissue Microarrays (TMA) (FIGURE 1C). Through this automated acquisition, we can increase the imaging speed and the highly resolved information obtained from a sample.

The Unit is involved in developing image processing and analysis pipelines, including 3D and high content analysis, and helping its users with novel protocol development for sample handling and preparation.

In December, Isabel Peset has joined the CNIO as new Head of the Unit, bringing more than 10 years of experience in implementing optical microscopy methods in cell biology, oncology and drug discovery studies. ■

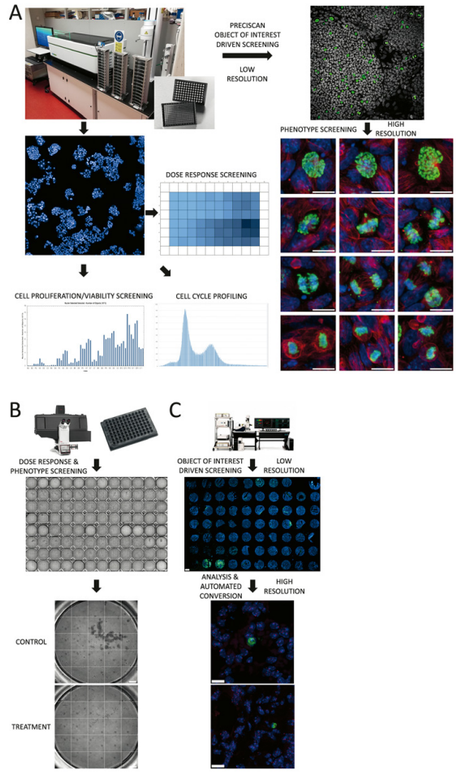


**FIGURE 1** Developing automated imaging workflows. (**A**) Examples of HCS methods. Left. HT nuclei detection enables different cellular analysis. Right. Mitotic phenotypic screening using the PreciScan feature. (**B** and **C**) Examples of semi-automated HT feeding. (**B**) Dose response screening with complete well mosaic acquisition. (**C**) Tissue microarray screening with driven acquisition for high-resolution imaging. Data provided by MJ. Bueno, C. Sayago, A. El Bakkali and P. Martínez, respectively.

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# PROTEOMICS CORE UNIT

Marta Isasa (since October)
Core Unit Head

Technicians
Fernando García (TS) *, Julia Isabel Morales (TS) *, Cristina Sayago (since April) (TS) *, Jana Sánchez (until April) (TS) *, Pilar Ximénez de Embún (TS) *, Eduardo Zarzuela (TS) *

**Titulado Superior* (Advanced Degree)

## OVERVIEW

Proteomics is acquiring a critical role in the comprehensive understanding of human biology. The fast development in mass spectrometry-based proteomics instrumentation and data analysis pipelines has helped the scientific community to dig (even) deeper into the proteome. In the last decade, the main output of differential proteomics studies has evolved from long lists of proteins to the generation of new hypotheses, allowing proteomics to become functional. For example, cancer proteomics has unravelled key data in mechanistic studies on tumour growth and metastasis, contributing to the identification of clinical biomarkers and novel therapeutic targets. Several cancer proteome databases have been established and are being shared worldwide. The CNIO Proteomics Core Unit develops and applies state-of-the-art proteomics, informatics, and related technologies, for direct interrogation of protein expression, modification, and function in cell-based models of human cancer. We aim to provide valuable guidance for experimental strategies, which are critical for cancer research success.





## RESEARCH HIGHLIGHTS

In collaboration with the Experimental Oncology Group, the Unit has measured stoichiometric changes in the RHC complex due to 8 RAF1 and 1 CDC37 single mutations. We observed that the modification of key interface residues between both RAF1 and CDC37 proteins reduced RAF1 protein levels present in the complex. Global analysis of protein phosphorylation was also performed, and novel RAF1, CDC37 and HSP90 phosphorylation sites were elucidated when forming this complex. Together with the Cell Division and Cancer Group, the Unit performed a global proteome analysis of neural differentiation in CDC14-null cells and elucidated UTF1 *in vitro* phosphorylated sites. The Unit also teamed up with the Breast Cancer Clinical Research Unit to reveal a new physical interactor of Filamin A, CLIP170, which plays a role in microtubule stabilisation and may explain the increased sensitivity to paclitaxel in tumours with elevated CDK4. With the Microenvironment and Metastasis Group, the Unit characterised plasma circulating small extracellular vesicles derived from melanoma patients compared to proteins detected in plasma samples. In collaboration with the Medical University of Dresden (Germany), the Unit used Tandem Mass Tag (TMT) isobaric labelling proteomics and phosphoproteomics to identify a novel treatment approach for RTK/MAPK pathway altered in gastric cancer patients. With M. Serrano's group at *IRB* Barcelona, we used label-free proteomics to reveal the profound changes of the lysosomal proteome in senescent cells and studied the "surfaceome" of 2 diploid primary fibroblasts and 2 cancer cell lines in response to the senescence inducers doxorubicin and palbociclib. Aiming to investigate the effect of different variables in the performance of proteome-wide phosphoprotein analysis protocols, the Unit has formed part of a multicentre collaboration launched by *ProteoRed-ISCIII*. Finally, the Unit setup a new cross-linking mass spectrometry-based workflow to fit the needs of the Structural Biology Programme (FIGURE 1). This emerging technology interrogates protein structure and helps reveal novel protein-protein interactions. The protocol, robust and widely applicable, is based on protein cross-linking with MS-cleavable reagents, enzymatic digestion followed by high pH fractionation, and LC-MS/MS analysis. The output allows the identification of cross-links, assessing spatial and morphology constraints for recombinant purified proteins and complexes. ■

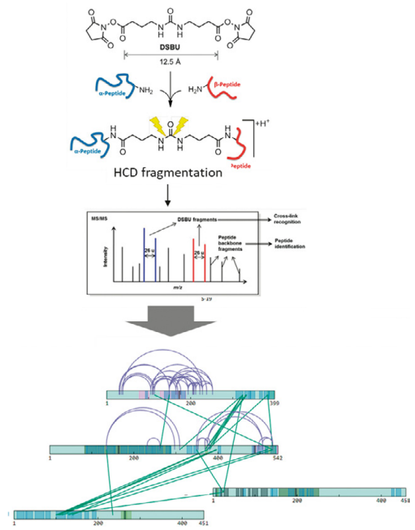


**FIGURE 1** Schematic showing the workflow used for cross-linking-based mass spectrometry. Covalently bound peptides derived from cross-linked proteins are identified, providing 3D structure analysis of proteins and protein complexes.

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# HISTOPATHOLOGY CORE UNIT

Vacant
Core Unit Head

Research Scientist
Eduardo José Caleiras

## OVERVIEW

Pathology is the branch of science dedicated to the study of the structural, biochemical, and functional changes in cells, tissues, and organs that underlie disease. The Histopathology Unit offers support and expertise in a range of services from paraffin embedding and tissue sections to histochemical staining, research and diagnostic immunohistochemistry (IHC) testing, antibody validation, *in situ* hybridisation techniques (including mRNA *in situ* detection using RNAScope), and tissue microarray generation. Other value-added services offered by the Unit's highly skilled technicians include laser capture microdissection, slide digitalisation, image analysis, and quantification. The Unit also collaborates with CNIO researchers in the histopathological characterisation of animal models of disease, providing them with the necessary expert pathological advice. Finally, the Unit offers its portfolio of services to other institutions, including hospitals, research centres and private companies.

**"The pathological analysis of mouse and human tissues provided by the Unit, applying a broad array of histochemical and immuno-histochemical techniques, is critical to the progress of oncology research projects run at the CNIO."**





Technicians
Nuria Cabrera, María Gómez, Patricia González, Verónica Neva, Andrea Romero (PEJ)*, Zaira Vega

*Student in Practice*
Daniel Marban (March-June) (*Instituto Técnico de Estudios Profesionales*, Madrid, Spain)

**Plan de Empleo Joven* (Youth Employment Plan, until March)

## RESEARCH HIGHLIGHTS

During 2022, the Unit significantly increased its workload compared to the previous years. Thus, about 30,000 paraffin blocks of tissue samples were generated, and nearly 25,000 histological techniques and over 22,000 immunohistochemistry techniques were delivered. This represents an increase of approximately 30% over the levels of 2021.

We also made significant progress in the digitisation of our material with about 15,400 slides, which represents approximately 54% of the stains generated. In addition, the Unit supports the CNIO Groups with the digital analysis of the images, training researchers in the use of the Zen imaging software.

Furthermore, we consolidated the application of *in situ* hybridisation technology to research projects at the CNIO, focusing on mRNA detection using RNAScope technology. As many as 402 cases were analysed, some of them with double staining, using the Ventana-Roche automated platform for IHC staining. This technique enables the detection of specific mRNAs directly in formalin-fixed, paraffin-embedded (FFPE) tissue sections, thus bringing a spatial dimension to gene expression analysis.

In 2022 the Unit was awarded a grant through the call *Ayudas a Proyectos de Colaboración Público-Privada* from the Ministry of Science and Innovation (*MCI*), for a project in collaboration with the company MedLumics and the *Universitat Pompeu Fabra*. The project focuses on the development of a system to treat auricular fibrillation using irreversible electroporation. The role of the Unit in the project focuses on the analysis of the pathological features and the mechanisms mediating cell death in the cardiac tissue upon auricular fibrillation ablation.

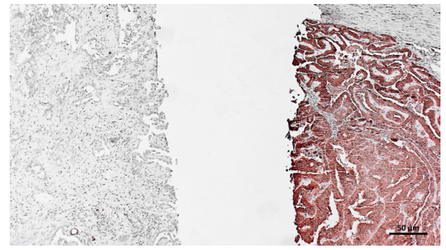

**FIGURE 1** Detection of S-(2-Succinyl)-Cysteine (2SC) by immunohistochemistry in papillary carcinoma of the kidney. On the right, a case showing fumarate deficiency and the consequent accumulation of 2SC. On the left, another case of the same tumour type without fumarate deficiency. Courtesy of Cristina Rodríguez, Hereditary Endocrine Cancer Group.

The high quality of the techniques run by the Unit continues to be endorsed by External Quality Assessment Schemes. In this respect, our histochemical techniques were evaluated by UK NEQAS. Similarly, NordiQC and SEAP (*Sociedad Española de Anatomía Patológica*) evaluated a subset of our IHC techniques under different modules, including general markers, breast cancer markers, and PD-L1; these all obtained very good scores.

Training and outreach activities are also a key component of the Unit's activities. In the lab we hosted 1 vocational training student in anatomical pathology (*Formación Profesional de Grado Superior en Anatomía Patológica*) undertaking a practical module for 3 months. In addition, the Unit participated in a master's course in oncology research. ■

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# ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
*Vivotecnia* Management & Services

The Animal Facility's primary responsibility is the supply, husbandry, and quality control of laboratory animals used by the Research Programmes in their experimental protocols. The strict compliance to national, EU, and international recommendations regarding the use and care of animals in research is paramount to the CNIO.

The Animal Facility provides CNIO researchers with all the support required to work with mouse models, in compliance with the highest standards of animal care and welfare. The Animal Facility was established to assist researchers in the development and analysis of *in vivo* models as tools in cancer research. We are currently collaborating with as many as 27 CNIO Research Groups, Sections, and Units.

All the work carried out by the Animal Facility complies with both national and EU legislation — RD53/2013 and EU Directive 2010/63/UE — for the protection of animals used for research experimentation and other scientific purposes. Experimental procedures and projects are reviewed by the Research Ethics and Animal Welfare Committee of the *Instituto de Salud Carlos III*, as well as by the Institutional Animal Care and Use Committee (IACUC). The *Orden ECC/566/2015* stipulates that all animal procedures are to be carried out by qualified people with accreditation issued by the competent authority. The Animal Facility offers CNIO's new staff a course focused on work with laboratory animals, complementary to the online courses that are a requisite to gain access to the facility.

**"Ensuring high standards in animal welfare is a critical factor to guarantee the quality of animal-based research and, as such, providing those high standards is one of the main missions of our Animal Facility."**

In accordance with our commitment to maintaining the highest possible standards in relation to animal research, the CNIO joined the Agreement on Openness on Animal Research, promoted by the Federation of Scientific Societies in Spain (COSCE) in collaboration with the European Animal Research Association (EARA), launched in September 2016. An institutional statement on the use of animals for research can be consulted on the CNIO website.

The high standards achieved by the CNIO with regard to the use and care of animals for experimentation have been recognised by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. This is a private non-profit organisation that promotes the humane treatment of animals in science through voluntary accreditation and assessment programmes. AAALAC accreditation, considered one of the top international recognitions in this field, was first obtained in October 2016. In 2022, the Animal Facility programme was reviewed and full accreditation was renewed. The Animal Facility's Head was also elected as AAALAC Ad Hoc Consultant, to assist members of the Council on Accreditation in evaluating animal care and use programmes. In addition, the Assistant Veterinarian was recently elected as vice-treasurer of the Spanish Society for Laboratory Animal Sciences (SECAL). SECAL is the most prominent scientific society in the field of laboratory animals in Spain, devoted to advancing the scientific understanding of the use, care, and welfare of laboratory animals.

Our Animal Facility has the capacity to house 19,000 type IIL cages. Our mouse lines are maintained and bred in the Facility's barrier area, which assures Specific Pathogen Free (SPF) health status. Microbiological and environmental parameters in the animal areas are constantly monitored. All mouse strains housed in the barrier are either generated within the barrier or introduced by rederivation. We also have an additional area with a capacity for 1,800 type II cages for the use of non-replicative strains of adenovirus, lentivirus, and retrovirus, as well as for xenograft models. In this area, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are always manipulated in Type II biosafety cabins.

Daily operations and husbandry procedures are highly automated to safeguard our personnel from any associated risks. Robotic devices perform the potentially hazardous tasks such as the processing of dirty bedding, the washing and filling of cages and bottles, etc. These automated systems maximise productivity and ensure quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility currently harbours nearly 40,000 mice representing more than 3,000 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying close to 400 gene targeted alleles and more than 200 transgenic integrations. The Facility also provides access to more than 50 tool strains, including constitutive and inducible Cre strains, Flp strains, reporter strains, and others.

The Animal Facility offers the possibility of running a broad number of experimental procedures in the premises. These include the use of gamma irradiation, UV light and volatile carcinogenic agents; surgical procedures, behavioural studies, and non-invasive blood pressure measurement; a laboratory animal monitoring system (Oxylet) that enables tracking a number of physiological parameters for metabolic profiling and phenotyping of mouse models; and a climate chamber (HPPlife) that allows mice to be kept under controlled environmental conditions of temperature, humidity, and light, beyond the standard conditions established at the SPF barrier area.

Additionally, the monitoring of the mouse models through non-invasive imaging technologies is provided by the Molecular Imaging Unit, which has all its image acquisition instruments within the Animal Facility. Likewise, the work of the Mouse Genome Editing Unit is performed in a laboratory inside the SPF barrier. Finally, the necropsy laboratory is equipped with instruments for the haematological and biochemical analysis of blood and urine, which complement the pathology and clinical diagnostics.

In addition to mice, the Animal Facility hosts a colony of rats to generate monoclonal antibodies against mouse antigens, as well as for a project of the Experimental Therapeutics Programme aimed at testing the safety of some anti-tumour compounds. ■