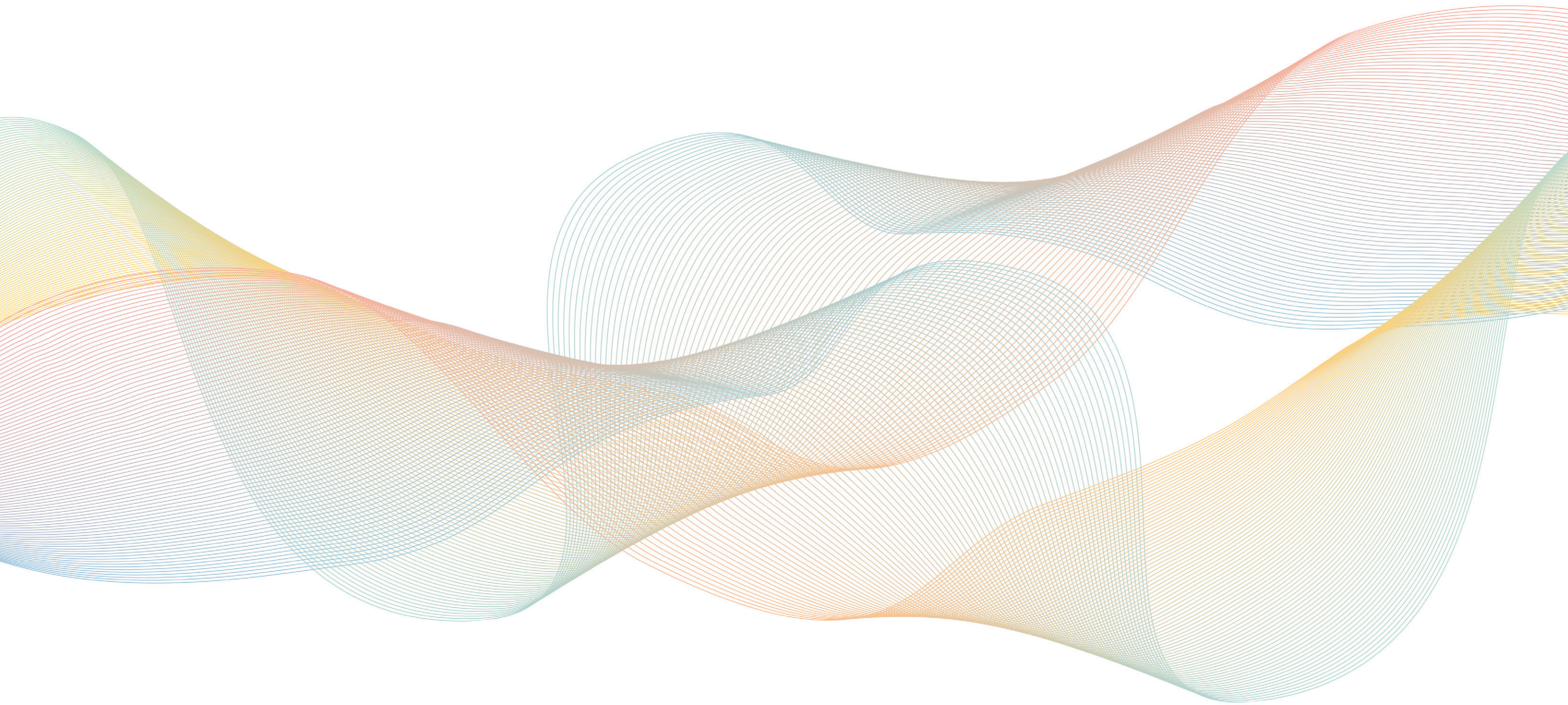
SPANISH NATIONAL
CANCER RESEARCH CENTRE, CNIO

ANNUAL REPORT 2024



cnio stop cancer

EXCELENCIA SEVERO OCHOA
2012 - 2024

ANNUAL REPORT 2024

SPANISH NATIONAL
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cnio stop cancer

GOBIERNO DE ESPAÑA | MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES | Instituto de Salud Carlos III

EXCELENCIA SEVERO OCHOA
01/2012-12/2024
04/2025-03/2031

GOBIERNO DE ESPAÑA | MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES | AGENCIA ESTATAL DE INVESTIGACIÓN

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LETTER FROM THE DIRECTOR

FERNANDO PELÁEZ Acting Director

Over the past decade, the CNIO has firmly established itself as one of the world's leading cancer research centres. It now enters a new phase marked by strong scientific momentum and sustained productivity, as well as by some critical challenges. We acknowledge Maria A. Blasco for her 14 years of service as Scientific Director as we begin a new chapter—one that will demand clear strategic vision and thoughtful allocation of resources to uphold our privileged position at the forefront of cancer research. The CNIO is navigating this transition period—until a new Scientific Director is appointed—by doing what it does best: generating knowledge to advance the prevention, diagnosis, and treatment of cancer.

This Annual Report provides a summary of the research activities carried out throughout 2024. We welcomed new research groups, celebrated our participation in ambitious international programmes, and laid the groundwork—in terms of human resources, technologies, and new projects—to explore the new frontiers in cancer research opened up by artificial intelligence. The list of achievements continues to be truly remarkable—not only in terms of generating relevant scientific knowledge, some of which has had a direct impact on cancer patients and has been published in high-profile journals—but also through success in securing competitive research grants, the transfer of knowledge to the private sector via contracts and collaborations with industrial partners (including revenues from the exploitation of our intellectual property assets), and the dissemination of scientific results through seminars, workshops, and events aimed not only at specialised audiences but also at the general public.

As this edition closes, we are also proud to celebrate the recent announcement that the CNIO has been awarded the Severo Ochoa Centre of Excellence accreditation for the fourth consecutive time. This prestigious recognition, granted to "leading centres and units that stand out for the relevance and international impact" of their research, will remain in effect for the next six years.

As Acting Scientific Director, and after more than 16 years as Director of the Biotechnology Programme, I can confidently say that the CNIO is fortunate to have a quality that is sometimes difficult to fully capture with conventional indicators: the ideas, creativity, dedication, and drive of its scientists, technical and support staff—its human capital. My sincere thanks to all. ■

ORGANISATION OF RESEARCH 2024

MARIA A. BLASCO DIRECTOR

BASIC RESEARCH

MOLECULAR ONCOLOGY PROGRAMME

Óscar Fernández-Capetillo Programme Director
Paloma Olave, Cristina Álvaro, Lucía Ámez Secretaries

Maria A. Blasco
Telomeres and Telomerase Group – Humanism and Science Foundation

Mariano Barbacid
Experimental Oncology Group

Óscar Fernández-Capetillo
Genomic Instability Group

Felipe Cortés-Ledesma
Topology and DNA Breaks Group

Ana Losada
Chromosome Dynamics Group

Juan Méndez
DNA Replication Group

María S. Soengas
Melanoma Group

Francisco X. Real
Epithelial Carcinogenesis Group

Nabil Djouder
Growth Factors, Nutrients and Cancer Group

Eva González-Suárez
Transformation and Metastasis Group

Manuel Valiente
Brain Metastasis Group

Guadalupe Sabio
Organ Crosstalk in Metabolic Diseases Group

Gonçalo Bernardes (since October)
Translational Chemical Biology Group

Héctor Peinado
Microenvironment and Metastasis Junior Group

Alejo Efeyan
Metabolism and Cell Signalling Junior Group

María Casanova-Acebes
Cancer Immunity Junior Group

STRUCTURAL BIOLOGY PROGRAMME

Óscar Llorca Programme Director
Belén Bañeres Secretary

Óscar Llorca
Macromolecular Complexes in DNA Damage Response Group

Iván Plaza-Menacho
Kinases, Protein Phosphorylation and Cancer Junior Group

Rafael Fernández Leiro
Genome Integrity and Structural Biology Junior Group

Solip Park
Computational Cancer Genomics Junior Group

Geoff Macintyre
Computational Oncology Junior Group

Lucas Tafur (since October)
Structural Mechanisms of Cell Growth Junior Group

Marcos Díaz-Gay (since November)
Digital Genomics Junior Group

Ramón Campos-Olivas
Spectroscopy and Nuclear Magnetic Resonance Unit

Fátima Al-Shahrour
Bioinformatics Unit

Jasminka Boskovic
Electron Microscopy Unit

Inés Muñoz
Protein Crystallography Unit

Jorge L. Martínez-Torrecuadrada
Protein Production Unit

PATIENT-ORIENTED RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Vacant Programme Director
Gema Moreno Secretary

Mercedes Robledo
Hereditary Endocrine Cancer Group

Núria Malats
Genetic and Molecular Epidemiology Group

Sandra Rodríguez-Perales
Molecular Cytogenetics Unit

Maria Currás
Familial Cancer Clinical Unit

Anna González-Neira
Human Genotyping-*CEGEN* Unit

CLINICAL RESEARCH PROGRAMME

Miguel Quintela-Fandino Programme Director
María Luisa Anguita Secretary

Miguel Quintela-Fandino
Breast Cancer Clinical Research Unit

Luis J. Lombardía
Molecular Diagnostics Unit

Luis Álvarez-Vallina
H12O-CNIO Cancer Immunotherapy Clinical Research Unit

Luis Paz-Ares
H12O-CNIO Lung Cancer Clinical Research Unit

Joaquín Martínez-López
H12O-CNIO Haematological Malignancies Clinical Research Unit

Antonio Pérez-Mártínez
IdiPAZ-CNIO Pediatric Onco-Hematology Clinical Research Unit

INNOVATION

ROKE I. ORUEZABAL DIRECTOR OF INNOVATION
M. Carmen Rodríguez Secretary

BIOTECHNOLOGY PROGRAMME

Fernando Peláez Programme Director
Celia María Ramos Secretary

Marta Isasa
Proteomics Core Unit

Orlando Domínguez
Genomics Core Unit

Isabel Peset
Confocal Microscopy Core Unit

Francisca Mulero
Molecular Imaging Core Unit

Sagrario Ortega
Mouse Genome Editing Core Unit

Giovanna Roncador
Monoclonal Antibodies Core Unit

Vacant
Histopathology Core Unit

Lola Martínez
Flow Cytometry Core Unit

Isabel Blanco
Animal Facility (*Vivotecnia* Management & Services)

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor Programme Director
Natalia Catalá Secretary

Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
CNIO-Lilly Cell Signalling and Immunometabolism Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Irene Herrera Head

BIOBANK

MARÍA JESÚS ARTIGA Acting Head

Basic Research

MOLECULAR ONCOLOGY PROGRAMME

ÓSCAR FERNÁNDEZ-CAPETILLO Programme Director

The overall aim of the Molecular Oncology Programme is to gain a fundamental understanding of the mechanisms that drive cancer onset and progression, and to use this information to set the principles for innovative cancer therapies. To do so, we have Groups covering a wide range of topics in cancer research, such as DNA and chromosome stability (Maria A. Blasco, Óscar Fernández-Capetillo, Felipe Cortés-Ledesma and Ana Losada), oncogenes and cell cycle kinases (Mariano Barbacid), DNA replication (Juan Méndez), melanoma (María S. Soengas), metabolism and cell signalling (Nabil Djouder and Alejo Efeyan), immunotherapy (María Casanova), epithelial carcinogenesis (Francisco X. Real) and metastasis (Manuel Valiente, Eva González-Suárez and Héctor Peinado). In its origins, the Programme had a strong orientation towards the basic mechanisms that regulate cell growth and death. However, throughout the years, we have been progressively expanding into other areas of emerging interest, such as metabolism, metastasis, and immunology. In 2024, we have further broadened our expertise by incorporating two additional Groups working on inter-organ communication (Guadalupe Sabio) and chemical biology (Gonçalo Bernardes). I wish you both a fruitful and exciting time at CNIO and very much look forward to your discoveries.

"I am firmly convinced that our new Groups will not only contribute to great science but also trigger novel and exciting collaborations that will broaden the horizons of many of our teams."

During 2024, our scientists kept reporting exciting discoveries, and here I summarise a few examples of their works. Ana Losada's Group continues to explore how STAG2 deficiency contributes to carcinogenesis by modulating chromatin structure, a discovery pioneered at the CNIO by the work of Francisco X. Real. Real's Group is also involved in international efforts related to clinical trials for novel treatments of urothelial cancer. The Group of Nabil Djouder has revealed data about how cold temperatures impact hepatocellular carcinoma, as well as other interesting discoveries around signalling pathways that modulate obesity and pluripotency. Also related to the liver, the Group of Alejo Efeyan revealed an intriguing role for mTOR in coordinating how cells are positioned in this organ during development. They were also able to provide, for the first time, evidence demonstrating that hyperactivation of mTOR accelerates ageing in mammals. In the context of ageing, Maria Blasco's Group keeps an active line of research aimed at deciphering the cell types that contribute to organ fibrosis, in this case renal fibrosis. The Group of Juan Méndez made interesting insights into how DNA replication is coordinated during pluripotency and how cells ensure that DNA replication occurs once, and only once, per cell division. Héctor Peinado made further insights about how NGFR signalling might be a target of interest for cancer therapy, now linking it to the immune system. In addition, he also reported novel ideas on how pancreatic cancer cells might hide from immune cells. Manuel Valiente's team has made exciting discoveries regarding the mechanisms that limit the efficacy of immunotherapies in brain metastases, as well as contributed to the development of a fascinating technology that can use light to visualise brain lesions with minimal invasiveness. In our own Group, we keep trying to understand fundamental principles that drive age-related diseases such as neurodegeneration, and our data suggest an important contribution from aggregates of ribosomal proteins. In addition, we reported novel inhibitors of the SETD8 methyltransferase and the mutations that sensitise cancer cells to these agents. Finally, Guadalupe Sabio's team has revealed novel mechanisms that regulate muscle/brain communication during exercise, as well as key mediators of heat production from brown fat. She now has plans and a host of genetic tools to investigate the contribution of these and other inter-organ signalling events in the context of cancer development. ■

TELOMERES AND TELOMERASE GROUP – HUMANISM AND SCIENCE FOUNDATION

Maria A. Blasco
Group Leader

Research Scientists
Isabel López de Silanes, Rosa M. Marión, Paula Martínez

Post-Doctoral Fellows
Giuseppe Bosso, Sonia Burgaz, María Isabel Espejo, Buyun Ma, Sarita Saraswati

Graduate Students
Jaime Fiel (since October), José Carlos González, Óscar Laguía, Jessica Louzame, Amparo Sánchez

Technicians
David Hernández (TS)*, Raúl Sánchez-Vázquez (TS)*, Rosa M. Serrano

Titulado Superior (Advanced Degree)

Students in Practice
Giulia Cardena (July- Sept) (Erasmus +, Urbino Carlo Bo University, Italy), Jaime Fiel (until Sept) (Master's Thesis, Francisco de Vitoria University, Madrid, Spain), Tiia Kärkkäinen (Sept-Dec) (Emil Aaltonen Foundation Postdoc Fellowship, MNCN, Madrid, Spain), Abigail López-Freire (May-Aug) (Predoctoral Fellowship, Yale University, CT, USA), Martina Marini (July- Sept) (Erasmus, Urbino Carlo Bo University, Italy), Claudia Sánchez (Aug-Oct) (UAM, Madrid, Spain), Selin Yozgyur (Sept-Nov) (Erasmus, Biruni University, Istanbul, Turkey), Samuel L. Zaid (Jan-June) (Bachelor's Degree Final Project, UAM, Madrid, Spain)

Visiting Students/Scientists
Marwa M. Abu-Serie Ali (until April) (GEBRI, SRTA-City, Alexandria, Egypt) (Science by Women Programme), Saba Nategui (until July) (Master's Thesis, Univ. of Groningen, The Netherlands), Xinying Zhou (May-Nov) (Pre-doc Fellowship, Southern Medical University, Guangzhou, China)

OVERVIEW

One of the most universal characteristics of cancer cells is their immortality. We study the mechanisms by which tumour cells are immortal and normal cells are mortal. Over 95% of all types of human cancers exhibit the enzyme telomerase, absent in normal cells in the body. Telomeres, nucleoprotein complexes at the ends of chromosomes, are essential for chromosome protection and genomic stability. Their progressive shortening as the organism ages leads to ageing. If telomeres are altered, the regenerative capacity of adult stem cells is maimed.

"We provided useful insights into the understanding of the pathophysiology of malignancies driven by oncogenic *BRAF*^{V600E} and the *in vivo* consequences of novel ERK pathway-targeted anti-cancer therapies."

Our research focuses on:

1. Mouse models to validate telomeres and telomerase as therapeutic targets for cancer and age-related diseases.
2. Interplay between telomeres and DNA repair pathways.
3. Role and regulation of non-coding telomeric RNAs (TERRA).
4. Telomerase gene therapy in *telomere syndromes* and age-related diseases.
5. Role of telomerase and telomeres in:
   - adult stem cell biology
   - nuclear reprogramming of differentiated cells to induced pluripotent stem cells (iPS cells).

RESEARCH HIGHLIGHTS

Involvement of renal fibroblasts in dysfunctional telomeres-associated kidney fibrosis

Tubulointerstitial fibrosis, associated with chronic kidney disease (CKD), is a global health care problem. Renal fibrosis is characterised by a slow deterioration of the functional renal parenchyma; this sustained kidney dysfunction is associated with an irreversible loss of kidney cells and nephrons, a hallmark of CKD, which can ultimately lead to end-stage renal disease.

We had already reported that short and dysfunctional telomeres, arising from the genetic ablation of the *Trf1* gene encoding a telomere-biding factor, led to the acquisition of a mesenchymal phenotype associated with interstitial renal fibrosis, recapitulating human disease. The cells of origin of kidney fibrosis associated with this telomere dysfunction, however, were yet to be established. Given the significant role of TRF1 as one of the initiator cells of renal interstitial fibrosis, we aimed to address its role in kidney fibroblasts. To identify said role, we induced telomere dysfunction in mice by deleting *Trf1* specifically in renal fibroblasts in both short-term and long-term life-long experiments. We found out that short-term *Trf1* deletion in renal fibroblasts per se was not sufficient to trigger kidney fibrosis, but it did suffice to induce inflammatory responses, extracellular matrix deposition, G2/M cell cycle arrest, fibrogenesis, and vascular rarefaction. Long-term persistent deletion of *Trf1* in fibroblasts, however, resulted in kidney fibrosis accompanied by an elevated urinary albumin-to-creatinine ratio and a decline in mouse survival rates. These cellular responses led to the transition of fibroblasts to myofibroblasts via the macrophage-to-myofibroblast transition (MMT), endothelial-to-mesenchymal transition (EndMT), and partial epithelial-to-mesenchymal transition (EMT), ultimately causing kidney fibrosis at the humane endpoint (HEP) when the deletion of *Trf1* in fibroblasts is maintained throughout the lifespan of mice (Figure 1).

We demonstrated for the first time that telomere dysfunction in renal fibroblasts activates an inflammatory cascade, vascular rarefaction, and G2/M cell cycle arrest, causing fibroblasts to undergo the above-mentioned various transitions in the context of acute kidney injury and maladaptive repair, exacerbating the classical features observed in CKD patients.

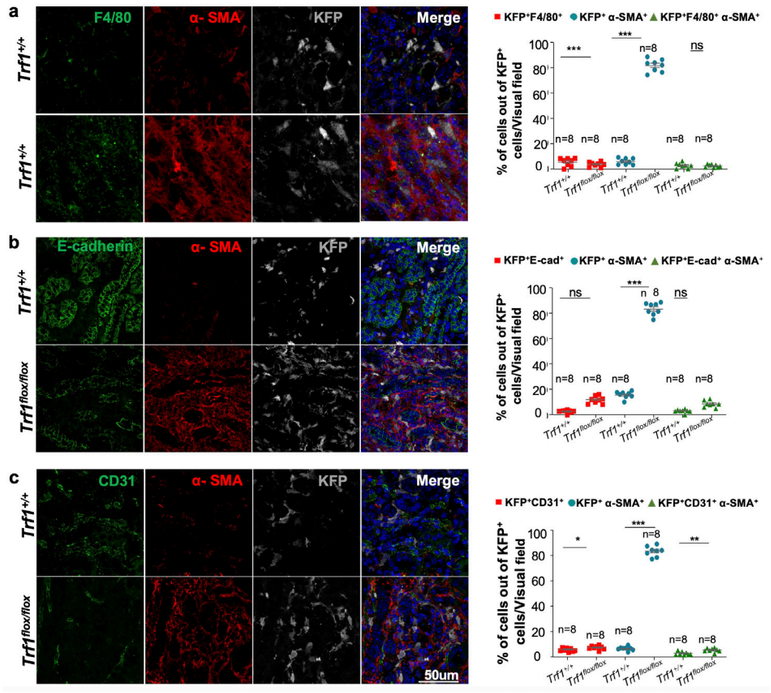

FIGURE 1 Persistent long-term fibroblast-specific deletion of *Trf1* leads to acquisition of a mesenchymal phenotype via MMT, partial EMT, and EndMT at HEP. Representative images and quantifications of **a**) immunolabelling for F4/80, α-SMA and KFP in *Trf1*$^{+/+}$ and *Trf* $^{flox/flox}$ kidneys, **b**) immunolabelling for E-cadherin, α-SMA and KFP in *Trf1*$^{+/+}$ and Trf1$^{flox/flox}$ kidneys, **c**) immunolabelling for CD31, α-SMA and KFP in *Trf1*$^{+/+}$ and *Trf1*$^{flox/flox}$ kidneys. Nuclei are stained with DAPI (blue).

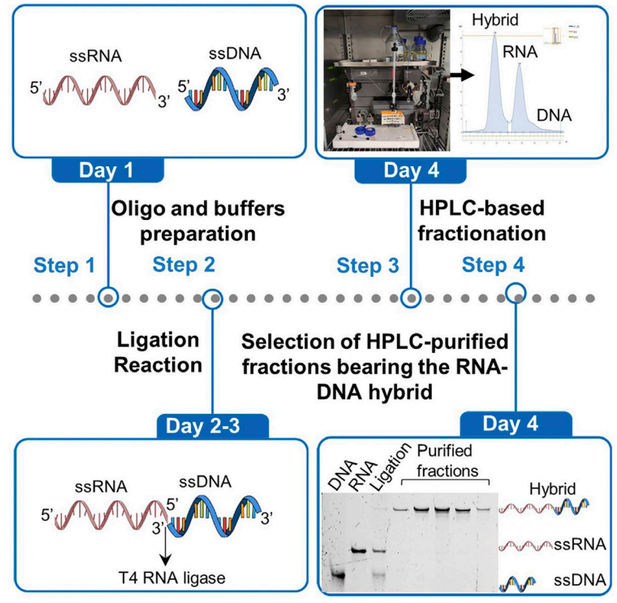

FIGURE 2 Graphical abstract of protocol for the generation and purification of high molecular weight RNA-DNA hybrids.

Our findings contribute to a better understanding of the role of dysfunctional telomeres in the onset of the profibrotic alterations that lead to kidney fibrosis. Early detection and accurate assessment of the cell types, responsible for kidney fibrosis, are crucial for the application of timely interventions as a primary measure and the discovery of new cell-targeted therapies to treat CKD.

A 4-step protocol for the generation and purification of high molecular weight RNA-DNA hybrids

RNA-DNA covalent hybrids (RDHs) are employed in an ample variety of applications in biology, including the stabilisation of small interference RNA molecules, generation of transcription factor targeting chimeras, design of ricin inhibitors, and the identification of single nucleotide polymorphisms. RDHs are present in nature and manufacturable by means of chemical or enzymatic reactions. Most molecular biology companies, however, find the synthesis of RDHs longer than 120 nucleotides challenging. Pertinent to these challenges was the fact that detailed protocols for the production and purification of RDHs, which would circumvent the limitations due to the hybrids size, were so far lacking in the literature. In response, we have developed the very first optimised protocol for the generation and isolation of high-grade purified high molecular weight 5'-RNA-DNA-3' covalent hybrids. Our 4-step protocol makes use of T4 RNA ligase I, followed by high-performance liquid chromatography (HPLC) purification. Our protocol (Figure 2) is executable in standard molecular biology laboratories. ■

PUBLICATIONS

- Bosso G, Cintra-Herpst AC, Laguía O, Adetchessi S, Serrano R, Blasco MA (2024). Differential contribution for ERK1 and ERK2 kinases in BRAF^{V600E}-triggered phenotypes in adult mouse models. *Cell Death Differ* 31, 804-819.
- Saraswati S, Martínez P, Serrano R, Mejías D, Graña-Castro O, Álvarez Díaz R, Blasco MA (2024). Renal fibroblasts are involved in fibrogenic changes in kidney fibrosis associated with dysfunctional telomeres. *Exp Mol Med* 56, 2216-2230.
- Ma B, Martínez P, Sánchez-Vázquez R, Blasco MA (2024). Telomere dynamics in human pluripotent stem cells. *Cell Cycle* 22, 2505-2521.
- Laguía O, Bosso G, Martínez-Torrecuadrada J, Miguel-Amil S, Fernández-Leiro R, Blasco MA (2024). Protocol for the generation and purification of high-molecular-weight covalent RNA-DNA hybrids with T4 RNA Ligase. *STAR Protoc* 5, 102930.

PATENTS

- Blasco MA, Saraswati S, Martínez P. Telomerase reverse transcriptase therapy for kidney fibrosis and non-human animals thereof. International Publication Number: *WO2023138791A1*. National Phase Entry (2024).
- Blasco MA, Bär C, Bosch F, Formentini I, Bobadilla M. Telomerase reverse transcriptase-based therapies. International Publication Number: *WO2016020345A1*. National Phase Entry (2024).

AWARDS AND RECOGNITION

- Full Member (*Académica de número*) of the Royal Spanish National Academy of Pharmacy. Madrid (Spain).

EXPERIMENTAL ONCOLOGY GROUP

Mariano Barbacid
Group Leader

Research Scientists
Sara García-Alonso, Carmen Guerra

Post-Doctoral Fellows
Vasiliki Liaki, Carolina Villarroya (until Aug.)

Graduate Students
Domingo Acosta (since Sept.), Gonzalo M. Aizpurua, Sara Barrambana, Oksana Brehey, Laura de la Puente, Ana María Fernández, Ana Galván, Lucía Lomba, Blanca Rosas (since Sept.), Pian Sun, Elena Zamorano

Technicians
Rebeca Barrero, M. Carmen González

(TS)*, Silvia Jiménez, Teresa Laguna (since Feb.) (TS)*, Alejandra López, Marta San Román, Raquel Villar

**Titulado Superior* (Advanced Degree)

Undergraduate Students
Mario Castillo (Jan-June) (Master's Thesis, Univ. Autónoma de Madrid, Spain), Javier Montes (Jan-June) (Master's Thesis, Univ. Autónoma de Madrid, Spain), Laura Navarro (April-June) (Technician's in training (FCT), IES José Luis Sampedro, Madrid, Spain), Blanca Rosas (Jan-July) (Master's Thesis, Univ. Autónoma de Madrid, Spain)

External Associates
Matthias Drosten (Centro de Investigación de Cáncer (CIC), Salamanca, Spain), Monica Musteanu (Univ. Complutense de Madrid, Spain), Juan Velasco (Eli Lilly, Alcobendas, Spain), Carolina Villaroya (CBM Severo Ochoa, Madrid, Spain)

OVERVIEW

The main thrust of our laboratory is to identify therapeutic strategies against KRAS mutant lung and pancreatic tumours using genetically engineered mouse strains. We recently reported that ablation of KRAS oncogenes induces complete tumour regressions with no signs of tumour resistance. Unfortunately, KRAS inhibitors, either specific for selected mutations or panKRAS inhibitors, only result in partial tumour regressions. Moreover, this limited therapeutic activity is thwarted by the rapid appearance of tumour resistance. Five years ago, we reported that concomitant ablation of two targets involved in KRAS signalling, RAF1 and EGFR, led to the complete disappearance of a limited number of small pancreatic tumours. Yet, most tumours, especially those exhibiting sizes larger than 100 mm^3, did not respond to this therapy. We have now identified a third target, STAT3, whose combined ablation with RAF1 and EGFR led to the complete disappearance of all pancreatic tumours regardless of size. More importantly, these mice remained tumour free for up a year. We are now trying to validate this therapeutic strategy using pharmacological approaches with the ultimate goal of translating these results to a clinical scenario.

"Genetic studies have identified a therapeutic strategy that completely eliminated experimental pancreatic tumours. Validation of this therapy with pharmacological approaches will make it possible to translate these results to a clinical scenario."

RESEARCH HIGHLIGHTS

Lung Cancer

Lung cancer is the leading cause of cancer mortality worldwide. KRAS oncogenes are responsible for at least a quarter of lung adenocarcinomas (LUAD), the main subtype of lung cancer. After four decades of intense research, selective inhibitors of KRAS oncoproteins are finally reaching the clinic. Yet, their effect on overall survival is limited due to the rapid appearance of drug resistance, a likely consequence of the high intra-tumoural heterogeneity characteristic of these tumours. We have attempted to identify those functional alterations, which result from KRAS oncoprotein expression during the earliest stages of tumour development. Such functional changes are likely to be maintained during the entire process of tumour progression regardless of additional co-occurring mutations. Single cell RNAseq analysis of murine alveolar type 2 (AT2) cells, expressing a resident KRAS oncogene, revealed impairment of the type I Interferon pathway, a feature maintained throughout tumour progression. This alteration was also present in advanced murine and human tumours harbouring additional mutations in the p53 or LKB1 tumour suppressors. Restoration of type I interferon signalling, by IFN-β or constitutive active STING expression, had a profound influence on the tumour microenvironment, switching them from immunologically "cold" to immunologically "hot" tumours. Therefore, enhancement of the type I IFN pathway predisposes KRAS mutant lung tumours to immunotherapy treatments, regardless of co-occurring mutations in p53 or LKB1. Furthermore, restoration of type I interferon signalling in non-immunogenic KRAS mutant lung tumours also alters their tumour microenvironment from immunologically "cold" to "hot", thus enhancing responses to immunotherapy regardless of co-occurring p53 or LKB1 loss.

In our murine models, IFN-β and, to a lesser extent, STINGV154M expression were able to attract infiltration of CD8+ and CD4+ T cells, dendritic cells, and NKT cells. Neutrophil infiltration was higher in the KL than in the KP tumours, an observation consistent with previous data indicating that massive neutrophil infiltration, reduced T cell content, and down-regulation of MHC-I molecules are features of KRAS/LKB1-driven LUAD. Moreover, IFN-β but not STINGV154M expression was able to promote a reduction in the content of neutrophils, probably a good prognostic factor due to their immunosuppressive nature. Finally, in both p53 or LKB1 mutant LUAD tumours, IFN-β-induced type I IFN pathway stimulation or activation of STING along with α-PD-1 treatment had a significant antitumoural effect, indicating that the enhancement of the type I IFN pathway is likely to predispose KRAS mutant lung tumours to immunotherapy treatments. Indeed, several clinical trials are currently being conducted to investigate the benefits of using IFN-based therapies in combination with immune checkpoint inhibitors, such as the use of STING agonists, Toll-like receptors agonists, or IFN-β expression systems. In summary, our results suggest that it is necessary to use stimulators of the type I IFN pathway in order to enhance current immunotherapy protocols.

Pancreatic Cancer

Mouse pancreatic ductal adenocarcinomas (PDAC) are defined by their differential response to RAF1 and EGFR ablation as well as by their distinct transcriptional profiles that correlate with the classical and basal subtypes of human pancreatic tumours. These transcriptional profiles represent different stages of tumour development, most likely induced by the tumour microenvironment. Furthermore, we have observed hybrid states and transitions between these tumour stages, an intratumour heterogeneity and cellular plasticity that may have a significant impact on the effectiveness of forthcoming therapeutic strategies. Indeed, the percentage of tumours sensitive to RAF1/EGFR ablation decreased markedly with tumour progression.

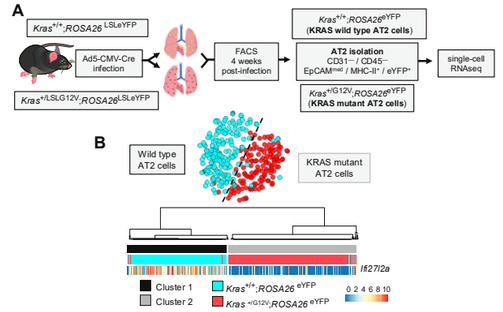

FIGURE 1 scRNAseq data reveals only two cell populations separating KRAS wildtype from KRAS^{G12V} expressing AT2 cells. (**A**) Schematic representation of the experimental design. (**B**) Consensus clustering. Most of the KRAS wild type AT2 cells (*Kras*$^{+/+}$;*ROSA26*$^{eYFP/eYFP}$) were grouped in cluster 1, while the KRAS mutant AT2 cells (*Kras*$^{+/G12V}$;*ROSA26*$^{eYFP/eYFP}$) were grouped in cluster 2.

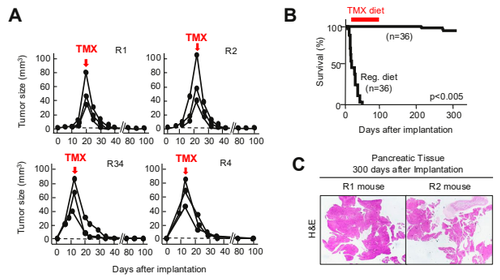

FIGURE 2 Concomitant ablation of *Raf1*, *Egfr* and *Stat3* leads to complete and durable tumour regression. (**A**) Tumour volume present in C57BL/6 mice (n=3) orthotopically implanted with four RAF1/EGFR Resistant KPeFC;*Raf1*$^{L/L}$;*Egfr*$^{L/L}$;*Stat3*$^{L/L}$ tumour cell lines exposed to a TMX diet at the indicated times (red arrow). (**B**) Kaplan-Meier survival curve of tumour-bearing mice. Two mice died due to tumour-unrelated causes. (**C**) Representative images of H&E-stained sections of pancreatic tissue of mice sacrificed 300 days post-implantation. No tumoural or stromal tissue could be detected.

We have now demonstrated that the acquisition of tumour resistance upon loss of RAF1/EGFR expression is mediated by activation of the transcription factor STAT3. Indeed, RAF1/EGFR resistant PDAC tumour cells die upon downregulation of STAT3 expression. Moreover, ectopic expression of a constitutively active isoform of STAT3 induced resistance to RAF1/EGFR elimination in sensitive tumour cells. Finally, Cre-mediated recombination of *Raf1*, *Egfr* and *Stat3* conditional alleles induces the complete regression of orthotopic PDAC tumours with no evidence of tumour resistance.

Previous studies have illustrated that STAT3 is an important player in PDAC development and progression. Genetic and biochemical evidence indicates that STAT3 activation, in the absence of RAF1 and EGFR expression, is not mediated by its classical pathway involving IL6 and the JAK kinases. Instead, STAT3 is activated via phosphorylation of its Tyr^{705} residue by FYN, a member of the SRC family of tyrosine protein kinases. Indeed, downregulation of FYN expression led to the rapid death of resistant RAF1/EGFR tumour cell lines presumably due to the absence of STAT3 activation. Yet, how loss of RAF1/EGFR expression leads to the activation of the FYN kinase, and possibly other members of the SRC family, remains to be resolved. Additionally, downregulation or ablation of STAT3 alone had no effect on PDAC maintenance and progression. Moreover, pancreatic tumour cells maintain their oncogenic properties as long as they retain expression of one of the three signalling nodes, RAF1, EGFR or STAT3. These observations suggest the existence of a complex signalling network involving these independent nodes. Unveiling how they interact among themselves, to ultimately contribute to tumour development, should help us to better understand the molecular events involved in KRAS signalling, at least in pancreatic tumour cells. ■

PUBLICATIONS

- Fernández-García F, Fernández-Rodríguez A, Fustero-Torre C, Piñeiro-Yáñez E, Wang H, Lechuga CG, Callejas S, Álvarez R, López-García A, Esteban-Burgos L, Salmón M, San Román M, Guerra C, Ambrogio C, Drosten M, Santamaría D, Al-Shahrour F, Dopazo A, Barbacid M, Musteanu M (2024). Type I interferon signaling pathway enhances immune-checkpoint inhibition in KRAS mutant lung tumors. *Proc Natl Acad Sci USA* 121, e2402913121.
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PATENT

- Guerra C, Liaki V. Triple combined therapy inhibiting EGFR, RAF1, and STAT3 against pancreatic ductal adenocarcinoma. PCT application (2024). *PCT/EP2024/052345*.

AWARDS AND RECOGNITION

M. Barbacid:
- 2024 'Alumni UCM Ilustre', Universidad Complutense de Madrid, Spain.
- 2024 Honorary Member, Royal Academy of Medicine of the Basque Country, Bilbao, Spain.
- 'Premio Virtuoso 2024', Spain.
- 'Premio Consalud 2024' Science Research Award and Opening Lecture, Madrid, Spain.
- 2024 Gold Medal Award, European Association for Cancer Research (EACR), Rotterdam, the Netherlands.
- Keynote Speaker, 59th Congress of the Norwegian Bioscience Society, Storfjell, Norway.
- Commencement Speech, XXXVII Summer Courses of the Universidad Complutense, El Escorial, Madrid, Spain.

C. Guerra:
- IX Carmen Delgado/Miguel Pérez-Mateo Award for Basic Research, Spain.
- Invited speaker, X Anniversary of the Asociación Cáncer de Páncreas, Spain.

S. García-Alonso:
- 'Alumni Destacada 2024', Universidad de León, Spain.
- ELLE Hope Award – Ciencia, Madrid, Spain.
- Premio Hipatia-Mujeres en Ciencia (Talento Joven), Madrid, Spain.
- FORBES Awards 2024: 100 mujeres más influyentes de España; 35 mujeres líderes en tecnología de España.
- TOP100 mujeres líderes 2024, MAGAS-El Español.
- Inaugural Lecture, XII Congreso de Investigación Biomédica, Universidad de Valencia, Spain.
- Keynote Lectures: Embassy of Spain in Paris, France; Fundación de Estudios Médicos (FEM), Murcia, Spain; Embassy of Spain in Stockholm, Sweden; Universidad de Deusto, Bilbao, Spain; XXIII Asamblea General de la AMIT (Asociación de Mujeres Investigadoras y Tecnológicas, Madrid, Spain.
- Invited keynote, XXXVII Summer Courses of the Universidad Complutense, El Escorial, Madrid, Spain.
- Invited speaker, "Sesión AECC" - 46º Congreso de la Sociedad Española de Bioquímica y Biología Molecular (SEBBM), A Coruña, Spain.
- Faculty Member: XXIII Escuela de Biología Molecular "Eladio Viñuela Margarita Salas" (June 2024) and "La aventura de divulgar ciencia en español con éxito: claves y herramientas" (July 2024), Universidad Internacional Menéndez Pelayo, Santander, Spain.

GENOMIC INSTABILITY GROUP

Óscar Fernández-Capetillo
Group Leader

Research Scientists
Vanesa Lafarga, Matilde Murga

Post-Doctoral Fellow
Ivó Hernández

Graduate Students
Amin El Manchoud (since September), Gema López, Mario López, Jorge Mota, Belén Navarro, Anabel Sáez

Technicians
Marta E. Antón, Alicia González (TS)*, Sara Rodrigo

**Titulado Superior* (Advanced Degree)

Students in Practice
Carlota Cradona (July-Sept.) (Bachelor's Degree Final Project, Universidad Francisco de Vitoria, AECC, Madrid, Spain), Amin El Manchoud (Jan.-June) (Master's Student, UAM, Madrid, Spain),
Ana Martínez (Jan.-July) (Master's Student, UAM, Madrid, Spain)

Visiting Scientist
Rosario Padilla (Sept.-Dec.) (Hospital Universitario de Móstoles, Spain)

OVERVIEW

The Genomic Instability Laboratory is interested in the molecular basis underlying cancer, and other age-related pathologies, with the ultimate goal to apply this knowledge in the development of novel strategies to treat these diseases. Our initial investigations focused on replicative stress (RS), a type of DNA damage, which is particularly abundant in certain cancer types. Our research on RS responses revealed important mechanistic insights into the impact of RS on cancer and ageing. Indeed, we pioneered the concept that targeting ATR could be particularly toxic for tumours with high levels of RS, and developed ATR inhibitors that have provided experimental support to this hypothesis. In recent years, our Group has developed an increasing interest in improving the efficacy of cancer chemotherapies by developing strategies that can target therapy-resistant cells.

"In 2024, our Group has provided novel and improved strategies to overcome multidrug resistance and discovered a new senolytic drug targeting persister cancer cells."

RESEARCH HIGHLIGHTS

Overcoming multidrug resistance in cancer therapy

In a previous study (Sanchez-Burgos *et al.*, *EMBO Mol Med* 2022), we found that drugs activating the Integrated Stress Response (ISR) could overcome multidrug resistance (MDR) in FBXW7-deficient cells, a common mutation in human cancer. Surprisingly, several of these drugs were tyrosine kinase inhibitors with different targets like B-RAF or EGFR. Our research suggests that the antitumour effect of some medically used kinase inhibitors may be - at least partly - due to their ability to activate the ISR rather than by inhibition of their supposed targets. To find novel ISR-activating drugs, we conducted an image-based chemical screen using a fluorescent ISR reporter. This revealed that benzimidazoles are potent ISR activators and can kill MDR cancer cells both *in vitro* and *in vivo* (Figure 1). We are now exploring how benzimidazoles activate the ISR and seeking additional methods to activate this apoptotic pathway to target therapy-resistant cancer cells.

The one-two-punch approach in cancer

Incomplete eradication of cancer cells during initial treatment leads to tumour relapse and secondary disease, often with a poorer prognosis. There are several reasons that explain why some cancer cells can endure the initial treatment. One of the ways by which cancer cells can escape therapy-induced cell death is by entering senescence, a state of permanent growth arrest that limits the expansion of these premalignant clones. However, while senescence can contribute to limit the growth of malignant cells, it can also fuel tumour progression through the activation of an inflammatory programme. In this context, an emergent concept in cancer therapies is that of the "one-two-punch", whereby an initial chemotherapy that triggers senescence is followed shortly thereafter with a second treatment that targets senescent cells. Such an approach aims to maximise the efficacy of the initial treatment thereby reducing tumour relapse. The identification of drugs that can target senescent cells (a.k.a., senolytics) and thus elicit the second punch, has become an important part of our research activities. An example of this line of research is our discovery of *Rocky*, an efficient and potent novel senolytic *in vitro* an *in vivo* (Figure 2). Beyond characterising the efficacy of *Rocky* as a second punch, part of our work has been devoted to deciphering its mechanism of action, for which we have combined proteomic and transcriptomic analyses, and genetic screens. Our research in the coming years is likely to be devoted to exploring the full potential of this approach, and to develop novel and potent drugs that can maximise the efficacy of cancer therapies and thus reduce the frequency of tumour relapse. ■

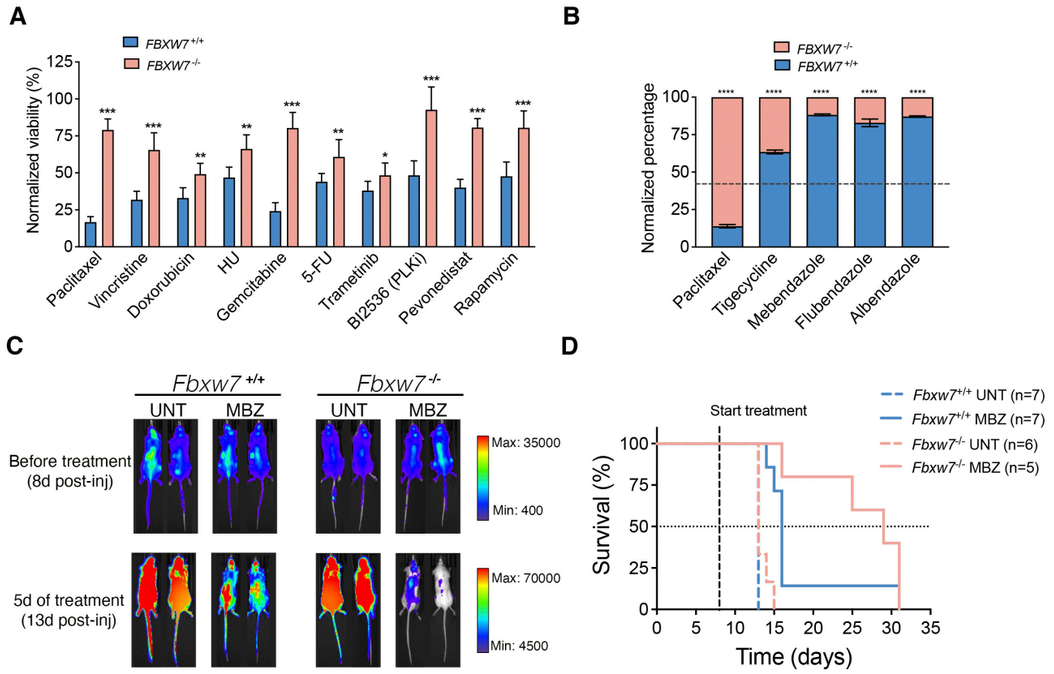

FIGURE 1 Activating the ISR as a strategy to overcome multidrug resistance. (**A**) Normalised viability of *FBXW7*$^{+/+}$ and *FBXW7*$^{-/-}$ DLD1 colorectal cancer cells after 72h of exposure to the indicated drugs, illustrating the multidrug resistance of FBXW7 deficient cells. (**B**) Percentage of viable *FBXW7*$^{+/+}$ and *FBXW7*$^{-/-}$ DLD1 cells grown in co-culture at a 1:1 ratio, 72h after treatment with the indicated drugs. Tigecycline was used as a positive control as it activates the ISR and preferentially kills *FBXW7*$^{-/-}$ cells. (**C**) Representative intravital imaging examples of the luciferase signal coming from Fbxw7$^{+/+}$ or Fbxw7$^{-/-}$ murine acute myeloid leukaemia (AML) cells inoculated in immunocompetent mice. Images were taken before starting the treatment and 5 days after treatment with mebendazole (MBZ) or the corresponding vehicle (UNT). (**D**) Kaplan-Meier survival curve of mice inoculated with Fbxw7$^{+/+}$ or Fbxw7$^{-/-}$ AML cells, either untreated (UNT, dashed lines) or treated with mebendazole (MBZ, solid lines).

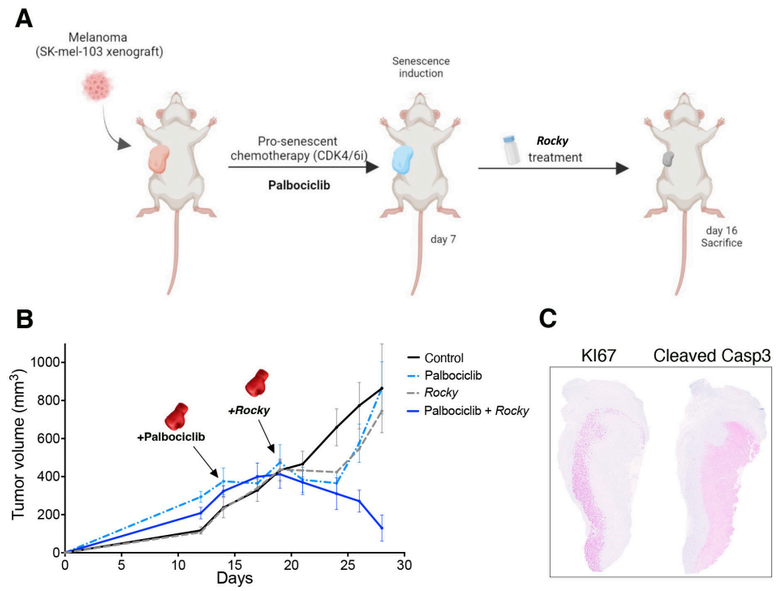

FIGURE 2 *Rocky* is an effective "second punch" *in vivo*. (**A**) Schematic representation of one of the xenograft models used to test the efficacy of *Rocky* following a "one-two-punch" scheme. (**B**) Evolution of tumour volumes from the experiment described above. Control groups included mice that were treated only with palbociclib or *Rocky*. (**C**) Immunohistochemical staining of xenograft sections of mice treated sequentially with palbociclib and *Rocky*, illustrating that the apoptosis triggered by *Rocky* (cleaved caspase 3) is restricted to the areas of the tumour that have ceased to proliferate (Ki67 negative).

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AWARDS AND RECOGNITION

- Elected Member of CIBERNED (*Centro de Investigación Biomédica en Red. Enfermedades Neurodegenerativas*), Spain.

TOPOLOGY AND DNA BREAKS GROUP

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Technicians
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Students in Practice
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**Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

***Titulado Superior* (Advanced Degree)

OVERVIEW

We have a broad interest in understanding how DNA topoisomerase activity is regulated to integrate different aspects of genome dynamics, how an imbalance in these processes can lead to the appearance of pathological DNA breaks, and how cells specifically respond to these lesions to maintain genome stability. In this sense, topoisomerase-induced DNA breaks are emerging as important drivers of oncogenic transformation. Moreover, since drugs that target topoisomerase activity are widely used chemotherapeutic agents, our discoveries have direct implications in cancer treatment.

We therefore have a strong focus on the different cellular processes that influence topoisomerase activity, as a potential source of endogenously-occurring and chemotherapeutically-induced DNA breaks, but also on the different mechanisms that participate in the repair of these lesions, and how the outcome of these repair events can compromise genome integrity and fuel oncogenic processes.

"We have generated a genetic catalogue of how each human gene affects the outcome of DNA break repair processes and proven its potential to decode mutational signatures found in cancer."

RESEARCH HIGHLIGHTS

DNA double-strand breaks (DSBs) are particularly dangerous lesions that disrupt the continuity of the DNA molecule. They represent important threats for genome integrity and are linked to severe pathologies such as cancer, immune, and neurological disorders. Furthermore, DSB-inducing agents as well as inhibitors of DSB-repair factors and pathways are widely used in cancer therapy. More recently, the development of CRISPR-Cas methodologies for gene editing has further expanded the general interest in DSB repair mechanisms. A comprehensive understanding of how DSB repair mechanisms operate to give rise to specific mutational outcomes is therefore an area of extraordinary interest with profound implications for human health, including cancer biology and treatment, as well as in our efforts towards a full control over CRISPR-Cas gene-editing technologies.

We have combined the power of in-pool massive genetic screening and the profiling of DSB repair outcomes to interrogate, within a single experiment, the involvement of each of more than 18-thousand human genes, covering virtually the entire genome, in DSB repair. This genetic catalogue of human DSB repair (REPAIRome; Figure 1) represents an invaluable resource for the scientific community and promises to become a reference consulting tool for the DNA repair and genome instability fields. As examples of its potential application, it has allowed us to uncover:

1. Novel functions and relationships of known DSB repair factors. We found that, unexpectedly, XLF and PAXX, two accessory proteins of the non-homologous end-joining (NHEJ) pathway of DSB repair that were thought to cooperate redundantly in the final ligation step that restores the integrity of the DNA molecule, oppositely affect the repair outcome. Thus XLF favours repair involving incorporation of nucleotides at the repair junction (insertions), while PAXX channels repair towards the removal of nucleotides from the ends, and therefore the loss of sequence at the junction (deletions). Based on a detailed molecular and structural characterisation of these functions, we propose a model in which XLF and PAXX compete for DNA ends and affect the structure of NHEJ and ligation complexes to determine the access of DNA polymerases, responsible for the insertions, or nucleases, that result in deletions. This changes the current paradigm of how accessory ligation factors operate, putting them in the context of recent structural models of NHEJ.
2. Novel DSB repair factors. We found that HLTF, a multifunctional protein with reported helicase and ubiquitin ligase activities with known involvement in the metabolism of replication forks and repair of replication associated DNA damage, also functions in DSB repair, favouring NHEJ-mediated insertions. In particular, we demonstrate that HLTF removes nucleic acid/protein adducts from DSB ends to facilitate their repair, in this case the Cas9 ribonucleoparticle that we use for DSB induction. These results have implications for gene-editing approaches, and suggest that aberrant nucleoprotein structures may arise at DSBs and influence their repair.
3. Novel chromatin-related functions involved in DSB repair. We found that the SAGA chromatin-remodelling complex is involved in the microhomology-mediated end-joining (MMEJ) alternative repair pathway. Interestingly, cancer cells are known to strongly rely on MMEJ, so inhibitors of this pathway constitute a promising strategy for cancer treatment.
4. The molecular aetiology of an orphan mutational signature found in cancer. We demonstrated that ID11, a mutational signature of unknown molecular aetiology and highly enriched in clear-cell renal carcinoma, is caused by insertional NHEJ and associated to loss of the VHL tumour suppressor and hypoxic conditions. This could represent interesting vulnerabilities for these, as well as constituting a proof-of-concept on the potential of massive DSB profiling to decode cancer-associated mutational processes. ■

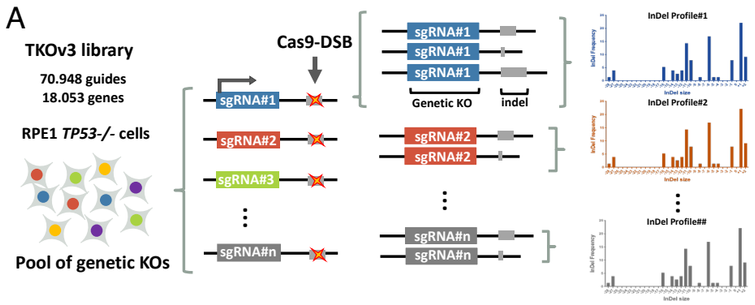

FIGURE 1 REPAIRome: a genetic catalogue of human DSB repair. (**A**) Scheme of the REPAIRome approach for massive parallel DSB repair profiling. (**B**) Network of factors identified in the REPAIRome based on similarities between repair patterns. Nodes represent genes and edges represent correlation in repair patterns. Insertion/deletion ratio is represented with the colour code.

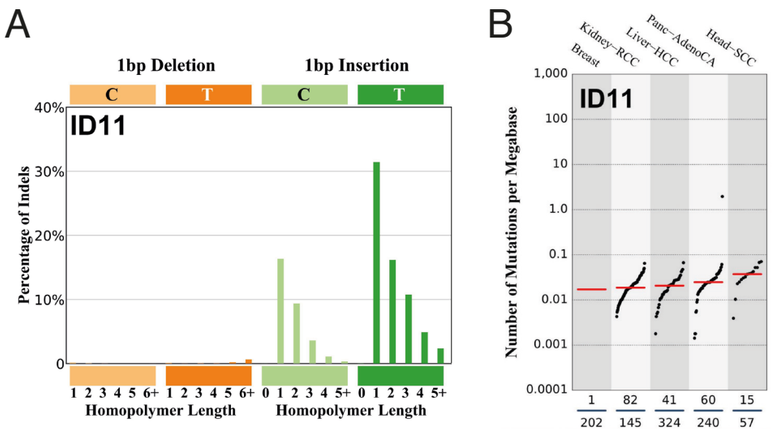

FIGURE 2 Molecular aetiology of ID11 mutational signature. (**A**) Representation of ID11 mutational signature (COSMIC database). (**B**) Prevalence and activity of ID11 in the cancer types in which it is observed (COSMIC database).

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- Manguso N, Kim M, Joshi N, Al Mahmud MR, Aldaco J, Suzuki R, Cortes-Ledesma F, Cui X, Yamada S, Takeda S, Giuliano A, You S, Tanaka H (2024). TDP2 is a regulator of estrogen-responsive oncogene expression. *NAR Cancer* 6, zcae016.
- López de Alba E, Salguero I, Giménez-Llorente D, Fernández-Sanromán A, Casajús-Pelegay E, Terrón-Bautista J, Barroso-González J, Bernal JA, Macintyre G, Fernández-Leiro R, Losada A, Cortés-Ledesma F (2024). A comprehensive genetic catalog of human double-strand break repair. *bioRxiv*. doi: 10.1101/2024.08.03.606369.
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- Cortés Ledesma F, García Expósito L, López de Alba E, Muñoz Barrera M, Gómez Marín C. Improved padlock probes for nucleic acid detection by Hyperbranched Rolling Circle Amplification. PCT application (2024). *PCT/EP2024/057987*.
- Cortés Ledesma F, Gómez Marín C, Muñoz Barrera M, López de Alba E, García Expósito L, Martínez Cañas JC. Nucleic acid detection method. International Publication Number: *WO2024003165A1*. National Phase Entry (2024).

CHROMOSOME DYNAMICS GROUP

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Ana Cuadrado

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Bioinformatician
Daniel Giménez (TS)*

**Titulado Superior* (Advanced Degree)

Technician
Miriam Rodríguez

OVERVIEW

Our research focuses on a protein complex named cohesin, which engages DNA to mediate sister chromatid cohesion, a process essential for chromosome segregation and faithful DNA repair by homologous recombination. Cohesin also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation, DNA replication and recombination. Two variant cohesin complexes, carrying either the STAG1 or the STAG2 subunit, are present in all somatic vertebrate cells. While cells require a single complex for viability, both are necessary to fulfill embryonic development. Mutations in cohesin genes, particularly in *STAG2*, have been found in several tumour types, including bladder cancer, Ewing sarcoma and acute myeloid leukaemia. Germline mutations in cohesin, and its regulatory factors, are also at the origin of developmental syndromes collectively known as cohesinopathies, such as Cornelia de Lange Syndrome (CdLS). Our goal is to understand how cohesin works, how it is regulated, and how its dysfunction contributes to cancer and other human diseases.

"We have determined how STAG2 loss modifies the chromatin interactome of Ewing sarcoma cells and provided a list of potential biomarkers and therapeutic targets."

RESEARCH HIGHLIGHTS

Contributions of cohesin-Stag1 and cohesin-STAG2 to early embryonic development

Cohesin mediates 3D genome organisation by binding to chromatin and extruding DNA loops that become stabilised at multiple sites along the genome, most notably at sites bound by CTCF. In this way, the complex facilitates contacts between promoters and distal enhancers while restricting such interactions within topologically associating domains (TADs). Cohesin-STAG1 and cohesin-STAG2 present different chromatin association dynamics that dictate their specific contributions to genome folding.

Changes in genome folding accompany the extensive epigenetic remodelling that takes place upon fertilisation. The poorly defined TADs observed in the zygote only become mature after the 8-cell stage. How the two cohesin complexes contribute to these early stages of embryonic development remains unknown. We have previously reported that *Stag1* or *Stag2* knock out (KO) mouse embryos die by mid-gestation. However, it is unclear whether the defects that cause this lethality are the consequence of alterations at earlier stages. Moreover, these were zygotic KOs, in which maternally deposited *Stag1* and *Stag2* gene products could form functional complexes. We have now used confocal microscopy to analyse the behaviour of STAG1 and STAG2 from the zygote to the blastocyst stage and the relevance of their maternal contribution. We have found clear differences in the relative abundance of the two paralogs at these stages, in part due to the persistence of maternal STAG1 beyond the 2-cell stage (Figure 1). STAG2 accumulation in the more differentiated cells that result from the first cell-fate decision at blastocyst stage was also observed. Importantly, neither paralog is essential for viability when the other is present. However, transcriptome analyses are currently underway to identify alterations that may lead to defects during gastrulation. These studies will help us to understand the molecular pathways driving developmental abnormalities in cohesinopathy patients, particularly those that carry mutations in *STAG1* and *STAG2*.

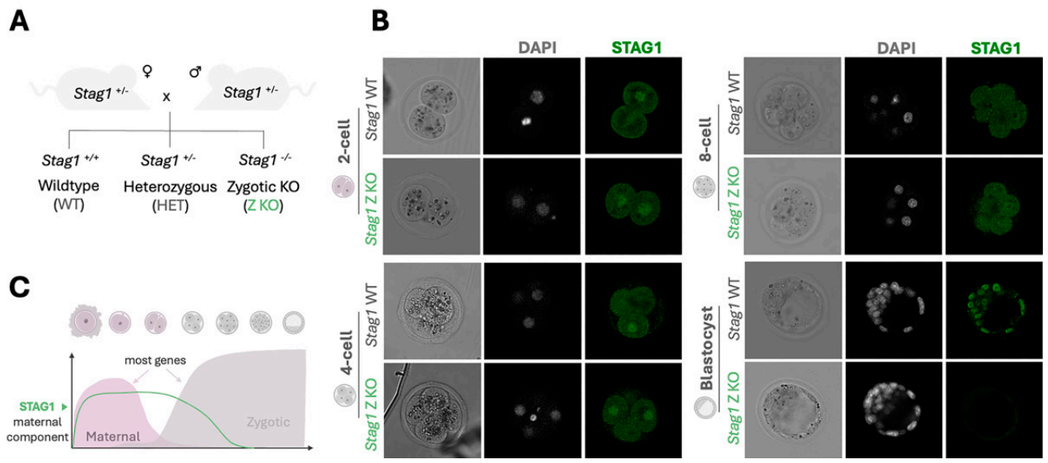

FIGURE 1 Unusual persistence of maternally contributed *Stag1* gene product in early embryos. **A**. Mating scheme to obtain *Stag1* (Zygotic) KO embryos. **B**. STAG1 staining (green) in these embryos and their wildtype (WT) counterparts. **C**. During maternal-to-zygotic transition at 2-cell stage, most maternally deposited gene products are cleared, but not STAG1.

STAG2 loss in Ewing sarcoma rewires chromatin contacts

Ewing sarcoma (EWS) is a paediatric bone cancer driven by a fusion protein, most often EWS::FLI1, which acts as a neomorphic transcription factor. It is a highly aggressive cancer with a 5-year survival rate of less than 30% in patients that present metastasis. The prognosis is generally better for patients with localised tumours at diagnosis, but around 25% of these patients do not respond well to therapy and have poor survival. Among the few recurrent mutations identified in EWS, in addition to the oncogenic fusion, are those that inactivate *STAG2*. These mutations are often present in the most aggressive EWS tumours.

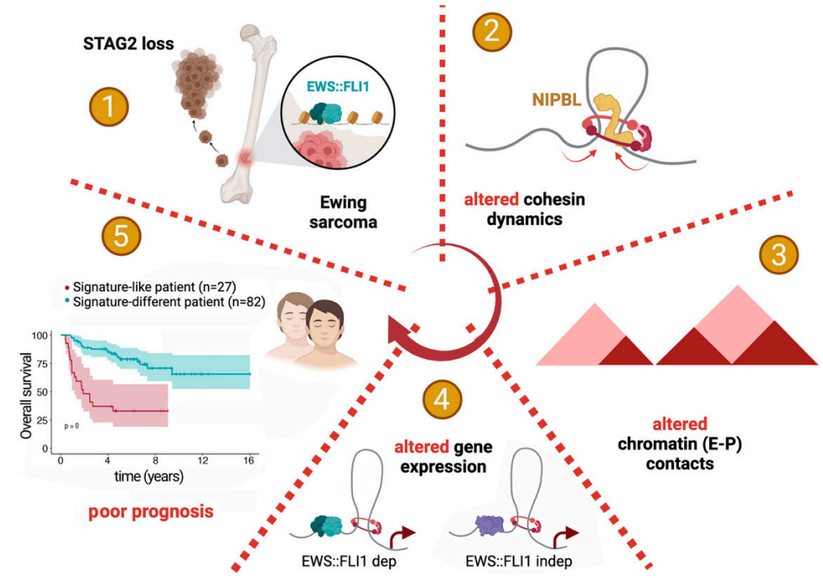

FIGURE 2 Mechanistic understanding of the consequences of STAG2 loss in Ewing sarcoma. Cohesin/NIPBL imbalance upon STAG2 loss in Ewing sarcoma cells alters chromatin contacts, which contributes to altered transcription of oncogene-dependent and -independent genes and promotes an aggressive phenotype.

To understand how loss of cohesin STAG2 facilitates the acquisition of the aggressive phenotype, we generated isogenic EWS cell lines with and without STAG2. Firstly, we note that STAG1 is unable to compensate for STAG2 deficiency and overall levels of chromatin-bound cohesin are severely reduced. In contrast, levels of the cohesin processivity factor NIPBL remain unchanged, likely affecting DNA looping dynamics (Figure 2). Genomic profiling and high-resolution chromatin interaction data, from Capture Hi-C analyses, indicated that cohesin-STAG2 facilitates communication between EWS::FLI1-bound long GGAA repeats, acting as neoenhancers, and their target promoters. Changes in CTCF-dependent chromatin contacts involving signature genes, unrelated to EWS::FLI1 binding, were also identified. Integration of transcriptomic data from patients and cellular models led to the identification of a STAG2-dependent gene signature associated with worse prognosis. These results suggest that STAG2 loss modifies the chromatin interactome of Ewing sarcoma cells resulting in altered transcription of EWS::FLI1-dependent and independent genes. We are investigating how these alterations promote metastasis and/or resistance to chemotherapy. ■

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- Giménez-Llorente D, Cuadrado A, Andreu MJ, Sanclemente-Alamán I, Solé-Ferran M, Rodríguez-Corsino M, Losada A (2024). STAG2 loss in Ewing sarcoma alters enhancer-promoter contacts dependent and independent of EWS::FLI1. *EMBO Rep.* 25, 5537-5560.
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DNA REPLICATION GROUP

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Group Leader

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Post-Doctoral Fellow
Sergio Muñoz

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Visiting Graduate Student
Ran Tong (since April) (Centro de Biología Molecular Severo Ochoa, Madrid, Spain)

Student in Practice
Javier Castillo (since November) (Master's Thesis, Universidad Complutense de Madrid, Spain)

OVERVIEW

The DNA Replication Group focuses on the molecular mechanisms underlying genome duplication in normal and tumoural cells. We are interested in the cellular responses to replication stress, a phenomenon known to induce genomic instability. As part of this effort, in the past year, we investigated how cells avoid the accumulation of over-replicated DNA, one of the causes of abnormal gene amplification in tumour cells. We also described the molecular adaptations of the replisome that take place in early embryonic stem cells during pluripotency transitions. Changes in DNA replication dynamics are required for efficient cell reprogramming, which is a promising tool in regenerative medicine. In addition, we reported the biochemical limitations of high-throughput screenings designed to identify SARS-CoV-2 RNA polymerase inhibitors and continued to characterise the role of primase-polymerase PRIMPOL in the replicative tolerance of damaged DNA.

"We have described how homologous recombination protein RAD51 prevents DNA over-replication in cancer cells, reducing the risk of oncogene amplification."

RESEARCH HIGHLIGHTS

A new mechanism to prevent DNA over-replication from re-activated origins

Most of the known mechanisms that prevent genome over-replication restrict the activity of origin-licensing proteins to prevent the re-activation of origins that have already been used. However, origin re-activation is not uncommon in cancer cells, and we investigated whether additional controls restrict the extension of re-replicated DNA in the event of origin re-activation (Figure 1). Through genetic screening of cells forced to re-activate origins, we found that re-replication is limited by RAD51 and enhanced by FBH1, a RAD51 antagonist. In the presence of chromatin-bound RAD51, forks stemming from re-fired origins are slowed down, leading to fork reversal. Eventual re-initiation of DNA synthesis mediated by PRIMPOL creates ssDNA gaps that facilitate the partial elimination of re-duplicated DNA by MRE11 exonuclease. Our study uncovers a safeguard mechanism to protect genome stability in the event of origin reactivation (Muñoz *et al.*, 2024a).

Rewiring of DNA replication mediated by MRE11 during cell de-differentiation

Following on our previous identification of replication origins in mouse embryonic stem cells (mESCs; Jodkowska *et al.*, *Nucleic Acids Res* 50, 12149-65, 2022), we focused on the changes in DNA replication dynamics observed when 'primed' mESCs (resembling the post-implantation epiblast) are de-differentiated to the 'naive' state that resembles the pre-implantation inner cell mass. Primed-to-naive de-differentiation can be triggered by MEK and GSK3 inhibitors and involves a slowdown of replication forks and the compensatory activation of dormant origins. Using iPOND ("isolation of proteins on nascent DNA") coupled to mass spectrometry; we identified key changes in replisome composition that may be responsible for these effects. Naive mESC forks are enriched in MRE11 nuclease that is recruited to newly synthesised DNA in response to transcription-replication conflicts. Its downregulation or chemical inhibition in naive mESCs is sufficient to restore the fork rate seen in primed cells. Transcriptomic analyses indicated that MRE11 exonuclease activity is required for a complete primed-to-naive transition (Figure 2), demonstrating a direct link between replicative dynamics and the mESC de-differentiation process (Ubieto-Capella *et al.*, 2024).

Validation of SARS-CoV-2 RdRp inhibitors

Viral RNA-dependent RNA polymerases (RdRp) are promising targets for the design of small-molecule inhibitors. However, due to worldwide virtual screenings for potential SARS-CoV-2 RdRp inhibitors being conducted without validation of the identified hits, we tested a set of putative inhibitors in biochemical assays based on fluorometric or electrophoretic detection of RdRp activity. Notably, we found that many small compounds interfere with the binding of fluorophores to dsRNA, making fluorometric-based RdRp assays prone to false-positive hits. Furthermore, reported RdRp inhibitors, such as suramin, display inhibitory activity to many nucleic acid-binding proteins. Combined, these findings underscore the requirement for independent validation methods for alleged SARS-CoV-2 RdRp inhibitors (Llanos *et al.*, 2024).

DNA replication and RS in other cellular contexts

Other projects developed or completed in 2024 include: (i) a collaborative study with Dr A. Aguilera (CABIMER, CSIC, Sevilla, Spain) about the role of SIN3A deacetylase at stalled replication forks (Muñoz *et al.*, 2024b); (ii) a collaborative study with Dr C. Martínez-Alonso (CNB, CSIC, Madrid, Spain) about a ternary complex formed by PARP1-DIDO3-DHX9 that prevents replicative stress caused by R-loops (Futterer *et al.*, 2024); (iii) a high-resolution microscopy study of the nuclear localisation of PrimPol primase, in collaboration with Dr E. Rothenberg (NYU, New York, USA); (iv) the mapping of replication origins in human cells by CUT&RUN techniques; and (v) the function of AURKA kinase in the regulation of DNA replication. ■

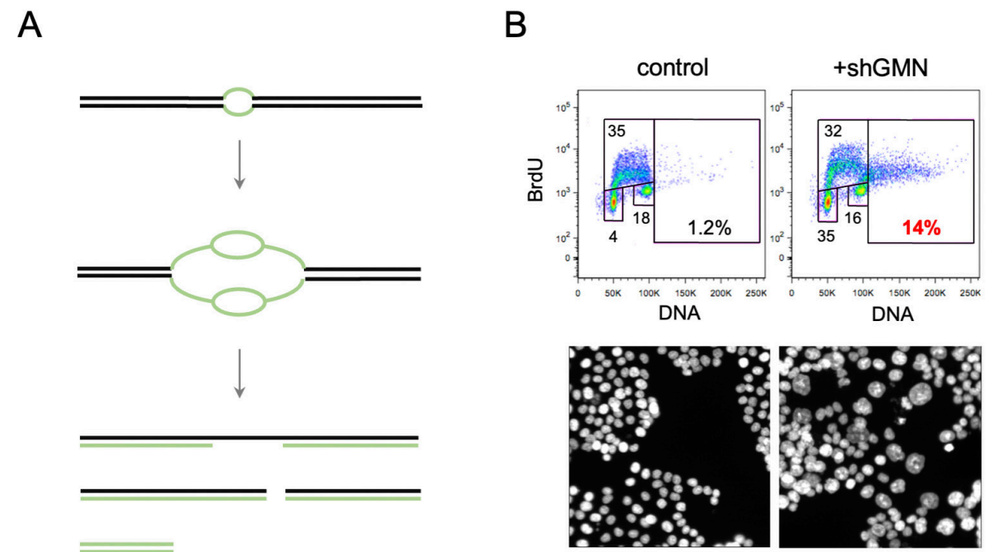

FIGURE 1 A cellular system to study DNA over-replication. (**A**) Schematic of an event of replication origin re-activation leading to ssDNA gaps, DNA breaks, and extra DNA fragments. (**B**) In HCT116-shGMN cells, GMN downregulation causes DNA over-replication (top) and increased nuclear size (bottom). Full article: Muñoz *et al.* (2024a).

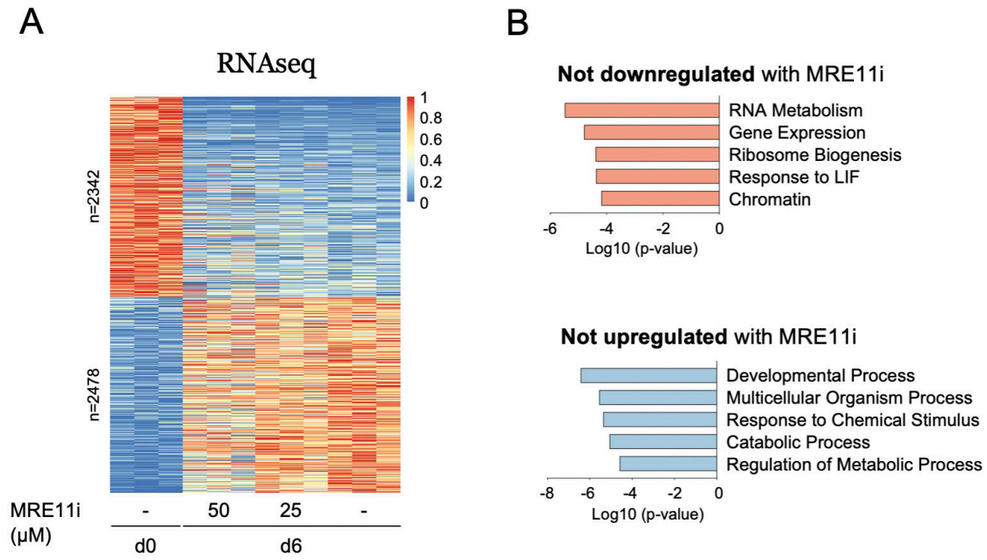

FIGURE 2 MRE11 nuclease is required for primed-to-naive mESC de-differentiation. (**A**) Differentially expressed genes in a 2i mESC de-differentiation assay (day 0 vs day 6) in the absence or presence of MRE11 inhibitor. (**B**) Analysis of pathways that failed to be downregulated (top) or upregulated (bottom) in the presence of MRE11i. Full article: Ubieto-Capella *et al.* (2024).

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MELANOMA GROUP

María S. Soengas
Group Leader

Research Scientists
Nuria Gago, Mariya London (since October)

Post-Doctoral Fellows
Adriana Sanna (until September), Chao Zhang

Graduate Students
Pablo Casado (since September), Guillermo de la Vega, Georgia Vasiliki Gkountana (until July), Naiara Juan-Larrea, Teresa Martí, Sergio Oterino (until October), Thelma Poluha, José Antonio Torres

Technicians
Tonantzin G. Calvo, Marta Contreras

(TS)*, Viktor Georgiev (since October), Cynthia Mucientes (TS)*

**Titulado Superior* (Advanced Degree)

Student in Practice
Alexandra Shirikova (until June) (Master's Thesis, Universidad Autónoma de Madrid, Spain)

Visiting Scientist
M. José Alonso (until June) (Univ. Santiago de Compostela, Spain)

Clinical Collaborators
Daniela Cerezo (Dermatology, Hospital 12 de Octubre, Madrid, Spain), José L Rodríguez-Peralto (Pathology, Hospital 12 de Octubre, Madrid, Spain), Pablo Ortiz-Romero (Dermatology, Hospital 12 de Octubre, Madrid, Spain)

OVERVIEW

The main objective of our Group is to identify and validate new drivers and therapeutic targets in melanoma, the most aggressive form of skin cancer. We are particularly interested in mechanisms that account for the unique ability of this tumour type to bypass immune recognition and be able to generate metastasis from lesions barely over one millimetre in depth (publications in *Nature, Cancer Cell, Nature Cell Biology, Nature Communications*, among others). Our laboratory has also reported the first-in-class lymphoreporter (*MetAlert*) mice for non-invasive imaging of pre-metastatic niches in melanoma (*Nature*). These systems led to the identification of new mechanisms of immune resistance (*Nature Medicine, Nature Cancer*) and the generation of nanoparticle-based treatments (*Cancer Cell, EMBO Mol Med*), with derivatives now being tested in clinical trials. These studies are performed in the context of large cohorts of patient-associated datasets, with the ultimate goal of defining physiological relevance.

"By combining expression and functional analyses of mechanisms underlying immune suppression in melanoma, we have identified new tumour drivers and potential therapeutic targets in this otherwise highly metastatic disease."

RESEARCH HIGHLIGHTS

The long-term goals of our Group are:

1. To monitor and characterise the mechanisms of melanoma initiation and progression, with emphasis on whole-body imaging techniques.
2. To determine and target signalling cascades that turn immunologically "hot" melanomas into "cold" and refractory tumours.
3. To develop new therapeutic strategies to overcome immune suppression and immune tolerance in melanoma.
4. To assess immunomodulatory drivers in other cancer types.

New drivers of melanoma progression and immune suppression

A main objective of our group is to understand and target mechanisms that define the inherent aggressiveness of malignant melanoma. We address this unmet need using genetic and functional studies in melanocytic cell lines, mouse models and tissue specimens, but also by performing cross-cancer type analyses. We have previously identified mechanisms of vesicular trafficking, autophagy, and RNA-associated metabolism that promote melanoma metastasis by mechanisms distinctively enriched in melanoma with respect to over 25 malignancies (Alonso-Curbelo *et al.*, *Cancer Cell* 2014; García-Fernández *et al.*, *Autophagy* 2016; Perez-Guijarro *et al.*, *Nat Commun* 2016; Cifdaloz *et al.*, *Nat Commun* 2017; Karras *et al.*, *Cancer Cell* 2019). Moreover, and in collaboration with Sagrario Ortega at CNIO, we developed the first '*Melanoma-MetAlert*' murine strain for spatio-temporal analyses of premetastatic niches *in vivo* (Olmeda *et al.*, *Nature* 2017). These '*MetAlert*' animals, in combination with human tissue specimens, revealed the growth factor Midkine (MDK) as a tumour-secreted pro-metastatic factor that promotes a systemic expansion of the lymphatic vasculature (neolymphangiogenesis) at early stages of melanoma progression. Subsequently, we uncovered new immune suppressive functions of MDK. First, we demonstrated that MDK promotes a transcriptional rewiring of macrophages, ultimately resulting in CD8$^+$ T cell dysfunction (Cerezo-Wallis *et al.*, *Nat Medicine* 2020). Secondly, as summarised in Figure 1A, MDK was found to be a multilayered inhibitor of antigen-presenting cells, in particular conventional type 1 dendritic cells (cDC1). Specifically, we discovered that MDK acts in a systemic manner, compromising cDC1 differentiation and interfering with all main stages in the action of these cells (antigen uptake, antigen processing, cross-priming, and activation of CD8$^+$ T cells). Furthermore, patients expressing high levels of the MDK-"educated" DCs acquired a tolerogenic expression profile that defined poor prognosis in metastatic melanoma patients (Figure 1B). Together, these data provide insight into how melanomas acquire an immunologically cold or refractory phenotype (Catena *et al.*, *Nature Cancer*, in press).

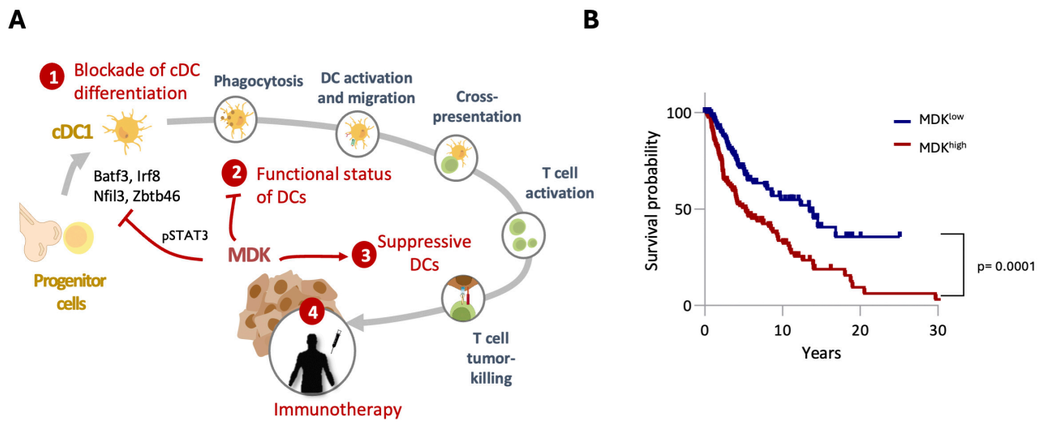

FIGURE 1 Impact of MDK-educated dendritic cells on patient prognosis and resistance to immune therapy. (**A**) Shown is a summary of inhibitory roles of MDK on the systemic inhibition of cDC1 differentiation (1), function (2), induction of suppressive features (3), and resistance to immune checkpoint blockade (4). (**B**) Differential overall survival of metastatic melanoma patients with high vs. low MDK-educated DC signature.

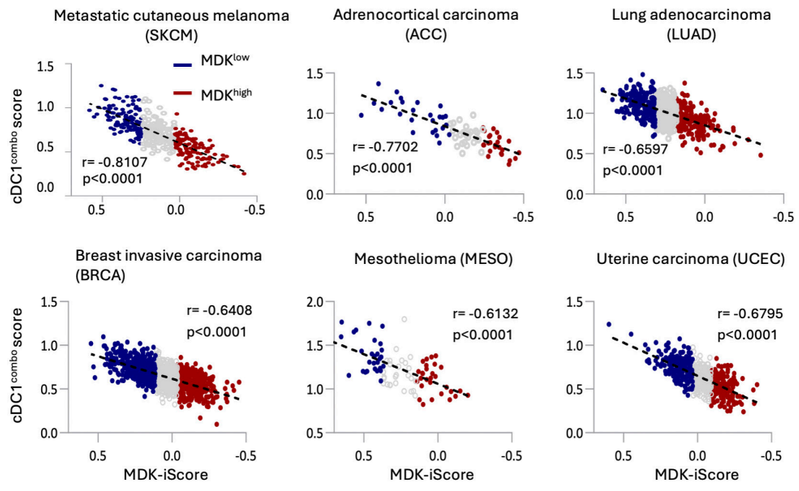

FIGURE 2 Impact of immune modulatory roles of MDK beyond melanomas. Shown is the overall survival of patients expressing high or low levels of cDC1-associated genes (cCD1^{combo} score) identified in the indicated datasets from The Cancer Genome Atlas.

Targeting mechanisms of immune suppression in melanoma, with implications for other cancer types

The above results support MDK as a therapeutic target. In support of this hypothesis, genetic blockade of MDK favoured the antitumoural effect of the CD-associated CD40 agonist treatment. This was the case also for immune checkpoint blockade (ICB). Importantly, the MDK-educated DC signature was also found to correlate with the resistance to ICB in 10 independent cohorts of human patients. To further assess the applicability of these results, we mined a large set of datasets encompassing different cancer types. As summarised in Figure 2, an inverse correlation between MDK- and cDC1-associated signatures was observed in a variety of tumour types, broadening the therapeutic implications of MDK in immune-refractory malignancies (Catena *et al.*, *Nature Cancer* in press). Finally, our expertise in functional studies of tumour promotion has helped in collaborative studies to describe new drivers of melanoma progression associated with vesicular trafficking (Abrahamian *et al.*, *Nat Commun* 2024) and in characterising immunomodulatory signals in neuroblastoma (Strijker *et al.*, *Eur J Cancer* 2025). ■

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AWARDS AND RECOGNITION

- 'Premio Maruja Mallo', Ortega-Marañón Foundation, Spain.
- Piel Sana Foundation-AEDV Dermatology Research Award, Piel Sana Foundation-The Spanish Academy of Dermatology and Venereology (AEDV), Spain.
- International Zendal Award in recognition of lifetime achievements in the life sciences, Spain.

EPITHELIAL CARCINOGENESIS GROUP

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Group Leader

Research Scientist
Miriam Marqués

Post-Doctoral Fellows
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Graduate Students
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Technicians
Ruth Josefina Micha, Leticia Rodríguez

Students in Practice
Mireia Andueza (until June) (Master's Thesis, UAM, Madrid, Spain), Óscar España (until June) (AECC Traineeship, Spain), Ignacio Moreno (June-Dec.) (Master's in Bioinformatics, ISCIII-ENS, Madrid, Spain), Hugo Schodet (since Nov.) (University of Toulouse, France)

Visiting Scientists
Alba Angelina (until Dec.) (UCM, Madrid, Spain), Brice Chanez (until March) (Institut Paoli-Calmettes, Marseille, France), Luís C. Fernández (Universidad Europea de Madrid, Spain), Mark Kalisz (CIBER, Madrid, Spain), Gabriel Piedrafita (UCM, Madrid, Spain), Alejandro Rego (Feb.-March) (IBIMA, Málaga, Spain)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and bladder carcinoma, taking a disease-orientated approach. These tumours present very distinct challenges. We learn from patient samples, cultured cells/organoids, and genetically modified mice. To translate the findings, we bring this knowledge to a "population" level —leveraging on information and samples from large patient cohorts—together with Núria Malats (CNIO).

"We have joined efforts with a team of Spanish oncologists and researchers to generate the first large spatial transcriptomic map of bladder cancer in the context of a highly innovative prospective clinical trial."

PDAC has a dismal prognosis even when diagnosed early. We aim at dissecting the molecular mechanisms involved in the very early steps of tumour development. We harness the power of genetic mouse models to answer questions that cannot be tackled in humans. A main hypothesis is that cell differentiation is an early and potent tumour suppressor mechanism. Understanding the contribution of early molecular events is crucial to design better strategies for prevention and early tumour detection.

Bladder cancer is a highly heterogeneous tumour, presenting a wide range of clinical problems. We aim at acquiring knowledge about the underlying biology that might be leveraged towards improved tumour subclassification, prediction of outcome, and therapy.

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

Genome-wide association studies (GWAS) have identified common genetic variants associated with PDAC risk. Several of them are associated with genes involved in acinar cell biology, including cell differentiation (e.g., the transcription factors *NR5A2* and *HNF1A*), ER stress response (e.g., *XBP1*), and inflammation. These observations have strengthened the notion, pioneered by our laboratory, that understanding tissue homeostasis is critical to better capture the earliest steps in cancer development. We have extensively studied the role of NR5A2 using heterozygous mice and have shown that *Nr5a2* heterozygous mice display a pre-inflammatory phenotype that predisposes to pancreatitis and cancer. This phenotype can be rescued by antibiotic treatment and is associated with selective changes in Trp metabolites arising from the synergistic interaction of host and microbial enzymes. The role of these metabolites is currently being examined.

Another major GWAS hit maps to the *CTRB1/2* locus encoding chymotrypsinogen, a major pancreatic protease. We have generated, with Sagrario Ortega (CNIO), a new mouse strain recapitulating the putative causal variant (exon 6 deletion) and showed that it predisposes to damage induced by high fat diet, caerulein pancreatitis, and cancer. The pancreas of mice with the *Ctrb1* exon 6 deletion is histologically normal but displays dramatic ultrastructural alterations including ER cisternal dilation, cytoplasmic aggregates, and CTRB1 nuclear inclusions. Strikingly, some acinar cells appear spared despite the germline nature of the mutation. Mutant mice age well and do not display an overt phenotype, likely reflecting the similarly mild effects occurring in humans carrying this variant. Nevertheless, their pancreas displays persistent ER stress that can be partially alleviated using TUDCA and Sulindac. Understanding the resilience capacity of the pancreas in mice may provide clues about how tissues tolerate molecular damage, and should help design precision prevention strategies in mutation carriers.

Urothelial bladder carcinoma genetics, biology, and clinical translation

We focus on understanding new tumour suppressor genes that are mutated in bladder cancer that we have identified through exome sequencing: *STAG2* and *RBM10*. *STAG2* codes for a cohesin subunit; using a *Stag2* conditional mutant mouse, we have now uncovered a novel mechanism through which STAG2-cohesin cooperates with the DREAM complex to maintain urothelial quiescence. These findings provide a molecular understanding to the positive selection of *STAG2*-mutant clones in the normal urothelium of organ donors. *RBM10* codes for a splicing regulator and together with J. Valcárcel (CRG, Barcelona) we are analysing how mutations contribute to bladder cancer.

In collaboration with a group of medical oncologists and urologists led by E. Grande (MD Anderson Hospital, Madrid), and with Núria Malats (CNIO) and E. Porta (Inst. Josep Carreras, Badalona), we have conducted a prospective clinical trial (DUTRENEO, EudraCT: 2017-002246-6) exploring novel strategies to increase the response rate to immune checkpoint blockade in this tumour. The study design has highlighted the challenges of such an approach. The multi-omics analyses associated with the trial, and - especially - the use of spatial transcriptomics of pre-treatment samples, have allowed the discovery of new biomarkers associated with response to immunotherapy and chemotherapy. For example, transcriptomic heterogeneity in the context of low genomic differences (i.e., plasticity) stands as a marker predictive of response to chemotherapy, while the distance between tumour and adaptive immune cells is associated with response to immunotherapy. We have also uncovered several ligand-receptor pairs that, when expressed in specific cell populations, are associated with response to immunotherapy. These new concepts need to be translated into simpler assays for clinical implementation. ■

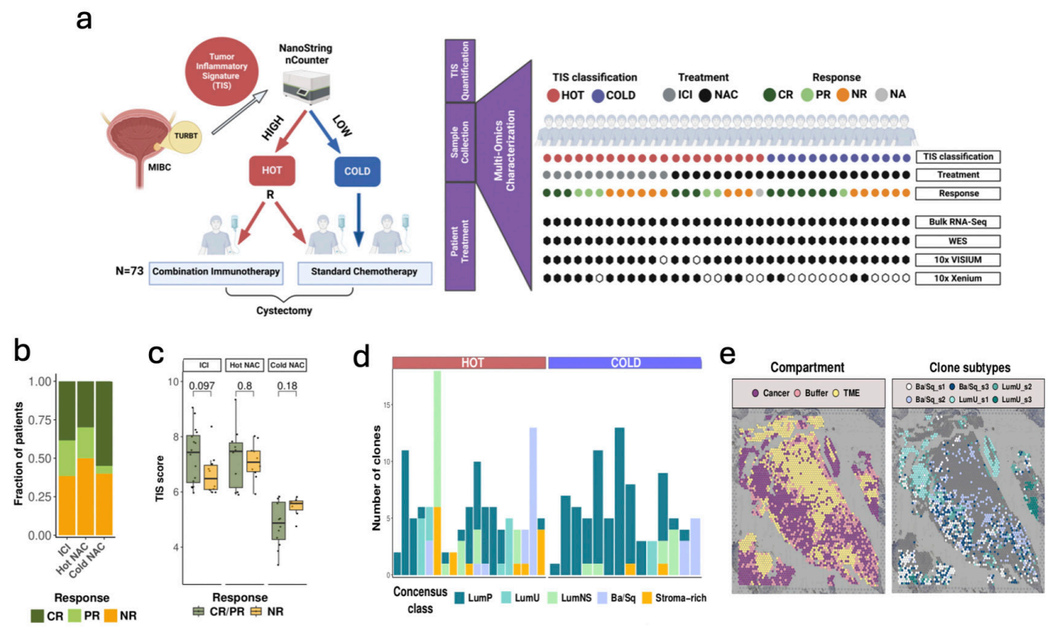

FIGURE 1 DUTRENEO study design and multiomics analyses reveal molecular subtype heterogeneity. (**a**) Tumours (n=73) were classified as hot or cold based on an inflammatory (TIS) signature. Patients with hot tumours were randomised to receive anti-PDL1/anti_CTLA4 antibodies or cisplatin-based chemotherapy; patients with cold tumours received standard chemotherapy. Multiomics analysis of pre-treatment tumour samples included whole exome sequencing (n=44), bulk RNA sequencing (n=57), and spatial transcriptomics (n=35). (**b**) Barplot showing the fraction of patients who had complete (CR), partial (PR) or no (NR) pathological response in each treatment arm. (**c**) Boxplot showing that the TIS threshold does not discriminate well between responders and non-responders in any of the treatment arms. (**d**) Spatial transcriptomics and inferCNV reveal the presence of multiple tumour cell clones in each sample, coloured by the assigned consensus molecular subtype. (**e**) Distribution of the tumour and microenvironmental (TME) compartments in the sample of a patient who received chemotherapy (left) and distribution of the predicted clones according to the assigned molecular subtype (right).

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Book Chapter

- Berca C, Real FX, Marqués M (2024). In vitro bladder cancer models. In M.A. Knowles & L. Dyrskjøt (Eds.), *Biology of Bladder Cancer* (pp 343-368). Springer Cham. DOI: 10.1007/978-3-031-68505-7.

PATENT

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Algorithm Registration

- Villoslada-Blanco P, Alonso L, Sabroso-Lasa S, Maquedano M, Estudillo L, Real FX, López De Maturana E, Malats N. Algorithm registry for a consensus molecular classifier for pancreatic ductal adenocarcinoma. Title of the digital file: 240620_PDACMOC.zip. Registered in Blockchain (Ethereum, Alastria and Minchain) on 21/6/2024 (Hash available upon request).

AWARDS AND RECOGNITION

- Member, Lustgarten Foundation-AACR Pancreatic Cancer Research Grants Committee.

GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

Nabil Djouder
Group Leader

Research Scientists
Rayan Naser, Sladjana Zagorac

Graduate Students
Mariana Angulo, Marta Foronda (since March), Rosa Gallo, Irene Herranz, Yizhi Liu, Carlos Martínez, Maria Del Mar Rigual, Paula Sánchez, Karla Santos, Zhaoshuo Wang

Technician
Marta Benítez

Students in Practice
Inés Calderón (January-June) (Master's Thesis, Universidad Autónoma de Madrid, Spain), Giuseppina Pennacchio (until March) (Erasmus + Fellowship, University of Naples, Italy)

OVERVIEW

While cancer research has largely focused on the role of mutated genes, our laboratory investigates the crucial influence of environmental factors, such as diet and exercise, on disease development and progression, with a particular emphasis on cancer. Moreover, recognising the intricate interplay between diet, the nervous system, and the immune system, we are exploring their roles in cancer development, aligning with the emerging fields of cancer neuroscience and neuroimmunomodulation.

We concentrate on diseases of the gastrointestinal tract, including liver diseases (NASH, cirrhosis, hepatocellular carcinoma) and intestinal diseases (colitis, colorectal cancer), while also investigating the mechanisms of obesity, a significant risk factor for these disorders. Utilising genetically engineered mouse models and advanced technologies, our research encompasses tissue regeneration, metabolic dysregulation, inflammatory processes, and early embryonic development to understand fundamental disease mechanisms. Ultimately, we aim to guide the development of novel medicines, particularly immunomodulatory therapies, to combat these challenging diseases.

"Our laboratory utilises novel preclinical mouse models to investigate how dietary factors contribute to the development and progression of diseases, including cancer, particularly those associated with obesity and metabolic disorders, with a focus on understanding the underlying mechanisms."

RESEARCH HIGHLIGHTS

Employing a multidisciplinary approach that integrates genetically engineered mouse models with other model systems, cutting-edge technologies (including cell biology with organoid culture and quantitative imaging, biochemistry, and functional genomics), alongside human data analysis, our laboratory has dedicated significant effort over the past years to elucidate the molecular, cellular, and pathophysiological mechanisms that connect environmental stresses to disease pathogenesis. We focus on dissecting the origins of digestive system diseases arising from unhealthy diets, nutrient imbalances, and sedentary lifestyles, all of which can contribute to severe inflammatory conditions (Figure 1). Recognising the intricate physiological interconnections within the digestive system, where organs are not only directly impacted by environmental stressors, but also influence each other through exocrine and/or endocrine functions, we have made the following major discoveries:

Mechanisms of obesity

Our groundbreaking work has elucidated the mechanisms by which certain nutrients can trigger inflammatory responses and contribute to the development of various disorders. Recent research has linked inflammation, particularly IL-17A signalling, to obesity and autoimmune disorders, demonstrating a crucial role for IL-17A in the pathogenesis of hepatitis and liver disease-induced hepatocellular carcinoma. These findings have garnered significant attention from pharmaceutical companies, investigating IL-17A blockers as potential therapeutic interventions for these conditions.

Our recent work has made a novel discovery: POMC neurons express IL-17A. Furthermore, a reduction in POMC neuron numbers correlates with decreased IL-17A expression and a concomitant decline in adipocyte gene expression associated with obesity. These data reveal an unexpected crosstalk between IL-17A-producing POMC neurons and the endocrine system in regulating obesity.

Our ongoing research aims to further elucidate how specific nutrients can exert pro-inflammatory effects and contribute to the development of obesity. Additionally, we will dedicate significant efforts to identifying the specific inflammatory cell populations that drive obesity and its associated metabolic disorders.

Mechanisms of intestinal diseases and colorectal cancer

Colorectal cancer (CRC) is a multi-step neoplastic process initiated by APC mutations, leading to the formation of adenomatous polyps. These polyps subsequently progress to malignancy through the acquisition of p53 loss and other genetic and epigenetic alterations. Our research focuses on comprehending the mechanisms underlying CRC initiation, the transitional mutations leading to the transformation of polyps into malignant carcinomas, and the subsequent development of metastatic disease. Furthermore, we are investigating the underlying mechanisms of immune evasion in colorectal cancer, particularly focusing on the resistance of these cancers to immune checkpoint inhibitors, which have limited clinical efficacy.

Mechanisms of liver regeneration

Our successful research in tissue regeneration has spurred our interest in elucidating the intricate mechanisms governing liver regeneration following both acute and chronic liver injury. By deciphering the underlying molecular pathways, we aim to unlock novel therapeutic targets for combating various liver diseases, significantly enhance liver restoration after surgical resection, and ultimately expand the available transplantation options for patients suffering from liver diseases.

Role of cold temperature in liver cancer

We demonstrated that chronic cold exposure in a mouse model of hepatocellular carcinoma (HCC), the most common and one of the most aggressive liver cancers, surprisingly prolongs lifespan, improves liver health, and suppresses tumour development. This beneficial effect is associated with restoring NAD+ levels, which are typically depleted in HCC. Our findings suggest that cold therapy may have potential as a novel and promising strategy for preventing and treating HCC and potentially other cancers.

Mechanisms of totipotency-to pluripotency transition

The smooth and precise transition from totipotency to pluripotency is a critical step in early embryonic development, giving rise to pluripotent stem cells with the capacity to differentiate into all cell types. We demonstrated that endogenous retroviruses (ERVs) play a crucial role in this process, acting as modulators of the pluripotent factors OCT4 and SOX2 during lineage specification. Our findings highlight the symbiotic coevolution of ERVs with their host cells, ensuring the precise and timely progression of early embryonic development.

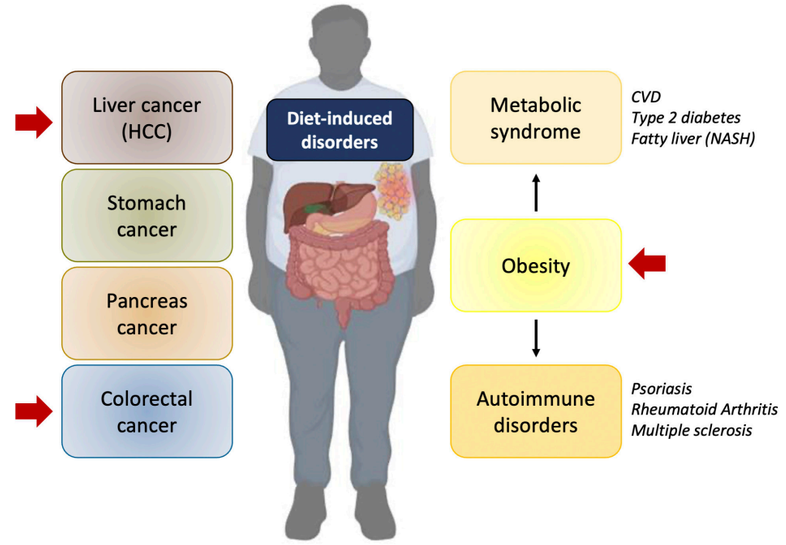

FIGURE 1 Representation of some of our current and future research directions.

Structure of the URI prefoldin-like complex

One of our future goals is to further determine the functions of the URI prefoldin-like complex by unravelling its structural organisation through advanced techniques such as electron microscopy, crystallography, and biophysical techniques. ■

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AWARDS AND RECOGNITION

- Member of the European Association for the Study of Diabetes (EASD).
- Member of *SEOM*, the Spanish Society of Medical Oncology (*SEOM*).

TRANSFORMATION AND METASTASIS GROUP

Eva González Suárez
Group Leader

Research Scientists
Marta Barradas, María Jiménez

Post-Doctoral Fellow
Macarena Pozo

Graduate Students
Alexandra Barranco, Alejandro Collado, Aránzazu Gómez, Jaime Redondo, Mario Rodríguez (since April), Alejandro Sánchez Juan, Andrea Vethencourt (Clinical Oncologist at *ICO/IDIBELL*, Barcelona, Spain), Samuel Vives (until August)

Mouse Technician
Víctor López

Students in Practice
Anastasios Triantafyllou (until May) (Erasmus + student, Univ. of Thessaly, Greece)

OVERVIEW

In the Transformation and Metastasis Group, we aim to understand normal mammary gland development and the key events that lead to tumour initiation, progression, and metastasis, and to identify novel therapeutic targets to combat breast cancer. We use complementary tools, including primary cell cultures and organoids, lineage tracing mouse models, and clinical samples with the goal of translating basic knowledge into clinically relevant findings.

"Neoadjuvant denosumab is an immune-enhancing agent in early breast cancer."

"Basal lineage infidelity triggered by Rank-driven epigenetic mechanisms contributes to the generation of pre-invasive lesions and their transition to invasive breast cancer."

RESEARCH HIGHLIGHTS

Results from the window-of-opportunity D-Biomark clinical trial

D-BIOMARK is a prospective, randomised window-of-opportunity clinical trial assessing the biological effects of the anti-RANKL monoclonal antibody denosumab, in patients with HER2-negative early breast cancer. Denosumab demonstrated its ability to reduce serum free RANKL levels. However, a reduction in tumour cell proliferation or cell survival was not observed. A denosumab-driven increase in tumour infiltrating lymphocytes (TILs) was observed, particularly in the luminal B-like population. Denosumab led to increased TILs in both pre-menopausal and post-menopausal women with luminal tumours. RANK protein expression in tumour and stroma was associated with markers of tumour aggressiveness, but an increase in TILs was observed in the experimental arm irrespectively of RANK and RANKL expression in tumour or stromal cells. The D-BIOMARK trial highlights the potential of denosumab as an immune-enhancing agent in early HER2-negative breast cancer. (Vethencourt A *et al.*, *Breast Cancer Research*, Doi: 10.1186/s13058-025-01996-w).

Rank signalling drives basal cell-lineage infidelity leading to mammary tumourigenesis

Rank signalling regulates mammary gland development and epithelial cell differentiation. The Rank receptor is expressed by mammary basal and luminal cell populations, but unlike luminal cells, the contribution of basal Rank signalling to mammary gland homeostasis remains poorly studied. Combining time-regulated, basal-specific Rank expression with lineage tracing strategies, we unveiled that Rank signalling controls basal cell identity in postnatal mammary glands. Enhanced basal Rank disrupts basal and luminal cell identity, resulting in aberrant luminal-like differentiation of basal cells, defective lactation, and the appearance of premalignant lesions composed of a basal-derived hybrid population with luminal/alveolar features, which ultimately generate basal and luminal breast adenocarcinomas. Mechanistically, phospho-proteomic, transcriptomic and chromatin analyses support that basal Rank activation triggers the loss of tumour suppressive epigenetic regulators, leading to chromatin remodelling, disruption of basal identity, and tumourigenesis. We have uncovered a basal Rank gene signature that can be predictive of progression from *in situ* to invasive adenocarcinomas and associates with poor prognosis in breast cancer patients, particularly in those diagnosed with luminal adenocarcinomas, underlining the clinical relevance of our findings. Our results reinforce that basal lineage infidelity, triggered by Rank signalling, contributes to the generation of pre-invasive lesions and transition to invasive breast cancer. (Redondo *et al.*, under review in *Nature Communications*). ■

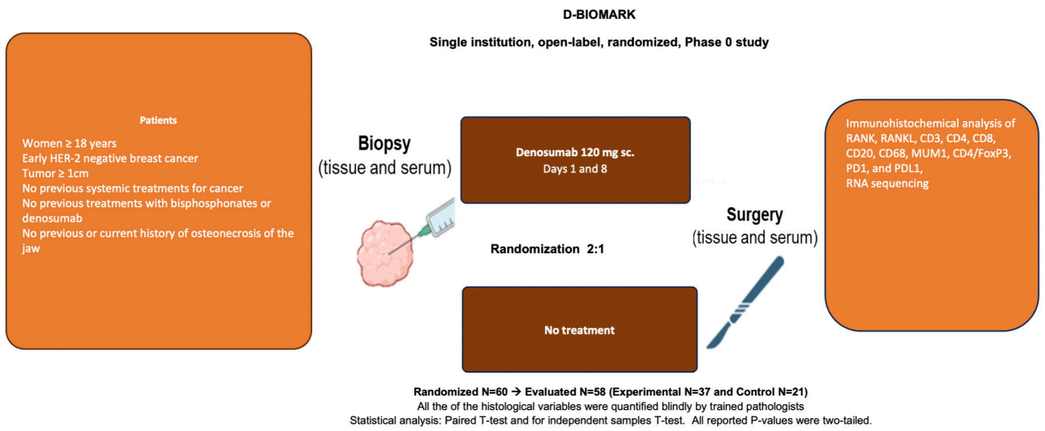

FIGURE 1 Methodology of data presented from the D-BIOMARK clinical trial. Inclusion and exclusion criteria are summarised alongside the patient's journey through the trial, which involved randomisation at a 2:1 ratio to receive two doses of denosumab before surgery (experimental arm) or no treatment (control arm). Paired biopsies collected at surgery and baseline for each patient were compared, and various immunohistochemical stains were performed for paired analysis.

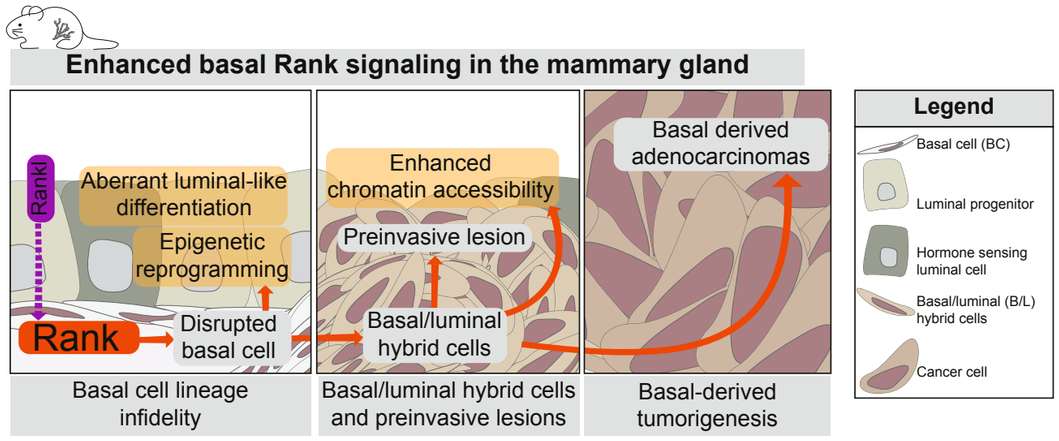

FIGURE 2 Enhanced basal Rank signaling in the mammary gland.

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Book Chapter

- Pérez-Chacón G, Santamaría PG, Redondo-Pedraza J, González-Suárez E (2024). RANK/RANKL signaling pathway in breast development and cancer. In T Sørlie T & RB Clarke (Eds), *A Guide to Breast Cancer Research: From Cellular Heterogeneity and Molecular Mechanisms to Therapy* (*Advances in Experimental Medicine and Biology* vol. 1464, pp. 309-335). Springer Cham. ISBN 978-3-031-708749-9.

AWARDS AND RECOGNITION

Eva González Suárez:
- Chair, GEICAM Meeting, Madrid, Spain.
- Selected for Chair for Gordon Conference of Mammary Gland Biology in 2026.
- Keynote speaker, EurOPDX Workshop, VHIO Barcelona, Spain.
- Speaker: 28th MetNET Meeting, Barcelona; Joint meeting CNIO-IRB Lleida. Spain.
- Invited Speaker: Gordon Conference of Mammary Gland Biology, El Ciocco, Italy; Centro de Investigaciones Biológicas, CSIC, Madrid; Instituto de Neurociencias, Alicante. Spain.
- Organiser and speaker of SenesceX Workshop, CNIO, Madrid, Spain.
- Oral Presentations: Oncobell Symposium, Barcelona; Frontiers in Immunomodulation, CNIO, Madrid. Spain.

Macarena Pozo:
- Postdoctoral Contracts: Juan de la Cierva JDC2023-053002; Amigos del CNIO. Spain.
- Selected for oral presentations at the Jornada Amigos del CNIO L@s Fuertes, El Espinar; and CNIO Friends Day, Madrid. Spain.

Mario Rodríguez del Collado:
- FPU contract, FPU23/02826, Spain.

Alejandro Collado:
- Speaker IBIS, Sevilla, Spain.
- ENBDC Zoom talk, the European Network of Breast Development and Cancer.
- Research stays: Alberto Pascual group (IBIS, Sevilla, Spain); Vincent Prevot group (INSERM, Lille, France).

Alexandra Barranco:
- Excellent *Cum Laude* PhD Thesis defence. *RANK pathway as a key regulator of the tumour-immune crosstalk and tumour immunity*. Universidad de Barcelona, Spain.

Jaime Redondo Pedraza:
- Organiser and Oral Presenter, 15th annual European Network of Breast Development and Cancer (ENBDC) Workshop, Weggis, Switzerland.

Aranzazú Gómez:
- CAM Predoctoral Contract (PIPF-2022SAL-GL-26079), Comunidad de Madrid, Spain.
- Flash Talk at CNIO Lab Day, Madrid, Spain.

BRAIN METASTASIS GROUP

Manuel Valiente
Group Leader

Research Scientist
Raul Bardini (since October)

Post-doctoral Fellows
Mariam Al-Masmudi, Lluís Cordón (until 19.11.2024), Leticia Cuarental (since October), Pedro García, Neibla Priego

Graduate Students
Mohmeh Abdalfttah (since September), Laura Adriana Álvaro, Jimena Benzal, Ana de Pablos, Carolina Hernández, Irene Salgado, Juan José Vázquez

Technicians
Patricia Baena, Diana P. Retana (TS)*, Oliva A. Sánchez, Catalina Vela

**Titulado Superior* (Advanced Degree)

Lab Administrative Manager
Jorge G. Ortiz

Students in Practice
Andrea Rojas (until 20.12.2024) (Master's Thesis, Universidad Autónoma de Madrid, Spain), Ana Sartorius (until 29.11.2024) (Master's in Bioinformatics, ENS-ISCIII, Madrid, Spain)

Visiting Scientists
Ariane Steindl (until 30.09.2024) (Medical University of Vienna, Austria), Eduard Teixidor (until 28.06.2024) (ICO Girona - Institut Català d'Oncologia, Spain)

OVERVIEW

Brain metastasis is the most common neurological complication of cancer and, in spite of the progress made with local (i.e., surgery and radiation) and systemic (i.e., targeted therapy, immunotherapy) therapies, prognosis remains poor. Indeed, the increased incidence of brain metastases is partially due to systemic therapies that work extra-cranially, but do not provide the same therapeutic benefit in the brain. We study why and how cells from different cancer types (breast cancer, lung cancer, and melanoma) are able to access the brain, survive, and colonise this vital organ. We dissect the biology of these processes *in vivo* using experimental models and patient-derived material to challenge this unmet clinical need. Our research has identified novel brain metastasis mediators, characterised the metastasis-associated microenvironment, designed better experimental models, and explored novel methods to target brain metastasis as well as to prevent or revert the frequent impact of metastasis on brain function.

"We found an improved strategy to provide immunotherapy to patients with symptomatic brain metastases by combining immune checkpoint blockade antibodies with inhibitors of local immunosuppression."

RESEARCH HIGHLIGHTS

Astrocytes are key local immunomodulators that dampen benefits of immunotherapy against brain metastasis

As immune checkpoint blocking (ICB) antibodies are broadly used in Oncology, this immunotherapy shows different responses depending on the cancer type but also on the specific organ where cancer cells are located during systemic dissemination. When in the brain, cancer cells are not equally affected, especially when immunotherapy is provided to clinically symptomatic patients. We have found that a subpopulation of STAT3+ astrocytes, which are induced by established metastases, act as a physical and chemical barrier surrounding metastases, and protect them from CD8+ T cells reaching the brain from the periphery. In particular, we dissected the immunosuppression mechanism by discovering that astrocyte-derived TIMP1 binds to CD63 receptor on activated CD8+ T cells, which dampens its cytotoxicity against the metastasis. Importantly, this mechanism has been functionally validated in patient samples *ex vivo* using RENACER fresh neurosurgeries. Moreover, a more effective immunotherapy is possible by combining ICB with the STAT3 inhibitor silibinin, which could be guided and followed up by TIMP1 levels as a liquid biopsy biomarker. Thus, our findings involve a novel strategy to use immunotherapy against brain metastases through the identification of an astrocyte subpopulation with a major role in immunomodulation.

Disruptive technology using light could improve detection of brain metastases

Light properties include the generation of the Raman effect, which means that depending on the molecular structure of an irradiated sample, its chemical composition will create spectra uniquely associated with such specific structure. Together with an international and interdisciplinary team of physicists, engineers, neuroscientists, and cancer researchers, we have been able to build and test the first prototype of a device compatible with performing Raman spectroscopy *in vivo* in experimental models. The applications of such approach are immense as it allows to interrogate tissues without the need of using exogenous markers (i.e., GFP). For instance, we have used it in our brain metastasis models to define the area of the brain occupied by the metastasis and the interface where invasive fronts are located. There we can clearly differentiate healthy versus cancerous tissue, which would be very relevant, for instance, to guide more accurate neurosurgery techniques. The completion of this project (NanoBRIGHT) has granted CNIO recognition as an Innovation Radar-associated centre of the European Union.

The Cancer-Neuroscience field gets established as a novel and highly necessary discipline

How come is it possible to study brain tumours without knowing how they impact neuronal communication? Neurocognitive dysfunction is one of the most prevalent symptoms experienced by patients with brain metastases, yet research on this topic to date has not been prioritised. Fortunately, this is no longer the case, as the field of Cancer-Neuroscience has bloomed and several laboratories worldwide have started to discuss this subject, creating a critical mass to address the many unknowns of the impact of tumours on the nervous system. ■

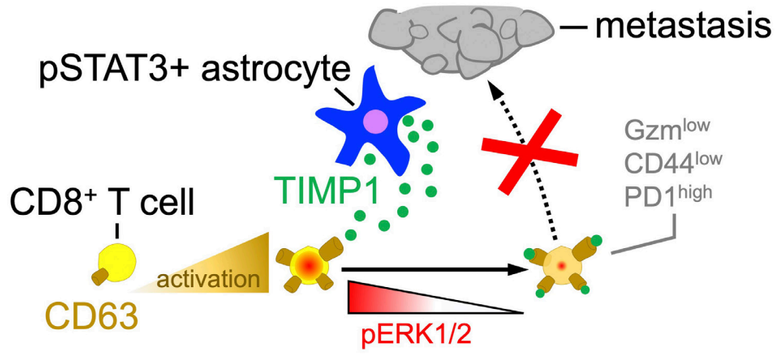

FIGURE 1 STAT3+ pro-metastatic astrocytes impair the cytotoxic activity of CD8+ T cells that reach brain metastases. The schema represents the molecular pathway involved, which includes TIMP1 production by STAT3+ astrocytes and the CD63 receptor in CD8+ T cells.

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- Priego N*, de Pablos-Aragoneses A, Perea-García M, Pieri V, Hernández-Oliver C, Álvaro-Espinosa L, … Caleiras E, García F, García-Martín S, Graña-Castro O, García-Mulero S, Serrano D, Velasco-Beltrán P, Jiménez-Lasheras B, Egia-Mendikute L, Rupp L, Stammberger A, Meinhardt M, Chaachou-Charradi A, Martínez-Saez E, …. Al-Shahrour F, Saftig P, Sanz-Pamplona R, Schmitz M, Crocker SJ, Calvo A, Palazón A, RENACER, Valiente M* (2024). TIMP1 mediates astrocyte-dependent local immunosuppression in brain metastasis acting on infiltrating CD8+ T cells. *Cancer Discov*. PMID: 39354883. (*) Co-corresponding author.
- Amit M, Anastasaki C, Dantzer R, Demir IE, Deneen B, Dixon KO, Egeblad M, Gibson EM, Hervey-Jumper SL, Hondermarck H, Magnon C, Monje M, Na'ara S, Pan Y, Repasky EA, Scheff NN, Sloan EK, Talbot S, Tracey KJ, Trotman LC, Valiente M, Van Aelst L, Venkataramani V, Venkatesh HS, Vermeer PD, Winkler F, Wong RJ, Gutmann DH, Borniger JC (2024). Next directions in the neuroscience of cancers arising outside the CNS. *Cancer Discov* 14, 669-673.
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- Raymant M, Astuti Y, Alvaro-Espinosa L, Green D, Quaranta V, Bellomo G, Glenn M, Chandran-Gorner V, Palmer DH, Halloran C, Ghaneh P, Henderson NC, Morton JP, Valiente M, Mielgo A, Schmid MC (2024). Macrophage-fibroblast JAK/STAT dependent crosstalk promotes liver metastatic outgrowth in pancreatic cancer. *Nat Comm* 15, 3593.
- Dankner M, Maritan SM, Priego N, Kruck G, Nkili-Meyong A, Nadaf J, Zhuang R, Annis MG, Zuo D, Nowakowski A, Biondini M, Kiepas A, Mourcos C, Le P, Charron F, Inglebert Y, Savage P, Théret L, Guiot MC, McKinney RA, Muller WJ, Park M, Valiente M, Petrecca K, Siegel PM (2024). Invasive growth of brain metastases is linked to CHI3L1 release from pSTAT3-positive astrocytes. *Neuro Oncol* 26, 1052-1066.
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- Jagust P, Powell AM, Ola M, Watson L, de Pablos-Aragoneses A, García-Gómez P, Fallon R, Bane F, Heiland M, Morris G, Cavanagh B, McGrath J, Ottaviani D, Hegarty A, Cocchiglia S, Sweeney KJ, MacNally S, Brett FM, Cryan J, Beausang A, Morris P, Valiente M, Hill ADK, Vareslija D, Young LS (2024). RET overexpression leads to increased brain metastatic competency in luminal breast cancer. *J Natl Cancer Inst* 116, 1632-1644.

PATENT

- Zhu L, Graña-Castro O, Salgado-Crespo I, Valiente M. Signature for the prognosis of brain metastasis relapse. PCT application (2024). *PCT/EP2024/069048*.

AWARDS AND RECOGNITION

Manuel Valiente:
- *X Premio Constantes y Vitales* Award "Joven talento en Investigación biomédica", Spain.
- *AECC Coordinados* 2023 (Coordinated project. Role: coordinator), Spain.
- Grant reviewer, Luxembourg National Research Fund (FNR).
- Treasurer and Member of the Board, European Association of Neuro Oncology (EANO).
- Jimena Benzal: *CAM* PhD Fellowship from the Community of Madrid, Spain.
- Leticia Cuarental: 'CNIO Friends' Postdoctoral Fellowship, Spain.

Neibla Priego:
- Ana de Pablos Aragoneses: Thesis defence (November 2024).
- Oral presentation, *ASEICA* Congress 2024, Zaragoza, Spain.
- Co-organiser (Scientific Committee Member), "The neuroscience of Cancer. Breaking Borders Series 2024", Bologna, Italy.
- Patricia Baena: 'Best Oral Presentation' in the XXXV National AETEL Congress, Valladolid, Spain.

ORGAN CROSSTALK IN METABOLIC DISEASES GROUP

Guadalupe Sabio
Group Leader

Research Scientist
Alfonso Mora

Post-Doctoral Fellows
Alba Concepción, Cintia Folgueira (CNIC), Ayelén Santamans (Univ. de Valencia), Juan Ignacio Jiménez-Loygorri (since October)

Graduate Students
Ana Belén Alonso (since April), Clara Bonacasa (since August), Beatriz Cicúendez (since October), Nauzet Deniz-Eyre (since September), Javier Pérez (since December), Irene Ruiz-Garrido (CNIC)

Technicians
Luis Leiva (TS)*, Marta León (TS)*, M. Elena Rodríguez

**Titulado Superior* (Advanced Degree)

Students in Practice
Irene Corchero (until May) (Master's Thesis, UAM, Madrid, Spain), Adriana de Bonis (until June) (Master's Thesis, UCM, Madrid, Spain), Daniel Matamala Luengo (since November) (Master's Thesis, UCM, Madrid, Spain), Ángela Sánchez Fernández (AECC fellow summer practice in the lab)

Visiting Scientists
Magdalena Leiva (Universidad Autónoma de Madrid, Spain), Rafael Romero (until March) (CNIC, Madrid, Spain)

OVERVIEW

The Organ Crosstalk in Metabolic Diseases Group is dedicated to understanding how metabolic alterations and obesity trigger other secondary diseases such as cancer, diabetes, and cardiovascular diseases. Our research takes a holistic approach, aiming to comprehend how obesity disrupts the communication between organs. In this context, we have found that the activation in different organs of several stress kinases during obesity affects the development of tumours.

"This year, our Group discovered that MCJ, a mitochondrial protein, plays a key role in regulating oxygen sensing by both the mitochondria and cardiomyocytes, and identified p38α and p38γ as a switch in our muscles for the desire to exercise."

The Group focuses on four main aspects induced by obesity:

1. The alteration of adipose tissue and, consequently, the secretion of adipokines.
2. The onset of chronic inflammation, which is associated with an increased risk of cancer.
3. Cell metabolism alteration as a driver of disease.
4. The role of exercise in disease prevention and progression.

RESEARCH HIGHLIGHTS

This year, our Group discovered that MCJ, a mitochondrial protein, plays a key role in regulating oxygen sensing by both the mitochondria and cardiomyocytes. We explored its potential as a therapeutic target for cardiac complications in pulmonary hypertension (PH). As PH puts immense pressure on the right side of the heart, often leading to heart failure, our aim was to identify ways to protect the heart under these conditions. First, we examined how MCJ levels behave in low-oxygen environments, such as those experienced in chronic lung diseases like COPD, or when the heart faces pressure overload. We found that MCJ levels increase significantly under these hypoxic conditions. To understand the impact of this, mice lacking MCJ were generated and exposed to chronic low-oxygen scenarios. What we discovered was striking: the absence of MCJ preserved the function of the right ventricle. We saw that the hearts of mice lacking MCJ were protected from damage and maintained proper function, even when the lungs were undergoing harmful remodelling. We found that without MCJ, the heart produced more reactive oxygen species (ROS), which surprisingly triggered protective pathways like mTOR and HIF-1α. Normally, ROS are known for causing damage, but in this case, they seemed to help the heart precondition itself to deal with low oxygen levels more effectively. By silencing MCJ specifically in the heart in mice with COPD, we preserved the cardiac function despite the lung damage. Through these experiments, our Group demonstrated that targeting MCJ could offer a novel way to protect the heart from the detrimental effects of pulmonary hypertension and chronic hypoxia.

Our Group also identified p38α and p38γ as a *switch* in our muscles for the desire to exercise. We found that p38α and p38γ are activated in skeletal muscle during exercise. In mice lacking p38α in muscle, there was an increase in spontaneous physical activity, higher energy expenditure, and protection against diet-induced obesity and related conditions like diabetes and liver steatosis. Interestingly, we discovered that this protection depends on p38γ activation, which became more active in the absence of p38α. Furthermore, this increased activity was linked to higher IL-15 levels in the muscle and bloodstream. When we overexpressed active p38γ in muscle, mice showed even more voluntary physical activity and improved exercise performance, suggesting that p38γ not only increases activity levels but also boosts endurance. Additionally, we demonstrated that IL-15 released from muscle plays a crucial role in this effect by signalling in the brain's motor cortex. Blocking IL-15 signalling in this brain area reduced activity and worsened glucose metabolism. Our findings highlight a muscle-brain communication pathway where exercise-induced p38γ activation leads to IL-15 secretion, promoting physical activity, and offering a potential strategy for treating metabolic diseases like obesity, as we observed that IL-15 levels are lower in people with obesity.

We also investigated how the immune system influences obesity and energy balance. In humans with obesity, we observed that the p38 pathway is highly active in $CD8^+$ T cells that infiltrate adipose tissue. This activation is problematic because $CD8^+$ T cells are known to promote inflammation, and their increased presence in fat tissue contributes to the chronic low-grade inflammation characteristic of obesity. Specifically, we found elevated levels of p38α, an important component of this pathway, along with its upstream activators.

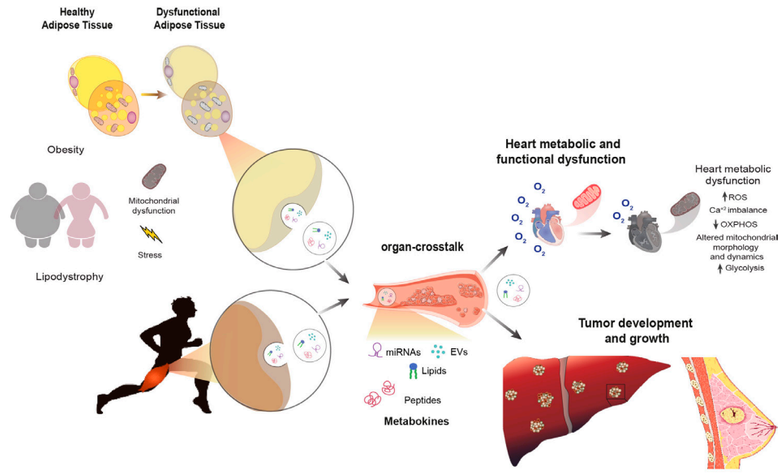
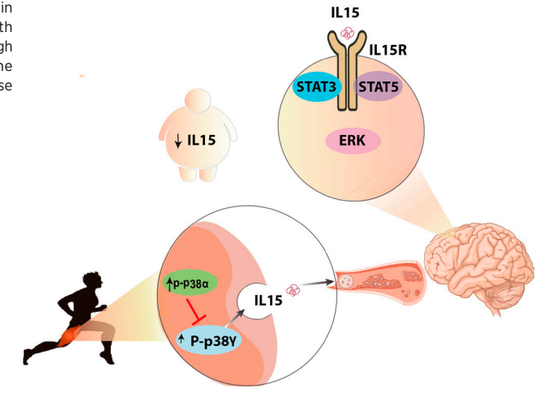


FIGURE 1 In obesity and with sedentary life, the adipose tissue becomes enlarged and experiences mitochondrial dysfunction, with the accumulation of stress factors that disrupt its normal function. The dysfunctional adipose tissue releases various signalling molecules that negatively affect other organs, such as the heart or liver and can trigger cardiovascular diseases and cancer.

FIGURE 2 The proteins p38α and p38γ as a switch in muscles for our desire to exercise. When we exercise, both kinases are activated in the muscle, and p38γ controls the secretion of IL-15, which reaches the brain and regulates our motivation to exercise. On the other hand, p38α, when activated in the muscle, inhibits p38γ, with both controlling voluntary exercise through the activation of STAT/ERK in the brain. IL-15 levels are lower in obese individuals.



At the same time, the expression of molecules that normally turn off or regulate p38, such as the phosphatase DUSP1, was reduced. This imbalance suggests that in obesity, p38 signalling is activated in $CD8^+$ T cells, driving inflammation and disrupting metabolic health. We discovered that by blocking p38 activation in T cells, the mice became resistant to obesity, even when fed a high-fat diet. These mice had lower levels of inflammation in their adipose tissue and showed signs of better energy management, such as higher thermogenesis (heat production) in brown fat. One of the key findings is that the absence of p38 activation in regulatory T cells (Tregs) led to an increase in the production of IL-35, which helped promote fat burning and reduced inflammation. Overall, we found that targeting the p38 pathway in Tregs could be a promising strategy for treating obesity by enhancing the body's ability to burn fat and reducing inflammation. Our study also highlights the potential of IL-35 as a therapeutic target to improve metabolic health. ■

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AWARDS AND RECOGNITION

Guadalupe Sabio:
- "Juan Florencio Macías Núñez" Award for best scientific career, the FIIPERVA Foundation Chair, Spain.
- Member of the Royal Academy of Veterinary Medicine, Spain.
- 'One Health' National Award 2024, Spain.
- 'ABC Health Award' 2024, Spain.

Cintia Folgueira:
- "Entre Mulleres 2024" Award, Ayuntamiento de Lugo, Spain.
- IBSA Foundation Fellowship in Endocrinology, Spain.
- EFSD Novo Nordisk Rising Star Award, The European Foundation for the Study of Diabetes (EFSD).

TRANSLATIONAL CHEMICAL BIOLOGY GROUP

Gonçalo Bernardes (since October)
Group Leader

OVERVIEW

The Translational Chemical Biology Group bridges the gap between organic chemistry and biology to unlock fundamental biological knowledge and pave the way for the next generation of targeted therapeutics.

In chemistry, the term 'chemoselectivity' refers to the ability of a reagent (a substance or compound that triggers a reaction) to single out a specific chemical group, ignoring other reaction pathways. Organic chemists have exploited this property to develop new catalysts and streamline the chemical synthesis of complex synthetic targets. Chemical biology takes this a step further. The Group uses this background in organic chemistry to harness the inherent reactivity of functional groups within biological macromolecules like DNA, RNA, and proteins to create biological probes and novel therapeutic agents.

"Our Group has made contributions at the interface of chemistry and cancer biology. Our ability to use chemistry principles has provided new methods for *de novo* biology, which are central to several translational clinical programmes."

RESEARCH HIGHLIGHTS

Our research uses chemistry principles to address questions of importance in life sciences and molecular medicine.

Recent examples of emerging areas in our Group include:

- Development of small-molecule RNA degraders which were readily adapted to create a bifunctional molecule approach for degradation of specific secondary and tertiary RNA structures linked to pathologies, such as COVID-19 or cancer.
- Development of *de novo* mini protein drug conjugates for targeted cancer therapy.
- Development of immunodiagnostic plasma amino acid residue biomarkers to detect cancer early and predict treatment response. ■

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AWARDS AND RECOGNITION

- 2024 Corday-Morgan Mid-Career Prize for Chemistry, The Royal Society of Chemistry, UK.
- Biogen Lectureship 2024, Boston College, USA.

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Research Scientist
Susana García

Post-Doctoral Fellow
Marta Hergueta

Graduate Students
Enrique Bastón, M. Victoria Del Castillo (since March), Juan García-Agulló

OVERVIEW

Our Group focuses on understanding how the tumour microenvironment influences cancer progression, metastasis, and treatment resistance. Specifically, we investigate how extracellular vesicles, obesity, and specific receptors like NGFR shape interactions within the tumour microenvironment, modulating immune responses and cellular dormancy states. By elucidating these mechanisms, we aim to identify potential therapeutic targets to inhibit metastatic growth and overcome resistance to current treatments. This research is crucial for developing strategies to prevent metastasis, improve patient outcomes, and advance personalised therapies in oncology.

"Our Group has advanced insights into how tumour microenvironment factors drive metastasis and therapy resistance, identifying novel therapeutic targets for effective cancer treatment and prevention."

Technicians
Sara Sánchez-Redondo, Vanesa Santos

Students in Practice
Lucía Garrido (January-June)

(Graduate Student, UCM, Madrid, Spain), Rodrigo Barber (July-August) (AECC Summer Traineeship, UAM, Madrid, Spain)

Visiting Scientists
Mireia Gómez (January-April) (Institut Curie, Paris, France), Laura Nogués (July-December) (Universidad Autónoma de Madrid, Madrid, Spain)

RESEARCH HIGHLIGHTS

Exploring the relevance of damaged DNA secretion in extracellular vesicles. It is well established that DNA can be shed in extracellular vesicles (EVs). Interestingly, we have found that damaged DNA (dDNA) can also be packaged into EVs, though the mechanisms and significance of this process remain largely unexplored. Our laboratory focuses on determining the biological relevance of dDNA secretion in EVs. Additionally, we aim to understand the impact of dDNA-containing EVs on the tumour microenvironment and their potential influence on tumour evolution.

Targeting NGFR: novel therapeutic approaches to combat tumour progression and therapy resistance. The nerve growth factor receptor (NGFR) has been implicated in metastasis and therapy resistance across various tumour types. We are investigating NGFR inhibitors as potential anti-tumour agents and assessing their efficacy. Notably, we recently identified NGFR overexpression at the invasive front of melanoma cell spheroids, associated with the activation of phosphorylated Myosin II (pMLC2) (Figure 1A, 1B). Our goal is to elucidate the molecular mechanisms driving NGFR-dependent tumour progression, metastasis and therapy resistance, aiming to develop novel therapeutic strategies.

Deciphering the impact of high fat diet on breast cancer metastasis. We have found that a high-fat diet (HFD) promotes platelet activation, tumour-platelet interaction (Figure 1C), and metastatic cell homing by increasing vascular permeability and upregulating fibronectin expression in pre-metastatic niches (*manuscript under review*). However, it remains unclear whether HFD influences disseminated tumour cell (DTC) dormancy. Our next goal is to investigate how a HFD affects DTC behaviour and to elucidate the underlying biological mechanisms involved. ■

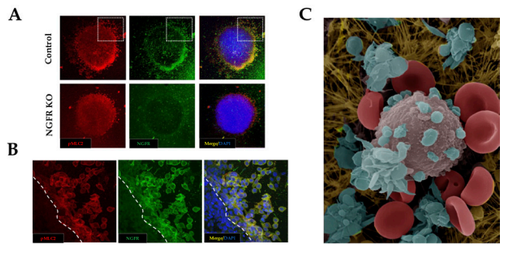

FIGURE 1 (**A**) In melanoma cell spheroids of A375P control cells, NGFR co-localises with pMLC2 at the invasive front. Note that invasive properties are lost in NGFR knockout (KO) conditions (lower panels). Staining includes pMLC2 (red), NGFR (green), and DAPI (blue). (**B**) High-magnification (63×) confocal image of the invasive front (dashed line) in control A375P spheroids. (**C**) Scanning electron microscopy image showing tumour-platelet interaction. The original image was pseudocoloured to highlight platelets in blue, tumour cells in grey, erythrocytes in red, and extracellular matrix in yellow.

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METABOLISM AND CELL SIGNALLING JUNIOR GROUP

Alejo Efeyan
Junior Group Leader

Research Scientist
Bárbara Martínez

Post-Doctoral Fellow
Yurena Vivas

Graduate Students
Lucía de Prado, Lucas Fellmann (since March),
Elena Fernández, Elena Sánchez

Technicians
María Cuerda (until July), Jon Lasa (TS)*, Alba Sanz

Titulado Superior (Advanced Degree)

Students in Practice
Emma Nyberg (since Sept) (Master's Thesis, Uppsala University, Sweden), Sofía García (March-July) (Erasmus +, Université Catholique de Louvain, Belgium), Georgia Caragianni (Feb-June) (Erasmus +, National and Kapodistrian University of Athens, Greece)

Visiting Scientists
Ana Ortega (Centro de Biología Molecular Severo Ochoa, Madrid, Spain), Sebastian Thompson (IMDEA Nanociencia Institute, Madrid, Spain)

OVERVIEW

In the Metabolism & Cell Signalling Lab we study the links between nutrients, cancer and ageing. All our cells integrate signals emanating from the abundance of intracellular nutrients and the nutritional state of the entire organism. Integration of these signals is key to adjusting metabolic functions, as well as for energy storage and expenditure. Importantly, the components of these signalling cascades are generally corrupted in cancer, and are the drivers of metabolic complications of chronic nutrient overload. Conversely, dietary restriction regimes are extremely efficacious interventions against tumorigenesis and to delay the process of ageing; albeit we still ignore the fundamental molecular underpinnings of such protective effects. We combine mouse genetics and cell biological tools to gain insight into the genetic and environmental corruptions of nutrient signalling cascades, aiming to conceive therapeutic interventions in the context of cancer, obesity, and the process of ageing.

"We have found that elevated nutrient signalling drives tissue damage and the process of ageing in multiple organs, and this pushes a vicious cycle of inflammation that further precipitates tissue damage."

RESEARCH HIGHLIGHTS

We have undertaken several efforts, using mouse genetic tools, to understand the negative effects of increased nutrient signalling. One such mouse genetic tool, RagC^{ON} mice, have in every cell, increased nutrient signalling to mTORC1, the master regulator of metabolism. This increase is moderate, and mimics the effect that chronic nutrient overload would have in our bodies. In monitoring these mice over their lifetime, we observed a 20% shortened longevity, and multiple features of premature ageing (Figure 1). While the mTORC1 pathway has been extensively linked to ageing by means of pharmacological and genetic manipulation in yeast, worms, and flies, our RagC^{ON} mice are the first genetic system in mammals to exhibit a premature ageing phenotype. This uniqueness is explained by the fact that this genetic perturbation only results in a moderate increase in signalling, unlike all previous models, which result in strong activation of the mTORC1 pathway and early-onset lethalities. As such, RagC^{ON} mice allow the interrogation of how elevated nutrients contribute to ageing. We found that the deregulation in nutrient signalling leading to shortened longevity has two components: 1. Slow, but steady multi-organ damage, resulting in organ dysfunction, premature senescence, and release of inflammatory cytokines. 2. In response to these cytokines, myeloid cells, and neutrophils in particular, rather than repairing the organ damage, cause further inflammation and inflammatory damage. These two steps feed forward one another, perpetuating and boosting organ dysfunction and premature ageing in mice with increased nutrient signalling (Figure 1). ■

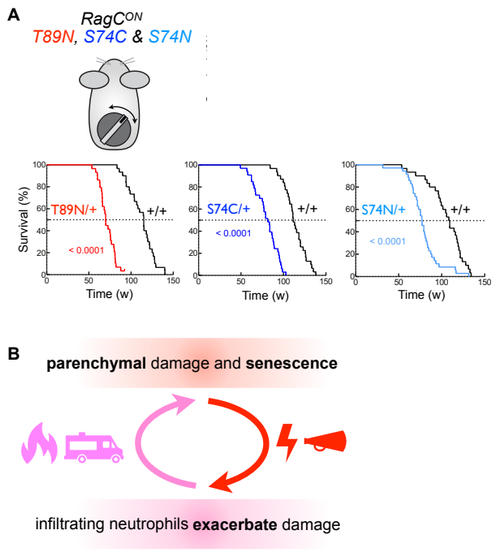

FIGURE 1 **A**. Three strains of RagC^{ON} mice were generated: RagC$^{T89N/+}$, RagC$^{S74C/+}$ and RagC$^{S74N/+}$, showing shortened longevity. **B**. Summary: Increased nutrient signalling results in parenchymal damage and senescence, resulting in the attraction of inflammatory cells that contribute to extensive damage, and further precipitate dysfunction and ageing.

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AWARDS AND RECOGNITION

- Yurena Vivas was recipient of an "Asun Almajano" Fellowship from the Spanish Leukemia and Lymphoma Foundation.
- Bárbara Martínez received an AECC Seed Ideas Grant from the Spanish Association Against Cancer (AECC).

CANCER IMMUNITY JUNIOR GROUP

María Casanova-Acebes
Junior Group Leader

Research Scientist
Alba de Juan

Post-Doctoral Fellows
Alejandra Aroca (since May), Raquel González-Novo (since November),
Ana Mantrana (since September), Sarai Martínez

Graduate Students
Eduardo Garvín, Jan Hochstadt, Mariola Munárriz

Technicians
David Cáceres (TS)*, Mónica Gómez, María Nogales (TS)*

**Titulado Superior* (Advanced Degree)

Student in Practice
Sandra Barrantes (until July) (Harper Adams University, Newport, Shropshire, UK)

OVERVIEW

Our laboratory investigates how myeloid cells shape the microenvironment of solid tumours (lung, ovarian and breast cancer metastasis). We use unbiased approaches, such as single-cell RNA sequencing (scRNAseq), to identify novel biomarkers within this cell compartment that can be harnessed into the clinic to dampen immunosuppression. Our work also uncovers new physiological cues on the regulation of circadian immunity in lung cancer, clonal haematopoiesis in tumour-associated macrophage activity, as well as macrophage-fibroblast crosstalk in lung tumours.

This year, our Group made significant strides in advancing cancer research by contributing to the understanding of lung tumour biology, particularly with the identification of universal fibroblasts as key regulators of T-cell infiltration in non-small-cell lung cancer (NSCLC). This breakthrough discovery will pave the way to explore novel treatment approaches, all of which aim to improve early detection, treatment outcomes, and the overall response to immune checkpoint blockade.

"We have identified universal fibroblasts as key regulators of T-cell infiltration in NSCLC, paving the way to explore novel treatment approaches."

RESEARCH HIGHLIGHTS

In 2024, we consolidated our laboratory and incorporated 2 new members. Ana Mantrana joined the lab to work on fibroblasts' circadian role in immunity, and together with David Cáceres (MSc in Bioinformatics and Computational Biology), they will develop analytical tools to understand how BMAL1-controlled programmes promote the recruitment of myeloid cells in lung cancer (funded by ERCStG2023). We also successfully obtained funding for one of the PhD students in the lab, Jan Hochstadt, supported by the Comunidad Autónoma de Madrid PhD Fellowship Programme PIPF-2023/SAL-GL-29563. Jan's PhD focuses on the co-evolution of cancer-immune cells in breast metastasis.

In October 2024, we successfully organised a CNIO-CaixaResearch Frontiers Meeting focused on Immunomodulation and Cancer Therapy. This was a world-leading meeting on immunotherapies in 2024, which gathered top speakers for 3 days full of vibrant discussions. One of the award-wining short talks was given by Eduardo Garvín, a second year PhD student in the laboratory.

Throughout this year, we also established new collaborations with the Instituto Valenciano de Oncología's Biobank to study the impact of clonal haematopoiesis of indeterminate potential (CHIP) in macrophages, and with the Hospital Universitario Marqués de Valdecilla (HUMV) to interrogate the impact of immune checkpoint blockade on immune cell functional rhythmicity. These collaborations will collect critical human data for our preclinical models of CHIP and lung cancer circadian immunity.

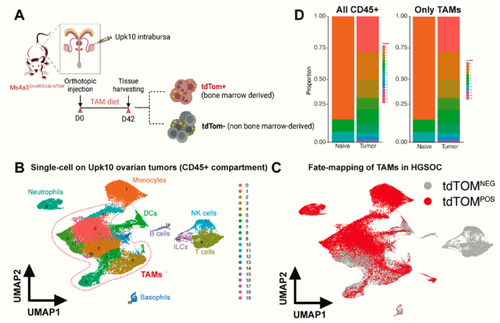

FIGURE 1 Tumour-associated macrophages of bone marrow origin dominate the immune microenvironment of ovarian tumours. (**A**) Experimental outline of *Ms4a3*$^{CreERT2\text{-}LSL\text{-}RosatdTom}$. (**B**) UMAP plot showing single-cell transcriptome of Upk10 tumours. (**C**) tdTomato transcript expression is projected into the clusters identified in B.(**D**) Quantification of all CD45+ cells in naïve and Upk10 tumours and TAM cluster identification.

Finally, our laboratory gathered together with the SOSCLC-AECC consortia to help in the identification of new macrophage targets for the treatment of small-cell lung cancer. ■

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PUBLICATIONS AT OTHER INSTITUTIONS

- Park MD, Le Berichel J, Hamon P, Wilk CM, Belabed M, Yatim N, Saffon A, Boumelha J, Falcomatà C, Tepper A, Hegde S, Mattiuz R, Soong BY, LaMarche NM, Rentzeperis F, Troncoso L, Halasz L, Hennequin C, Chin T, Chen EP, Reid AM, Su M, Cahn AR, Koekkoek LL, Venturini N, Wood-Isenberg S, D'souza D, Chen R, Dawson T, Nie K, Chen Z, Kim-Schulze S, Casanova-Acebes M, Swirski FK, Downward J, Vabret N, Brown BD, Marron TU, Merad M (2024). Hematopoietic aging promotes cancer by fueling IL-1α-driven emergency myelopoiesis. *Science* 386, eadn0327.

AWARDS AND RECOGNITION

María Casanova-Acebes:
- Associate Editor of the *Journal of Experimental Medicine* (*JEM*).

STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA Programme Director

The Programme's research areas and strategic goals

Many of the breakthroughs that are helping patients would not have been possible without earlier discoveries on the fundamental biological processes influencing cancer. The primary mission of the Structural Biology Programme (SBP) is to deepen our understanding of core mechanisms that drive cancer onset and progression. For this, we make use of the opportunities provided by advances in structural and computational biology. In addition, we design tools that can ultimately benefit patients.

Lucas Tafur from the University of Geneva joined our faculty in October 2024 as leader of the "Structural Mechanisms of Cell Growth Group". Lucas is using cryo-EM, biochemistry and cell biology to investigate the molecular mechanisms by which specific nutrients, growth factors, and other signals regulate cell growth and metabolism.

In 2024, the CNIO received funding from the European fund NextGeneration EU to incorporate scientists and trainees in the field of Big Data and Artificial Intelligence (AI). As part of this institutional project, SBP has incorporated two new Junior Group leaders and their teams. Marcos Diaz-Gay is leading the "Digital Genomics Group", which aims to improve our understanding of the mechanisms behind the accumulation of genomic alterations in human tumours in order to benefit cancer diagnosis, prognosis, and treatment selection. Roger Castells-Graells joined the Programme in 2025 to lead the "Biomolecular Design and Structural Nanomedicine Group". Roger combines AI and cryo-EM to design new proteins that never previously existed as new tools in research and drug discovery.

With these new recruitments, SPB is now consolidated with a critical mass that will allow us to tackle complex challenges in cancer research. The new recruits are young and bring new ideas and renewed energy to succeed in a very active area of research combining AI with structural biology and cancer genomics.

In 2024, SBP comprised 7 Groups and 5 Units organised according to 2 major strategic lines: (a) structural biology and (b) computational and cancer genomics. The goal of the strategic line in structural biology is to determine the structure and molecular mechanisms of proteins and macromolecular complexes implicated in cancer and support drug discovery efforts. The strategic line in computational and cancer genomics makes use of bioinformatics, computational biology, and cancer genomics to better understand the complexity of cancer, predict therapy responses, design new therapeutic strategies, and develop new tools. In 2025, the Groups working in computational and cancer genomics, as well as the Bioinformatics Unit, split from SBP to become part of a new Programme focusing on cancer genomics. Thus, in 2025, SBP consists of 5 Groups and 4 Units focused on structural biology.

> "Our research has generated knowledge on fundamental processes in cancer and the proteins involved. We have used computational tools to better understand cancer and have advanced new methodologies."

Summary of milestones & major achievements during 2024

During 2024, scientists at SBP made interesting discoveries in several areas of cancer research. The Kinases, Protein Phosphorylation and Cancer Group has characterised the oncogenic CCDC6-RET fusion, a driver and therapeutic target in lung and thyroid cancers. The Macromolecular Complexes in DNA Damage Response Group discovered mechanisms that regulate the formation of microtubules in cell division, and characterised how some compounds inhibit ATPases relevant in cancer. The Genome Integrity and Structural Biology Group developed new tools to help determine the structure of proteins using cryo-EM. The Computational Cancer Genomics Group revealed previously uncharacterised classes of cancer-predisposing genes. The Computational Oncology Group contributed to the development of new methods based on single-cell sequencing. In addition, Units at SBP provided specialised support in cryo-EM, NMR, protein purification, protein crystallography, bioinformatics and biophysics, making important contributions as collaborations with groups at CNIO. ■

MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Óscar Llorca
Group Leader

Research Scientists
Javier Coloma, Ana Isabel Hernández, Ángel Rivera, Marina Serna

Post-Doctoral Fellows
Ana González, María I. Daudén, Andrés López, María Martínez, Clara Reglero (since September)

Graduate Students
Carmen García, Nayim González, Álvaro López (since September), Juan Tasis (since October)

Student in Practice
Celia Varela (until June) (Universidad Autónoma de Madrid; AECC, Spain)

OVERVIEW

Our Group uses cryo-electron microscopy (cryo-EM) to determine the 3D structure of large macromolecular complexes of relevance in cancer. Structural information, in combination with molecular and cell biology and biochemistry, is used to propose how these molecules work and increase our understanding of the molecular basis of cellular pathways related to cancer. Most of our efforts are currently focused on two major areas of research: the study of chaperones essential for the activation of several macromolecular complexes relevant in cancer such as mTORC1; and the study of complexes implicated in the repair of DNA damage and in genomic instability. In collaboration with other groups, we are also studying the mechanisms that regulate microtubule nucleation for the assembly of the mitotic spindle during cell division.

"We have discovered how cells start building the microtubules that form the mitotic spindle during cell division, and how the aminopyrazolone CB-6644 inhibits an ATPase involved in mTORC1 activation."

RESEARCH HIGHLIGHTS

Visualising how microtubules are constructed during cell division

Cell division depends on microtubules, dynamic structures built during mitosis with the help of γTuRC, a protein complex that acts as a template for microtubule nucleation. However, γTuRC is naturally found in an open, inactive form, and its transformation into a closed, functional ring is essential for its role in microtubule formation. In collaboration with Thomas Surrey at the Centre for Genomic Regulation (CRG), Jens Lüders at the IRB-Barcelona, and Pablo Guerra at the IBMB-CSIC's Electron Cryo-microscopy Platform at JEMCA, we have uncovered how γTuRC undergoes this critical conformational change during cell division.

Using a combination of cryo-electron microscopy (cryo-EM), artificial intelligence, and biochemical methods, we captured the step-by-step process by which γTuRC closes to form a ring and becomes a perfect template for microtubule growth. The CRG group played a pivotal role by slowing down and halting microtubule assembly in its early stages, allowing us at the CNIO to generate a "molecular movie" of the nucleation process by using cryo-EM and image processing of over a million images of microtubules, in various stages of the nucleation process (Figure 1).

Furthermore, to date, the mechanisms by which regulators bind to γTuRC and affect the nucleation of microtubules were poorly understood. Our work revealed that the regulator protein CDK5RAP2 is critical for γTuRC activation. CDK5RAP2 binds to five specific sites on γTuRC, and these interactions turn the complex into a more flexible structure, which is now more likely to undergo the conformational changes required for converting the ring into a perfect template for microtubule nucleation.

The implications of this research, published in 2024 as part of two manuscripts, extend beyond basic biology. Microtubules are a key target in cancer therapies, but current cancer drugs act on all microtubules, affecting both cancerous and healthy cells, resulting in side effects. By understanding γTuRC's role in nucleation and its regulatory mechanisms in greater detail, and by determining the structures of the complexes involved, we can envision the development of strategies that disrupt microtubule formation specifically in dividing cells.

Inhibition of ATPases required in the assembly and activation of mTORC1

RUVBL1 and RUVBL2 are ATPases involved in numerous cellular processes such as chromatin remodelling, DNA repair, and mRNA decay. They are also an essential component of R2TP, a chaperone complex required for activating kinases such as mTOR and ATR, which are implicated in pathways associated with cancer progression. The RUVBL1/2 complex is required for the assembly of mTORC1 and mTORC2 complexes. This function relies on their ATPase activity, making these proteins attractive targets for cancer therapy, as their inhibition affects cancer cells, particularly those with hyperactive mTORC1 activity.

CB-6644, a selective allosteric inhibitor of RUVBL1/2 ATPase activity, represents a promising compound to characterise the potential of these ATPases as targets in cancer. However, the molecular mechanisms and structural basis of how CB-6644 inhibits RUVBL1/2 was not known. Using cryo-EM and biochemistry, we found that CB-6644 works by binding to the interface between RUVBL1 and RUVBL2 in the hetero-hexameric RUVBL1/2 complex, locking it in an ATP-bound conformation and preventing ATP hydrolysis (Figure 2). This action disrupts the dynamic conformational changes required for their activity. This mechanism may interfere with RUVBL1/2-mediated assembly of client complexes, such as mTORC1 and other PIKK kinases, and destabilise cancer-related pathways reliant on RUVBL1/2 activity. Our findings make CB-6644 a valuable tool for studying RUVBL1/2 functions and a potential foundation for targeting mTOR activity. ■

FIGURE 1 Mechanism of microtubule nucleation by γTuRC. (**A**) Cryo-EM combined with AI tools revealed the structure of several stages during the nucleation of microtubules by γTuRC. (**B**) Only in the active conformation, γTuRC becomes a perfect template to nucleate the formation of microtubules.

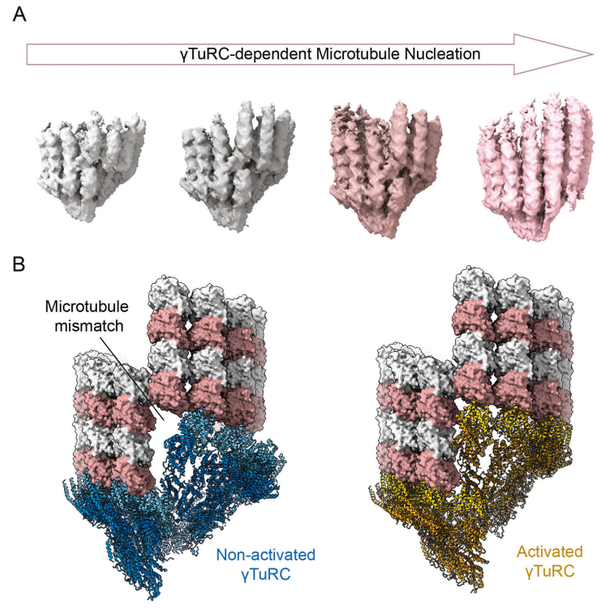
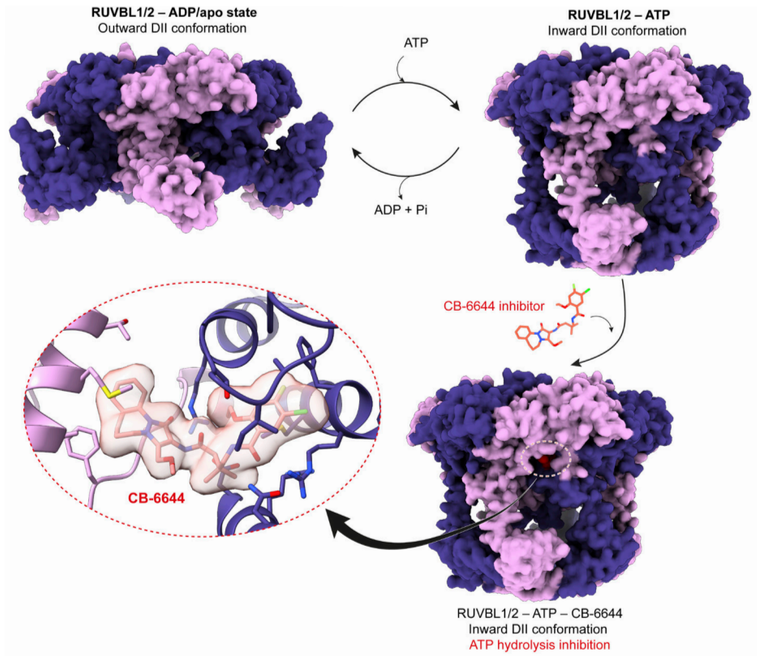


FIGURE 2 Structure of the RUVBL1/2 ATPase in complex with CB-6644. ATP binding to RUVBL1/2 induces large conformational changes in Domain II (DII) that CB-6644 recognises by interacting at the interface between two subunits. CB-6644 traps this ATP-bound conformation and prevents ATP hydrolysis.



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- Rullo-Tubau J, Martinez-Molledo M, Bartoccioni P, Puch-Giner I, Arias Á, Saen-Oon S, Stephan-Otto Attolini C, Artuch R, Díaz L, Guallar V, Errasti-Murugarren E, Palacín M, Llorca O (2024). Structure and mechanisms of transport of human Asc1/CD98hc amino acid transporter. *Nat Commun* 15, 2986.
- Serna M, Zimmermann F, Vineethakumari C, Gonzalez-Rodriguez N, Llorca O, Lüders J (2024). CDK5RAP2 activates microtubule nucleator γTuRC by facilitating template formation and actin release. *Dev Cell* 59, 3175-3188.e8.
- Murga M, Lopez-Pernas G, Soliva R, Fueyo-Marcos E, Amor C, Faustino I, Serna M, Serrano AG, Díaz L, Martínez S, Blanco-Aparicio C, Antón ME, Seashore-Ludlow B, Pastor J, Jafari R, Lafarga M, Llorca O, Orozco M, Fernández-Capetillo O (2024). SETD8 inhibition targets cancer cells with increased rates of ribosome biogenesis. *Cell Death Dis* 15, 694.
- García- Martín C, López-Perrote A, Boskovic J, Llorca O (2024). Mechanism of allosteric inhibition of RUVBL1-RUVBL2 ATPase by the small molecule CB-6644. *Cell Rep Phys Sci* 5, 101982.
- López-Perrote A, Serna M, Llorca O (2024). Maturation and assembly of mTOR complexes by the HSP90-R2TP-TTT chaperone system: molecular insights and mechanisms. *Subcell Biochem* 104, 459-483.

AWARDS AND RECOGNITION

- Marina Serna: 'José Tormo' Award 2024 by the Molecular Biology and Biochemistry Spanish Society (SEBBM) and BRUKER Española S.A., Spain.

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellow
Julia M. Contreras

Graduate Student
Saray Aragón (since March)

OVERVIEW

Rational and precise targeting of oncogene-driven signalling is a crucial and yet outstanding challenge in cancer research. Understanding the structural and molecular bases of oncogene activation and signalling is key for the design and development of better therapeutics. Our research focuses on the structural and molecular understanding of protein kinase function: how protein kinases are activated and regulated by post-translational modifications and allosteric inputs, and how they assemble into macromolecular protein complexes to transmit signals inside the cell. We put a special emphasis on how these mechanisms are corrupted in cancer, due to oncogenic mutations and other oncogenic insults. Crucially, such atomic and molecular information can be translated into the design and development of next generation protein kinase inhibitors for targeted and personalised therapies.

We apply an integrated and multidisciplinary approach, combining molecular biology to generate suitable constructs; protein biochemistry and biophysics for protein purification, quality assessment and functional evaluation; mass spectrometry (MS) to identify and quantify post-translational modifications; X-ray crystallography for the 3D-visualisation of proteins; and *Drosophila* as an *in vivo* model for data validation. Furthermore, we use structure-guided drug discovery and MD simulation approaches to exploit structural and functional vulnerabilities for drug design and development.

Visiting Graduate Student
Yanara Astudillo (until May) (Universidad Tecnológica Equinoccial - Fundación Carolina, Quito, Ecuador)

Technicians
Yanara Astudillo (since September), Laura Valentina Morantes (since September)

Students in Practice
Rocío Calderón (August-October) (Universidad Autónoma de Madrid, Spain), Anna Paula Correa (August-December) (Instituto Tecnológico de Morelia, Mexico),
David Herrero (July-August) (Universidad Autónoma de Madrid, Spain)

RESEARCH HIGHLIGHTS

Our main strategic lines are:

1. Structural and molecular determinants that control protein kinase function and phosphorylation. Auto-phosphorylation controls the transition between discrete functional and conformational states in protein kinases, yet the structural and molecular determinants underlying this fundamental process remain unclear. We have recently proven a self-autonomous mechanism for c-Src regulation driven by autophosphorylation. We demonstrated that c-terminal Tyr 530 is a de facto c-Src autophosphorylation site and identified a critical c-terminal palindrome phospho-motif that controls the interplay between substrate- and enzyme-acting kinases during the autophosphorylation process (Cuesta-Hernández and Contreras *et al.*, *Nature Commun* 2023). This work is fundamental to the design and development of next generation tyrosine kinase inhibitors targeting allosteric and non-catalytic functions for targeted and personalised cancer therapies.

2. Structure, function, and pharmacology of RET kinase-gene fusion products. Gene fusion products are known drivers in human cancers and are current drug targets for personalised therapy. A second strategic line in the lab is focused on elucidating the functional and structural determinants for several RET oncogenic fusion products (Figure 1). We advanced projects on CCDC6-RET and KIF5B-RET fusion products, both drivers and therapeutic targets in lung (NSCLC) and thyroid cancers. We performed a careful and detailed structure and functional characterisation, revealing striking and unexpected regulatory mechanisms and interactions – not previously known nor envisioned for the wild-type protein – with important implications for drug discovery. In particular, we dissected, for the first time, the auto-activation mechanism of the CCDC6-RET fusion product, and found that CCDC6-RET is a highly active dimeric protein in solution (Figure 1), showing a striking dual ATP and ADP dependency (Martin-Hurtado *et al.*, under revision). In the case of the KIF5B-RET, we are looking at the specific interaction between this fusion product and key components of the cytoskeleton.

3. *Structure-guided drug discovery for next generation protein kinase inhibitors*. A third main research line focuses on the exploitation of structural and functional vulnerabilities in RET and c-Src for the rational design and development of next generation tyrosine kinase inhibitors. ■

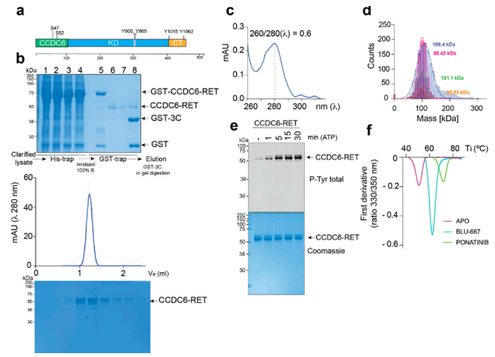


FIGURE 1 Purification and quality check of a recombinant CCDC6-RET fusion product. (**a**) Schematic diagram of the CCDC6-RET fusion product depicting functional domains. (**b**) In tandem immobilised metal affinity (Ni^{2+}), glutathione-conjugated gravity flow chromatography and in-gel 3C-protease digestion followed by size exclusion chromatography (sec) using a Superdex 200 3.2/300 column. (**c**) Absorbance spectra (260-310 nm) with max at 280 nm and indicated (260/280 nm) ratio. (**d**) Mass photometry profiles (counts vs mass in kDa) of CCDC6-RET at different concentrations in colour code: 50 nM (magenta), 25 nM (blue), 10 nM (green), 5 nM (orange). (**e**) WB of samples from a time-course autophosphorylation experiment with CCDC6-RET (1 μM) in the presence of ATP (1 mM) and $MgCl_2$ (2 mM) for 0–30 min using a total anti-phospho-tyrosine antibody. The total amount of protein was visualised by Coomassie staining. (**f**) DSF profile (IF) of CCDC6-RET in apo state and bound to BLU-667 and Ponatinib.

PUBLICATIONS

- Lucena-Agell D *et al.* (incl. Contreras J, Plaza-Menacho I) (2024). PM534, an optimized target protein interaction strategy through the colchicine site of tubulin. *J Med Chem* 67, 2619-2630.
- Plaza- Menacho I (2024). Redox takes control. *Elife* 13, e99765.
- Martín-Hurtado A, Contreras J, Sánchez-Wandelmer J, Zarzuela E, Muñoz IG, Muñoz J, Isasa M, Plaza-Menacho I. The oncogenic CCDC6-RET fusion product is a dual ATP and ADP-dependent kinase that functions via *cis*-phosphorylation. *bioRxiv*. https://doi.org/10.1101/2025.01.14.632894.

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
Junior Group Leader

Research Scientist
Maria Dolores Moreno

Post-Doctoral Fellow
Elena Jiménez

Graduate Students
Ester Casajús, Samuel Míguez

OVERVIEW

Safeguarding genetic information is essential to prevent malignant transformation. Two critical cellular processes maintain it free from errors: DNA replication and DNA repair. Importantly, genetic information can be damaged or lost when these processes do not function correctly, ultimately leading to disease. Deregulation and malfunction of the protein machinery that safeguards our genome are hallmarks of cancer, yet it remains unclear how this occurs at the molecular level. The devil is in the detail, and we aim to understand what goes wrong with these molecular machines, and when, so we can act to correct it and prevent it from happening.

"Macromolecules are like real-life machines. To understand how they work, we study their structures using cryo-EM. Beyond basic research, this provides the necessary information for drug development."

These macromolecules are like real-life machines, equipped with intricate mechanisms that enable them to carry out their activities. We use cryo-electron microscopy and biochemistry in an integrative approach to understand how they operate. Beyond fundamental research, this structural information delivers the necessary details for drug development.

Technicians
Elena Blanco (since March), Carlos Chacón (since October) (TS)*, Araceli Grande (TS)*

Titulado Superior (Advanced Degree)

Students in Practice
Victor Arranz (until June) (Master's Thesis, UAM, AECC, Madrid, Spain), Carlos Chacón (until September) (Master's Thesis, UAM, Madrid, Spain), David Collado (February-May) (Bachelor's Degree Final Project, UAM, Madrid, Spain), Santiago Gómez (May-July) (Internship, Université Paris-Saclay, France)

Visiting Scientist
Diego Alonso (until July) (Universidad Politécnica de Madrid, Spain)

RESEARCH HIGHLIGHTS

DNA replication & repair – focus on mitochondria

Mitochondrial DNA (mtDNA) replication is crucial for human health. Deficiencies in the function of mtDNA replication machinery underlie various debilitating, multi-systemic, mitochondrial disorders and have been directly linked to the onset of neurodegenerative diseases, including multiple forms of ataxia, Parkinson's disease, Alzheimer's disease, and several types of cancer. However, the mechanisms by which the integrity of the mitochondrial genome is maintained, through the balance of DNA replication, repair, degradation, and organelle dynamics, remain unclear. We are keen to understand these pathways due to their implications for ageing and disease, particularly in cancer.

Genome integrity – focus on telomeres

Telomeres are essential nucleoprotein structures that protect the ends of chromosomes. These structures are shaped by the protective shelterin complex, that specifically binds to telomeric DNA repeats. Shelterin comprises six proteins – TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 – and their correct arrangement and function protect telomeres from degradation and activation of persistent DNA Damage Response. Shelterin function is, therefore, crucial for telomere and genome integrity. Despite the key role of the shelterin complex in cell viability and tissue homeostasis, as well as its potential use as a target for anti-cancer therapeutic strategies, its mechanistic details and architecture are poorly understood.

Biochemistry & cryo-EM

By combining *in vitro* reconstitution and native purification of protein-DNA complexes, and taking advantage of recent developments in cryo-EM imaging, we capture these protein machines in different functional states to study their structures. This information can help unveil their molecular mechanisms, rationalise pathological mutations and their physiological consequences, and aid in developing future cancer therapeutic strategies. ■

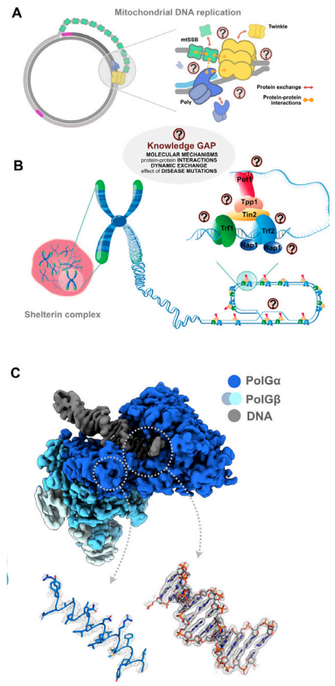


FIGURE 1 (**A**) Mitochondrial DNA replication machinery. (**B**) The shelterin complex protects and regulates enzymatic activities at telomeres. Several aspects of their regulation and molecular mechanisms through which they exert their activities remain unknown. (**C**) Structure of PolG bound to DNA.

PUBLICATIONS

- Del Caño-Ochoa F, Ramadane-Morchadi L, Eixerés L, Moreno-Morcillo M, Fernández-Leiro R, Ramón-Maiques S (2024). Disruption of CAD oligomerization by pathogenic variants. *J Mol Biol* 436, 168832.
- Llanos S, Di Geronimo B, Casajús E, Blanco-Romero E, Fernández-Leiro R, Méndez J (2024). Interference of small compounds and Mg2+ with dsRNA-binding fluorophores compromises the identification of SARS-CoV-2 RdRp inhibitors. *Sci Rep* 14, 28250.
- González-Rodríguez N, Areán-Ulloa E, Fernández-Leiro R (2024). A web-based dashboard for RELION metadata visualization. *Acta Crystallogr D Struct Biol* 80, 93-100.
- Laguía O, Bosso G, Martínez-Torrecuadrada J, Míguez-Amil S, Fernández-Leiro R, Blasco MA (2024). Protocol for the generation and purification of high-molecular-weight covalent RNA-DNA hybrids with T4 RNA ligase. *STAR Protoc* 5, 102930.

COMPUTATIONAL CANCER GENOMICS JUNIOR GROUP

Solip Park
Junior Group Leader

Post-Doctoral Fellow
Seokjin Ham (since March)

Graduate Students
Jaejun Lee, Manuel Moradiellos, Alejandro Palacios (since March) (PEJ, CAM) *

** Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

Technician
Adrián Maqueda

Master's Students
Yiran Du (until July) (Master's Thesis, Univ. Autónoma de Madrid, Spain), María Legarreta (May-December) (Master's Programme in Bioinformatics, ENS-ISCIII, Madrid, Spain)

Visiting Scientists
Sojeong Jeon (until February) (Dong-Eui University, South Korea), Guillermo Palou (April-July) (Instituto de Investigación Biomédica de Barcelona, Spain)

OVERVIEW

Through big data analysis on cancer patients, we aim to address a fundamental question: *do identical genes or mutations exhibit the same effects in different cellular contexts*? The optimal fitness level of a cancer gene can vary based on the context, and to deepen our understanding, we apply quantitative methods to explore it. First, we investigate how the fitness landscape shifts between primary and metastatic cancers by analysing the combination of alterations. Next, we seek to unravel how the position of mutations affects protein interaction partners and how these perturbations impact phenotypic outcomes. For this, we build a model that leverages state-of-the-art AI-based structural predictions and multi-omics data. Through this interdisciplinary approach, our goal is to gain insights into the diverse modes of action that genes may assume, ultimately contributing to the development of patient-specific treatments and preventive strategies.

"Our goal is to decipher how identical mutations yield diverse outcomes across cellular environments, driving the development of treatments tailored to the cancer state and mutation context."

RESEARCH HIGHLIGHTS

Mapping cancer gene dynamics through state-specific interactions

Metastatic cancer, a major cause of mortality, has been understudied compared to primary tumours, leaving gaps in our understanding of how cancer genes adapt between these states. We analysed the association between mutations and copy-number alterations in 25,000 tumour samples from both primary and metastatic cancers. Our findings showed that cancer genes display distinct interaction strengths across these states, with 27.45% of genes, including *ARID1A*, *FBXW7*, and *SMARCA4*, shifting between one-hit and two-hit drivers (Figure 1 a-c). Interaction strengths varied by cancer state and treatment conditions, revealing seven state-specific interactions. We also identified 38 primary-specific and 21 metastatic-specific high-order interactions, enriched in cancer hallmarks, indicating unique tumour progression mechanisms. These findings highlight dynamic tumour progression mechanisms and underscore the importance of considering cancer state in research and treatment strategies for precise therapeutic interventions (Figure 1 d).

FIGURE 1 State-specific cancer fitness landscape. (**a**) Gene classification based on their preferences for two-way interactions. (**b**) The number of genes assigned to each gene class in primary and metastatic tumours. (**c**) The number of genes categorised as consistent and perturbed. (**d**) Impact of genomic alterations on cancer patient prognosis. (**e**) Assessing position-specific interaction perturbations (TP53 pilot).

Position-specific perturbed interaction network

Despite advancements in big data for analysing cancer biology, the context-specific roles of genes and variants in tumour progression remain unclear. Each cancer gene has an optimal activity level for cancer fitness, so the impact of mutations, like those in TP53, varies across cancer types. TP53 mutations, common in cancer, not only inactivate tumour-suppressing functions but also introduce gain-of-function (GOF) activities that drive tumour progression. To investigate the position-specific effects of TP53 mutations, we are collaborating with M. Oren (Weizmann Institute of Science, Rehovot, Israel). We integrated multiple approaches, including AI-based structure predictions, stability assessments, and functional impact analyses to prioritise variants likely to disrupt protein-protein interactions. Our findings revealed a negative correlation between mutation frequency and Gibbs free energy (ΔΔG), suggesting that frequent TP53 mutants tend to destabilise the TP53 structure. These results underscore the value of combining structural and functional analyses, highlighting how common TP53 mutations may alter protein stability and interaction networks. Looking ahead, we plan to simulate variant effects on the protein-protein interaction network and integrate this with drug screening results to explore variant-specific therapeutic responses (Figure 1 e). ■

PUBLICATION

- Maqueda-Real A, Ollé-Monràs L, Park S. Mapping cancer gene dynamics through state-specific interactions. *bioRxiv*. https://doi.org/10.1101/2025.01.08.631858.

PATENT

- Park S, Yoon SS, Koh Y, Song S. Novel cancer predisposition genes derived from Mendelian disorder-related gene group. PCT application (2024). *PCT/KR2024/020749.*

COMPUTATIONAL ONCOLOGY JUNIOR GROUP

Geoff Macintyre
Junior Group Leader

Research Scientist
Patricia González-Santamaría

Post-Doctoral Fellows
Bárbara Hernando, Marina Torres

Graduate Students
Blas Chaves-Urbano, María Escobar-Rey, Ángel Fernández-Sanromán, David Gómez-Sánchez (until April), Joe Thompson, Sylvie Van Genderen (since July)

Technician
Alice Nicole Cádiz

Master's Students
M. Teresa Castillo (until June) (Master's Thesis, Universidad Complutense de Madrid, Spain), Noelia Sánchez (since May) (Master's Programme in Bioinformatics, ENS-ISCIII, Madrid, Spain)

OVERVIEW

In the Computational Oncology Group, we are tackling some of the deadliest cancers by targeting the causes of chromosomal instability (CIN). By therapeutically targeting CIN, we aim to improve patient outcomes.

Our main research areas include:

- → Using model systems to develop therapeutic strategies to target CIN.
- → Predicting therapy response using CIN signatures in patient biopsies.
- → Developing single cell sequencing approaches for ultrasensitive CIN detection.
- → Modelling tumour evolution and CIN.

"We are turning our insights on chromosomal instability into innovation by developing new AI methods to enable better cancer treatment."

We aim to apply these technologies at the earliest stages of tumour development in patients with premalignant lesions, with the goal of preventing aggressive, difficult to treat cancers.

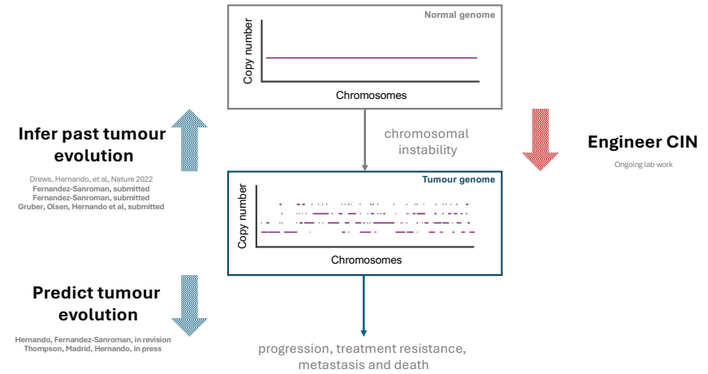
RESEARCH HIGHLIGHTS

One of the key highlights for the Computational Oncology Group in 2024 was receiving major funding to drive our research efforts forward. The Ministerio para la Transformación Digital y de la Función Pública awarded us €1.3 million for an Excellence in AI project, where we will advance our technology for predicting resistance to chemotherapy to the clinic. In collaboration with Hospital Universitario 12 de Octubre, we will acquire the necessary accreditation to enable use of the technology in future clinical trials. We were also fortunate to be part of the team awarded €10 million by the Asociación Española Contra el Cáncer to improve outcomes in small cell lung cancer. Our team will provide expertise in data management, genomics, and AI alongside 40 other research groups across Spain.

In 2024 we welcomed a new PhD student, Sylvie Van Genderen, who will bring her medical training insights to study the interaction between cells with chromosomal instability and the immune system.

Some of our major research in 2024 is now wrapped up for submission, the highlight being our collaborative work as part of the Pan-Prostate Cancer Group. In collaboration with researchers across Europe, USA and Australia, we performed three studies – the first where we inferred the evolutionary history of primary prostate cancer from whole-genome sequence data of 1001 patients – the second where we tracked the clonal evolution of metastatic cancer from 103 patients – and the third where we identified the mutational processes driving prostate cancer evolution using an integrated signature approach. These studies and others in the lab have advanced our understanding of how early chromosomal instability contributes to disease initiation and progression (Figure 1). This is causing an exciting shift in the lab where we are now focussing on a new phase of research where we can begin to build models which can predict how tumours evolve. ■

FIGURE 1 An overview of how the Computational Oncology Group is studying chromosomal instability (CIN) and tumour evolution. Recent work submitted for publication is highlighted in bold. Ongoing efforts in the lab are focussed on forward engineering CIN and tumour development.



PUBLICATIONS

- Schneider MP, Cullen AE, Pangonyte J, Skelton J, Major H, Van Oudenhove E, Garcia MJ, Chaves Urbano B, Piskorz AM, Brenton JD, Macintyre G, Markowetz F (2024). scAbsolute: measuring single-cell ploidy and replication status. *Genome Biol* 25, 62.
- Loras A, Gil-Barrachina M, Hernando B, Perez-Pastor G, Martinez-Domenech A, Mahiques L, Pitarch G, Valcuende-Cavero F, Ballester-Sanchez R, Marques-Torrejon MA, Martinez-Cadenas C (2024). Association between several immune response-related genes and the effectiveness of biological treatments in patients with moderate-to-severe psoriasis. *Exp Dermatol* 33, e15003.

PATENTS

- Macintyre G, Gómez-Sánchez D, García Lopez D, de Galard H. Genomic sequencing analysis. PCT application (2024). *PCT/EP2024/070580*.
- Macintyre G, Hernando B. Detection of chromosomal instability. PCT application (2024). *PCT/ EP2024/082483*.
- Macintyre G, Hernando B, Fernández Sanromán Á, Paz-Ares L, Zugazagoitia J, Gómez-Sánchez D, Goya JM. Prediction of gene amplification. PCT application (2024). *PCT/ EP2024/082564*.
- Macintyre G, Drews R, Markowetz F, Hernando B. Method of characterising a DNA sample. *WO2023057392A1*. National Phase Entry (2024).

STRUCTURAL MECHANISMS OF CELL GROWTH JUNIOR GROUP

Lucas Tafur (since October)
Junior Group Leader

OVERVIEW

Cells need to adjust their growth rate and metabolism in response to the availability of resources in their environment. This mechanism utilises evolutionarily conserved multi-component protein complexes to detect and transduce extra- and intracellular information to specific protein effectors. Correct coordination and function of these complexes is essential in human physiology, as defects have been associated with cancer, metabolic diseases and ageing.

"Structures of protein complexes that regulate growth have revealed unexpected similarities with other cellular components, but also novel features that can be exploited therapeutically."

Using state of the art cryo-electron microscopy, biochemistry and cell biology, our Group investigates the molecular mechanisms by which specific nutrients, growth factors, and other signals regulate cell growth and metabolism. By understanding how the protein complexes involved work, we aim to help in the development of novel and improved therapeutic strategies for the prevention and treatment of different types of cancer and associated diseases.

RESEARCH HIGHLIGHTS

We are interested in understanding the molecular details of cellular adaptation to the environment. Currently, our work is divided into two main research areas:

Lysosomal nutrient-sensing complexes

Nutrients regulate cell growth primarily via a highly conserved pathway converging on the mechanistic Target of Rapamycin Complex 1 (mTORC1). In response to amino acids, mTORC1 is activated on the lysosomal membrane, where it can phosphorylate distinct substrates located in different parts of the cell. Several complexes couple the nutrient status of the cell with growth by regulating mTORC1 activity; however, the molecular mechanisms by which they achieve this task are not completely understood. We are interested in understanding the molecular details underpinning this regulation. We have recently determined cryo-EM structures of the yeast amino acid-sensing SEA complex, which is a main regulator of TORC1, revealing unprecedented insights into its function (Figure 1).

Cellular response to starvation and stress

In the absence of nutrients, cells must downregulate processes that stimulate cell growth and promote those that serve to recycle macromolecules. In addition, when encountering other stressful environments, cells have to upregulate protective mechanisms to survive. Crucially, these responses involve the precise coordination among different organelles and protein complexes. Nevertheless, the structural and functional interplay between complexes from different organelles, and their regulation by nutritional and environmental cues, is not well understood. We want to understand how these complexes interact, spatially and temporally, to organise the cellular response to starvation and stress in the short and long term. ■

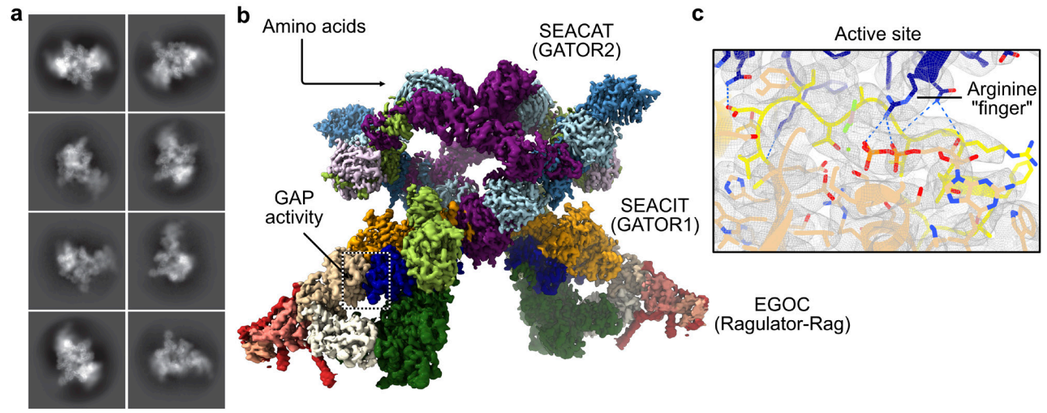


FIGURE 1 Cryo-EM structure of the amino acid-sensing SEA complex. (**a**) 2D class averages. (**b**) Composite cryo-EM map indicating subcomplexes. (**c**) Zoom of the active site, with high-resolution density allowing the modelling of interactions.

PUBLICATION

- Tafur L*, Bonadei L, Zheng Y, Loewith R* (2024). Structure and function of the yeast amino acid-sensing SEA-EGOC supercomplex. *BioRxiv*. doi: 10.1101/2024.10.05.616782. (*) Co-corresponding authors.

DIGITAL GENOMICS JUNIOR GROUP

Marcos Díaz-Gay (since November)
Junior Group Leader

OVERVIEW

In the Digital Genomics Group we are in pursuit of better understanding the mechanisms behind the accumulation of genomic alterations in human tumours, and transforming this knowledge into actionable insights for cancer diagnosis, prognosis, and treatment selection.

We specialise in developing cutting-edge computational methodologies, powered by artificial intelligence (AI), to characterise the molecular processes driving tumour development. By analysing the genomic patterns left by the different processes generating somatic mutations, known as mutational signatures, and integrating them with patient-specific clinical, epidemiological, and germline genetic data, we aim to implement prevention strategies and develop novel clinical biomarkers. Our approach seeks to enhance the current understanding of the mechanisms underlying cancer initiation and progression, ultimately improving patient outcomes.

We are also committed to applying these innovative methods to diverse cohorts of cancer patients. By doing so, we aim to advance our understanding of the dynamics and evolution of tumours in different populations across the world, exploring variations that may inform more precise and equitable approaches to cancer care. ■

PUBLICATIONS AT OTHER INSTITUTIONS

- Senkin S, Moody S, Díaz-Gay M, Abedi-Ardekani B, Cattiaux T, Ferreiro-Iglesias A, Wang J, Fitzgerald S, Kazachkova M, Vangara R, Le AP, Bergstrom EN, Khandekar A, Otlu B, Cheema S, Latimer C, Thomas E, Atkins JR, Smith-Byrne K, Cortez Cardoso Penha R, Carreira C, Chopard P, Gaborieau V, Keski-Rahkonen P, Jones D, Teague JW, Ferlicot S, Asgari M, Sangkhathat S, Attawettayanon W, Świątkowska B, Jarmalaite S, Sabaliauskaite R, Shibata T, Fukagawa A, Mates D, Jinga V, Rascu S, Mijuskovic M, Savic S, Milosavljevic S, Bartlett JMS, Albert M, Phouthavongsy L, Ashton-Prolla P, Botton MR, Silva Neto B, Bezerra SM, Curado MP, Zequi SC, Reis RM, Faria EF, de Menezes NS, Ferrari RS, Banks RE, Vasudev NS, Zaridze D, Mukeriya A, Shangina O, Matveev V, Foretova L, Navratilova M, Holcatova I, Hornakova A, Janout V, Purdue MP, Rothman N, Chanock SJ, Ueland PM, Johansson M, McKay J, Scelo G, Chanudet E, Humphreys L, de Carvalho AC, Perdomo S, Alexandrov LB, Stratton MR, Brennan P (2024). Geographic variation of mutagenic exposures in kidney cancer genomes. *Nature* 629, 910-918.
- Bergstrom EN, Abbasi A, Díaz-Gay M, Galland L, Ladoire S, Lippman SM, Alexandrov LB (2024). Deep learning artificial intelligence predicts homologous recombination deficiency and platinum response from histologic slides. *J Clin Oncol* 42, 3550-3560.
- Herrera-Pariente C, Bonjoch L, Muñoz J, Fernàndez G, Soares de Lima Y, Mahmood R, Cuatrecasas M, Ocaña T, Lopez-Prades S, Llargués-Sistac G, Domínguez-Rovira X, Llach J, Luzko I, Díaz-Gay M, Lazaro C, Brunet J, Castillo-Manzano C, García-González MA, Lanas A, Carrillo M, Hernández San Gil R, Quintero E, Sala N, Llort G, Aguilera L, Carot L, Diez-Redondo P, Jover R, Ramon Y Cajal T, Cubiella J, Castells A, Balaguer F, Bujanda L, Castellví-Bel S, Moreira L (2024). CTNND1 is involved in germline predisposition to early-onset gastric cancer by affecting cell-to-cell interactions. *Gastric Cancer* 27, 747-759.
- Fixman B, Díaz-Gay M, Qiu C, Margaryan T, Lee B, Chen XS (2024). Validation of the APOBEC3A-mediated RNA single base substitution signature and proposal of novel APOBEC1, APOBEC3B, and APOBEC3G RNA signatures. *J Mol Biol* 436, 168854.

SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas
Unit Head

Technician
Clara M. Santiveri (TS)*

Titulado Superior (Advanced Degree)

OVERVIEW

This Unit focuses on the technical and scientific management of Nuclear Magnetic Resonance (NMR) spectroscopy and molecular biophysics instrumentation available at the Structural Biology Programme. It provides CNIO researchers with equipment and experimental support for biophysical techniques in studies of molecules involved in cancer. This includes: the *in vitro* characterisation of the structure and dynamics of proteins by NMR; and the characterisation of the affinity and kinetics of protein interactions with other biopolymers and small molecules of physiological relevance, or that could represent initial hits in drug discovery, and research compounds for biophysical and functional studies. Furthermore, we use NMR to screen libraries of fluorinated fragments against macromolecular targets, and to characterise the metabolic profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples.

"In 2024, in collaboration with the Telomeres and Telomerase and the Epithelial Carcinogenesis Groups, we used NMR to characterise the metabolic impact of genetic alterations of telomere components and of antibiotic administration, respectively, on mice faecal water extracts, to complement the physiological and cancer-related phenotypic observations investigated by these groups."

RESEARCH HIGHLIGHTS

The Unit provides a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, a fluorimeter, a nanoDSF (differential scanning fluorimetry) device, isothermal titration and differential scanning calorimeters, a circular dichrograph, dynamic (DLS) and multi-angle static light scattering (MALS) equipments, two biosensor instruments: surface plasmon resonance (SPR) and biolayer interferometry (BLI), and a multiple-well microplate reader with numerous technologies. Research groups mostly from, but not limited to (i.e., the Experimental Oncology, Growth Factors, Nutrients and Cancer, and Melanoma Groups, as well as H12O-CNIO Cancer Immunotherapy and Haematological Malignancies Clinical Research Units) the Structural Biology Programme have used these technologies throughout the year.

The Unit hosts a 700 MHz NMR spectrometer that is equipped with probes and a sample changer to run up to 120 samples automatically. This provides medium throughput for screening small molecule protein binders (together with the Experimental Therapeutics Programme), as well as for metabolite quantification that this year was done in collaboration with the Cell Division and Cancer, Epithelial Carcinogenesis, Growth Factors, Nutrients and Cancer, Metabolism and Cell Signalling, Telomeres and Telomerase, and Transformation and Metastasis Groups (Molecular Oncology Programme). Collectively with our client groups, we will continue implementing sample preparation protocols and developing spectroscopic and analytical tools to characterise metabolites present in different biological samples. For example, in collaboration with the Epithelial Carcinogenesis Group, we characterised the impact of antibiotic treatment on the metabolic profiles of mice faecal water extracts. Most signals corresponding to products of bacterial metabolism, such as short chain fatty acids (see Figure 1), completely disappear upon antibiotic treatment. These observations allow not only to verify the extent and effect of the treatment, but also to identify bacterial metabolites that are potentially required for the development of inflammation and cancer. ■

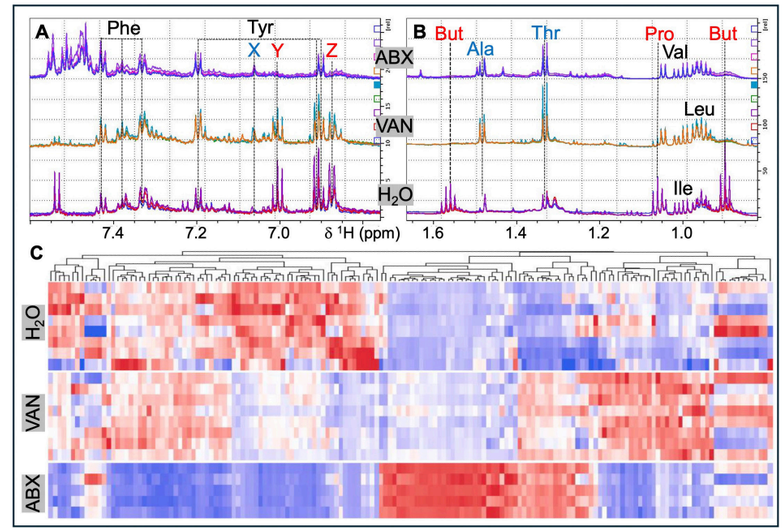


FIGURE 1 NMR profiling of the metabolic changes in mice faecal extracts upon antibiotic treatment. Superposition of (**A**) aromatic and (**B**) aliphatic regions of three representative spectra corresponding to untreated (H_2O), Vancomycin (VAN), and antibiotic-cocktail (ABX) treated samples. Illustrative signals increased in the H_2O group are labeled in red (butyrate and propionate), increased upon treatments in blue, and not significantly affected in black; unassigned signals are labelled X, Y, Z. (**C**) Heatmap of the relative intensity variations in the 161 most intense NMR signals after clustering (upper dendrogram). For each NMR signal (columns), dark red and blue correspond to twofold and half values with respect to the average value of all samples (rows).

PUBLICATION

- Márquez-Moñino MÁ, Santiveri CM, de León P, Camero S, Campos-Olivas R, Jiménez MÁ, Sáiz M, González B, Pérez-Cañadillas JM (2024). The ALS drug riluzole binds to the C-terminal domain of SARS-CoV-2 nucleocapsid protein and has antiviral activity. *Structure*. PMID: 39541975.

BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Research Scientist
Michael Tress

Post-Doctoral Fellows
Dido Carrero, Óscar Lapuente

Graduate Students
Mohmed Abdalfttah (since Sept), María González (since July), María José Jiménez (until Oct), José Manuel Lamas (since Sept), Miguel Maquedano

Bioinformaticians (TS) *
Ruth Álvarez, Daniel Cerdán, Elias Miguel Da Costa (since April), Tomas Di Domenico, Gonzalo Gómez, Pablo González, Elena Piñeiro, Laura Serrano, Francisco J Soriano, Sara Vidal (since June), Francisco J Villena (since Dec)

'Titulado Superior (Advanced Degree)

Master's Students
Irene Benito (May-Dec) (Master's Prog. in Bioinformatics, ENS-ISCIII, Madrid, Spain), María González (until April) (Master's Thesis, UAM, Madrid, Spain), José M Lamas (Feb-Sept) (Master's Thesis, UPM, Madrid, Spain), Francisco J Villena (May-Dec) (Master's Prog. in Bioinformatics, ENS-ISCIII, Madrid, Spain)

Visiting Scientists
David Cáceres (until March) (Hosp. 12 de Octubre, Madrid, Spain), Guillermo M Sánchez-Cid (since March) (Fund. Parque Científico de Madrid, Spain)

OVERVIEW

Bioinformatics is essential for understanding cancer genomics and shaping future cancer therapies. Bioinformatics approaches can transform vast quantities of biological data into comprehensive models, providing insights into cancer and the genotype-phenotype relationships crucial for identifying cancer-driving alterations and therapeutic targets.

The CNIO Bioinformatics Unit (BU; https://bioinformatics.cnio.es/) is a multidisciplinary team that uses computational methods to solve scientific questions. BU's objectives include developing computational tools for integrating biological and clinical data, analysing cancer genomes to identify biomarkers and drug response mechanisms, providing bioinformatics support for data analysis, maintaining CNIO's scientific computing facilities, and offering training in bioinformatics methodologies.

"In 2024, we introduced spatial Beyondcell to analyse therapeutic profiles of tumours at a spatial level, enabling insights into tumour biology and improving our understanding of treatment responses."

BU is an active node of ELIXIR (https://elixir-europe.org/), leading the Cancer Data Focus Group to enhance cancer genome analysis across Europe.

RESEARCH HIGHLIGHTS

This year, we published over 10 peer-reviewed articles as a result of our ongoing research projects and scientific collaborations. The full list of our activities is available on our website: https://bioinformatics.cnio.es/. In 2024, we provided a framework and expertise for the systematic analysis and interpretation of cancer genomes (Nikolski *et al.* 2024) and collaborated to establish the 1+Million Genomes Minimal Dataset for Cancer (Riba *et al.* 2024). Additionally, our Beyondcell method was applied to spatial transcriptomics data to predict drug sensitivity in breast cancer patients (Jiménez-Santos *et al.* 2024). We also launched a line of research focused on analysing long-read sequencing data, from Oxford Nanopore Technologies, to enhance cancer genomics and epigenomics studies, and initiated pilot projects on telomere variations and drug resistance in multiple myeloma. In addition, we upgraded the CNIO HPC cluster with three GPUs to support AI-based analysis tools.

The BU has also been actively involved in the European network ELIXIR (https://www.elixir-europe.org/), leading the ELIXIR Cancer Data Focus Group. BU also participates in and co-coordinates the Spanish National Network on Brain Metastases (RENACER; https://renacerbrainmet.org/) and the ISCIII IMPaCT-Data project (https://impact-data.bsc.es/). We co-lead working packages (WPs) 3 and 5 of the IMPaCT-VUSCan project, aimed at studying genetic variants that influence hereditary cancer predisposition. Also, we co-lead WP5 of the EOSC4Cancer project (https://eosc4cancer.eu/), which aims to create a federated, interoperable system for accessing and analysing cancer genomes across Europe, involving cancer research centres, hospitals, and supercomputing centres. Finally, our training and knowledge-transfer activities include co-directing with the Barcelona Supercomputing Center (BSC) the MSc programme from ENS-ISCIII: "*Bioinformática y Ciencia de Datos en Medicina Personalizada de Precisión y Salud*" (https://masterbioinformatica.com/). ■

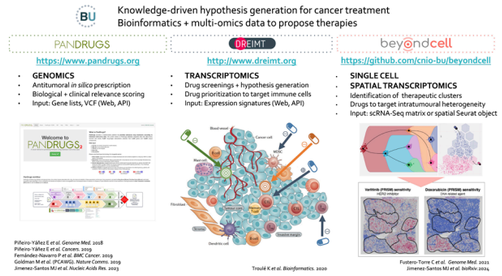


FIGURE 1 Overview of the CNIO Bioinformatics Unit's (BU) research focus. The BU generates knowledge-driven hypotheses for cancer treatment by integrating bioinformatics approaches with multi-omics data, proposing personalised therapies to optimise treatment strategies.

SELECTED PUBLICATIONS*

- Riba M *et al.* (incl. Al-Shahrour F) (2024). The 1+million genomes minimal dataset for cancer. *Nat Genet* 56, 733-736.
- Chaib S *et al.* (incl. Llanos S, Al-Shahrour F) (2024). The efficacy of chemotherapy is limited by intratumoral senescent cells expressing PD-L2. *Nat Cancer* 5, 448-462.
- Nikolski M, Hovig E, Al-Shahrour F, Blomberg N, Scollen S, Valencia A, Saunders G (2024). Roadmap for a European cancer data management and precision medicine infrastructure. *Nat Cancer* 5, 367-372.
- López-Gil JC *et al.* (incl. García-Silva S, Gómez-López G, Guerra C) (2024). The Peptidoglycan Recognition Protein 1 confers immune evasive properties on pancreatic cancer stem cells. *Gut* 73, 1489-1508.
- Rodriguez JM, Abascal F, Cerdán-Vélez D, Gómez LM, Vázquez J, Tress ML (2024). Evidence for widespread translation of 5' untranslated regions. *Nucleic Acids Res* 52, 8112-8126.
- Fernández-García F, Fernández-Rodríguez A, Fustero-Torre C, Piñeiro-Yáñez E, Wang H, Lechuga CG, Callejas S, Álvarez R, López-García A, Esteban-Burgos L, Salmón M, San Román M, Guerra C, Ambrogio C, Drosten M, Santamaría D, Al-Shahrour F, Dopazo A, Barbacid M, Musteanu M (2024). Type I interferon signaling pathway enhances immune-checkpoint inhibition in KRAS mutant lung tumors. *Proc Natl Acad Sci U S A* 121, e2402913121.
- Monteagudo M, Calsina B, Salazar-Hidalgo ME, Martínez-Montes ÁM, Piñeiro-Yáñez E, Caleiras E, Martín MC, Rodríguez-Perales S, Letón R, Gil E, Buffet A, Burnichon N, Fernández-Sanromán Á, Díaz-Talavera A, Mellid S, Arroba E, Reglero C, Martínez-Puente N, Roncador G, ... Rodriguez-Antona C, Currás-Freixes M, Al-Shahrour F, Cascón A, Leandro-García LJ, Montero-Conde C, Robledo M (2024). MAML3-fusions modulate vascular and immune tumour microenvironment and confer high metastatic risk in pheochromocytoma and paraganglioma. *Best Pract Res Clin Endocrinol Metab* 38, 101931.
- Jiménez-Santos MJ, García-Martín S, Rubio-Fernández M, Gómez-López G, Al-Shahrour F (2024). Spatial transcriptomics in breast cancer reveals tumour microenvironment-driven drug responses and clonal therapeutic heterogeneity. *NAR Cancer* 6, zcae046.

*please see BU´s web site for a list of all publications.

AWARDS AND RECOGNITION

Fátima Al-Shahrour:
- Scientific Advisory Board Member, the European Bioinformatics Institute (EMBL-EBI).
- Evaluation Panel Member, Institución Catalana de Investigación y Estudios Avanzados (ICREA), Spain.

ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Post-Doctoral Fellow
Johanne Le Coq

OVERVIEW

The principal mission of the Electron Microscopy (EM) Unit is to provide scientific and technical support to researchers, helping them address their scientific questions through various transmission electron microscopy techniques. We focus on sample preparation for cryo-electron microscopy (cryoEM) and negative staining methods. Our activity also includes data collection and assistance with image processing, such as 2D analysis and 3D reconstruction. We guide researchers in selecting the most appropriate EM techniques and assist with sample preparation. Additionally, we produce our own EM grids to ensure better quality control and reduce costs. Comprehensive training on the operation of our microscopes and auxiliary equipment is also available. More advanced structural studies are typically conducted through research collaborations.

"In the Electron Microscopy Unit, our main effort is to ensure efficient access to all of our infrastructure. We provide essential training for the use of our microscopes and auxiliary equipment, enabling researchers to make full use of our facilities."

RESEARCH HIGHLIGHTS

We have provided research support to several groups at the CNIO. In collaboration with the Growth Factors, Nutrients, and Cancer Group, we optimised cryo-EM grids and collected data for structural studies of the URI complex. In addition, with the same Group, we are studying the structural integrity of bile ducts in their mutant mice. In collaboration with the Metabolism and Cell Signalling Group, we are studying potential mitochondrial alterations in macrophages derived from their animal models. We are also collaborating with the Transformation and Metastasis Group on their project, investigating the role of RANK/RANKL signalling in metabolism related to breast cancer.

We have maintained close collaborations with all the groups in the Structural Biology Programme, providing support for single-particle EM grid preparation, cryo-EM grid screening, data collection, and 2D and 3D analysis of different samples. Notably, we contributed to several projects led by the Macromolecular Complexes in DNA Damage Response Group involving EM grid preparation, data collection, and analysis for studies on the RNA helicase DDX11, the RuvBL complex from *Arabidopsis thaliana* (in collaboration with D. Alabadí from Universitat Politècnica de València), and lncRNA (in collaboration with M. Huarte from CIMA, Universidad de Navarra). We also supported work on heteromeric amino acid transporters in collaboration with M. Palacín and J. Fort from the IRB Barcelona.

In collaboration with the Genome Integrity and Structural Biology Group, we carried out cryo-EM grid screening and data collection of numerous samples. With the Kinases, Protein Phosphorylation and Cancer Group, we optimised EM grids, collected data, and processed the PTC1 Kinase, while also preparing and imaging EM grids for the KIF5B-RET kinesin sample.

Outside CNIO, we are collaborating with E. Lara (CNIC) to study mitochondrial structure in brown adipose tissue of KO CnAbeta1 mice. ■

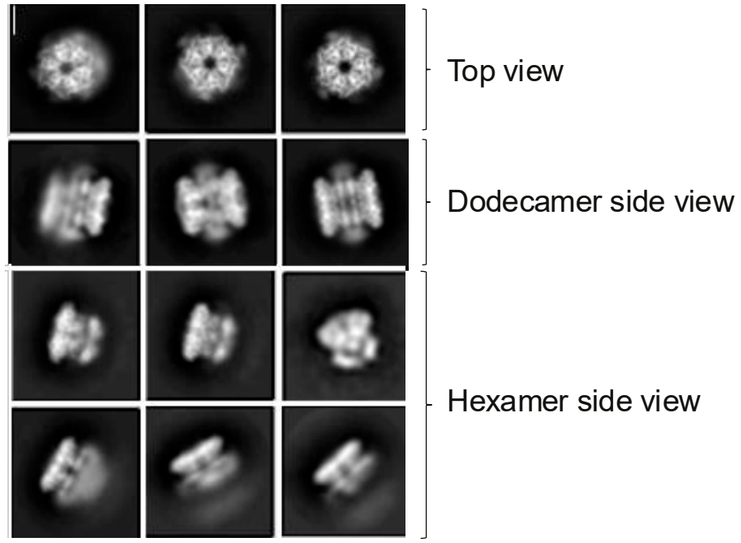


FIGURE 1 Reference-free 2D class averages of human RUVBL1/2 in the presence of ATP revealed different oligomerisation states and conformational heterogeneity within the complex.

PUBLICATION

García-Martín C, López-Perrote A, Boskovic J, Llorca O (2024). Mechanism of allosteric inhibition of RUVBL1-RUVBL2 ATPase by the small molecule CB-6644. *Cell Rep Phys Sci* 5, 101982.

PROTEIN CRYSTALLOGRAPHY UNIT

Inés Muñoz
Unit Head

OVERVIEW

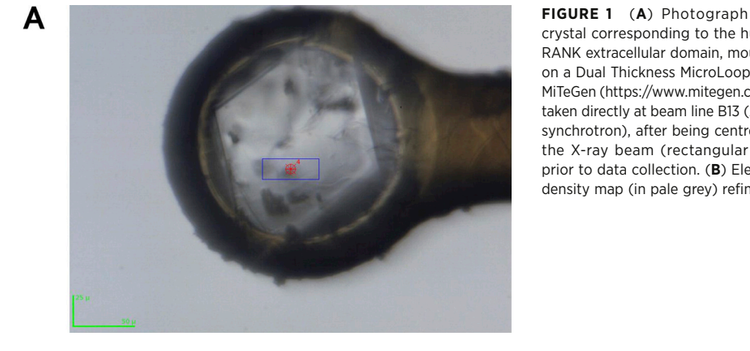
The Protein Crystallography Unit is a core facility that provides specialised services focused on the structural investigation of biological systems at various levels of resolution and length scales. This is executed through a series of techniques, using synchrotron radiation, ranging from the identification of changes occurring in tissues, cells, and organelles (Fourier Transform Infrared spectroscopy, cryo-soft x-ray tomography, and phase-contrast X-ray imaging) to the characterisation of proteins and macromolecular complexes in-solution by SAXS (Small Angle X-ray Scattering), or at atomic resolution by X-ray crystallography.

"Detailed imaging of the relationship between structure and function of tissues, cells, organelles, macromolecules at various levels, is fundamental to life science and pharmaceutical projects."

RESEARCH HIGHLIGHTS

The Unit was engaged in several internal collaborations with other CNIO groups (the Transformation and Metastasis Group; Kinases, Protein Phosphorylation and Cancer Group; and the H12O-CNIO Cancer Immunotherapy Clinical Research Unit), providing them with recombinant proteins that were used for protein crystallography and SAXS analysis and, in some cases, for other biophysical, biochemical, cell-based functional assays and cryoEM studies. In addition, as in previous years, consultation and support were given to CNIO groups in need of our expertise in protein production, protein crystallisation and crystallography, as well as structural analysis and drug-discovery projects.

Throughout 2024, the Unit also continued working on its own scientific projects and maintained collaborations with various external groups in Spain at the Department of Crystallography and Structural Biology (IQF-CSIC, Madrid), and the Pharmacology and Therapeutics Department at Roswell Park Cancer Institute (Buffalo, USA). ■



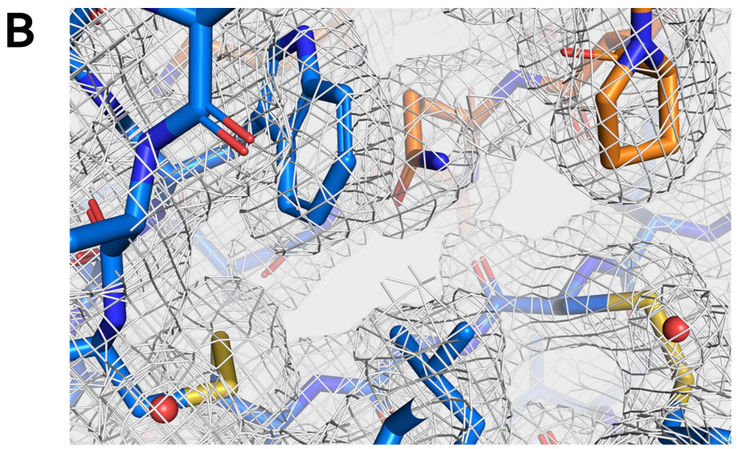


FIGURE 1 (**A**) Photograph of a crystal corresponding to the human RANK extracellular domain, mounted on a Dual Thickness MicroLoop from MiTeGen (https://www.mitegen.com/), taken directly at beam line B13 (ALBA synchrotron), after being centred on the X-ray beam (rectangular box) prior to data collection. (**B**) Electron density map (in pale grey) refined to 2.0 Å resolution (1 sigma contour) for the first reported crystal structure of the human RANK extracellular dimerization domain solved at CNIO by the Protein Crystallography Unit. The protein representation was done in stick, where each chain was coloured in steel blue and orange, respectively.

PUBLICATIONS

- Lama R, Wu W, Mavis CK, Ruiz FM, Querol-García J, Martin D, Chemler SR, Chandra D, Goodrich DW, Hernandez-Ilizaliturri FJ, Muñoz IG, Wang X (2024). Small-molecule MMRi36 induces apoptosis in p53-mutant lymphomas by targeting MDM2/MDM4/XIAP for degradation. *Front Oncol* 14, 1462231.
- Lama R, Fose JM, Martín D, Muñoz IG, Wang ES, Sung PJ, Chemler SR, Wang X (2024). Novel inhibitors for MDM2-MDM4 E3 ligase potently induce p53-indepedent apoptosis in drug-resistant leukemic cells. *Molecules*. PMID: 39795242.

PROTEIN PRODUCTION UNIT

Jorge L. Martínez-Torrecuadrada
Unit Head

Technicians
María Rivas (TS)*, David Sánchez (since March) (PEJ, CAM)**

Titulado Superior (Advanced Degree)
**Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

OVERVIEW

The roles of numerous proteins, particularly those linked to cancer, remain largely unexplored. Advancing biomedical research requires mapping these proteins' structures, understanding where and how they are distributed within tissues and cells, and uncovering their specific functions. However, a major obstacle is the difficulty of producing proteins of interest in sufficient quality and quantity for thorough analysis, which slows down research.

"The Protein Production Unit handles all steps of protein expression, from cloning and optimising to purification, providing high-quality, soluble proteins to accelerate biochemical research."

The Protein Production Unit was created to tackle this challenge. This core lab provides expertise and advanced technology to develop streamlined methods for producing high quality recombinant proteins. These proteins serve as essential resources for a range of downstream applications such as the production of highly specific antibodies, detailed biophysical and biochemical analyses, functional studies, and structural investigations. Ultimately, this Unit aims to accelerate cutting-edge cancer research at CNIO and support external groups.

RESEARCH HIGHLIGHTS

Over the past year, the Protein Production Unit successfully completed more than 25 protein production projects for CNIO groups and external collaborators. These projects focused on identifying optimal expression conditions and purifying target proteins for applications such as antibody production, functional assays, and biophysical characterisation. Notably, in collaboration with the Melanoma Group, we continued producing active human Midkine for drug development, and expressed several recombinant full-length monoclonal antibodies and scFv antibody fragments for further characterisation and therapeutic purposes.

The Unit also produced various antibody formats, including bispecific T cell engagers (BiTEs) and IgG-like full-size bispecific antibodies, for the H12O-CNIO Cancer Immunotherapy Clinical Research Unit. Other successful protein productions included TREM1 and B7H3 (CD276), in collaboration with the Cancer Immunity Group and the IdiPAZ-CNIO Pediatric Onco-Hematology Clinical Research Unit, respectively, for generating highly specific monoclonal antibodies, and GapR in partnership with the Topology and DNA Breaks Group for functional characterisation.

Our collaborations extended to external groups such as Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CiMUS) in Santiago de Compostela, Spain; Institut de Recerca Germans Trias i Pujol (IGTP) in Badalona, Spain; Instituto de Investigación del Hospital de la Princesa in Madrid, Spain; and Institut de Química Avançada de Catalunya (IQAC) in Barcelona, Spain, among others.

In addition to these projects, the Unit pursued innovative research to develop new nanobody-based theragnostic tools. These tools, radiolabelled with gallium-68 for PET imaging and lutetium-177 for therapy, were tested in various cancer mouse models in collaboration with the CNIO Molecular Imaging Unit and *Centro de Investigación del Cancer* (CIC) in Salamanca, Spain. ■

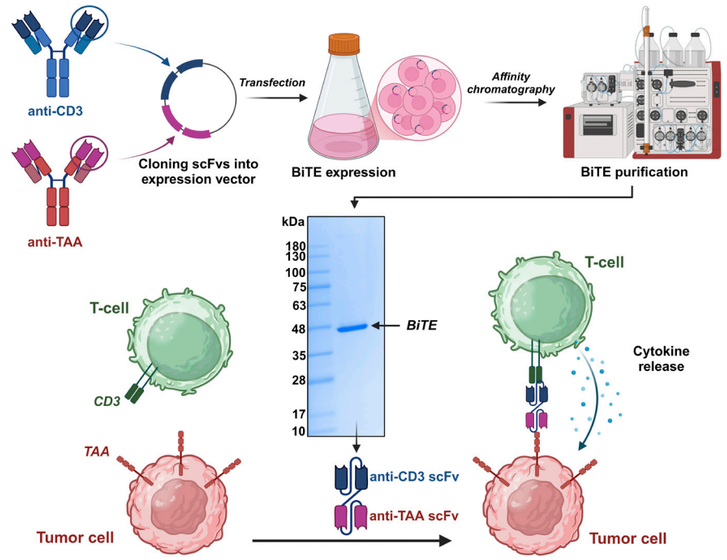


FIGURE 1 Schematic representation of bispecific T-cell engager (BiTE) production in mammalian cells, consisting of two single-chain variable fragments (scFvs): one targeting a tumour-associated antigen (TAA) and the other targeting CD3 on T cells. The mechanism of action is illustrated below.

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AWARDS AND RECOGNITION

- 'The Bill Eckelman Award for the best Nuclear Medicine and Biology Paper 2024', Elsevier, The Netherlands: *Target engagement of an anti-MT1-MMP antibody for triple-negative breast cancer PET imaging and beta therapy*.

Patient-Oriented Research

HUMAN CANCER GENETICS PROGRAMME

The Human Cancer Genetics Programme (HCGP) is a translational research programme focusing on genetics, genomics, pharmacogenetics, molecular cytogenetics, and the environmental bases of human cancer.

The Programme's interests focus on (1) identifying new genetic and non-genetic factors related to cancer predisposition and risk, to guide risk-stratified screening strategies for personalised cancer prevention and treatment, and (2) understanding the molecular mechanisms involved in cancer to facilitate the development of targeted therapies and early diagnostics.

In 2024, the HCGP consisted of two Research Groups: Hereditary Endocrine Cancer (HECG) and Genetic and Molecular Epidemiology (GMEG); along with three Units: Human Genotyping-CEGEN, Molecular Cytogenetics, and the Familial Cancer Clinical Unit (FCCU). Additionally, the Programme includes a Familial Cancer Consultancy at the Hospital Universitario de Fuenlabrada to evaluate families affected by cancer and provide genetic counselling.

The Programme collaborates closely with the clinical community to foster cooperation in genetic diagnosis and research and to promote training and education. In 2024, there were 1204 visits at the Familial Cancer Consultancy (630 new cases and 574 patients receiving results), and the HCGP performed 2098 genetic diagnoses (436 HECG and 1662 FCCU) and carried out 1686 cytogenetic studies. Regarding research, the Programme's members engage in collaborative studies involving a network of more than 33 hospitals from our National Health System and medical/research societies to conduct collaborative studies and facilitate clinical translation. The Programme additionally provides professionals and students from different national and international research centres with opportunities to join, either as visitors or for training visits consisting of short-term stays of 1 to 3 months. Five national and three international visitors, along with six medical residents from Spanish hospitals were hosted in 2024. In terms of education, two vocational training, one Bachelor's, three Master's, and 11 PhD students worked on their research projects.

The Programme has established research collaborations with national and international groups; this is clearly demonstrated by the co-authorships of its publications and the key roles held by several of the Programme's members in consortia and international projects. In 2024, for example, GMEG members, led by Núria Malats, in collaboration with C. Van Eijck from Erasmus Medical Centre, Rotterdam, conducted a study published in *GUT* that demonstrated pancreatic cancer in women is more sensitive to gemcitabine-based neoadjuvant chemoradiotherapy, resulting in longer survival after resection compared to men.

Especially noteworthy is the contribution to IMPaCT of several group members of the HCGP, an initiative of the Instituto de Salud Carlos III to promote Precision Medicine in our country.

Milestones and major achievements of the HCGP in 2024 include the following:

- → Núria Malats was awarded with the project "Implementation of the IA-PMPD algorithm for predicting pancreatic cancer metastasis in tertiary hospitals and demonstration of its real-time performance (AI-PMPD.v2)". PI: N. Malats [*Programa de IA Excelente 2024*, Ministerio para la Transformación Digital y de la Fundación Pública, co-funded by 'NextGenerationEU'].
- → GMEG contributed to the bladder cancer field through an EU-based collaborative study to identify an integrated genomic signature of early-stage bladder cancer.
- → Mercedes Robledo was appointed Focus Area Lead (Basic) for ESE's Adrenal and Cardiovascular Endocrinology Focus Area, the European Society of Endocrinology (ESE).
- → Mercedes Robledo leads the NMG9 National Mirror Group focused on the Cancer Use Case of the 1+MG initiative.
- → Mercedes Robledo led an international study that allowed us to identify MAML3 pheochromocytoma-specific vulnerabilities beyond Wnt pathway dysregulation, such as a rich vascular network, and overexpression of PD-L1 and CD40, suggesting potential therapeutic targets (work published in *Best Pract Res Clin Endocrinol Metab*).
- → Mercedes Robledo participated in two clinical consensuses on diagnosis and management of pheochromocytoma and paraganglioma in children and adolescents (*Nat Rev Endocrinol*) and of patients with high risk of developing metastases (*Nat Rev Endocrinol*).
- → Sandra Rodríguez-Perales participated in the Phase 1/2 FANCOLEN-1 trial, which demonstrated promising outcomes for treating Fanconi anaemia-A without genotoxic conditioning (work published in *The Lancet*).
- → The Human Genotyping Unit is participating in the Genome of Europe (GoE) Project in collaboration with the ISCIII, CIBER and CNAG. The GoE project (https://b1mg-project.eu/1mg/genome-europe) aims to establish the European reference genome database, a vital resource for the advancement of public health. The Unit will be responsible for DNA extraction from participants in the Spanish IMPaCT cohort initiative (https://cohorte-impact.es/) and for whole genome sequencing together with CNAG, supporting Spain's commitment to the 1+ Million Genomes (1+MG) initiative.

One of the CNIO's main strategic initiatives at present is the consolidation of a new Cancer Genomics Programme. HCGP groups will become part of the new Programme. We are confident that, under this new strategic action, the HCGP Groups and Units will continue to grow and further develop research in cancer genomics. ■

HEREDITARY ENDOCRINE CANCER GROUP

Mercedes Robledo
Group Leader

Research Scientists
Alberto Cascón, Luis Javier Leandro

Post-Doctoral Fellows
Ester Arroba, Alberto Díaz (CIBERER, Madrid), Cristina Montero, Clara Reglero (until August)

Graduate Students
Javier de Nicolás, Sara Mellid (until October), Carlos Valdivia, Natalia Martínez (CIBERER, Madrid)

Technicians
Alicia Arenas (until July) (TS)*, Mariolga Berrizbeitia (TS)*, Rocío León, Gabriela Roberta Radu (PEJ, CAM)** (until October), Milton E. Salazar (since April) (TS)*

*_Titulado Superior_ (Advanced Degree)
** _Plan de Empleo Joven de la Comunidad de Madrid_ (Youth Employment Plan, Community of Madrid)

Visiting Scientist
Cristina Rodríguez (Instituto de Investigaciones Biomédicas Sols-Morreale (IIBM), Madrid, Spain)

OVERVIEW

Our Group is mainly interested in identifying genetic risk factors involved in endocrine tumour susceptibility. Through a comprehensive analysis of tumour genomic features, we have been able to propose diagnostic and prognostic markers to identify altered pathways that could be targeted with therapeutics, and to identify new major susceptibility genes.

We are also interested in defining markers associated with differences in anticancer drug response and toxicity. We are applying "omic" analyses to a large series of clinically well-characterised patients. The aim is to identify new therapeutic approaches to personalise cancer treatment. These efforts will collectively improve the diagnosis, prognosis, and treatment of patients.

"We uncovered a unique microenvironment with rich vasculature and a unique immune profile in MAML3-PPGLs, offering new opportunities for targeted therapies, and identified a new RCC (renal cell carcinoma) molecular subtype with clinical implications."

RESEARCH HIGHLIGHTS

MAML3-fusions modulate vascular and immune tumour microenvironment and confer high metastatic risk in pheochromocytoma and paraganglioma (PPGL)

PPGLs are rare neuroendocrine tumours (incidence 3-8:10^6). Around 20-25% of patients develop metastases, for which there is an urgent need of prognostic markers and therapeutic stratification strategies. The presence of *MAML3*-fusion is associated with increased metastatic risk, but neither the processes underlying disease progression nor the targetable vulnerabilities have been addressed. We have compiled a cohort of 779 patients with a fusion prevalence of 4%, which is the largest MAML3 series reported so far. *MAML3*-tumours showed an increased expression of neuroendocrine-to-mesenchymal transition markers, MYC-targets, and angiogenesis-related genes, ultimately leading to a differential tumour microenvironment with unique vascular and immune profiles. Importantly, our findings identified *MAML3* tumour-specific vulnerabilities, such as Wnt-pathway dysregulation, a rich vascular network, and overexpression of PD-L1 and CD40, which are suitable therapeutic targets. As PD-L1 overexpression is a characteristic of *MAML3* related PPGLs, targeting NK-DC axis activation in these tumours could be a promising strategy to improve anti-PD1/PD-L1 therapy effectiveness (Figure 1).

Expression of Hsa-miR-139-5p as a clinically feasible prognostic marker for differentiated thyroid cancer

Our previous research found that miR139-5p is significantly downregulated in aggressive thyroid tumours and affects the MAPK and PI3K pathways, crucial for differentiated thyroid cancer (DTC). MiR139-5p has since emerged as a promising prognostic marker, but its clinical use requires standardised quantification and cut-off values. To address this, we developed a qPCR-based method with a retrospective cohort of 60 patients, establishing cut-off points by standardising miR139-5p expression to normal thyroid tissue. We also optimised a chromogenic *in situ* hybridisation (ISH) assay for automated detection, in collaboration with the CNIO Histopathology Core Unit. ISH data correlated well with qPCR results, showing the lowest miR139-5p expression in areas with a high Ki-67 proliferation index, a known high-risk marker (Figure 2). Our study confirms miR139-5p as a reliable prognostic marker and offers a practical tool for its evaluation to enhance risk assessment in DTC patients.

Identification of a novel papillary renal cell carcinoma (pRCC) molecular subtype characterised by HIF-pathway overactivation

pRCC is a molecularly heterogeneous form of renal cancer. Current therapy shows diverse efficacy, stressing the potential for molecular-based treatment selection. By merging and analysing the multi-omic characteristics of two pRCC cohorts (TCGA-KIRP and a Spanish series; total of 281 cases), we identified a subset of pRCC tumours with hypoxia-inducible factors (HIF) transcriptional activity. This "HIF-active" group was characterised by low expression of Krebs cycle genes and mitochondrial activity, a pro-angiogenic tumour microenvironment (TME), and high immune infiltration. Clinically, "HIF-active" cases exhibited higher tumour stage and worse overall survival. The accumulation of L-2-hydroxyglutarate, an oncometabolite able to inhibit HIF prolyl hydroxylases and prevent the degradation of HIFα, was identified as the mechanism leading to pseudohypoxia. The discovery of this new molecular subgroup of aggressive pRCC tumours offers novel insights for potential treatments and enhances therapeutic precision. ■

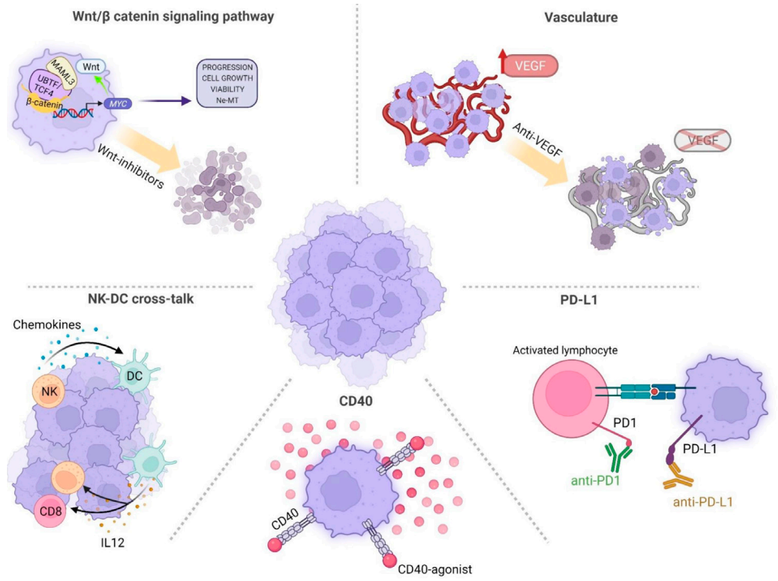

FIGURE 1 MAML3-tumour vulnerabilities uncovered in this study, susceptible for clinical implementation.

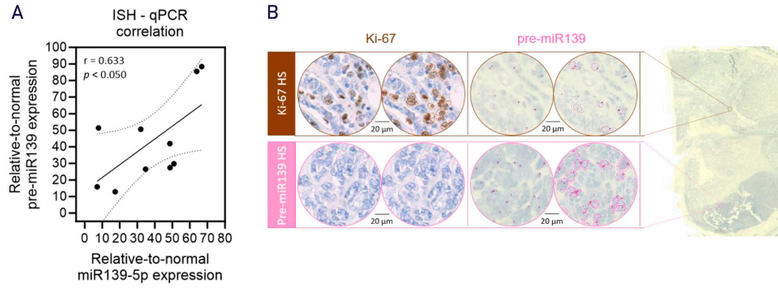

FIGURE 2 Analysis of miR139 expression by ISH in DTC sections replicates qPCR results and inversely correlates with the Ki-67 proliferation index. (**A**) Pearson correlation between miR139-5p values from qPCR (X-axis) and ISH (Y-axis) (n=10), with the regression line and 95% confidence interval (dotted lines). (**B**) IHC and ISH images of Ki-67 (left) and pre-miR139 (right) from hotspots of one DTC. Raw images (left) and classifier-processed images (right) are shown. The far right displays the full section and hotspot locations.

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AWARDS AND RECOGNITION

Mercedes Robledo was elected as:

- Co-leader of one of the National Mirror Groups of the 1+MG initiative, the European '1+ Million Genomes' Initiative.
- Basic Science Focus Area Lead for the Adrenal and Cardiovascular Endocrinology Focus Area, the European Society of Endocrinology.

GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

Núria Malats
Group Leader

Research Scientist
M. Evangelina López de Maturana

Post-Doctoral Fellows
Carlos Castilla (*CIBERONC*), Brune de Dreuille, Sarah Klauß (since Aug., TUM), Pablo Villoslada-Blanco

Graduate Students
Mireia Andueza (since Feb.), Jiangchuan He, Francisco J Jurado, Alberto Langtry (until Sept.), Victor M. Sobrino (*UPM*), Nannan Xue (China Scholarship Council)

Technicians
Lola Alonso (TS)˚, Raquel Benítez (TS)˚(Project Manager), Ekaterina Demidova (since Aug.), Lidia Estudillo, Lucas Friedman (TS)˚, Ane Moreno (TS)˚ (*CIBERONC*), María Olano (TS)˚, Laura Paniagua, Sergio Sabroso (TS)˚

˚*Titulado Superior* (Advanced Degree)

Master's Students
Tamara Said El Artah (May-Dec.) (ISCIII-ENS, Madrid, Spain), Adrián Santiso (since Sept.) (UAM, Madrid, Spain)

Student in Practice
Alicia Herráez (March-July) (Bachelor's Degree Final Project, UPM, Madrid, Spain)

Visiting Scientists
Patricia Aymerich (Erasmus University Rotterdam, the Netherlands), Isabel A. Martín (Univ. CEU San Pablo, Madrid, Spain), Casper Van Eijck (Erasmus University Rotterdam, the Netherlands)

OVERVIEW

Research in the Genetic and Molecular Epidemiology Group (GMEG) focuses on bladder and pancreatic cancer. We aim to identify aetiological agents and genetic pathways and apply findings in clinical and public health settings.

We utilise various biomarkers/omics and image-derived data to characterise better exposures, patterns of genetic susceptibility, and cancer outcomes. While multimodal data generation presents a unique opportunity in this context, integrating it poses significant challenges. GMEG explores methodological issues in integrative epidemiologic studies by applying artificial intelligence (AI).

"Multimodal data integration, which encompasses epidemiological, clinical, omics, and image-derived data, enhances the performance of algorithms that predict pancreatic cancer survival."

The strategic goals of the Group are to:

- → Identify non-genetic and genetic factors and their interactions associated with cancer development and progression and with its molecular/omics subphenotypes.
- → Develop and apply AI/statistical/informatics tools to model the risk and course of cancer patients by integrating epidemiological, clinical, omics, and image-derived information.
- → Evaluate clinical and public health interventions for cancer control by using newly developed biomarkers and algorithms.

RESEARCH HIGHLIGHTS

Research findings

In 2024, GMEG initiated fieldwork for new European-funded **pancreatic cancer** (PC) projects, including PANCAID and GUIDE.MRD, in collaboration with various hospitals in Madrid. As part of the European-funded PANCAIM project, we conducted preliminary multimodal data integrative analyses to predict the overall survival of PC. We observed that pathomics was the data modality that accounted for the most variation in the overall survival trait (Figure 1). Continuing the atopy-pancreatic cancer research stream, we concluded our analyses of IgE and eosinophils highlighting that a low type-2 immune response mediates the protective effect of atopy on pancreatic cancer (Figure 2). We also completed a Mendelian randomisation analysis concluding that atopic conditions are not causally linked to a decreased risk of pancreatic cancer. GMEG has begun fieldwork on the PanGen-ASTHMA study to thoroughly investigate the mechanisms underlying the protective effect of atopy on pancreatic cancer risk. To further explore the immune-related factors associated with PC risk and prognosis, we completed two analyses on the impact of genetic variation in HLA-I/II and the complement system on PC risk and survival. We also advanced our investigation into the microbiome (oral and gut) and metabolome (serum, urine, and stool) associated with pancreatic cancer risk, noting significant correlations among these data layers. In addition, we developed and validated a consensus molecular classifier for pancreatic cancer (PDAConsensus) that encompasses both tumour and stroma RNA features. Finally, we developed, validated, and patented a deep learning model that accurately predicts local and distant metastases in patients diagnosed with pancreatic ductal adenocarcinoma, using contrast-enhanced computed tomography images of the primary tumour. On **bladder cancer** (BC), GMEG is collaborating with Ravid Straussman's Lab at WIS, Israel, to determine the role of the urine and tumour microbiome in BCG treatment response; with GUARD and seven hospitals in Spain to conduct a prospective study in high-risk BC; with David Berman at Queen's University, Ontario, on a pathomics study aimed at improving BC grading; and with the Epithelial Carcinogenesis Group at CNIO to identify biomarkers of immunotherapy in BC. We also concluded a study on BC risk factors and genetic susceptibility according to tumour molecular subtypes.

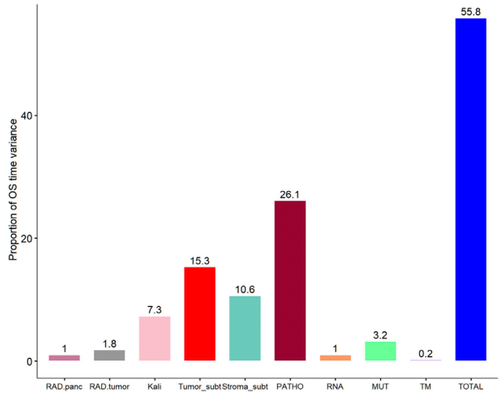
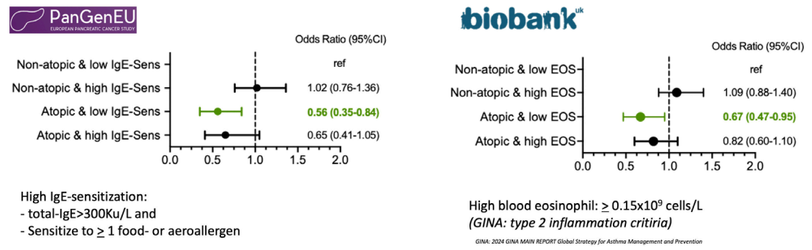


FIGURE 1 Percentage of the overall survival (OS) time variance explained by every multimodal layer included in the multimodal model: radiomics features of the normal pancreas and the tumour (RAD.panc and RAD.tumour), Kalimuthu classification (Kali), molecular classification of tumour and stroma (Tumour_sub and Stroma_sub, respectively), pathomics (PATHO), transcriptomics (RNA), DNA mutations (DNA), and microbiome (TM). TOTAL is the percentage of the OS time variance explained by all the layers together.

FIGURE 2 Atopic individuals with low levels of type-2 biomarker have reduced risk of PDAC.



Controlled covariates: sex, age, smoking status, alcohol consumption, medical centre, body mass index, diabetes status, autoimmune disease status, corticosteroid use, PDAC family history

Methodological contributions

During the past year, members of the GMEG have been involved in a review of methodological aspects of integrative studies on liquid biopsy and cancer, resulting in guidelines for future studies aimed at building multi-feature algorithms for various clinical aims. The Group has also entered the field of generative artificial intelligence (AI) by creating pancreatic cancer whole-section image tiles from RNA-seq data using the RNA-CDM model. We also explored how the proportion and distribution of missing data influence model performance, specifically the Youden Index. Our findings indicate that most diagnostic metrics decrease by 20–30% compared to models with complete data.

Translational activities

GMEG actively supports several clinical trials on immunotherapy in BC at the methodological level. We continue to sustain the Spanish Familial PC Registry (PanGen-FAM) and the European Registry of PC (PancreOS) under the umbrella of Pancreatic Cancer Europe (PCE). We chair the Spanish Alliance for Pancreatic Cancer Research (ALIPANC), to accelerate the translation of research results into the clinical and public health domains. We lead the Research Work Stream of the PCE multistakeholder platform, and are moving ahead on increasing PC awareness. We contributed to the European Alliance for Personalized Medicine (EAPM) series of expert interviews, to ascertain the current status of the uptake of advanced molecular diagnostics/NGS for quick and efficient genetic profiles of tumour cells across EU member states. ■

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Algorithm Registration

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MOLECULAR CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Research Scientist
Raúl Torres

Graduate Students
Alejandro Alonso, Alejandro Nieto, Pilar Puig

OVERVIEW

The Molecular Cytogenetics and Genome Editing Unit focuses on deciphering the impact of chromosomal rearrangements on cancer progression, and discovering novel therapeutic targets. Chromosomal rearrangements, structural changes in chromosomes, are frequent hallmarks of cancer. Using advanced CRISPR genome editing alongside cytogenetic techniques, the Unit develops models that replicate these genetic alterations observed in tumours. This approach aims to deepen our understanding of cancer mechanisms, potentially driving the creation of innovative treatments. Additionally, the Unit offers cutting-edge molecular cytogenetics and genome editing tools to CNIO researchers and external collaborators, facilitating diverse projects in clinical and basic research. With a commitment to technological advancement, the Unit integrates and develops new methodologies in the field.

"We use gene editing to model and target chromosomal and genetic alterations, advancing cancer insights and treatment tools. Our Unit provides cutting-edge cytogenetic and CRISPR technologies."

Technicians
M. Carmen Martín, Cristian Moreno, Francisco José Moya (TS)*

**Titulado Superior* (Advanced Degree)

Students in Practice
María Fernández (until May) (Univ. CEU San Pablo, Madrid, Spain), Alejandra Jaen (April-June) (Technicians in Training (FCT), IES José Luis Sampedro, Madrid, Spain), Mónica C. Rodríguez (Jan.-Sept.) (Master's Thesis, UAM, Madrid, Spain), Sophie L. Vuillemin (June-Sept.) (Master's Thesis, ETH Zúrich, Switzerland)

Visiting Scientist
Paula Díaz (until October) (*Instituto i+12*, Madrid, Spain)

RESEARCH HIGHLIGHTS

Despite significant progress in treatment, cancer remains a major cause of mortality worldwide, particularly due to aggressive, therapy-resistant types. Innovative approaches such as immunomodulatory therapies show promise by activating the immune system against cancer. However, these therapies often face obstacles as tumours can evade immune responses. New methods, such as inducing immunogenic cell death (ICD) via DNA damage, could initiate a sustained immune attack on tumours. Among these strategies, CRISPR shows great potential by allowing precise DNA targeting, which could amplify ICD, enhancing therapeutic efficacy while reducing side effects. Targeting oncogene amplifications (OAs) with CRISPR is especially promising, as these amplifications are prevalent in aggressive cancers and contribute to therapeutic resistance. We are focusing on neuroblastoma (NB), where high-risk cases often have *MYCN* amplification.

Our Unit is also advancing the use of Optical Genome Mapping (OGM), an innovative method that leverages ultra-long DNA molecule analysis for high-resolution, genome-wide assessment of cytogenetic variations. We are currently evaluating OGM, alongside traditional methods like karyotyping and FISH, across diverse tumour samples. OGM shows significant promise as a complementary approach for the detailed cytogenetic characterisation of cancer cells, offering a cost-effective alternative that can detect complex cytogenetic rearrangements not easily captured by standard techniques. This dual approach of Cas13-mediated fusion oncogene (FO) targeting, and OGM's comprehensive mapping capabilities, represents a pioneering advancement in cancer diagnostics and targeted therapy. ■

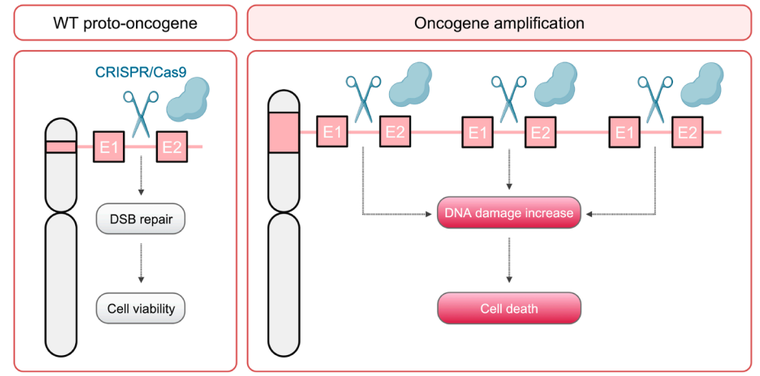

FIGURE 1 CRISPR/Cas9 selectively induces cell death in cells with oncogene amplification. The left panel illustrates the wild-type (WT) proto-oncogene configuration, where CRISPR/Cas9-induced DNA double-strand breaks (DSBs) are efficiently repaired, maintaining cell viability. The right panel demonstrates the scenario in oncogene-amplified cells, where a single sgRNA CRISPR/Cas9 induces multiple DSBs across several gene copies (E1, E2 and En regions). This accumulation of DNA damage exceeds the cell's repair capacity, leading to increased DNA damage and triggering cell death. This approach selectively targets amplified oncogenes while sparing non-amplified genes in normal cells.

PUBLICATIONS

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PATENT

- Malats N, Bork P, Kartal E, Molina E, Rodríguez S, Estudillo L, Real FX, Schmidt TSB, Zeller G, Wirbel J, Maistrenko OM. Faecal microbiota signature for pancreatic cancer. International Publication Number: *WO2023052486A1*. National Phase Entry (2024).

AWARDS AND RECOGNITION

- Board Member of SETGyC´s Executive Committee, The Spanish Society for Gene and Cell Therapy (SETGYC).

FAMILIAL CANCER CLINICAL UNIT

Maria Currás
Clinical Unit Head

Post-Doctoral Fellow
Bruna Calsina

Technicians
Alicia Barroso (since September), Ana I. Corao (until March), Verónica García (TS)*, Eduardo Gil, Miguel Á. Grillo, Fátima Mercadillo,

Pilar Redondo, Milton E. Salazar (until March) (TS)*

Titulado Superior (Advanced Degree)

Student in Practice
Alejandro de la Cruz (April-June) (Technicians in training (FCT), IES Rosa Chacel, Madrid, Spain)

OVERVIEW

Although 70% of cancers are sporadic, 5-10% are hereditary (HC, with a known genetic cause), and the remaining 15-20% are familial (FC, characterised by aggregation within a family, but without a known genetic cause). Our Unit focuses on the genetic diagnosis and translational research of HC and FC, which together represent 25-30% of all tumours.

Our activity is divided into two main areas:

1. **Genetic Diagnosis Service** - using Next-Generation Sequencing (NGS) on DNA obtained from peripheral blood and saliva, we provide diagnosis for HC and FC. Approximately 33% of our activity is dedicated to the Familial Cancer Clinic of the University Hospital of Fuenlabrada (FCC-UHF), while the remaining 67% supports other hospitals across Madrid, Castilla La Mancha, and the rest of Spain. In 2024, 61% of the genetic diagnoses were conducted in index cases, while 39% involved predictive testing in relatives, to guide early diagnosis and clinical surveillance at an asymptomatic stage. During this last year, 260 urgent studies were completed within six weeks to guide surgery and/or treatment decisions. In addition, up to 50 extensions of previous studies were carried out to include novel susceptibility genes described in recent years. Furthermore, we successfully established the *MLH1* promoter hypermethylation testing service and NGS on DNA from tumour samples (for deceased patients).

2. **Research.** We secured funding for the CRIGAMI project.

RESEARCH HIGHLIGHTS

Clinical and diagnostic activity. In 2024, 1104 patients were evaluated, and 1767 genetic studies were conducted at the Familial Cancer Clinical Unit (FCCU), reflecting a 6% increase in the number of clinical visits. A total of 462 germline pathogenic/likely pathogenic (P/LP) variants were detected. Identifying P and LP variants allow patients and their families to benefit from appropriate clinical follow-up, early tumour detection at an asymptomatic stage and/or targeted treatment.

Over the past year, we integrated into the Unit's diagnostic routine, the diagnosis of healthy individuals with deceased relatives affected by cancer using a novel 600 genes NGS panel. When tumour samples were not available, we performed the study in peripheral blood of the apparently healthy relatives of those genes that would change the clinical follow-up if altered, achieving genetic diagnosis in 11%. This has enabled these individuals to benefit specifically from prophylactic surgeries and/or more comprehensive clinical follow-up.

FCCU is a member of the technical committee dedicated to organising and improving the hereditary cancer care in the Community of Madrid. This initiative was launched following the publication of the common Catalogue of Genetic Tests by the National Health System in 2024, along with the establishment of the Medical Genetics and Clinical Laboratory Genetics specialities by the Spanish Ministry of Health.

Population-based screening of a variant in *CDH1* and the use of the microbiota in diffuse gastric cancer as an early diagnostic biomarker. In this project, we aim to establish a genetic screening for an LP variant in the *CDH1* gene associated with a high risk of diffuse gastric cancer, in a specific town in Spain. In addition, we plan to explore new non-invasive biomarkers to detect the disease in asymptomatic carriers at an early, treatable and curable stage.

IMPaCT-GENóMICA and IMPACT-VUSCan projects. During 2024, the FCCU has contributed to the VUSCan project with three new families, unresolved by our genetic diagnostic workflow. These families presented a high aggregation of cancer cases: breast, pilomatrixoma, and colon. In addition, our participation in these collaborative projects included the analysis and interpretation of genomic data to prioritise candidate variants that could explain the families' phenotype, as well as the elaboration of genetic reports and clinical recommendations as part of a consortium that includes groups from several Spanish institutions as well as other CNIO groups.

SpadaHC: Spanish variant database for hereditary cancer. We are one of the most active participants in SpadaHC among the 18 participating laboratories, with newly identified genetic variants and updates in the classification of previously reported ones, based on the variant classification criteria updated by the different international expert groups. In February 2024, SpadaHC stored 1.17 million variants from 4306 patients and 16343 laboratory classifications. An initial analysis of the shared data identified 84 genetic variants with clinically relevant classification discrepancies, which were resolved through a three-phase strategy as detailed in the corresponding publication. ■

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HUMAN GENOTYPING-*CEGEN* UNIT

Anna González-Neira
Unit Head

Graduate Students
Carla Cortina (since February), Hugo Tejera

Bioinformaticians
Laura Martínez (until December) (TS)*, Guillermo Pita (TS)*

Technicians
Charo Alonso, Núria Álvarez, Patricia González (until December) (TS)*, Belén Herráez, Rocío Núñez (TS)*

Student in Practice
Courtney Saqueton (until May) (Fulbright Predoctoral Researcher, USA)

**Titulado Superior* (Advanced Degree)

OVERVIEW

Our Unit provides state-of-the-art genotyping and sequencing services designed to meet a wide range of research needs, constantly innovating to adapt to evolving demands. In 2023, the Centre enhanced its research capabilities with the acquisition of the cutting-edge NovaSeq X Plus System. This advanced sequencing platform has positioned the Centre at the forefront of scientific progress, enabling the introduction of new sequencing services at a lower cost.

"We aim to improve breast cancer risk assessment, implement personalised screening strategies, and enhance the safety of therapeutic interventions."

Our research is dedicated to identifying genetic factors that influence breast cancer susceptibility, treatment response, and drug toxicity. We aim to refine personalised breast cancer risk assessment, develop groundbreaking strategies for early detection, and enhance the precision and safety of cancer treatments. Furthermore, we are committed to integrating these insights into clinical practice, ensuring that our findings translate into tangible improvements in patient care through more personalised and effective approaches to cancer prevention, diagnosis, and treatment.

RESEARCH HIGHLIGHTS

Participation in the Genome of Europe (GoE) project. The Unit participates in the GoE project in collaboration with ISCIII, CIBER and CNAG, as well as in the PMP24 GoIMPaCT project. The GoE project (https://b1mg-project.eu/1mg/genome-europe) aims to establish the European reference genome database, a vital resource for advancing public health. In the short term, this initiative will enable the quantification of genetic diversity and heterogeneity across European populations, improving variant interpretation and genetic risk assessment. In the long term, it will serve as a key tool for identifying genetic-phenotypic associations, and will become a cornerstone of genomic health programmes in Europe, driving significant progress in personalised medicine and public health initiatives. The Unit will be responsible for extracting DNA from the first 20,000 participants enrolled in the Spanish IMPaCT Cohort initiative (https://cohorte-impact.es/) and sequencing the whole genome of half of them, supporting Spain's commitment to the 1+ Million Genomes (1+MG) initiative.

Polygenic risk score in breast cancer varies across European ancestry populations. This study evaluated the 313-variant polygenic risk score (PRS313) across different European ancestry populations. The findings revealed significant variations in PRS distribution, even among individuals of European descent. This genetic heterogeneity could affect risk classification if not properly accounted for, potentially leading to risk overestimation in some individuals and underestimation in others — factors that may influence clinical decision-making. This study concludes that to improve breast cancer risk prediction, the implementation of PRS313 in European ancestry populations must include population-specific calibration. This approach will enhance the accuracy of risk assessment and optimise clinical management (Yiangou K *et al.*, 2024). To address this issue in the Spanish population, an ongoing project, recently funded through the PMP24 call and led by the Unit in collaboration with the University of Santiago de Compostela, will work in conjunction with most breast cancer screening programmes in the Spanish Autonomous Communities.

Analysis of the BRIDGES sequencing dataset: evaluating co-observation as a predictor of variant pathogenicity. Co-observation of a gene variant, with a pathogenic variant in another gene explaining disease presentation, is typically considered evidence against pathogenicity in standard classification guidelines. However, multiple expert panels agree that this type of evidence does not apply to breast cancer predisposition gene variants. To investigate this, sequencing data from 55,815 individuals diagnosed with breast cancer in the BRIDGES project were analysed. Statistical analysis identified depletion of co-observation of pathogenic variants in the BRCA1, BRCA2, and PALB2 genes, compared to other genes commonly included in clinical breast cancer gene panels. In addition, the data showed that the identification of a variant of uncertain significance (VUS) in BRCA1, BRCA2 or PALB2, in an individual with a pathogenic variant in another breast cancer gene, could be used to provide supporting evidence against pathogenicity for that VUS (Davidson AL *et al.*, 2024). ■

PUBLICATIONS

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- Vega-Alonso T, ... González-Neira A (2024). Health and wellbeing status of the long-lived individuals of the Spanish LONGECYL cross-sectional study. *Arch Public Health* 82, 77.
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PATENT

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CLINICAL RESEARCH PROGRAMME

MIGUEL QUINTELA-FANDINO Programme Director

The Clinical Research Programme (CRP) has two main aims: 1) to translate preclinical research into novel clinical care standards; and 2) to address novel clinical oncology challenges with preclinical research. The specific areas of work include: 1) developing novel agents; 2) study of mechanisms of action of novel compounds and tackling drug resistance; 3) moving forward in the field of biomarkers, functional taxonomy, and precision medicine; and 4) developing novel therapeutic approaches in immuno-oncology. Currently, two functional objectives summarise the new operating model: A) generating synergies with ongoing research lines in the basic research programmes; and B) creating a bi-directional bridge closing interactions between the CNIO and tertiary cancer hospitals.

"The Clinical Research Programme aims to improve cancer care by developing novel agents and personalising therapeutic approaches on the basis of novel biomarkers."

The clinical activity of the CRP's Clinical Units takes place through agreements with tertiary hospitals (Hospital 12 de Octubre (H12O), Hospital La Paz (HLP), and Hospital de Fuenlabrada). These agreements foster the interaction between clinicians and scientists and allow scientists from all CNIO Programmes to participate in translational research studies. The number of ongoing collaborations between the Units of the CRP and CNIO Research Groups from other Programmes now involve 50 projects and 12 coordinated grants, which translate into the high translational research activity of the Centre. During 2024, 13 residents in medical oncology, from different Spanish hospitals, completed their 3-month optional visiting stays at CNIO.

This year, 2024, we are pleased to mention the following research highlights. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, demonstrated for the first time the therapeutic role of anti-fibrogenics in breast cancer. The H12O-CNIO Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, contributed to several immunotherapy registration trials for new standards of care in lung cancer. The H12O-CNIO Haematological Malignancies Clinical Research Unit, led by Joaquín Martínez-López, developed a CAR-T therapy against multiple myeloma, a highly unmet clinical need, and made advances in the field of liquid biopsy, also in myeloma. The Molecular Diagnostics Unit, headed by Luis Lombardía, continued providing support to hospitals in the diagnosis of different malignancies, performing >500 diagnoses.

The two recently incorporated senior groups to the CRP - the H12O-CNIO Cancer Immunotherapy Clinical Research Unit, led by Luis Álvarez-Vallina, and the IdiPaz-CNIO Pediatric Onco-Hematology Clinical Research Unit, led by Antonio Pérez-Martínez - have already started to make important contributions. The first academic bi-specific, antibody-secreting, cellular therapy against myeloma cells (STAb cells) was engineered by the Cancer Immunotherapy Clinical Research Unit and is currently being prepared for early phase clinical testing. Also, in the cellular therapy field, the Pediatric Onco-Hematology Clinical Research Unit continued developing novel approaches targeting difficult-to-treat childhood cancers. Finally, the Clinical Research Programme will expand its current size during 2025 by incorporating two novel research groups. ■

BREAST CANCER CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Clinical Research Unit Head

Research Scientists
María José Bueno, Leonardo Garma, Silvana A. Mouron

Clinical Research Fellow
Desirée Jiménez

Post-Doctoral Fellows
Nuria Moreno (since October), Ana María Roncero (until September)

Graduate Students
Gozeel Binte (since December), Marina Rodríguez (since May)

Technicians
Verónica Jiménez, Aída Morillas (since June), Manuel Muñoz, Ángela Sánchez (until July) (TS)*

**Titulado Superior* (Advanced Degree)

Students in Practice
Marta de la Hoz (July-August) (Master's Degree Final Project, UAH, Spain), Alba Lozano (July-August) (AECC Summer Traineeship, UCM, Madrid, Spain)

Visiting Scientists
Rocío Moreno (Hospital Universitario 12 de Octubre, Madrid, Spain), Berta Nassarre (Peaches Biotech, Madrid, Spain)

OVERVIEW

The Breast Cancer Clinical Research Unit (BCCRU) focuses on the translational interface of therapeutic development. Breast cancer is a heterogeneous disease and, thus, there are large inter-patient variations in terms of disease course, prognosis, relapse, and resistance to conventional or targeted therapeutics. Our activities are directed towards personalised treatment and range from preclinical models to correlative studies and clinical trials.

"At the Breast Cancer Clinical Research Unit, we are focused on individualising therapy for patients with advanced breast cancer."

Our current research areas are to:

- → Study the implications of hypoxia for immunotherapies.
- → Understand the individual factors regulating response to immunotherapy in breast cancer, taking advantage of an advanced, personalised "tumouroid" platform.
- → Tackle mechanisms of resistance against novel therapies in advanced breast cancer.
- → Incorporate our findings into concept-driven clinical trials.
- → Develop the new discipline of High-Definition Oncology.

RESEARCH HIGHLIGHTS

We started a new major line of research – High-Definition Oncology (HDO). Currently, precision oncology is largely based on genomics and performs poorly outside the context of matching targeted agents with oncogene addiction driving alterations. Outside of this context, next-generation sequencing (NGS) panels are able to provide a solution in less than 10% of the cases. The problem is that many factors explain inter-patient heterogeneity beyond genomics. These factors include, for example, the patient's exposome (exposure to factors such as diet or the environment, which translate into changes in the plasma metabolome); microbiome; plasma proteome; individual germline genetic variations that drive different pharmacodynamic or pharmacokinetic traits; co-morbidities or concurrent medications; and habits, mood or cognitive factors, among others. In addition, current medical approaches are based on single point-observations or probing at distant timepoints, obtaining only snapshots of what it is in reality a disease trajectory. To account for this multitude of factors, we launched a project consisting of longitudinal omic sampling, combined with electronic data capture from medical records and continuous physiologic monitoring via a smartwatch, and habits/diet/quality of life tracking through an application built ad hoc and installed in the patients' smartphones. The ultimate goals of this grant-supported research are to: 1) establish and understand female patients' disease trajectories in advanced breast, lung or colorectal cancer; and 2) build a prototype of a cancer "Patient Digital Twin". During 2024, the recruitment of patients into the HDO study has thrived, and as of December 2024, 127 women had joined the trial.

Scientific studies often unfold in unexpected ways. During sample processing in the HDO study, specifically while trying to compute biological age based on methylation arrays (to have this parameter available for predictive purposes), we realised that currently existing "epiclock" models had been developed using previous generations of methylation arrays. We evaluated the compatibility of existing epigenetic clocks with the EPICv2 methylation array, a next-generation platform that replaces prior models like the 450k and EPICv1 arrays. While epigenetic clocks such as Horvath, Hannum, and PhenoAge are pivotal for predicting biological age, the partial overlap of probes between older and newer arrays distorts their accuracy, with errors reaching up to 25 years. To address this, we developed a new model compatible across EPICv2 and prior platforms, using elastic net regression and CpG probes selected for linear and quadratic age associations. The model demonstrated high accuracy in predicting chronological age and minimised technical and intra-subject variability. It also replicated known epigenetic age acceleration in cancer patients and radiation-exposed survivors. Furthermore, when applied to studies reporting epigenetic age acceleration correction in response to "anti-ageing" interventions, we found that this claimed "reversal" of epigenetic age was not evident with this new generation model, questioning whether former conclusions in this regard were accurate. This work provides a robust, cross-platform solution for epigenetic ageing research and raises critical questions about the biological relevance of current epigenetic clocks. The main results are summarised in Figure 1.

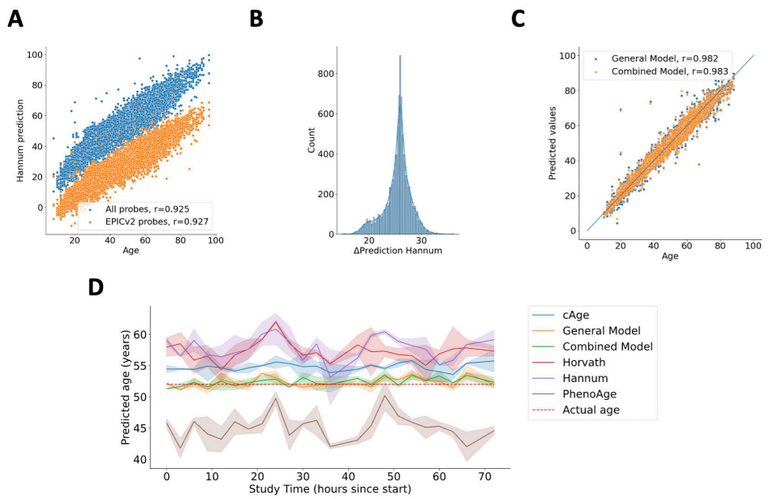

FIGURE 1 Development of a novel Epi-Clock compatible with EPIC2. (**A**) Magnitude of the error of the Hannum Phenoage clock when run in previous versions (orange) or in the current (blue) EPIC2 array. As compared to the chronologic date, the predicted bio-age drifts on average >20 years (**B**). In (**C**), we show the accuracy of our Epi-clock model, which has a linear correlation with chronological age. (**D**) Previous Epi-clocks were unstable: for example, circadian replicas provided up to 10-year variation in the test results for the same individual, depending on which time of the day the sample was drawn. Our model (in orange) provides a stable result regardless of the time or number of technical replicas.

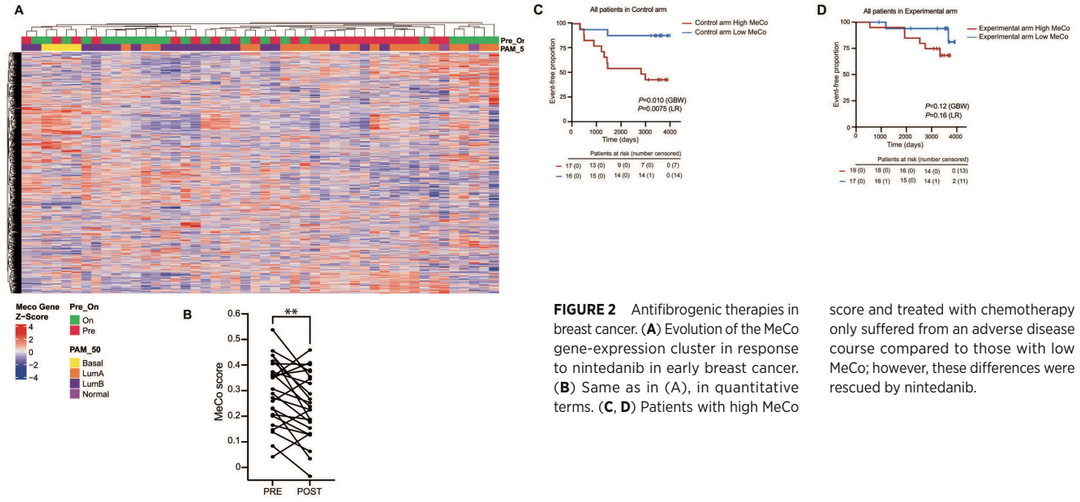

FIGURE 2 Antifibrogenic therapies in breast cancer. (**A**) Evolution of the MeCo gene-expression cluster in response to nintedanib in early breast cancer. (**B**) Same as in (A), in quantitative terms. (**C**, **D**) Patients with high MeCo score and treated with chemotherapy only suffered from an adverse disease course compared to those with low MeCo; however, these differences were rescued by nintedanib.

Another area of relevance, within the field of treatment personalisation, is the role of diet as an adjuvant treatment in cancer management. Traditionally, cancer nutrition has focused only on cancer cachexia or other tangential aspects such as anorexia or dysgeusia. However, in recent years, robust evidence (at various preclinical and clinical levels) has been generated about how specific metabolic modulations can actually have therapeutic effect in cancer. As a result of cancer mutations, tumours harbour metabolic alterations that render nutrients essential for the tumour but disposable for healthy cells; additionally, some nutrients can specifically damage the tumour and be innocuous to healthy cells. We have created an algorithm that takes into account the tumour type, treatment type, known mutations, co-morbidities, certain microbiota parameters, and even patient dietary preferences, which is able to deliver a unique set of dietary patterns for each patient. Through the translation of these patterns into a specific diet, we can deliver therapeutic nutrition to each patient so that efficacy is boosted and toxicity diminished. This algorithm – LUMICA V. 1.0 – was licensed to a spin-off company created at CNIO in 2023 – *TNC Nutrición Terapéutica*. We are proud to announce that in Q4 2024, we started the first clinical trial comparing a personalised therapeutic diet with a control diet (Mediterranean diet counselling) in women's cancers; this trial aims to demonstrate that patients allocated to the tailored diet will experience better treatment outcomes (prolonged disease-free progression and decreased toxicity).

Finally, in the field of targeted therapies, we studied the role of antifibrogenics. We investigated the role of tumour extracellular matrix (ECM) stiffness and mechanical conditioning (MeCo) as biomarkers for predicting response to antifibrotic therapy in HER2-negative breast cancer. Using data from a randomised phase II trial, we explored the effects of nintedanib, an antifibrotic tyrosine kinase inhibitor, in combination with paclitaxel. High MeCo scores, indicative of increased ECM stiffness, were associated with poor outcomes and higher risk of relapse in the control arm. Nintedanib effectively reduced MeCo scores by 25% during a two-week run-in phase, improving event-free survival (EFS) in high-MeCo patients. Low-MeCo patients maintained favourable outcomes regardless of treatment. The study establishes MeCo as a predictive biomarker for selecting patients that are likely to benefit from nintedanib, offering a personalised approach with minimal toxicity and cost. These findings represent the first clinical evidence supporting antifibrotic therapy in breast cancer, paving the way for targeted interventions in tumours characterised by pathological stiffness (Figure 2). ■

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MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

Student in Practice
Carlos A. Sánchez (April-June) (IES Antonio Machado, Madrid, Spain)

OVERVIEW

The Molecular Diagnostics Unit (MDU) aims to provide quality molecular tests for patients with cancer through the Spanish National Health System (NHS). MDU offers sensitive assays to detect genetic alterations enabling clinicians to better manage their patients by monitoring minimal residual disease after cancer remission, or by predicting their response to therapy. The Unit is committed to enhancing its testing capabilities by updating existing assays or implementing new ones. MDU also provides support, with its technology and expertise, to CNIO's Units and Research Groups by developing novel diagnostic solutions, and collaborates with national and international consortia to standardise and control molecular diagnostics quality. Additionally, MDU is committed to training and mentoring students, technicians, and medical residents in molecular techniques, fostering a knowledgeable workforce in the field of cancer diagnostics.

"Over the course of 2024, the growing interest in molecular tests for diagnostic purposes, has allowed us to establish research collaborations with clinicians who strive to improve the management of their patients with cancer."

Visiting Scientists
David S. Juliao Caamaño (April-June) (Hospital General Universitario Gregorio Marañón, Madrid, Spain), Manal Mohamed Elsayed Ahmed (September-November) (National Research Centre, Egypt) (Science by Women Programme)

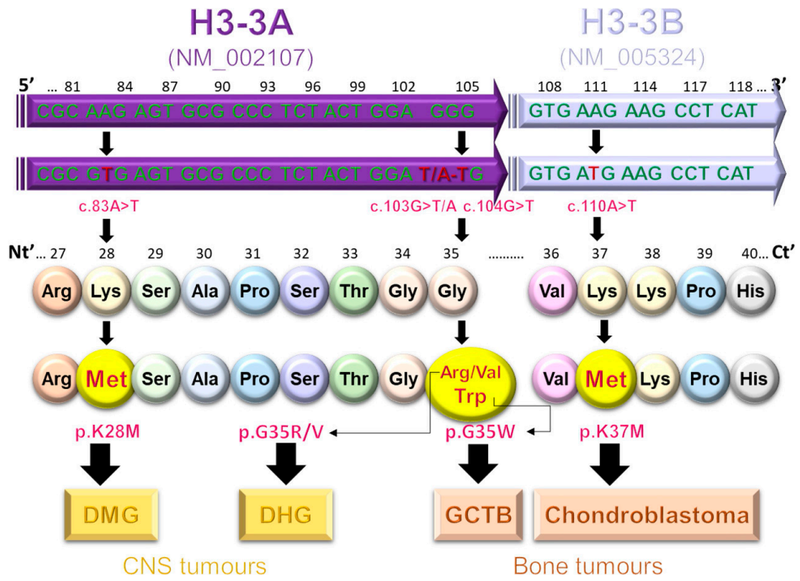
CORE UNIT HIGHLIGHTS

During 2024, our catalogue grew with the addition of three new diagnostics assays based on Sanger sequencing. The first one allows the detection of driver mutations in *H3-3A* and *H3-3B* genes, which encode identical H3.3 histones but with specific amino acid residue alterations that give rise to distinct tumours. Consequently, this test may be helpful as a diagnostic tool for patients with bone or nervous system tumours (see Figure 1). The second test enables the detection of gain-of-function mutations in the *PDGFRB* gene (Platelet Derived Growth Factor Receptor Beta) concurrent with paediatric myofibroma, the most frequent fibrous tumour in children, and improving their treatment, as these alterations are sensitive to tyrosine kinase inhibitors like imatinib. The last assay was implemented to detect activating mutations in the *CTNNB1* gene, which encodes β-Catenin. Since alterations in *CTNNB1* are present in almost all cases of adamantinomatous craniopharyngiomas, this test can help diagnose this pituitary tumour as is already established in other neoplasms like medulloblastomas or colorectal and ovarian cancers.

Additionally, MDU extended its internal technical support to CNIO's Research Groups (e.g., Genome Integrity and Structural Biology Group) and Units (e.g., Familial Cancer Clinical Unit). We also started a collaboration with oncologists from the Gregorio Marañón University Hospital in Madrid. In this project, we will perform a retrospective analysis, seeking to assess the impact of two molecular biomarkers (i.e., TERT promoter mutations and MGMT promoter methylation) on the estimated prognosis and survival of patients with glioblastomas.

Lastly, owing to our regulatory and educational commitments, we devoted significant effort, respectively, to develop standardised protocols, complying with the new European IVDR (*In Vitro* Diagnostic Regulation) guidelines, and to host and coach two high school pupils during 2024; one medical resident, and a visiting scientist from Egypt awarded by the "Women for Africa" Foundation. ■

FIGURE 1 Detection of specific point mutations in *H3F3A* and *H3F3B* genes is facilitating the diagnosis of different and infrequent malignancies of the bones and the central nervous system (CNS). DMG: Diffuse Midline Glioma; DHG: Diffuse Hemispheric Glioma; GCTB: Giant Cell Tumour of Bone.



H12O-CNIO CANCER IMMUNOTHERAPY CLINICAL RESEARCH UNIT

Luis Álvarez-Vallina
Clinical Research Unit Head

Research Scientists
Belén Blanco, Anáis Jiménez

Post-Doctoral Fellows
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Graduate Students
Francisco J. Arroyo, Elena Barba, Eva García, Marina Gómez, Susana Luengo, Jaime Franco (since Dec.), Lucía Rivas, Laura Rubio (until July), Alejandro Segura (until April), Miriam Velasco

Technician
María de la Yedra Pacheco

Students in Practice
Jaime Franco (Jan.-July) (Master's Degree Final Project, Univ. Complutense de Madrid, Spain), Marina Hermosilla (March-July) (Master's Degree Final Project, Univ. Francisco de Vitoria, Madrid, Spain)

Visiting Scientists
Manal Mohamed Elsayed Ahmed (June-Sept.) (National Research Centre, Egypt) (Science by Women Programme), Aida Falgás (Jan.-April) (Josep Carreras Leukaemia Research Institute, Barcelona, Spain), Philipp Lapuhs (June-Oct.) (Universidad de Santiago de Compostela, Spain)

OVERVIEW

Immune evasion is a critical step in cancer progression in which tumour cells modulate the host immune system to evade destruction. Our Unit focuses on understanding the molecular and cellular mechanisms of cancer immune evasion to develop more effective and safer cancer immunotherapies. The Cancer Immunotherapy Clinical Research Unit has several areas of interest:

- → Reinvigorating endogenous tumour-specific T cell responses through the design of multi-specific antibodies against a combination of immunomodulatory targets, and the development of innovative RNA-delivery strategies. Preclinical and early clinical data show that this is a promising approach to enhance the clinical benefit of conventional checkpoint blockers.
- → Generating tumour reactive "artificial" T cell effectors by redirecting T cell activity towards cancer cells, targeting tumour-associated antigens (TAAs) with bispecific T cell-engaging (TCE) antibodies and/or membrane-anchored chimeric receptors (chimeric antigen receptors and/or chimeric costimulatory receptors).
- → Developing multi-targeted approaches for the simultaneous detection of extracellular and intracellular tumour antigens.
- → The Provision of personalised cancer treatments by bringing new immuno-oncology drugs and adoptive cell therapies to the clinic.

"At the Cancer Immunotherapy Clinical Research Unit, we aim to develop immunotherapies that synergistically stimulate different components of the patient´s immune system against cancer."

RESEARCH HIGHLIGHTS

Next generation T cell-redirecting immunotherapies: the "STAb-T concept"

The "STAb-T strategy" is a novel adoptive cell therapy (ACT) developed by our Unit based on the *in vivo* Secretion of TCE Antibodies (STAb) by engineered T cells (FIGURE 1a). The secreted TCE antibodies redirect T cells against cancer cells expressing a specific tumour-associated antigen (TAA). STAb-T cells offer several potential advantages over current T cell redirection strategies (FIGURE 1): the *in vivo* secretion results in effective concentrations of the TCE, and T cell recruitment is not restricted to engineered T cells, as in the case of CAR-T cell approaches. Polyclonal recruitment by TCEs of both engineered and unmodified bystander T cells, present in the tumour microenvironment, could lead to a significant boost in antitumour T cell responses (FIGURE 1). Several single-targeted STAb-T immunotherapies have already demonstrated preclinical activity against various haematological malignancies. CD19- and BCMA-specific STAb-T cells have been shown to be more effective than CAR-T cells in inducing specific cytotoxicity, preventing tumour escape *in vitro* and inhibiting tumour progression *in vitro* and *in vivo* (Diez-Alonso L,...., Álvarez-Vallina L. *Sci Transl Med,* 2024).

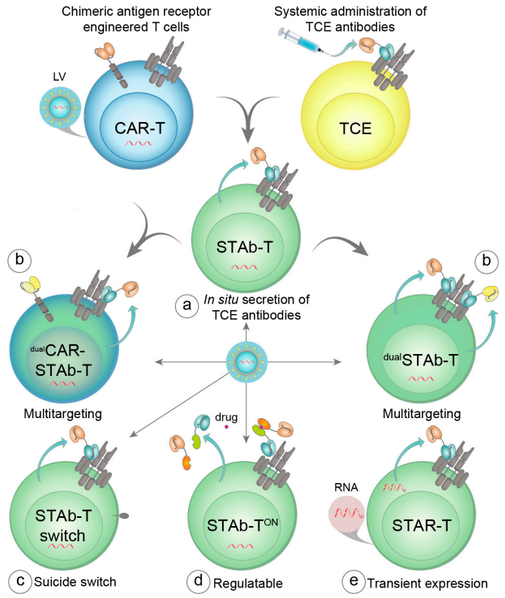


FIGURE 1 Schematic diagram summarising the current T cell-redirecting strategies (autologous gene-modified CAR-T cells and systemic injection of purified TCE), next-generation STAb-T cells (**a**), and evolution of the "STAb-T concept". Dual-target strategies (**b**) based on the combination of different redirection mechanisms, CAR and STAb, or on the combination of two STAb strategies against different TAAs. STAb-T cells expressing a safety switch (**c**) and regulatable STAb-T cells based on a molecular ON switch that heterodimerise in the presence of a priming drug (**d**). RNA-electroporated human primary STAb-T (STAR) cells temporarily secrete bispecific TCEs (**e**). LV, lentiviral vector.

Evolution of the STAb-T immunotherapy

An increasing number of dual-targeting strategies are being developed to overcome the tumour heterogeneity and antigen downregulation associated with single-target therapies, and we are pioneering dual-target immunotherapies combining CAR and STAb mechanisms of action (CAR-STAb-T cells), or "dual STAb-T cell only" (FIGURE 1b). The use of T cell-redirecting strategies can result in serious adverse effects, such as cytokine release syndrome and neurotoxicity. We are working to provide systems to eliminate infused STAb-T cells in the event of toxicity, such as the addition of a safety switch to selectively deplete modified STAb-T cells upon administration of a clinically approved monoclonal antibody (FIGURE 1c). However, this approach involves the irreversible endpoint of a very expensive and time-consuming product, in most of the cases before the desired therapeutic effect is achieved. New strategies to control and modulate the activity of engineered STAb-T cells could greatly accelerate their clinical development. We are generating switchable STAb-T cells based on a split TCE. Formation of the functional TCE is dependent on a molecular ON-switch which heterodimerises in the presence of a priming agent (FIGURE 1d).

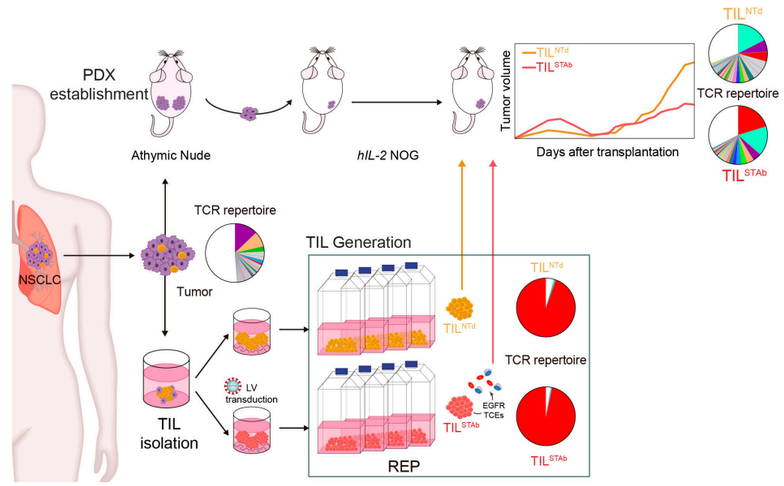
STAb-T immunotherapy for solid tumours

Most T cell-redirecting strategies against solid tumours have shown only modest therapeutic activity; and the risk of uncontrollable off-tumour toxicities, due to the difficulty in defining truly tumour-specific antigens, hinders their application in the treatment of non-haematological cancers. We have optimised the electroporation of human primary T cells with mRNA encoding TCE. Such mRNA-modified STAb-T (STAR-T) cells (FIGURE 1e) transiently secrete bispecific TCEs capable of recruiting both modified and bystander T lymphocytes to specifically eliminate tumour cells, and efficiently control solid tumour growth *in vivo* in various xenograft models. Adoptive transfer of tumour-infiltrating lymphocytes (TIL) has shown remarkable results in melanoma, but only modest benefit in other tumours. The required *ex vivo* TIL expansion process may induce changes in the clonal composition of T cells, which could likely compromise the tumour reactivity of TIL preparations and ultimately the success of TIL therapy. We have generated TIL secreting TCE (TIL^{STAb}) and evaluated their efficacy in a lung cancer patient-derived xenograft model (in which tumour fragments and TILs from the same patient were transplanted into immunodeficient mice) and we demonstrated antitumour activity when administered intratumourally and systemically (FIGURE 2) (Jiménez-Reinoso A,...., Álvarez-Vallina L. *Oncoimmunology,* 2024).

Early clinical trials

Our Unit, in collaboration with the Haematology and Medical Oncology Departments of the Hospital Universitario 12 de Octubre, has launched 3 independent clinical trials funded by the Carlos III Health Institute: a phase I, first-in-human clinical trial to evaluate the safety of STAb-T19 cells (genetically modified autologous T lymphocytes secreting CD19xCD3 bispecific antibodies) for B cell malignancies; a phase I, first-in-human clinical trial to evaluate the safety of intratumoural administration of P32-specific Chimeric Antigen Receptor (CAR)-engineered T cells for progressive high-grade gliomas; and a phase 1, first-in-human trial to evaluate the safety and tolerability of genetically modified autologous T lymphocytes secreting BCMAxCD3 bispecific antibodies in relapsed/refractory multiple myeloma. ■

FIGURE 2 Generation and preclinical validation of STAb-TIL immunotherapy. LV, lentiviral vector; NSCLC, non-small cell lung cancer; PDX, patient-derived xenograft; REP, rapid expansion protocol; TCR, T cell receptor; TIL, tumour-infiltrating lymphocytes.



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AWARDS AND RECOGNITION

Luis Álvarez-Vallina:

- 'Premio Admirables' 2024 in research awarded by Diario Médico, Spain.
- 'Premio *ex aequo* Constantes y Vitales' for Best Biomedical Research in 2024 awarded by La Sexta and Fundación AXA, Spain.
- Best Doctors in Spain 2024 in Immunology awarded by El Confidencial.
- 'III Beca Dr Baselga' awarded by the Fundación FERO, Spain.
- Belén Blanco received the Award for 'Best Poster' at the 10th GETICA´s Forum on Translational Immunology and Immunotherapy of Cancer (FIC Cancer 11), Seville, Spain.
- Rodrigo Lázaro received the Award for 'Best Oral Communication' at the 13th EuroMAbNet Meeting, Montpellier, France.
- Francisco Javier Arroyo received an Award for 'Best Oral Communication' in the XII Scientific meeting of the Society of Immunology of the Community of Madrid, Majadahonda, Spain.
- Ivana Zagorac received an Award for 'Best Poster' at the CNIO-CaixaResearch Frontiers Meeting «Frontiers in Immunomodulation and Cancer Therapy" (2nd edition), Madrid, Spain.

H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis G. Paz-Ares
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Graduate Students
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Technicians
Vera Adradas, Eva Álvarez, Nuria Carrizo, Patricia Cozar (until Aug.), María Cuerda (since Sept.), Laura García, Beatriz Gil, José M. Gracia, Marta Jiménez, Patricia Llamas, María del Mar López (since July), Elsa Moras (since July), Patricia Plaza (until Aug.), Cristina Ramírez (since Nov.), Belén Revuelta (until Oct.), Jacinto Sarmentero, Rocío Suárez, César Vélez

Students in Practice
Ana I. Báscones (Jan-Sept.) (Master's Thesis, UCM, Madrid, Spain), Pablo Fernández (Jan-June) (Master's Thesis, UCM, Madrid, Spain), Paula Leal (July-Dec.) (Master´s Thesis, ENS-ISCIII, Madrid, Spain), Irene Pazos (Jan-Aug.) (Master's Thesis, UCM, Madrid, Spain)

Visiting Scientist
Daniel Curto (July-Sept.) (Hospital Universitario 12 de Octubre, Madrid, Spain)

OVERVIEW

Lung cancer remains the most frequent cause of cancer-related deaths worldwide. Our Unit focuses on the study of lung cancer, with a pragmatic orientation, always aiming to solve the problems of lung cancer patients. We specifically focus on two research areas: the identification of novel molecular biomarkers for diagnostic, prognostic, and predictive purposes; and the development of novel treatment approaches, including targeted therapies and immunotherapeutics. For example, we have contributed to elucidating the molecular determinants of EGFR or FGFR oncogenicity and have discovered biomarkers that may guide the efficacy of inhibitors of those receptors in lung cancer. We have continued developing an extensive platform of patient-derived xenografts (PDXs) and organoids (PDOs) of non-small-cell and small cell lung cancers to evaluate emerging therapeutic strategies. Finally, our Unit has extensive experience in taking new drugs (pemetrexed, erlotinib, nivolumab, tarlatamab, and many others) to the clinic, as well as in conducting practice-changing trials in the fields of personalised cancer care and immuno-oncology.

"Our Unit has significantly contributed to the dissection of the immune microenvironment of SCLC that resulted in the discovery of novel targets and mechanisms to promote immunogenicity in this context. We have led randomised clinical trials with novel immunotherapies and other agents as monotherapies or in combination (e.g., the ADC Sacituzumab Govitecan in previously treated patients with advanced NSCLC)."

RESEARCH HIGHLIGHTS

Sacituzumab Govitecan as a therapeutic option for advanced lung cancer patients unresponsive to standard treatments

In this phase III clinical trial, our group demonstrated the increased efficacy of the Antibody-Drug Conjugate (ADC) Sacituzumab Govitecan (SG) compared to docetaxel in treating advanced non–small cell lung cancer (NSCLC) that has progressed after standard therapies. This treatment targets cells expressing the trophoblast cell-surface antigen 2 (trop2) and delivers a topoisomerase I inhibitor specifically to these cells. 603 patients with stage IV disease, divided into SG-treated and docetaxel-treated, showed a modest but not significant improvement in overall survival (OS) (11.1 months versus 9.8 months, respectively) with an increase in 12-month OS from 36.72% in docetaxel-treated to 46.59% in the SG-treated, and showed fewer adverse effects. Interestingly, a clinically meaningful improvement in OS was observed in patients who were nonresponsive (SD/PD) to their last anti–PD-(L)1–containing regimen, increasing median OS from 8.3 to 11.8 months in SG-treated patients (Figure 1). This suggests that SG may be a particularly promising option for patients unresponsive to anti–PD-(L)1 treatment.

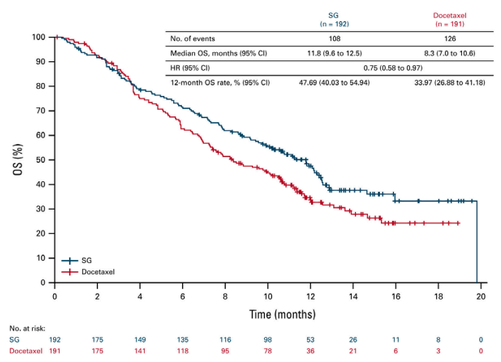

FIGURE 1 OS in patients nonresponsive (SD/PD) to the last anti-PD-(L)1-containing regimen treated with Sacituzumab Govitecan (SG) compared to docetaxel. The HRs and 95% CIs for the subgroup analyses were calculated using the unstratified Cox proportional hazards model without any adjustment.

Exploring immune pathways and metabolic vulnerabilities as key factors in chemoimmunotherapy response for extensive-stage small cell lung cancer

We investigate transcriptomic characteristics and potential biomarkers linked to the response to chemoimmunotherapy in patients with extensive-stage small cell lung cancer (ES-SCLC). Using multi-region transcriptomic analysis of samples from 58 patients in the IMfirst and CANTABRICO phase IIIb trials, our group identified notable intratumoural heterogeneity. Approximately 44% of patients displayed multiple transcriptomic subtypes within their tumours. However, we did not find a direct association between these transcriptomic subtypes or subtype heterogeneity with patient outcomes. Most of the therapeutic targets analysed did not show expression that was specific to a particular subtype.

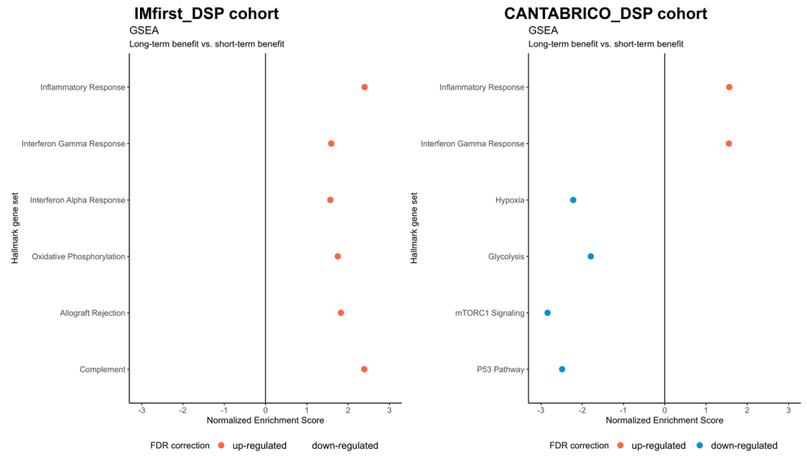

FIGURE 2 GSEA of tumour samples from patients with long-term benefit versus short-term benefit using the MSigDB Hallmark gene set collection in the IMfirst_DSP cohort and the CANTABRICO_DSP cohort. Significance corrected by the Benjamini-Hochberg FDR method.

Interestingly, patients with longer treatment responses had tumours enriched with IFNγ-related immune pathways, suggesting that preexisting immune activation might play a positive role in treatment efficacy. In contrast, pathways related to hypoxia and glycolysis were associated with resistance to chemoimmunotherapy. These findings imply that therapeutic strategies focusing on enhancing immune responses, particularly IFNγ-driven immunity and antigen presentation, as well as targeting metabolic vulnerabilities, may improve outcomes for patients with ES-SCLC. This study underscores the complexity of ES-SCLC and suggests that factors related to the immune microenvironment may have a greater impact on treatment response than transcriptomic subtypes alone. ■

PUBLICATIONS

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AWARDS AND RECOGNIGTION

Luis Paz-Ares:

- Heine H. Hansen Lectureship Award for Small Cell Lung Cancer, International Association for the Study of Lung Cancer – IASLC, 2024.

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head

Research Scientists
Miguel Gallardo, María Hernandez-Sanchez, María Linares, María Velasco

Clinical Investigators
Rosa Ayala, Ana Jiménez, Ricardo Sánchez

Post-Doctoral Fellows
Pedro Aguilar (until December), Almudena García, Larissa A Haertle, Alejandra Leivas, Alba Rodríguez, Ana Rodríguez, Antonio Valeri

Graduate Students
Noemí Álvarez, Raquel Ancos, Pablo Blanco, Eva Castellano, Laura Córdoba, Jessica Encinas (until April), Roberto García, Alba Garrote, Miguel Ángel Marugal, Álvaro Otero

Technicians
Andrés Arroyo, Natalia Sofía Buenache, Paula Matías (since June), Miguel Ángel Navarro, Alejandra Ortiz

Student in Practice
Lucía de la Mata (until June) (Master´s Degree Final Project, Universidad Complutense de Madrid, Spain)

OVERVIEW

The Haematological Malignancies Clinical Research Unit focuses on the study of haematological diseases, both neoplastic and dysplastic, with a special interest in multiple myeloma. We apply cutting-edge technologies such as next-generation sequencing (NGS) and artificial intelligence (AI) to discover new prognostic, diagnostic and treatment resistance biomarkers. In the Haematological Malignancies Clinical Research Unit at CNIO we investigate:

- → Microbiota: Its role in myeloma and treatment response.
- → AI integration: To improve the measurable residual disease (MRD) detection.
- → Mechanoreception: Its impact on the immune system.
- → RNA biology: As orchestrator of the haematopoietic stem cell fate.
- → Immunotherapy: NK-CARs, BITES, T-CARs, and immune checkpoint inhibitors.

“Our results suggest that short-chain fatty acids regulated by gut microbiota might have beneficial effects in multiple myeloma evolution and response to treatment.”

RESEARCH HIGHLIGHTS

Short-chain fatty acid production by gut microbiota predicts treatment response in multiple myeloma

We have identified changes in gut microbiota composition in the progression of multiple myeloma and its response to treatment. Specifically, we observed a reduction in short-chain fatty acid (SCFA) producers in patients with active disease and in those with poor prognosis after treatment, which was reflected in changes to SCFA-related metabolic pathways. Importantly, some of these changes were associated with a greater overall survival. Indeed, serum levels of the SCFAs, specifically butyrate, inversely correlated with disease progression and were directly associated with a better response. These changes were validated in a larger cohort and might serve as predictors of disease progression and drug response.

Measurable residual disease (MRD) dynamics in multiple myeloma and the influence of clonal diversity analysed by artificial intelligence

Here we investigated the prognostic value of MRD, MRD dynamics, and clonal diversity in a large cohort of patients with multiple myeloma (MM).

We found that achieving a very deep response by Ig genes sequencing (<10−6) was associated with a significantly longer progression-free survival (PFS) and overall survival (OS).

We also identified a pattern of MRD dynamics that discriminated patients with an excellent outcome from those with a poor outcome better than MRD measurement at a single time point. Finally, we found that clonal diversity of the Ig sequences was associated with a longer PFS in patients with MM. Our retrospective study found that achieving a very deep and sustained MRD response (a pattern of MRD that is decreasing over time) and higher clonal diversity are all associated with a better prognosis in patients with MM. These findings suggest that MRD results combined with clonal diversity assessment could be used to identify patients who are at risk of relapse and could be targeted for earlier treatment intervention. ■

FIGURE 1 Gut metagenome metabolic pathways showing discriminative KEGG metabolic pathways among relapsed multiple myeloma patients (RRMM) compared with those in complete remission (CRMM).

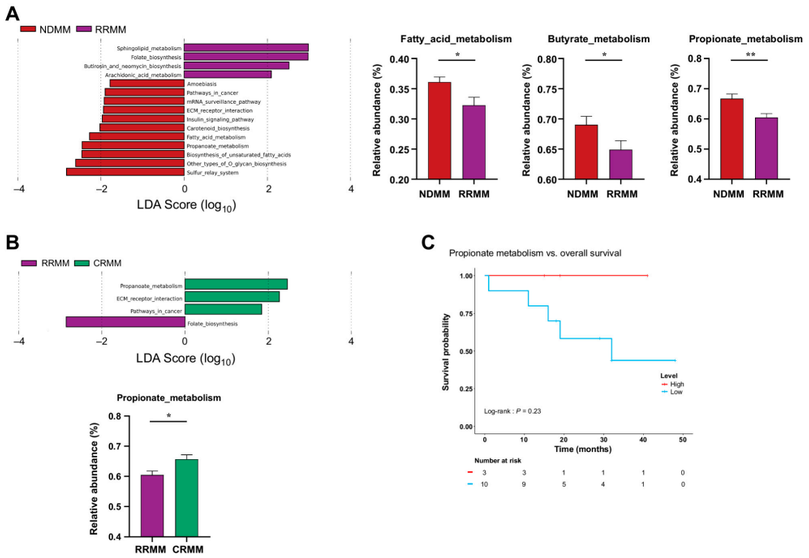
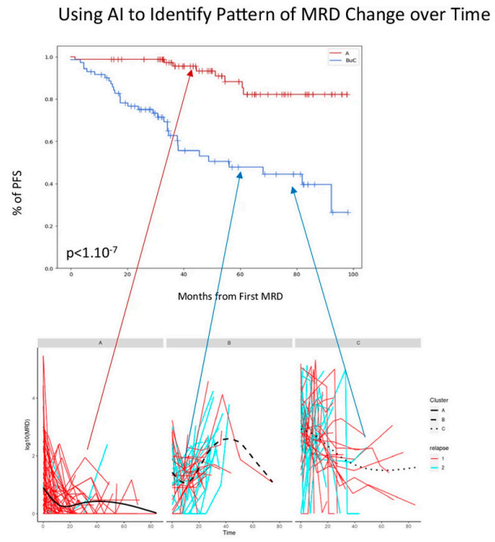


FIGURE 2 Kaplan–Meier curves showing the PFS of different MRD dynamics patterns identified in multiple myeloma patient outcomes.



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- Sonneveld P *et al.* (incl. Martinez-Lopez J; PERSEUS Trial Investigator) (2024). Daratumumab, bortezomib, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med* 390, 301-313.
- Mahon FX *et al.* (incl. Martinez-Lopez J) (2024). European stop tyrosine kinase inhibitor trial (EURO-SKI) in chronic myeloid leukemia: final analysis and novel prognostic factors for treatment-free remission. *J Clin Oncol* 42, 1875-1880.
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AWARDS AND RECOGNITION

- Joaquín Martínez-López: AES 2024 Health Research Project (PI24/01528), Spain.
- Miguel Gallardo: AES 2024 Health Research Project (PI24/01042), Spain.
- María Hernández-Sánchez: PID 2024 Knowledge Generation Project (PID2023-149241OA-I00), Spain.
- María Velasco-Estévez: AES 2024 Health Research Project (PI24/01614), Spain.
- Miguel Ángel Marugal: i+12 2024 Predoctoral Research Training Grant, Spain.
- Alba Garrote: PIPF-CAM 2024 Predoctoral Research Student Contract, Community of Madrid, Spain.

PATENT

- López Rodríguez ML, Benhamú Salama B, Vazquez Villa MH, Algar Lizana S, Sánchez Merino A, Gallardo Delgado M. NPM1-depending leukemia agents. International Publication Number: *WO2023052354A1*. National Phase Entry (2024).

IDIPAZ-CNIO PEDIATRIC ONCO-HEMATOLOGY CLINICAL RESEARCH UNIT

Antonio Pérez-Martínez
Clinical Research Unit Head

Clinical Investigators
Mikel Fernández (since February), Víctor Galán, Antonio J Muñoz, Ignacia Ceballos (since April, Hospital la Paz), Andrés Gómez (Hospital la Paz)

Post-Doctoral Fellows
Cristina Aguirre, Halin Bareke, Elisa Izquierdo (since July), Adriana Mañas, Jordi Minguillón, Andrés París, Odelaisy León Triana

Graduate Students
Karima Al-Akioui, Laura Clares, Beatriz García (since September), Adrián Ibáñez (since June), Marta Ibáñez, Carmen Mestre, Sara Naharro, Alfonso Navarro, Beatriz Ruz, Clara Venegas

Technicians
Lidia Pertiñez (TS)*, Natalia Riestra (TS)*

**Titulado Superior* (Advanced Degree)

Students in Practice
Rebeca Bulaman (July-Dec.) (Medical Student, Maastricht University, Limburg, The Netherlands), Sara Carpintero (April- Dec.) (UAH, Madrid, Spain), María Octavia de Santisteban (Jan.-June) (Master's Thesis, UCM, Madrid, Spain), Esther Díaz Maroto (since Sept.) (Master's Thesis, UCM, Madrid, Spain), Beatriz Madroñal (since July) (Bachelor's Thesis, CEU, Madrid, Spain), Alicia Martín (Jan.-July) (Master's Thesis, UAM, Madrid, Spain), Andrea Matesanz (since Nov.) (Bachelor's Thesis, UAH, Madrid, Spain), Lucía Sánchez (Jan.-June) (Bachelor's Thesis, UAM, Madrid, Spain), Claudia Suanzes (since Sept.) (Bachelor's Thesis, CEU, Madrid, Spain), Mario Zafra (Feb.-April) (Bachelor's Degree Final Project, UCM, Madrid, Spain), Pere Marsillach (Feb.-May) (Universitat Rovira i Virgili, Tarragona, Spain)

OVERVIEW

Cancer is the leading cause of death in children and adolescents in developed countries. While advanced cell therapies have transformed treatment, for some types of blood cancers and solid tumours there are no efficient CAR-T cell approaches available. We aim to develop cutting-edge research with direct clinical impact to improve childhood cancer prognosis by minimising side effects through the development of new therapies or the refinement of existing ones.

Our research lines include:

- → The use of haematopoietic stem cell transplantation as a platform for cell therapy.
- → The improvement of human stem cell progenitors transplantation.
- → The use of memory T lymphocytes as adoptive immunotherapy.
- → The use of both activated, memory-like NK Cells and CAR T cells in paediatric oncology.
- → The study of the efficacy and feasibility of cell therapy against infectious diseases.
- → The induction of immunological tolerance in solid organ transplantation by mixed chimerism through cell therapy.

"In 2024 we launched two world-first clinical trials employing NK cell therapy (SANKOMA) and CAR T-cell therapy (CAR4SAR) to address advanced sarcoma — an urgent, unmet clinical need. To date, 8 patients have been enrolled: 5 in the SANKOMA trial and 3 in the CAR4SAR trial."

RESEARCH HIGHLIGHTS

Our team unites healthcare professionals and researchers to address paediatric cancer in three areas: prevention, diagnosis, and treatment.

Prevention

Cancer development is influenced by environmental factors, particularly in genetically predisposed individuals. Ionising radiation, linked to leukaemia and CNS tumours, is the most significant in childhood. This project integrates genetics with environmental modulation to identify risks and develop personalised prevention and counselling strategies for the 10% of paediatric patients with genetic predispositions. The Paediatric Environmental Health Green Sheet is now digitised and routinely completed. In 2024, the Unit hosted a workshop with environmental healthcare providers at La Paz University Hospital (HULP) with the proposal to establish a Paediatric Environmental Health Unit specialised in paediatric oncology: "A Future of Comprehensive and Planetary Health for Childhood and Adolescent Cancer Survivors" for Environmental Health Day.

Diagnosis

We have implemented an integrative molecular profiling platform with three objectives: i) customised next-generation sequencing (NGS) at diagnosis and relapse (mut4Child) (Figure 1); ii) germline NGS for identifying cancer predisposition syndromes; and iii) evaluating liquid biopsy for diagnosis and monitoring in Retinoblastoma and CNS tumours. This programme has enhanced diagnostic and prognostic capabilities, disease monitoring, access to novel therapies at relapse, and early intervention for predisposition syndromes, delivering significant societal benefits.

Within the Unit, tumour sample sequencing has increased from 83 in 2023 (39 leukaemias, 44 solid tumours) to 144 in 2024 (48 leukaemias, 96 solid tumours). The mut4Child panel has been updated to include newly identified childhood cancer genes and those listed in the Ministry of Health´s portfolio of genetic services. With this project, we have been able to directly impact patient survival: at the end of 2024, we were able to successfully (and rapidly) detect the fusion FUS::TCFP2 in an adolescent girl with intracranial spindle cell rhabdomyosarcoma as a manifestation of a second tumour after a first diagnosis of AML at 6 months. The discovery of this targetable genetic alteration allowed us to design a personalised treatment with a third generation ITK inhibitor (lorlatinib) in combination with proton therapy that resulted in complete disease remission (January 2024).

Treatment

In 2024, our preclinical research had been translated into two clinical trials: SANKOMA, a Phase I/II, Multicentre, Open-Label Clinical Trial of Activated NK Cell Infusion for the Treatment of Children, Adolescents, and Young Adults with Sarcomas; and

FIGURE 1 (**A**) Mut4Child work pipeline and main goals. (**B**) Example of the fusion gene detected with mut4C custom panel, and the bioinformatics pipeline developed in the lab.

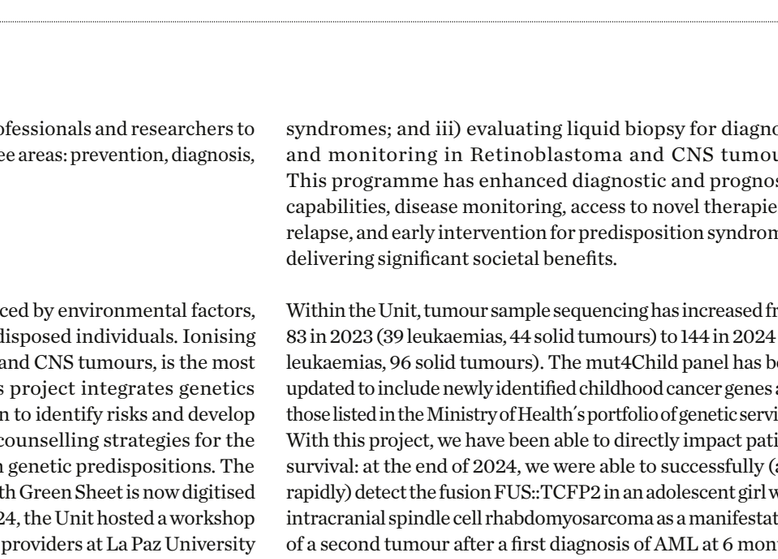
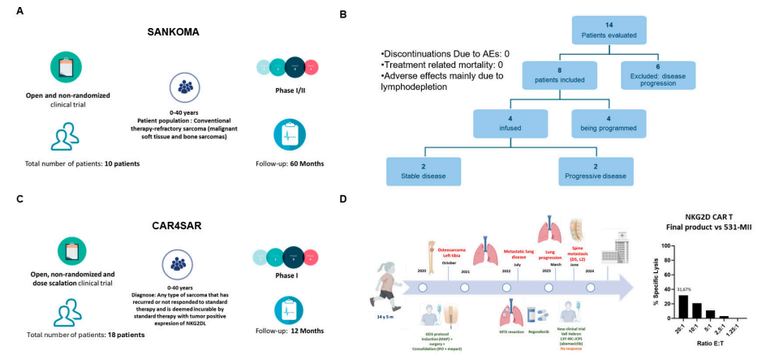


FIGURE 2 (**A**) SANKOMA study design. (**B**) Current status of the clinical trial at Hospital Universitario La Paz. (**C**) CAR4SAR study design. (**D**) Schematic representation of the first CAR4SAR patient clinical record before inclusion. Histogram shows specific cytotoxic capacity of the infused product (CAR T NKG2D).



CAR4SAR, a Phase I Trial of Memory T Cells Expressing an NKG2D Chimeric Antigen Receptor in Children, Adolescents, and Young Adults with Advanced Sarcoma.

The **SANKOMA** trial aims to recruit 10 patients with refractory sarcomas. To date, five patients have received activated, expanded NK cell infusions. Biological samples are being collected to advance understanding of NK cell behaviour *in vivo*, identify treatment success markers, and uncover immune evasion mechanisms in sarcomas. Preliminary findings have confirmed the feasibility and safety of haploidentical NK cell therapy combined with lymphoablative chemotherapy and low-dose radiation (2Gy) to tumours and metastases (Figure 2).

The **CAR4SAR** trial is a pioneering trial in adoptive cell therapy, using allogeneic T cells from first-degree relatives to create CAR T cells without the need for genetic engineering to prevent graft-versus-host disease. We use memory ($CD45RA^-$) T cells, less alloreactive than naïve T cells, simplifying donor selection and manufacturing. Given the limited success of CAR T cell therapies in solid tumours, this trial's clinical and biological findings could guide the optimisation of these therapies. It may also provide insights into effective, safe dosing and delivery methods to improve treatment strategies (Figure 2).

Under a compassionate use programme, we conducted a pioneering study in Europe on the safety and efficacy of tandem **anti-CD19/CD22 CAR T** cells in a cohort of 11 patients with relapsed or refractory B-cell acute lymphoblastic leukaemia who had received multiple prior treatments. Complete remission was achieved on day +28 post-infusion in 9/11 (81%) patients (8 with negative minimal residual disease, 72%), and 6/8 (75%) received HSCT as consolidative therapy within three months post-infusion. Two patients with early relapse after tandem anti-CD19/CD22 CAR-T therapy received rescue treatment and HSCT. At the 18-month follow-up, overall survival (OS) was 72%. This study has since been concluded and followed by the launch of the Corregir: REALL_CART clinical trial, which officially opened in June 2025. In addition, a new trial of solid organ induction by mixed haematopoietic chimerism using HSCT, DUALGRAFT, is expected to commence recruitment soon. ■

PUBLICATIONS

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- Ibáñez-Navarro M *et al.* (incl. Navarro-Zapata A, Mestre-Durán C, Ferreras C, Martínez-López J, Pérez-Martínez A) (2024). NKG2D-CAR memory T cells target pediatric T-cell acute lymphoblastic leukemia *in vitro* and *in vivo* but fail to eliminate leukemia initiating cells. *Front Immunol* 14, 1187665.
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AWARDS AND RECOGNITION

Antonio Pérez-Martínez:

- *III Edición Premios Imparables Sanitarios*. Honorary recognition to Antonio Pérez-Martínez for his role as "Unstoppable 2024" in the research category of Innovative Drugs and Therapies. Spain.
- Member of the Spanish Group for Transversal Oncology and Research in Orphan and Infrequent Tumors (GETTHI).
- Evaluation Committee on the Innovation of Human Cells and Tissues, as representative of the Spanish Network of Advanced Therapies and expert in the field. National Transplant Organization, Spanish Ministry of Health.
- Adriana Mañas received a Research Contract from the Miguel Servet Programme, Carlos III Health Institute, Spain.

Innovation

ROKE I. ORUEZABAL
Director of Innovation

Lola Pérez
Innovation Manager

Carlos Alonso
Innovation Facilitator

CNIO's Research Groups share a strong commitment towards innovation and public-private collaboration. Three relevant structures go hand-in-hand with researchers in order to increase the impact and generate innovative technological developments: the Biotechnology Programme, the Experimental Therapeutics Programme, and the Technology Transfer and Valorisation Office (TTVO).

International cooperation with the biotech and pharma industry is shown by the 269 transfer agreements (including research, licence, biological material and data transfer agreements) managed by the TTVO; 68% of these are with international entities. Besides the strengthened collaborations with pharmaceutical companies such as Loxo@Lilly (USA), Moderna Therapeutics (USA), Janssen (USA), and AstraZeneca (UK) (among others), the CNIO, with the Experimental Therapeutics Programme in the lead, was also successful in establishing and progressing alliances with foreign biotech companies and public research institutions, giving added value to the CNIO's small-compound library. This reflects the fact that 42% of the patents in CNIO's portfolio have been licensed out, an astonishing achievement for a public research institution. Once again, the CNIO broke another barrier thanks to its Biotechnology Programme, signing contracts with industry with a value over €1.1 million, especially in the monoclonal antibodies field.

The volume of collaborative research agreements signed in 2024 represents almost €2 million in revenue for CNIO. This increases the annual valuation of our collaborations, with net income from the licensing of CNIO assets in 2023 amounting to €1.7 million (9% increase). Among the new licence agreements signed in 2024, in addition to cell lines and antibodies, it is worth highlighting the continuation of strategic alliances with research foundations such as PETHEMA, as well as with companies such as L´Oreal, Moderna, Vega Oncotargets, Tempos Microscopy or Tailor Bio. Among the most outstanding achievements this year with the private sector are revenues of around €700,000 from the licensing of compounds generated by ETP to biotech companies, and the successful completion of a Phase I clinical trial with one of CNIO's compounds.

Currently, the CNIO's active patent portfolio comprises 43 patent families. In 2024, one intellectual property registration and one priority patent co-owned by ISCIII and CNIO were filed. In addition, one divisional patent has been applied for in the US by the patent licensee, seven patents entered the international phase (PCT), and seven patent families entered the national phase. As in previous years, we monitored both the possible inventions derived from the work of CNIO scientists and the Open Innovation Programmes of the main pharmaceutical companies in order to find potential matches.

With regard to the funding of research projects with a strong innovation component, it is worth noting that, in 2024, two projects of excellence in artificial intelligence (AI) were awarded funding from the European Union through NextGeneration EU as part of the State Plan for Artificial Intelligence. These projects focus on the market introduction of AI-based products and services: "Advancing AI for Precision Oncology", led by Geoffrey Macintyre, with a budget of €1,304,584.15; and "Implementation of the AI-PMPD Algorithm in Tertiary Hospitals for the Prediction of Pancreatic Cancer Metastasis and Real-Time Performance Demonstration", led by Núria Malats, with a budget of €793,505.98.

In addition, in 2025, the Spanish State Research Agency will provide funding for four new public-private collaboration projects co-funded by the European Union (ERDF): "Development of the First STAb Cellular Immunotherapy for Solid Tumors (STABSOLID)", led by Luis Álvarez Vallina in collaboration with STAB THERAPEUTICS SL, with a budget of €273,816.50 allocated to the CNIO; "Phase II Study in Patients with Newly Diagnosed Nodular Basal Cell Carcinoma (SPOTLIGHT 204)", led by Maria S. Soengas in collaboration with HIGHLIGHT THERAPEUTICS SL, with a budget of €991,311.10; "LUMICare: A Comprehensive Personalized Medicine Solution for the Care of Female Cancer Patients, Based on Dietary, Lifestyle and Emotional Well-being Interventions", led by Miguel Quintela in collaboration with EVIDENCE-BASED BEHAVIOR SL, with a budget of €351,796.50; and "FEMHEALTH: Precision Nutrition for the Improvement of Menopause-Associated Symptoms", led by Miguel Quintela in collaboration with PRECISION FORHEALTH SL, with a budget of €143,165.80. Furthermore, Fundación "la Caixa" awarded a grant of €150,000 to the Caixa*Impulse* innovation project proposed by Felipe Cortés and entitled "CRISPR-Cas Endonuclease Chain Reaction: A Revolution in Genetic Testing".

The Investors Day event, organised at the CNIO, showcased 10 CNIO projects to around 15 venture capital firms. As a result of this event, a framework collaboration agreement was signed with one of the venture capital firms, and communication channels were established with the remaining firms to keep them informed about the progress of the projects presented and to provide updates on new projects as they emerge.

The European Commission awarded the "Horizon Results Booster" grant to two of the Centre's innovation projects: MetPlatform and CRISPR-Cas Endonuclease Chain Reaction (ECR). This grant provided the following consulting services: BPD Business Plan Development; G2M Commercial Feasibility: Feasibility studies to assess potential business plans; and G2M Access to Non-European Funding.

Furthermore, in 2024, the CNIO established alliances with two key institutions within the innovation ecosystem. First, it formed a partnership with the Cluster for Technological Innovation and Talent in Biomedical and Biotechnological Technologies (CITT-BIO) of the Community of Madrid, through which the CNIO became a member of the Cluster. Secondly, the CNIO started a collaboration with EIT HEALTH by becoming a member of the Advisory Committee of InnPulso Salud. InnPulso Salud is an EIT HEALTH initiative aimed at fostering a culture of innovation and entrepreneurship in the healthcare sector in Spain.

Finally, the TTVO at the CNIO has been officially registered in the Registry of Knowledge Transfer Offices (KTO) of the Ministry of Science, Innovation and Universities, in accordance with Royal Decree 984/2022 of 22 November.

As a research institution of excellence, the CNIO has developed a strong commitment to innovation and public-private collaboration, a commitment that will impact our society in the form of new therapies and new hope for families. ■

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 new experimental procedures. The Programme consists of nine 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.

"Advances in cancer research run in parallel with technological developments. Access to cutting-edge technologies, assisted by expert staff, is key to guarantee the research excellence of the CNIO."

The necessary upgrade of CNIO's technological capabilities continues being one of the main areas where we focus our efforts. For instance, the Genomics Unit is now offering deep sequencing services using the Illumina NovaSeq X-Plus, the latest technology in the field, which was acquired in late 2023. Likewise, during 2024, a major investment was made in the acquisition of a state-of-the-art Thermo Astral mass spectrometry platform for proteomics analysis, which allows a much higher sensibility and analysis speed than precedent MS models. This new instrument allows the Proteomics Unit to engage with ambitious and complex procedures, such as plasma proteins analysis in large numbers of clinical samples, which are expected to have a significant impact in the development of future research projects at the Centre. Additionally, the Biotechnology Units participated in the call for infrastructures launched by the MICIU in 2024, obtaining financial support for the acquisition of essential innovative scientific infrastructure, such as a spectral cell sorter and a spatial biology platform based on fluorescence microscopy for multiplex detection of markers on tissue samples.

The Programme is also very active in attracting funding from external sources, including contracts and agreements with private companies and public institutions owing to our innovation related activities based on the technologies mastered by our Core Units. For example, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit continue representing a relevant funding source for the CNIO. This year the total income derived from these licenses was again close to €1.5 million - an impressive achievement placing the CNIO as a true reference point in this field worldwide.

Training activities are also an essential part of our mission. Beyond training new CNIO staff in a broad array of technologies (e.g. flow cytometry, advanced microscopy, image analysis, animal experimentation, etc.), our Core Units hosted as many as 15 external students in different steps of their academic career, who stayed as interns at the Centre for variable periods of time.

Last but not least, 2024 was again a very productive year for the Programme in terms of scientific achievements. The contribution of the Core Units to the overall research performance of the CNIO is reflected in nearly 30 publications co-authored by members of the Units, many of them in top journals. ■

PROTEOMICS CORE UNIT

Marta Isasa
Core Unit Head

Technicians
Fernando García (TS)*, Laura C. Woods (TS)*, Pilar Ximénez de Embún (until September) (TS)*, Eduardo Zarzuela (TS)*

**Titulado Superior* (Advanced Degree)

Master's Student
Esther Miguelez (May-July) (Maastricht Univ., The Netherlands)

OVERVIEW

Proteomics has become a cornerstone of modern biological research, offering dynamic insights that extend beyond genomics. Advances in mass spectrometry, bioinformatics, and data analysis have transformed proteomics into a powerful tool for deciphering disease mechanisms, identifying biomarkers, and discovering therapeutic targets. By systematically navigating the proteome's vast complexity, researchers can uncover hidden layers of protein expression, modifications, and interactions, providing a comprehensive view of cellular processes and their regulation.

The CNIO Proteomics Core Unit is at the forefront of this transformation, leveraging cutting-edge technologies to tackle fundamental questions in cancer biology. The recent acquisition of the Orbitrap Astral mass spectrometer marks a major milestone, delivering unprecedented sensitivity, depth, and throughput for large-scale, high-resolution proteomic studies. Through close collaboration with CNIO investigators, we integrate quantitative proteomics, functional analyses, and advanced bioinformatics to drive high-impact discoveries that advance cancer research and precision medicine.

"Within the complexity of the proteome lies the key to understanding life itself"
– Ruedi Aebersold, ETH Zurich.

RESEARCH HIGHLIGHTS

Streamlining requests: a new integrated system

In 2024, the CNIO Proteomics Core Unit implemented a new intranet request form, improving efficiency and communication between users and the Unit. This system ensures that all required information is submitted in a standardised and structured manner, ensuring seamless coordination and real-time tracking of incoming entries.

Optimising acquisition methods for the Orbitrap Astral

The CNIO Proteomics Core Unit has optimised Orbitrap Astral acquisition methods to enhance sensitivity, reproducibility, and throughput. Method development included gradient optimisation, column selection, and phosphopeptide quantification, ensuring robust performance across diverse samples. In global proteomics, over 9,000 protein groups are identified within a 1-hour gradient. For phosphoproteomics, the Astral achieves a 3-fold increase, quantifying 40,000+ phosphorylation sites from just 100 µg of lysate (Figure 1). These advancements expand dynamic range, improve accuracy, and strengthen quantitative reliability, solidifying the Astral as a next-gen platform for cancer proteomics.

Automating data processing and streamlining analysis

The Orbitrap Astral's high speed, sensitivity, and deeper proteome coverage generate exponentially larger datasets, requiring more efficient processing. In response, the CNIO Proteomics Core Unit is automating data processing and refining analysis pipelines. Efforts focus on reducing downtime after data acquisition and enhancing pipelines with automated normalisation, statistical validation, and visualisation tools for quantitative proteomics and interactomics. These initiatives are ongoing, with continuous improvements to boost efficiency, scalability, and reproducibility. ■

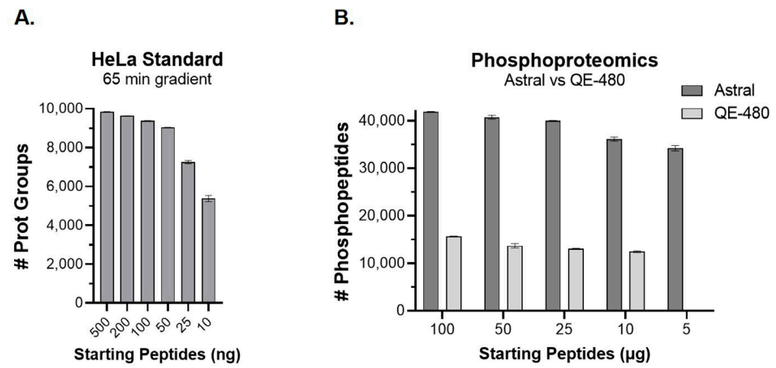

FIGURE 1 Enhanced proteome and phosphoproteome coverage with the Orbitrap Astral. (**A**) Protein identification in HeLa standard samples using different amounts of starting peptides. Data were acquired using an Orbitrap Astral mass spectrometer with a 65-minute gradient. (**B**) Comparison of phosphopeptide identification between the Orbitrap Astral and Orbitrap QE-480 mass spectrometers using different amounts of starting HEK293T peptides.

PUBLICATIONS

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GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Laura Conde, Rodrigo Gómez, Guadalupe Luengo, Jorge Monsech, Ángeles Rubio

OVERVIEW

The Genomics Unit offers centralised research services and expert consultation in the expansive fields of genomics and genetics. These services cover a broad range of applications, from traditional to the latest genomic technologies, and contribute to uncovering biological complexity, therapeutic targets, and prognostic biomarkers. By leveraging their capacity to scrutinise entire genomes and their activities, these technologies can reveal the full spectrum of structural and functional features. This includes mutation landscapes, protein binding to chromosomal locations, understanding chromatin structure, and deciphering molecular programmes such as transcriptomic RNA profiles, either in bulk within a tumour or at the single-cell level. Next-generation sequencing (NGS) is a mainstay of these applications, while more traditional methodologies like Sanger capillary DNA sequencing are also offered. In addition, the Unit manages a genotyping service for genetic models.

"Both of our generic and boutique services in the fields of genomics and genetic technologies are adapted to meet the needs of CNIO scientists, from basic housekeeping activities to advanced explorations of biological complexity."

RESEARCH HIGHLIGHTS

The Genomics Unit is a specialised laboratory that provides researchers with access to advanced genomic technologies and expertise. It plays an important role in supporting a wide range of scientific research, from basic biological studies to clinical applications. With its array of molecular services, the Unit contributes to the dissection of biological complexity and the understanding of the genetic basis of diseases. It employs next-generation sequencing (NGS) technologies for various purposes, both at the structural and functional levels. For structural analysis, we perform genome or exome tumour characterisations, mutation repertoire interrogations, analysis of binding sites for relevant protein factors in chromosomal DNA and variations in chromatin folding, and exploration of on/off functions. For functional analysis, transcriptional profiles are investigated to decipher tumour compositions, uncover therapeutic targets, assess response to treatment, and predict disease course.

Several technologies to study the heterogeneity of the transcriptome of individual cells are available. Both cells in suspension and tissue sections can be analysed. The analysis of cell suspensions provides higher resolution and detects more genes, and therefore markers, than the analysis of solid tissue sections. For this application, two variants of microdroplet, emulsion-based, single-cell analysis are implemented, each capable of analysing up to tens of thousands of cells from a given sample: the dominant 10xGenomics platform and the more affordable PIPseq, from Fluent BioSciences/Illumina. For spatial transcriptomics, a term coined to describe the determination of single cell transcriptomes in tissue sections, the Visium HD spatial gene expression platform from 10xGenomics is used.

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

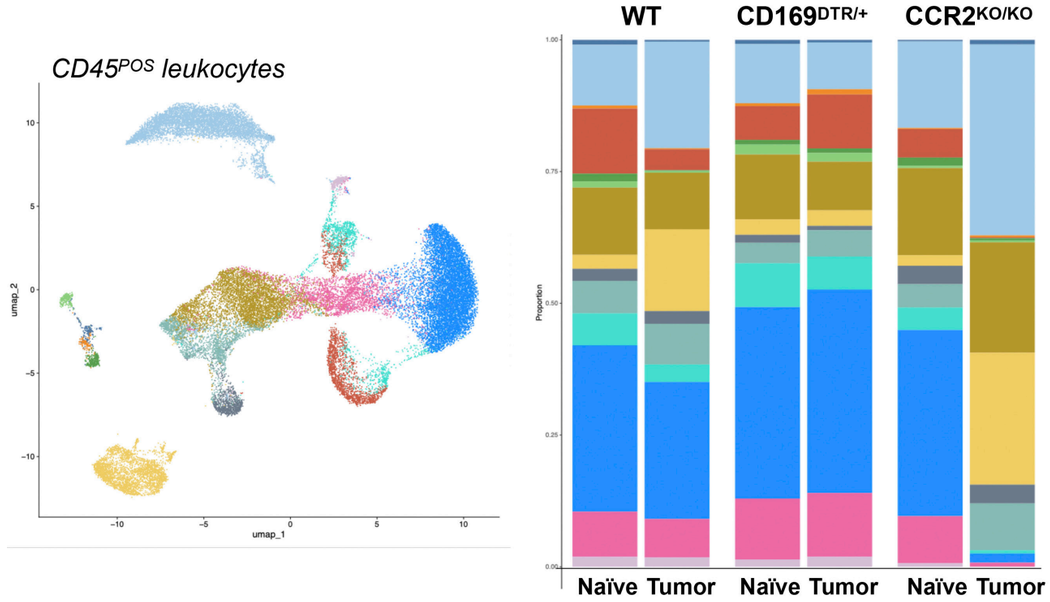

FIGURE 1 Haematopoietic cells from lung tumours by single-cell transcriptomic analysis. An early sample fixation procedure was used to preserve neutrophils and granulocytes. The plot on the left shows the different immune cell clusters, with each dot representing an individual cell grouped by its own gene expression profile. The graph on the right shows the fractions of different clusters in mice with different treatments and genotypes. Data kindly provided by E. Garvin from the Casanova-Acebes laboratory at the CNIO.

CONFOCAL MICROSCOPY CORE UNIT

Isabel Peset
Core Unit Head

Technicians (TS)*
Ana Cayuela, Jesús Gómez, Manuel Pérez

Titulado Superior (Advanced Degree)

OVERVIEW

One of the greatest challenges in oncology research is the detailed study of specific markers, expression patterns, and individual cells in the tumour and its microenvironment. Optical microscopy has become a key technology in the field to boost our understanding of the cellular and molecular mechanisms driving cancer, as well as to explore potential therapeutic strategies.

The Confocal Microscopy Unit (CMU) provides scientific advice and technical support through cutting-edge imaging technologies, focusing on cancer biology and other biomedical research areas. CNIO research groups have access to state-of-the-art equipment and a High-end BioImage Analysis platform.

"The CMU is committed to providing a deep understanding of molecular mechanisms involved in tumour progression and treatment responses by applying advanced microscopy methods."

Our team is also dedicated to the development and implementation of innovative imaging techniques that can have an impact on CNIO scientific projects. Training and science dissemination are key elements of our mission. We organise courses, seminars, and lab visits aimed at advancing optical microscopy culture.

Students in Practice
Daniel Anchuela (since October) (Master's Thesis, Urv Etse, Tarragona, Spain), Dayanhara Tarazona (April-June) (Technicians in Training (FCT), IES José Luis Sampedro, Madrid, Spain), Lorena Villafruela (April-June) (Technicians in Training (FCT), IES Jaime Ferrán Clúa, Madrid, Spain)

RESEARCH HIGHLIGHTS

In 2024, the Confocal Microscopy Unit (CMU) has continued to provide imaging solutions to support research across the CNIO, with a particular emphasis on expanding the **BioImage Analysis** capabilities. The team has played a pivotal role in various scientific questions, contributing highly valuable insights by integrating cutting-edge imaging technologies such as laser scanning confocal (Leica Sp8 and Sp5), live-cell imaging (Leica Thunder), super-resolution (Leica STED-SP8), and high-content (Revvity Opera Phenix Plus) together with advanced image analysis tools (https://github.com/cnio-cmu-BioimageAnalysis/).

One of the most significant accomplishments this year was the integration of immunofluorescence multiplexing techniques and the development of complex image analysis workflows for comprehensive tissue analysis. In collaboration with the Histopathology Unit, we optimised automated multiplex staining protocols, allowing the visualisation of 6 cellular markers within the same tissue section. Additionally, we have explored the High-Plex immunofluorescence techniques for the simultaneous visualisation of 20-30 cellular markers through hosting a spatial biology platform. The true value of these high-dimensional imaging datasets has come through the development of powerful deep learning algorithms tailored to extract both quantitative and spatial information.

Altogether, the Unit's efforts have contributed to improving the CNIO BioImage Analysis capabilities, implementing high-performance computing resources and developing powerful advanced image analysis pipelines, with a special focus on spatial biology analysis. Through the application of these methodologies, we can map how cellular markers are distributed within the tissue, revealing insights into the tumour heterogeneity and the spatial relationships between different cell types. ■

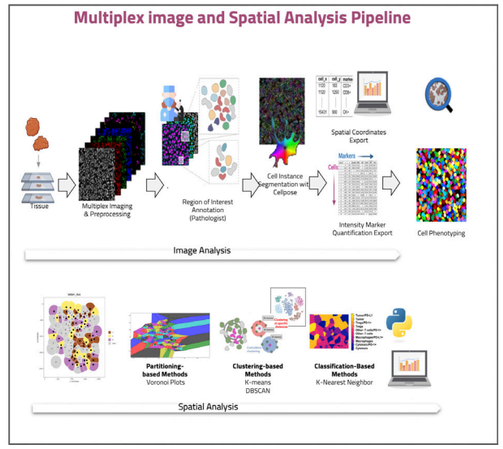

FIGURE 1 Advanced image analysis of multiplex or high-plex stained images allows for the identification of distinct cell populations, their spatial distribution, and interactions within the tissue. Extracting these spatial and molecular signatures helps to uncover tissue heterogeneity, revealing patterns of cellular organisation and disease-specific features.

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MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Post-Doctoral Fellow
Bárbara Beatriz Báez (since April)

Technicians
Tatiana Álvarez, Guillermo Garaulet (TS)*, Guillermo Medrano (TS)*, Walter Navidad (since May), Luis Ordoñez, Judith Rey (until May) (TS)*, Gloria Visdomine

**Titulado Superior* (Advanced Degree)

OVERVIEW

Molecular imaging is a cutting-edge medical imaging technique that enables the visualisation, characterisation, and measurement of biological processes at the molecular and cellular levels in living organisms. By integrating principles from chemistry, biology, physics, and advanced technology, molecular imaging offers a detailed understanding of the biochemical and physiological processes in tissues and organs. Preclinical molecular imaging techniques are non-invasive, allowing researchers to track disease progression and treatment responses in the same animal subjects over time.

"Molecular imaging is a transformative tool in biomedical research, giving scientists insights into the molecular and cellular processes *in vivo*."

The Molecular Imaging Unit at CNIO provides broad support to research groups, offering state-of-the-art equipment and technologies. These tools are essential for early disease detection, monitoring disease progression, evaluating treatment effectiveness, and deepening our understanding of disease mechanisms at the molecular level - ultimately advancing medical research and improving patient outcomes.

RESEARCH HIGHLIGHTS

Leading-Edge Technology

The Molecular Imaging Unit leads in advanced imaging technologies, assisting CNIO Research Groups with state-of-the-art molecular imaging techniques. Our equipment includes:

- → A micro-PET-CT (Positron emission Tomography) system (eXplore Vista) from GE.
- → A CT (computer tomography) (CompaCT) from SEDECAL.
- → Two ultrasound systems (Vevo 3100) from Fujifilm VisualSonics.
- → A densitometer system (Lunar PixiMus) from GE.
- → Two optical imaging devices (IVIS Lumina III) from PerkinElmer.

Expert Team

Our dedicated team of imaging experts provides comprehensive imaging analysis ranging from basic to highly complex studies. We also offer hands-on training in optical imaging, ensuring that our users are proficient operatives with these advanced technologies.

Innovative Research

Our Unit actively supports ground-breaking research and continuously develops new imaging tools through funded research projects, maintaining technological leadership in the field. Current projects include:

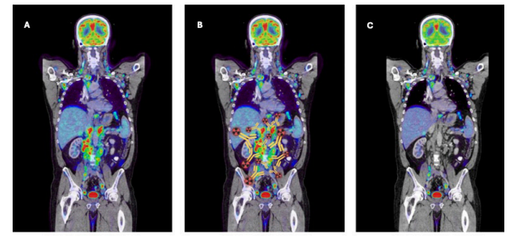

FIGURE 1 Scheme of theragnosis with radiolabelled antibodies. **A**: ImmunoPET Imaging with 68Ga labelling Ab showing multiple cervical, thoracic and abdominopelvic metastatic dissemination. **B**: Scheme of the beta emitter 177Lu targeting the same lesions visualised at the ImmunoPET imaging. **C**: ImmunoPET imaging with 68Ga showing the decrease in the number of metastases after radioimmunotherapy.

- → Comunidad de Madrid Grant (RENIM 2): A collaboration with top centres in molecular imaging and nanomedicine, this project focuses on developing and optimising molecular imaging probes, particularly for PET and optical imaging. It aligns with cutting-edge trends in medical research.
- → EU Infrastructures grant of a 3T MRI System: Thanks to a Next Generation EU infrastructures grant, the acquisition of a 3T MRI system will significantly enhance CNIO's research capabilities.

These projects highlight our commitment to innovation and collaboration in medical imaging and cancer research. The combination of advanced equipment, partnerships with leading research institutions, and pioneering techniques like ImmunoPET (Figure 1) firmly establishes our Unit as a leader in the molecular imaging field. ■

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AWARDS & RECOGNITION

- 'The Bill Eckelman Award for the best Nuclear Medicine and Biology Paper 2024', Elsevier, The Netherlands: *Target engagement of an anti-MT1-MMP antibody for triple-negative breast cancer PET imaging and beta therapy*.
- Scientific Advisor of the Distributed Network of Biomedical Imaging (*REDIB ICTS*), Spain.
- Faculty and Mentor of IDEA2 and Catalyst 2024 Programmes with MIT (Massachusetts Institute of Technology, USA).
- CDTI Technology Expert, Centro de Desarrollo Tecnológico Industrial, Spain.

MOUSE GENOME EDITING CORE UNIT

Sagrario Ortega
Core Unit Head

Technicians
Beatriz Escobar (TS)*, Carmen Gómez, Melani Margullón, Jaime Muñoz (TS)*, Patricia Prieto (TS)*, Pierfrancesco Vargiu (TS)*

Titulado Superior (Advanced Degree)

OVERVIEW

Cancer encompasses a diversity of complex diseases that arise from genetic and epigenetic alterations in cells, leading to the development of malignant traits that enable these cells to evade normal physiological control. Genetic factors related to the function of different processes in the body such as metabolism, circulation, immunity and ageing, determine, in many cases, susceptibility to cancer development and therapeutic outcome. In addition, lifestyle factors like diet and smoking also affect cancer incidence. To address this intricate complexity, reliable genetically modified animal models offer a holistic "whole-body" context for studying cancer. The laboratory mouse is the best animal model to study the genetic basis of cancer, both because of its similarity to humans and for its versatility for genetic manipulation. Precise and targeted manipulation of the mouse genome, using state-of-the-art genome editing tools, enables the creation of genetic models that are pivotal for understanding the molecular basis of tumourigenesis and for the preclinical evaluation of innovative and more effective cancer therapies.

"The Unit's expertise in the creation of genetic mouse models contributes significantly to the quality and impact of scientific research at the CNIO. These models play a critical role in advancing the understanding of cancer and the development of therapeutic strategies."

RESEARCH HIGHLIGHTS

In 2024, the Unit made significant contributions to two publications cited below. In collaboration with the Cell Division and Cancer Group led by Marcos Malumbres, we generated two conditional loss-of-function models for the *Ppp2r2a* and *Ppp2r2d* genes encoding two of the four B-regulatory subunits of the PP2A-B55 phosphatase, B55α and B55δ, respectively. PP2A-B55 is the major CDK1 counteracting phosphatase in cells during mitotic exit, and is important in the regulation of entry into mitosis. The B55α and B55δ subunits are widely expressed in tissues, whereas the expression of the other two subunits, B55β and B55γ, is mostly restricted to the brain. A previously generated embryonic stem cell (ESC) clone (EPD0328_1_G08; KOMP repository) was injected into C57Bl6 blastocysts to generate conditional floxed mice for Ppp2r2a. For Ppp2r2d _lox allele generation, a targeting vector was used to flox exon 3 of the Ppp2r2d gene by electroporation into V6.4 (129 x C57Bl6J) ESCs. These two conditional models have shown that these two B55 isoforms (α and δ) play overlapping roles in cell proliferation, controlling chromosome individualisation and clustering during mitosis (Sanz-Flores *et al.* 2024).

In collaboration with the Growth Factors, Nutrients and Cancer group led by Nabil Djouder, the Unit has contributed to the study of the role of the unconventional prefolding RPB5 interactor (URI), a component of the nutrient and growth factor signalling cascade in early mouse embryo development. The generation of conditional overexpression and loss-of-function mouse models, together with the fine-tuning of URI expression in embryos and ESCs by different methods, has led to the identification of a critical role for URI in the transition from totipotency to pluripotency (Figure 1). URI interacts both with the pluripotency factors Sox2 and Oct4, preventing their proteosome-mediated degradation, and with the retroviral-encoded protein MERVL-gag expressed in the 2-cell stage embryo. Binding of MERVL-gag to URI releases Sox2 and Oct4 from the complex, exposing them to the proteasome. Therefore, URI plays a central role in determining cell fate in the early embryo, at least in part by modulating the availability of critical developmental factors (De la Rosa *et al.* 2024). ■

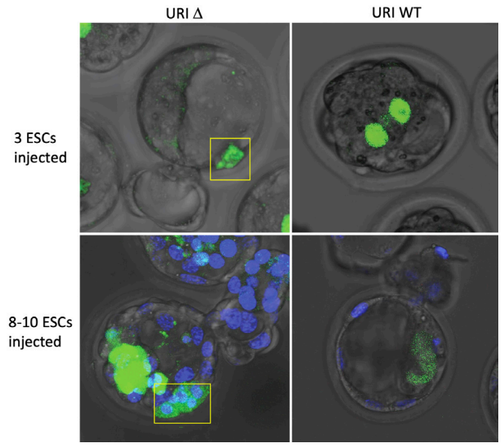

FIGURE 1 The absence of URI enhances the totipotency properties of ESCs. The chimera formation assay shows the increased capacity of the URID ESCs (GFP⁺) to contribute to both the trophectoderm (yellow rectangles) and the inner cell mass in the embryo. Two different conditions are shown: 2-3 cells or 8-10 cells injected in 8-cell embryos.

PUBLICATIONS

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- Sanz-Flores M, Ruiz-Torres M, Aguirre-Portolés C, El Bakkali A, Salvador-Barberó B, Villarroya-Beltri C, Ortega S, Megías D, Gerlich DW, Álvarez-Fernández M, Malumbres M (2024). PP2A-B55 phosphatase counteracts Ki-67-dependent chromosome individualization during mitosis. *Cell Rep* 43, 114494.
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MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
Scherezade Jiménez-Villa, Lorena Maestre (TS)*, Ana I. Reyes, Carmen San Juan (since February), María Villa (until April)

**Titulado Superior* (Advanced Degree)
***Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

OVERVIEW

Monoclonal antibodies (mAbs) represent a major breakthrough in biomedical science, including providing precise and versatile tools for cancer research. Their high specificity and reproducibility have made them indispensable for advancing our understanding of cancer cell biology. Beyond their research applications, mAbs play a critical role in oncology, contributing to accurate cancer diagnosis and the development of targeted therapies that improve patient treatment and outcomes.

The Monoclonal Antibodies Unit at CNIO specialises in the generation of mAbs utilising hybridoma technology, especially in the production of high-quality antibodies in mice and rats. We are specialised in mAb characterisation and validation to ensure optimal specificity, sensitivity, and performance of the mAbs in different applications (immunohistochemistry, flow cytometry, Western blotting, and immunofluorescence). We also offer medium-scale mAb production to support advanced research applications. Additionally, the Unit performs *Mycoplasma* testing for the cell culture facility and generation of recombinant antibodies.

"The Monoclonal Antibodies Unit is an essential resource for CNIO research groups, offering specialised expertise in mAb production and validation, and providing the necessary tools for innovative research and a deeper understanding of cancer cell biology."

RESEARCH HIGHLIGHTS

During the last 24 years, the Monoclonal Antibodies Unit has generated a large number of mAbs, directed against more than 250 different antigens, mostly targeting molecules for which mAbs are not commercially available. Many of these mAbs (67) 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 140 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:

Optimising antibody production: the shift to recombinant technologies. The Monoclonal Antibodies Unit is expanding its portfolio by incorporating the capability to convert hybridoma-based antibodies into a recombinant format. This strategic advancement represents a significant step forward in our commitment to excellence, enabling us to enhance antibody production with greater precision, efficiency, and reliability. The transition to recombinant technology offers several key advantages that align with our commitment to delivering high-quality mAbs. One of the most significant benefits is the improvement in production consistency and scalability. Unlike traditional hybridoma methods, which can be prone to genetic drift and variability over time, recombinant antibodies ensure batch-to-batch reproducibility, leading to a more stable and uniform product. This enhanced control over production parameters then translates into superior performance in research, diagnostic, and therapeutic applications. Additionally, this approach supports ethical and sustainable practices by minimising reliance on animal-derived materials.

Implementing antibody sequencing at CNIO. The Monoclonal Antibodies Unit aims to establish antibody-sequencing capabilities in collaboration with the Genomics and Bioinformatics Units at Cnio. Implementing this technology offers numerous advantages, including the ability to preserve and reproduce valuable antibody clones, optimise antibody engineering, and facilitate the transition to recombinant production. Additionally, sequencing enhances quality control by ensuring genetic stability and identifying potential mutations that could impact performance. By integrating antibody sequencing at Cnio, we will strengthen our research and development capabilities, enabling the creation of high-quality, customised antibody solutions to support a wide range of biomedical applications. This initiative strengthens our commitment to innovation and collaboration in advancing antibody technologies. ■

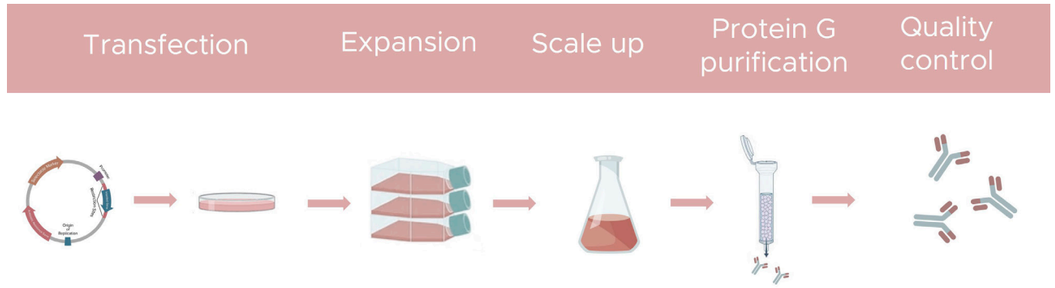

FIGURE 1 Schematic diagram illustrating the essential stages involved in the production process of recombinant antibodies.

PUBLICATIONS

- Menéndez V *et al.* (incl. Roncador G) (2024). Mapping the spatial dynamics of the CD4+ T cell spectrum in classical Hodgkin lymphoma. *Mod Pathol* 37, 100551.
- Monteagudo M *et al.* (incl. Caleiras E, Rodríguez-Perales S, Roncador G, Currás-Freixes M, Al-Shahrour F, Robledo M) (2024). MAML3-fusions modulate vascular and immune tumour microenvironment and confer high metastatic risk in pheochromocytoma and paraganglioma. *Best Pract Res Clin Endocrinol Metab* 38, 101931.
- Carreras J, Roncador G, Hamoudi R (2024). Ulcerative colitis, LAIR1 and TOX2 expression, and colorectal cancer deep learning image classification using convolutional neural networks. *Cancers (Basel)* 16, 4230.
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- Felipe I *et al.* (incl. Martínez-Torrecuadrada J, Roncador G, Muñoz J, Real FX) (2024). BPTF cooperates with MYCN and MYC to link neuroblastoma cell cycle control to epigenetic cellular states. *BioRxiv*. doi: 10.1101/2024.02.11.579816.

HISTOPATHOLOGY CORE UNIT

Vacant
Core Unit Head

Research Scientist
Eduardo José Caleiras

Technicians
Nuria Cabrera, Carlos Garrido (since February), María Gómez, Patricia González, María Martínez (July - October), Verónica Neva (until November), Zaira Vega

Students in Practice
Mario Fernández (April-June)
(Technicians in Training (FCT), IES Antonio Machado, Madrid, Spain), María Martínez (April-June) (Technicians in Training (FCT), IES Rosa Chacel, Madrid, Spain)

OVERVIEW

Pathology is the branch of science focused on studying the structural, biochemical, and functional changes in cells, tissues, and organs that underlie disease processes. The Histopathology Unit provides comprehensive support and expertise across a wide range of services, including paraffin embedding, tissue sectioning, histochemical staining, and both research and diagnostic immunohistochemistry (IHC). Additional capabilities include antibody validation and *in situ* hybridisation techniques — such as RNA *in situ* detection using RNAScope® and BaseScope® — as well as tissue microarray construction.

"The pathological analysis of mouse and human tissues carried out by the Unit, using a broad array of histochemical and immunohistochemical techniques, is essential for the advancement of oncology research projects conducted at the CNIO."

The Unit's highly skilled technicians also offer value-added services such as laser capture microdissection, slide digitisation, image analysis, and quantitative assessments. Furthermore, the Unit collaborates closely with CNIO researchers in the histopathological characterisation of disease models in animals, offering expert pathological guidance. Its full portfolio of services is also available to external institutions, including hospitals, research centres, and private companies.

RESEARCH HIGHLIGHTS

In 2024, the Unit increased its workload compared to the previous year. The number of histological techniques performed surpassed 15,000 - representing a 10% increase over 2023. Similarly, around 15,000 immunohistochemistry (IHC) techniques were conducted, 20% of which involved double or triple immunostaining.

As many as 70 new antibodies were optimised and made available to users, expanding the Unit's portfolio to over 925 antibodies suitable for IHC staining in mouse, human, and xenograft tissues. Additionally, a total of 14,400 slides were digitised during 2024, marking a 5% increase from 2023. Approximately 15% of this digitised material was analysed with the support of the Unit, which also provides training to researchers in the use of various image analysis software platforms.

In 2024, we also consolidated the use of *in situ* hybridisation technologies focused on mRNA detection in paraffin-embedded tissue sections through the RNAScope® platform. A total of 454 cases were analysed — 20% more than the previous year — some of them involving double staining using the Ventana-Roche automated IHC platform.

The Unit continued participating in the project aimed at developing a system for the treatment of atrial fibrillation through irreversible electroporation, in partnership with the company MedLumics and the *Universitat Pompeu Fabra*. The Unit contributes to this project by analysing the pathological features and mechanisms underlying cell death in cardiac tissue following ablation procedures.

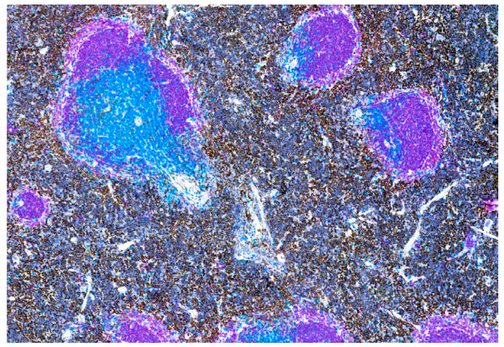

FIGURE 1 Example of triple IHC staining: In the white pulp of the spleen, T lymphocytes are identified by CD3 staining (Teal, blue), and B lymphocytes by CD45R staining (purple). In the red pulp, macrophages are detected using F4/80 immunostaining (DAB, brown).

The high quality of the Unit's techniques continues to be validated by External Quality Assessment Schemes. Our histochemical techniques were evaluated by UK NEQAS, while a subset of IHC techniques were assessed under various modules by NordiQC and the Spanish Society of Pathology (SEAP), all receiving excellent evaluations.

The Unit is also engaged in training activities. This year, we hosted one vocational training student for a three-month practical placement. Additionally, members of the Unit participated in two Master's courses focused on oncological research. ■

PUBLICATIONS

- Priego N *et al.* (incl. Caleiras E, Al-Shahrour F, RENACER, Valiente M) (2024). TIMP1 mediates astrocyte-dependent local immunosuppression in brain metastasis acting on infiltrating CD8+ T cells. *Cancer Discov*. PMID: 39354883.
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- Plata-Gómez AB *et al.* (incl. Caleiras E, Graña-Castro O, Muñoz J, Sabio G, Efeyan A) (2024) Hepatic nutrient and hormone signaling to mTORC1 instructs the postnatal metabolic zonation of the liver. *Nat Commun* 15,1878.
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- García-Sancha N *et al.* (incl. González-García P, Caleiras E, Peset I) (2024) Cabergoline as a novel strategy for post-pregnancy breast cancer prevention in mice and human. *Res Sq* [Preprint]. PMID: 38405932.

FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

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

**Titulado Superior* (Advanced Degree)

OVERVIEW

Flow cytometry is a fast and multiparametric technology, indispensable in the field of cancer. It allows for the identification, quantification and isolation of defined subpopulations, based on the expression levels of fluorescent markers at the single cell level, as well as to measure quantitatively cell proliferation and cell death rates upon genetic modifications or drug treatments.

Our aim is to provide CNIO Groups with technical and scientific advice on the use of flow cytometry needed to answer their biological questions, developing new methodologies or pipelines when needed.

We currently have 4 analysers and 3 high-speed cell sorters with different optical configurations to cater to user needs, plus other small instruments to help standardise sample preparation. We offer extensive training so users can independently operate the analysers, while the Unit staff operate the cell sorters, which can separate up to 4- or 6- defined populations simultaneously, depending on the nozzle type, as well as perform single cell cloning and index sorting. We can accept human samples to sort under BSL2 regulations.

"Thanks to a collaboration agreement with Becton Dickinson, we installed a FACS Discover S8 in September, which allows us to visualise cells of interest in real-time and sort out subsets based on correlation of fluorescent markers plus imaging features."

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators setting up and optimising flow cytometry techniques relevant to their research projects. 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 Potential, 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.
- → Platelets studies.
- → Extracellular vesicles detection (microvesicles and exosomes).
- → CTC detection and isolation.
- → Single cell sorting for OMICs analysis.

We further advanced projects involving cytometry within the CNIO community with the implementation of a FACS Discover S8 in September, thanks to a collaboration agreement with Becton Dickinson. This cell sorter, a disruptive new technology that combines spectral cell sorting with imaging capabilities, will improve several current lines of research. In CAR-T research, we can look at the immune synapses and sort cells for further characterisation. Additionally, we will open new avenues of research through better identification and more precisely sorting single cell subtypes, thanks to the possibility of visualising the cells of interest in real time, while looking at changes in morphology, co-localisation, internalisation and/or aggregation.

We continued to optimise our multicolour flow cytometry panels, incorporating new dyes with improved performance for characterising the immune response in various samples from haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Also, single cell deposition using index sorting into 96 or 384 PCR plates to do single OMICs techniques is now part of our routine portfolio. We continue to expand our training capacities, with many more workshops and small practical analysis sessions to provide our users with more tools to successfully perform their flow cytometry experiments. ■

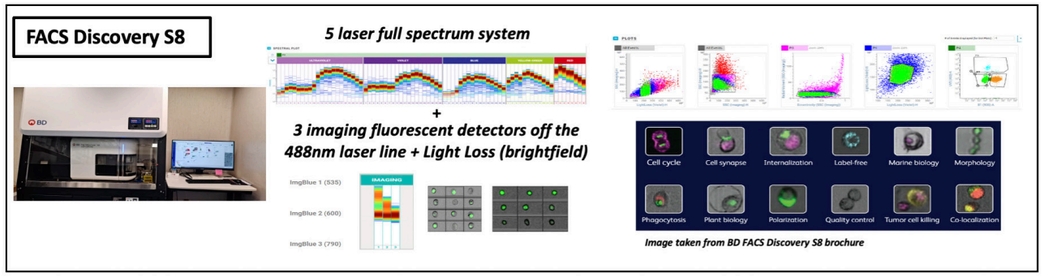

FIGURE 1 The power of the full spectrum system, together with the imaging features available on the FACS Discover S8, opens new avenues for deciphering tumour heterogeneity and profiling immune responses.

PUBLICATIONS

- Pastor-Fernández A *et al.* (incl. Efeyan A, Martinez L) (2024). Sexual dimorphism in the antitumor immune responses elicited by the combination of fasting and chemotherapy. *Cancer Commun (Lond)* 44, 508-513.
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AWARDS AND RECOGNITION

- Lola Martínez was invited to run the Spectral Cytometry course sessions at the prestigious International Annual Course in Cytometry (47th edition) in Madison, Wisconsin (USA).
- The Unit has been awarded an infrastructure grant (EQC2024-007953-P) – funded by the National Science Agency (AEI/10.13039/501100011033/), the Spanish Ministry of Science, Innovation and Universities, and the EU through FEDER – to acquire a spectral cell sorter.

ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services

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

"Excellence in science when using animal experimental models must be based on the upmost respect to animal welfare."

The Animal Facility provides CNIO researchers with all the necessary support to work with mouse models while ensuring the highest standards of animal care and welfare. It was established to assist researchers in the development and analysis of *in vivo* models as tools for cancer research. Currently, the Facility collaborates with approximately 30 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 – concerning 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 Animal Facility also offers new CNIO staff a training course focused on working with laboratory animals, complementary to the legally required online courses (Orden ECC/566/2015) needed to access the facility. In addition, a new course has been established in collaboration with ISCIII and CNIC, aimed at providing ongoing training for users, to ensure improved animal management and regulatory compliance.

The high standards achieved by the CNIO, in the use and care of animals for experimentation, are recognised by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, a private, non-profit organisation that promotes the humane treatment of animals in science, through voluntary accreditation and assessment programmes. AAALAC accreditation, one of the highest international recognitions in this field, was first obtained in October 2016. Since 2022, the Head of the Animal Facility has served as an AAALAC Ad Hoc Consultant, assisting Council members on Accreditation in evaluating animal care and use programmes.

As part of its commitment to maintaining the highest possible standards in 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) and the Spanish Society for Laboratory Animal Sciences (SECAL), launched in September 2016. An institutional statement on the use of animals for research is available on the CNIO website.

The Head of the Animal Facility also serves as Coordinator of the Spanish Animal Welfare Body Network (ROEBA) and is the designated Spanish contact person for the European Network of Animal Welfare Bodies (ENAWB). This European network, endorsed by FELASA and supported by the European Commission, is dedicated to fostering a culture of care and promoting the ethical and humane treatment of animals.

The Animal Facility has the capacity to house 19,000 type IIL cages. Mouse lines are maintained and bred in the Facility's barrier area, ensuring a Specific Pathogen-Free (SPF) health status. Microbiological and environmental parameters in the animal areas are continuously monitored. There is also an additional area with a capacity for 1,800 type II cages, designated 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 Individually Ventilated Caging (IVC) units integrated into the building's ventilation system. Mice are always handled in Type II biosafety cabinets.

Daily operations and husbandry procedures are highly automated to protect personnel from potential risks. Robotic devices handle tasks such as processing dirty bedding, washing and filling cages and bottles, among others. These automated systems maximise productivity and ensure consistent quality standards. All records related to breeding protocols and animal inventory are computerised and stored in a customised web-based application accessible via the CNIO intranet.

The Animal Facility currently houses nearly 40,000 mice, representing approximately 3,800 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm. These include nearly 500 gene-targeted alleles and more than 300 transgenic integrations. The Facility also provides access to over 50 tool strains, including constitutive and inducible Cre strains, Flp strains, reporter strains, and others.

The Animal Facility offers a wide range of experimental procedures on-site, including gamma irradiation, UV light exposure, the use of volatile carcinogenic agents, surgical procedures, behavioural studies, non-invasive blood pressure measurement, and a laboratory animal monitoring system (Oxylet) for tracking various physiological parameters in mouse model phenotyping. Additionally, the Facility houses a climate chamber (HPPlife) that allows the maintenance of mice under controlled environmental conditions of temperature, humidity, and light, beyond the standard conditions of the SPF barrier area.

Furthermore, the monitoring of mouse models using non-invasive imaging technologies is carried out by the Molecular Imaging Unit. The Mouse Genome Editing Unit also operates within a laboratory inside the SPF barrier. Finally, the necropsy laboratory is equipped with instruments for haematological and biochemical analysis of blood and urine, complementing pathology and clinical diagnostics. ■

EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director

The following highlights summarise some of the main achievements of the Experimental Therapeutics Programme (ETP) during 2024:

FOXO Activators (in collaboration with Refoxy Pharmaceuticals GmbH). ETP-CNIO discovered several FOXO activators. Importantly, Refoxy Pharma in-licensed some molecules of this project under a license agreement signed in 2023. The license contemplated upfront and milestone related payments. The first payment to CNIO has been made in 2024.

PI3K inhibitors. We developed a series of advanced preclinical PI3K inhibitors. The CNIO and an undisclosed company reached a license agreement for these molecules and the corresponding patent. The agreement includes significant upfront and milestone payments from the company to CNIO along with royalties on sales. During 2024, the licensing agreement reached a significant milestone with the completion of the IND studies and the initiation of Phase 1 clinical trials. The corresponding payments have been made to CNIO. These molecules will contribute to the clinical development of next-generation PI3K inhibitors by the company in the coming years.

PIM Inhibitors (sublicense from Inflection Biosciences to Mysthera Therapeutics). In 2023, Mysthera Therapeutics acquired Inflection Biosciences' rights to selective PIM inhibitors developed by ETP-CNIO. Over the last year, the CNIO has received the corresponding payment as result of this sublicense agreement.

Mastl Inhibitors (MASTL-is) (in collaboration with M. Malumbres´ Group, CNIO). In 2024, we advanced in the optimisation of our MASTL-is and PROTACs. We focused our attention on the characterisation and selection of molecules with the best inhibition/degradation potency, selectivity, ADME-T, and pharmacokinetics profiles. We have already tested some molecules in cell viability assays using several therapeutic settings. Overall, we have obtained promising results. We have identified several sensitive cancer cell lines and combination agents that will serve to establish future *in vivo* models to perform PK-PD studies.

TRF1 Inhibitors (TRF1-is) (in collaboration with Maria A. Blasco's Group, CNIO). In 2024, we focused our activities on the chemical optimisation of direct-TRF1 inhibitors previously identified and validated by EMSA experiments. We have now improved the potency and selectivity of our TRF1-is in comparison with the initial hit molecules. The binding of some TRF1-is to F.l.-hTRF1 was also validated by MicroScale Thermophoresis (MST). Selected TRF1-is will be tested in the Telomeres and Telomerase Laboratory in a TRF1-dependent cell phenotypic assay. We will continue the medicinal chemistry exploration of the current TRF1-is to further improve their properties.

"ETP continues to give support to the Drug Discovery and Chemical Biology projects at CNIO and to collaborate with external partners to discover new therapeutic agents."

SETD8 Inhibitors (in collaboration with Óscar Fernández-Capetillo's Group, CNIO). In 2024, we developed a physiological-relevant high throughput cellular assay that measures monomethylation of H4K20. A screening campaign using the CNIO ETP 5k library is underway. Identified cellular hits will be validated against SETD8 biochemically to confirm direct inhibition. These molecules will be starting points for future medicinal chemistry exploration.

Others. ETP has worked in the early phases of other internalised projects: RANK (Eva González-Suárez, CNIO), and those under strategic alliances signed with the CRG-UIC and the IRB, NUDIX5 (R. Wright) and Molecular Glue Degraders (C. Mayor-Ruiz), respectively. ETP also provided support to several CNIO Groups in exploratory projects or contributed with internally synthesised tool compounds: Felipe Cortés-Ledesma, María S. Soengas, Juan Méndez, Manuel Valiente, Mariano Barbacid, Maria A. Blasco, Ana Losada, Óscar Fernández-Capetillo, Nabil Djouder, and Ivan Plaza. ■

MEDICINAL CHEMISTRY SECTION

Sonia Martínez
Section Head

Staff Scientists
Ana Belén García, Cristina Gómez, Esther González, Sonsoles Rodríguez, Terry Pascal Tomakinian, Carmen Varela

OVERVIEW

The Medicinal Chemistry Section plays a central role in the development of novel therapeutic agents for the treatment of cancer, and strives to bridge the gap between basic science and clinical application. Medicinal Chemistry is an interdisciplinary discipline that combines the principles of chemistry, biology, and pharmacology to design, synthesise and optimise small molecules with the potential to impact cancer treatment.

Collaboration is a hallmark of our approach at the Medicinal Chemistry Section and of the Experimental Therapeutics Programme to which it belongs. The team collaborates with other CNIO research groups to validate their target hypotheses with small molecules, or to generate potential drugs with validated, *in vivo* proofs of concept. The Section also collaborates with external institutions and pharmaceutical companies to translate scientific discoveries into potential clinical applications. Indeed, several projects have been licensed to biotech or pharmaceutical companies.

"We are pleased to announce that the licensing agreement for ETP-PI3K compounds has reached a significant milestone with the completion of the IND studies and the initiation of Phase 1 clinical trials."

RESEARCH HIGHLIGHTS

Telomeric repeat binding factor 1 (TRF1) inhibitors

Project in collaboration with María A. Blasco from the CNIO Telomeres and Telomerase Group. During this year, we identified several hits that affect the binding between telomeric DNA and TRF1 using electrophoretic mobility shift assay (EMSA). We further confirmed the interaction of some of these hits with TRF1 by quantifying the thermophoretic movement of fluorescent molecules in response to a temperature gradient, using MicroScale Thermophoresis (MST), an immobilisation-free technology for measuring biomolecular interactions. Going forward we continue our activities with this chemical series to optimise the compounds and further evaluate their activity in cells.

Microtubule-associated serine/threonine protein kinase-like (MASTL) inhibitors

This project has been undertaken in collaboration with Marcos Malumbres from the CNIO Cell Division and Cancer Group. This project has two distinct objectives: the search for small molecule inhibitors and the search for degraders of MASTL. Having identified potent Mastl inhibitors, our objective is to generate backup series with the further aim of enhancing our intellectual property and assessing how different scaffolds influence drug-like properties. Regarding degraders, the fine optimisation of advanced PROTACS with favourable degradation profiles across different cell lines is ongoing, and we have initiated the generation of Mini-PROTACs based on the N-end rule pathway for protein degradation. The N-end rule pathway, which is mediated by the UBR family of E3 ubiquitin ligases, enables the recognition and polyubiquitylation of proteins bearing N-terminal degradation signals (N-degrons), which encompass a range of amino acids. This ultimately results in the proteasomal degradation of the protein. Figure 1 provides a detailed overview of the flowchart for Mastl degraders.

RANK Antagonists as a novel therapeutic approach for the treatment of triple negative breast cancer (TNBC) patients

This collaboration, with Eva González-Suarez, from the CNIO Transformation and Metastasis Group, aims to develop small molecules that specifically target RANK receptor. We have supported hit validation activities: supervising the acquisition of compounds, assessing their quality, and contributing to determine the mechanism of action of identified hits in the alternative cellular screening set up.

NUDT5 Inhibition

In collaboration with R Wright (Universitat Internacional de Catalunya) and the GRG, we aim to optimise a hit that inhibits the ATP generating activity of NUDT5 in a biochemical assay. Several analogues have been acquired, and their quality assessed internally. Structure Activitity Relationships (SARs) are being generated, including the characterisation of some *in vitro* ADMET properties, to guide the design of proprietary inhibitors.

In addition to the drug discovery activities outlined above, we are assisting several research groups by synthesising small series of analogues of their initial hits. This is being done in order to generate SARs and validate their results. In particular, we are carrying out a small chemical exploration of a MIDKINE growth factor hit in collaboration with the Melanoma Group, and around a URI prefolding-like complex hit in collaboration with the CNIO Growth Factors, Nutrients and Cancer Group. Furthermore, in 2024 we were involved in the synthesis of chemical probes and reference compounds for the following CNIO groups: Brain Metastasis, Genomic Instability, Topology and DNA Breaks, and Growth Factors, Nutrients and Cancer. ■

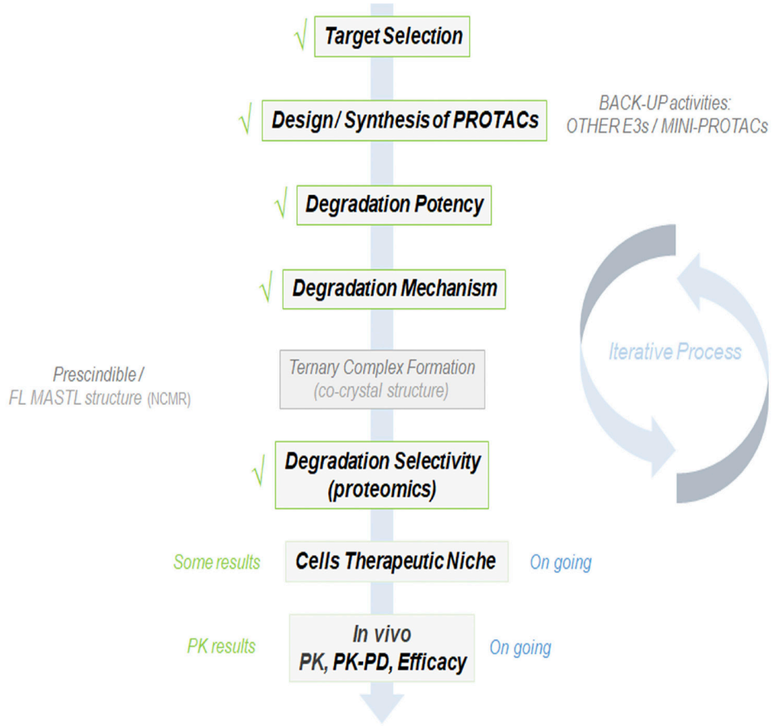


FIGURE 1 The flowchart illustrates the methodology employed for the identification and characterisation of MASTL degraders and current activities.

PUBLICATION

- Murga M, Lopez-Pernas G, Soliva R, Fueyo-Marcos E, Amor C, Faustino I, Serna M, Serrano AG, Díaz L, Martínez S, Blanco-Aparicio C, Antón ME, Seashore-Ludlow B, Pastor J, Jafari R, Lafarga M, Llorca O, Orozco M, Fernández-Capetillo O (2024). SETD8 inhibition targets cancer cells with increased rates of ribosome biogenesis. *Cell Death Dis* 15, 694.

PATENT

- Pastor J, Blanco-Aparicio C, Martínez S, Albarrán MI, Alvarez RM, Cebriá A, Cebrián DA, García AB, Gómez CA, González E, Hernández Encinas E, Martín JI, Ramos FJ, Riesco RC. Compounds. International Publication Number: *WO2023099072A1*. National Phase Entry (2024).

BIOLOGY SECTION

Carmen Blanco
Section Head

Research Scientists
Pablo Aparicio, Marta San Martín (since February)

Post-Doctoral Fellow
Elena Hernández (until May)

Graduate Student
Lucía Cañizares (until November) (PEJ, CAM)*

Technicians
M. Isabel Albarrán (until December) (TS)**, Antonio Cebriá (TS)**, Claudia Díaz (since February) (PEJ, CAM)*, Elena Gómez-Casero (TS)**

Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Community of Madrid)
***Titulado Superior* (Advanced Degree)

Students in Practice
Mariclara Ciccarelli (since October) (Erasmus, University of Naples Federico II, Italy), Lucía González (until June) (Master's Degree Final Project, Universidad Complutense de Madrid, Spain), Miguel Rodríguez (February-May) (Bachelor's Degree Final Project, Universidad Complutense de Madrid, Spain)

OVERVIEW

Our group conducts targeted early drug discovery projects to identify and develop small molecules as potential therapeutics against oncogenic targets. After target validation, the starting point of these projects is a screening campaign using scalable and robust assays that reflect target interaction and biological relevance; these can be either biochemical or cellular assays. Biochemical assays are simpler, with fast turnarounds but lack context. Conversely, cellular assays are more physiologically relevant, more complex to interpret, and have a slower turnaround. In a project flowchart, typically we run a biochemical HTS screen followed by validation in a target-dependent cellular assay. However, this sometimes fails to identify potential hits, at which point we move to a more complex cellular assay that measures the target activity in cells. In the latter case, the identification of a hit then requires a target deconvolution task to determine whether its mechanism of action is direct, or through modulation of another pathway.

"We are pleased to announce that the licensing agreement for ETP-PI3K compounds has reached a significant milestone with the completion of IND studies and the initiation of a Phase 1 clinical trial."

RESEARCH HIGHLIGHTS

Microtubule-associated serine/threonine protein kinase-like (MASTL)

Project undertaken in collaboration with the CNIO Cell Division and Cancer Group. In 2024, we characterised around 120 new compounds (MASTL-i and MASTL PROTACs) in our screening panel assays (biochemical with human full-length MASTL, MASTL cell engagement and MASTL degradation). We focused on characterising and selecting our MASTL-is and MASTL PROTACs with the best degradation potency and proteome-wide selectivity to test their effect on proliferation. We looked to validate their effect in prostate cell lines with high CIN, in breast cancer cell lines in combination with radiation, and also with an agnostic approach to identify sensitive cell lines. We have obtained promising results. We also started to characterise other types of MASTL degraders such as miniPROTACs, based on the N-end rule pathway for protein degradation.

Telomeric repeat binding factor 1 (TRF1)

Project carried out in collaboration with the CNIO Telomeres and Telomerase (TT) Group. We are focusing on the identification of inhibitors of TRF1 binding to ds telomeric DNA (dstelDNA). Non-radioactive EMSA experiments have been set up as functional assays to validate selected compounds from different previous screening campaigns with an Alpha assay. New compounds from different chemical series were synthesised to improve their potency and properties and characterised by EMSA. The binding of active compounds to F.l.-hTRF1 by EMSA was validated by a label-free MicroScale Thermophoresis (MST) biophysical assay. Selected TRF1 inhibitors will be tested in the TT Laboratory in a TRF1-dependent cell phenotypic assay. Figure 1 provides a detailed overview of the flowchart for the identification and characterisation of direct TRF1 inhibitors.

SET domain containing lysine methyltransferase 8 (SETD8)

Project in collaboration with the CNIO Genomic Instability and Macromolecular Complexes in DNA Damage Response Groups. Our aim is to develop novel SET8 inhibitors as new therapeutic agents. We have established a cellular assay that measures monomethylation of H4K20 in an HTS format. A screening campaign with CNIO´s ETP-5K library is underway. Confirmed hits identified in this more physiologically relevant cellular assay will be tested in a SETD8 biochemical assay to verify that they are direct inhibitors of SETD8 methyl transferase activity, and to rule out that modulation of H4K20me1 is not an indirect effect. Biochemically validated hits will be starting points for SAR exploration and characterisation.

Collaborations with other CNIO Groups

ETP-Biology performed formulation studies of selected compounds in collaboration with the H12O-CNIO Cancer Immunotherapy Clinical Research Unit.

ETP-Biology also provided support in hit validation activities for the Topology and DNA Breaks and Melanoma Groups. Moreover, we collaborated with the Experimental Oncology and Computational Oncology Groups by giving support in phenotypic cellular screening campaigns. Finally, we helped the Transformation and Metastasis Group in cellular screening campaigns with two different CNIO libraries and in hit validation activities.

Strategic alliances with other institutions (CRG/UIC and IRB)

Collaboration with CRG/UIC (R. Wright): We continued to characterise, in terms of ADME-T, selected compounds to generate SAR information for a NUDIX5 inhibitor previously identified by the researcher. ■

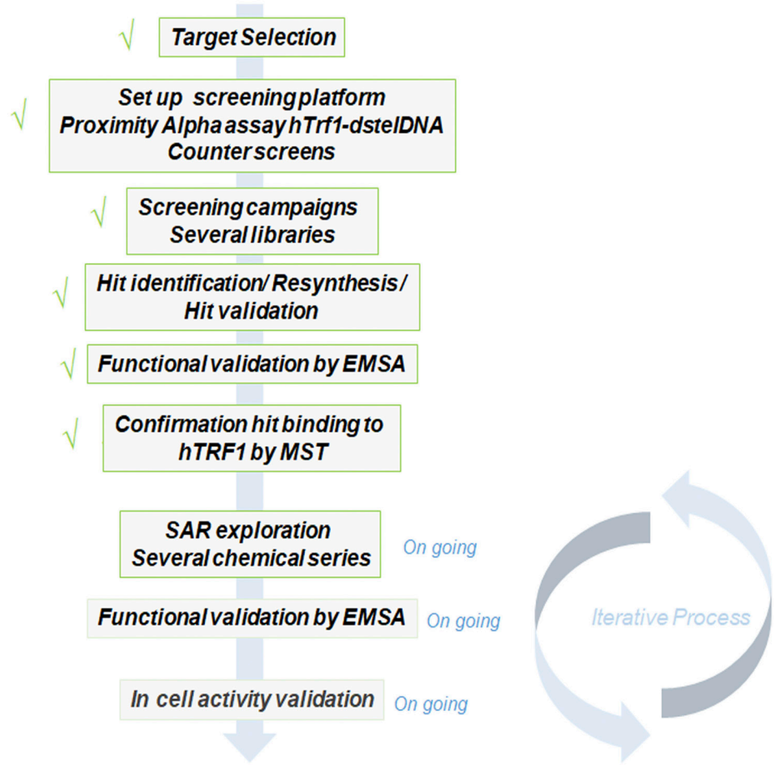


FIGURE 1 Flowchart illustrating the methodology used to identify and characterise TRF1 direct inhibitors of binding to dstelDNA and current activities.

PUBLICATION

- Murga M, Lopez-Pernas G, Soliva R, Fueyo-Marcos E, Amor C, Faustino I, Serna M, Serrano AG, Díaz L, Martínez S, Blanco-Aparicio C, Antón ME, Seashore-Ludlow B, Pastor J, Jafari R, Lafarga M, Llorca O, Orozco M, Fernández-Capetillo O (2024). SETD8 inhibition targets cancer cells with increased rates of ribosome biogenesis. *Cell Death Dis* 15, 694.

PATENT

- Pastor J, Blanco-Aparicio C, Martínez S, Albarrán MI, Alvarez RM, Cebriá A, Cebrián DA, García AB, Gómez CA, González E, Hernández Encinas E, Martín JI, Ramos FJ, Riesco RC. Compounds. International Publication Number: *WO2023099072A1*. National Phase Entry (2024).

CNIO – LILLY CELL SIGNALLING AND IMMUNOMETABOLISM SECTION

Susana Velasco
Section Head

OVERVIEW

Our laboratory, in collaboration with Lilly, works on the identification and validation of cellular models of Chromosomal instability (CIN) and novel dependencies as a result of CIN. We apply bespoke functional genomic screens to uncover novel vulnerabilities, in combination with *in vitro* and *in vivo* approaches, to obtain a complete understanding of the role of CIN in tumour development and anti-tumour response. Each potential target goes through a validation process using xenografts, allografts, and mouse models developed at the CNIO. Our work includes the use of non-invasive *in vivo* imaging technologies, and histochemical characterisation of tumours, using a variety of intrinsic and extrinsic tumour markers. The final step is the validation in human samples using tumour tissue arrays.

Research Scientists
Sonia Hernández Tiedra, Eva P. Lospitao, Carolina Maestre, Gloria Martínez Del Hoyo

Technicians
Jenniffer Patricia Condo, Laura Diezma, Roberto Gómez (TS)*, Alicia Luengo, Sandra Peregrina (TS)*

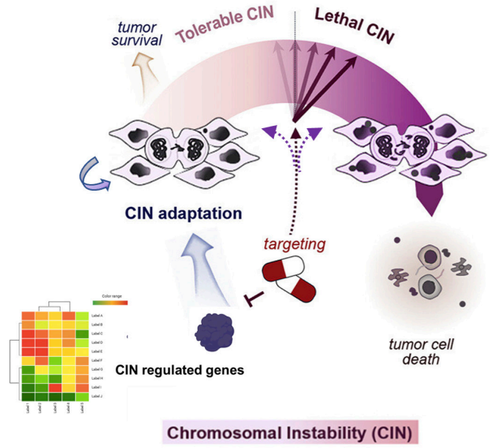
Bioinformatician
Teresa Laguna (since Feb.) (TS)*

**Titulado Superior* (Advanced Degree)

RESEARCH SUMMARY

Chromosomal instability (CIN) and whole genome duplication (WGD) are a hallmark of human cancer and are associated with poor prognosis, metastasis, and therapeutic resistance (Figure 1), being present in up to 80% of human cancers. CIN results from errors in chromosome segregation during mitosis, leading to structural and numerical chromosomal abnormalities, including loss or amplification of DNA segments, rearrangements, extrachromosomal DNA, and micro-nuclei formation. These abnormalities lead to the activation of oncogenes or the inactivation of tumour suppressor genes as well as other genes aiding in the processes of metastasis, drug resistance, and immune escape. The causes of CIN are diverse, including mitotic errors, replication stress, homologous recombination deficiency (HRD), and telomere crisis. The lack of clinically approved drugs and precise experimental models that directly target CIN, highlights the necessity for identification of CIN targeting drugs.

Our goal is to find novel therapies that would make tumours bearing CIN more susceptible to destruction, either with the use of single agents, or acting synergistically with other anti-tumour therapies. Exploring how to better target these mechanisms would lead to better and more efficient therapeutic options, including more personalised therapies. ■



Modified from Dhital et al., 2023, Cell Reports Medicine 4

FIGURE 1 Chromosome instability (CIN): Identify vulnerabilities in cancer cell line models with various forms of genomic instability. CIN cells undergo different mechanisms of adaptation. Our goal is to find novel therapies that would make tumours bearing CIN more susceptible to elimination using novel and known therapeutic strategies.

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Irene Herrera
Director

Technology Transfer Manager
Irene Pino

Administrative Technician
Rocío Manzano

The Technology Transfer and Valorisation Office (TTVO) contributes to translating CNIO research and innovation for society's benefit, by ensuring appropriate protection of intellectual property and by channelling the technologies that arise from our research to companies and entrepreneurs for further development and beneficial social impact.

"Our Office acts as a bridge between basic science and business, helping to turn discoveries into tangible benefits for patients and society."

The activities of the TTVO during 2024 focused on monitoring the CNIO's scientific developments; identifying new inventions; protecting and managing industrial and intellectual property at the CNIO; managing contracts with other institutions and industry; and, finally, commercialising and exploiting CNIO's assets to promote a positive impact on the biomedical industrial sector and society, both through exploitation licences and through the creation of spin-off companies.

TTVO manages a portfolio of 43 active patent families, and provides advice and assistance during the drafting of patent documents, their filing, and the prosecution process. In 2024, one priority patent application, co-owned with Instituto de Salud Carlos III (ISCIII), was filed. This new patent is a method for determining the risk of developing liver disease using single nucleotide polymorphisms. The CNIO also registered using Blockchain as a consensus classifier for PDAC tumours (including the stroma). Moreover, seven PCT (Patent Cooperation Treaty) applications for international extension were also filed in 2024. Licensed patents to companies make up 42% of the CNIO portfolio.

Over the course of the year, the TTVO managed 269 agreements (MTAs, CDAs, research collaborations, licences, etc.). The majority of these agreements (68%) were established with international entities, which is an indicator of the internationalisation of CNIO's research activity. Through collaborations with industry, €2 million were secured for research activities.

Among the most outstanding agreements signed in 2024 with the private sector is an addendum to extend the collaboration framework contract with the pharmaceutical company Eli Lilly for an additional three years; Eli Lilly finances the research activity of the Cell Signalling and Immunometabolism laboratory led by Susana Velasco.

Other relevant agreements with the private sector include the collaboration with Moderna Therapeutics (USA) for a project with the Telomeres and Telomerase Group directed by Maria A. Blasco. Also worth mentioning are research agreements signed with other companies such as Tailor Bio (UK) for a research project with the Computational Oncology Group led by Geoff Macintyre; Vega Oncotargets (Spain) for a project with the Experimental Oncology Group, led by Mariano Barbacid; an agreement with the PETHEMA Foundation for a project with the Proteomics Unit, led by Marta Isasa; and another one with Tempos Microscopy SL for a project with the Genomic Integrity and Structural Biology Group, led by Rafael Fernández Leiro.

Patents and unpatented research tools (murine lines, cell lines and antibodies) are licensed to provide financial return to CNIO. In 2024, the posted net income generated in 2023 from CNIO asset licenses, amounted to €1.7 million (about € 1.1 million from monoclonal antibodies).

In 2024, TTVO participated in the *Transfiere* Forum together with the ISCIII and in the Cell & Gene Therapy International Europe Conference. On Investors Day at CNIO, 10 CNIO projects were presented to several venture capital firms. As a result of this event, a collaboration framework agreement was signed with one of the venture capital firms, and by 2025, a collaboration contract for shared-risk technological development will be signed with a consulting firm in the biomedical health sector.

Finally, after an evaluation process, the Spanish Ministry of Science, Innovation and Universities included the CNIO in the new Registry of Knowledge Transfer Offices (KTOs), in accordance with Spanish Royal Decree 984/2022, of 22 November. This will allow the CNIO to compete for funding from public state programmes that are aimed at financing, directly or indirectly, these Offices.

All of the achievements mentioned here stand as a testament to the excellence and hard work of CNIO scientists and to CNIO's unwavering encouragement of innovation and technology transfer activities. ■

Biobank

BIOBANK

M. Jesús Artiga
Acting Director

Research Scientist
Nuria Ajenjo

Technicians
Daniel Alba (TS)*, Inmaculada Almenara, Pilar Caro (TS)*, José Miguel Escolano (TS)*, Sergio Fernández (since April) (TS)*, Elena Molina (since April) (TS)*, Cecilia Sobrino

Student in Practice
Mónica Ruiz (September-December) (Technicians in Training (FCT) IES Ríos Rosas, Madrid, Spain)

Visiting Scientist
Inmaculada de Melo-Martín (Weill Cornell Medicine, Medial College – Cornell Univ., New York, USA)

**Titulado Superior* (Advanced Degree)

OVERVIEW

The main goal of CNIO Biobank is to facilitate access to high-quality human samples and their associated data for cancer research and related diseases, ensuring that both the acquisition and use comply with legal and ethical principles safeguarding donors' rights.

CNIO Biobank is a cross-service platform for CNIO researchers, and the broader scientific community, offering a wide range of services that cover all stages of research project management requiring human samples. These services include sample processing, collection management, quality, ethical and legal consultancy, acquisition and design of valuable research collections, and negotiation with stakeholders to find suitable samples or assist in obtaining ethical approval for research projects.

"Access to biological samples and their information is essential for advancing biomedical research, improving diagnoses and treatments. The future of biomedical research is, therefore, linked to biobanks."

CNIO Biobank is authorised by the *Consejería de Sanidad de la Comunidad Autónoma de Madrid* and registered in the *Registro Nacional de Biobancos del Instituto de Salud Carlos III* (Ref: B.848).

RESEARCH HIGHLIGHTS

Collections of sample and data

CNIO Biobank houses almost 51,000 samples from more than 9,000 donors. These samples correspond to the historical archive collections (tumoural and non-tumoural tissue samples of more than 7,300 cases); patient-derived xenograft (PDX) collection (243 cases); COVID-19 collection (700 cases); brain metastasis (RENACER) collection, which has grown to 367 cases from 19 hospitals; and a prospective cohort from the Spanish Cabin Crew Association (AETCP), with 146 cases. Furthermore, the Biobank's Virtual Catalogue includes 8,064 images from TMAs, histological & IHQ images.

FIGURE 1 CNIO Biobank conducts sample processing not only for its own collections intended for research projects, but also offers a range of specialised sample processing services for research groups.

Services to researchers

- → Transfer of samples to research projects: 2,230 samples supported 9 research projects, and 149 samples were provided for technical validations. This activity led to 2 publications in Q1 and 1 in Q3 journals, with an average impact factor of 12.4.
- → Sample processing services: We have provided 7 technical support services for sample processing.
- → Custody of collections: We provide custody and management service for collections, ensuring sample traceability and GDPR compliance.
- → Ethical-legal advice to researchers: We have handled 43 ethical and legal consultations, 18 through the canSERV project, and assisted CNIO researchers in obtaining ethical approval for 11 projects from the ISCIII Research Ethics Committee.

Participation in new cooperative projects

- → ISCIII Biomodels and Biobanks Platform: *Plataformas ISCIII de apoyo a la I+D+I en Biomedicina y Ciencias de la Salud*, AES 2021-2023, PT23/000065.
- → EvolveBBMRI: Accelerating datafication for support of EU health priorities, greening of biobanks and integrated approach to "One Health", HORIZON-RIA action, H2020, Project's Grant Agreement No. 101131701.

Organisation and participation in training and dissemination activities

CNIO Biobank contributes to and organises training and dissemination activities (like FCT programme, European Researcher's Night, "*Feria Madrid es Ciencia*"). ■

PUBLICATIONS

- Priego N *et al.* (incl. de Pablos-Aragoneses A, Perea-García M, Hernandez-Oliver C, Alvaro-Espinosa L, Rojas A, Sanchez O, Caleiras E, Garcia F, Garcia-Martin S, Grana-Castro O, Al-Shahrour F, Renacer Group R, Valiente M) (2024). TIMP1 mediates astrocyte-dependent local immunosuppression in brain metastasis acting on infiltrating CD8+ T cells. *Cancer Discov*. PMID: 39354883.
- Diz-de Almeida S *et al.* (incl., Alvarez N, Artiga MJ, González-Neira A, Pita G, Ortega-Paino E) (2024). Novel risk loci for COVID-19 hospitalization among admixed American populations. *Elife* 13, RP93666.
- de Melo-Martín I, Ortega-Paíno E (2024). Biobanking legislation in Spain: advancing or undermining its ethical values? *Biopreserv Biobank* 22, 242-247.

AWARDS AND RECOGNITION

Nuria Ajenjo:
- Collaborator of the General Secretariat for Research of the Spanish Ministry of Science, Innovation and Universities.
- Member of the Research Ethics Committee of the National Institute of Health Carlos III (ISCIII), Spain.

Daniel Alba:
- Collaborator in the Cancer Working Group of the Spanish Society of Epidemiology (SEE).

Elena Molina:
- Technical expert by the Spanish National Accreditation Body (ENAC) for the ISO 20387 standard.

CNIO Offices

DEAN'S OFFICE

María S. Soengas
Dean for Academic Affairs

PARTICIPANTS
Personnel in training:
Alejandro Alonso

Ana Belén Alonso
Cristina Bodas
Bruna Calsina
Lluís Cordón
Guillermo de la Vega
Ana de Pablos

The CNIO Dean's Office continues to play a central role in advancing one of the Centre's core missions: to foster the professional and academic development of the next generation of researchers. Young scientists — ranging from undergraduates to PhD students, postdoctoral fellows, medical residents, and visiting researchers — make up more than 60% of the CNIO's personnel. Through strategic collaborations with universities and hospitals, we work to promote academic-clinical partnerships and provide a multidisciplinary training environment.

Our efforts are focused on ensuring that CNIO trainees receive support in both their scientific and personal growth. To this end, we oversee regular PhD thesis committee meetings and coordinate weekly Progress Report seminars. In addition, and representing a cornerstone of the Dean's Office, our training activities are largely shaped in collaboration with the CNIO Student Association (**CNIOSA**) and, newly reinstated in 2024, the CNIO Postdoctoral Fellows Association (**CNIO-PDA**). Both Associations play a central role in coordinating and promoting new initiatives. For example, a major highlight of this year's CNIO-PDA and CNIOSA activities was the organisation of the "**Road to Competitive Funding**" workshops, tailored for junior predoctoral and postdoctoral fellows, as well as for senior postdoctoral researchers and staff scientists. Several projects stemming from these workshops are underway and we expect even more to come. We thank Fundación Occident for their generous support of this important initiative.

We also celebrated the **European Researchers' Night**, now a CNIO tradition (this was our 11th edition!). As part of a Europe-wide Horizon 2020 Marie Skłodowska-Curie Action, this event is key to bringing science closer to society. Over 100 volunteers organised talks and hands-on experiments so that visitors could "Meet a scientist and become a scientist". We hosted

Irene Felipe
Bárbara Hernando
Jan Hochstadt
Nayim González
Óscar Laguía
Lucía Lomba
Ernesto López
Belén Navarro
Thelma Poluha
María Ramal
Luis Rodrigo López
Elena Sánchez
Maria Solé
Joe Thompson
María Velasco

Staff/Faculty:
Ana Cuadrado

Rafael Fernández-Leiro
Eva González-Suárez
Ana Losada
Geoff Macintyre
Jorge L. Martínez
Mercedes Robledo

over 260 participants of all ages and organised live events on social media to showcase research and our laboratory life to a broad audience. The feedback was once again highly positive and preparations for the next edition are already underway.

Our annual **CNIO Lab Day** was also another highlight of 2024. We featured 14 short talks, 19 flash talks, and 4 poster sessions covering research from all the Scientific Programmes at the CNIO. This internal retreat emphasised the breadth and excellence of research at the Centre, which we all cherish. We were delighted to learn from guest speakers Merixell Rovira (IDIBELL, Barcelona) about work on stem cell niches in the pancreas and from Luis Quevedo (@LuisQuevedo) about the ins and outs of scientific communication. We extend our thanks again to the Fundación Occident, as well as Merck, Eppendorf, Clinisciences, Revvity, and Promega for their support.

We also proudly celebrated the **Director's List Awards**, which recognise exceptional contributions in 3 categories:

1. Awards for Excellence in Research by Predoctoral Fellows

We are grateful again to the Agüera-Nieto family for a generous donation in the name of their mother, Antonia Nieto, to support the PhD student authoring the article with the highest impact in a scientific journal. In 2024, the Antonia Nieto Award went to **Xavier Catena** for new insights on mechanisms of immune suppression in melanoma, published in the prestigious *Nature Cancer* journal. Additional awards in the PhD category went to **Ana Belén Plata-Gómez** for work on hepatic signalling and liver zonation (*Nature Communications*); **Silvia de la Rosa** for discoveries related to endogenous retroviruses and pluripotency (*Science Advances);* **Oleksandra Sirozh** for work on nucleolar stress (*Molecular Cell*); and **Sergio de la Rosa** for new discoveries on how endogenous retroviruses shape pluripotency specification in mouse embryos (*Science Advances*).

2. *Award for Excellence in Research by Postdoctoral Fellows/ Staff Investigators*

This award was granted to **Marina Serna Gil**, a research scientist in Óscar Llorca's Group, for multiple high-profile publications in *Developmental Cell, Science, and Cell Death & Disease*, exploring microtubule nucleation and cancer vulnerabilities.

"At the CNIO, we are committed to cutting-edge research, while fostering a culture that encourages our trainees to be rigorous and think independently so that they can best fulfil their potential as influential leaders."

3. Outstanding Contribution to Outreach and Awareness

The award went to **Irene Felipe**, a postdoctoral fellow in the Epithelial Carcinogenesis Group, for her sustained and enthusiastic participation in public engagement. Her activities ranged from co-organising the European Researcher's Night at the CNIO to participating in the Feria de la Ciencia at IFEMA, as well as numerous outreach events outside the CNIO.

The Lab Day also featured awards from the Dean's Office: three awards for the Best Oral presentations, three for the Best Posters, and one for T-Shirt Design, all portraying the mission of the CNIO to get research closer to the bedside.

In summary, we are as proud as ever of the achievements of our young investigators at the CNIO, and we thank all those public and private contributors that have helped fuel their efforts. As always, we are committed to being a valuable resource to both the scientific community and society at large. ■

CNIO WISE DIVERSITY OFFICE

Isabel López de Silanes
Coordinator

The CNIO Women in Science (WISE) Office was established in 2012. Our main objectives are to raise awareness about the importance of gender equality in science and society; and to help correct imbalances in the career ladder within the CNIO community, especially in leadership positions. The WISE Diversity Office is composed of CNIO volunteers from all the areas represented at the Centre, including the Director.

In 2024, the WISE Diversity Office continued to work actively to make the CNIO a better place to work and to reconcile work and private life. It was also part of the negotiating Committee to renew the Equality Plan. In addition, the Office was involved in preparing the Human Resources Excellence in Research Award (HRS4R) from the European Commission.

As in the past, we organised the WISE seminar series, in which we invite numerous top female leaders from different areas to give a talk. The following talks were held during the year:

- → H.E. Dr Sarmila Parajuli Dhakal, Ambassador of Nepal to Spain. Title: "Bridging Borders Through Science: Insights from Nepal". 09/01/2024.
- → Mina Sohn, CEO and founder of Koala company. Title: "Power of Listening to Your Inner Voice". 08/04/2024.
- → Clara Montoya, artist. Title: "Ignotas y Resueltas". 07/03/2024.
- → Eleonora Viezzer, full professor at the University of Seville. Title: "Nuclear Fusion: Bringing the Sun down on Earth". 12/03/2024.
- → Nuria Oliver, Director of the ELLIS Alicante Foundation. Title: "My Personal Story in AI". 07/06/2024.
- → Rocío Hervella, founder and CEO of PRODUCTOS SOLUBLES, S.A. PROSOL. Title: "What Does Coffee Have to Do with the CNIO? ". 17/06/2024.

Members
Maria A. Blasco
Maria Jesús Alcamí
Isabel López de Silanes
Lola Martínez
Francisca Mulero
Fernando Peláez
Sandra Rodríguez-Perales

- → Roundtable with the NetWomening Association, dedicated to supporting Afghan women in Spain. Title: "Women Without Borders: Struggles and Hope from Afghanistan to Spain". 18/11/20204.

All the talks are recorded and available to the public in the CNIO YouTube channel.

For International Women's Day, the WISE Office organised two seminars. The first with Clara Montoya, the first artist-in-residence at the CNIO. The second was given by Eleonora Viezzer, a leading scientist in the field of nuclear fusion (see the list of seminars for more details).

In the spirit of promoting STEM careers among young women and breaking gender stereotypes, several scientists gave seminars at different high schools on the occasion of February 11, 2024, International Day of Women and Girls in Science. The WISE Office also has a long-standing collaboration with STEM Talent Girl, an educational programme that aims to awaken, develop, and strengthen the STEM vocation among ESO students. As part of this collaboration, our Coordinator, Isabel López de Silanes, gave a masterclass to around 100 teenagers in March 2024. The title of the masterclass was "Combatiendo el cáncer y el envejecimiento como científica". ■

Here at the WISE Diversity Office, we share what the writer J.K. Rowling said: "We do not need magic to transform our world. We carry all of the power we need inside ourselves already."

Facts & Figures

COMMUNICATIONS

MÓNICA G. SALOMONE Director

Communications
Pilar Gil

Science Communication and Social Media
Esther Sánchez

Sharing CNIO discoveries with society is the main task of CNIO's Communications Office. We feel compelled to contribute to the public's scientific culture, while at the same time conveying an institutional image consistent with that of a top-level research centre.

Specifically, our work is structured around the following areas:

- → Defining the overall communication and branding strategy.
- → Monitoring news, social conversation, and the media ecosystem.
- → Identifying and producing engaging CNIO stories to be disseminated through the media and CNIO's own channels.
- → Managing media relations.
- → Organising outreach activities aimed at both the educational community and general public.
- → Supporting other offices, groups, or units—especially the Philanthropy Office, with whom we publish the *CNIO Friends* newsletter.

Our communication strategy aims to reinforce the message that CNIO is one of the world's leading cancer research centres. As such, it attracts scientific talent and competitive funding from national and supranational institutions, both public and private. CNIO is also a major source of innovation, capable of transforming knowledge into tangible advances for society.

"We reinforce CNIO's image as a world reference Centre in cancer research by sharing its discoveries with society in an understandable and attractive way."

We also focus on the educational community. For example, CNIO's participation in the *Madrid es Ciencia* fair has become one of our annual highlights—thanks mainly to the generosity of CNIO researchers who volunteer their time.

As a result of these and other efforts, CNIO maintains an active and ongoing dialogue with society. At the CNIO Communications Office, we work to ensure this relationship is fruitful and enriching for everyone. ■

In today's media landscape, the public not only consumes information from traditional newspapers and TV, but increasingly from independent sources and even automated systems with little or no human oversight. As a result, public exposure to disinformation is on the rise —something we always consider when selecting, shaping, and distributing our content.

For traditional media we share CNIO's scientific breakthroughs via press releases, images, and graphics. We strive for a clear and engaging style, always maintaining scientific accuracy and avoiding hype, while providing well-contextualized information.

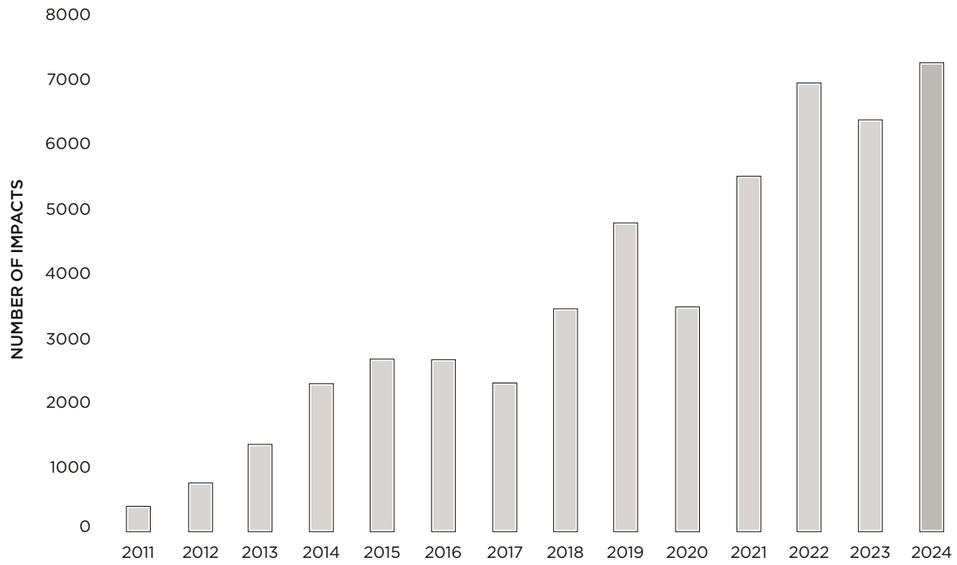
For CNIO's own platforms—especially social media—we adapt our content for broader audiences, sharing scientific concepts and sparking curiosity. When we speak directly to the general public, we emphasise visual language, including, whenever possible, short videos in which CNIO researchers explain their work themselves.

At events such as the World Cancer Research Day celebration at CaixaForum (Madrid), we engage with the public directly. In the 2024 edition, the conference on Artificial Intelligence and cancer research by Alfonso Valencia (Barcelona Supercomputing Center, BSC) was particularly well received by both the audience and the media.

CNIO MEDIA IMPACT

TRADITIONAL MEDIA COVERAGE

OVERALL TRADITIONAL MEDIA COVERAGE



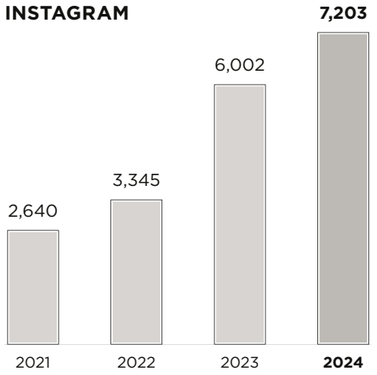
PRESS RELEASES

	2020	2021	2022	2023	**2024**
SCIENTIFIC PUBLICATIONS	18	26	21	36	**26**
INSTITUTIONAL	36	23	46	81	**83**
TOTAL:	54	49	67	117	**109**

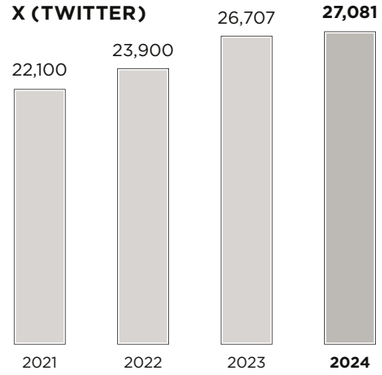
SOCIAL MEDIA

SOCIAL MEDIA AS A TOOL TO INTERACT WITH OUR AUDIENCE

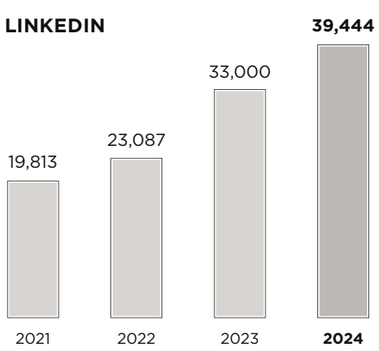
INSTAGRAM



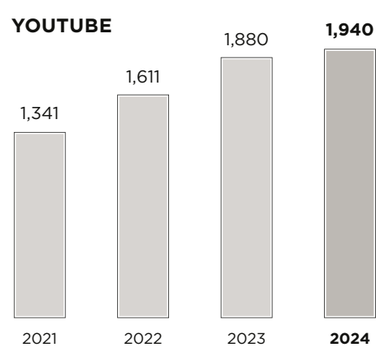
X (TWITTER)



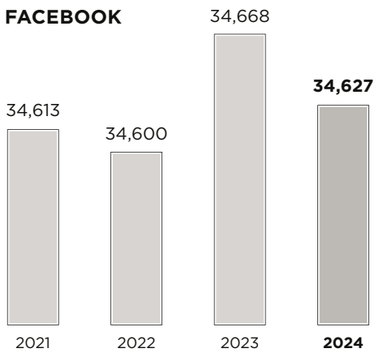
LINKEDIN



YOUTUBE



FACEBOOK



DEVELOPMENT & PHILANTHROPY

Development & Philanthropy Officer
Mercedes Antona

JESSICA ROSE Director

2024 was another successful year for the Philanthropy Office as we raised over €1m for cancer research. As always, every single euro raised directly supports cancer research. The '***CNIO Friends Contracts'*** programme funds 100% of the salaries of the CNIO Friends post-doctoral researchers, and ensures every single contribution directly funds the salaries of our research Fellows, be it €1 or €10,000.

"Philanthropy allows individuals, patient associations, foundations and companies to make a real difference in the lives of cancer patients. It is only research that can enable better diagnosis, treatment and prevention of cancer: every donation to the CNIO makes a difference."

Last year we increased our philanthropic programme strategy to include fundraising for the '***CNIO Friends Projects'*** programme, which includes larger gifts to support other areas of our cancer research. These larger 6- and 7-figure gifts can fund specific projects, research areas, equipment, or scientific collaborations at the CNIO. In 2024, we were delighted to attract some new partnerships to fund equipment, an essential area of support for the Centre. This is an area that we plan to increase in 2025, and we look forward to working with our scientific colleagues to determine their funding needs for the future.

To recognise and acknowledge World Cancer Day on February 4th, we launched our annual campaign in conjunction with the Offices of Communication and Institutional Image to encourage greater awareness and new donations to the CNIO. This year we were joined by comedian José Mota as the face of the campaign and as in previous years we had the invaluable collaboration of our partner JCDecaux and their digital billboards that helped to disseminate the campaign across Spain.

We launched our annual ***'CNIO Friends Contracts'*** call for 2024 which resulted in hiring **7 new research fellows**. In addition, we were delighted to partner once again with both Fundación Domingo Martínez and La Roche-Posay (via L'Oréal Groupe) who each funded a second fellow, after the successful completion of their previous funded post-doctoral fellowships. As a result, at the end of the year a second call was opened for two additional research fellows. The new Fundación Domingo Martínez and La Roche-Posay Fellows will start their post-docs in 2025. The CNIO Friends Programme has raised over €5.52 million in donations and pledges since its inception.

This year, the CNIO was both the beneficiary and an integral part of the Siempre Positivo campaign. Football coach Louis van Gaal and Maria Blasco starred in a short documentary SIEMPRE+POSITIVO, directed by Nacho Solana and produced by Morena Films and IPG Mediabrands. The campaign was conceived by the creative Jorge Martínez and developed in conjunction with other partners including Cøllage, Diario AS and the Sociedad Española de Radiodifusión Española (SER). In addition to the film, the initiative has created a charity fashion collection with the motto " SIEMPRE+POSITIVO " produced by the Spanish fashion brand El Ganso. All proceeds from the sale of this collection are being donated to the CNIO to the recruitment of research talent through the 'CNIO Friends Contracts' Programme.

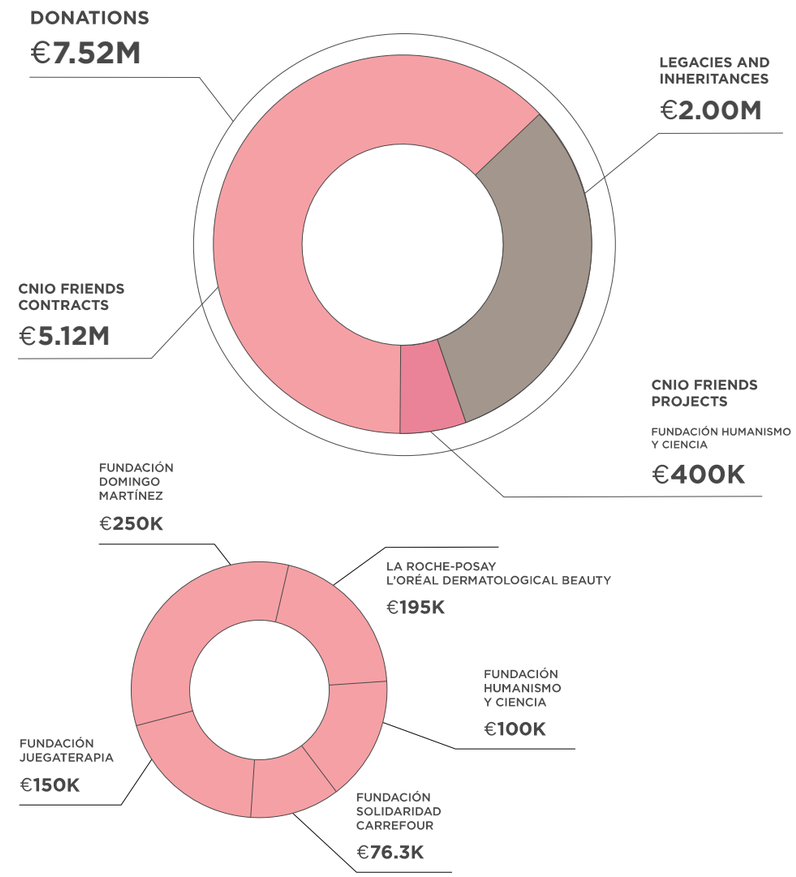
In 2024, we were supported by several new and existing collaborating partners and cancer patient associations including Brother Iberia, L@s Fuertes, Asociación Española Contra el Cáncer Alicante, Supermercados Manper, Rosae, El Arbol de la Vida, AFECC-Asociación Atención Integral de Afectados de Cáncer (AEFF), Asociación Oncológica Juan Víctor, Asociación Pedalovida, Ayuntamiento Torreperogil, Fundación Seve Ballesteros, Ingenieria de Sistemas para la Defensa de España, S.A., S.M.E., M.P (ISDEFE), and others. We also continued to develop the new collaboration with the Federación Española de Municipios y Provincias (FEMP) responsible for all the councils of Spain to develop a country-wide network of support for cancer research.

We enjoyed our annual *CNIO Friends Day* in June, welcoming around 100 Friends to the CNIO to hear from the philanthropy funded researchers and visit our laboratories. As always, our researchers were delighted to be able to demonstrate the impact of their research directly to their supporters.

The legacies and inheritances programme continues to grow, allowing individuals to contribute to the CNIO that will benefit cancer patients and their families for generations to come. All legacies and inheritances to the CNIO provide funding for existing research groups at the CNIO, and we have raised over €2 million since 2015 with an estimation of an additional €2 million pending to be executed.

We are very proud that every euro raised helps to enable the brilliant cancer research at the CNIO. The Philanthropy Office remains committed to building on our successful partnerships and proactively cultivating new donors who wish to join the quest to stop cancer. ■

DONATIONS RECEIVED AND PLEDGED TO THE CNIO



COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies and is used to finance not only the Centre's outstanding R&D activities, but also strategic actions in Innovation, together with Industry partners. The funding is also used to support other relevant activities related to dissemination and scientific outreach aimed at promoting public awareness. In 2024, CNIO researchers were involved in 148 projects that received extramural funding.

CNIO is actively participating in 67 collaborative projects: 17 are international collaborative projects (1 of which is coordinated by the CNIO), and 50 are collaborative projects conducted at the national level (20 of them are coordinated by the CNIO). The international collaborative projects are funded by the European Union through the Research & Innovation Framework Programmes Horizon 2020 (2014-2020) and Horizon Europe (2021-2027), as well as the EU4Health Programme (2021-2027) and the Digital Europe Programme; the US National Institutes of Health (NIH); the Ramón Areces Foundation in collaboration with the Weizmann Institute of Science; the Mark Foundation for Cancer Research; and Worldwide Cancer Research. At national level, collaborative projects have received important public grants from Strategic Health Action, managed by the Carlos III Health Institute (ISCIII); the State Research Agency, Spanish Ministry of Science, Innovation and Universities (AEI/MCIU); and the R&D Activities Programmes of the Community of Madrid.

In addition to these collaborative projects, researchers at the CNIO have attracted funding for projects carried out by individual groups. In 2024, 8 of these projects received international funds, while 73 of them received national funding (mainly from the AEI/MCIU, the ISCIII, private foundations, as well as significant funding from the State Secretariat for Digitalization and Artificial Intelligence, for the implementation of R&D projects in Artificial Intelligence also with funding from the European Union, NextGenerationEU/PRTR). The international individual projects are funded by the European Union (5 European Research Council [ERC] grants and 3 Marie Skłodowska-Curie Actions).

Private funders and charities have also recognised the excellence of our scientific projects, among them, the Scientific Foundation of the Spanish Association Against Cancer (*FC AECC*), CRIS Cancer Foundation, the Ramón Areces Foundation, La Marató de TV3 Foundation, the BBVA Foundation, or "la Caixa" Banking Foundation

INTERNATIONAL GRANTS: COLLABORATIVE PROJECTS

EU FRAMEWORK PROGRAMMES FOR RESEARCH & INNOVATION (HORIZON 2020 AND HORIZON EU)

EUROPEAN UNION

INNOVATIVE HEALTH INITIATIVE (IHI)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Nuria	GUIDE.MRD: GUIding multi-moDal thErapies against MRD by liquid biopsies (Ref.: 101112066)

RESEARCH INFRASTRUCTURES

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ajenjo, Nuria	EvolveBBMRI: Accelerating datafication for support of EU health priorities, greening of biobanks and integrated approach to "One Health" (Ref.: 101131701)
Al-Shahrour, Fátima	EOSC4CAncer: A European-wide foundation to accelerate Data-driven Cancer Research (Ref.: 101058427)

MARIE SKŁODOWSKA-CURIE ACTIONS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Losada, Ana	MSCA Doctoral Network Cohesinet: Cohesin and its regulators: from chromosome dynamics and nuclear architecture to human diseases (Ref.: 101072505)
Real, Francisco X.	ITN TranSYS: Translational SYStemics: Personalised Medicine at the Interface of Translational Research and Systems Medicine (Ref.: 860895)
Soengas, María S.	MADRIDNIGHT: Researchers and citizens: facing together the European challenges (Ref.: 101061343)
Soengas, María S.	MADRIDNIGHT: Researchers and citizen creating together a better future (Ref.: 101162110)

HEALTH PROGRAMME

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ajenjo, Nuria	REACT: Respiratory Host-Pathogen Interaction (Ref.: 101057129)

MISSION CANCER

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria Peinado, Héctor	HORIZON-MISSON PANCAID: PANcreatic CAncer Initial Detection via liquid biopsy (Ref.: 101096309)

FET OPEN – NOVEL IDEAS FOR RADICALLY NEW TECHNOLOGIES

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	NanoBRIGHT: BRInGing nano-pHoTonics into the brain (Ref.: 828972)

TRUSTED DIGITAL SOLUTIONS AND CYBERSECURITY IN HEALTH AND CARE)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria; Real, Francisco X.	PANCAIM: Pancreatic cancer AI for genomics and personalized Medicine (Ref.: 101016851)

EU4HEALTH PROGRAMME (2021-2027)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	EU4HEALTH Jane-2: Joint Action on Networks of Expertise on Cancer (Ref.: 101183265)

DIGITAL EUROPE PROGRAMME - GENOMIC DATA INFRASTRUCTURE (GDI)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González-Neira, Anna	GoE: Genome of Europe (Ref.: 101168231)

US NATIONAL INSTITUTES OF HEALTH (NIH)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Real, Francisco X.	Role of the smooth muscle layer in bladder cancer biology and progression: a systems and experimental approach (Ref.: R21CA266660)
Tress, Michael	GENCODE: Integrated human genome annotation: generation of a reference gene set (Ref.: U24 HG007234)

***FUNDACIÓN RAMÓN ARECES*/CNIO/ WEIZMANN INSTITUTE OF SCIENCE**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria (Coordinator); Real, Francisco X.	MIT-BC Study: Tumor Microbiome and Immune profiles as predictors of Treatment response in high-risk Non-Muscle Invasive Bladder Cancer
Park, Solip (Coordinator)	Comprehensive identification of position-specific mutant p53 protein-protein interactions and their implications for cancer

MARK FOUNDATION FOR CANCER RESEARCH

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	Chemical hijacking of E3 ligases for the selective targeting of oncogenic mTOR signaling

WORLDWIDE CANCER RESEARCH

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor (Coordinator)	Defining the role of obesity in the regulation of disseminated tumor cell homing and dormancy (Ref.: 24-0197)

INTERNATIONAL GRANTS: INDIVIDUAL PROJECTS

EUROPEAN UNION

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	ERC Advanced Grant SHELTERINS: Targeting Shelterin Proteins in Cancer (Ref.: 882385)
Casanova, María	ERC Starting Grant INN-TIME: Co-option of host circadian rhythms in cancer (Ref.: 101115811)
González, Eva	ERC Proof of Concept Grant TargetRANK: Targeting RANK receptor as a novel therapeutic strategy in triple negative breast cancer (Ref.: 101062190)
Soengas, María S.	ERC Advanced Grant METALERT-STOP: Imaging, characterizing and targeting metastatic niches in melanoma (Ref.: 884699)
Valiente, Manuel	ERC Consolidator Grant ALTER-Brain: Metastasis-associated altered molecular patterns in the brain (Ref.: 864759)

MARIE SKŁODOWSKA-CURIE ACTIONS, POSDOCTORAL FELLOWSHIPS (MSCA-PF)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	MSCA NutFLirsFA: NUTRIENT FLUCTUATIONS AND THE INTEGRATIVE STRESS RESPONSE AS METABOLIC CELL FATE DETERMINANTS IN B CELL LYMPHOMA (Ref. 101108401)
Llorca, Óscar	MSCA WAC-mTOR-OC: Targeting R2TP/TTT/WAC/ mTOR complex in ovarian cancer. (Ref.: 101105041)
Quintela, Miguel A.	MSCA HD-BRECA: Integrating longitudinal multi-modal profiling of metastatic breast cancer patients for high-definition oncology (Ref.: 101155328)

NATIONAL GRANTS: COLLABORATIVE PROJECTS

INSTITUTE OF HEALTH CARLOS III / *INSTITUTO DE SALUD CARLOS III (ISCIII)* STRATEGIC HEALTH ACTION / *ACCIÓN ESTRATÉGICA EN SALUD (AES)*

RESEARCH PROJECTS IN HEALTH / *PROYECTOS DE INVESTIGACIÓN EN SALUD* [1]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González-Neira, Anna (Coordinator)	Transcriptome-wide association study of breast cancer women: a new strategy to identify novel risk genes for anthracycline-induced cardiotoxicity in the era of precision medicine (Ref. PI21/00795)
Robledo, Mercedes (Coordinator)	Characterization of new drivers associated with development and progression of rare endocrine and neuroendocrine tumors. Predictive markers of sensitivity to treatment (Ref.: PI20/01169)

SUB-PROGRAMME OF GRANTS FOR RESEARCH SUPPORT PLATFORMS IN HEALTH SCIENCES AND TECHNOLOGY / *SUBPROGRAMA DE AYUDAS PARA PLATAFORMAS DE APOYO A LA INVESTIGACIÓN EN CIENCIAS Y TECNOLOGÍAS DE LA SALUD* [1]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ajenjo, Nuria	*Plataforma de Biobancos y Biomodelos*, (Ref.: PT20/0070)
Ajenjo, Nuria; Ortega, Sagrario	*Marco colaborativo y cartera de servicios de biobanco y biomodelos del Centro Nacional de Investigaciones Oncológicas (CNIO)* (Ref.: PT23/00065)

IMPACT PROJECTS: PRECISION MEDICINE INFRASTRUCTURE ASSOCIATED WITH SCIENCE AND TECHNOLOGY[1]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	IMPaCT-Data Science (Ref.: IMP/00019)
González-Neira, Anna	IMPaCT-Genomic Medicine (Ref.: IMP/00009)

JOINT INTERNATIONAL COLLABORATIVE ACTIONS: ERA-NETS[2]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ERA PerMed: Personalized multimodal therapies for the treatment of lung cancer (Ref.: AC20/00114)
Casanova, María	ERANET TRANSCAN-3 LipidMac: Exploiting lipid-laden macrophages to overcome resistance to cancer immunotherapy (Ref.: AC22/00044)
Valiente, Manuel (Coordinator)	ERANET TRANSCAN-3 RISEBrain: Reverting immune suppression to elicit brain metastasis control (Ref.: AC22/00019)

PRECISION PERSONALIZED MEDICINE RESEARCH PROJECTS (STRATEGIC HEALTH ACTION)[2]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima; Llorca, Óscar; Robledo, Mercedes; Rodríguez, Sandra	IMPaCT_VUSCan: Development and implementation of a functional genomics platform for undiagnosed hereditary cancer (ref.: PMP22/00064)
Quintela, Miguel A. (Coordinator)	Integrating longitudinal patient-generated data and multi-omic profiling for comprehensive precision oncology in womens' cancers (Ref.: PMP22/00032)

1 This Programme is cofunded by the European Union

2 Funded the European Union "NextGenerationEU"/PRTR". *Only applicable to calls in 2022*

STATE RESEARCH AGENCY. MINISTRY OF SCIENCE, INNOVATION AND UNIVERSITIES / *AGENCIA ESTATAL DE INVESTIGACIÓN. MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES*

NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION

STRATEGIC LINES PROJECTS (PUBLIC-PRIVATE COLLABORATIVE PROJECTS)[3]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano (Coordinator)	Patient-derived pancreatic tumor organoids: a better predictive alternative to animal models (Ref. PLEC2022-009255)
Malumbres, Marcos (Coordinator)	A new patient-derived circulating micrometastases-on-chip platform for drug screen and validation (microMETonChip) (Ref.: PLEC2021-008106)
Malumbres, Marcos	Ultrasensitive optoplasmonic immunoassay platform (Oncodeeplasm) for early detection of breast cancer based on protein biomarkers at the deep region of the blood proteome (Ref.: PLEC2021-007892)
Paz-Ares, Luis (Coordinator)	Sensitization to immunotherapy through manipulation of tumor transcription (Ref. PLEC2022-009241)

PUBLIC-PRIVATE COLLABORATIVE PROJECTS / *PROYECTOS DE COLABORACIÓN PÚBLICO-PRIVADA*[3]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Álvarez-Vallina, Luis	CONDICOS: Conditional 4-1BB Costimulation exploiting Crosspriming in cancer (Ref.: CPP2022-009762)
Álvarez-Vallina, Luis	EFFESO: Immune check point-conditional 4-1BB co-stimulation for effective and safe cancer immunotherapy (Ref.: CPP2022-009765)
Álvarez-Vallina, Luis	*Desarrollo de la primera inmunoterapia celular STAb para tumores sólidos (STABSOLID)* (Ref.: CPP2023-010827)
Blasco, Maria	Development of a novel gene therapy for the treatment of Idiopathic Fibrosis (Ref.: CPP2021-008483)
Barbacid, Mariano	Genetic, pharmacological and computational approaches to identify Precision therapies in pancreatic cancer (Ref.: CPP2022-009609)
Peláez, Fernando	An effective and safe system for the treatment of Atrial Fibrillation through Irreversible Electroporation (Ref.: CPP2021-008480)
Quintela, Miguel A.	*LUMICare, una solución integral de medicina personalizada para el cuidado de pacientes de cáncer de la mujer basada en la intervención dietética, de hábitos de vida y de bienestar emocional* (Ref.: CPP2023-010721)
Quintela, Miguel A.	*FEMHEALTH: Nutrición de precisión para la mejora de los síntomas asociados a la menopausia* (Ref.: CPP2023-010803)
Soengas, María S.	*Estudio de fase II en pacientes con Carcinoma Basocelular nodular recién diagnosticado - SPOTLIGHT 204* (Ref.: CPP2023-010610)

CHALLENGES-RESEARCH PROJECTS OR KNOWLEDGE GENERATION / *PROYECTOS RETOS-INVESTIGACIÓN O DE GENERACIÓN DE CONOCIMIENTO (COORDINATED PROJECTS MODALITY)*[4]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Isasa, Marta	CERTACS-2: Mechanisms of chemosensitization to HER2-targeted therapies by CERT-degraders using multi-omics approaches (Ref.: PID2023-146670OA-C22; subproject of project PID2023-146670OA-C20)

3 Funded by MCIU/AEI/10.13039/501100011033 and the European Union "NextGenerationEU"/PRTR". *Only applicable to calls in 2021 and 2022*

4 This Programme is cofunded by the European Union

EXCELLENCE NETWORKS / *REDES DE EXCELENCIA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cortés Ledesma, Felipe (Coordinator)	Research Network Chromodyst: Chromosome dynamics and stability (Ref.: RED2022-134961-T)

COMMUNITY OF MADRID / *COMUNIDAD AUTÓNOMA DE MADRID*

Comunidad de Madrid

R&D ACTIVITIES PROGRAMME IN BIOMEDICINE:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González, Eva (Coordinator); Djouder, Nabil	*Programa SenesceX-CM: Senescencia celular en fisiología y enfermedad* (Ref.: S2022/BMD-7393)
Malumbres, Marcos; Barbacid, Mariano	*Programa i-LUNG 2.0* (Ref.: S2022/BMD-7437)
Mulero, Francisca	*Programa RENIM-CM: Red Madrileña de Nanomedicina en Imagen Molecular* (Ref.: S2022/BMD-7403)
Robledo, Mercedes (Coordinator); Al-Shahrour, Fátima	*Programa iTIRONET-CM: Estudio de la heterogeneidad celular y del entorno inmunológico en las patologías tiroideas: cáncer y enfermedad autoinmune* (Ref.: S2022/BMD-7379)

SYNERGY PROJECTS:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos (Coordinator)	*Proyecto scCANCER-CM: Convergencia tecnológica para el análisis biofísico y bioquímico de células individuales en la progresión del cáncer de mama* (Ref.: Y2020-BIO-6519)
Ortega, Sagrario (Coordinator); Blasco, Maria	*Proyecto COVID-PREclinical-MODels: Modelos Animales para el Estudio de la Covid-19. Desarrollo Pre-Clínico de Estrategias Preventivas y Terapéuticas* (Ref.: Y2020/BIO-6576)

SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / *FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (FC AECC)*

fundación científica asociación española contra el cáncer

COORDINATED GROUPS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor (Coordinator)	Reactivation of anti-tumor immune cell responses by functionalized nanoparticles in melanoma (Ref.: PRYCO223002PEIN)
Valiente, Manuel (Coordinator); Al-Shahrour, Fátima; Artiga, María J.	RENACER, the National Network of Brain Metastasis, as a strategy to challenge brain metastasis-associated lethality, therapeutic resistance and impact in quality of life (Ref.: PRYCO234528VALI)

ERA-NETS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	ERANET TRANSCAN-3 LipidMac: Exploiting lipid-laden macrophages to overcome resistance to cancer immunotherapy (Ref.: TRNSC213885CASA)
Valiente, Manuel (Coordinator)	ERANET TRANSCAN-3 Reverting immune suppression to elicit brain metastasis control (Ref.: TRNSC213878VALI)

"LA CAIXA" BANKING FOUNDATION / *FUNDACIÓN BANCARIA "LA CAIXA"*

"la Caixa" Foundation

HEALTH RESEARCH PROGRAMME:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	Single cell mapping of tumor-immune coevolution in organ-specific breast cancer metastasis (Ref.: HR23-00392)
Efeyan, Alejo (Coordinator)	NUTRITHELIUM: Decoding the paracrine control of metabolic fitness by endothelial nutrient signaling (Ref.: HR21-00046)
Fernández-Leiro, Rafael (Coordinador)	Mito4Neuro: Integrative characterization of the mitochondrial DNA replisome in health and disease (Ref.: HR24-00604)
Llorca, Óscar	ASC4Neuro: Amino acid transporter structure to target glutamate transmission in neuro diseases (Ref.: HR20-00081)
Llorca, Óscar	lncRNAs-RS-CRC: Understanding lncRNAs in replicative stress and colorectal cancer: from cancer biology to single-molecules (Ref.: HR21-00176)
Llorca, Óscar	LncRNAs modulating DNA damage and repair: towards novel therapies for hepatocellular carcinoma (Ref.: HR24-00341)
Real, Francisco X.	RBM10across: RBM10, a novel splicing regulator and tumor suppressor: from mechanisms to therapies (Ref.: HR21-01208)
Sabio, Guadalupe (Coordinator)	Mitochondrial metabolism, at the heart of disease (Ref.: HR24-00581)
Zugazagoitia, Jon	IL7R_LungCan: IL7R in lung cancer development, metastasis and resistance to immune checkpoint inhibitor therapy (Ref.: HR21-00761)

LA MARATÓ DE TV3 FOUNDATION / *FUNDACIÓ LA MARATÓ DE TV3*

Fundació La Marató de TV3

YOUNG INVESTIGATOR AWARDS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	Organ-specific biomarkers and therapies to improve the management of brain metastasis (Ref.: 141/C/2019)

ASTRAZENECA FOUNDATION / *FUNDACIÓN ASTRAZENECA*

Fundación AstraZeneca

YOUNG INVESTIGATOR AWARDS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	*Descifrando las bases moleculares y celulares de la comunicación intercelular en el cáncer de próstata letal: LETHALCHAT*

HNA FOUNDATION / *FUNDACIÓN HNA*

hna Fundación hna

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Cristina (until 3 October 2023)	*Estudio farmacogenómico de la toxicidad de Trastuzumab-Deruxtecan en pacientes con cáncer de mama*

NATIONAL GRANTS: INDIVIDUAL PROJECTS

INSTITUTE OF HEALTH CARLOS III / *INSTITUTO DE SALUD CARLOS III (ISCIII)* STRATEGIC HEALTH ACTION / *ACCIÓN ESTRATÉGICA EN SALUD (AES)*

GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES · Instituto de Salud Carlos III

RESEARCH PROJECTS IN HEALTH[5]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cascón, Alberto	Identification of novel susceptibility genes in head and neck paragangliomas by transcriptomic profiles and whole genome sequencing (Ref.: PI22/01490)
Fernández, Lucía	Exosomes derived from NKG2D CAR T cells (Exo-NKG2D CAR) as therapeutic strategy to treat pediatric CNS tumors (Ref. PI21/01049)
Guerra, Carmen	The stroma as a therapeutic target of pancreatic cancer (Ref.: PI19/00514)
Malats, Nuria	Deciphering the complex relationship between asthma/ allergy and pancreatic cancer risk (Ref.: PI21/00495)
Quintela, Miguel A.	Immuno-priming Triple-Negative Breast Cancer taking advantage of tumor signaling aberrations (Ref.: PI22/00317)
Rodríguez, Sandra	Use of CRISPR/Cas13 system for a programmable diagnosis and inhibition of fusion oncogenes (Ref.: PI20/01837)
Rodríguez, Sandra	*Mejora del tratamiento del cáncer mediante activación in vivo de muerte celular inmunogénica usando de forma combinada LNP y CRISPR dirigidas contra amplificaciones de oncogenes* (Ref.: PI23/01932)

TECHNICAL DEVELOPMENT IN HEALTH[5]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cortés, Felipe	*Reacción en cadena de endonucleasa CRISPR-Cas para un diagnóstico ultrasensible en punto de atención* (Ref.: DTS23/00147)

STATE RESEARCH AGENCY. MINISTRY OF SCIENCE, INNOVATION AND UNIVERSITIES / *AGENCIA ESTATAL DE INVESTIGACIÓN. MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES*

GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION

CENTRES OF EXCELLENCE "SEVERO OCHOA" AND UNITS "RAMIRO DE MAEZTU "SUB-PROGRAMME / *SUBPROGRAMA DE APOYO A CENTROS DE EXCELENCIA 'SEVERO OCHOA' Y UNIDADES 'RAMIRO DE MAEZTU'*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Center of Excellence "Severo Ochoa" (Ref.: CEX2019-000891-S)

R&D EXCELLENCE PROJECTS / *PROYECTOS DE I+D EXCELENCIA*[6]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Leiro, Rafael	CRYOTELO: Structural and molecular characterisation of the shelterin complex (Ref.: PID2020-120258GB-I00)

CHALLENGES-RESEARCH PROJECTS OR KNOWLEDGE GENERATION / *PROYECTOS RETOS-INVESTIGACIÓN O DE GENERACIÓN DE CONOCIMIENTO*[6]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	CLONTHERTUME: Development of computational multi-omics strategies for targeting therapeutically the tumour and tumour microenvironment heterogeneity (Ref.: PID2021-124188NB-I00)

5 This Programme is cofunded by the European Union · EUROPEAN UNION · European Regional Development Fund

6 This Programme is cofunded by the European Regional Development Fund (ERDF), "A way of making Europe" from year 2020 on · EUROPEAN UNION · European Regional Development Fund

CHALLENGES-RESEARCH PROJECTS OR KNOWLEDGE GENERATION / *PROYECTOS RETOS-INVESTIGACIÓN O DE GENERACIÓN DE CONOCIMIENTO*[6]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	PERSCAN: Personalized medicine in pancreatic cancer (Ref.: PID2021-124106OB-I00)
Casanova, María	FibroMac: Macrophage-fibroblast crosstalk in cancer (Ref. PID2021-122292NA-I00)
Cortés, Felipe	super-TOP: Physipathological implications of DNA supercoiling and topoisomerase function as master regulators of genome dynamics (Ref.: PID2020-119570RB-I00)
Cortés, Felipe	TOPO-GEN: Genome dynamics and instability: the force and the dark side of DNA topoisomerases (Ref.: PID2023-153302OB-I00)
Djouder, Nabil	MECHANOCIR: From cirrhosis to hepatocellular carcinoma: a mechanobiology perspective (Ref.: PID2021-122695OB-I00)
Efeyan, Alejo	RAGPARAPAN: Nutrient Rag GTPase signaling axis as a paracine determinant of pancreatic inflammation and carcinogenesis (Ref.: PID2022-136413OB-I00)
Fernández-Capetillo, Óscar	DIEHARD: Overcoming resistance to cancer therapies (Ref.: PID2021-128722OB-I00)
González, Eva	SYSTEMIC-RANK: Systemic and myeloid RANK in mammary gland homeostasis and breast cancer: beyond the epithelium. SYSTEMIC-RANK (Ref.: PID2020-116441GB-I00)
González, Eva	MENOCAN: Menopause and cancer: RANK as a metabolic regulator (Ref.: PID2023-152798OB-I00)
Llorca, Óscar	mTOR-chaperone: Structural and molecular basis for mTOR complex 1 (mTORC1) assembly and activation by the R2TP-HSP90 chaperone system (Ref.: PID2020-114429RB-I00)
Llorca, Óscar	PIKK_structure: Structural Bases and Mechanisms for the Assembly and Activation of PIKK Kinases (Ref.: PID2023-146110NB-I00)
Losada, Ana	COHESIN3D: Cohesin functions in development, differentiation and disease (Ref.: PID2019-106499RB-I00)
Losada, Ana	2COHESIN: Molecular mechanisms of cohesin STAG1 and STAG2 specific functions (Ref.: PID2022-139333NB-I00)
Macintyre, Geoffrey J.	INDUCIN: Induced models of chromosomal instability for drug development in cancer (Ref.: PID2022-137042OB-I00)
Méndez., Juan R.	FLEXI-REP: Flexibility in the DNA replication program in mammalian cells (Ref.: PID2022-142771NB-I00)
Park, Solip	CancerFitness: Systematic analysis of the cancer fitness landscape for cancer genes across cancer types (Ref.: PID2019-109571RA-I00)
Park, Solip	CompCancer: Comprehensive understanding of genomic alterations in cancer (Ref.: PID2022-141202OB-I00)
Pastor, Joaquín	MASTL-DEGs: MASTL DEGRADERS (Ref.: PID2023-150522OB-I00)
Otano, Itziar	Immune evasion in oncogene-addicted NSCLC subgroups (Ref.: PID2022-143169OB-I00)
Peinado, Héctor	OUTANERVE: Role of NGFR regulating the immunoevasive phenotype of melanoma metastasis initiating cells (Ref.: PID2020-118558RB-I00)

6 This Programme is cofunded by the European Regional Development Fund (ERDF), "A way of making Europe" from year 2020 on · EUROPEAN UNION · European Regional Development Fund

CHALLENGES-RESEARCH PROJECTS OR KNOWLEDGE GENERATION / *PROYECTOS RETOS-INVESTIGACIÓN O DE GENERACIÓN DE CONOCIMIENTO*[6]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	EVdamaged: Analysis of the role of extracellular vesicles in the control of DNA damage in melanoma (Ref.: PID2023-147503OB-I00)
Plaza, Iván	ESFRRET: Functional and structural characterization of KIF5B-RET rearrangements (Ref.: PID2020-117580RB-I00)
Plaza, Iván	SFUPOS: Structural, functional and pharmacological studies of the hyper-phosphorylated form of c-Src in human cancer (Ref.: PID2023-152471OB-I00)
Real, Francisco X.	PDAC-MolPrev: An integrative approach towards the prevention of pancreatic cancer using mouse models and genomic tools (Ref.: PID2021-128125OB-I00)
Sabio, Guadalupe	METABOkines: Organ crosstalk in metabolism: involvement of the p38MAPK pathway (Ref.: PID2022-138525OB-I00)
Soengas, María S.	MEL_IMAGE_TREAT: Imaging and targeting metastatic niches in melanoma (Ref.: PID2020-117621RB-I00)
Soengas, María S.	IMMUNO-MEL-MET: IMMUNOMODULATORY SIGNALS AS DRIVERS AND TARGETS OF (PRE)METASTATIC NICHES IN MELANOMA (Ref.: PID2023-147213OB-I00)
Tafur, Lucas	SEAC-STRUC: Structural basis of TORC1 regulation by the SEA complex (Ref.: PID2023-147101NA-I00)
Valiente, Manuel	METneural: Crosstalk between brain metastasis and neural circuits (Ref.: PID2021-124582OB-I00)

EUROPEAN EXCELLENCE INITIATIVE/ *PROYECTOS EUROPA EXCELENCIA*[7]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	INN-TIME: Co-option of host circadian rhythms in cancer (Ref.: EUR2023-143451)

SCIENTIFIC EQUIPMENT AND INFRASTRUCTURES / *EQUIPAMIENTO E INFRAESTRUCTURAS CIENTÍFICAS*[8]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	*Fortalecimiento de la capacidad científica-computacional en el CNIO: Implementación de un Clúster de Computación de Alto Rendimiento (HPC) para el desarrollo de la IA y la oncología computacional* (Ref.: EQC2024-008222-P)
Martínez, María D.	*Implementación de "sorting" espectral con imagen de subpoblaciones celulares murinas y humanas, para posteriores estudios "ómicos.* (Ref.: EQC2024-007953-P)
Mulero, Francisca	High Resolution Magnetic resonance 3T based in superconducting magnet wihtout helium as criogenizer (Ref.: EQC2021-006797-P)
Peset, Isabel	*Implementación de una plataforma de Biología Espacial para estudios de interacciones celulares complejas en el contexto del tejido tumoral* (Ref.: EQC2024-008582-P)

6 This Programme is cofunded by the European Regional Development Fund (ERDF), "A way of making Europe" from year 2020 on

7 *Funded by MCIU/AEI/10.13039/501100011033 and the European Union "NextGenerationEU"/PRTR"*

8 *This Programme is cofunded by the European Union. NextGenerationEU/PRTR in 2021, and ERDF in 2023*

STATE SECRETARY FOR DIGITALIZATION AND ARTIFICIAL INTELLIGENCE. MINISTRY FOR DIGITAL TRANSFORMATION AND PUBLIC SERVICE / *SECRETARÍA DE ESTADO DE DIGITALIZACIÓN E INTELIGENCIA ARTIFICIAL. MINISTERIO PARA LA TRANSFORMACIÓN DIGITAL Y DE LA FUNCIÓN PÚBLICA*

R&D PROJECTS IN ARTIFICIAL INTELLIGENCE[9]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Macintyre, Geoffrey J.	*Promoción del avance de la IA para la oncología de precisión*
Malats, Núria	*Implementación en hospitales terciarios del algoritmo IA-PMPD para la predicción de metástasis de cáncer de páncreas y demostración de su rendimiento a tiempo real*

SPANISH FOUNDATION FOR SCIENCE AND TECHNOLOGY / *FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y TECNOLOGÍA (FECYT)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barthelemy, Isabel	Access to bibliografic data bases: web of Science (Ref.: MDG-24-11706)

SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / *FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (FC AECC)*

GRANTS FOR RESEARCH PROJECTS IN CANCER:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Álvarez-Vallina, Luis	Generation of dual STAb-T cells targeting intracellular and cell surface tumor-associated antigens to overcome the clonal heterogeneity of solid tumors (Ref.: PRYGN234844ALVA)
Djouder, Nabil	Elucidating the role of liver cirrhosis in the development of hepatocellular carcinoma: towards novel therapeutic strategies (Ref.: PRYGN211184NABI)
Losada, Ana	Identification of a gene signature associated with aggressive Ewing Sarcoma for diagnostic and therapeutic purposes (Ref.: PROYE20046LOSA)
Quintela, Miguel A.	Assessment of the stimulated immune signaling pathways status and its relationship with response to immunotherapies and ADCs in triple-negative breast cancer (Ref.: PRYGN234888QUIN)
Real, Francisco X.	STAG2 and FGFR3: cooperation with the DREAM complex in bladder cancer (Ref.: PRYGN223005REAL)

AECC LAB GRANTS:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Sandra	Programmable detection and inhibition of fusion oncogenes using CRISPR/Cas13 (Ref.: LABAE20049RODR)

"SEED" IDEAS RESEARCH PROJECTS:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Martínez, Bárbara	Metabolic shuttling of epigenetic factors (Ref.: IDEAS246908MART)

9 *Funded by the European Union "NextGenerationEU"/PRTR*

"LA CAIXA" BANKING FOUNDATION AND LUZÓN FOUNDATION / *FUNDACIÓN BANCARIA "LA CAIXA" Y FUNDACIÓN LUZÓN*

HEALTH RESEARCH PROGRAMME (NEURODEGENERATIVE DISEASES)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	RNALS: Modulating nucleolar activity and stress responses as a therapeutic strategy in ALS (Ref.: HR22-00890)

"LA CAIXA" BANKING FOUNDATION / *FUNDACIÓN BANCARIA "LA CAIXA"*

HEALTH RESEARCH PROGRAMME (CANCER)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	CancerTelomeres: Targeting Telomeres in Cancer (Ref.: HR18-00023)
Cortés, Felipe	DNA topology and topoisomerases during oncogenesis: mechanisms and therapeutic opportunities (Ref.: HR24-00980)
González, Eva	Role of RANK in myeloid cells and tumor development (Ref.: HR23-00361)
Soengas, María S.	IMMUMELANOMA: Immunomodulatory drivers in melanoma progression and therapy response (Ref.: HR20-00465)
Valiente, Manuel.; Al-Shahrour, Fátima.; Artiga, María J.	Establishment of a precision medicine pipeline to personalize the treatment of brain metastasis (Ref.: HR23-00051)

CAIXAIMPULSE PROJECTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cortés, Felipe; Fernández-Leiro, Rafael	CRISPR-Cas Endonuclease Chain Reaction: a revolution in point-of-care genetic testing (Ref.: CI24-20447)

CRIS FOUNDATION AGAINST CANCER / *FUNDACIÓN CRIS CONTRA EL CÁNCER*

EXCELLENCE GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	Network of myeloid vulnerabilities at metastatic site (Ref.: PR_TPD_2020-09)
Velasco, María	Deciphering the bone marrow niche: the role of mechanoreceptor PIEZO1 in haematopoiesis and leukaemia (Ref.: PR_TPD_2022-21)

SPANISH ASSOCIATION OF PANCREATOLOGY / *ASOCIACIÓN ESPAÑOLA DE PANCREATOLOGÍA*

CARMEN DELGADO/MIGUEL PÉREZ-MATEO GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Guerra, Carmen	Risk factors and prevention strategies of pancreatic ductal adenocarcinoma
Malats, Núria	Marcadores microbianos para el diagnóstico del adenocarcinoma ductal de páncreas

FERO FOUNDATION / *FUNDACIÓN FERO*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	Macrophage-fibroblast cell to cell circuit modulation in NSCLC

GETNE GROUP / *GRUPO GETNE*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Montero, Cristina	*Identificación de marcadores moleculares de respuesta a tratamiento con inhibidores tirosina-quinasa en cáncer* (Ref.: G2212)

BBVA FOUNDATION / *FUNDACIÓN BBVA*

SCIENTIFIC RESEARCH TEAMS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	IMMUNO-KETOCAR: The cholinergic immunosurveillance of ketogenic diet in the treatment of hepatocellular carcinoma (Ref.: EIC21-1-243)

RAMÓN ARECES FOUNDATION / *FUNDACIÓN RAMÓN ARECES*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Artiga, María J.	*Red Nacional de Metástasis Cerebral: Implantación, Desarrollo y Coordinación* (Ref.: CIVP20S10662)

GRUPO ESPAÑOL MULTIDISCIPLINAR DE MELANOMA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Use of circulating extracellular vesicles and ctDNA from lymphatic fluid exudate obtained postlymphadenectomy as surrogate markers of minimal residual disease in melanoma patients

LASEXTA AND AXA FOUNDATION / *LASEXTA Y FUNDACIÓN AXA*

"CONSTANTES Y VITALES" AWARDS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	Award granted on the 2024 call, in the modality of "Young Talent in Biomedical Investigation"

LEUKEMIA & LYMPHOMA FOUNDATION (FUNDED THANKS TO MARÍA ASUNCIÓN ALMAJANO SALVO FOUNDATION) / *FUNDACIÓN LEUCEMIA Y LINFOMA*

"ASUN ALMAJANO" GRANT

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Vivas, Yurena	*La señalización de nutrientes y la síntesis lisosomal en el linfoma folicular*

EDUCATION AND TRAINING PROGRAMMES

One of the CNIO's principal goals is to increase its training capacity in order to give students and professionals the opportunity to advance their careers in the healthcare sector. The CNIO obtains essential grants for the training of new professionals and attracts funds to hire personnel-in-training. Coordinating specific training programmes, establishing collaborations with different universities and institutions, and managing grant funding and training contracts to hire personnel-in-training, are fundamental to guarantee the training of new researchers and the continuity of high-level research projects.

During 2024, the CNIO obtained funding to hire personnel-in-training from several national and international public institutions such as the State Research Agency of the Spanish Ministry of Science, Innovation and Universities (AEI/MCIU), the Community of Madrid, the Institute of Health Carlos III, and the China Scholarship Council, most of them co-funded by European Structural and Investment Funds. Funding also came from private funders, including the Spanish Association Against Cancer *(AECC)*, "la Caixa" Banking Foundation, and the *Cris* Foundation.

In 2024, CNIO signed several new agreements with Spanish Universities and other institutions, namely with the Universidad Politécnica de Madrid, the Universidad Miguel Hernández, and the Universidad Complutense de Madrid. Addenda were also signed to update and extend the existing agreements with the Universities of Alfonso X el Sabio, UCM, URJC, Lleida and Extremadura. In addition, agreements have been signed with 10 other organisations.

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2020	2021	2022	2023	2024
Training of PhD students	109	135	130	130	**127**
Post-doctoral training	52	62	55	57	**58**
Training for MDs	7	10	12	9	**9**
Laboratory training for MSc/BSc students	85	106	136	115	**105**
Laboratory training for technicians	5	9	17	14	**13**

TRAINING OF BSC/MSC STUDENTS

The CNIO is committed to training junior scientists at the onset of their careers. To this end, the Centre has established a Programme that offers BSc and MSc students the opportunity to gain hands-on practical laboratory experience by working on ongoing research projects in one of the CNIO Groups. CNIO offers 2 types of short-term laboratory training:

- An annual Summer Training Programme for undergraduate students, from any country, who are in their final year of biomedical studies. The Programme encompasses 8 weeks of full-time laboratory training (292.5 hours). During this time, the students actively participate in research projects in one of the CNIO Groups. During 2024, 4 students from Spain participated in this programme.
- Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO's individual Research Groups or Units. This year, 105 students participated in these programmes, of whom 4 ended up joining the CNIO as pre-doctoral students.

TRAINING OF PHD STUDENTS

The training of PhD students in cutting-edge cancer research is of key importance to the CNIO. The Centre offers many opportunities for bright and dynamic university graduates, of all nationalities, to pursue an ambitious PhD project. To attest this, 12 students obtained their PhD degrees in 2024 and 22 others joined the CNIO in the same year. Over 17% of the students working at the CNIO in 2024 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

Since 2008, the "la Caixa" Foundation has offered international fellowships to PhD students to enable them to carry out their thesis projects in biomedical research in Spanish centres of excellence, such as the CNIO. In 2024, 3 pre-doctoral students received a doctoral fellowship from the INPhINIT programme of the "la Caixa" Foundation to join CNIO.

The distribution of students across the CNIO's Research Programmes in 2024 was as follows: 73 % of students worked in the Molecular Oncology Programme, 15% in the Structural Biology Programme, 9 % in the Human Cancer Genetics Programme, and 3% in the Clinical Research Programme.

FUNDING OF PHD TRAINING	NO.
SPANISH ORGANISATIONS	108
State Research Agency / *Agencia Estatal de Investigación* (*AEI*). Ministry of Science, Innovation and Universities / *Ministerio de Ciencia, Innovación y Universidades* (Predoctoral Fellowships)	53
State Research Agency / *Agencia Estatal de Investigación* (*AEI*). Ministry of Science, Innovation and Universities / *Ministerio de Ciencia, Innovación y Universidades* (I+D Projects)	7
Spanish Association Against Cancer (*AECC*) / *Fundación Científica de la AECC* (I+D Projects)	5
Spanish Association Against Cancer (*AECC*) / *Fundación Científica de la AECC* (Fellowships)	11
Institute of Health Carlos III / *Instituto de Salud Carlos III* (*ISCIII*) (Fellowships)	1
Institute of Health Carlos III / *Instituto de Salud Carlos III* (*ISCIII*) (I+D Projects)	1
Cris Foundation / *Fundación Cris*	2
Community of Madrid / *Comunidad de Madrid*	13
Bionam Biotech AIE	1
The Spanish Hematology and Hemotherapy Society / Fundación Española de Hematología y Hemoterapia	1
CNIO	4
"la Caixa" Banking Foundation/ *Fundación Bancaria* "la Caixa" (I+D Projects)	2
"la Caixa" Banking Foundation/ *Fundación Bancaria* "la Caixa" (Predoctoral fellowships)	7
INTERNATIONAL ORGANISATIONS	19
AXA	2
China Scholarship Council (CSC)	3
European Research Council	9
NIH	2
MERCK	1
Marie Skłodowska-Curie actions of the European Commission	2
TOTAL	**127**

POST-DOCTORAL TRAINING

One of the CNIO's prime objectives is to attract young researchers, who have recently obtained their PhD or MD degrees, and offer them highly attractive research projects at the forefront of cancer research.

In 2024, 48 postdoctoral fellows worked at the CNIO. It is noteworthy that about a quarter of these fellows came from outside Spain, many of them from very prestigious international institutions.

Thanks to donations received through the 'CNIO Friends' platform, the 9th call of the 'CNIO Friends' Postdoctoral Contract Programme, launched in 2024, resulted in the recruitment of 7 scientists for a 2-year period each.

FUNDING SOURCES OF POSTDOCTORAL CONTRACTS	NO.
SPANISH ORGANISATIONS	41
State Research Agency / *Agencia Estatal de Investigación (AEI)*. Ministry of Science, Innovation and Universities / *Ministerio de Ciencia, Innovación y Universidades (Postdoctoral fellowships)*	4
State Research Agency / *Agencia Estatal de Investigación (AEI)*. Ministry of Science, Innovation and Universities / *Ministerio de Ciencia, Innovación y Universidades (I+D Projects)*	3
Spanish Association Against Cancer (*AECC*) / *Fundación Científica de la AECC (Fellowships)*	5
"la Caixa" Banking Foundation / *Fundación Bancaria "la Caixa" (Postdoctoral Junior Leader- INCOMING)*	1
"la Caixa" Banking Foundation / *Fundación Bancaria "la Caixa" (I+D Projects)*	3
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)*	1
Cris Cancer Foundation (*CRIS*) / *Fundación Cris Contra el Cáncer (CRIS)*	1
Community of Madrid / *Comunidad de Madrid*	3
CNIO	18
Banco Santander Foundation / *Fundación Banco Santander*	1
BBVA Foundation / *Fundación BBVA*	1
INTERNATIONAL ORGANISATIONS	17
AIRC	1
European Commission	1
European Research Council	7
Janssen	2
Marie Skłodowska-Curie actions of the European Commission	4
ESMO	1
Worldwide Cancer Research UK	1
TOTAL	**58**

POSTGRADUATE PROGRAMMES

In addition, the CNIO – in collaboration with academic institutions across Spain – offers access to a variety of postgraduate programmes that cover the areas of Cellular & Molecular Biology, Molecular Biomedicine, Biotechnology, Biocomputing, Clinical & Applied Cancer Research, and Therapeutic Targets.

Official Postgraduate Programmes in Molecular Biosciences

The majority of the international postgraduate trainings offered at CNIO are developed in collaboration with the Faculty of Medicine and the Faculty of Sciences at the Autonomous University of Madrid (UAM). These trainings fall under 4 official Postgraduate Programmes, namely, the Doctorate in Molecular Biosciences, the Master in Biomolecules & Cell Dynamics, the Master in Molecular Biomedicine, and the Master in Biotechnology. CNIO also collaborates with the UAM as a partner institution of UAM's Doctoral School (EDUAM) and is a member of its Management Committee.

Master's in Bioinformatics and Data Science for Precision Medicine and Personalized Health

The Master's in Bioinformatics and Data Science for Precision Medicine and Personalized Health is organised together with the National School of Health of the National Institute of Health Carlos III (*Escuela Nacional de Sanidad del Instituto de Salud Carlos III, ENS-ISCIII*).

Official Master's Degree in Therapeutic Targets of Cell Signalling: Research and Development

CNIO collaborates with the Department of Biochemistry and Molecular Biology at the University of Alcalá de Henares (*UAH*) for the *Máster Oficial en Dianas Terapéuticas en Señalización Celular: Investigación y Desarrollo.*

LABORATORY TRAINING FOR TECHNICIANS

This training programme has been developed for students of Anatomical Pathology, Clinical Diagnostic Laboratory, and Archiving/Recording and is organised through agreements with 19 institutions that provide secondary education for laboratory technicians in Spain. It provides students with hands-on knowledge of cellular and molecular biology techniques. The programme consists of 14 weeks (370-400 hours) of laboratory training. In 2024, 13 students participated in this programme at CNIO.

TRAINING FOR MDS

In line with the CNIO's commitment to bridging the 'bench to bedside' gap, the Centre offers 3 training opportunity programmes to MDs and other healthcare professionals. The training usually consists of a 3-month period during residency. In 2024, 9 medical residents from 5 different hospitals enjoyed the benefits of rotations within the different Groups and Units at CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2024, the Ramón y Cajal Programme supported 7 scientists. Established in 2001 by the former Spanish Ministry of Science and Technology (now the State Research Agency of the Spanish Ministry of Science, Innovation and Universities), this special initiative aims to encourage Spanish or foreign scientists working abroad to return to or relocate to Spain. Successful candidates are selected based on their potential capacity to lead independent projects and groups, or to successfully contribute to ongoing research in existing groups.

VISITING RESEARCHERS PROGRAMME

The Fundación Occident (previously known as Fundación Jesús Serra) aims to help eminent international specialists work together with CNIO researchers for a few months in order for them to expand their knowledge in areas of common interest. During 2024, Yardena Samuels, from the Weizmann Institute of Science (Israel), and Tak Mak, from the University of Toronto (Canada), were the beneficiaries of the Fundación Occident's Visiting Researchers Programme.

Occident Fundación

'SCIENCE BY WOMEN' PROGRAMME

Thanks to the 'Science by Women' Programme, launched by the Spanish 'Fundación Mujeres por África', Manal Mohamed Elsayed Ahmed, from the National Research Centre, Egypt, was awarded a grant to join the CNIO's H12O-CNIO Cancer Immunotherapy Clinical Research Unit for a 6-month stay as a visiting scientist. Manal also worked in the Molecular Diagnostics Unit from September to November 2024.

FUNDACIÓN MUJERES POR ÁFRICA

SCIENTIFIC EVENTS

MEETINGS & CONFERENCES

CNIO-CaixaResearch Frontiers Meetings (CFM)

CNIO-CaixaResearch Frontiers Meetings are premier international conferences organised by the CNIO and "la Caixa" Foundation. These gatherings are dedicated to exploring cutting-edge aspects of cancer research, providing a unique platform for intensive and dynamic exchange and debate on scientific ideas. Each event features approximately 20 globally recognised leaders in oncology who present their latest findings over two and a half days. Participants benefit from a collaborative environment that fosters the exchange of experiences, ideas, and best practices across various sectors, facilitates networking with like-minded researchers, and offers opportunities to engage with keynote speakers. The conferences also include enriching extracurricular programmes and provide insights into the latest developments in the field. Additionally, up to 100 participants are selected through a widely publicised call for applications, chosen for their potential to contribute significantly to the conferences through presentations of hot topics as posters or short talks.

In 2024, we arranged 2 CFMs: 1) **Molecular Chaperones in Cancer and Protein Quality Control**. Molecular chaperones are essential for ensuring proper protein folding, stability, and function in both healthy and diseased states, including cancer. Despite considerable progress, the precise molecular mechanisms governing chaperone activity and their involvement in cancer progression are still not fully understood. This conference highlighted recent advances in unravelling the structure and function of molecular chaperones, particularly HSP90, using model organisms. It also explored how chaperones interact with other proteins in cancer-related networks, their role in cancer evolution, and strategies to better target chaperones for therapeutic purposes. By bringing together researchers and clinicians from various fields, the event fostered collaboration and deepened our understanding of the pivotal role chaperones play in cancer biology. 2) **Frontiers in Immunomodulation and Cancer Therapy: second edition.** The immune system has the ability to recognise and kill tumour cells. However, tumours may invade the immune system generating an immunosuppressive tumour microenvironment, making tumours resistant to immunotherapies. This conference focused on and discussed recent findings on mechanistic insights into the immune escape machinery, immunotolerance, and immunomodulation. It addressed how tumours can escape the immune system by creating an immunosuppressive tumour microenvironment and how tumours become resistant to immunotherapy. The conference also presented an overview of pre-clinical and emerging clinical advances targeting the immune system for the treatment of cancer or cancer-associated disorders.

MOLECULAR CHAPERONES IN CANCER AND PROTEIN QUALITY CONTROL
10 – 12 JUNE 2024

ORGANISERS

- **Gabriela Chiosis**, Memorial Sloan Kettering Institute, NY, US - **Nabil Djouder**, CNIO, Madrid, Spain - **Judith Frydman**, Stanford University, US - **Oscar Llorca**, CNIO, Madrid, Spain - **Paul Workman**, The Institute of Cancer Research, London, UK.

SESSIONS

- Protein Quality Control I / II
- Chaperones, Molecular Mechanisms and Structure
- Chaperones in disease and Chaperonotherapy I / II

FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY (2ND EDITION)
16-18 OCTOBER 2024

ORGANISERS

- **Maria Casanova-Acebes**, CNIO, Spain - **Nabil Djouder**, CNIO, Spain - **Luis Álvarez-Vallina**, CNIO, Spain - **David Sancho**, CNIC, Spain - **Gaia Trincucci**, JEM - Rockefeller University Press, US.

SESSIONS

- Targeting the tumor microenvironment
- Immune system engineering and vaccination strategies in tumors
- Immunomodulation and immunotolerance
- Metabolism in immunology
- Neuroimmunomodulation

PANCAID 2ND GENERAL ASSEMBLY MEETING
17-19 JANUARY 2024

ALIPANC FIRST SCIENTIFIC MEETING
5 MARCH 2024

ORGANISER

- **Alejo Efeyan,** CNIO, Madrid, Spain

With the attendance of 85 researchers from 36 groups distributed throughout Spain. The event consisted of scientific presentations, including clinical, translational, and basic research, with the aim of nourishing, connecting, and strengthening pancreatic cancer research.

CHROMODYST ANNUAL MEETING (NATIONAL NETWORK CHROMOSOME DYNAMICS AND STABILITY)
25 – 27 SEPTEMBER 2024

CO-ORDINATED BY

- **Felipe Cortés**, CNIO, Madrid, Spain

JORNADA SEFF 2024
FARMACOGENÉTICA COMO SOPORTE A LA DECISIÓN CLÍNICA
16 NOVEMBER 2024

AI MEETS CANCER RESEARCH
11-12 NOVEMBER 2024

ORGANISING COMMITTEE

- **Maria A. Blasco**, Director of the Spanish National Cancer Research Centre (CNIO) - **Raul Rabadan**, Director of Mathematical Genomics Program (PMG), Dept. Systems Biology & Biological Informatics, Columbia University Irving Medical Center - **Anil Rustgi**, Director of the Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center (HICCC) - **Alfonso Valencia**, Director of Life sciences dept. ICREA research professor, Barcelona Supercomputing Center (BSC).

This symposium was an exchange of ideas to develop collaboration and innovation in cancer research and education.

TRAINING COURSES AND WORKSHOPS

The CNIO is committed to disseminating the latest advances in cancer research with the wider community, including healthcare professionals and emerging scientists, helping them to stay up to date on recent developments in specialised techniques. This is achieved through training courses and practical workshops led by CNIO scientists and technologists.

FLOW CYTOMETRY INDUCTION COURSE: FUNDAMENTALS, APPLICATIONS, DATA ANALYSIS AND DATA PRESENTATION
15-16 JANUARY 2024, 27-28 FEBRUARY 2024,
25-26 MARCH 2024, 29-30 APRIL 2024, 3-4 JUNE 2024,
8-9 JULY 2024, 9-10 SEPTEMBER 2024,
22-23 OCTOBER 2024, 26-27 NOVEMBER 2024

SPEAKERS

- **Julia García-Lestón,** Flow Cytometry Unit Technician. CNIO
- **Jamie McCarthy**, European Applications Specialist. DeNovo Software
- **Irene Fernández Delgado**, Flow Cytometry Unit. CNIO
- **Lola Martinez,** Head of the Flow Cytometry Unit. CNIO

SCIENTIFIC COMMUNICATION COURSE
15 JANUARY 2024

- Pitches, Posters and Presentations Workshop

ORGANISER

- **CNIOSA**

SPEAKER

- **Laura Moro, PhD** (Science Writer & Science Communication Consultant)

ADVANCE MULTICOLOR COURSE
13-14 FEBRUARY 2024

SPEAKERS

- **Laura Ferrer**, Scientific Solutions Manager - Medical & Scientific Affairs at BD
- **Lola Martinez**, Head of the Flow Cytometry Unit. CNIO
- **Ana Vieira & Patricia Suárez**, European Technical Application Specialists. Cytek Biosciences
- **Jamie McCarthy**, European Application Specialist. DeNovo Software
- **Serena di Cecilia**, Senior European Application Specialist. FlowJo, BD Biosciences

MULTICOLOR FLOW CYTOMETRY WORKSHOP
20-21 MAY 2024

SPEAKERS

- **Oliver Burton**, Senior Scientist, Liston-Dooley Lab. University of Cambridge
- **Lola Martinez**, Head of the Flow Cytometry Unit. CNIO
- **Ana Vieira**, European Technical Application Specialists. Cytek Biosciences
- **Jamie McCarthy**, European Application Specialist. DeNovo Software
- **Ioannis Panetas**, European Application Specialist. FlowJo, BD Biosciences

ADVANCE MULTICOLOR COURSE GOOD PRACTICES IN HIGH DIMENSIONAL FLOW CYTOMETRY
30 SEPTEMBER – 1 OCTOBER 2024

SPEAKERS

- **Laura Ferrer-Font**, Scientific Solutions Manager - Medical & Scientific Affairs at BD
- **Oliver Burton**, Senior Scientist, Liston-Dooley Lab. University of Cambridge
- **Lola Martinez**, Head of the Flow Cytometry Unit. CNIO
- **Ana Vieira**, European Technical Application Specialists. Cytek Biosciences
- **Jamie McCarthy**, European Application Specialist. DeNovo Software
- **Serena di Cecilia**, European Application Specialist. FlowJo, BD Biosciences

CONSORTIA SENESCEX WORKSHOP
28 OCTOBER 2024

ORGANISERS

- **Eva González Suárez** and **Nabil Djouder**, CNIO, Spain

CNIO DISTINGUISHED SEMINARS

The Distinguished Seminars Series aims to bring exceptional, internationally recognised scientists to present seminars and engage with researchers at CNIO. These seminars are recurring events, open to the public, and typically take place on Fridays at noon in the CNIO Auditorium. Each series features renowned scientists who discuss topics of broad interest to the CNIO community.

In total, CNIO hosted 20 distinguished speakers in 2024.

DATE	SPEAKER	ORGANISATION	TITLE
FEBRUARY			
09/02/2024	**Cristina Mayor**	IRB Barcelona, Spain	Targeted protein degradation: drug discovery opportunities & overcoming resistance mechanisms in cancer
16/02/2024	**Mariam Jamal-Hanjani**	University College London, UK	Insights into lung cancer evolution and metastasis in TRACERx and PEACE
23/02/2024	**Sjors Scheres**	MRC Laboratory of Molecular Biology, Cambridge, UK	Molecular pathology of neurodegenerative diseases by cryo-EM of amyloids
MARCH			
08/03/2024	**Lindsay Hinck**	University of California, Santa Cruz, US	Consequences of Physiological DNA Damage in the Breast and Uterus
15/03/2024	**Joseph Schlessinger**	Yale University School of Medicine, New Haven, US	Cell Signaling By Receptor Tyrosine Kinases; From Basic Principles To Cancer Therapies
22/05/2024	**Andrés Hidalgo**	Spanish National Centre for Cardiovascular Research - CNIC, Madrid, Spain	Multidimensional neutrophils

DATE	SPEAKER	ORGANISATION	TITLE
APRIL			
12/04/2024	**Ailong Ke**	Molecular Biology and Genetics, Cornell University, New York, US	New Frontiers in CRISPR-Cas Biology – RNA-guided Proteases and Ancestorial Cas9s in Transposons
26/04/2024	**Tony Wyss-Coray**	Stanford University, Stanford, US	Young blood for old brains
MAY			
10/05/2024	**Jacob Hanna**	RenewalBio Ltd.; Weizmann Institute of Science, Rehovot, Israel	Synthetic Ex Utero Embryogenesis: from Naive Pluripotent Stem Cells to Bona Fide Embryo-Models
24/05/2024	**Jose Ignacio Martín Subero**	August Pi i Sunyer Biomedical Research Institute, Barcelona, Spain	Learning from the past to predict the future: epigenetic memory in lymphoid tumors
JUNE			
07/06/2024	**Michelle Monje-Deisseroth**	Howard Hughes Medical Institute, Stanford University, US	Neuron-glial interactions in health and disease: from cognition to cancer
14/06/2024	**Renato Ostuni**	Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milano, Italy	Molecular and spatial control of tumor-associated macrophages
SEPTEMBER			
13/09/2024	**Alberto Kornblihtt**	Institute for Physiology, Molecular Biology and Neurosciences (IFIBYNE CONICET-UBA), University of Buenos Aires, Argentina	Chromatin, transcription, alternative splicing and the cure of an hereditary disease
27/09/2024	**Eunjung (Alice) Lee**	Boston Children's Hospital & Harvard Medical School, Boston, US	Multifaceted Role of Retrotransposons in Human Cancer
OCTOBER			
04/10/2024	**Denes Hnisz**	Max Planck Institute for Molecular Genetics, Berlin, Germany	Transcriptional condensates in health and disease
11/10/2024	**Ketan J. Patel**	MRC Weatherall Institute of Molecular Medicine, MRC-MHU, Oxford, UK	How an endogenous mutagen leads to clonal haematopoiesis
NOVEMBER			
15/11/2024	**Mikhail Shapiro**	California Institute of Technology, Pasadena, US	Talking to cells: biomolecular ultrasound for non-invasive imaging and control of cellular function
22/11/2024	**Daniela Quail**	McGill University, Montreal, Canada	Fuelling cancer through the tumour immune microenvironment
29/11/2024	**Xavier Trepat**	Institute for Bioengineering of Catalonia; ICREA, Barcelona, Spain	Mechanobiology of the immunocompetent tumor ecosystem
DECEMBER			
20/12/2024	**Carola García de Vinuesa**	Crick Institute, London UK	Insights into mechanisms of B cell-driven autoimmunity

AD-HOC SEMINARS

In addition to the CNIO Distinguished Seminar Series, the CNIO also organises a variety of *ad hoc* seminars throughout the year. These seminars are designed to foster academic collaboration, promote intellectual growth, and provide opportunities for networking both within the academic community and across institutions. In 2024, CNIO researchers organised a total of 47 ad hoc seminars.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
04/01/2024	**Miguel Bernabé Rubio**	Centre for Gene Therapy & Regenerative Medicine (CGTRM), London UK	Mechanical factors induce Myc-dependent dedifferentiation of adult epidermal cells
16/01/2024	**Javier Rey Barroso**	Institut de Pharmacologie et Biologie Structurale, Centre National de la Recherche Scientifique,Toulouse, France	Immune cells on the move : dynamics in homeostasis, aging and leukemia
17/01/2024	**Gauthier Chassang**	Lawyer in EU and International Law Inserm – CERPOP, Faculté de Médecine, Toulouse - France	European Ethical Principles Driving Innovation in AI and Machine Learning for Health
29/01/2024	**Raúl Bardini Bressan**	Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) University of Copenhagen, Denmark	Dissecting the aetiology of paediatric high-grade gliomas in human stem cell-based models
FEBRUARY			
20/02/2024	**Edgar Bernardo Vasco**	Centre for Genomic Regulation (CRG), Barcelona, Spain	HNF1A as a central player in endocrine and exocrine pancreas homeostasis
26/02/2024	**Jacky Goetz**	National Institute of Health and Medical Research, INSERM, Paris, France	Intravascular Forces shape Tumor Metastasis
26/02/2024	**Daniel Salas**	Leiden University Medical Centre (LUMC), The Netherlands	Mass-Spectrometry Proteomics Tools and its application for Breast and Ovarian Cancer and Immunotherapy
29/02/2024	**Basil Greber**	UCR - The Institute of Cancer Research, London, UK	High-resolution structures of the human CDK-activating kinase bound to inhibitors: Harnessing the power of cryo-EM for discovery of cancer therapeutics
MARCH			
06/03/2024	**Francisco M. Barriga**	Group Leader - Cancer Genome Engineering Lab Vall D´Hebron Institute of Oncology (VHIO), Barcelona, Spain	Dissecting the Function of Copy Number Alterations in Cancer
11/03/2024	**Alfonso Troyano and Barbara Ottolini**	Oxford Nanopore Technologies	What You're Missing Matters! Methlyation, Adpative Sampling, Single Cells omics, and Trascriptome applications using ONT
APRIL			
15/04/2024	**Manuel Perucho**	Sanford Burnham Prebys Medical Discovery Institute (SBP), Professor Emeritus, La Jolla, California, US	Serendipity, DNA fingerprinting, and cancer discoveries
18/04/2024	**Catherine Alix-Panabières**	PU-PH – Oncology Institut Universitaire de Recherche Clinique (IURC) Director of the Liquid Biopsy Laboratory, University Medical Centre of Montpellier (CHU), France WG Leader – European Liquid Biopsy Society (ELBS), France	Liquid Biopsy: From Discovery to Clinical Application
22/04/2024	**Karlene Cimprich**	Stanford University School of Medicine, Stanford, US	The Causes and Consequences of Replication Stress

DATE	SPEAKER	ORGANISATION	TITLE
30/04/2024	**Isidro Cortés-Ciriano**	European Bioinformatics Institute - European Molecular Biology Laboratory (EMBL-EBI)	Computational cancer genome analysis: insights into the mechanisms underpinning cancer genome complexity and applications to early detection
MAY			
09/05/2024	**Juan A. Gabaldón**	Barcelona Supercomputing Centre (BSC) and Institute for Research in Biomedicine (IRB) Life Sciences (BSC) and Mechanisms of Disease (IRB) Associate Professor, Pompeu Fabra University, Barcelona, Spain	Microbiome and colorectal cancer: potential roles and clinical potential
10/05/2024	**Mark A. Febbraio**	Monash Institute of Pharmaceutical Sciences, Monash University. Australia	Role of Exercise and Extracellular Vesicles in the Prevention of Cancer
13/05/2024	**Ke Yuan**	Machine Learning and Computational Biology School of Computing Science, University of Glasgow School of Cancer Sciences, University of Glasgow Cancer Research UK Scotland Institute, UK	Learning deep representations of cancer tissue without supervision
23/05/2024	**Isidoro Cobo**	Assistant Professor, Division of Clinical Immunology & Rheumatology, Associate Scientist, CAMBAC (Comprehensive Arthritis, Musculoskeletal, Bone and Autoimmunity Center), Department of Medicine, Heersink School of Medicine The University of Alabama at Birmingham, US	Particle uptake by macrophages triggers bifurcated transcriptional pathways that differentially regulate inflammation and lysosomal gene expression
27/05/2024	**Abraham Acevedo**	University Hospital of Canarias, Tenerife, Spain	New models and mechanisms of amyotrophic lateral sclerosis (ALS)
27/05/2024	**Eduard Porta**	Josep Carreras Leukaemia Research Institute, Barcelona, Spain	Spatial transcriptomics in cancer research: from Cancer Hallmarks to clinical biomarkers
30/05/2024	**Javier Batista Pérez**	ADT S.L.R.S., Rome, Italy	Unlocking Multiomics Landscapes: Balancing Resolution and Bias for Optimal Spatial Insights
31/05/2024	**Serap Erkek Özhan**	Izmir Biomedicine and Genome Center, Izmir, Turkey	Epigenetic regulation in bladder cancer
JUNE			
06/06/2024	**Sanguk Kim**	Pohang University of Science and Technology (POSTECH), South Korea	Network-based interpretable machine learning predicts cancer patient response to immune checkpoint inhibitors
17/06/2024	**Pablo San Segundo Acosta**	Post-doctoral researcher Sara Borrel, Functional Proteomics Laboratory, Carlos III Health Institute, Majadahonda, Madrid, Spain	Structural insights into bacterial NADPH oxidases and archeal CO2-fixing megacomplexes
25/06/2024	**Jae Bum Kim**	Director, Center for Adipocyte Structure & Function. National Leading Researcher Initiatives. Korea	Adipose Tissue Remodeling and Its Role in Energy Metabolism
JULY			
03/072024	**Juan Manuel Schvartzman**	-	Polyamines regulate cell fate by changing the accessibility of histone tails
04/07/2024	**Juan Enríquez Traba**	Lüscher Lab, University of Geneva, Switzerland	Dissociable control of motivation and reinforcement by distinct ventral striatal dopamine receptors
22/07/2024	**Leticia Cuarental**	Erasmus Medical Center, Netherlands	Fosl1: an oncogene regulating kidney diseases
23/07/2024	**Miguel Lafarga**	University of Cantabria, Santander, Spain	*Fundamentos metodológicos y aplicaciones del análisis ultraestructural en Biomedicina*
23/07/2024	**Susana Vázquez Torres**	Institute for Protein Design, Department of Biochemistry, University of Washington, US	De novo design of protein binders to bioactive peptides: from hormones to snake toxins
SEPTEMBER			
12/09/2024	**Amri Wandel**	University of Jerusalem, Israel	Astrobiology: looking for biochemistry and life on other planets

DATE	SPEAKER	ORGANISATION	TITLE
16/09/2024	**Albert Antolín**	Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain	Harnessing machine learning and chemical biology to better precision oncology
19/09/2024	**Alejandro Ocampo**	Epiterna SA, Epalinges, Switzerland	Developing novel approaches to target the biology of aging based on epigenetic reprogramming
OCTOBER			
04/10/2024	**Albana Gatelli**	Institute of Physiology, Molecular Biology and Neuroscience (IFIBYNE)- CONICET (National Research Council of Argentina). University of Buenos Aires (UBA), CABA, Argentina	New Molecular Therapeutic Targets in Breast Cancer: Tumor cell and Microenvironment interplay
07/10/2024	**Paola Falletta**	University Vita-Salute San Raffaele, Milan, Italy	What doesn't kill it makes it stronger: how Integrated Stress Response fuels Breast Cancer metastasis
14/10/2024	**Gaia Trincucci**	Deputy Editor from Journal of Experimental Medicine, US	Scientific publishing at JEM: What, How and Why
18/10/2024	**Harihar Basnet**	Tsinghua University, Beijing, China	Investigating transcriptomic plasticity in cancer and beyond using Flura-seq
24/10/2024	**Sanne Belle Schagen**	The Netherlands Cancer Institute, Amsterdam, The Netherlands	Improving the care of cancer patients who are faced with cognitive problems
29/10/2024	**Denis Wirtz**	Johns Hopkins University, Baltimore, US	3D Multi-omic cellular maps and CAR T cells engineered to infiltrate solid tumors
30/10/2024	**Irene Martínez Rio**	Applications Speacialist BD	Transforming Cell Analysis: The new BD FACSDiscover tm S8 Cell Sorter with BD CellView
NOVEMBER			
11/11/2024	**Isabel Espinosa Medina**	HHMI - Janelia Research Campus, Ashburn, US	Development of the Nervous System – Organ connection: New tools to visualize cell histories in vivo
14/11/2024	**Antonia Tomás Loba**	Biomedical Research Institute of Murcia (IMIB), Spain	Chronodisruption and chronosomes: the overall effect of social jetlag
19/11/2024	**Beatriz Ocaña**	Mathematical Oncology Lab, University of Castilla la Mancha, Ciudad Real, Spain	Brain metastasis: from mathematical models to clinical applications
21/11/2024	**Nada Kalaany**	Harvard Medical School, US	How do cancers overcome their metabolic challenges?
25/11/2024	**Ilse Ariadna Valtierra Gutierrez, Paula Jáuregui and Laura Sanchez-Burgos**	Senior Editor in the Cancer team at Nature Communications; Senior Editor at Nature Microbiology and Nature Immunology and Associate Editor in the Cancer team at Nature Communications	Publishing at Nature Press
DECEMBER			
16/12/2024	**Juan José Montero Valderrama**	Technical University Munich, Munich, Germany	Uncovering cancer vulnerabilities through novel non-Coding, multidimensional, and phenotypic screening technologies
16/12/2024	**Susana Vázquez Torres**	Institute for protein design, University of Washington	De Novo Design of protein binders to bioactive peptides: from hormones to lethal snake toxins

WOMEN IN SCIENCE SEMINARS

In 2024, the WISE Office invited and welcomed several top female and male leaders from different areas to tell us about their career path experiences.

DATE	SPEAKER	ORGANISATION	TITLE
09/01/2024	H.E. Dr. Sarmila Parajuli Dhakal	Ambassador of Nepal to Spain	Bridging Borders Through Science: Insights from Nepal
07/03/2024	Clara Montoya	CNIO Artistic Residences 2023	*Ignotas y Resueltas*
12/03/2024	Eleonora Viezzer	Department of Atomic, Molecular and Nuclear Physics, University of Seville, Spain	Nuclear fusion: Bringing the Sun down on Earth
08/04/2024	Mina Sohn	Founder & CEO of Koala Company	Power of listening to your inner voice
07/06/2024	Nuria Oliver	Director of the ELLIS Alicante Foundation, Alicante, Spain	My personal story in AI
08/11/2024	Manal M E Ahmed	Medical Research and Clinical Studies Institute, National Research Centre (NRC), Egypt	The Intersection of Molecular Diagnostics and Immunotherapy: A Path to Personalized Medicine

SCIENCE DISSEMINATION EVENTS

WORLD CANCER RESEARCH DAY
"LA INTELIGENCIA ARTIFICIAL SE UNE A LA CIENCIA QUE BUSCA CURAR EL CÁNCER"
24 SEPTEMBER 2024

ORGANISER

- **Centro Nacional de Investigaciones Oncológicas (CNIO)**

WITH THE SUPPORT OF:

- **"la Caixa" Foundation**

LECTURES:

- *"La importancia de los datos y la computación en la investigación en cáncer"*
  Alfonso Valencia, director of Life Sciences at the Barcelona Supercomputing Center (BSC). Professor at ICREA.
- *"El impacto presente y futuro de la IA"*
  Nuria Oliver, co-founder and vice-president of ELLIS.

ROUND TABLE:

- **Ignacio Astilleros**, director of Strategy, Sales and Marketing at Openchip. Member of CNIO Friends.
- **Maria A. Blasco**, director of the Spanish National Cancer Research Centre-CNIO.
- **Miguel Calero Lara**, deputy director general for applied services, Training and Research of the Carlos III Institute of Health (ISCiii).
- **Alfonso Valencia**, director of Life Sciences at the Barcelona Supercomputing Center (BSC). Professor at ICREA.
- **Chair: Pampa García Molina**, science journalist. Director of the Science Media Center Spain.

EUROPEAN RESEARCHERS' NIGHT 2024
CNIO: CONOCE A LOS CIENTÍFICOS, SÉ UN CIENTÍFICO
CNIO: MEET THE SCIENTISTS, BECOME A SCIENTIST
27 SEPTEMBER 2024

The Centre opened its doors to the public to show its commitment to society and to promoting scientific culture.

Between 5 p.m. and 9 p.m., 3 groups of people participated in a science experiment with young volunteer researchers from the Centre, who guided each group step by step through the process of carrying out a **science experiment** at home using everyday household products. The scientists also explained what a researcher's work consists of and answered questions and addressed people's doubts. Altogether, 254 people and 60 volunteers signed up for the day.

Our objective is to teach people who we are and how we work at the CNIO, one of the best cancer research centres in the world. During the Researchers' Night at the CNIO, people had the opportunity to discover and do science, no matter what their age. The activities began with a video presentation, with live connections to different areas of the centre, and a welcome from a group of scientists from the CNIO who shared their personal experiences about how they decided to dedicate themselves to cancer research. Next, we conducted a DNA extraction experiment. Attendees also had the chance to visit different laboratory stands that we had prepared with various activities and equipment, so that they could learn how we study, how we identify the mutations that lead to diseases like cancer, and how we search for better treatments. During the event, they had the opportunity to ask questions, and the CNIO team really enjoyed trying to answer them.

In addition, the CNIO did an Instagram 'live' at 8pm for those who wanted to connect live, two researchers were able to chat with them and answer their questions.

On 4 October, we held a Webinar for high schools (10:15 a.m. till 11:00 a.m.). Designed to allow secondary and high school students to connect from their classrooms, this initiative enabled them to ask questions and learn more about three young researchers.

The 2024 edition in Madrid is coordinated by the madri+d Foundation for Knowledge and funded by the European Union under Horizon Europe, the EU 's Research and Innovation Program, through the Marie Skłodowska-Curie Actions with grant agreement number 101.162.110.

OPEN DOORS DAY: EVERYONE UNRAVELLING CANCER
14 NOVEMBER 2024

The CNIO also dedicates considerable effort to bringing science and society closer together; one of these endeavours is its collaboration with the madri+d research network for the organisation of the Madrid Science Week (XXIV Semana de la Ciencia y la Innovación, 4-17 November 2024).

In November 2024, the CNIO participated in the Science and Innovation Week under the motto '*Todos y todas desarmando al cancer*' ('Everyone Unravelling Cancer'). The event was held online with over 41 attendees, who took the opportunity to learn more about a top research institution like the CNIO.

The Madrid Science and Innovation Week is a science outreach and citizen participation event organised by the Community of Madrid through the madri+d Foundation for Knowledge, with the aim of actively engaging society in the processes of research and development in science, technology, and innovation.

GOVERNANCE

BOARD OF TRUSTEES

→ Honorary President

- **Diana Morant Ripoli**
  Minister of Science, Innovation and Universities
  Ministra de Ciencia, Innovación y Universidades

→ President

- **Eva Ortega-Paíno**
  Secretary-General for Research, Ministry of Science, Innovation and Universities
  Secretaria General de Investigación del Ministerio de Ciencia, Innovación y Universidades

→ Vice-President

- **Marina Anunciación Pollán Santamaría**
  Director of the National Institute of Health Carlos III
  Directora del Instituto de Salud Carlos III

→ Appointed Members

- **Javier Padilla Bernáldez**
  Secretary of State for Health, Ministry of Health
  Secretario de Estado de Sanidad, Ministerio de Sanidad

- **María Eloísa del Pino Matute**
  President of the Spanish National Research Council (*CSIC*)
  Presidenta de la Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC)

- **Borja Monreal Gainza**
  Director of Public Policy at the Prime Minister´s Office
  Director del Departamento de Políticas Públicas del Gabinete de la Presidencia del Gobierno

- **Agustín González González**
  Deputy Director-General for Networks and Cooperative Research Centres, National Institute of Health Carlos III
  Subdirector General de Redes y Centros de Investigación Cooperativa del Instituto de Salud Carlos III

- **Vincenç Juan Verger**
  Managing Director of Health Research, Training and Accreditation, the Balearic Islands
  Director General de Investigación en Salud, Formación y Acreditación de las Islas Baleares

- **Blanca Ares González**
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*In compliance with the Spanish Transparency Legislation (Spanish Law 19/2013, of December 9), the following information is hereby provided:
-At the close of the financial year, the accumulated remuneration received by the Top Management of the Foundation – the CNIO´s Director plus the Managing Director – amounted to a total of €311,588 in 2024 (€363,641 in 2023). This amount was received as base salary and position-based supplements: €249,099 (€236,915 in 2023); variable remuneration: €61,304 accrued during 2023 (€118,434 in 2023, accrued during 2021 and 2022); and 0.5% in back pay: €1,185 accrued for 2023.
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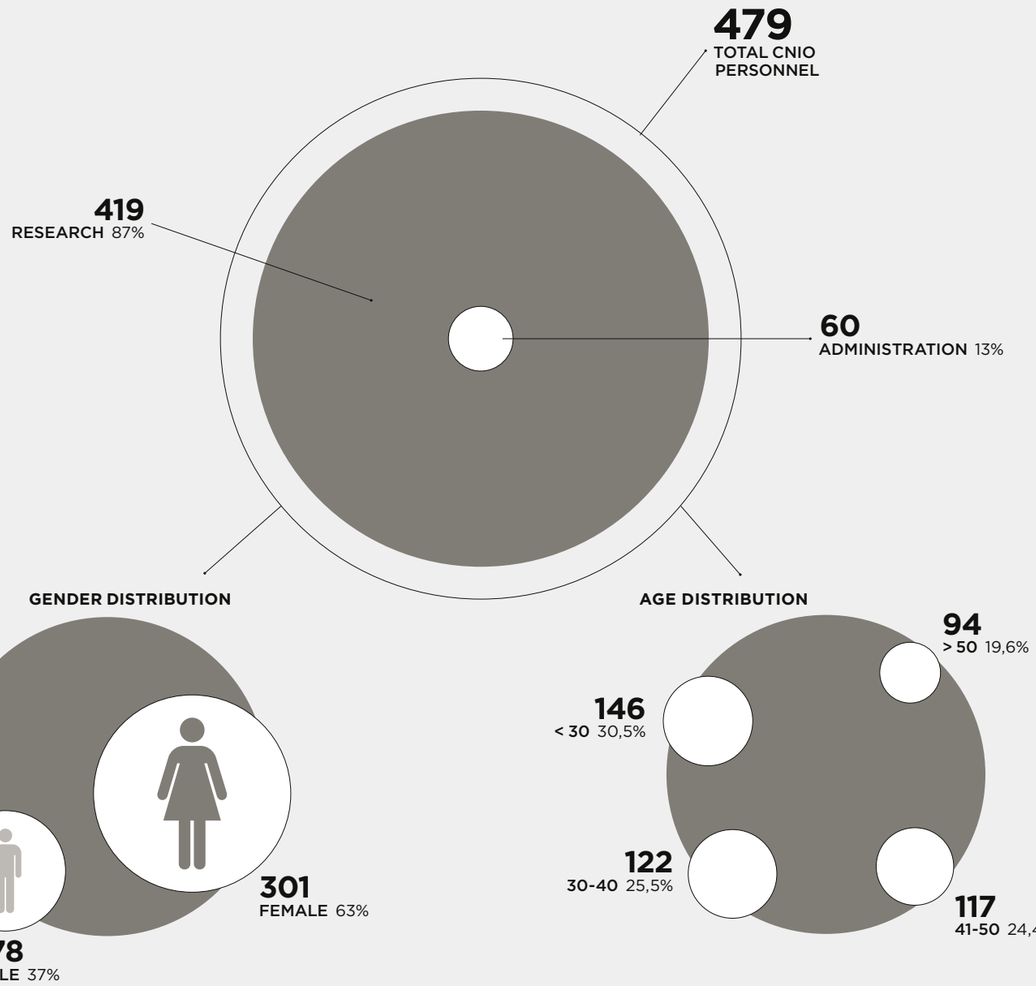

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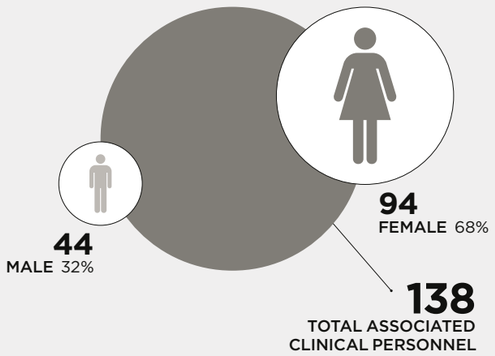

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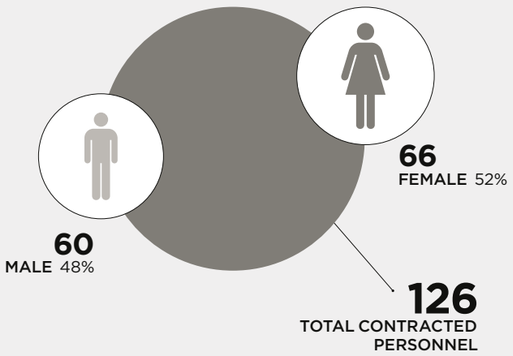

CNIO SCIENTIFIC PERSONNEL 2024

TOTAL SCIENTIFIC PERSONNEL **419**

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DISTRIBUTION BY PROFESSIONAL CATEGORY

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RESEARCH SCIENTISTS 13,4%	56
POST-DOCTORAL FELLOWS 11,5%	48
GRADUATE STUDENTS 28,9%	121
TECHNICIANS 35%	147

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS	FEMALE	51%	**24**
	MALE	49%	**23**
RESEARCH SCIENTISTS	FEMALE	75%	**42**
	MALE	25%	**14**
POST-DOCTORAL FELLOWS	FEMALE	69%	**33**
	MALE	31%	**15**
GRADUATE STUDENTS	FEMALE	55%	**66**
	MALE	45%	**55**
TECHNICIANS	FEMALE	67%	**99**
	MALE	33%	**48**
TOTAL SCIENTIFIC PERSONNEL	FEMALE		**264**
	MALE		**155**

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