**ANNUAL
REPORT 2023**

SPANISH NATIONAL
CANCER RESEARCH CENTRE, CNIO

ANNUAL
REPORT 2023

CONTENTS

"In 2023 we reached an historical maximum in the number of publications in journals with an impact factor greater than 15 since the start of CNIO operations."

MARIA A. BLASCO
Director

FOREWORD
MARIA A. BLASCO Director

I feel proud to open this foreword by remarking that 2023 was once again excellent for our scientific productivity. We authored a total of 197 papers, 33 of which were published in journals with an impact factor between 10 and 15, and 59 publications in journals with an impact factor greater than 15. The latter figure represents the maximum of the historical series from the beginning of CNIO operations.

According to the *Nature Index,* considering our scientific contributions in the natural sciences and healthcare sector, the CNIO ranked second among cancer-focused singular centres in Europe and holds the 9th position worldwide. Likewise, the SCImago ranking places CNIO in the 9th position among cancer centres worldwide. These indicators provide evidence of the success of our scientific activity in cancer research and our leadership at the international level.

The CNIO External Scientific Advisory Board (SAB) plays a critical role in guiding the strategic plans of the CNIO, as well as in reviewing our Research Groups. During 2023, the SAB reviewed the Groups in 3 of the CNIO Research Programmes (Molecular Oncology, Structural Biology and Clinical Research). Upon concluding their visit to the CNIO on April 24-25, they delivered a report that highlights the scientific excellence of the Centre, acknowledging its prominent position at the forefront of basic and translational cancer research worldwide, and commending the senior leadership of the CNIO and its Scientific Director for maintaining the CNIO's scientific productivity and prominence, despite the considerable challenges imposed by the recent pandemic.

The adaptation of the Centre to the constant evolution of science, by incorporating new research groups working in emerging fields in cancer research, is key to maintaining our competitive edge. Along these lines, a process of selecting new groups for the Molecular Oncology Programme resulted in our incorporating Guadalupe Sabio, formerly Group Leader at the Spanish National Cardiovascular Centre (*CNIC*), by the end of 2023. Guadalupe Sabio is a well-known expert in the

TOTAL NUMBER OF PUBLICATIONS

Legend: CNIO First author — CNIO Collaboration

TOP PUBLICATIONS

Legend: IF > 15 — IF > 10 - 15
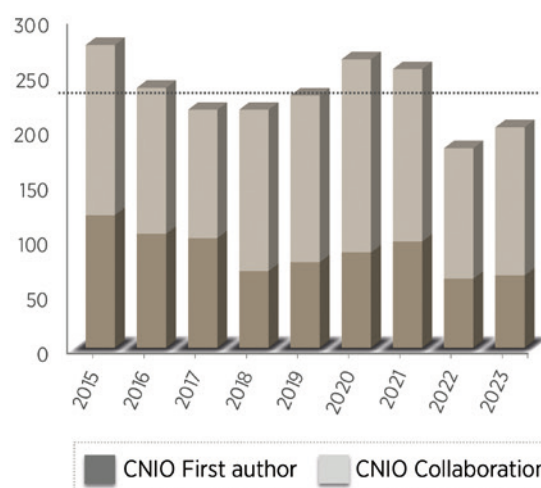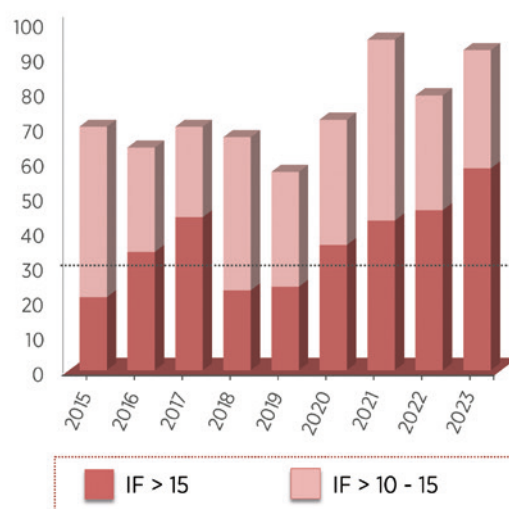
field of the pathologies related with metabolism and obesity, including cancer, and at the CNIO she is leading the new Organ Crosstalk in Metabolic Diseases Group. Likewise, Antonio Pérez, Head of the Service of Paediatric Haemato-oncology at the *Hospital La Paz*, joined the CNIO to lead a joint Unit focused on the paediatric cancer field, to reinforce our Clinical Research Programme. Antonio Pérez is a reference in the application of cell therapy in paediatric cancer, including NK cells, transplant of haematopoietic stem cells, and advanced therapies with CAR-T cells.

Another candidate who will join the CNIO in the near future is Gonçalo Bernardes, at present Chemical Biology Professor at the University of Cambridge, UK. Prof Bernardes has focused his successful research career on the application of chemical biology methods and approaches to better understand cancer-related processes. During 2023 he was beneficiary of the *Jesús Serra* Foundation (now *Fundación Occident*) Visiting Researchers Programme, spending several months at the CNIO during a sabbatical stay. He was the recipient of a grant from the *ATRAE* programme of the *MCIU*, aimed to attract researchers of recognised international prestige to research centres in Spain.

In addition, a selection process was put into place during 2023 to incorporate a new Junior Group Leader to the Structural Biology Programme. The process was concluded with the proposal to recruit Lucas Tafur, a bright young scientist who is currently a senior researcher at the University of Geneva. At the CNIO he will develop a project focused on the study of the mTORC1 complex, highly relevant in the regulation of cancer and metabolism from a structural biology perspective. He is expected to be incorporated into the CNIO in September 2024.

Regarding our activity in the innovation field, in 2023, the CNIO filed 7 priority patent applications, 2 PCT applications for international extension, and 3 for national phases. Our efforts in promoting collaborations with industry resulted in more than €4M secured through research agreements with key partners from the pharmaceutical and biotech sectors. As much as 63% of CNIO agreements with the private sector were established with international entities. Additionally, the net income from royalties derived from the licensing of CNIO assets quantified in 2023 (corresponding to the sales in 2022) reached €1.64M, which represents an increase of 26% over the levels achieved in the previous year. Moreover, a new spin-off company was launched in 2023 based upon the results from our research activities. Thus, the CNIO is participating in a new company, *TNC Terapia*, together with the *Fundación para la Investigación Biomédica Hospital 12 de Octubre* (*FIBH12O*). Both institutions are co-owners of the LUMICA technology, which aims to use precision nutrition as a therapeutic tool against cancer and derives from the work of the Breast Cancer Clinical Research Unit, headed by Miguel A. Quintela. These

achievements represent a clear reflection of the leadership and influence of the CNIO and our researchers worldwide in the innovation field.

Our drug discovery programme (the Experimental Therapeutics Programme) continues working on the co-development of drug candidates from novel targets validated by CNIO investigators, contributing to the generation of new therapeutic agents to fight cancer and ageing-related diseases, which also represent assets for the CNIO.

Furthermore, we continue supporting and promoting the participation of CNIO researchers in national and international calls for proposals from public agencies and private foundations that fund projects with a strong innovation component. Thus, in 2023 two CNIO Groups were awarded grants from the new National *AEI* Innovation calls, co-funded by the EU Reconstruction Funds "NextGenerationEU". Miguel A. Quintela is coordinating the project "Integrating longitudinal patient-generated data and multi-omic profiling for comprehensive precision oncology in women's cancers"; and Mercedes Robledo is participating in the project "Development and implementation of a functional genomics platform for undiagnosed hereditary cancer (IMPaCT_VUSCan)". It is also worth mentioning the project "GUIDE.MRD: GUIding multi-moDal thErapies against MRD by liquid biopsies", which received a grant from the EU call "Innovative Health Initiative Joint Undertaking (IHI-JU)" that was awarded to Núria Malats as principal investigator, with a total budget of €17.6M, out of which €1.27M will fund the research developed at the CNIO.

Our training programmes are among the key elements of the Centre's strategic plan, as an essential part of our mission. To provide high-quality training to the new generations of biomedical researchers is one of our priorities, and we warmly thank our sponsors contributing to support our training initiatives.

As another initiative related to attracting talent, each year the CNIO continues hosting visiting scientists from both national and international institutions. In addition to the above mentioned case of Gonçalo Bernardes, recipient of a grant from the *Jesús Serra* Foundation's Visiting Researchers Programme, 3 female African scientists, awarded with grants from different editions of the "Science by Women" programme of the *Mujeres por Africa* Foundation, came to CNIO to spend sabbatical stays of 6 months as Visiting Scientists: Marwa Muhammad Abu-Serie Ali (7th edition), from GEBRI, SRTA-City (Egypt); Mai Tolba (5th edition), from Ain Shams University (Egypt); and Nwodo Ngozi (8th edition), from the University of Nigeria.

We continued with all our ongoing activities related to the communication and discussion of scientific results and

advances, through the organisation of our ongoing series of seminars, conferences and workshops. In 2023 we organised 2 new editions of the prestigious CNIO-CaixaResearch Frontiers Meetings, which always count on the participation of key leaders in a broad array of fields. Two conferences were held in 2023, focusing on the topics of Genome Organisation and Stability (May 22-23) and Metastasis (November 6-8).

The "Philosophy and Science" symposia series that began in 2019, with the support *of Banco Sabadell* Foundation, held its 5th edition in 2023. Under the title "The catastrophe of the loss of biodiversity", a panel of philosophers and scientists discussed the reasons behind this increasingly accelerated process and the associated challenges, as well as the potential strategies to prevent or revert such loss.

CNIO news also continued attracting the interest of the media, marking over 6,300 appearances in press (printed and online), an increase of nearly 40% over the previous year. In addition to covering some of our most relevant research findings, the general media were also attracted by other CNIO initiatives, such as described below.

On the occasion of World Cancer Day on February 4, we organised a campaign of fundraising and awareness, with the collaboration of the actress Nathalie Seseña, focusing on conveying the strong links between human health and planet health. The success of the campaign contributed to significant presence of CNIO in the media, which in turn generated a significant number of new donors for our "CNIO Friends" philanthropic platform.

The Institutional Image and Outreach Office leads several projects aiming to generate society's trust and attention. Upon the departure of the former Office Coordinator, Amparo Garrido, we incorporated a new Director, Juan de Nieves, an expert in the field of visual arts, with a long trajectory as curator of museums and exhibitions, at both national and international levels.

In 2023, the 6th edition of our "CNIO Arte" project, co-funded by the *Banco Santander* Foundation, which explores the common territories between scientific research and artistic creation, brought together the visual artist Amparo Garrido and Elizabeth Blackburn, the latter recipient of the Nobel Prize of Medicine and Physiology (2009) for her work on telomeres, structures that protect the chromosomes and that are involved in ageing processes at the cellular and organismal levels. The pieces created by the artist, 2 pictures and 1 video, were exhibited at the CNIO throughout the year. For the 3rd year, we had our own space at the contemporary art fair ARCO (February 22-26), where the work of Amparo Garrido was exhibited, attracting a lot of interest from the public and the media. CNIO Arte 2023 was also presented at the Guggenheim

CNIO Arte 2023 encounter at the Guggenheim Museum in Bilbao. July 5, 2023. In this image: CNIO Director Maria A. Blasco (right), Nobel laureate Liz Blackburn connected virtually by Zoom (stage screen), and CNIO Arte 2023 featured artist Amparo Garrido (left).

Museum in Bilbao. Beyond the social visibility and impact, CNIO Arte generated, for the 6th consecutive year, a donation of €100,000 that contributed directly to our "CNIO Friends" philanthropic initiative.

Derived from the success of this initiative, a proposal was developed jointly with the *Instituto Cervantes* (*IC*), to exhibit the works of the successive CNIO Arte editions in the different *IC* sites across the world. Through this initiative, the exhibition "*Excelentes*: Dialogues between Art and Science" was opened on February 2 at the *IC* in New York. The exhibition, which offers a selection of works from the CNIO Arte programme across the years, including photographs, videos, drawings, and collages, was on display until April 15 and moved later to the Spanish Embassy in Washington (from April 27 to September 20) and then to the *IC* site in Chicago (from October 25). This exhibition will tour across other *IC* sites during 2024.

Furthermore, aiming to increase the impact of this initiative, we organised the 4th edition of the "Art and Science Symposia" series, which brings renowned professionals from both fields to participate in an open and enriching discussion on a selected topic. The symposium was held jointly with the presentation of the 6th edition of CNIO Arte, under the title "The thread of life in arts and sciences", chaired by Carlos Jiménez (Emeritus Professor of Aesthetics, historian, and art critic).

Another related activity established in 2023 was the "CNIO Artistic Residences" initiative, which received financial support from the *FECYT*. A young artist, Clara Montoya, was selected through an open call to spend several months at the CNIO. The aim is to achieve an understanding of our research activities and, based on this experience, to create an artwork inspired by or reflecting the work developed at the CNIO. As a result of the project, Clara Montoya created the work "*Ignota* 2023", which is now being exhibited at the CNIO, and was also presented in ARCO. Based on the success of this initiative, a second edition of the CNIO Artistic Residences was launched in 2023. A call was opened to select a new artist, who will develop his/her residence at the CNIO in 2024.

The fundraising strategy of the CNIO is implemented through our Philanthropy and Development Office, aiming to generate an additional income stream for our research activities. Donations from members of the public, corporate organisations, associations, and foundations go directly to the "CNIO Friends Contracts" philanthropic initiative, to fund the salaries of new post-doctoral research fellows recruited through a highly competitive international call each year. From 2016 to date, philanthropic donations to the CNIO have made it possible to incorporate 35 new researchers, and from the call that was open in 2023, we expect to fill 8 new positions. We are deeply grateful to our donors and CNIO Friends for their ongoing support to our research programme. After all, philanthropy is a tool that enables all members of society to have a direct impact on our mission to stop cancer.

Our commitment to gender equality continued during 2023 through the activities spearheaded by our CNIO Women and Diversity Office (WISE), an effort of CNIO volunteers that facilitates cultural changes as well as institutional improvements. The Office continued bringing outstanding female speakers who are leaders in a broad array of fields to the "WISE Seminars" series. Open to the general public, this initiative aims to inspire the audience by sharing new perspectives and visions on the role of women in society. Aligned with our goal of fostering the STEM careers among the youngsters and breaking gender stereotypes, on February 11, International Day of the Women and Girls in Science, the WISE Diversity Office organised an event with several secondary schools, which included, among other activities, a talk by Sara García, who described how she became a researcher at the CNIO as well as an astronaut at the ESA. The office was also involved in the follow-up of the CNIO's first Equality Plan, preparing protocols to prevent situations of harassment and other conflicts, as well as to protect women during the pregnancy and lactation periods, among other documents. The WISE Diversity Office also worked together with the Workers Council and other CNIO areas to elaborate the CNIO's second Equality Plan, to be approved during 2024.

Lastly, I do not want to end this foreword without mentioning the appointment of the CNIO Biobank Director, Eva Ortega-Paíno, as new Secretary General for Research by the Spanish Government in late 2023. Since joining the CNIO in 2018, Eva has been instrumental in expanding the activities and role of the Biobank, which under her leadership has reached a new level of excellence. We feel proud of having had Eva as a member of our team and wish her all the success in her new role at the *MCIU*.

To conclude, it is a pleasure for us to convey to society the report of our activities and achievements during 2023 and to emphasise again our commitment to continue dedicating our best efforts to fulfil our mission of conducting research of excellence in oncology, translating scientific knowledge into clinical practice, and transferring the technology developed at the CNIO to the productive sector for the years to come. ∎

OSCAR FERNÁNDEZ-CAPETILLO
Vice-Director

"Year after year, scientists from CNIO manage to make important discoveries that help us advance in our understanding of cancer and its treatment."

Dear colleagues, 2023 was the hottest year on record, which is a reminder to all of us that humanity needs to be creative in finding novel solutions to big problems. At CNIO, we all do our share in trying to make new discoveries to advance in the war against cancer. To prepare for this foreword, I always take the time to review the work that came out of your labs. And the picture always makes me feel that I am in good company. In 2023, we made progress in all of our areas: from new bioinformatics pipelines for drug-response predictions to an atomic-level view of the machines that repair broken DNA. Other exciting work includes the development of innovative cell-based immunotherapies, unravelling the basis for resistance to RAS inhibition, the potential of targeting RANK signalling in breast cancer, or contributing to the most comprehensive genomic characterisation of rare tumours. This selection is a necessarily incomplete collage, since a full description of all of our discoveries would demand a much longer text than this one. Apologies for the work that I miss, but take my word that I do keep an eye on your discoveries. Of course, CNIO is not just a collection of papers, and there are many areas to which we contribute, with a particular mention to those who help to bring science to society. Thank you all for your efforts and best of luck with your experiments. ■

ORGANISATION OF RESEARCH

MARIA A. BLASCO DIRECTOR

ÓSCAR FERNÁNDEZ-CAPETILLO VICE-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
– *Fundación Humanismo y Ciencia*

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 (since November)
Organ Crosstalk in Metabolic Diseases 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

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

TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Vacant Programme Director
Gema Moreno Secretary

Mercedes Robledo
Hereditary Endocrine Cancer Group

Maria Currás
Familial Cancer Clinical Unit

Núria Malats
Genetic and Molecular Epidemiology Group

Anna González-Neira
Human Genotyping-*CEGEN* Unit

Sandra Rodríguez-Perales
Molecular Cytogenetics Unit

CLINICAL RESEARCH PROGRAMME

Miguel Quintela-Fandino Acting Programme Director
María Luisa Anguita Secretary

Miguel Quintela-Fandino
Breast Cancer Clinical Research Unit

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

Luis J. Lombardía
Molecular Diagnostics Unit

Antonio Pérez-Mártínez (since September)
IdiPAZ-CNIO Pediatric Onco-Hematology
Clinical Research Unit

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

Luis Paz-Ares
H12O-CNIO Lung Cancer 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

Giovanna Roncador
Monoclonal Antibodies Core Unit

Orlando Domínguez
Genomics Core Unit

Vacant
Histopathology Core Unit

Isabel Peset
Confocal Microscopy Core Unit

Lola Martínez
Flow Cytometry Core Unit

Francisca Mulero
Molecular Imaging Core Unit

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

Sagrario Ortega
Mouse Genome Editing Core Unit

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor Programme Director
Natalia Catalá Secretary

Sonia Martínez
Medicinal Chemistry Section

Susana Velasco
CNIO-Lilly Cell Signalling and
Immunometabolism Section

Carmen Blanco
Biology Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Irene Herrera Head

BIOBANK

EVA ORTEGA-PAÍNO DIRECTOR

Basic Research

MOLECULAR ONCOLOGY PROGRAMME

ÓSCAR FERNÁNDEZ-CAPETILLO   Programme Director

Research at the Molecular Oncology Programme (MOP) aims to discover the genetic determinants that contribute to cancer onset and progression, as well as to provide new ideas and tools for the development of innovative therapies for cancer patients. 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, Alejo Efeyan and Guadalupe Sabio); immunotherapy (Maria Casanova); epithelial carcinogenesis (Francisco X. Real); and metastasis (Manuel Valiente, Eva González-Suárez and Héctor Peinado). In 2023, Marcos Malumbres' Group left CNIO to join the VHIO community in Barcelona. I am grateful to have shared many years of science at the MOP with him, and he will certainly be missed. At the same time, Marcos is a very original and creative scientist, and I am confident that he will help to strengthen the importance of basic research at the VHIO.

During 2023, our scientists reported relevant contributions in many areas, and here I provide a few selected examples of their work. For instance, Mariano Barbacid's Group provided some of the first mechanistic insights as to how resistance to RAS inhibitors might arise and showed that RAS depletion might be more efficacious than its inhibition. On a related theme, and in collaboration with C. Blanplain's team (*Université Libre de Bruxelles*, Belgium), Juan Méndez's Group revealed how the EMT influences the response to genotoxic chemotherapies. The Malumbres' Group reported on novel roles for phosphatases and kinases, previously related to the cell cycle, to aspects such as pluripotency or nutrient signalling. Eva González-Suárez's Group keeps making significant advances on the potential of targeting RANK signalling, particularly in the context of breast cancer. In addition, the Group led by Maria A. Blasco presented evidence that supports the potential of targeting telomeres from cells in the tumour microenvironment for cancer therapy. Héctor Peinado and his team identified how a factor related to angiogenesis might be a biomarker and potentially a target for the treatment of some aggressive soft-tissue sarcomas. Ana Losada's team made important conceptual advances in our understanding of how different cohesin complexes are assembled, critical for overall genome structure. The Group of Francisco X. Real maintains an important focus on elucidating the role that transcription factors play in pancreatic cancer onset and progression and, in 2023, they showed that *NFIC* is important for coordinating the transcriptional response

"Basic and fundamental research has been behind most cancer therapies that have reached patients. And we, at the MOP, are doing our share to contribute to this effort. Keep it up."

to ER stress and suppressing the occurrence of pancreatic adenocarcinomas. To end, I would like to single out work done by Manuel Valiente's Group, since it pioneered in showing how cancer cells in brain metastases interact with neurons and how this can potentially explain their impact on the cognitive decline observed in cancer patients. In summary, scientists at the MOP keep making important contributions to their fields of research, some of which open new areas and directions for others to follow in the future.

Our scientific excellence is exemplified by the recognition that our scientists receive. Notable examples for 2023 include the *Premio Nacional de Investigación* awarded to Mariano Barbacid, and the *Fundación Banco de Sabadell* Award to Manuel Valiente. Congratulations to both, once again. In addition, Maria Casanova received an ERC Starting Grant for her research on circadian regulation of immune responses. Best of luck to her in making the most of this important support. To end, and while we, the Principal Investigators, often receive most of the attention, this should not hide the fact that these recognitions largely rely on the hard work done by the students, technicians, postdocs, and investigators in our Groups. Thanks to you all. ∎

TELOMERES AND TELOMERASE GROUP – *FUNDACIÓN HUMANISMO Y CIENCIA*

Maria A. Blasco
Group Leader

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

Post-Doctoral Fellows
Giuseppe Bosso, Sonia Burgaz (since September), María Isabel Espejo, Buyun Ma, Arpita Saha (until November), Sarita Saraswati

Gradutate Students
José Carlos González, Óscar Laguía, Jessica Louzame, Amparo Sánchez, Raúl Sánchez

Technicians
David Hernández (TS)*, Rosa M. Serrano

Titulado Superior (Advanced Degree)

Students in Practice
A. Carolina Cintra (until July) (Erasmus+, Tras-os-Montes e Alto Douro University, Vila Real, Portugal), Mehtap Gal (July-Sept.) (Erasmus+, Yıldız Technical University, Istanbul, Turkey), Z. João Morgado Esteves (June-Aug.) (CNIO Summer Student, *Univ. Federal de Goiás*, Goiânia, Brazil), I. Patricia

Paniagua (June-July) (*Univ. Francisco de Vitoria*, Madrid, Spain), Claudia Sánchez (June) (*UAM*, Madrid, Spain), Betül Tire (Oct.-Dec.) (Erasmus+, Akdeniz University, Antalya, Turkey), Samuel L. Zaid (July-Sept.) (*UAM*, Madrid, Spain), Aleksanra Zielinska (July-Sept.) (Erasmus+, Medical University of Lodz, Poland)

Visiting Students/Scientists
Marwa M. Abu-Serie Ali (since Nov.) (Gebri, Srta-City, Alexandria, Egypt) (Science by Women Programme), Andrea Domínguez (July-Aug.) (Manchester Univ., UK), Helena García (June-Aug.) (Univ. of Skövde, Sweden), Ysa Hernández (June-Aug.) (Johns Hopkins University, Baltimore, USA)

OVERVIEW

Immortality is one of the most universal characteristics of cancer cells. We study the mechanisms by which tumour cells are immortal and normal cells are mortal. Telomerase is an enzyme present in over 95% of all types of human cancers and absent in normal cells in the body. Telomeres, nucleoprotein complexes located at the ends of chromosomes, are essential for chromosome protection and genomic stability. Telomeres shorten progressively with organism ageing, leading to ageing. If telomeres are altered, adult stem cells have a maimed regenerative capacity.

Our research focuses on:

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

"Removing immortality from cancer cells by targeting their telomeres is a yet unexploited therapeutic strategy in the fight against cancer that we showed might be effective to treat non-small cell lung carcinoma."

RESEARCH HIGHLIGHTS

Telomerase deficiency and dysfunctional telomeres in the lung tumour microenvironment impair tumour progression in lung cancer

Lung cancer is the leading cause of cancer death. The long-term ineffectiveness of current therapies and late-stage diagnosis result in a five-year survival rate of about 20%. Non-small-cell lung cancer (NSCLC) accounts for 85% of lung cancer-associated deaths. Focusing on the so-called tumour microenvironment, a set of cells and factors surrounding the tumour and playing a crucial role in the development of cancer and the response to therapies, we used a combination of NSCLC mouse models and patient-derived xenografts to address the effects of telomerase deficiency and the anti-tumour activity of 6-thio-2′-deoxyguanosine (6-thio-dG), a nucleoside analogue that leads to telomere dysfunction, genomic instability, and cell death.

We showed in mice that telomerase deficiency and 6-thio-dG-induced telomere dysfunctionality reduced lung tumour implantation and vascularisation, and increased DNA damage response, cell cycle arrest and apoptosis, while it reduced proliferation, inflammation, and lung tumour immunosuppression and invasion. 6-thio-dG-treated human NSCLC xenografts exhibited increased telomere damage, cell cycle arrest and apoptosis, as well as reduced proliferation, resulting in reduced tumour growth. Targeting telomeres might thus be an effective therapeutic strategy in NSCLC.

A link between short telomeres in alveolar type II cells and lung fibrosis in post COVID-19 patients with cancer

The severity of COVID-19 increases with each passing decade of life, suggesting that organismal ageing contributes to the fatality of the disease. We and others previously showed that COVID-19 severity correlates with shorter telomeres, a molecular determinant of ageing, in patients' leukocytes. Lung injury, a predominant feature of acute SARS-CoV-2 infection, can further progress to lung fibrosis in post-COVID-19 patients. Short or dysfunctional telomeres in alveolar type II (ATII) cells suffice to induce pulmonary fibrosis in mouse and humans. Our analyses of telomere length and histopathology of lung biopsies from a cohort of alive post-COVID-19 patients and a cohort of age-matched controls with lung cancer have now revealed a loss of ATII cellularity and the presence of shorter telomeres in ATII cells, concomitant with a marked increase in fibrotic lung parenchyma remodelling in post-COVID-19 patients. Our findings uncovered a link between presence of short telomeres in ATII cells and long-term lung fibrosis sequelae in post-COVID-19 patients.

Expanding the hallmarks of ageing

Ageing research explores the decline in function of organisms during adulthood. In a joint effort with 4 other groups, in 2013 we suggested 9 molecular, cellular, and systemic hallmarks of ageing: telomere attrition, DNA instability, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Ten years on, we have now updated the previous hallmarks, introduced some reorganisations, and included the following 3 new ones: disabled macroautophagy, chronic inflammation, and dysbiosis, bringing the updated hallmarks of ageing to a total of 12 (FIGURE 1). All the hallmarks we have settled upon have fulfilled the following 3 premises: (1) their age-associated manifestation, (2) the acceleration of ageing by experimentally accentuating them, and, most decisively, (3) the opportunity to decelerate, stop, or reverse ageing by therapeutic interventions on them. These hallmarks are interconnected among each other, as well as to the recently proposed hallmarks of health, which include organisational features of spatial compartmentalisation, maintenance of homeostasis, and adequate responses to stress (FIGURE 2). ■
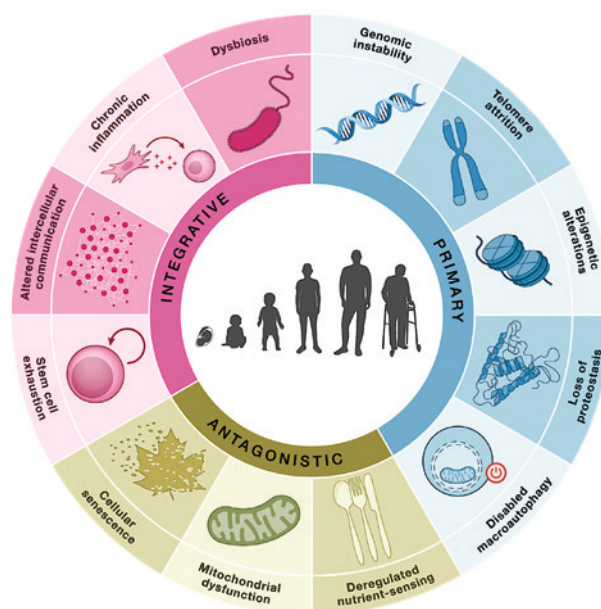
FIGURE 1 The hallmarks of ageing. Scheme compiling the proposed 12 hallmarks of ageing. These hallmarks are grouped into 3 categories: primary, antagonistic, and integrative.
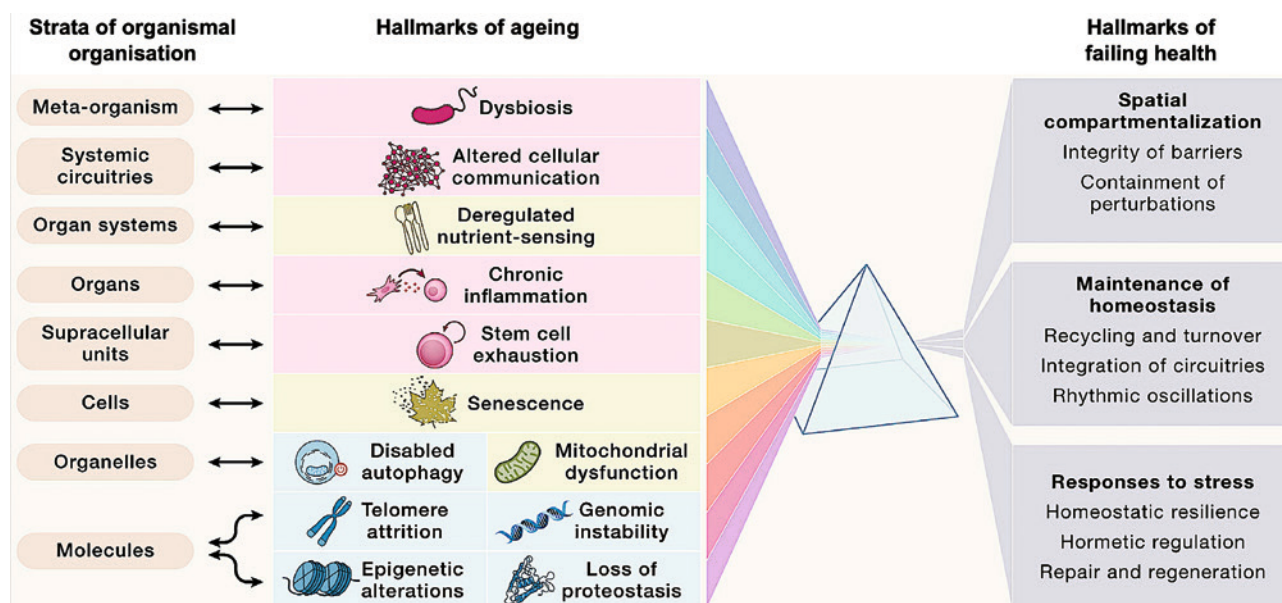
FIGURE 2 Integration of hallmarks. All 12 proposed hallmarks of ageing are functionally related amongst each other. They are also interconnected to the 8 hallmarks of health and, finally, to the 8 proposed strata of organismal organisation and create a multidimensional space of interactions that may contribute to explain some important features of the ageing process.

▸ **PUBLICATIONS**

▸ López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2023). Hallmarks of aging: an expanding universe. *Cell* 186, 243-278.
▸ Piñeiro-Hermida S, Bosso G, Sánchez-Vázquez R, Martínez P, Blasco MA (2023). Telomerase deficiency and dysfunctional telomeres in the lung tumor microenvironment impair tumor progression in NSCLC mouse models and patient-derived xenografts. *Cell Death Differ* 30, 1585-1600.
▸ Martínez-Balsalobre E, García-Castillo J, García-Moreno D, Naranjo-Sánchez

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OVERVIEW

The main thrust of our laboratory is to identify therapeutic strategies against KRAS mutant lung and pancreatic tumours. In recent years, inhibitors against KRAS oncoproteins selective for some of their mutations such as G12C and G12D, as well as panKRAS^{G12X} inhibitors active against all mutations, have been either approved by the FDA (sotorasib and adagrasib) or are undergoing clinical trials. Yet, their clinical efficacy is far from what was expected. In lung cancer patients, sotorasib does not increase overall survival compared to standard chemotherapy regimens due to the rapid appearance of tumour resistance. We have used genetically engineered mouse models of lung and pancreatic tumours to compare the therapeutic efficacy of KRAS ablation with that of KRAS inhibition, and to interrogate the molecular mechanism responsible for tumour resistance. Whereas ablation of KRAS oncogenes eliminates both lung and pancreatic tumours completely with no signs of tumour resistance, KRAS inhibition results in the rapid appearance of resistance as previously observed in human tumours. We are currently exploring whether inhibiting KRAS signalling at independent nodes within its downstream or upstream signalling pathways, as well as in orthogonal pathways, will not only increase tumour responses but also prevent the appearance of tumour resistance.

"Increasing the efficacy of therapeutic strategies aimed at KRAS mutant lung and pancreatic tumours represents a major challenge for precision medicine in cancer."

RESEARCH HIGHLIGHTS

Kras oncogene ablation induces complete regression of advanced *Kras*/*Trp53* driven lung adenocarcinomas

We have interrogated whether continuous expression of the *Kras* oncogene was essential for tumour progression and maintenance as well as for the appearance of tumour resistant cells. To this end, we modified the *Kras*[FSFG12V] allele by inserting *loxP* sites flanking those first exon sequences that encompass the G12V activating mutation. In addition, we added 2 independent loci encoding the inducible CreERT2 recombinase. The resulting mice, *Kras*[+/FSFG12Vlox];*Trp53*[F/F];*Rosa26-CreERT2*[KI/KI];*Tg.hUBC-CreERT2*[+/T] (K[G12Vlox]PC2) developed lung tumours with the same incidence and latency as their parental strain when exposed to Adeno-FLPo particles. Exposure of 76 K[G12Vlox]PC2 mice harbouring 156 lung tumours ranging in size from 0.13 to 43 mm^3 to a tamoxifen (TMX) diet for 1 month led to the complete regression of almost two-thirds of the tumours. Tumour regression was even more evident when mice were examined after 2 months of TMX exposure, when the percentage of complete regressions increased to 84%. Tumour monitoring was extended for up to 6 months of TMX exposure. At this time point, only 2 mice carried CT+ tumours. Mice were allowed to thrive until the time when they had to be sacrificed at the humane end point. The lungs of these mice failed to display detectable lung tumours. Thus, ablation of *Kras* oncogene expression in advanced lung tumours not only induced complete tumour regressions but also prevented the appearance of *Kras* resistant tumours.

The therapeutic effect of *Kras* oncogene ablation is independent of the mutational burden of the tumours

Unlike human lung tumours, *Kras*-driven genetically engineered mouse lung adenocarcinomas display few additional mutations. Hence, we interrogated the therapeutic effect of *Kras* oncogene ablation in tumours induced by urethane, a chemical carcinogen known to induce hundreds of mutations. For this purpose, we used a *Kras* conditional (floxed) strain to which we added the CreERT2 loci described above. The resulting mice, *Kras*[lox/lox];*Rosa26-CreERT2*[KI/KI];*Tg.hUBC-CreERT2*[+/T] (K[lox]C2), were exposed to urethane at 4 weeks of age. Once tumours were detected by CT analysis, tumour-bearing K[lox]C2 mice (n=21, 111 CT[+] tumours) were subjected to a continuous TMX-containing diet to mediate recombination of the *Kras*[lox] alleles. *Kras* ablation dramatically decreased tumour burden after 1 month of TMX exposure, reaching 64.9% of complete responses, a result similar to that observed in the K[G12Vlox]PC2 tumour model. Extended exposure to the TMX diet for an additional month resulted in increased percentage of those tumours undergoing complete responses (89%).

No progressive or stable disease was identified in this trial. Surviving K[lox]C2 mice were allowed to thrive. More than half of them survived 4 to 10 additional months. None of these mice displayed signs of tumour relapse. Mice sacrificed at humane end points failed to reveal detectable lung tumours. These results illustrate that the complete tumour regressions in the absence of tumour resistance observed upon KRAS ablation is independent of the mutational burden of the tumours.

Pharmacological inhibition of oncogenic KRAS signalling induces rapid tumour resistance

To study the effect of KRAS inhibition in GEM tumour models, we generated a *Kras*[FSFG12C] allele by replacing the G12V by a G12C mutation using homologous recombination in ES cells. Intranasal infection of *Kras*[+/FSFG12C];*Trp53*[F/F] mice (K[G12C]P) with Adeno-FLPo particles induced the development of lung adenocarcinomas indistinguishable from those previously observed in the *Kras*[FSFG12V];*Trp53*[F/F] mice. Continuous exposure of these tumour-bearing mice to sotorasib for 1 month (100 mg/kg), a dose equivalent to that used in the CodeBreaK100 clinical trial, resulted in the appearance of resistant tumours in all treated animals after 4 to 12 weeks of treatment. Resistant tumours did not differ from untreated controls, with the exception of the degree of apoptotic cells that remained elevated. Finally, resistant tumours displayed a clear trend towards higher histological grades.

Sotorasib resistant tumours display amplification of the *Kras*[G12C] allele and elevated levels of drug metabolism pathways

To identify the mechanisms associated with resistance to sotorasib treatment, we submitted resistant tumours to WES analysis. We could not detect any of the driver mutations described in a fraction of human resistant tumours. However, we identified robust amplifications of the genomic region of chromosome 6 encompassing the *Kras* locus in the resistant tumours analysed, suggesting that amplification of the mutant *Kras*[G12C] allele is a major driver of sotorasib resistance in this experimental model.

To further characterise these sotorasib-resistant tumours, they were submitted to RNAseq analysis. Gene Set Enrichment Analysis (GSEA) of differential gene expression revealed upregulation of gene sets involved in the metabolism of drugs by cytochrome P450 (CYP450) and glutathione-S-transferases (GSTs), as well as proliferation-related pathways. These results suggest that resistance could, at least partially, emerge as a consequence of an altered metabolism of sotorasib, resulting
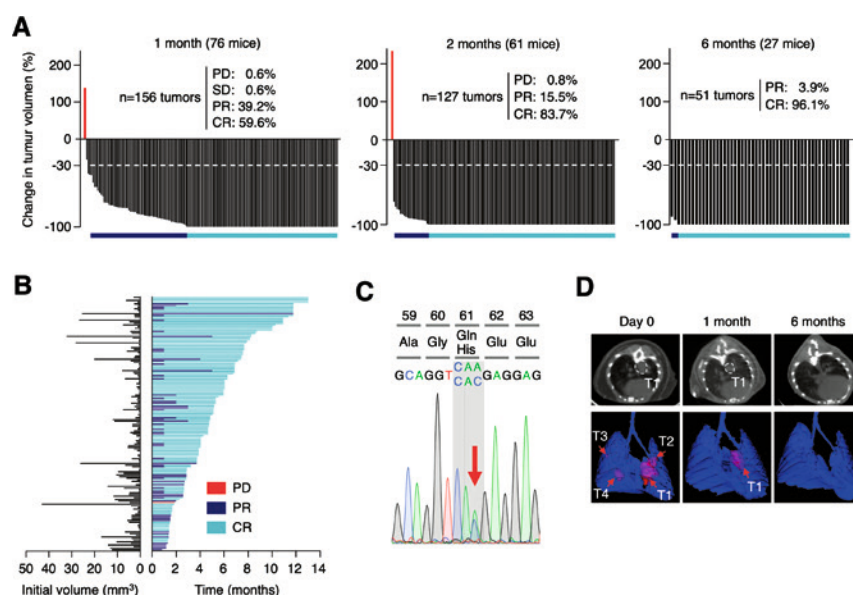
FIGURE 1 Genetic ablation of *Kras*[G12V] in lung tumours induces complete tumour regression and prevents the appearance of resistant tumours. (**A**) Waterfall plots representing the change in tumour volume of tumours exposed to a TMX diet for 1, 2, and 6 months as determined by CT scans. (PD) progressive or (SD) stable disease, (PR) partial or (CR) complete tumour regression is depicted in the figure. A growing tumour lacking the resident *Kras*[G12Vlox] oncogene is depicted in red. (**B**) Initial tumour size (left) and duration of response (right) from individual tumours represented in (A). (**C**) Sequencing chromatogram depicting the p.Q61H mutation in the wild-type *Kras* allele present in the single tumour indicated in red. (**D**) Lungs depicting tumour response after 1 and 6 months of TMX exposure.

in its detoxification and reducing its effect in tumour cells. To determine whether these results could be translated to human tumours, we implanted pieces derived from a patient-derived KRAS[G12C]-positive xenograft (PDX) lung tumour model in immunocompromised mice. Four mice carrying a PDX piece in each flank were treated with vehicle, whereas 3 mice implanted with 6 pieces were treated with 100 mg/kg of sotorasib. These PDX tumours became resistant to the drug at about 150 days of treatment and were subsequently submitted to WES analysis. Interestingly, we did not identify amplifications in the *Kras* oncogene or additional driver mutations as previously described in clinical samples. Yet, RNAseq analysis also revealed upregulation of a pathway related to the metabolism of xenobiotics. In sum, our results revealed that inhibition of the molecular events responsible for tumour resistance, at least in this experimental model, will be difficult to be overcome using pharmacological strategies. Instead, they suggest that resistance to KRAS inhibitors might be prevented, or at least ameliorated, by achieving a more robust inhibition of KRAS signalling, mimicking the results obtained upon *Kras* ablation. ∎

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▸ Macaya I, Roman M, Welch C, Entrialgo-Cadierno R, Salmon M, Santos A, Feliu I, Kovalski J, Lopez I, Rodriguez-Remirez M, Palomino-Echeverria S, Lonfgren SM, Ferrero M, Calabuig S, Ludwig IA, Lara-Astiaso D, Jantus-Lewintre E, Guruceaga E, Narayanan S, Ponz-Sarvise M, Pineda-Lucena A, Lecanda F, Ruggero D, Khatri P, Santamaria E, Fernandez-Irigoyen J, Ferrer I, Paz-Ares L, Drosten M, Barbacid M, Gil-Bazo I, Vicent S (2023). Signature-driven repurposing of Midostaurin for combination with MEK1/2 and KRASG12C inhibitors in lung cancer. *Nat Commun* 14, 6332-6350.

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▸ **PUBLICATION AT OTHER INSTITUTIONS**

▸ Sánchez-Fdez A, Matilla-Almazán S, Montero JC, Del Carmen S, Abad M, García-Alonso S, Bhattacharya S, Calar K, de la Puente P, Ocaña A, Pandiella A, Esparís-Ogando A (2023). The WNK1–ERK5 route plays a pathophysiological role in ovarian cancer and limits therapeutic efficacy of trametinib. *Clin Transl Med* 13, e1217.

▸ **PATENT**

▸ Barbacid M, Guerra C, Vasiliki L (2023). Triple combined therapy inhibiting EGFR, RAF1, and STAT3 against pancreatic ductal adenocarcinoma. *EP23382078.6.*

▸ **AWARDS AND RECOGNITION**

M. Barbacid:

▸ *XXI Premio Madrid a la Iniciativa en Ciencia e Investigación*, Spain.

▸ *XVII Premio Francisco Cobos a la Investigación Biomédica*, Spain.

▸ Molecular Oncology Plenary Lecture, FEBS Congress 2023 (Tours), France.

▸ Closing Lecture of the 40th International Congress of the Spanish Association for Cancer Research (*ASEICA*), Spain.

▸ Honorary Member of *ASEICA*.

S. García-Alonso:

▸ Silver Medal of the Order of May 2nd, Community of Madrid, Spain.

▸ *Hija Predilecta de la ciudad de León*, Spain.

▸ List of the 100 most influential women in Spain; List of the 23 changemakers. *FORBES*.

▸ Senate Award for the promotion of science and innovation (nominated), Senate of Spain.

▸ Ada Bryon Award (Junior), *Universidad de Deusto* (Madrid), Spain.

▸ UU Prize Innovative Leadership, *WstartupC – Royal Academy of Engineering* (Madrid), Spain.

▸ *AMMDE* Aerospatial Award, *Asociación Multisectorial de Mujeres Directivas y Empresarias* (Madrid), Spain.

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OVERVIEW

The Genomic Instability Laboratory is interested in understanding the molecular mechanisms causing cancer and other age-related diseases, in order to provide the knowledge needed to develop novel treatments for these diseases. Initially, we focused on the study of replicative stress, a type of DNA damage that fuels genomic instability and is present in many types of cancer. Those studies led to important contributions to basic research and also led to the development of potent and selective ATR inhibitors that were transferred to the pharmaceutical industry for clinical development. Subsequent to elucidating the mechanisms of resistance to ATR inhibition by genetic screens, our Group gradually developed an interest in understanding how cancer cells develop resistance to therapies, and how we can target therapy-resistant cancer cells. In addition, we are actively involved in exploring the contribution of nucleolar stress to cancer and neurodegeneration.

"In 2023, we discovered new biomarkers that predict sensitivity to SETD8 inhibitors and significantly advanced in our understanding of nucleolar stress as a driver of ageing and neurodegeneration."

RESEARCH HIGHLIGHTS

Targeting SETD8 in tumours with high rates of ribosome biogenesis

A large number of the driver mutations found in tumour cells occur in genes related to chromatin regulation, a fact particularly relevant for paediatric tumours, which frequently harbour mutations linked to cell fate and differentiation. These findings have revitalised the efforts to develop drugs targeting epigenetic regulators ("epidrugs") and today, epigenetics is a very active area in the development of cancer therapies. In this regard, SETD8 is a histone methyltransferase known to play important roles in DNA replication and repair, and is overexpressed in a wide range of cancers. Moreover, SETD8 has been identified as a specific vulnerability of several tumours of bad prognosis, such as neuroblastoma or MYC-driven medulloblastoma. This research triggered additional efforts to develop SETD8 inhibitors, and several compounds have already been generated. However, the available molecules present poor pharmacological properties and none have progressed to clinical development. In our Group, we have discovered novel SETD8 inhibitors (SETD8i) and performed the first steps to characterise them. In 2023, we completed several CRISPR screens using both chemical and genetic strategies to target SETD8. These efforts revealed that the toxicity of SETD8 inhibitors is highest in cells with increased levels of nucleolar activity. Accordingly, these compounds are particularly efficacious for the killing of cancer cells with high MYC or mTOR activity. Conversely, their toxicity is alleviated upon MYC depletion or rapamycin treatment (FIGURE 1).

Overall, our work provides novel SETD8 inhibitors and has helped to identify biomarkers that can be used to select patients most likely to respond to this therapy.

Nucleolar stress as a driver of ageing

Ribosome biogenesis is the most energy-demanding activity in a cell and takes place in the nucleolus. Accordingly, abnormalities in nucleolar activity or structure, collectively known as nucleolar stress (NS), have often been found in patients with several human diseases such as cancer or neurodegeneration. Although P53 contributes to NS toxicity, this stress ultimately kills cells by P53-independent mechanisms that remain to be deciphered. To investigate how NS triggers cellular toxicity, our Group used (PR)n arginine-rich peptides, found in patients with amyotrophic lateral sclerosis (ALS) and other neurodegenerative pathologies, as inducers of this perturbation. We previously showed that PR-peptides accumulate at nucleoli and impair rRNA processing. These observations led us to hypothesise that reduced amounts of mature rRNA molecules could trigger an accumulation of free r-proteins. Indeed, proteomic analyses of the ribosome free-fraction of cells expressing (PR)97 peptides allowed us to confirm a significant increase in the levels of free r-proteins. Conversely, (PR)97-resistant cells have lower rates of ribosome biogenesis. Furthermore, targeting r-protein synthesis by mTOR inhibition or MYC depletion, the 2 main known regulators of ribosome biogenesis, alleviates (PR)n toxicity
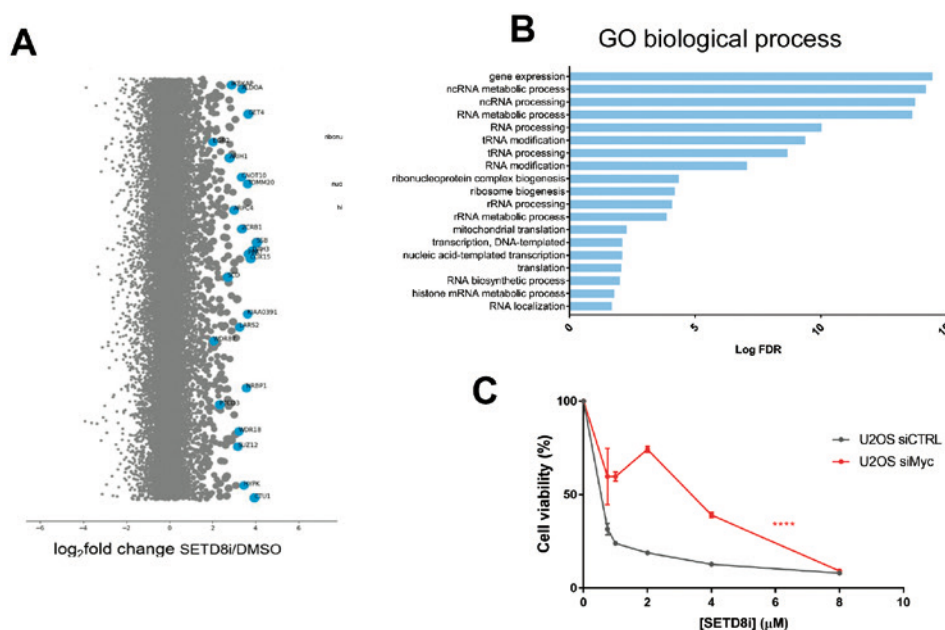
FIGURE 1 The toxicity of SETD8 inhibitors correlates with nucleolar activity. (**A**) Results of a CRISPR screen in U2OS cells exposed to SETD8 inhibition. (**B**) GO terms reflect that aspects related to nucleolar activity and ribosome biogenesis are enriched among the factors that modulate the sensitivity to SETD8 inhibition. (**C**) Depletion of MYC by siRNA in U2OS cells increases the resistance to SETD8 inhibition.
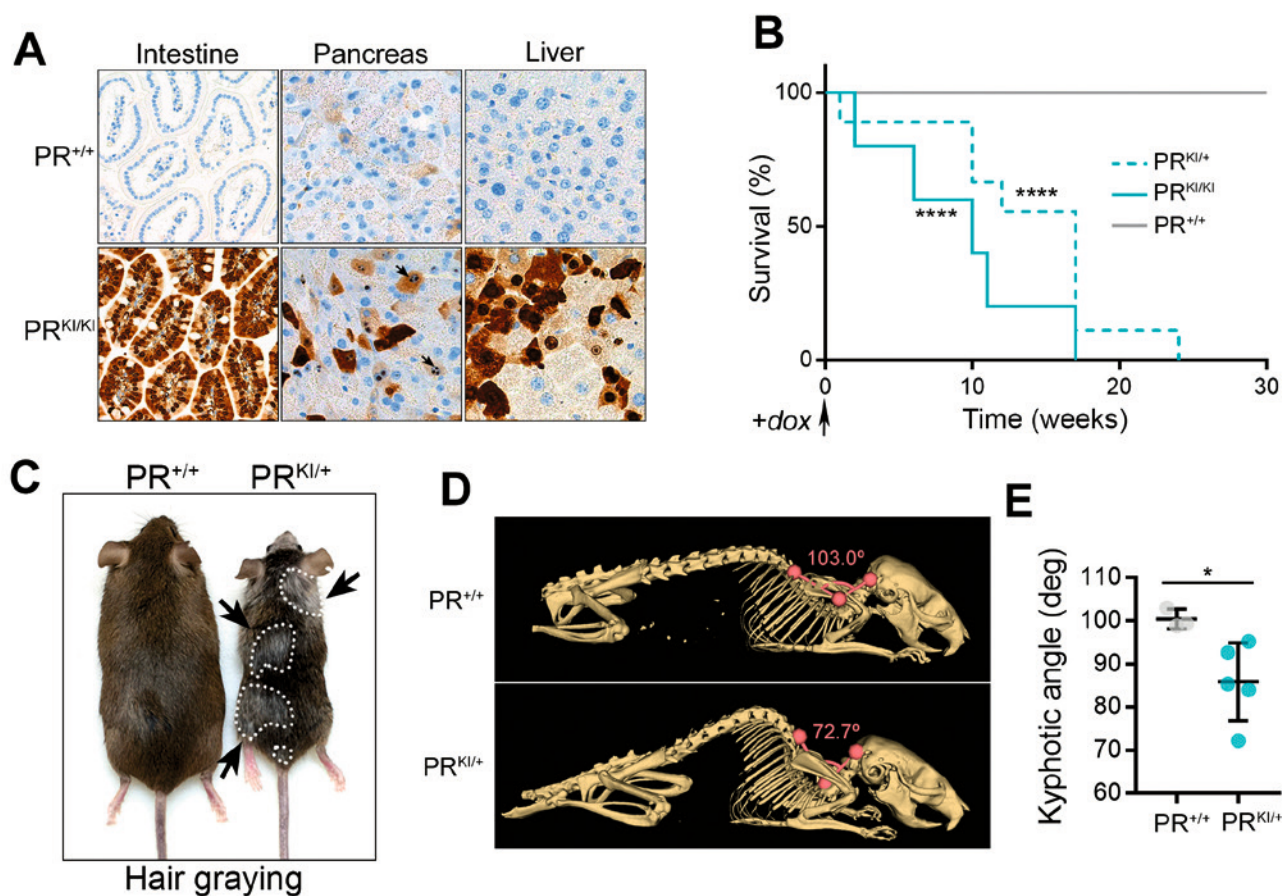
FIGURE 2 (PR)97 expression accelerates ageing in mice. (**A**) Representative images illustrating the inducible expression of (PR)97 peptides in PR$^{KI/+}$ mice. (**B**) Survival curve of mice upon inducible expression of (PR)97 peptides. (**C**) Representative images illustrating hair greying in PR$^{KI/+}$ mice. (**D, E**) Images (D) and quantification (E) of the kyphotic angle in control and PR$^{KI/+}$ mice.

in several cell lines. In mice, systemic expression of (PR)97 drives widespread NS and accelerated ageing (FIGURE 2), which is alleviated with rapamycin. Importantly, we discovered that the generalised accumulation of free r-proteins is a common outcome of NS, independent of its source. Overall, our work reports a unifying model to explain how NS kills cells independently of P53 and provides the first *in vivo* evidence to illustrate that NS accelerates ageing in mammals. ∎

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▸ Sayago C, Sánchez-Wandelmer J, García F, Hurtado B, Lafarga V, Prieto P, Zarzuela E, Ximénez-Embún P, Ortega S, Megías D, Fernández-Capetillo O, Malumbres M, Munoz J (2023). Decoding protein methylation function with thermal stability analysis. *Nat Commun* 14, 3016.

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▸ Kanellis DC, Zisi A, Skrott Z, Lemmens B, Espinoza JA, Kosar M, Björkman A, Li X, Arampatzis S, Bartkova J, Andújar-Sánchez M, Fernandez-Capetillo O, Mistrik M, Lindström MS, Bartek J (2023). Actionable cancer vulnerability due to translational arrest, p53 aggregation and ribosome biogenesis stress evoked by the disulfiram metabolite CuET. *Cell Death Differ* 30, 1666-1678.

▸ Schlegel J, Porebski B, Andronico L, Hanke L, Edwards S, Brismar H, Murrell B, McInerney GM, Fernandez-Capetillo O, Sezgin E (2023). A multiparametric and high-throughput platform for host-virus binding screens. *Nano Lett* 23, 3701-3707.

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OVERVIEW

We have a general 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.

Recently, we opened a new research line aimed at developing novel methods for sequence-specific nucleic-acid detection based on CRISPR-Cas technology, with the idea of producing sensitive and versatile genetic diagnostic kits and devices that can be implemented in a point-of-care setting.

> "We have developed 2 different technologies with unprecedented sensitivity for nucleic acid detection, which will constitute the basis for future point-of-care testing of genetic cancer biomarkers."

RESEARCH HIGHLIGHTS

A topological model for the estrogen transcriptional response

Estrogen response is a well-characterised mechanism of transcriptional regulation with important implications in breast and ovarian cancer that involves an acute response with strong changes in chromatin organisation. We therefore reasoned that it could be an interesting process to study the contribution of DNA topoisomerases. By applying novel methodology developed in the laboratory to measure topoisomerase activity, we uncovered the topological principles regulating estrogen response (FIGURE 1). Thus, under non-induced conditions, topoisomerases maintain low levels of topological stress (supercoiling) in enhancer regions. However, upon exposure to estrogen, recruitment of the estrogen receptor (ER) to target enhancers results in a local inhibition of topoisomerase activity and an accumulation of supercoiling that increases the contacts with their target promoters and, therefore, subsequent transcriptional stimulation. This provides a first example in which topoisomerase activity is physiologically downregulated to promote long-range regulatory chromatin contacts and gene expression. The manuscript reporting these findings is currently under review. We will now explore the direct implications of this model in breast and ovarian cancer, with the aim of developing more potent and safer therapeutic treatments.

Novel method for point-of-care genetic testing

The capacity of CRISPR-Cas systems being programmed to recognise specific nucleic acid sequences has boosted their biotechnological applications. One of them is the detection of the genetic material of pathogens or genetic markers in diagnosis. Systems for the detection of specific nucleic acid sequences based on CRISPR-Cas technology have recently been developed and promise to revolutionise point-of-care diagnostics in the near future. These systems rely on the fact that, upon recognition and cleavage of the desired target, which is highly specific and easily programmable, the Cas protein becomes activated with a sequence-independent, unscheduled nucleolytic activity that can be easily detected with nuclease reporter substrates, whose signal can therefore be used as a readout for the presence of the given nucleic acid of interest.

These CRISPR-Cas diagnostics, however, despite their great specificity and versatility, are currently limited by their levels of sensitivity, which are outside the range of the concentrations required for diagnostic purposes, and currently rely on pre-amplification of the target sequences by methods such as PCR or LAMP. This introduces a complication to the reactions, limiting their current use in point-of-care applications. We developed and patented a conceptually novel solution that, instead of amplifying the target nucleic acid, focuses on boosting Cas activation, so that the reaction is carried out in a single step at room temperature, providing an ideal setting for point-of-care diagnostics. The trick is to establish a chain reaction of Cas endonuclease activity (Endonuclease Chain Reaction, ECR; read "easier") that reaches high levels of signal, even in the presence of very low amounts of the target sequence of interest. In analogy to PCR, one can use end-point measurements for qualitative detection of a nucleic acid of interest, or real time reactions for quantitative analysis, as shown in FIGURE 2. Due to its versatility in the detection of any nucleic acid of interest, this invention should constitute the platform for the development of a wide range of specific genetic testing kits and devices, including pathogen and genetic marker detection. Our next steps will be to adapt the methodology to the detection of genetic cancer biomarkers. ■

▸ **PUBLICATION**

▸ Terrón-Bautista J, Martínez-Sánchez MM, López-Hernández L, Vadusevan AA, García-Domínguez M, Williams RS, Aguilera A, Millán-Zambrano D, Cortés-Ledesma F (2023). Topological regulation of the estrogen transcriptional response by ZATT-mediated inhibition of TOP2B activity. *bioRxiv*. PMID: 38328138.

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▸ Cortés Ledesma F, García Expósito L, López de Alba E, Gómez Marín C, Muñoz Barrera M (2023). Improved padlock probes for nucleic acid detection by Hyperbranched Rolling Circle Amplification. *EP23382281.6*.

▸ Cortés Ledesma F, Gómez Marín C, Muñoz Barrera M, López de Alba E, García Expósito L, Martínez Cañas JC. Nucleic acid detection method. PCT application (2023). *PCT/EP2023/067698*.
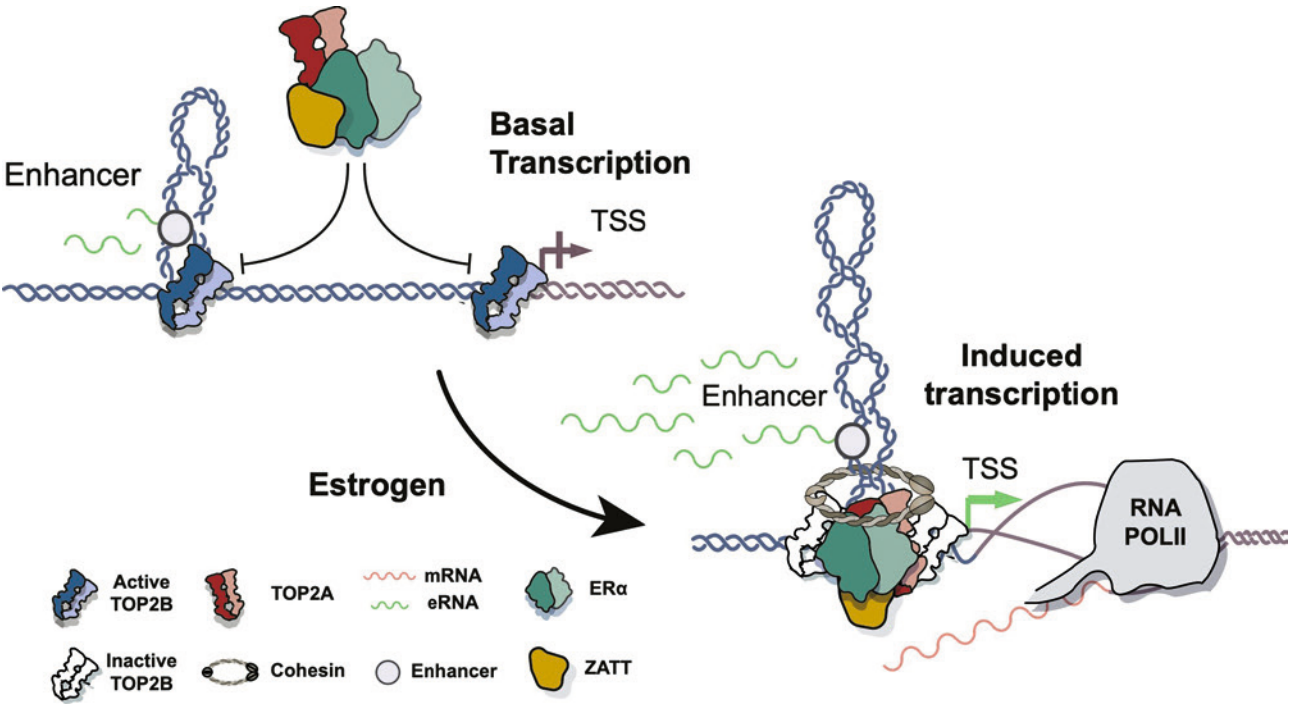
FIGURE 1 Model to explain the role of topoisomerases in estrogen response. TOP2B removes supercoiling caused by basal transcription. Upon estrogen exposure, ER, together with TOP2A and ZATT, inhibits TOP2B, allowing the accumulation of supercoiling that mediates enhancer-promoter contacts.
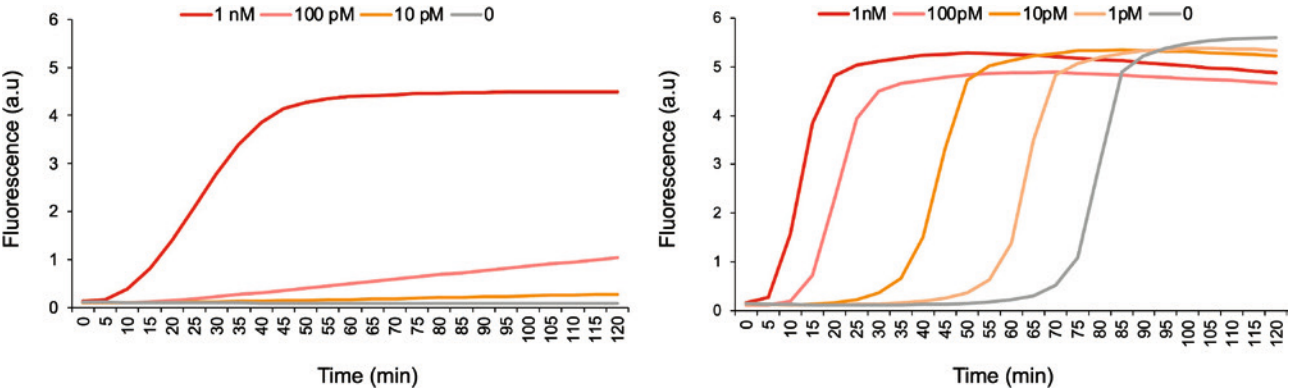
FIGURE 2 ECR method for sensitive nucleic-acid detection with CRISPR-Cas. Direct detection of decreasing concentrations of a nucleic acid sequence of interest with CRISPR-Cas (left), compared to our improved method with chain amplification of Cas endonuclease activity (ECR) (right).

CHROMOSOME DYNAMICS GROUP

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OVERVIEW

Our research focuses on a protein complex named cohesin that embraces 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, most prominently 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 identified a 13-gene signature that recognises cases with poor prognosis among Ewing sarcoma patients without detectable metastases at the time of diagnosis."

RESEARCH HIGHLIGHTS

Different NIPBL requirements of cohesin-STAG1 and cohesin-STAG2

Cohesin mediates 3D genome organisation by binding to chromatin and extruding DNA loops that become stabilised at several locations 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 topological associated domains (TADs). In recent years, we reported that the two versions of cohesin carrying either STAG1 or STAG2 make some specific contributions to 3D genome architecture. Moreover, we proposed that their different chromatin association dynamics underlie this specificity. STAG2 is more often found to be associated with the cohesin unloading factor WAPL, while cohesin-STAG1 is more stably retained at CTCF-bound sites. We have now found that the two complexes also respond differently to limited availability of NIPBL, the putative cohesin loader.

NIPBL activates the cohesin ATPase and is essential for loop extrusion by cohesin *in vitro*. Using a flow cytometry assay to measure chromatin-bound proteins and chromatin immunoprecipitation (ChIP) to map cohesin binding sites along the genome, we found that cohesin-STAG1 increases on chromatin and further accumulates at CTCF positions after NIPBL knock down, while cohesin-STAG2 diminishes genome-wide. Despite the presence of cohesin-STAG1 on chromatin, in situ Hi-C analyses reveal that loop formation is severely impaired. Based on these data, we propose that NIPBL is not required for initial association of cohesin with chromatin, as currently thought. Instead, NIPBL is an essential processivity factor for loop extrusion by cohesin. Given the more dynamic behaviour of cohesin-STAG2, this complex would have a stronger requirement of NIPBL-dependent loop extrusion activity to reach CTCF-bound stabilisation sites. Cohesin-STAG1 would get stabilised at CTCF sites even under low NIPBL levels, although in that condition is unable to form long loops. These results add to our understanding of the different behaviour of cohesin-STAG1 and cohesin-STAG2. More importantly, they provide a new perspective on the role of NIPBL on cohesin dynamics that needs to be considered when thinking of potential therapies for CdLS patients, most of which carry mutations in *NIPBL* (FIGURE 1).
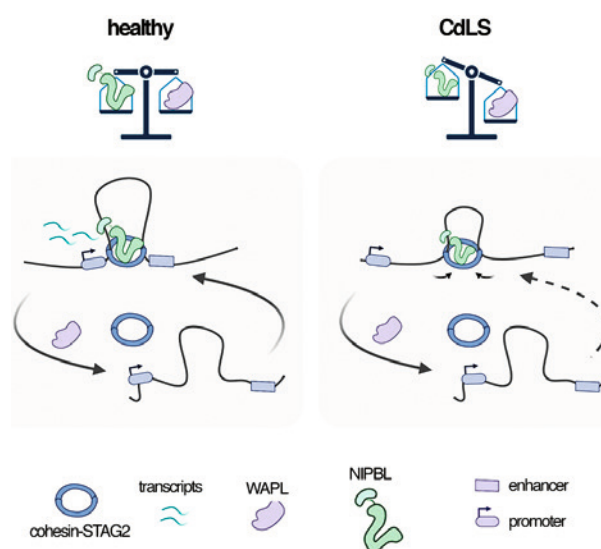
FIGURE 1 Model for the consequences of *NIPBL* mutation in CdLS. Proper balance between loop-extruding cohesin-STAG2, bound by NIPBL, and WAPL-mediated release of this complex is important for transcriptional regulation (healthy, left). In CdLS, NIPBL function is impaired (dashed arrow) resulting in reduced gene expression (CdLS, right).

A STAG2 dependent gene signature to predict aggressive Ewing sarcoma

Ewing sarcoma (EWS) is the second most frequent type of bone cancer in children and young adults. It is driven by a fusion protein, most often EWS-FLI1, which encodes a neomorphic transcription factor that rewires the transcriptome of the cell initiating the tumour. It is a highly aggressive cancer with a 5-year survival below 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 show poor survival. Among the few recurrent mutations identified in EWS, in addition to oncogenic fusion, are those that inactivate *STAG2*. These mutations are often present in the most aggressive EWS tumours, suggesting that loss of cohesin STAG2 facilitates the acquisition of the aggressive phenotype. We have therefore asked whether we can identify a STAG2-dependent gene signature to predict the prognosis of patients that do not present detectable metastases when they are first diagnosed.

We generated isogenic EWS cell lines with and without STAG2 and compared their transcriptomes with those of EWS patients carrying or not *STAG2* mutations. Out of 233 genes commonly deregulated after STAG2 loss, we selected 68 genes that showed significant correlation with patient survival. The gene list was further reduced based on proteomic data and additional features such as druggability. The final gene signature consists of 13 genes and identifies cases with worse prognosis among patients that present localised disease at diagnosis (FIGURE 2). We are currently exploring the contribution of these genes to the metastatic phenotype of EWS cells. ■
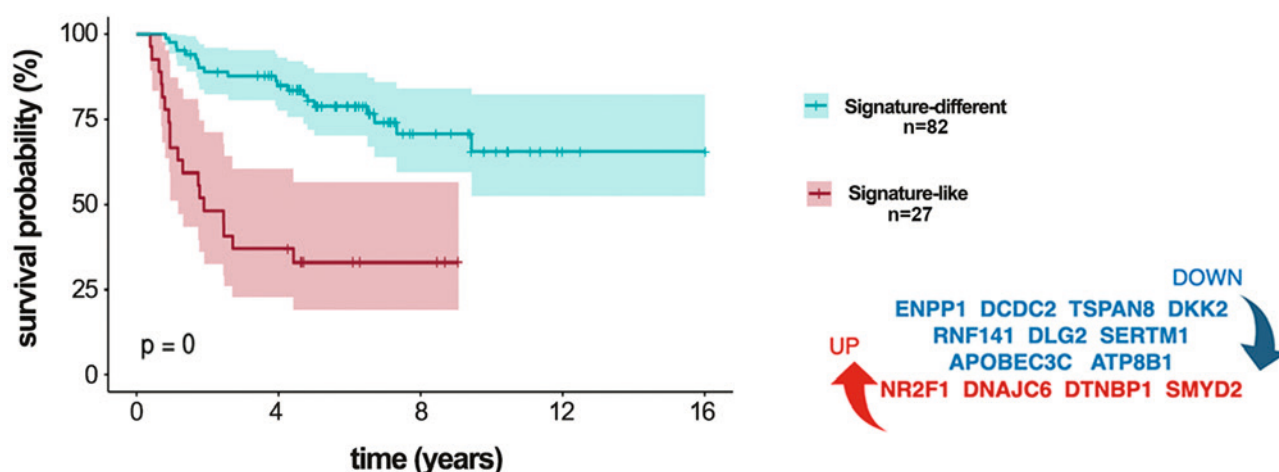
FIGURE 2 A 13-gene signature to predict outcome of Ewing sarcoma patients. Overall survival for patients with localised Ewing sarcoma stratified by expression of the 13-gene signature comprising 9 up-regulated genes (in blue) and 4 down-regulated genes (in red) whose expression depends on cohesin-STAG2.

▸ **PUBLICATIONS**

▸ Alonso-Gil A, Cuadrado A, Giménez-Llorente D, Rodríguez-Corsino M, Losada A (2023). Different NIPBL requirements of cohesin-STAG1 and cohesin-STAG2. *Nat Commun 14*, 1326.
▸ Alonso-Gil D, Losada A (2023). NIPBL and cohesin: new take on a classic tale. *Trends Cell Biol 33*, 860-871.
▸ Giménez-Llorente D, Portela M, Losada A, Manzanares M, Andreu MJ (2023). A protocol to study three-dimensional genome structure in individual mutant preimplantation mouse embryos. *STAR Protoc 4*,102267.
▸ Alonso-Gil D, Losada A (2023). Protocol to analyze chromatin-bound proteins through the cell cycle using Chromoflow flow cytometry. *STAR Protoc 4*,102568.

DNA REPLICATION GROUP

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OVERVIEW

Our Group studies the fundamental mechanisms of DNA replication and how the replicative process adapts to cell type-specific transcriptional programmes and chromatin organisation. We also investigate the cellular responses to replication stress (RS), a phenomenon caused by endogenous or exogenous factors that slow down DNA replication forks and may result in DNA damage and genomic instability. Our long-term goal is to develop strategies to minimise RS in normal cells and enhance it in cancer cells to increase their vulnerability. In 2023, we addressed the molecular changes that underlie the rewiring of DNA replication during pluripotency transitions in mouse embryonic stem cells. We also described how regulation of replication origin activity influences the acquisition of chemotherapy resistance in tumour cells undergoing epithelial-to-mesenchymal transition. Finally, we showed how DNA replication is blocked by a new combination therapy for diffuse large B cell lymphoma.

"We have described how the activation of new replication origins in response to chemotherapy mediates the acquisition of resistance in epithelial tumour cells."

RESEARCH HIGHLIGHTS

A rewiring of DNA replication during cell pluripotency transitions

In previous years, we had mapped the positions of replication origins in mouse embryonic stem cells (mESCs) in the *primed* pluripotency state that resembles the post-implantation epiblast (Jodkowska K *et al.*, 2022, *Nucleic Acids Res* 50, 12149-12165). We are now studying the adaptation of the DNA replication programme when primed mESCs are de-differentiated to the naive state, which resembles the pre-implantation inner cell mass. We have found that the primed-to-naive mESC transition, triggered in cultured cells by MEK and GSK3 inhibitors (2i), entails a significant slowdown of replication forks, a higher frequency of asymmetric fork progression, 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 are likely responsible for these effects. For instance, naive mESC forks are enriched in proteins involved in DNA recombination and repair, notably MRE11 nuclease, while primed mESC forks are enriched in factors related to translation initiation, ubiquitin-dependent protein metabolism and cell cycle progression. We are investigating the causal links between the alteration in DNA replication dynamics and the capacity of mESC to be reprogrammed into earlier pluripotency states.

Activation of dormant replication origins in response to cisplatin/5-FU facilitates chemotherapy resistance

The development of resistance by tumour cells constitutes a major problem in anticancer therapy. Epithelial-to-mesenchymal transition (EMT) is one of the cellular processes that has been linked to chemotherapy resistance by mechanisms that are not well understood. We collaborated with C. Blanpain (*Université Libre de Bruxelles*, Belgium) to report that skin squamous carcinoma cells undergoing EMT are highly resistant to anti-cancer therapy both *in-vivo* and *in-vitro*. RhoJ, a small GTPase that is preferentially expressed in EMT cancer cells, enhances the response to replicative stress and activates the DNA damage response, allowing tumour cells to repair DNA lesions induced by chemotherapy. This response includes the activation of extra DNA replication origins in response to fork slowdown caused by cisplatin and 5-FU (FIGURE 1). The effects of RhoJ on DNA replication, combined with its regulation of nuclear actin polymerisation, regulate EMT-associated resistance to chemotherapy (Debaugnies M *et al.*, 2023).

A combination therapy in large diffuse B-cell lymphomas effectively blocks DNA replication

Diffuse large B cell lymphoma (DLBCL) is the most common aggressive B cell lymphoma. DLBCL is normally treated with chemotherapy, but a substantial proportion of patients do not respond or relapse after treatment. In a collaborative study with V.G. de Yébenes and A. Ramiro (*CNIC*, Madrid), we have observed that the combination of tumour suppressor microRNA miR-28 with BTK inhibitor ibrutinib induces a specific transcriptional cell-cycle arrest programme that impairs DNA replication in DLBCL cells. Single-molecule analysis revealed that miR-28 was sufficient to reduce replication origin activation, while ibrutinib restricted the compensatory acceleration of replication forks (FIGURE 2). Notably, the same transcriptional signature repressed by miR-28-plus-ibrutinib combination therapy appears downregulated in DLBCL patients with better survival (Fortes T *et al.*, 2023).

DNA replication and RS in other cellular contexts

Other current projects include: (i) a structural and functional characterisation of PRIMPOL primase, specialised in re-initiation of DNA synthesis at stalled forks; (ii) the genome-wide identification of pre-replicative complexes in human cells using CUT&RUN; (iii) the identification of molecular mechanisms that prevent DNA over-replication and gene amplification; and (iv) the participation of mitotic kinase AURKA in the regulation of S phase progression. ∎
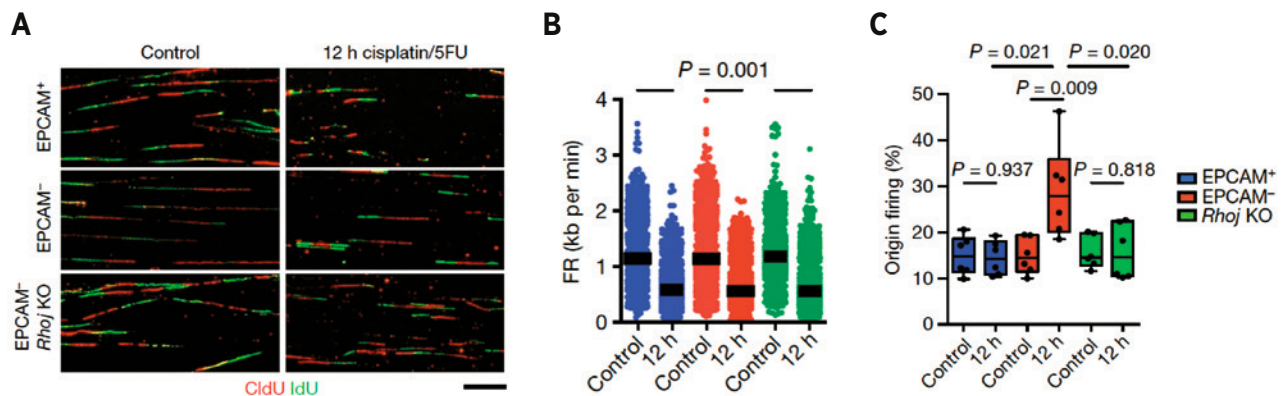
FIGURE 1 RhoJ promotes activation of new origins after chemotherapy. (**A**) Stretched DNA fibres derived from EPCAM+, EPCAM−, and EPCAM− RhoJ KO cells treated with cisplatin/ 5-FU for 12h when indicated. (**B**) Fork rate quantification. (**C**) Percentage of origin activation. Adapted from Debaugnies M *et al.* (2023).
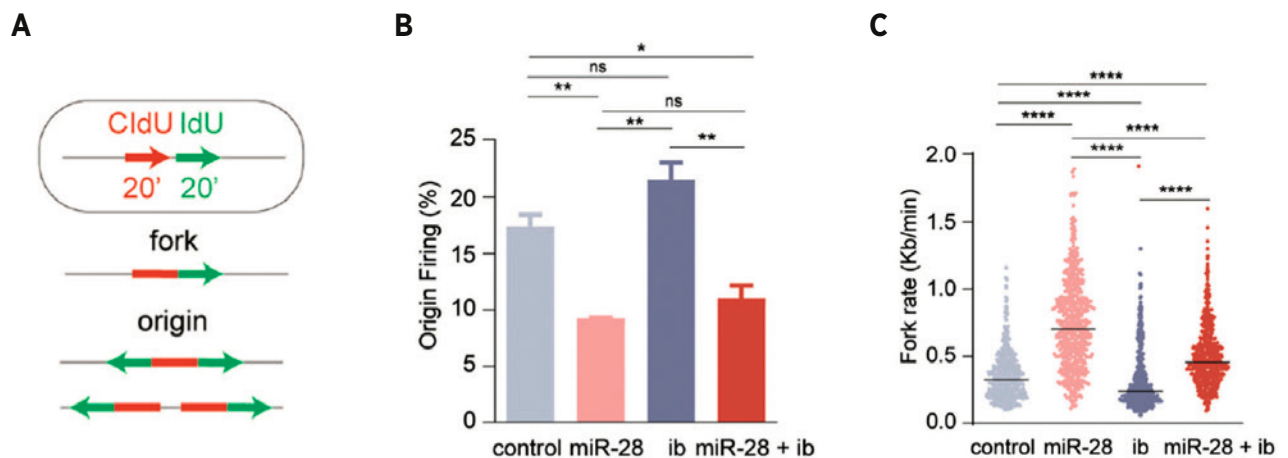
FIGURE 2 Efficient block of DNA replication by miR-28 + ibrutinib combination. (**A**) Schematic of DNA fibre assay in cells treated with miR-28, ibrutinib (ib) or the combination of both. (**B**) Frequency of origin activation. (**C**) Distribution of fork rate in the different conditions. Adapted from Fuertes T *et al.* (2023).

▸ **PUBLICATIONS**

▸ Debaugnies M, Rodríguez-Acebes S, Blondeau J, Parent MA, Zocco M, Song Y, de Maertelaer V, Moers V, Latil M, Dubois C, Coulonval K, Impens F, Van Haver D, Dufour S, Uemura A, Sotiropoulou PA, Méndez J, Blanpain C (2023). RhoJ controls EMT associated resistance to chemotherapy. *Nature* 616, 168-175.

▸ Fuertes T, Álvarez-Corrales E, Gómez-Escolar C, Ubieto-Capella P, Serrano-Navarro Á, de Molina A, Méndez J, Ramiro AR, de Yébenes VG (2023). miR-28-based combination therapy impairs aggressive B cell lymphoma growth by rewiring DNA replication. *Cell Death Dis* 14, 687.

MELANOMA GROUP

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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, being selectively deregulated in melanoma, may account for the unique ability of this tumour type to bypass immune recognition and generate metastasis already 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*) 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.

"Performing single cell analyses, we have identified different cellular states and new mechanisms of immune suppression in melanoma that will pave the way for potential diagnostic markers and therapeutic targets."

RESEARCH HIGHLIGHTS

The long-term goals of our Group are to:

1. Define the "fingerprint" that distinguishes melanomas from other cancer types.
2. Visualise and target melanoma progression at the whole-body level *in vivo*.
3. Determine and target signalling cascades that turn immunologically "hot" melanomas into "cold" and refractory tumours.
4. Develop new therapeutic strategies to overcome immune suppression and immune tolerance in melanoma.

New drivers of melanoma progression

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 through genetic and functional studies in melanocytic cell lines, mouse models, and tissue specimens, but also by performing cross-cancer type analyses. We previously identified mechanisms of vesicular trafficking, autophagy, and RNA-associated metabolism with protumorigenic functions in this disease that are not shared by 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). In addition, 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). *'MetAlert'* animals, in combination with human tissue specimens, revealed the growth factor MIDKINE (MDK) as a tumour-secreted pro-metastatic factor with a triple role in melanoma of: (1) driving a systemic expansion of the lymphatic vasculature (neolymphangiogenesis) at early stages of melanoma progression, (2) favouring tumour

cell adhesion and migration, and (3) promoting immune suppression (FIGURE 1). A quite surprising finding was the broad spectrum of action of the immune suppressive functions of MDK. First, we reported that MDK rewires macrophages, which instead of recognising and eliminating malignant cells, act as tumour promoters via the induction of dysfunctional CD8^{+} T cells (Cerezo-Wallis *et al.*, *Nat Medicine* 2020). More recently, we discovered an additional novel role of MDK as a potent suppressor of antigen presentation, namely by inhibiting the differentiation and function of dendritic cells (DCs), specifically those that are conventional type 1 (cDC1). Furthermore, we uncovered an MDK-associated signature in DCs that defines bad prognosis and resistance to immune checkpoint blockers actively used in human patients (Catena *et al.*, *BioRxiv* 2022; Catena *et al.*, submitted). In light of the tumour-promoting and immune-suppressive roles of MDK, we are now actively pursuing this protein as a therapeutic target. We previously reported dsRNA mimetics that repress MDK mRNA expression (Olmeda *et al.*, *EMBO Mol Med* 2021) and are now developing small molecule inhibitors and blocking antibodies.

Single cell analyses of tumour and immune compartments in melanoma

One of the challenges in the rational design of new therapies in melanoma is the marked inter- and intra-tumoural heterogeneity of these lesions. Recent studies have identified various cellular states in melanoma, but the underlying drivers are not well understood. Using scRNAseq, we recently addressed the impact of MDK at the cellular level, both in cutaneous lesions and at premetastatic sites in lymph nodes and the lungs (FIGURE 2). This approach revealed 6 transcriptionally distinct clusters in tumour implants driven by aggressive melanoma cells. Of these, MDK depletion was found to impinge
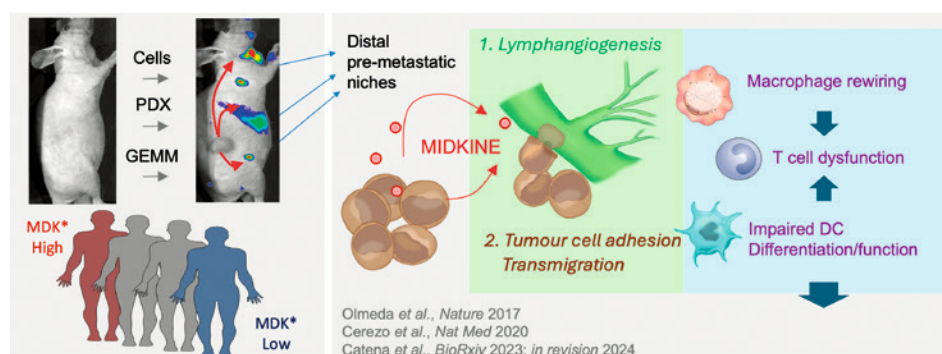
FIGURE 1 Identification of tumour drivers and immune modulators in melanoma. Combination of *MetAlert* mice and functional studies in patient biopsies for the discovery of lymphangiogenic factors, with roles in tumour cell metastasis and immune suppression, here illustrated for the growth factor Midkine.
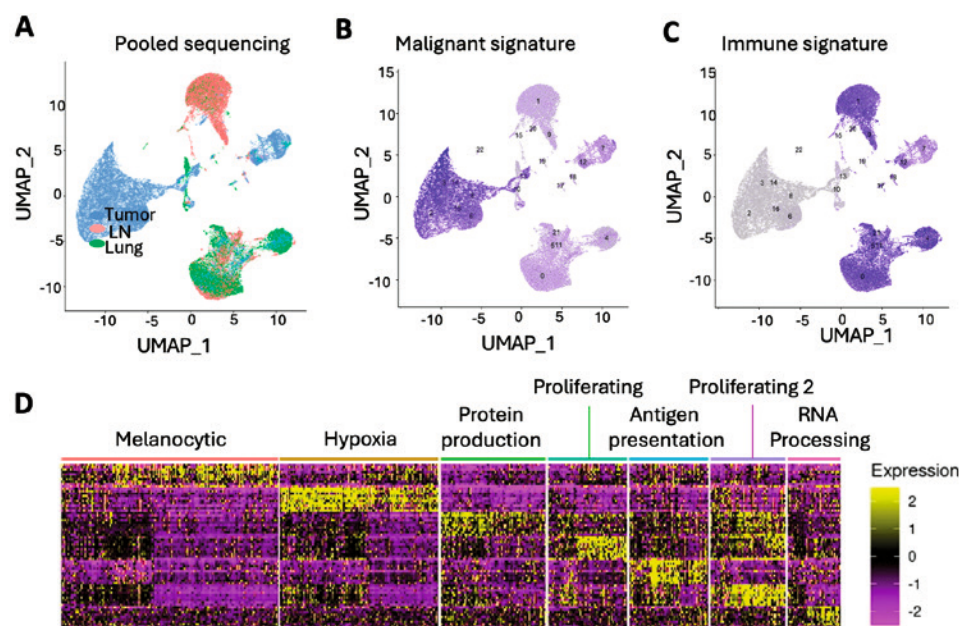
FIGURE 2 scRNAseq for a comprehensive analysis of the impact of MDK at primary vs premetastatic sites. (**A-C**) UMAP plots of scRNAseq data in tumour, lymph nodes, and lungs (A), with the corresponding separation of malignant cells (B) and immune cells (C). (**D**) Differential expression profile of MDK-associated tumour cellular states.

particularly on a population linked to antigen presentation and interferon response. Importantly, we identified distinct effects of MDK on the transcriptome of macrophages, DCs, and T cells, among others. These data provide new insight into how the secretome of tumour cells can impact at distal sites through a coordinated reprogramming of the expression profile of multiple immune cell types. Our expertise in immune suppression has also helped in collaborative studies to describe tumour-to-lung systemic effects of yet other immune modulators (i.e., IL22) in aggressive cancers (Briukhovetska *et al.*, *Immunity* 2023). ∎

EPITHELIAL CARCINOGENESIS GROUP

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OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and bladder carcinoma taking a disease-oriented approach. These tumours present very distinct clinical 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).

PDAC has a dismal prognosis even when diagnosed early. We aim at dissecting the molecular mechanisms involved in very early steps of tumour development, harnessing the power of mouse genetic editing. 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 presents with a very wide clinical and pathological heterogeneity. We aim at acquiring knowledge about the underlying biology that might be leveraged towards improved tumour subclassification, prediction of outcome, and therapy.

"A new mouse strain carrying an exon 6 deletion in Ctrb1 recapitulates the human variant associated with PDAC. A mutant, insoluble, CTRB1 truncated protein is present in the pancreas of these mice, associated with a dramatic ER stress phenotype."

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 *NR5A2* and *HNF1A* coding for transcription factors required for full acinar differentiation. A few other GWAS hits associate with genes involved in acinar function, such as *CTRB1/2* and *XBP1*. These observations have strengthened the notion, pioneered by our lab, that cell differentiation is the first tumour suppressor mechanism in the pancreas. Among the processes participating therein are inflammation and ER stress response. We have extensively studied the role of NR5A2 using heterozygous mice. Recently, we focused on a risk variant in *CTRB2* that has been finely mapped as a deletion in exon 6. We generated, with Sagrario Ortega (CNIO), a new mouse strain carrying the corresponding exon 6 deletion in *Ctrb1,* the mouse ortholog of the human gene. *Ctrb1*$^{\Delta/\Delta}$ mice are viable and display normal growth until adulthood. The mutant CTRB1 protein has a smaller molecular mass and is largely present in the insoluble fraction of pancreatic lysates, unlike the wild type protein. While the pancreas of 3-month-old homozygous mutant mice has a normal histological appearance, ultrastructural analysis shows dramatic alterations of the endoplasmic reticulum, with extensive cisternal dilation, abundant cytoplasmic aggregates, partial loss of zymogen granules, and even some nuclear inclusions. Strikingly, some acinar cells appear spared despite the germline nature of the mutation introduced. Similar, but less dramatic, changes occurred in heterozygous mice. RNA-Seq analysis of the pancreas of 3-month-old mice revealed a down-regulation of the acinar programme and an up-regulation of ER stress pathways, in agreement with the morphological changes. In addition, we found modest evidence of increased inflammatory pathway activity. We interpret these findings as an adaptation of acinar cells to stress, aimed at increasing cell viability. These mice are currently undergoing extensive phenotyping and we are assessing their ability to respond to a variety of insults including pancreatitis and high fat diet, among others.

Urothelial bladder carcinoma genetics, biology, and clinical translation

We focus on understanding 2 new tumour suppressor genes that are mutated in bladder cancer that we identified through exome sequencing: *STAG2* and *RBM10*. *STAG2* codes for a cohesin subunit and *RBM10* codes for a splicing regulator. We have generated conditional mouse models for these 2 genes and are exploring their role in development and urothelial biology as well as their cooperation with other cancer genes.

Bladder cancers that do not invade muscle largely maintain a luminal/urothelial phenotype characterised by the expression of transcription factors involved in the activation of this programme, such as GATA3 and FOXA1. However, upon invading muscle, a fraction of tumours loses this identity and displays a basal/squamous phenotype that is reminiscent of that of basal cells in the urothelium. This is generally associated with the loss of expression of luminal-type transcription factors. To acquire a more detailed understanding of the molecular mechanisms involved in this process, which is associated with more aggressive tumours, we set out to systematically identify novel transcriptional regulators that might participate in this process. Using bulk and single cell transcriptomic data from bladder cancer organoids and tumours classified as luminal or basal/squamous, we identified differentially expressed factors and searched for the enrichment of their binding motifs in accessible regions of chromatin and for experimental evidence in support of their binding. In addition, we considered their tumour specificity by comparing available information from tumours and normal urothelium. This integrative work has unveiled several new transcription factors that are candidates to participate in urothelial transformation, including FOXQ1, MECOM, TBX3, AP1 proteins, and TFAP2, among others.

In addition, we continue to collaborate with our clinical colleagues from the GUARD consortium in the conduct of clinical studies with a strong translational component. ∎

‣ **PUBLICATIONS**

‣ Koutros S, Kiemeney LA, Choudhury PP, Milne RL, Lopez de Maturana E, Ye Y, Joseph V, Dyrskjøt L, Florez-Vargas O, Figueroa J, Dutta D, Giles GG, Hildebrandt MAT, Offit K, Kogevinas M, Weiderpass E, McCullough ML, Freedman ND, Albanes D, Kooperberg C, Cortessis VK, Karagas MR, Johnson A, Schwenn MR, Furberg H, Bajorin DF, Cussenot O, Cancel-Tassin G, Benhamou S, Kraft P, Porru S, Bishop T, Matullo G, Fletcher T, Kumar R, Taylor JA, Gago-Dominguez M, Golka K, Purdue MP, McGlynn KA, Kitahara CM, Haiman CA, Greene MH, Rafnar T, Chatterjee N,* Chanock SJ,* Wu X,* Real FX,* Silverman DT,* Garcia-Closas M,* Stefansson K,* Prokunina-Olsson L,* Malats N,* Rothman N* (2023). Genome-wide association study of bladder cancer reveals new biological and translational insights. *Eur Urol* 84, 127-137. (*, equivalent contributions).

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‣ Marqués M,* Corral S,* Sánchez-Díaz M, del Pozo N, Martínez de Villarreal J, Schweifer N, Zagorac I, Hilberg F, Real FX (2023). Tumor and stromal cell targeting with Nintedanib and Alpelisib overcomes intrinsic bladder cancer resistance. *Mol Cancer Ther* 22, 616-629. (*, contributed equally).

‣ Font A, Domenech M, Ramirez JL, Marqués M, Benítez R, Ruiz de Porras V, Gago JL, Carrato C, Sant F, Lopez H, Castellano D, Malats N, Calle ML, Real FX (2023). Predictive signature of response to neoadjuvant chemotherapy in muscle invasive bladder cancer integrating mRNA expression, taxonomic subtypes, and clinicopathological features. *Front Oncol* 13, 1155244.

‣ Font A, Mellado B, Climent MA, Virizuela JA, Oudard S, Puente J, Castellano D, González-del-Alba A, Pinto A, Morales-Barrera R, Rodriguez-Vida A, Fer-

FIGURE 1 $Ctrb1^{\Delta/\Delta}$ pancreata recapitulate human $CTRB2$ exon 6 deletion: expression of a truncated CTRB1 protein that forms aggregates, leading to increased endoplasmic reticulum (ER) stress and down-regulation of the acinar programme. (**A**) Mice carrying $Ctrb1$ exon 6 deletion express a smaller, truncated, protein that forms aggregates. (**B**) Transmission electron microscopy reveals ER stress features in $Ctrb1^{\Delta/\Delta}$ pancreata, including cisternal swelling and intracisternal aggregates. (**C**) Volcano plot showing differentially expressed genes in the pancreas of $Ctrb1^{\Delta/\Delta}$ vs $Ctrb1^{+/+}$ mice (bulk RNA-seq). Down-regulated genes include mainly acinar enzymes whereas up-regulated genes are associated with ER stress.
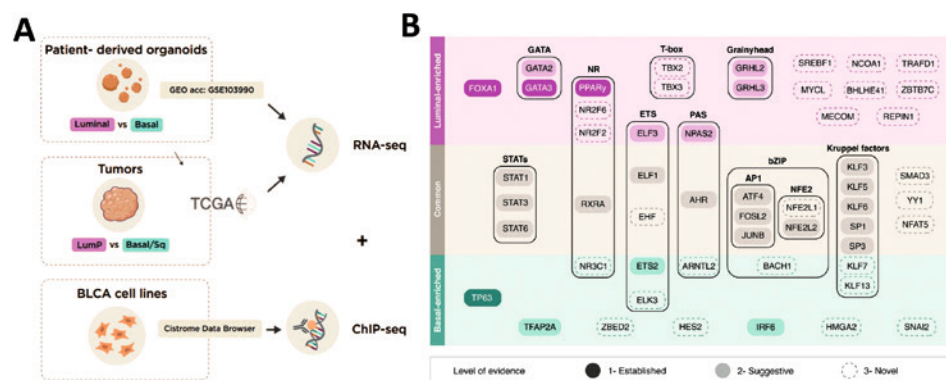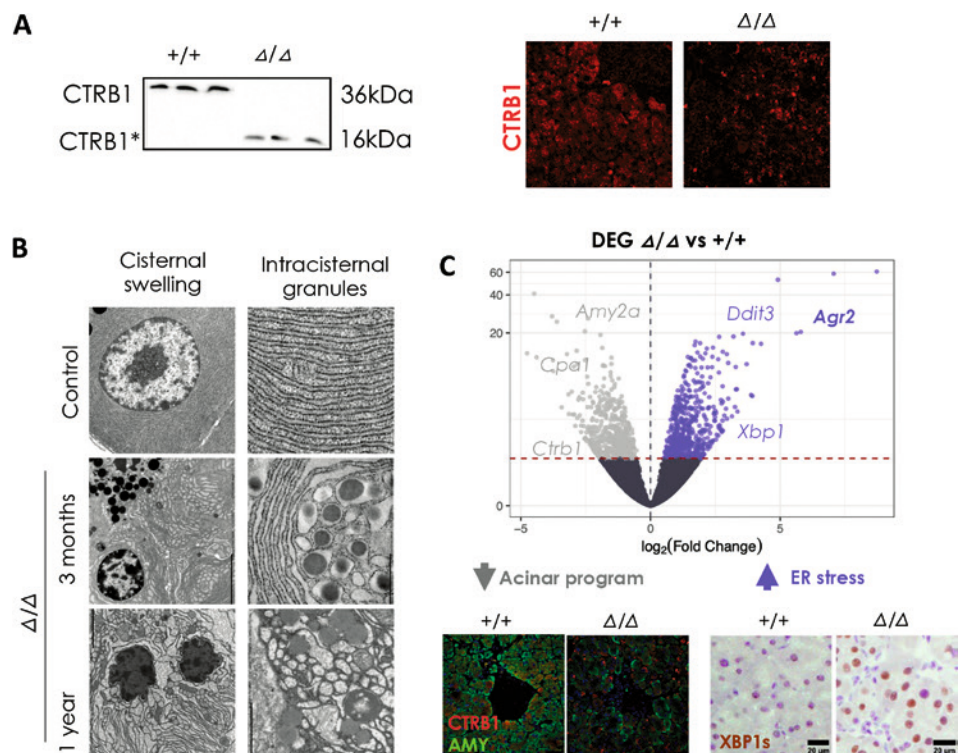
FIGURE 2 Identification of candidate transcription factors relevant for urothelial lineage identity and carcinogenesis.(**A**) Scheme of the approach used to systematically identify candidates integrating multi-omics data from different models. (**B**) Topological scheme depicting the involvement of well-established (dark background) and novel (discontinuous outline) candidate transcription factors participating in the luminal vs. basal differentiation programmes. Purple, "Luminal-enriched"; beige, "Common"; teal, "Basal-enriched" transcription factors.

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‣ King SD *et al.* (incl. Malats N, Real FX) (2023). Genetic susceptibility to non-alcoholic fatty liver disease and risk for pancreatic cancer: Mendelian randomization. *Cancer Epidemiol Biomarkers Prev* 32, 1265-1269.

‣ Rao N, Starrett, GJ, Piaskowski ML, Butler KE, Golubeva Y, Yan W, Lawrence SM, Dean M, Garcia-Closas M, Baris D, Johnson A, Schwenn M, Malats N, Real FX, Kogevinas M, Rothman N, Silverman D, Dyrskjøt L, Buck CB, Koutros S, Prokunina-Olsson L (2023). Association analysis of several common APOBEC-type mutations in bladder tumors suggests links to viral infection. *Cancer Prev Res* 16, 561-570.

‣ Caramelo B, Zagorac S, Corral S, Marqués M, Real FX (2023). Cancer-associated fibroblasts (CAFs) in bladder cancer: Origin, biology, and therapeutic opportunities. *Eur Urol Oncol* 6, 366-375.

‣ Radvanyi F, Real FX, McConkey D (2023). What is a bladder cancer molecular subtype? - Counterpoint. *Bladder Cancer* 9, 299-304.

Book Chapter

‣ López de Maturana E, Zagorac S, Malats N, Real FX (2023). Vitamin D and pancreatic cancer. In M. Hewison, R. Bouillon, E. Giovannucci, D. Goltzman, M. Meyer, J. Welsh (Eds.), *Feldman and Pike's Vitamin D Volume II: Health, Disease and Therapeutics* (5th ed., pp. 937-966). Elsevier Academic Press. ISBN: 9780323913386.

‣ **AWARDS AND RECOGNITION**

‣ Member, Scientific Advisory Board, *Fundación Científica de la Asociación Española Contra el Cáncer*, Madrid, Spain.

‣ Member, Scientific Advisory Board, *Instituto de Investigaçao e Inovaçao em Saude i3S*, Porto, Portugal.

GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

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Group Leader

Research Scientists
Rayan Naser (since March),
Sladjana Zagorac

Gradutate Students
Mariana Angulo, Sergio de la Rosa (until November), Rosa Gallo, Irene Herranz, Yizhi Liu (since September), Carlos Martínez, Maria del Mar Rigual, Paula Sánchez, Karla Santos, Zhaoshuo Wang (since October)

Technician
Marta Benítez

Students in Practice
Ali Ataman (June-September) (CNIO Summer Training Programme,

Istanbul Technical University, Turkey), Giuseppina Pennacchio (since October) (Erasmus + Fellowship, University of Naples, Italy)

Medical Residency – Visiting Scientist
Arturo Lecumberri (February-April) (*Hospital Universitario de Navarra,* Pamplona, Spain)

OVERVIEW

Over the last 2 decades, research has primarily focused on understanding the functions of mutated genes in cancer, neglecting the roles of environmental factors that can induce the expression of harmful proteins and tissue damage. These factors pose ongoing challenges, and their mechanisms in causing cancer-related pathologies are largely unknown. Identifying links between environmental stress and cancer progression is crucial for uncovering disease mechanisms and therapeutic targets.

Our laboratory employs genetically engineered mouse models and advanced technologies to investigate mechanisms of diseases associated with environmental stressors. We specifically study conditions related to toxic diets, nutrient imbalances, and sedentary lifestyles, which can lead to obesity and associated disorders, such as diseases from the digestive tract.

Our particular focus lies in diseases affecting the liver (non-alcoholic steatohepatitis, cirrhosis, and hepatocellular carcinoma), intestine (colitis and colorectal cancer), and pancreas (diabetes, pancreatitis and pancreatic cancer). These organs are primarily affected by environmental stressors, including nutrient overload and lack of physical activity, that can cause severe inflammatory conditions. In addition, their functions are interconnected and potentially regulated by the nervous system, through unknown mechanisms. Accordingly, we recently started to explore the intricate relationship between diet and the nervous and immune systems in aggressive cancers, including metastasis, a perspective we plan to emphasise further in the future, within the emerging field of cancer neuroscience and neuroimmunomodulation.

Furthermore, our research encompasses tissue regeneration (intestine and liver), the dysregulation of metabolic pathways in cancer initiation, inflammatory processes, and the initial stages of embryonic development, shedding light on fundamental mechanisms applicable to various diseases. Our goal is to guide the development of novel medicines, with a special focus on potential immunomodulatory therapies for these disorders.

"We continuously strive to generate new and unique preclinical mouse models to elucidate the mechanisms of diseases and capture the complexity of human disorders, with a particular focus on diseases associated with obesity and the digestive tract."

RESEARCH HIGHLIGHTS

Using genetically engineered mouse models, along with other model systems and cutting-edge technologies (including cell biology with organoid culture and quantitative imaging, biochemistry, and functional genomics), and human data, our laboratory has dedicated significant effort over the past years to comprehend the molecular, cellular, and pathophysiological mechanisms that connect environmental stresses to disease pathogenesis. In particular, we have studied the mechanisms of diseases associated with obesity and the digestive system, with a focus on liver and intestinal disorders. These conditions often stem from unhealthy diets, nutrient imbalances, and sedentary lifestyles, all of which can contribute to severe inflammatory conditions (see Figure 1). Organs of the digestive system are indeed primarily impacted by environmental stressors but are also physiologically interconnected and influenced through their exocrine and/or endocrine functions. Significant discoveries have been made, and several future research projects are planned as follows:

Mechanisms of obesity

Our groundbreaking work has uncovered the mechanisms behind the inflammatory properties of nutrients and their connection to various disorders. Our recent research has linked inflammation, particularly IL-17A, to obesity and autoimmune disorders, connecting them to hepatitis and liver disease-induced hepatocellular carcinoma. Our findings have gained significant attention from pharmaceutical companies exploring IL-17A blockers as potential treatments for these disorders. Our ongoing research aims to further understand how nutrients can be inflammatory by themselves leading to obesity. Additionally, we will dedicate special efforts to identify the specific inflammatory cells responsible for obesity and its associated metabolic disorders.

Diet, nutrients and cancer

We discussed in an extensive review how various diets could impact cancer development. This suggests that nutritional interventions could be beneficial for both the prevention and treatment of cancer.

Mechanisms of liver cancer progression

Environmental stress, nutrient overload and toxic diet can lead to chronic liver diseases, including cirrhosis, which may progress to hepatocellular carcinoma (HCC). To comprehend the influence of cirrhosis on HCC development, our work focuses on studying the mechanobiology of liver tissue at the molecular, cellular, and tissue levels. This involves investigating and genetically manipulating in mice the mechanical forces within and between various liver cells, as well as their interactions with microenvironments. Mathematical models and bioinformatics analyses will be used to complement our studies, with the ultimate goal of understanding the progression from an injured and diseased liver to a cancerous tissue.

Cell dormancy in HCC relapse

Despite numerous therapeutic strategies, cancer relapse is common, occurring months to years after treatment. Tumour relapse is thought to be driven by dormant, non- or slow-cycling resistant cells, yet conclusive proof-of-concept studies are lacking. Our laboratory aims to address this gap by utilising a genetically modified mouse model. We plan to label and track dormant cells to understand their role in HCC recurrence.

Mechanisms of intestinal diseases and colorectal cancer

Colorectal cancer (CRC) is a multi-hit neoplasia originating from APC mutation-induced adenomatous polyps, which progress to malignancy through the acquisition of p53 loss. Our research is focused on comprehending the mechanisms underlying CRC initiation and the transitional mutations leading to the transformation of polyps into malignant carcinomas. Additionally, we are prioritising the investigation of why the majority of colorectal cancers exhibit resistance to immune checkpoint inhibitors, which have proven ineffective in patient treatment.

Mechanisms of totipotency-to pluripotency transition

We are currently elucidating the mechanisms that govern the smooth and precise transition from totipotency to pluripotency, which is a crucial process in embryonic development. This transition generates pluripotent stem cells with the capability to form all cell types.

Structure of the URI prefoldin-like complex

One of our future goals is to determine the functions of the URI prefoldin-like complex by unravelling its structural organisation through advanced techniques such as electron microscopy. ■
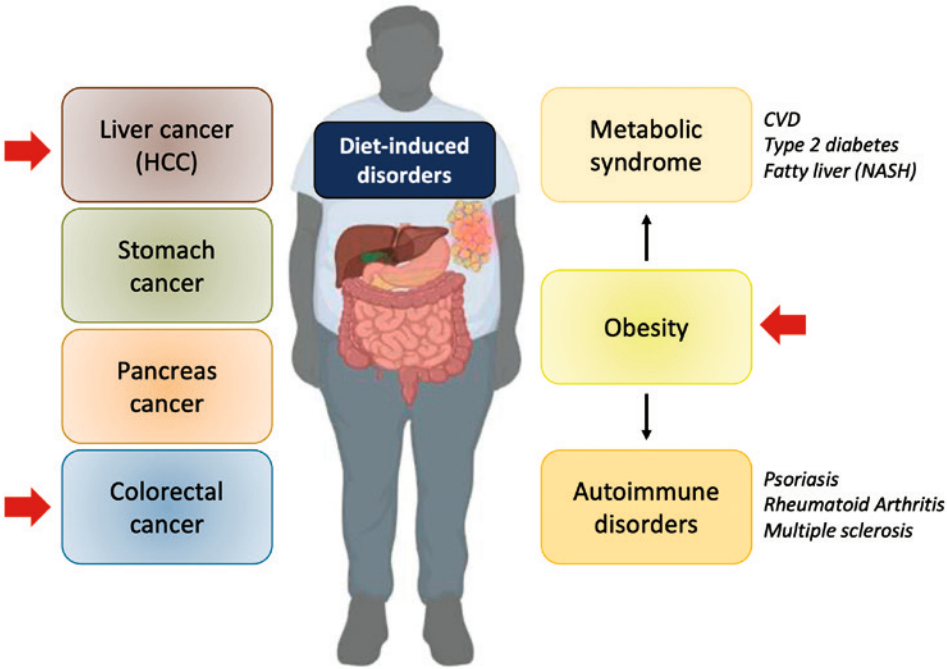
FIGURE 1 Representation of some of our current and future research directions.

▸ **PUBLICATIONS**

▸ Rigual MdM, Sánchez Sánchez P, Djouder N (2023). Is liver regeneration key in hepatocellular carcinoma development? *Trends Cancer* 9, 140-157.

▸ Martínez-Garay C, Djouder N (2023). Dietary interventions and precision nutrition in cancer therapy**.** *Trends Mol Med* 29, 489-511.

▸ Grazioso TP, Djouder N (2023). A mechanistic view of the use of cold temperature in the treatment of cancer. *iScience* 26, 106511.

▸ Grazioso TP, Djouder N (2023). The forgotten art of cold therapeutic properties in cancer: a comprehensive historical guide. *iScience* 26, 107010.

▸ **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

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Group Leader

Research Scientists
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Gema Pérez (until August)

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Aránzazu Gómez, Jaime Redondo,
Alejandro Sánchez Juan,

Andrea Vethencourt (Clinical Oncologist at *ICO/IDIBELI*, Barcelona, Spain), Samuel Vives (since November)

Technicians
Víctor López (Mouse technician), Gonzalo Soria (Bioinformatician, until July)

Student in Practice
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(Master's Thesis, *Univ. Francisco de Vitoria*, Madrid, Spain)

Visiting Scientists
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Juana Moro (September-December) (PhD student, *IBYME*, *CONICET*, Argentina), Anastasios Triantafyllou (since October) (Erasmus + student, Univ. of Thessaly, Greece)

OVERVIEW

Tumours exploit and manipulate for their benefit the same mechanisms that regulate homeostasis in healthy tissue. 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.

" Luminal Rank loss impairs lactation, awakening basal bipotency to restore functional milk production in parous glands."

"RANK pathway inhibitors can restore sensitivity to CDK4/6i and prevent acquired resistance in breast cancer."

RESEARCH HIGHLIGHTS

RANK is a poor prognosis marker and a therapeutic target in ER-negative postmenopausal breast cancer

Analyses of RANK and RANKL expression in more than 2000 breast tumours revealed that tumour RANK expression associated with poor prognosis in ER-negative breast cancer and in postmenopausal breast cancer patients. Gene set enrichment analysis (GSEA) showed that RANK protein expression in tumour cells in postmenopausal E- negative breast tumours was associated with multiple immune and metabolic pathways, suggesting that RANK signalling increases after menopause. Our results demonstrate that RANK expression is an independent biomarker of poor prognosis in postmenopausal patients with ER-negative breast cancer and support the therapeutic benefit of RANK pathway inhibitors in breast cancer patients with RANK-positive, ER-negative tumours after menopause. (Ciscar M *et al.*, *EMBO Mol Med* 2023).

Luminal Rank loss decreases cell fitness leading to basal cell bipotency in parous mammary glands

The Rank signalling pathway regulates mammary gland homeostasis and epithelial cell differentiation. By combining temporal/lineage specific *Rank* genetic deletion with lineage tracing techniques, we found that loss of luminal Rank reduces the luminal progenitor pool and leads to aberrant alveolar-like differentiation with high protein translation capacity in virgin mammary glands. These Rank-deleted luminal cells are unable to expand during the first pregnancy, leading to lactation failure and impairment of protein synthesis potential in the parous stage. The unfit parous Rank-deleted luminal cells in the alveoli are progressively replaced by Rank-proficient cells early during the second pregnancy, thereby restoring lactation. Transcriptomic analysis and functional assays point to the awakening of basal bipotency after pregnancy through the induction of Rank/NF-κB signalling in basal parous cell to restore lactation and tissue homeostasis. (Rocha AS, Collado-Solé A *et al.*, *Nat Commun* 2023).
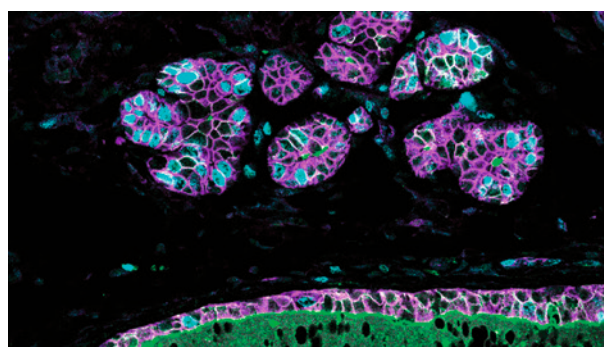
FIGURE 1 Mammary gland at G9.5 showing in pink luminal cells and in cyan PR expression.

Co-targeting RANK pathway treats and prevents acquired resistance to CDK4/6 inhibitors in luminal breast cancer

The combination of endocrine therapy (ET) and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors (CDK4/6i) was a hallmark in metastatic luminal breast cancer. However, intrinsic and acquired resistance affects long-term efficacy. We studied the role of the receptor activator of the nuclear factor-κB (RANK) pathway in CDK4/6i resistance. We found that RANK overexpression in luminal breast cancer is associated with intrinsic resistance to CDK4/6i, both *in vitro* and in mouse xenografts. Gene expression data from clinical trials and studies with palbociclib-resistant cell lines showed that RANK is upregulated after treatment with CDK4/6i, supporting a role in acquired resistance. Our study shows that RANK ligand (RANKL) inhibitors can restore sensitivity to CDK4/6i and prevent acquired resistance. (Gomes I *et al.*, *Cell Rep Med*, 2023).

▸ **PUBLICATIONS**

▸ Rocha AS·, Collado-Solé A·, Graña-Castro O, Redondo-Pedraza J, Soria-Alcaide G, Cordero A, Santamaría PG, González-Suárez E# (2023). Luminal Rank loss decreases cell fitness leading to basal cell bipotency in parous mammary glands. *Nat Commun* 14, 6213. *Co-first authors; #Corresponding author.

▸ Gomes I, Gallego-Paez LM, Jiménez M, Santamaria PG, Mansinho A, Sousa R, Abreu C, Suárez EG, Costa L, Casimiro S (2023). Co-targeting RANK pathway treats and prevents acquired resistance to CDK4/6 inhibitors in luminal breast cancer**.** *Cell Rep Med* 4, 101120.

▸ Ciscar M·, Trinidad EM·, Perez-Chacon G, Alsaleem M, Jimenez M, Jimenez-Santos MJ, Perez-Montoyo H, Sanz-Moreno A, Vethencourt A, Toss M, Petit A, Soler-Monso MT, Lopez V, Gomez-Miragaya J, Gomez-Aleza C, Dobrolecki LE, Lewis MT, Bruna A, Mouron S, Quintela-Fandino M, Al-Shahrour F, Martinez-Aranda A, Sierra A, Green AR, Rakha E, Gonzalez-Suarez E# (2023). RANK is a poor prognosis biomarker and a therapeutic target in ER-negative postmenopausal breast cancer. *EMBO Mol Med* 15, e16715. *Co-first authors; #Corresponding author.

▸ Cordero A·, Santamaría PG·, González-Suárez E# (2023). Rank ectopic expression in the presence of Neu and MMTV oncogenes alters mammary epithelial cell populations and their tumorigenic potential. *J Mammary Gland Biol Neoplasia* 28, 2. *Co-first authors; #Corresponding author.

▸ van Amerongen R *et al.* (incl. Suarez EG) (2023). Imagine beyond: recent breakthroughs and next challenges in mammary gland biology and breast cancer research. *J Mammary Gland Biol Neoplasia* 28, 17.

▸ Vethencourt A, Trinidad EM, Dorca Duch

Microglia Rank signalling regulates GnRH function and the hypothalamic-pituitary-gonadal axis

We have demonstrated a novel role of hypothalamic microglia in controlling reproductive hormones through Rank signalling. Congenital and microglia Rank depletion leads to severe hypogonadotropic hypogonadism (HH) in males and females, resulting from a direct alteration in gonadotropin-releasing hormone (GnRH) regulation. In addition, we identified rare sequence mutations of *RANK* in patients with congenital hypogonadotropic hypogonadism (CHH). Moreover, inducible Rank deletion during puberty and adulthood also leads to HH. Transcriptional profiling at single-cell level of hypothalamic microglia revealed the importance of Rank signalling in the maintenance of a functionally active homeostatic microglia. Our data have revealed the crucial role of microglia, mediated by Rank signalling, in regulating GnRH function and the hypothalamic-pituitary-gonadal (HPG) axis, which is essential for reproductive maturation and fertility. (Collado *et al.*, under review in *Nature*). ∎
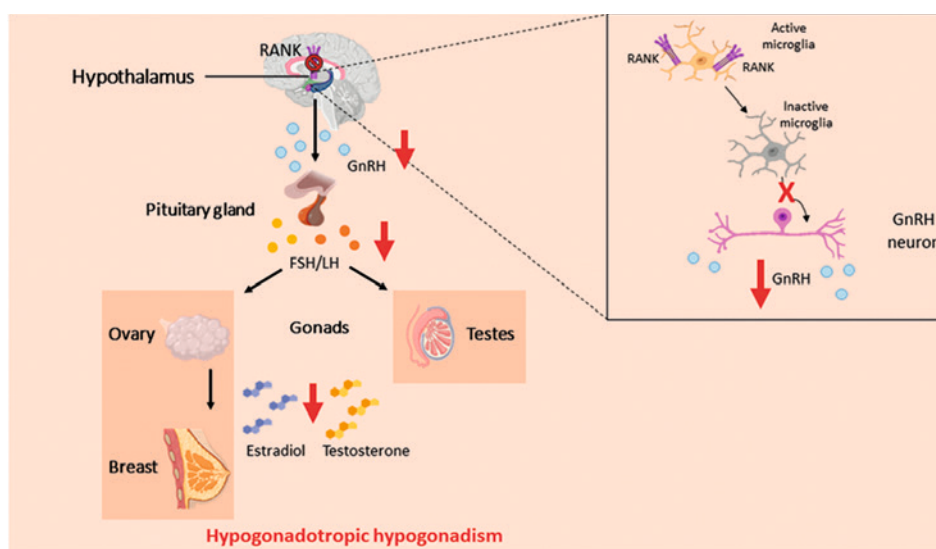
FIGURE 2 Defective HPG axis in microglia Rank null models.

E, Petit A, Soler-Monsó MT, Purqueras E, Brizzi ME, Matas Garcia M, Pérez-Chacón G, Jimenez M, Ciscar M, Stradella A, Iserte A, Gumà Martinez A, Ortega R, Gil MJ, Pernas Simon S, Falo C, Gonzalez-Suarez E (2023). 289P Results of the window-of-opportunity clinical trial D-BIOMARK: Study of biomarkers of the antitumor activity of denosumab and its role as a modulator of the immune response in early breast cancer. *Annals of Oncology* 34, suppl. 2, s300.

BRAIN METASTASIS GROUP

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Neibla Priego

Gradutate Students
Laura Adriana Álvaro, Jimena Benzal
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Pedro García, Carolina Hernández,
Irene Salgado, Juan José Vázquez
(since October)

Technicians
Patricia Baena, Virginia García-Calvo (until August), Diana P. Retana (TS)*, Oliva A. Sánchez, Catalina Vela (since November)

*Titulado Superior (Advanced Degree)

Lab Administrative Manager
Jorge Guillermo Ortiz

Students in Practice
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Andrea Rojas (since March) (Master's Thesis, Universidad Autónoma de Madrid, Spain)

Visiting Scientists
Petra Jagust (November) (Royal College of Surgeons in Ireland),

Valentina Pieri (March-November) (IRCCS Ospedale San Raffaele, Milan, Italy)
Ariane Steindl (since October) (Medical University of Vienna, Austria)

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 challenged mass effect as the only source of neurocognitive impairment in brain metastases and instead suggest that a molecular programme present in cancer cells could underlie such a process."

RESEARCH HIGHLIGHTS

How brain metastases impair neural communication

We published a manuscript that suggests an alternative way to look at the impact brain metastasis can cause on brain function. Rather than the compression and destruction of neurons in the peritumoral area derived from the mass effect as the tumour grows, we showed that the molecular profile of cancer cells might generate aberrant ways in which they influence surrounding intact neural circuits. By exploiting different brain metastasis models, we showed that they recapitulate the heterogeneous impact observed in patients with respect to the negative influence on neuronal communication. As such, we detected differences in the peritumoral electrophysiology from different models that correlated with the decrease in both inhibitory synapses as well as calcium activity. Furthermore, simply analysing brain activity associated with the presence of different brain metastasis models depicted a novel biomarker. In brief, computational analysis of brain activity using artificial intelligence demonstrates the possibility to predict the presence and the subtype of metastasis in the brain. Both aspects (the use of a novel biomarker as well as the molecular mediators of the impact on brain activity) are currently being followed-up to be exploited clinically through the National Network of Brain Metastasis, RENACER.

Progress on the co-evolution of the metastasis-associated microenvironment

Our programme exploiting the evolution of the microenvironment into a pro-tumoral niche to be exploited for novel therapies against brain metastases has been significantly expanded. We have deepened our knowledge on metastasis-associated astrocyte heterogeneity as well as on the process by which they are reprogrammed to acquire new roles as a strong local immunomodulatory cell type. Indeed, we have interrogated strategies to manipulate CD8^{+} T cells and evaluate their ability to target brain metastases using adoptive T cell transfer experiments. This strategy is giving us the opportunity to develop immunotherapies optimised to work on clinically relevant (symptomatic) brain metastases. The study of the plasticity of the microenvironment has been complemented by analysing a novel subpopulation of macrophages that emerges in the metastasis-associated microenvironment.

Development of preventive strategies against brain metastasis

Our systematic dissection of metastatic colonisation of the brain has made it possible to define different steps. By focusing on the early moments after extravasation, we are studying the process by which virtually all potentially metastasis-initiating cells rely on pre-existing vessels to survive, using the process termed vascular co-option. Deconstructing the crosstalk between co-opting metastatic cells and co-opted endothelial cells is giving us new molecular insights to develop preventive strategies to stop the development of micrometastases.

Consolidating RENACER as a pioneering strategy for a more efficient translation

The National Network of Brain Metastasis (RENACER) initiated in 2021 has expanded to 18 hospitals throughout the country, attracted competitive grants, and initiated clinical studies and trials (NCT05635734, NCT05689619) based on the use of Patient-Derived Organotypic Cultures (PDOC). Furthermore, the unique resource of patient-derived material generated over these years is allowing us to initiate research projects starting from findings in human data. ∎

▸ **PUBLICATIONS**

▸ Sanchez-Aguilera A**, Masmudi-Martín M**, Navas-Olive A, Baena P, Hernández-Oliver C, Priego N, Cordón-Barris L, Alvaro-Espinosa L, García S, Martínez S, Lafarga M; RENACER; Lin MZ, Al-Shahrour F, Menendez de la Prida L*, Valiente M* (2023). Machine learning identifies experimental brain metastasis subtypes based on their influence on neural circuits. *Cancer Cell* 41, 1637-1649.e11. (**) Shared authorship. (*) Corresponding author.

Journal cover.
See also:
· *Cancer Cell*
  DOI: https://doi.org/10.1016/j.ccell.2023.07.009 (30 August 2023).
· *Cancer Discovery*
  DOI: https://doi.org/10.1158/2159-8290.CD-RW2023-144 (8 September 2023).
· *Nature Reviews Cancer*
  DOI: https://doi.org/10.1038/s41568-023-00630-1 (27 September 2023).
· *"Highlights in Cancer Research"* by EACR (In press).

▸ Valiente M*, Ortega-Paino E* (2023). Updating cancer research with patient-focused networks. *Trends Cancer*. PMID: 37802739. (*) Corresponding author.
▸ Valiente M*, Sepúlveda JM*, Pérez A* (2023). Emerging targets for cancer treatment: S100A9/RAGE. *ESMO Open* 8, 100751. (*) Corresponding author.
▸ Zhu L, Miarka L, Baena P, Perea-García M, Valiente M* (2023). Protocol to generate murine organotypic brain cultures for drug screening and evaluation of anti-metastatic efficacy. *STAR Protoc* 4, 102194.

(*) Corresponding author.
▸ Ocaña-Tienda B, Pérez-Beteta J, Jiménez-Sánchez J, Molina-García D, Ortiz de Mendivil A, Asenjo B, Albillo D, Pérez-Romasanta LA, Valiente M, Zhu L, García-Gómez P, González-Del Portillo E, Llorente M, Carballo N, Arana E, Pérez-García VM (2023). Growth exponents reflect evolutionary processes and treatment response in brain metastases. *NPJ Syst Biol Appl* 9, 35.
▸ Zheng D, Pisano F, Collard L, Balena A, Pisanello M, Spagnolo B, Mach-Batlle
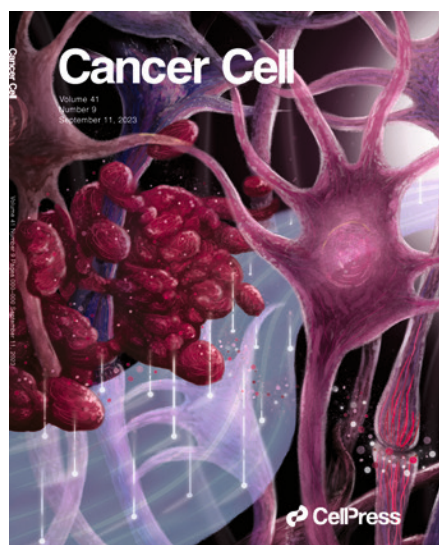
FIGURE 1 Cover of *Cancer Cell* depicting metastatic cancer cells colonising the brain (red mass). The transcriptomic profile of the cancer cells suggests that a molecular programme rather than or in addition to the mass effect may be a critical player. Metastatic cells affect neuronal activity by specific paracrine crosstalk, conceptualised as dropping signals. This in turn results in alterations of microcircuit operation (white trail).
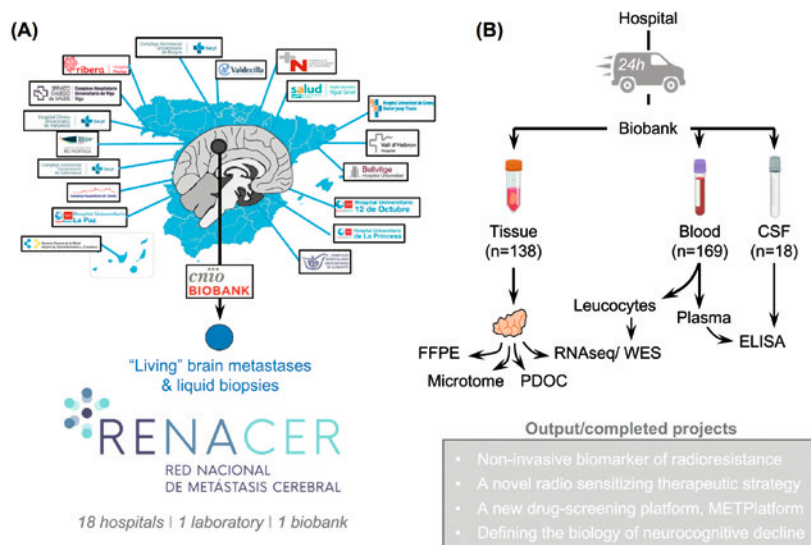
FIGURE 2 RENACER as a nation-wide effort to generate human brain metastases samples. CSF, cerebrospinal fluid; FFPE, formalin-fixed, paraffin-embedded; WES, whole exome sequencing.

R, Tantussi F, Carbone L, De Angelis F, Valiente M, de La Prida L, Ciracì C, De Vittorio M, Pisanello F (2023). Toward plasmonic neural probes: SERS detection of neurotransmitters through gold nanoislands-decorated tapered optical fibers with sub-10 nm gaps. *Adv Mater* 35, e2200902.
Journal cover.

▸ **PATENT**

▸ Zhu L, Graña-Castro O, Salgado-Crespo I, Valiente M (2023). Signature for the prognosis of brain metastasis relapse. *EP3382693.2.*

▸ **AWARDS AND RECOGNITION**

Manuel Valiente:
▸ Best Publication Award, *Cátedra UB-Atrys de Radioterapia Personalizada*, Spain.
▸ XVIII *Banco Sabadell* Award for biomedical research, Spain.
▸ *GEPAC* Award to *RENACER* (National Network of Brain Metastasis), Spanish Group of Cancer Patients (*GEPAC*).
▸ CaixaResearch Health 2023 (Individual project).
▸ AstraZeneca Research Grant 2023.
▸ Mariam Al-Masmudi Martín: *AECC* Postdoctoral Fellowship, Spain; selected oral presentation at the Heidelberg Conference on Cancer Neuroscience (Germany).
▸ Irene Salgado: *AECC* and *FPU* PhD Fellowships, Spain.
▸ Pedro García-Gómez: selected oral talks at the AACR Meeting in Orlando (USA), Keystone Symposia on Metastasis in Vancouver (Canada), and CNIO Frontiers Meeting "Metastasis" in Madrid (Spain). Thesis defence (April 2023).
▸ Neibla Priego: selected oral talks at the Keystone Symposia "The Resistant Tumor Microenvironment" in Vancouver (Canada), and CNIO Frontiers Meeting "Metastasis" in Madrid (Spain).

ORGAN CROSSTALK IN METABOLIC DISEASES GROUP

Guadalupe Sabio (since November)
Group Leader

Research Scientist
Alfonso Mora

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 these alterations occurring in obesity disrupt the communication between organs. In this context, we have found that during obesity several stress kinases are activated in different tissues, and that this activation can affect the development of a tumour.

Post-Doctoral Fellows
Alba C. Arcones, Cintia Folgueira, Rafael Romero, Ayelén Santamans

Gradutate Students
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Technicians
Luis Leiva (TS)*, Marta León, María Elena Rodríguez

*Titulado Superior (Advanced Degree)

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(Master's Thesis, *Universidad Autónoma de Madrid*, Spain)

Visiting Scientist
Magdalena Leiva (*Universidad Autónoma de Madrid*, Spain)

RESEARCH HIGHLIGHTS

The Group focuses on 3 main aspects induced by obesity:

1. *The alteration of adipose tissue and, consequently, the secretion of adipokines.* We have observed that in adipose tissue, during obesity, stress kinases are activated, the mitochondria become dysfunctional, and the circadian clock is altered. Our aim is to delve into whether these factors could serve as drivers of adipose tissue dysfunction during obesity and the associated comorbidities. The primary focus of our investigation lies in unravelling the endocrine function of adipose tissue, with particular attention to the distinctive role of brown adipose tissue in thermogenesis.

2. *The onset of chronic inflammation, which is associated with an increased risk of cancer.* We want to comprehend how stress kinases and alterations in metabolism within inflammatory cells impact the development of the disease.

3. *Cell metabolism alteration as a driver of disease.* Metabolism controls the functionality of cells in our body, as it is their means of obtaining ATP to carry out their functions or, in the case of tumours, to proliferate. Therefore, disruptions in cellular metabolism can serve as drivers of diseases, but the modulation of metabolism can also offer new therapies for cardiovascular diseases and cancer. By utilising animal models to manipulate metabolism, we aim to understand how metabolism is implicated in the development of diseases.

Our main research areas are:

→ Organ Interaction and Health-Related Disorders.
→ Adipose Tissue Dysfunction in Pathological Progression.
→ Chronic Inflammation and Cancer Association.
→ Cell Metabolism as a Driver of disease. ■
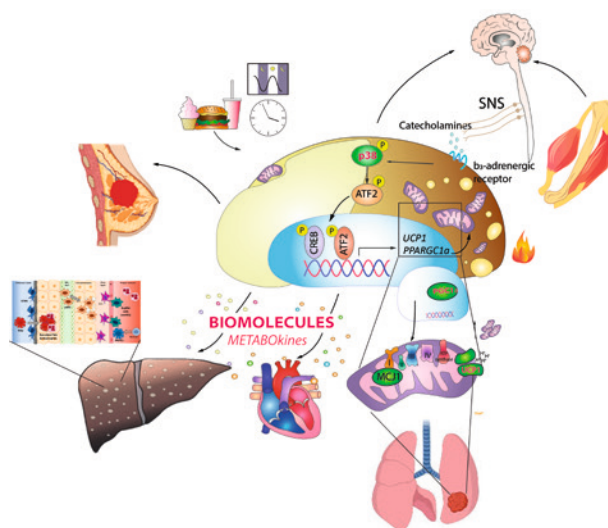
FIGURE 1 Schematic overview of our laboratory research.

▸ **PUBLICATIONS AT OTHER INSTITUTIONS**

▸ Romero-Becerra R, Cruz FM, Mora A, ..., León M, Rodríguez M E, Leiva-Vega L, ..., Jalife J, Sabio G (2023). p38γ/δ activation alters cardiac electrical activity and predisposes to ventricular arrhythmia. *Nat Cardiovasc Res* 2, 1204–1220.
▸ Crespo M, Nikolic I, Mora A, Rodríguez E, Leiva-Vega L, Pintor-Chocano A, ..., Leiva M, Sabio G (2023). Myeloid p38 activation maintains macrophage-liver crosstalk and BAT thermogenesis through IL-12-FGF21 axis. *Hepatology* 77, 874-887.
▸ Goikoetxea-Usandizaga N *et al.* (incl. Sabio G) (2023). The outcome of boosting mitochondrial activity in alcohol-associated liver disease is organ-dependent. *Hepatology* 78, 878-895.
▸ Ferreira V (2023). Hypothalamic JNK1-hepatic fatty acid synthase axis mediates a metabolic rewiring that prevents hepatic steatosis in male mice treated with olanzapine via intraperitoneal: Additional effects of PTP1B inhibition. *Redox Biol* 63, 102741.
▸ Fernández-Regueras M *et al.* (incl. Sabio G) (2023). Predominantly pro-inflammatory phenotype with mixed M1/M2 polarization of peripheral blood classical monocytes and monocyte-derived macrophages among patients with excessive ethanol intake. *Antioxidants (Basel)* 12, 1708.
▸ Ahmed O *et al.* (incl. Sabio G) (2023). Hepatic stellate cell activation markers are regulated by the vagus nerve in systemic inflammation. *Bioelectron Med* 9, 6.

▸ **AWARDS AND RECOGNITION**

▸ *Carmen y Severo Ochoa* Award in Molecular Biology, *Fundación Carmen y Severo Ochoa*, Spain.
▸ *Premio Extremadura en Getafe* Science Award, *la Casa Regional de Extremadura en Getafe*, Spain.
▸ "Mujeres que inspiran", Women Space Extremadura, Spain.
▸ *Juan Florencio Macias Nuñez* Award to the best scientific trajectory, *cátedra extraordinaria de la fundación FIIPERVA*, Spain.

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Staff Scientist
Susana García

Post-Doctoral Fellows
Marta Hergueta, Laura Nogués

Gradutate Students
Enrique Bastón, Juan García-Agulló, Alberto Hernández (until June)

OVERVIEW

Our Laboratory focuses on elucidating the crosstalk between tumours and their microenvironment throughout metastatic progression. Currently, we are examining the relationship between extracellular vesicles (EVs) and DNA damage responses (DDR). This project aims to uncover the potential role of EVs in controlling intrinsic/extrinsic DDR and their impact on tumour evolution and metastasis. Additionally, we are investigating the influence of obesity on pre-metastatic niche formation in breast cancer, with a specific emphasis on its adverse outcomes in triple-negative breast cancer patients. This study will help us to understand the relationship between obesity and the progression of breast cancer. Lastly, we are analysing the role of the nerve growth factor receptor (NGFR) in metastasis and therapy resistance in melanoma and head and neck cancer. We are interested in exploring the use of

"We are investigating the dynamic interplay between systemic factors and the tumour microenvironment in metastatic progression and therapy resistance."

NGFR inhibitors in combination with immunotherapy and chemotherapy to enhance therapeutic responses and reduce metastasis.

Technicians
Sara Sánchez-Redondo,
Vanesa Santos

Students in Practice
Jan Hochstadt (until August)
(Master's Thesis, *Univ. Autónoma de Madrid*, Spain), Noelia Lara (March-June) (*IES Jaime Ferrán Clúa*, San Fernando de Henares, Spain)

Visiting Scientist
María Mateos (February-April)
(*Complexo Hospitalario Universitario de Santiago de Compostela*, Spain)

RESEARCH HIGHLIGHTS

Exploring the link between EVs and DNA damage responses (DDR). EVs secreted by tumour cells contain a broad variety of biomolecules including DNA. Notably, we have identified molecules related to DDR within tumour-secreted EVs. Intriguingly, tumour-secreted EVs extrinsically impact DDR, leading to an increase in surrounding cells. Our project is dedicated to analysing the potential role of EVs in controlling DDR and their influence on tumour evolution and metastasis.

Impact of obesity in breast cancer pre-metastatic niche formation. Obesity, particularly in postmenopausal women and patients with triple-negative breast cancer (TNBC), is linked to unfavourable outcomes. Beyond its role in primary tumour initiation and growth, we found that obesity significantly influences metastasis. Our research is investigating the correlation between obesity, coagulation, and the formation of pre-metastatic niches in TNBC. Notably, a high-fat diet activates platelets and induces vascular leakiness, promoting tumour cell homing and metastasis (FIGURE 1). Additionally, we are exploring the correlation of body mass index, coagulation parameters, and disease outcomes in TNBC patients.

FIGURE 1 High-fat diet (HFD) promotes vascular leakiness, metastasis and tumour-platelet interaction. The panels, arranged from left to right, present diverse experimental setups: (**Left**) Immunofluorescence of tumour cells (highlighted in red) incubated with mouse platelets (depicted in green) obtained from mice on a normal diet (up) and HFD-fed mice (bottom). Intravital imaging acquired 1 hour after tail vein injection of GFP-labelled tumour cells (green) and platelets (red) from ND mice (top) and HFD-fed mice (bottom). Analysis of vascular permeability in lungs after Cy7-Dextran injection in lungs of ND (top) and HFD-fed mice (bottom). (**Right**) Bioluminescence imaging of the tumour cell homing assay in ND mice (top) and HFD-fed mice (bottom).

Deciphering the role of NGFR in metastasis and resistance to therapy. The nerve growth factor receptor (NGFR) has been involved in therapy resistance and metastasis in melanoma. We postulate that therapies against NGFR will not only enhance therapeutic responses but also reduce metastasis. We are currently investigating the use of NGFR inhibitors as anti-metastatic drugs and the combination with immunotherapy in melanoma. Moreover, we are studying the significance of NGFR in tumour development and metastasis in head and neck cancer, aiming to define its relevance in chemotherapy resistance. ∎

▸ **PUBLICATIONS**

▸ González-Muñoz T, Di Giannatale A, García-Silva S, Santos V, Sánchez-Redondo S, Savini C, Graña-Castro O, Blanco-Aparicio C, Fischer S, De Wever O, Creus-Bachiller E, Ortega-Bertran S, Pisapia DJ, Rodríguez-Peralto JL, Fernández-Rodríguez J, Pérez-Portabella CR, Alaggio R, Benassi MS, Pazzaglia L, Scotlandi K, Ratner N, Yohay K, Theuer CP, Peinado H (2023). Endoglin, a novel biomarker and therapeutical target to prevent malignant peripheral nerve sheath tumor Growth and metastasis. *Clin Cancer Res* 29, 3744-3758.

▸ Benito-Martin A, Nogués L, Hergueta-Redondo M, Castellano-Sanz E, Garvin E, Cioffi M, Sola-Castrillo P, Buehring W, Ximénez-Embún P, Muñoz J, Matei I, Villanueva J, Peinado H (2023). Mast cells impair melanoma cell homing and metastasis by inhibiting HMGA1 secretion. *Immunology* 168, 362-373.

▸ García-Silva S, Vico-Alonso C, Meyer L, Enderle D, Sanchez JA, Onteniente MDM, Noerholm M, Skog J, Rodríguez-Peralto JL, Ortiz-Romero PL, Peinado H (2023). Improved sensitivity in BRAFV600E detection in combined tissue and extracellular vesicle-based liquid biopsy in melanoma. *J Invest Dermatol* 143, 1606-1610.

▸ Burdiel M, Jiménez J, Rodríguez-Antolín C, García-Guede Á, ... Peinado H, Vera O, de Castro J, Ibáñez de Cáceres I (2023). MiR-151a: a robust endogenous control for normalizing small extracellular vesicle cargo in human cancer. *Biomark Res* 11, 94.

▸ Pericoli G *et al.* (incl. Peinado H) (2023). Inhibition of exosome biogenesis affects cell motility in heterogeneous sub-populations of paediatric-type diffuse high-grade gliomas. *Cell Biosci* 13, 207.

▸ Casadomé-Perales Á *et al.* (incl. Peinado H) (2023). Neuronal prosurvival role of ceramide synthase 2 by olidogendrocyte-to-neuron extracellular vesicle transfer. *Int J Mol Sci* 24, 5986.

▸ Couto-Rodriguez A *et al.* (incl. Peinado H) (2023). Platelet-derived extracellular vesicles as lipid carriers in severe allergic inflammation. *Int J Mol Sci* 24, 12714.

▸ González MI, ... Peinado H, Desco M, Salinas B (2023). Isolation of goat milk small extracellular vesicles by novel combined bio-physical methodology. *Front Bioeng Biotechnol* 11, 1197780.

▸ Ruiz-Llorente L, ... Peinado H, Bernabeu C (2023). Correlation between endoglin and malignant phenotype in human melanoma cells: analysis of hsa-mir-214 and hsa-mir-370 in cells and their extracellular vesicles. *Adv Exp Med Biol* 1408, 253-272.

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, Elena Fernández, Ana Belén Plata (until Oct.), Elena Sánchez

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 from the nutritional state of the entire organism. Integration of these signals is key for 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 drivers of the 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 are combining mouse genetics and cell biological tools to gain insight into the genetic and environmental corruptions of nutrient signalling cascades,

"We have found that the fluctuations in nutrient levels that occur with feeding cycles after birth operate as a GPS for the mammalian liver, and this spatial information is blurred in liver disease."

aiming to conceive therapeutic interventions in the context of cancer, obesity, and the process of ageing.

Technicians
María Cuerda (since March), Jon Lasa (since November), Alba Sanz

Titulado Superior (Advanced Degree)

Students in Practice
Paula Seghers (until May) (Undergraduate Student, *Universidad Politécnica de Madrid*, Spain), Andrea Pinilla (until March) (Undergraduate Student,

Universidad Industrial de Santander, Colombia)

Visiting Scientists
Cristina Lebrero (*Universidad de Valladolid*, Spain), Ana Ortega

(*Centro de Biología Molecular Severo Ochoa*, *CBMSO*, Madrid, Spain), Sebastian Thompson (*IMDEA Nanociencia* Institute, Madrid, Spain)

RESEARCH HIGHLIGHTS

The metabolic functions of the liver (control of circulating levels of glucose, lipids, and amino acids) are organised spatially in a phenomenon known as zonation. This zonation has most likely evolved in all mammals because some groups of hepatocytes are exposed to high and low levels of nutrients following feeding cycles, with others less so, and segregating these functions makes the liver operate more efficiently, just like the assembly line of a factory. The presence of cellular nutrients, such as amino acids and glucose, and systemic metabolic hormones such as insulin, are key regulators of liver functions by controlling the mTOR kinase, a master switch for cellular metabolism. We have generated mice with liver cells incapable of detecting drops in nutrient and insulin levels, hence with mTOR always active in the liver, regardless of mice being fed or fasted. We found that the inability of liver cells to respond to fluctuations in nutrients and insulin has no consequences during the formation of the liver *in utero* but impairs the establishment of the metabolic zonal identity of hepatocytes, a maturation process that takes place within the first weeks after birth. This finding suggests that the fluctuations in nutrient and hormonal signalling are a trigger for neonates to segregate liver functions only when needed: after birth and with the start of oral feeding cycles. At the molecular level, constitutive mTOR signalling impairs the action of the most important cue that controls zonation: Wnt/β-catenin. Importantly, the lack of postnatal establishment of metabolic zonation in the liver was recapitulated in a model of constant supply of nutrients by total parenteral nutrition in neonatal pigs. Collectively, our work shows a critical role of fluctuating nutrient levels in triggering the metabolic maturation of the mammalian liver and its functions. ■
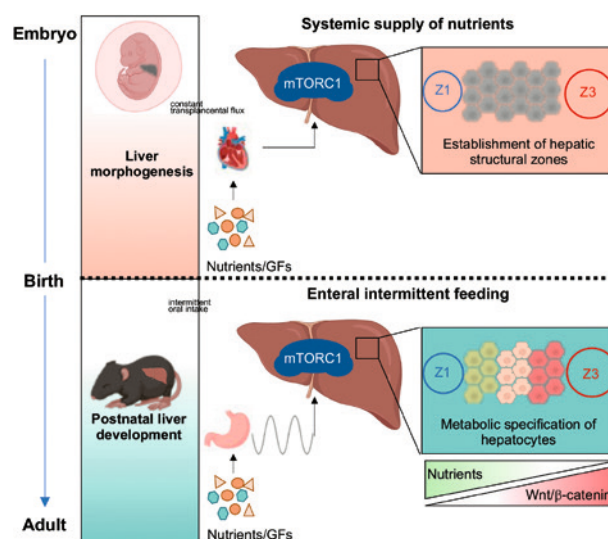
FIGURE 1 The liver is formed during embryonic development, but the segregation of metabolic functions is neither required nor established until after birth. With the start of feeding intermittency, fluctuations in nutrient and hormone signalling to mTOR instruct the maturation of liver zonation and the spatial segregation of liver functions.

▸ **PUBLICATIONS**

▸ Deleyto-Seldas N, Efeyan A (2023). AMPK knocks at the gate of GATOR. *Nat Metab* 5, 197-198.

▸ Kobialka P, Llena J, Deleyto-Seldas N, Munar-Gelabert M, Dengra JA, Villacampa P, Albinyà-Pedrós A, Muixi L, Andrade J, van Splunder H, Angulo-Urarte A, Potente M, Grego-Bessa J, Castillo SD, Vanhaese-broeck B, Efeyan A, Graupera M (2023). PI3K-C2β limits mTORC1 signaling and angiogenic growth. *Sci Signal* 16, eadg1913.

▸ García-Lizarribar A, Villasante A, Lopez-Martin JA, Flandez M, Soler-Vázquez MC, Serra D, Herrero L, Sagrera A, Efeyan A, Samitier J (2023). 3D bioprinted functional skeletal muscle models have potential applications for studies of muscle wasting in cancer cachexia. *Biomater Adv* 150, 213426.

▸ **AWARDS AND RECOGNITION**

▸ Yurena Vivas was recipient of a Marie Skłodowska-Curie Actions Individual Fellowship (MSCA-IF) from the European Union.

▸ Ana Belen Plata, excellent *Cum Laude* PhD Thesis defense in Molecular Biosciences, Autonomous University of Madrid, Spain.

▸ Elena Fernandez received a *CAM* PhD Fellowship from the Community of Madrid, Spain.

CANCER IMMUNITY JUNIOR GROUP

María Casanova-Acebes
Junior Group Leader

Research Scientist
Alba de Juan

Post-Doctoral Fellow
Sarai Martínez (since September)

Gradutate Students
Eduardo Garvín, Mariola Munárriz,
Jan Hochstadt (since December)

OVERVIEW

Myeloid cells are abundant in solid tumours. While their heterogeneity has been widely described, efficient ways of manipulating these cells are scarce. My laboratory focuses on the identification and therapeutic targeting of myeloid checkpoint programmes in cancer. By studying the microenvironment in which lung, ovarian, and breast cancer emerge, we examine how macrophages crosstalk with the stroma and how they modulate their malignant conversion into cancer-associated fibroblasts. As metastasis is the major cause of death in breast cancer, we explore the mechanisms by which neutrophils exacerbate metastasis malignancy. Lastly, understanding how tissue physiology is perturbed in cancer is key to investigating novel ways to modulate anti-tumour immunity: we do this by exploring the circadian biology of immune responses, interrogating how our diet modifies the

"Myeloid cells are not inherently tumour-supportive or tumour-opposing. Rather, cells from this compartment are highly plastic and their activity depends on the balance of signals within the tumour."

lipid metabolism of macrophages, and mining the dysfunctional properties of bone marrow-derived haematopoiesis.

Technician
Mónica Gómez

Bioinformatician
Gonzalo Soria (until July)

Student in Practice
María Nogales (until June) (Master's Student, *Universidad Complutense de Madrid*, Spain)

Visiting Student
Sandra Barrantes (January-July) (Harper Adams University, Newport, Shropshire, UK)

RESEARCH HIGHLIGHTS

During 2023, my Group successfully incorporated 3 new Ph.D. students: Mariola Munárriz and Eduardo Garvín, funded by "Severo Ochoa" Excellence and *Retos del Conocimiento* programmes, respectively; and Jan Hoschstad, funded by "La Caixa" Foundation Health Research Grant. Their projects are starting to bloom, and their work has already been presented at national symposia (*ASEICA* 40th Anniversary Meeting and the Annual *Sociedad Española de Inmunología* Congress). In addition, a new lab manager (Mónica Gómez) and 2 postdoctoral fellows joined the team: Alba de Juan (funded as a *Ramón y Cajal* Junior Fellow) and Sarai Martínez Pacheco (funded by the H2020 Transcan Network).

Ovarian tumours are massively infiltrated by macrophages, yet their origin and expansion remain unexplored. Clonal haematopoiesis is known to confer the advantageous expansion of certain haematopoietic stem or progenitor clones, and its presence correlates with increased inflammatory output from mutated cells. Interestingly, tumours in which WT and Tet2-deficient clones cohabit display increased macrophage numbers, as opposed to macrophages derived from a fully WT haematopoietic environment (FIGURE 1A-B). These macrophages full of lipids (or *lipid-laden macrophages* [LLM]) expressed higher levels of immunosuppressive molecules (PDL1, Arg1, among others) and a significant expansion at the tumour site. Given these observations, we are currently modulating an LLM immunosuppressive phenotype using different diets and combining this with immune checkpoint blockade (ICB). Interestingly, a high-fat diet turns ovarian Tet2-ICB-resistant tumours into ICB-sensitive. We are intrigued by this result and are currently exploring how this occurs mechanistically.

During 2023, we also obtained funding from the European Research Council (ERC StG2021). In this project, we are uncovering the mechanisms by which circadian rhythms of the immune system become dysfunctional in tumours. Our goal is
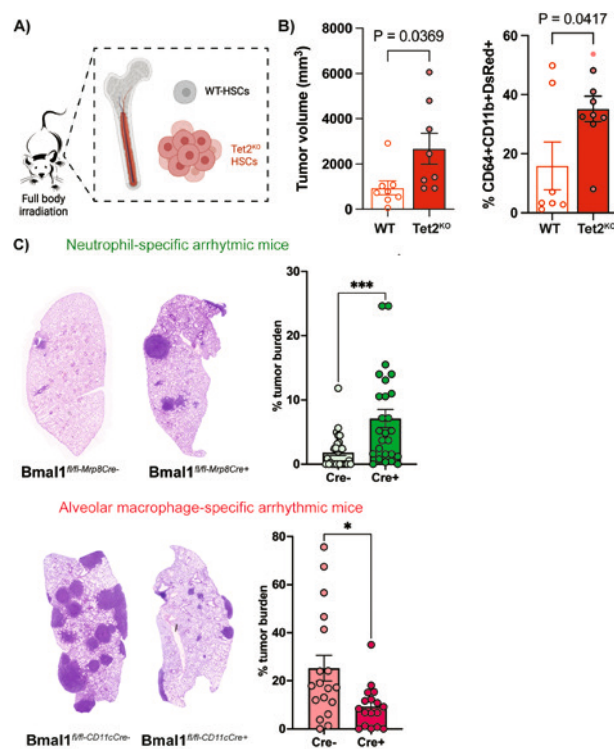
FIGURE 1 (**A**) Experimental layout for expanded haematopoietic clones in WT: Tet2[KO] mixed chimeras. (**B**) Tumoral burden and macrophage infiltrate in Upk10 ovarian tumours in WT and Tet2 chimeras. (**C**) Opposing anti-tumoral and pro-tumoral BMAL1-controlled programmes in tumour-associated myeloid cells (upper: tumoral lungs derived from neutrophil BMAL1-deficient mice; lower: tumoral lungs from macrophage BMAL1-deficient mice).

to understand ways to design *time-based* therapies to harness anti-tumour immunity and block time-dependent immune suppression. Interestingly, mice lacking circadian modulation in neutrophils and macrophages showed opposing trends when controlling tumour growth (FIGURE 1C), suggesting that BMAL1 controls specific programmes in particular subsets of myeloid cells. ∎

▸ **AWARDS AND RECOGNITION**

María Casanova-Acebes:
▸ Young Investigator Award, European Macrophage and Dendritic Cell Society.
▸ 2023 AstraZeneca Young Researcher Award, AstraZeneca Foundation, Spain.

▸ Academic (*Académica de Número*), *Academia Joven de España* (*AJdE*), Spain.

STRUCTURAL BIOLOGY PROGRAMME
ÓSCAR LLORCA Programme Director

The Programme's research areas and strategic goals

When a new therapy hits the headlines, it is often forgotten that most of these advances would never have happened without previous discoveries about the fundaments of the biological processes that influence cancer. The main mission of the Structural Biology Programme (SBP) is precisely that, enhancing our comprehension of the fundamental processes governing the origin and progression of cancer, using the immense opportunities provided by advances in structural and computational biology that are revolutionising cancer research. In addition, we design tools that can ultimately benefit patients. SBP comprises 5 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 to support drug discovery efforts. It comprises 3 Groups (Macromolecular Complexes in DNA Damage Response; Kinases, Protein Phosphorylation and Cancer; Genome Integrity and Structural Biology) and 4 Units (Electron Microscopy (EM); Spectroscopy and Nuclear Magnetic Resonance (NMR); Protein Crystallography; Protein Production). The strategic line in computational and cancer genomics consists of 2 Groups (Computational Cancer Genomics and Computational Oncology) and 1 Unit (Bioinformatics). Bioinformatics, computational biology, and cancer genomics are used to better understand the complexity of cancer, predict therapy responses, design new therapeutic strategies, and develop new tools. The Units at SBP provide support in cryoEM, NMR, protein purification, protein crystallography, bioinformatics and biophysics to all CNIO researchers, particularly to groups outside SBP that do not have the sufficient expertise in these methodologies.

Summary of milestones & major achievements

In 2023, the CNIO external Scientific Advisory Board (SAB) visited the CNIO and reviewed the Programme. We had the opportunity to present our research and future vision, which was recognised and appreciated by the SAB. In 2023, CNIO opened a call to recruit a new Junior Group Leader working on cryo-electron microscopy and structural biology. Outstanding candidates were evaluated by an ad-hoc panel of experts. We are very happy that Lucas Tafur, from Geneva University, will join our faculty in 2024 to study the structural

> **"Our research generated knowledge on fundamental processes in cancer and the proteins involved, and we developed tools to guide the selection of personalised cancer treatment."**

basis of mTORC1 activation and regulation. Scientists at SBP also made interesting discoveries in several areas of cancer research during 2023. The Kinases, Protein Phosphorylation and Cancer Group provided functional and structural evidence for a new mechanism regulating c-Src tyrosine kinase that could be potentially exploited to design c-Src inhibitors as anti-cancer drugs. The Macromolecular Complexes in DNA Damage Response Group, in collaboration with scientists at *CNB-CSIC* and *CIMA*, discovered that a long non-coding RNA named NIHCOLE, expressed by the hepatocellular carcinoma cells of patients with bad prognosis, provides an advantage to cancer cells by promoting the synapsis of 2 broken DNA ends and enhancing the efficiency of the DNA repair machinery. The Computational Cancer Genomics Group, in collaboration with Korean researchers, conducted a study that found a new type of cancer predisposition genes, and discovered the relationship of the PAH gene with lung and liver cancers. The Computational Oncology Group studied and discussed the existence of multiple mechanisms of resistance within individual patients with ovarian cancer and how this is a challenge for the treatment of the disease in advanced stages. The Bioinformatics Unit developed new tools to help in the interpretation of the long lists of tumour alterations detected in cancer patients and to guide during the selection of personalised treatment. The Unit also contributed extensively to the work of many CNIO groups, for example helping to use machine learning methods to identify subtypes of brain metastasis. The Protein Crystallography Unit contributed to the study of c-Src kinase and to the characterisation of neutralising antibodies that boost cytotoxic T cell responses. The Spectroscopy and NMR Unit helped with research regarding DLST mutations in pheochromocytoma and paraganglioma. The Electron Microscopy and the Protein Purification Units also contributed to the work of various groups in the SBP and the CNIO. ∎

MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Óscar Llorca
Group Leader

Research Scientists
Javier Coloma, Ana Isabel
Hernández, María Ibarra,
Andrés López, Ángel Rivera,
Marina Serna

Post-Doctoral Fellows
Sofía Cabezudo (until May),
Ana González, María Martínez

Gradutate Students
Natalia Cuervo (until October),
Carmen García, Nayim González

Student in Practice
Celia Varela (since February)
(Bachelor's Degree Final Project,
*Universidad Autónoma de Madrid;
AECC*, Spain)

Visiting Student
Aarón Terán (September-November)
(*Universidad Complutense de Madrid*,
Spain)

Visiting Scientist
Evangelina Nogales (September-
October) (Lawrence Berkeley
National Lab, USA)

OVERVIEW

Our Group uses cryo-electron microscopy (cryoEM) 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 then used to propose how these molecules work and increase our understanding of the molecular basis of cancer. Most of our efforts are currently focused on 2 major areas of research: i) the study of chaperones essential for the activation of several macromolecular complexes relevant in cancer such as mTORC1; and ii) 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 structure and mechanisms of several amino acid transporters, as well as the mechanisms that regulate microtubule nucleation for the assembly of the mitotic spindle.

"We have discovered a mechanism used by aggressive forms of hepatocellular carcinoma to repair broken DNA using a long non-coding RNA. Modulation of this RNA could serve as a therapeutic opportunity."

RESEARCH HIGHLIGHTS

Long non-coding RNA NIHCOLE regulates NHEJ in hepatocellular carcinoma

DNA double-strand breaks (DSBs) contribute to genomic instability and cancer development. Non-homologous end joining (NHEJ) is one of the main pathways to repair DSBs. NHEJ can also be exploited to sensitise cancer cells to treatments that induce DNA damage such as radiation.

The formation of a bridge (synapsis) between the 2 ends of a broken DNA is an essential step during the initiation of the repair of DSBs by the NHEJ pathway. Synapsis is facilitated by a multi-component protein complex built around the ring-shaped Ku70-Ku80 heterodimer, and it is regulated by several accessory factors using mechanisms still poorly understood. We investigated the roles of aprataxin-and-PNK-like factor (APLF) and the long non-coding RNA (lncRNA) NIHCOLE in NHEJ, as part of a research consortium involving a close collaboration with the groups headed by F. Moreno-Herrero at the *CNB-CSIC* in Madrid and P. Fortes at *CIMA* in Pamplona.

Our most significant finding has been to discover the mechanism by which lncRNA NIHCOLE provides an advantage to hepatocellular carcinoma (HCC) cells (FIGURE 1). HCC is a leading cause of liver cancer-related deaths worldwide. NIHCOLE is not a protein synthesised by a gene, but a long RNA molecule that does not code for a protein.

We discovered that NIHCOLE interacts with Ku70-Ku80 at both ends of the broken DNA, promoting DNA-end synapsis. Our model is that NIHCOLE serves as a glue that strengthens the bridge between the 2 pieces of the broken DNA, and this increases the efficiency of DNA repair by the NHEJ pathway. Interestingly, NIHCOLE is overexpressed and linked to poor prognosis and reduced survival rates in HCC patients. Depleting lncRNA NIHCOLE from HCC cells leads to the accumulation of DNA damage due to a specific reduction in the activity of the NHEJ pathway. We propose that the combination of DNA damaging agents, such as radio- or chemotherapy, with the modulation of NICHOLE could serve as a therapeutic opportunity for HCC.

Molecular architecture of the telomeric CST complex

Telomeres are structures found at the ends of linear chromosomes in eukaryotes. They consist of DNA repeats bound by multiple protein complexes, and they play a crucial role in preventing chromosome ends from being recognised as double-strand breaks by the cellular DNA repair machinery and from shortening during genome replication. The regulation
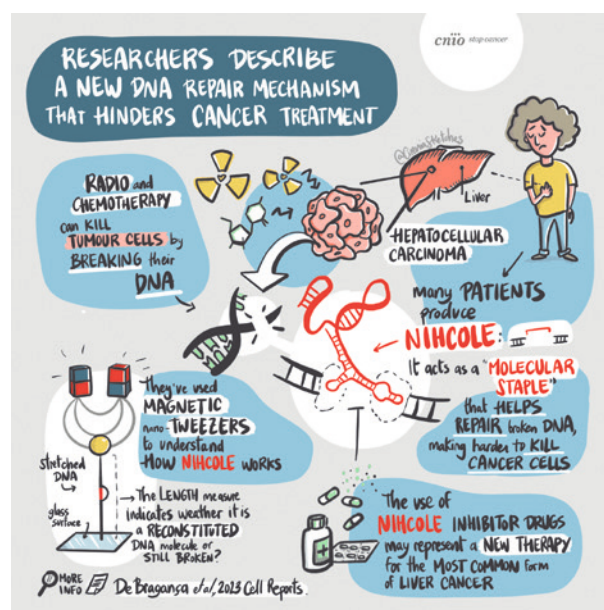
FIGURE 1   Illustration summarising the roles of lncRNA NIHCOLE in hepatocellular carcinoma and their potential implications in cancer treatment, describing also how the experiments were performed. This work is part of a collaboration with F. Moreno-Herrero (*CNB-CSIC*, Madrid) and P. Fortes (*CIMA*, Pamplona).

and maintenance of telomeres are essential pathways for preventing genomic instability associated with cancer and ageing.

One of our goals is to uncover the mechanism and structural basis of the CST complex in the homeostasis of telomeres. CST participates in regulating telomere extension and is also required to recruit DNA polymerase α-primase for converting part of the extended tail to dsDNA (C-strand fill-in). We are comparing the structure and function of complexes in humans and yeast to uncover core elements conserved across species, while discriminating adaptations specific for humans.

We have elucidated the molecular organisation of Cdc13, one of the components of the CST complex in the yeast *Candida glabrata*, using several biochemical and biophysical techniques (FIGURE 2). Cdc13 binds to the telomeric G-overhang during the G1 phase and recruits telomerase during progression into S-phase. We have found that Cdc13 forms a dimeric structure that requires dimerization of the OB2 domain. Dimerization enhances binding to telomeric sequences leading to the unfolding of ssDNA. Once bound to DNA, Cdc13 prevents the refolding of ssDNA through mechanisms involving all its
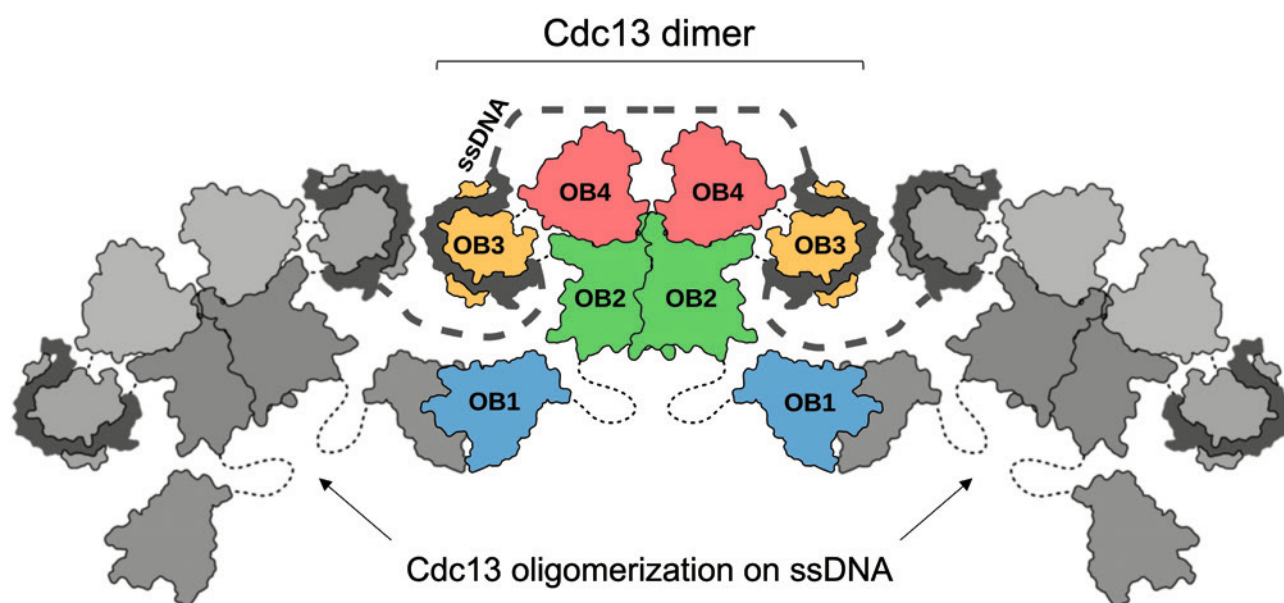
FIGURE 2 Model of the architecture *C. glabrata* Cdc13. Cdc13 forms a dimeric structure that enhances binding to telomeric sequences. Cdc13 oligomerises on ssDNA using the OB1 domain, and this oligomerisation unfolds the secondary structure of ssDNA.

domains. We propose the first detailed model for the molecular architecture of Cdc13 and how its structural organisation affects ssDNA binding. Our results suggest that the molecular architecture of CST varies between yeast and humans, as well as between different yeast species, but that some structural and functional elements are conserved. ∎

‣ **PUBLICATIONS**

‣ Coloma J*, Gonzalez-Rodriguez N, Balaguer FA, Gmurczyk K, Aicart-Ramos C, Nuero ÓM, Luque-Ortega JR, Calugaru K, Lue NF, Moreno-Herrero F*, Llorca O* (2023). Molecular architecture and oligomerization of *Candida glabrata* Cdc13 underpin its telomeric DNA-binding and unfolding activity. *Nucleic Acids Res* 51, 668-686. *Co-corresponding authors.

‣ Gallisà-Suñé N, Sànchez-Fernàndez-de-Landa P, Zimmermann F, Serna M, Regué L, Paz J, Llorca O, Lüders J, Roig J (2023). BICD2 phosphorylation regulates dynein function and centrosome separation in G2 and M. *Nat Commun* 14, 2434.

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KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellow
Julia M. Contreras

Gradutate Student
Ana Martín-Hurtado (until September)

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 (*Universidad Tecnológica Equinoccial - Fundación Carolina,* Quito, Ecuador)

Students in Practice
Francesco Maggiore (May-September) (Erasmus, University of Pavia, Italy), Laura V. Morantes (March-July) (*Universidad Industrial de Santander,* Colombia), Pilar Pérez (until May)

(*Universidad de Jaén,* Spain), Marina Rodríguez (until July) (Master's Thesis, *Universidad Autónoma de Madrid,* Spain), Alba Valle (March-June) (*Instituto Técnico de Estudios Profesionales*, Spain)

RESEARCH HIGHLIGHTS

Our main strategic lines are:

1. *Structural and molecular determinants that control protein 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 recently proved 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 (FIGURE 1) 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 HN and Contreras J *et al.*, *Nature Commun* 2023). This work is fundamental for the design and development of next generation tyrosine kinase inhibitors targeting allosteric and non-catalytic functions for targeted and personalised cancer therapies (see point 3). In a follow up study, we undertook a machine learning (AI) approach to identify aa sequence divergence (i.e., constrains) as markers for key determinants that control and/or regulate c-Src kinase function and druggability. Following this approach, we identified key residues that are highly divergent and that control the catalytic activity and function of c-Src, and we are currently performing a detailed structural and molecular characterisation of the regulatory mechanism.

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 dissecting the functional and structural determinants for several RET oncogenic fusion products. We have several 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 structural 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 (Martin-Hurtado *et al.* submitted, and Contreras *et al.* in preparation).

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. Our current paradigm is based on second generation RET inhibitors LOXO-292 and BLU-667, and last generation c-Src inhibitor TPX-0046, a poly-pharmacological compound that is able to inhibit RET as well. We are applying an integrated approach combining structural data, molecular docking, structure-guided molecular dynamics simulations, and screening with both virtual and chemical libraries, together with biophysical and biochemical tools for binding and functional validation. Following this approach, we identified an allosteric interface in RET with a good druggability score that can be potentially targeted with allosteric inhibitors. Furthermore, we found a cryptic and druggable pocket within the ATP-binding site that is exploited by LOXO-292 and BLU-667 *(*Shehata MA *et al., J Adv Res* 2023*)*. This information will be crucial to designing and developing highly specific third generation RET and c-Src inhibitors targeting allosteric and non-catalytic functions. Based on these results, we are designing new derivatives and optimising chemical scaffolds for next generation RET and c-Src inhibitors that exploit new druggable vulnerabilities, in collaboration with CNIO's Experimental Therapeutics Programme. ■
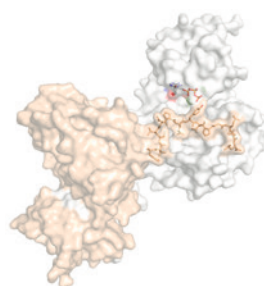
FIGURE 1 Asymetric c-Src dimer (PDB ID: 7OTE) capturing the intemolecular phosphorylation of the c-terminal Tyr 530 on the susbtrate kinase by the active kinase, from Cuesta-Hernández HN and Contreras J *et al*., *Nature Commun* 2023.

PUBLICATIONS

- Cuesta-Hernández HN, Contreras J, Soriano-Maldonado P, Sánchez-Wandelmer J, Yeung W, Martín-Hurtado A, Muñoz IG, Kannan N, Llimargas M, Muñoz J, Plaza-Menacho I (2023). An allosteric switch between the activation loop and a c-terminal palindromic phospho-motif controls c- Src function. *Nat Commun* 14, 6548.
- Shehata MA, Contreras J, Martín-Hurtado A, Froux A, Mohamed HT, El-Sherif AA, Plaza-Menacho I (2023). Structural and dynamic determinants for highly selective RET kinase inhibition reveal cryptic druggability. *J Advanced Res* 45, 87-100.
- Lucena-Agell D, Guillen MJ, Matesanz R, Alvarez-Bernad B, Hortiguela R, Avilés P, Martínez-Díez M, Santamaría-Nuñez G, Contreras J, Plaza-Menacho I, Giménez-Abián JF, Oliva MA, Cuevas C, Díaz JF. PM534, an optimized target protein interaction strategy through the colchicine site of tubulin. *J Med Chem*. PMID: 38294341.

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
Junior Group Leader

Research Scientist
Maria Dolores Moreno

Post-Doctoral Fellow
Elena Jiménez (since June)

Gradutate Students
Ester Casajús, Samuel Míguez

OVERVIEW

Safeguarding genetic information is essential to avoid malignant transformation. Two key cellular processes keep it free from errors: DNA replication and DNA repair. Importantly, genetic information may be damaged or lost when these processes do not work correctly, ultimately leading to disease. The deregulation and malfunction of the protein machinery that safeguards our genome are a hallmark of cancer, but it remains unclear how this happens at the molecular level. The devil is in the detail, and we aim to understand what and when something goes wrong with these molecular machines, so that we can act on it to correct it and prevent it from happening.

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

"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."

Technician
Araceli Grande (TS)*

*Titulado Superior (Advanced Degree)

Students in Practice
Emma Areán (until Sept.) (Master's Thesis, *Univ. Complutense de Madrid*, Spain), Victor Arranz (since Nov.) (Bachelor's Degree Final Project, *AECC*, Spain), Raquel Cuesta (until Sept.) (Master's Thesis, *Univ. Complutense de Madrid*, Spain), Carlos Chacón (since Nov.) (Master's Thesis, *Univ. Autónoma de Madrid*, Spain), Juan Lahoz (July-August) (Bachelor's Degree Final Project, *Univ. Francisco de Vitoria*, Spain), Paula Pérez (July-August) (*AECC* Summer Traineeship, *Univ. Complutense de Madrid*, Spain)

Visiting Scientist
Esther Cabañas (July-Dec.) (*CIB-CSIC*, Madrid, Spain)

RESEARCH HIGHLIGHTS

DNA replication & repair - focus on mitochondria

Mitochondrial DNA (mtDNA) replication is critical for human health. Deficiencies in the operation of mtDNA replication machinery underlie various devastating multi-systemic mitochondrial disorders and have been directly linked to the onset of neurodegenerative diseases, such as multiple types of ataxias, Parkinson's and Alzheimer's disease, and several cancer types. However, how the mitochondrial genome's integrity is maintained through the equilibrium between DNA replication, repair and degradation, and organelle dynamics remains unclear. We are interested in understanding these pathways because of their implications for ageing and disease, particularly their relationship to cancer.

Genome integrity - focus on telomeres

Telomeres are essential nucleoprotein structures that protect the end of our chromosomes. These structures are shaped by the protective shelterin complex that specifically binds to telomeric DNA repeats. Shelterin is composed of 6 proteins — TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 — and their proper 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 the recent developments in cryo-EM imaging, we can capture these protein machineries in different functional states to study their structures. With this information, we can unveil their molecular mechanisms, rationalise pathological mutations and their physiological consequences, and aid in the development of future cancer therapeutic strategies. ■
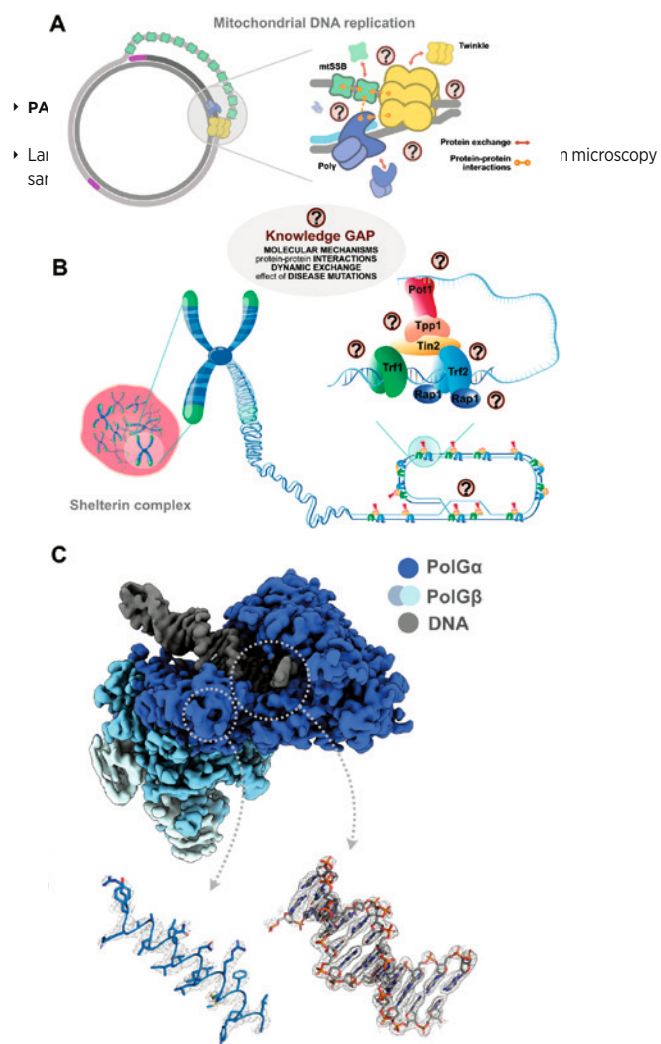
FIGURE 1 (**A**) Mitochondrial DNA replication machinery. (**B**) The shelterin complex shapes, protects, and regulates enzymatic activities at telomeres. Several key aspects of their regulation, and the molecular mechanisms through which both protein complexes exert their activities, remain unknown. (**C**) High resolution cryo-EM structures of these complexes allow us to study their molecular mechanisms.

▸ **AWARDS & RECOGNITION**

▸ *Premio E. Pérez Payá - SBE* Prize awarded by the Spanish Biophysical Society (*SBE*).
▸ Scientific Advisory Board Member, TEM-PoS – a cryo-EM service company, Spain.

COMPUTATIONAL CANCER GENOMICS JUNIOR GROUP

Solip Park
Junior Group Leader

Post-Doctoral Fellow
Seulki Song (until May)

Gradutate Students
Jaejun Lee, Pelayo González De Lena
(until March), Moustafa Ahmed
Mohamed Shehata (until February)

OVERVIEW

Cancer is a multifaceted disease influenced by multiple factors. Moreover, the impact of genomic alterations varies considerably depending on the cellular context. In the Computational Cancer Genomics Lab, we strive to decipher the context-dependent cancer fitness landscape. We do this by identifying novel cancer predisposition genes (CPGs), measuring variations in cancer fitness across cancer states, and constructing dynamic protein-protein interaction networks. Our research is rooted in cancer genomics, systems biology, and network medicine. First, our findings aim to provide a comprehensive understanding of tumour progression via novel CPGs. Second, after examining 250,000 primary and metastatic samples, we discerned specific cancer types/cancer genes with distinct fitness levels based on their cancer states. Through extensive data analysis, our goal is to address core questions in cancer genetics and to explore the practical and clinical implications of our genomic findings.

> **"We identify novel CPGs by integrating multi-omics beyond the classical CPGs. Using samples from 250,000 patients, we have been advancing our understanding of cancer fitness across different states."**

Technicians
Adrián Maqueda (since August), Manuel Moradiellos, Laia Ollé (until August)

Visiting Master's Students
Yiran Du (since September) (Master's Thesis, *Univ. Autónoma de Madrid*, Spain), Adrián Maqueda (until July) (Master's Thesis, *Univ. Autónoma de Madrid*, Spain)

Visiting Scientist
Sojeong Jeon (since December) (Dong-Eui University, South Korea)

RESEARCH HIGHLIGHTS

Identification of novel cancer predisposition genes (CPGs)

The roles of germline variants remain elusive and could be substantially underestimated. We presented compelling evidence indicating that Mendelian-disease associated genes could increase cancer risk similarly to CPGs. We proposed 4 potential classes of CPG-like OMIM genes that might indicate non-classical mechanisms of tumour progression (Song *et al.*, *Genome Medicine*). In addition, we formulated a machine learning method that leverages comprehensive feature integration to pinpoint novel CPGs. Significantly, this will be the pioneering study to comprehensively predict previously unidentified CPGs.

Cancer state-specific fitness landscape

While metastasis is a primary factor for decreased survival rates among cancer patients, our comprehension of metastasis remains nascent in comparison to that of primary tumours. Building on our prior discoveries (Park *et al.*, *Nature Commun*, 2021), we postulate that the optimal activity-fitness level of cancer genes varies based on the cancer's state, such as primary versus metastatic tumours. By analysing 250,000 samples, we have identified 5 cancer types with notable differences and pinpointed 16 genes exhibiting distinct perturbations on the cancer states. These insights are pivotal for grasping the variances in cancer fitness across cancer states and could greatly enhance our understanding of treatment response disparities.

Position-specific perturbed interaction network

Protein interaction partners for a specific mutant protein can shift depending on the mutation location within the protein. Furthermore, we expect that the clinical responses of these mutations will differ based on the mutations. In 2023, in collaboration with M. Oren (Weizmann Institute of Science, Rehovot, Israel) and JS. Yang (*CRAG*, Barcelona, Spain), we initiated a project focusing on p53 mutations, supported by a *Fundación Ramón Areces* Award. This endeavour holds the potential to revolutionise network medicine by introducing an edge-specific treatment methodology. ■
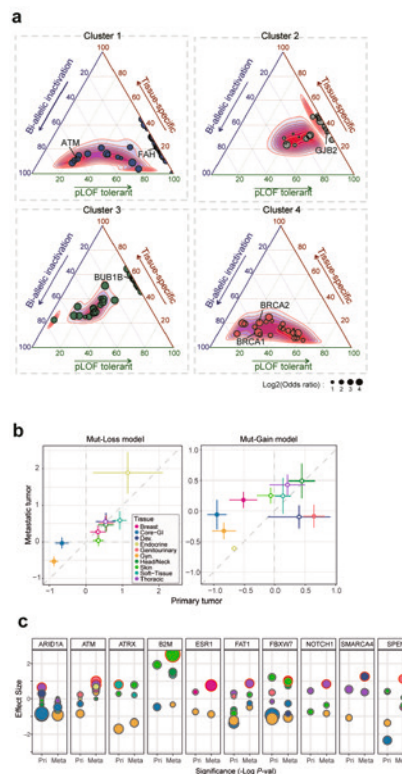
FIGURE 1 Context-dependent cancer fitness landscape. (**a**) Analysis of 116 CPG-like OMIM genes reveals 4 distinct classes. Distribution of the 3 features across the 4 classes. (**b**) Mean effect size between mutation and CNAs in primary and metastatic tumours across cancer types and (**c**) across cancer genes.

▸ **PUBLICATIONS**

▸ Song S, Koh Y, Kim S, Lee SM, Kim HU, Ko JM, Lee SH, Yoon SS, Park S (2023). Systematic analysis of disease-linked rare germline variants reveals new classes of cancer predisposing genes. *Genome Med* 15, 107.

▸ Vali-Pour M, Park S, Espinosa-Carrasco J, Ortiz-Martínez D, Lehner B, Supek F (2023). The impact of rare germline variants on human somatic mutation processes. *Nat Commun* 14, 5448.

▸ Koh Y, Kim H, Joo SY, Song S, Choi YH, Kim HR, Moon B, Byun J, Hong J, Shin DY, Park S, Lee KH, Lee KT, Lee JK, Park D, Lee SH, Jang JY, Lee H, Kim JA, Yoon SS, Park JK (2023). Genetic assessment of pathogenic germline alterations in lysosomal genes among Asian patients with pancreatic ductal adenocarcinoma. *J Transl Med* 21, 730.

▸ **PATENT**

▸ Park S, Yoon SS, Koh Y, Song S (2023). Novel cancer predisposition genes derived from Mendelian disorder-related gene group. *P22110SNU*.

▸ **AWARDS AND RECOGNITION**

▸ *CNIO-WIS* Collaborative Project awarded to Solip Park (CNIO) and Moshe Oren (the Weizmann Institute of Science). *Fundación Ramón Areces*, Weizmann Institute of Science and CNIO joint initiative for funding projects focused on precision medicine and cancer.

COMPUTATIONAL ONCOLOGY JUNIOR GROUP

Geoff Macintyre
Junior Group Leader

Research Scientist
Patricia González Santamaría (since June)

Post-Doctoral Fellows
Bárbara Hernando, Marina Torres (since October)

Gradutate Students
Blas Chaves, María Escobar,

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.

"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.

Ángel Fernández, David Gómez
(until December), Joseph S.
Thompson

Technician
Alice Nicole Cádiz

Master's Students
M. Teresa Castillo (since December)
(*Universidad Complutense de Madrid*,
Spain), Diego García (until March)
(*Universidad Politécnica de Madrid*,
Spain)

RESEARCH HIGHLIGHTS

2023 saw success for the Computational Oncology Group in terms of new funding and innovation. We also welcomed PhD student Joe Thompson, postdoctoral researcher Marina Torres, and senior staff scientist Patricia González Santamaria. Joe Thompson and Blas Chaves were both awarded highly competitive *la Caixa* PhD fellowships. Joe will be constructing a framework to guide the clinical approval of mutational signature-based biomarkers – an urgently needed approach to ensure patients see benefit from the biomarkers we and others are developing. Blas will be engineering cell lines to express particular types of chromosomal instability so we can better understand mechanisms and drug sensitivities. Bárbara Hernando was awarded a *la Caixa* Junior Leader fellowship which will allow her to build her independent career. The lab also secured a national grant and a collaborative project with UK-based start-up Tailor Bio to scale up our efforts to induce CIN across cell lines by editing over 500 DNA damage response genes in parallel.

Over 2023 the lab also began to solidify many of the new methodologies developed via the filing of 3 new patents. The first, developed in collaboration with Tailor Bio, is a method to extract robust genome-wide copy number profiles from tumour-only targeted gene panels. This work formed the basis of David Gomez's PhD thesis, which he submitted at the end of 2023 and promises to unlock the use of our CIN signature biomarkers in the clinic. The second method, developed by Bárbara, extends our CIN signature framework to quantify signatures for individual copy number events. This enables a range of downstream applications, including better understanding how CIN influences tumour evolution and enables detection of ongoing CIN. This method has also facilitated the development of the third patented methodology, where Ángel Fernández and Bárbara built a framework to forecast oncogene amplification. This framework represents a new class of biomarker, wherein selective pressures and mutation-generating processes are harnessed to anticipate future genomic alterations and thereby forecast tumour evolution (FIGURE 1). ■
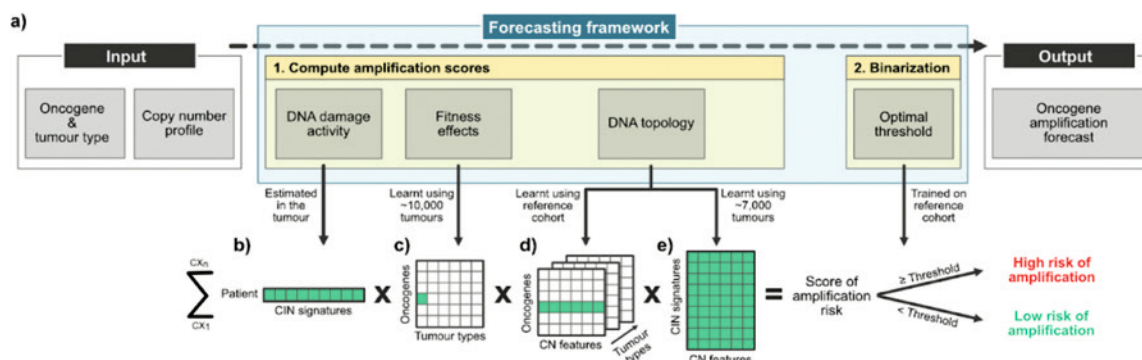
FIGURE 1 Schematic representation of our framework for forecasting oncogene amplifications (**a-e**).

▸ **PUBLICATIONS**

▸ Hernando B, Macintyre G (2023). A medley of resistance in ovarian cancers. *Nat Genet* 55, 361–362.
▸ Calsina B *et al.* (incl. Hernando B, Macintyre G) (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.
▸ Smith P *et al.* (incl. Macintyre G) (2023). The copy number and mutational landscape of recurrent ovarian high-grade serous carcinoma. *Nat Commun* 14, 5992.
▸ Meagher NS *et al.* (incl. García MJ) (2023). Profiling the immune landscape in mucinous ovarian carcinoma. *Gynecol Oncol* 168, 23 - 31.
▸ Vias M, *et al.* (incl. Hernando B, Macintyre G) (2023). High-grade serous ovarian carcinoma organoids as models of chromosomal instability. *Elife* 12, e83867.
▸ Thompson JS *et al.* (incl. Hernando B, Escobar-Rey M, Fernández-Sanromán Á, Macintyre G) (2023). Predicting response to cytotoxic chemotherapy. *BioRxiv*. doi:10.1101/2023.01.28.525988.

▸ **PATENTS**

▸ Macintyre G, Gómez-Sánchez D, de Galard Terraube H, García Lopez D (2023). Genomic sequencing analysis. *EP23382755.9*.
▸ Macintyre G, Hernando B (2023). Detection of chromosomal instability. *EP23383179.1*.
▸ Macintyre G, Hernando B, Fernández Sanromán Á, Paz-Ares L, Zugazagoitia J, Gómez-Sánchez D, Goya JM (2023). Prediction of gene amplification. *EP23383180.9*.

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: i) the structure and dynamics of proteins by NMR and ii) the affinity and kinetics of protein interactions with other biopolymers and small molecules that could represent initial hits in drug discovery or 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 2023, as a contribution to the collaboration between the Melanoma Group and the Experimental Therapeutics Programme, we characterised biophysically the interaction of several small molecules with the Midkine protein (MDK), a driver of melanoma metastasis and immune suppressor whose modulation by small molecule interactors may open new avenues for the future management of melanoma."

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) equipment, 2 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., DNA Replication, Experimental Oncology, Growth Factors, Nutrients and Cancer, Melanoma, and Transformation and Metastasis Groups, as well as the H12O-CNIO Cancer Immunotherapy Clinical Research Unit) the Structural Biology Programme used these technologies throughout 2023. For example, in collaboration with the Melanoma Group, using nanoDSF and SPR, we characterised biophysically the interaction of several small molecules with the Midkine protein (MDK), including a model of its natural binding partner heparin. We established that the recombinant protein provided by the Protein Production Unit is well-folded, monomeric and stable, and undergoes a 20 ºC thermal stabilisation upon binding the heparin mimic (FIGURE 1, panels A and B). The binding affinity of this protein-oligosaccharide complex measured from nanoDSF data matches what is measured by SPR (panels C, D and E). This research is useful for the discovery and development of small compounds that could modulate molecular interactions involving MDK and, therefore, may be of relevance to interfere with MDK functions and potentially contribute to melanoma management in the future.

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 in 2023 was done in collaboration with the Cell Division and Cancer, Epithelial Carcinogenesis, Growth Factors, Nutrients and Cancer, Metabolism and Cell Signalling, 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. ■
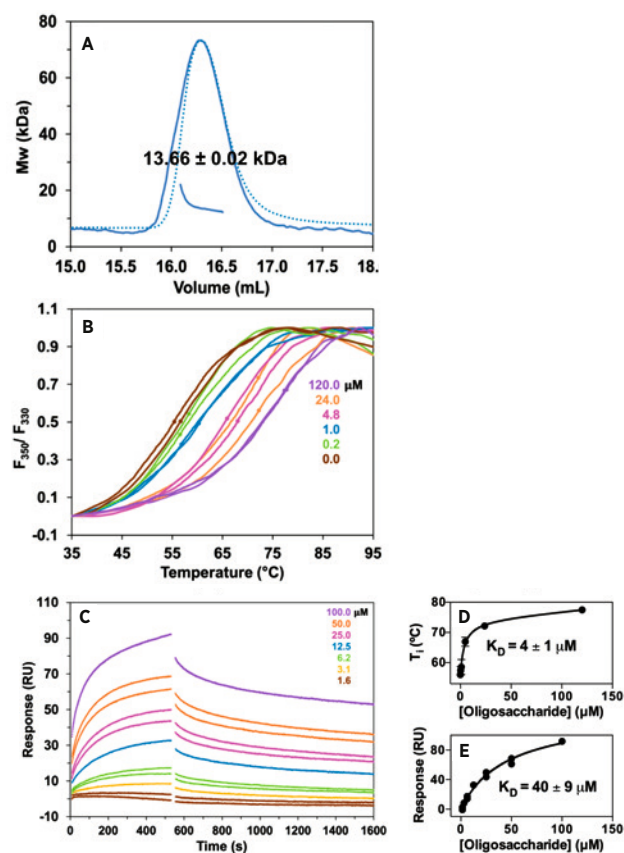
FIGURE 1 Characterisation of recombinant MDK protein and its interaction with a heparin model oligosaccharide, HO. (**A**) Superimposed MALS chromatographic traces of light scattering (solid lines), refractive index (dashed lines) and the calculated molecular weight. (**B**) NanoDSF thermal unfolding profiles in the absence and in the presence of increasing HO concentrations (most in duplicate), with calculated inflection temperatures (Ti) marked as dots. (**C**) Overlay of the SPR sensorgrams flowing increasing HO concentrations (some in duplicate) over immobilised MDK. (**D**, **E**) Estimation of binding affinity constants (K_D) from B and C experiments, respectively.

▸ **PUBLICATIONS**

▸ Mellid S, García F, Leandro-García LJ, Díaz-Talavera A, Martínez-Montes ÁM, Gil E, Calsina B, Monteagudo M, Letón R, Roldán-Romero JM, Santos M, Lanillos J, Valdivia C, Martínez-Puente N, de Nicolás-Hernández J, Jiménez S, Pérez-Martínez M, Honrado E, Coloma J, Cerezo A, Santiveri CM, Esteller M, Campos-Olivas R, Caleiras E, Montero-Conde C, Rodríguez-Antona C, Muñoz J, Robledo M, Cascón A (2023). DLST mutations in pheochromocytoma and paraganglioma cause proteome hyposuccinylation and metabolic remodeling. *Cancer Commun* 43, 838-843.

▸ Sanz-Castillo B, Hurtado B, Vara-Ciruelos D, El Bakkali A, Hermida D, Salvador-Barbero B, Martínez-Alonso D, González-Martínez J, Santiveri C, Campos-Olivas R, Ximénez-Embún P, Muñoz J, Álvarez-Fernández M, Malumbres M (2023). The MASTL/PP2A cell cycle kinase-phosphatase module restrains PI3K-Akt activity in an mTORC1-dependent manner. *EMBO J* 42, e110833.

BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Research Scientist
Michael Tress

Post-Doctoral Fellow
Óscar Lapuente (since May)

Gradutate Students
Santiago García (PEJ, CAM)`,
María José Jiménez, Laura Martínez
(until April)

OVERVIEW

Bioinformatics plays a crucial role in understanding the cancer genome and the future of cancer therapeutics. By utilising bioinformatics-based approaches, we can transform vast amounts of biological data into comprehensible models that provide a deep understanding of cancer and the complex genotype-phenotype relationships necessary to identify molecular cancer-driving alterations and novel therapeutic targets.

The Bioinformatics Unit (BU) at the CNIO has several goals: (i) developing novel computational methodologies and bioinformatics tools to integrate biological and clinical data, (ii) analysing genome data of cancer patients to identify new biomarkers and drug response mechanisms, (iii) providing bioinformatics support through data analysis and interpretation using computational and statistical methods, (iv) maintaining the scientific computing facilities at the CNIO, and (v) offering training in bioinformatics tools and methods.

> "In 2023, we introduced PanDrugs2, a bioinformatics method for profiling drug-responsive tumour cells and suggesting tailored treatments from integrated cancer multi-omics data."

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

Bioinformaticians (TS)**
Ruth Álvarez, Ester Arroba (Feb.-June), Dido Carrero (since June), Daniel Cerdán, Tomas Di Domenico,

Gonzalo Gómez, Pablo González (since Dec.), Elena Piñeiro, Laura Serrano (since March) Francisco J Soriano

**Titulado Superior (Advanced Degree)

Students in Practice
María González (Master's Thesis, *UAM,* Madrid, Spain), Pablo González and Marcos Rubio (May-Dec.) (Master's Prog.in Biocomputing, *ENS-ISCIII,* Madrid, Spain), Alessandro Gostoli (Feb.-June) (*Univ. Carlos III de Madrid*, Spain),

Angela Merici G Surya (June-Aug.) (CNIO Summer Training Programme, Indonesia International Institute for Life Sciences)

Visiting Scientist
David Cáceres (since April) (*Hosp. 12 de Octubre*, Madrid, Spain)

RESEARCH HIGHLIGHTS

In 2023, we published more than 10 peer-reviewed articles as a result of our ongoing research projects and scientific collaborations (you can find the full list of our activities on our website: https://bioinformatics.cnio.es/). We introduced PanDrugs2, our *in silico* prescription methodology for interpreting tumour molecular alterations. This updated version now allows multi-omics data to be integrated, enabling and assisting in the selection of tailored treatments in precision medicine (Jiménez-Santos *et al.* 2023) (FIGURE 1). Additionally, our group was involved in the most extensive genomic and immunogenomic analysis of metastatic pheochromocytoma/paragangliomas (Calsina *et al.* 2023). Moreover, our lab recently released an update on the annotation of human and mouse genes through the international GENCODE consortium (Frankish *et al.* 2023).

The BU is also actively involved in the European network ELIXIR (https://www.elixir-europe.org/), co-leading the ELIXIR Cancer Data Focus Group, which provides a framework and expertise for the systematic analysis and interpretation of cancer genomes. We also co-lead Work Package 5 of the EOSC4Cancer project (https://eosc4cancer.eu/) which aims to build a federated and interoperable system for accessing and analysing cancer genomes across Europe, involving cancer research centres, leading research groups and infrastructures, hospitals, and supercomputing centres. Additionally, the BU co-coordinates the *ISCIII* IMPaCT-Data project (https://impact-data.bsc.es/), focusing on leading training activities and genomics data management. Our training and knowledge-transfer activities include co-organising the MSc in "*Bioinformática aplicada a la medicina personalizada y la salud*" at the *ISCIII*. ∎
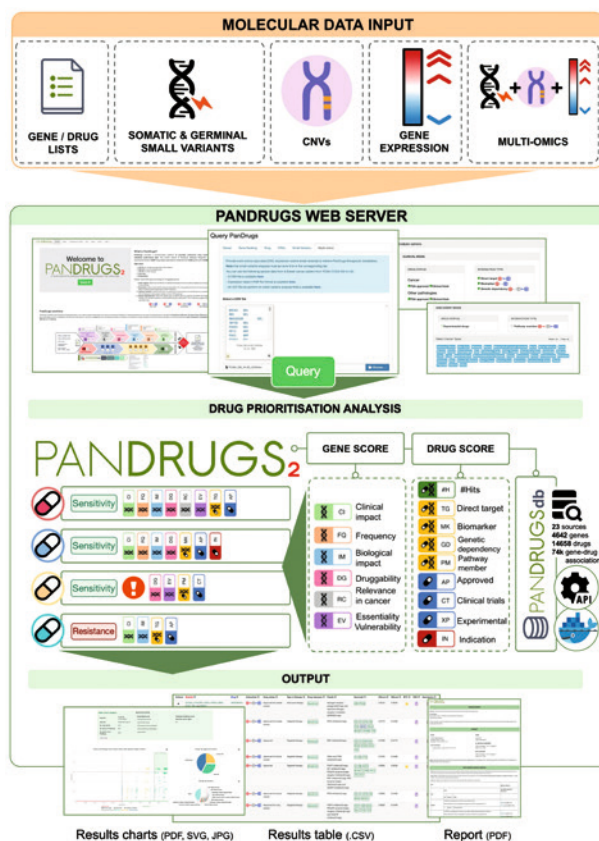
FIGURE 1 PanDrugs2 workflow. The PanDrugs2 web server allows uploading lists of genes, drugs, somatic and germline variants, copy-number alterations, and gene expression data for drug prioritisation analyses.

▸ **SELECTED PUBLICATIONS***

▸ Sanchez-Aguilera A *et al.* (incl. García S, Martínez S, Al-Shahrour F, Valiente M) (2023). Machine learning identifies experimental brain metastasis subtypes based on their influence on neural circuits. *Cancer Cell* 41, 1637-1649.

▸ Jiménez-Santos MJ, Nogueira-Rodríguez A, Piñeiro-Yáñez E, López-Fernández H, García-Martín S, Gómez-Plana P, Reboiro-Jato M, Gómez-López G, Glez-Peña D, Al-Shahrour F (2023). PanDrugs2: prioritizing cancer therapies using integrated individual multi-omics data. *Nucleic Acids Res* 51(W1), W411-W418.

▸ Frankish A *et al.* (incl. Cerdán-Vélez D, Gómez LM, Pozo F, Tress ML) (2023). GENCODE: reference annotation for the human and mouse genomes in 2023. *Nucleic Acids Res* 51(D1), D942-D949.

▸ Calsina B *et al.* (incl. Piñeiro-Yáñez E, Fustero-Torre C, García-Martín S, Rodríguez-Perales S, Domínguez O, Macintyre G, Currás-Freixes M, Rodríguez-Antona C, Cascón A, Leandro-García LJ, Montero-Conde C, Roncador G, Al-Shahrour F, Robledo M) (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.

▸ Salmón M *et al.* (incl. Fustero-Torre C, Rodríguez-Perales S, Mulero F, Al-Shahrour F, Martínez L, Domínguez O, Caleiras E, Ortega S, Barbacid M) (2023). Kras oncogene ablation prevents resistance in advanced lung adenocarcinoma. *J Clin Invest* 133, e164413.

▸ Jutzi JS *et al.* (incl. Jiménez-Santos MJ, Al-Shahrour F) (2023). CALR-mutated cells are vulnerable to combined inhibition of the proteasome and the endoplasmic reticulum stress response. *Leukemia* 37, 359-369.

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

▸ **AWARDS AND RECOGNITION**

▸ Member of the Scientific Advisory Board for *Institut Curie*, the Paris-Saclay Cancer Cluster (PSCC), and ELIXIR-Norway.

▸ Board Member for the *Sociedad Española de Biotecnología* (*SEBiot*) and the *Sociedad Española de Bioinformática y Biología Computacional* (*SEBiBC*), Spain.

ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Post-Doctoral Fellow
Johanne Le Coq

OVERVIEW

The primary goal of the Electron Microscopy (EM) Unit is to provide scientific-technical support to researchers in resolving their scientific questions using various transmission EM techniques. We routinely employ cryoEM and negative staining for sample preparation. Additionally, we perform data collection and assist with image processing, including 2D analysis and 3D reconstruction. Support is offered in selecting appropriate EM techniques and preparing samples. Furthermore, we manufacture our own sample supports (EM grids) to ensure better quality control and cost reduction. We also provide the training necessary for the use of our microscopes and auxiliary equipment. More advanced structural studies are typically carried out through research collaborations.

"In the Electron Microscopy Unit, our main effort is to ensure efficient access to all of our infrastructure. We also provide essential training for using our microscopes and auxiliary equipment."

RESEARCH HIGHLIGHTS

Over the past year, we provided research support to several Groups at the CNIO. In collaboration with the Metabolism and Cell Signalling Group, we started analysing mitochondrial alterations in their animal model. With the Transformation and Metastasis Group, we continued studying mitochondrial morphology in breast cancer animal models with RANK overexpression. Together with the Microenvironment & Metastasis Group, we examined different types of vesicles and, with the Experimental Oncology Group, we analysed the homogeneity of RAF1-HSP90-CDC37 in complex with additional protein. Finally, in collaboration with the Growth Factors, Nutrients and Cancer Group, we continued working on optimising single particle cryoEM structural analysis of the URI prefoldin complex.

We maintained our close collaboration with all the groups in the Structural Biology Programme. During 2023, our efforts were primarily focused on conducting single-particle EM grid preparation, cryoEM grid screening, negative staining and cryoEM data collection, 2D analysis and, in some cases, 3D analysis of numerous purified proteins, protein complexes, and RNA. For instance, we collaborated on several projects with the Macromolecular Complexes in DNA Damage Response Group involving EM grid preparation, data collection, and analysis of different samples. These include RNA helicase DDX11, finishing the structural study of the RuvBL complex in *Arabidopsis thaliana* (in collaboration with D. Alabadí from *Universitat Politècnica de València*), and continue deciphering the architecture of lncRNA (in collaboration with M. Huarte from *CIMA, Universidad de Navarra*). Additionally, in collaboration with the Genome Integrity and Structural Biology Group, we conducted cryoEM grid screening and data collection of numerous samples, and with the Kinases, Protein Phosphorylation, and Cancer Group, we optimised EM grids, performed data collection and processing for PTC1 kinase, and carried out EM grid preparation and imaging for the KIF5B-RET kinesin sample. ■
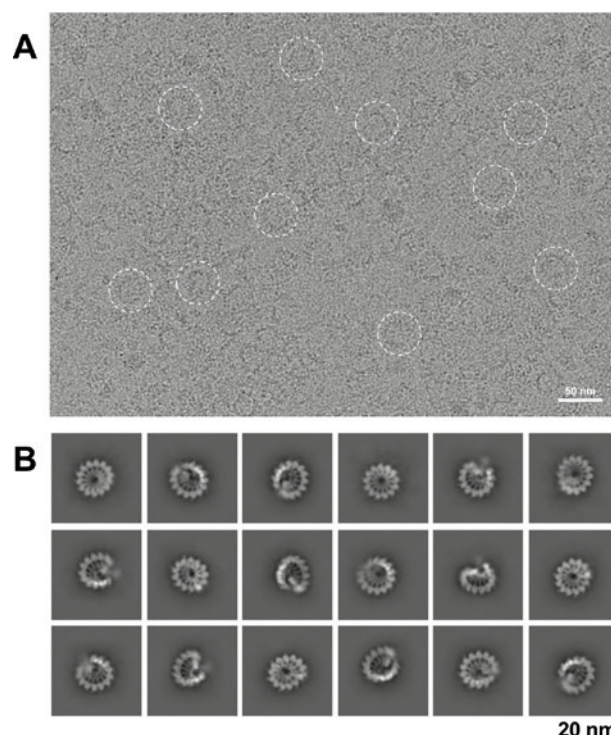
FIGURE 1 Single-molecule analysis of human gammaTuRC. Movies collected on our 200 kV microscope (**A**) and processed at 2D (**B**) demonstrate that our setup is a powerful tool for examining the structural features of protein and protein complexes.

PROTEIN CRYSTALLOGRAPHY UNIT

Inés Muñoz
Unit Head

Postdoctoral Fellow
Yudhi Nugraha (until March)

Technician
Pilar Redondo (until May)

OVERVIEW

The Protein Crystallography Unit is a core facility that provides on-demand services at different levels, from the cloning, expression, and purification of high-quality proteins to the determination of their 3D structures, with the purpose to fulfil the demands of our users and to understand the function of their protein targets. Thus, we produce proteins for different types of assays and structural determination at low resolution by small-angle X-ray scattering (SAXS) or at atomic resolution by X-ray crystallography. The latter includes protein co-crystallisation in the presence of inhibitors or small fragments, a method that we routinely combine with the quantification of protein thermal stability (thermofluor assay) to aid the drug discovery process.

"Understanding the three-dimensional crystal structure of proteins, including their behaviour in solution, is fundamental to life science and biopharmaceutical researchers."

Student in Practice
Daffa Adinegoro (until June)
(PhD student, Graduate School of
Bioagricultural Sciences, Nagoya
University, Japan)

RESEARCH HIGHLIGHTS

Our Unit works closely with the Experimental Therapeutics Programme on several projects in support of drug discovery. This includes the kinase domain of the human protein HASPIN for biochemical and structural analyses in the presence of new compounds developed in the Medicinal Chemistry Section.

The Unit is also engaged in several internal collaborations with other CNIO groups (Growth Factors, Nutrients and Cancer; Transformation and Metastasis; Experimental Oncology; Microenvironment and Metastasis; DNA Replication; Macromolecular Complexes in DNA Damage Response; and Kinases, Protein Phosphorylation and Cancer Groups; the H12O-CNIO Lung Cancer Clinical Research Unit; and the H12O-CNIO Cancer Immunotherapy Clinical Research Unit), providing recombinant proteins that were used for protein crystallography, SAXS or thermofluor assay analysis and, in some cases, for other biophysical, biochemical, cell-based functional assays and cryoEM studies.

Throughout 2023, the Unit also continued working on its own scientific project, supported by a grant from the *BBVA* Foundation. Carried out in collaboration with the H12O-CNIO Cancer Immunotherapy Clinical Research Unit, this research generated a new synthetic bispecific antibody capable of targeting the spike protein of the SARS-CoV-2 virus, inducing neutralisation while promoting T cell cross-priming. We were involved in solving the cryoEM structure, which shows how the trimerbody (TNT) binds the trimeric RBD spike ectodomain in a 1:1 equimolar ratio (FIGURE 1). In addition, the Unit maintained collaborations with various external groups in Spain: the Department of Crystallography and Structural
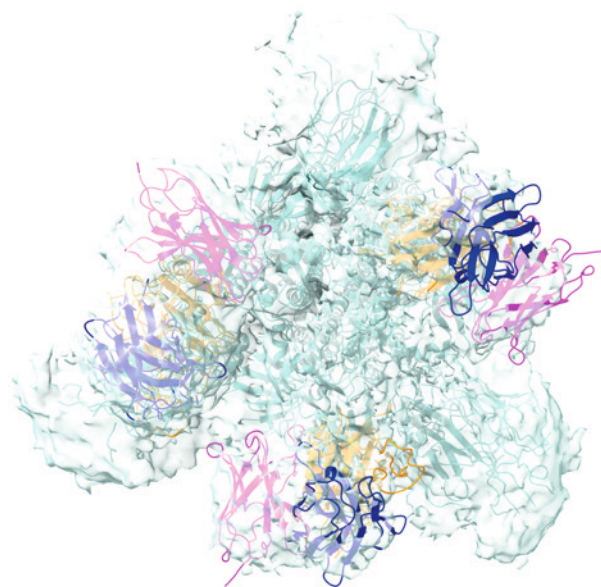
FIGURE 1 Top view of the spike protein/TN^T complex model showing TN^T embracing the spike protein in the 3-up RBD prefusion conformation. To enhance its visualisation, the spike protein was coloured in pale blue while its 3 RBD subunits were coloured in yellow. The $V_{HH}E$ and $V_{HH}V$ chains from the synthetic antibody are in purple and magenta, respectively. The cryoEM map is painted in light grey. The figure shows how each pair of antibody chains embraces one RBD, thus structurally demonstrating their neutralising effect.

Biology (*IQF-CSIC*, Madrid), and the Molecular Mechanisms and Experimental Therapy in Oncology Programme (*IDIBELL*, Barcelona). ∎

▸ **PUBLICATIONS**

▸ Cuesta-Hernández HN, Contreras J, Soriano-Maldonado P, Sánchez-Wandelmer J, Yeung W, Martín-Hurtado A, Muñoz IG, Kannan N, Llimargas M, Muñoz J, Plaza-Menacho I (2023). An allosteric switch between the activation loop and a c-terminal palindromic phospho-motif controls c-Src function. *Nat Commun* 14, 6548.
▸ Mur P, Viana-Errasti J, García-Mulero S, Magraner-Pardo L, Muñoz IG, Pons T, Capellá G, Pineda M, Feliubadaló L, Valle L (2023). Recommendations for the classification of germline variants in the exonuclease domain of POLE and POLD1. *Genome Med* 15, 85.
▸ Martínez-Caballero S, Freton C, Molina R, Bartual SG, Gueguen-Chaignon V, Mercy C, Gago F, Mahasenan KV, Muñoz IG, Lee M, Hesek D, Mobashery S, Hermoso JA, Grangeasse C (2023). Molecular basis of the final step of cell division in *Streptococcus pneumoniae*. *Cell Rep* 42, 112756.
▸ Lázaro-Gorines R, Pérez P, Heras-Murillo I, Adán-Barrientos I, Albericio G, Astorgano D, Flores S, Luczkowiak J, Labiod N, Harwood SL, Segura-Tudela A, Rubio-Pérez L, Nugraha Y, Shang X, Li Y, Alfonso C, Adipietro KA, Abeyawardhane DL, Navarro R, Compte M, Yu W, MacKerell AD Jr, Sanz L, Weber DJ, Blanco FJ, Esteban M, Pozharski E, Godoy-Ruiz R, Muñoz IG, Delgado R, Sancho D, García-Arriaza J, Álvarez-Vallina L (2023). Dendritic cell-mediated cross-priming by a bispecific neutralizing antibody boosts cytotoxic T cell responses and protects mice against SARS-CoV-2. *Adv Sci* 10, e2304818.

▸ **AWARDS AND RECOGNITION**

▸ Inés Muñoz was a member of the scientific panel that made the decision to begin construction, at the ALBA Synchrotron facility (Barcelona, Spain), of 2 new long-beamlines: Cryo bio-nano-imaging beamline (CORUS) and Coherent Diffraction Imaging beamline (CoDI).

PROTEIN PRODUCTION UNIT

Jorge L. Martínez-Torrecuadrada
Unit Head

Technician
María Rivas (since February) (TS)*

*Titulado Superior (Advanced Degree)

OVERVIEW

The biological functions of many proteins, especially those involved in cancer, still remain uncharted. Understanding their structures, tissue and cellular distributions, and functions is critical for biomedical progress. However, researchers often encounter the challenge of insufficient supply, as proteins of interest identified in a specific process can be very difficult to produce in high quality and quantity for analysis. This bottleneck hinders the pace at which they can be characterised. The Protein Production Unit is a core lab designed to address this issue. It offers expertise and state-of-the-art technologies to develop efficient processes for producing recombinant proteins. These proteins can be utilised in various downstream applications, including to generate highly specific antibodies, biophysical, biochemical or functional analyses, as well as structural studies. The ultimate goal is to accelerate cutting-edge cancer research for CNIO and external research groups.

> **"The Protein Production Unit offers expertise in all aspects of protein expression, from cloning to expression optimisation and protein purification, with the aim of providing high quality soluble proteins."**

RESEARCH HIGHLIGHTS

Last year, the Protein Production Unit completed more than 10 protein production projects from CNIO Groups and external collaborators. The projects aimed to determine optimal expression conditions and purify target proteins for antibody production, functional assays, and/or biophysical characterisation. In particular, we continued to produce active human Midkine in collaboration with the Melanoma Group for drug development and labelled the same protein with 15N for analysis by NMR spectroscopy. In addition, several scFv antibody fragments derived from monoclonal antibodies against Midkine were recombinantly expressed for further characterisation. We also successfully expressed a range of antibody formats and p32 protein for the H12O-CNIO Cancer Immunotherapy Clinical Research Unit, and produced Tn5 transposase and pAG-Hia5 for Directed Methylation and Long-read Sequencing (DiMeLo-seq), in collaboration with the Topology and DNA Breaks Group and the Telomeres and Telomerase Group, respectively. Furthermore, the Unit collaborated with external groups

such as Theodorescu Lab at Cedar Sinai Medical Center, Los Angeles, USA; Nanobiotechnology for Diagnostics group, Institute for Advanced Chemistry of Catalonia, Barcelona, Spain; Liver Disease Laboratory, CIC bioGUNE, Bizkaia, Spain; Vascular Research Laboratory, IIS-*Fundación Jiménez Díaz*, Madrid, Spain; and the *Instituto de Investigación Sanitaria Gregorio Marañón*, Madrid, Spain; among others. Finally, we continued our work with the Monoclonal Antibodies Unit producing recombinant proteins as antigens for the generation of monoclonal antibodies such as mouse IL4i1 or mouse PIGR.

As part of its research activities, the Unit has also worked on the development of antibody-based theragnostic tools, radiolabelled with zirconium-89 for PET and with lutetium-177 for therapy in a triple-negative breast cancer (TNBC) mouse model, teaming up with the Molecular Imaging Unit and *CIEMAT,* and supported by a grant from the *BBVA* Foundation. ■
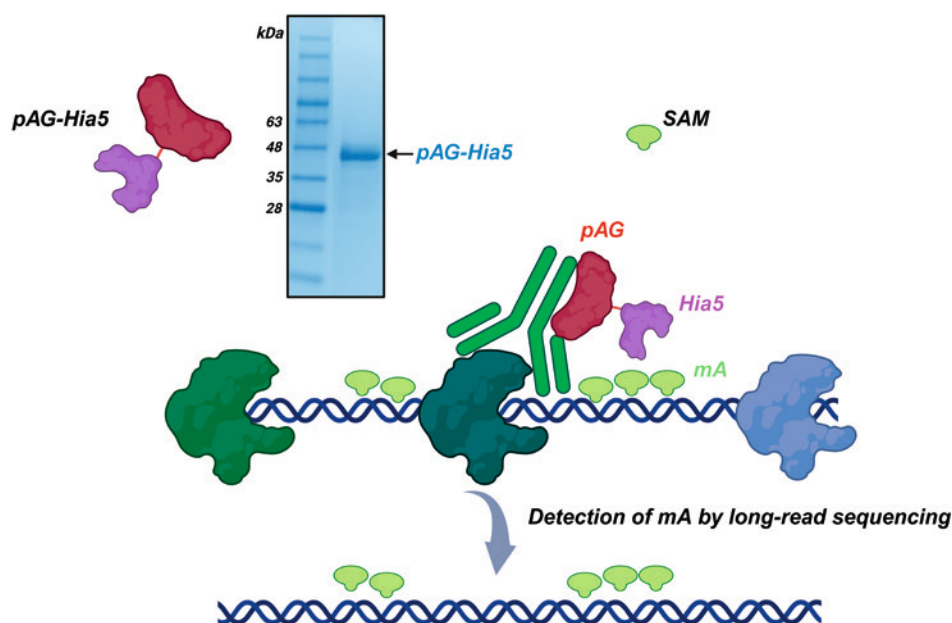
FIGURE 1 Schematic illustration of the use of recombinantly expressed pAG-Hia5 to map DNA-protein interactions by DiMeLo-seq. pAG-Hia5: The antibody-binding proteins A&G fused to the nonspecific deoxyadenosine methyltransferase Hia5. mA: N6-methyl-deoxyadenosine. SAM: S-adenosyl methionine. Created by BioRender.com.

Translational Research

HUMAN CANCER GENETICS PROGRAMME

The Human Cancer Genetics Programme (HCGP) is a translational research programme working on areas related to 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 in order to guide risk-stratified screening strategies towards personalised cancer prevention and treatment; and (2) understanding the molecular mechanisms involved cancer to facilitate the development of targeted therapies and early diagnostics.

In 2023, HCGP was composed of 2 Research Groups: Hereditary Endocrine Cancer (HECG) and Genetic and Molecular Epidemiology (GMEG); and 3 Units: Human Genotyping-*CEGEN*, Molecular Cytogenetics, and the Familial Cancer Clinical Unit. In addition, the Programme includes a Familial Cancer Consultancy at the *Hospital Universitario de Fuenlabrada* to evaluate families with cancer and provide genetic counselling.

 The Programme works in close collaboration with the clinical community to foster cooperation in genetic diagnosis and research, and to promote training and education. In 2023, there were 1,145 visits at the Familial Cancer Consultancy (579 new cases and 566 patients receiving results), and the HCGP performed 2,128 genetic diagnoses and carried out 2,102 cytogenetic studies. In terms of research, the Programme's members participated in collaborative studies involving a network of more than 30 hospitals from our National Health System, not only to conduct collaborative studies but also to facilitate clinical translation. The Programme also offers professionals and students from different national and international research centres the opportunity to join, either as visitors or for training visits consisting of short-term stays of 1 to 3 months. Three national and 7 international visitors, and 4 medical residents from different Spanish hospitals were hosted in 2023. In terms of education, 5 Bachelor's, 2 Master's, and 16 PhD students worked on their research projects.

The Programme has established research collaborations with national and international groups; this is well demonstrated by its publication record as well as the key roles held by several of the Programme's members in consortia and international projects. In 2023, for example, GMEG members, led by Núria Malats, in collaboration with C. Van Eijck from Erasmus Medical Centre, Rotterdam, undertook a study, published in *GUT*, that showed that 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 of several of the HCGP's group members to IMPaCT, an initiative of the *Instituto de Salud Carlos III* to Promote Precision Medicine in our country.

Milestones and major achievements of the HCGP in 2023 include the following:

→ GMEG was awarded an Innovative Health Initiative European project on liquid biopsy and cancer minimal residual disease.
→ GMEG contributed to the bladder cancer field through the identification of new genetic susceptibility variants revealing new biological and translational insights.
→ Mercedes Robledo led an international study published in *Nature Communications*. Focusing on metastatic pheochromocytoma, it was the first to identify differences in the tumour microenvironment dependent on the driver gene, which is of potential relevance for the treatment of patients with this disease.
→ Sandra Rodriguez-Perales and Raul Torres-Ruiz generated a human genome editing-based MLL::AF4 ALL model recapitulating key cellular and molecular leukemogenic features (work published in *Blood*).

One of the main strategic initiatives of the CNIO at present is the consolidation of a new Cancer Genomics Programme. HCGP groups will become part of the new Programme. I am confident that, under this new strategic action, the Programme will continue to grow and further develop research in the field of cancer genomics. ∎

Maria A Blasco, Director

HEREDITARY ENDOCRINE CANCER GROUP

Mercedes Robledo
Group Leader

Research Scientists
Alberto Cascón,
Cristina Rodríguez (until October)

Post-Doctoral Fellows
Ester Arroba (since June), Luis Javier
Leandro, Ángel Mario Martínez,
Cristina Montero, Clara Reglero
(since June), Alberto Díaz (*CIBERER*,
Madrid)

Gradutate Students
Javier De Nicolás, Javier Lanillos (until February), Natalia Martínez (*CIBERER*, Madrid), Sara Mellid, Maria Monteagudo, Carlos Valdivia

Technicians
Alicia Arenas (since February) (TS)*, Mariolga Berrizbeitia (since October) (TS)*, Rocío Letón, Gabriela Roberta Radu (PEJ, CAM)**

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

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 therapeutically targeted, 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 targeted and whole-exome next-generation sequencing 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 showed the utility of immunological parameters with prognostic value in PPGL, showed that DLST mutations remodel cellular succinyloma — being a promising therapeutic target — and defined novel RCC drug response predictive markers."

RESEARCH HIGHLIGHTS

DLST mutations in pheochromocytoma and paraganglioma (PPGL) cause proteome hyposuccinylation and metabolic remodelling

PPGLs are the most heritable tumours. One of the most recently identified PPGL susceptibility genes is *DLST*, a component of the OGDH complex that catalyses the conversion of alpha-ketoglutarate to succinyl-CoA in the tricarboxylic acid (TCA) cycle. In addition, *DLST* plays an understudied role in protein succinylation, a post-translational modification (PTM) that causes major chemical and structural changes to proteins, and has been shown to alter protein function. Accordingly, this PTM has been implicated in the development of several diseases, including cancer. Through an in-depth molecular characterisation of *DLST*-mutated PPGLs, we explored the underlying mechanisms of tumorigenesis, focusing on the role of succinylation. We found a dramatic decline in succinylation levels in the absence of DLST. Moreover, *DLST* mutations found in PPGLs remodel the cellular succinylome and cause a transcriptional shift from oxidative phosphorylation to a hypoxic cellular state. We concluded that global protein succinylation levels depend strongly on *DLST*, and proposed *DLST* as a promising therapeutic target for treating diseases linked to dysregulated succinylation.

Immunogenomics as a theranostic tool in the immunotherapy contexture of metastatic PPGL

The mechanisms triggering metastasis in PPGL are unknown, hindering therapeutic options for patients with metastatic tumours (mPPGL). Genomic profiling of a large cohort of mPPGLs allowed us to conclude that high mutational load, microsatellite instability, and somatic copy number alteration load are associated with *ATRX/TERT* alterations, and are suitable prognostic markers. Transcriptomic analysis defined signalling networks involved in the acquisition of metastatic competence and established a gene signature related to mPPGLs, highlighting *CDK1* as an additional mPPGL marker. Immunogenomics coupled with immunohistochemistry enabled us to identify a heterogeneous ecosystem at the level of the tumour microenvironment, linked to genomic subtype and tumour behaviour. Specifically, we identified a general immunosuppressive microenvironment in mPPGLs, the exception being *MAML3*-related tumours expressing *PD-L1*. We have discovered canonical markers of metastatic risk and suggested the utility of including immune parameters in clinical management for PPGL prognostication and identification of patients who might benefit from immunotherapy (FIGURE 1).

Novel predictive biomarkers for renal cancer therapy

Targeted therapy has improved the survival of patients with metastatic renal cell carcinoma (RCC). However, the large inter-patient variability in drug response stresses the urgent need to define novel predictive biomarkers. Here we provide 2 examples:

1. During clear cell RCC (ccRCC) tumour evolution, *VHL* inactivation is followed by secondary mutations linked to tumour progression. The mutational screening and transcriptomic analysis of large series of ccRCC showed that mutation of the chromatin remodeller genes *PBRM1* and *KDM5C* increases tumour angiogenesis and leads to higher benefit from antiangiogenic drug treatment. These mutations can modify the tumour microenvironment and might serve as predictors of antiangiogenic response.
2. mTOR inhibitors are used to treat RCC. Whole exome sequencing of 3 chromophobe RCC patients with an exceptional mTOR inhibitor sensitivity revealed that the deubiquitinase *USP9X* was the only shared mutated

▸ **PUBLICATIONS**

▸ Pamporaki C *et al.* (incl. Robledo M) (2023). Prediction of metastatic pheochromocytoma and paraganglioma: a machine learning modelling study using data from a cross-sectional cohort. *Lancet Digit Health* 5, e551-e559.

▸ Calsina B, Piñeiro-Yáñez E, Martínez-Montes ÁM, Caleiras E, Fernández-Sanromán Á, Monteagudo M, Torres-Pérez R, Fustero-Torre C, Pulgarín-Alfaro M, Gil E, Letón R, Jiménez S, García-Martín S, Martin MC, Roldán-Romero JM, Lanillos J, Mellid S, Santos M, Díaz-Talavera A, Rubio Á, González P, Hernando B, ... Rodríguez-Perales S, Domínguez O, Macintyre G, Currás-Freixes M, Rodríguez-Antona C, Cascón A, Leandro-García LJ, Montero-Conde C, Roncador G, García-García JF, Pacak K, Al-Shahrour F, Robledo M (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.

▸ Mellid S, García F, Leandro-García LJ, Díaz-Talavera A, Martínez-Montes ÁM, Gil E, Calsina B, Monteagudo M, Letón R, Roldán-Romero JM, Santos M, Lanillos J, Valdivia C, Martínez-Puente N, de Nicolás-Hernández J, Jiménez S, Pérez-Martínez M, Honrado E, Coloma J, Cerezo A, Santiveri CM, Esteller M, Campos-Olivas R, Caleiras E, Montero-Conde C, Rodríguez-Antona C, Muñoz J, Robledo M, Cascón A (2023). DLST mutations in pheochromocytoma and paraganglioma cause proteome hyposuccinylation and metabolic remodeling. *Cancer Commun (Lond)* 43, 838-843.

▸ Martin-Broto J *et al.* (incl. Robledo M) (2023). REGISTRI: Regorafenib in first-line of KIT/PDGFRA wild type metastatic GIST: a collaborative Spanish (GEIS), Italian (ISG) and French Sarcoma Group (FSG) phase II trial. *Mol Cancer* 22, 127.

▸ Roldán-Romero JM, Valdivia C, Santos M, Lanillos J, Maroto P, Anguera G, Calsina B, Martinez-Montes A, Monteagudo M, Mellid S, Leandro-García LJ, Montero-Conde C, Cascón A, Roncador G, Coloma J, Robledo M, Rodriguez-Antona C (2023). Deubiquitinase USP9X loss sensitizes renal cancer cells to mTOR inhibition. *Int J Cancer* 153, 1300-1312.

▸ Fischer A *et al.* (incl. Robledo M) (2023). Responses to systemic therapy in metastatic pheochromocytoma/paraganglioma: a retrospective multicenter cohort study. *Eur J Endocrinol* 189, 546-565.

▸ Santos M, Lanillos J, Caleiras E, Valdivia C, Roldan-Romero JM, ... Robledo M, Rodriguez-Antona C (2023). *PBRM1* and *KDM5C* cooperate to define high-angiogenesis tumors and increased antiangi-
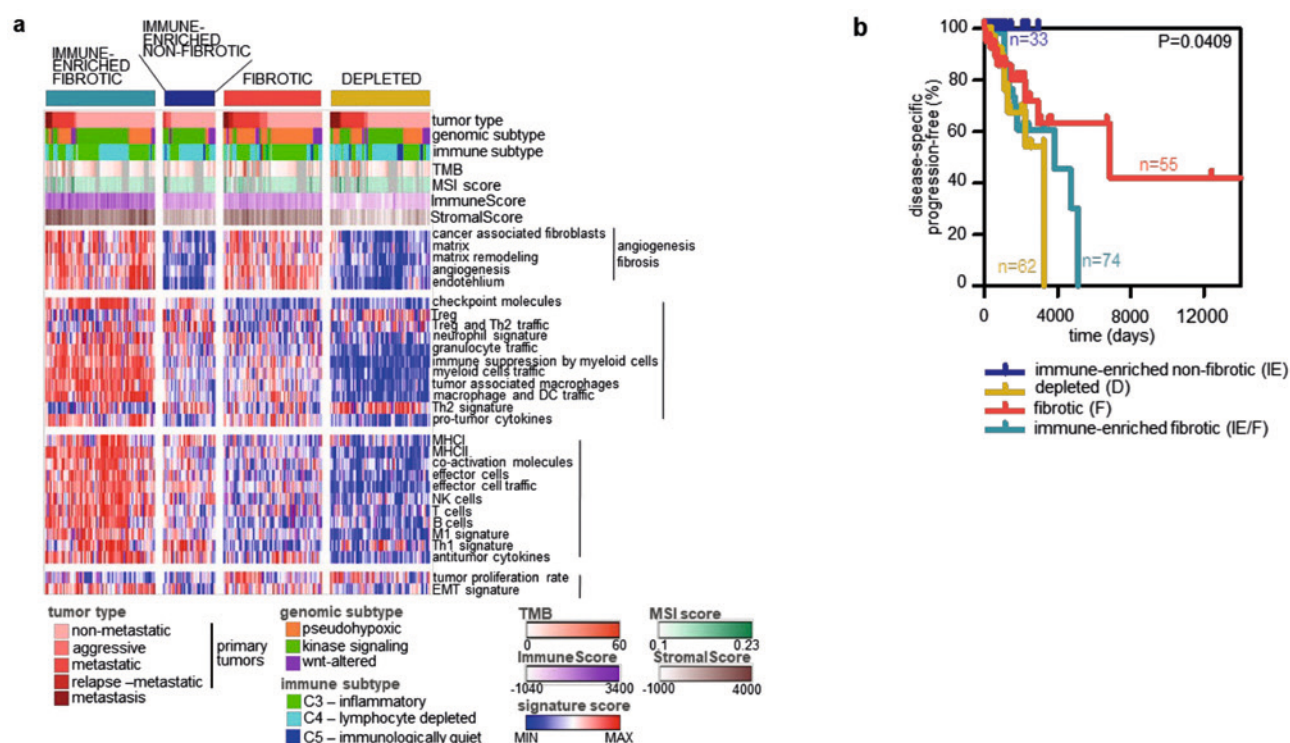
FIGURE 1 Immunogenomics as a theranostic tool in the immunotherapy contexture. (**a**) Heatmap of 267 PPGL tumours profiled by RNA-Seq and classified into 4 distinct TME subtypes. Genomic and clinical features are depicted in the legend. (**b**) Kaplan-Meier plot of time to progression in patients according to the primary tumour TME subtype (n=33 for IE, n=55 for F, n=74 for IE/F and n=62 for D). Only primary tumours from non-metastatic and metastatic patients are included. P-value was calculated using a log-rank test.

gene. Increased drug sensitivity in mutant cells was verified using cell line models. Proteomic analyses and immunofluorescence assays demonstrated a p62-mediated deregulation of autophagy in USP9X-depleted cells that has a synergistic effect with mTOR inhibitors. Thus, we defined *USP9X* as a potential novel marker of sensitivity to mTOR inhibitors and a target for cancer. ■

ogenic response in renal cancer. *Am J Cancer Res* 13, 2116-2125.

‣ Fischer A *et al.* (incl. Robledo M) (2023). PD-L1 and HIF-2α upregulation in head and neck paragangliomas after embolization. *Cancers (Basel)* 15, 5199.

‣ Kang EY *et al.* (incl. García MJ, Rodríguez-Antona C, Benitez J) (2023). CCNE1 and survival of patients with tubo-ovarian high-grade serous carcinoma: an ovarian tumor tissue analysis consortium study. *Cancer* 129, 697-713.

‣ Li M *et al.* (incl. Robledo M) (2023). Recurrent disease in patients with sporadic pheochromocytoma and paraganglioma. *J Clin Endocrinol Metab* 108, 397-404.

‣ Fischer A *et al.* (incl. Robledo M) (2023). Metastatic pheochromocytoma and paraganglioma: somatostatin receptor 2 expression, genetics, and therapeutic responses. *J Clin Endocrinol Metab* 108, 2676-2685.

‣ Li M *et al.* (incl. Robledo M) (2023). Regulation of epinephrine biosynthesis in HRAS-mutant paragangliomas. *Endocr Relat Cancer* 30, e230230.

‣ Gimenez-Roqueplo AP*, Robledo M*, Dahia PLM* (2023). Update on the genetics of paragangliomas. *Endocr Relat Cancer* 30, e220373.

‣ Mellid S, Gil E, Letón R, Caleiras E, … Robledo M, Cascón A (2023). Co-occurrence of mutations in *NF1* and other susceptibility genes in pheochromocytoma and paraganglioma. *Front Endocrinol (Lausanne)* 13, 1070074.

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‣ Karakaya S *et al.* (incl. Robledo M) (2023). Cytoplasmic HIF-2α as tissue biomarker to identify metastatic sympathetic paraganglioma. *Sci Rep* 13, 11588.

‣ Leandro-García LJ, Landa I (2023). Mechanistic insights of thyroid cancer progression. *Endocrinology* 164, bqad118.

‣ May T *et al.* (incl. Benitez J, Rodriguez-Antona C) (2023). Clinical parameters affecting survival outcomes in patients with low-grade serous ovarian carcinoma: an international multicentre analysis. *Can J Surg* 66, E310-E320.

‣ Piroli GG *et al.* (incl. Mellid S, Cascón A) (2023). Defective function of α-ketoglutarate dehydrogenase exacerbates mitochondrial ATP deficits during complex I deficiency. *Redox Biol* 67, 102932.

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GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

Núria Malats
Group Leader

Research Scientist
M. Evangelina López De Maturana

Post-Doctoral Fellows
Brune De Dreuille (since Nov.),
Pablo Villoslada-Blanco (since July), Carlos Castilla (since Nov.) (*CIBERONC*)

Gradutate Students
Jiangchuan He, Francisco J Jurado, Alberto Langtry, Víctor Sobrino (*UPM*), Casper Van Eijck (Erasmus Univ. Mc Rotterdam, until Nov.), Nannan Xue (CSC)

Technicians
Lola Alonso (TS)*,

Raquel Benítez (TS)*(Project Manager), Lidia Estudillo, Lucas Friedman (since June) (TS)*, Yaiza Gutiérrez, Ane Moreno (TS)* (*CIBERONC*), Vanesa Moreno (until Oct.) (TS)*, María Olano (TS)*, Laura Paniagua (since Dec.), Sergio Sabroso (TS)*

Titulado Superior (Advanced Degree)

Students in Practice
Sol Aletta (until Aug.) (Erasmus +, Rabdoud University, Nijmegen, The Netherlands), Vivian Guzmán (March-June) (*IES Mirasierra,* Spain), Teresa Hernández (March-July) (*ETSIT*, Madrid, Spain), Gabriele Scognamiglio (Feb.-June) (*Univ. Carlos III de Madrid*, Spain),

Elvira Urbano (March-May) (*UCM,* Madrid, Spain)

Visiting Scientists
Carmen Mota (April-July) (*Technische Universität München*, Germany), Isabel A. Martín (*Univ. CEU San Pablo*, Madrid, Spain)

OVERVIEW

The scope of the research carried out by the Genetic and Molecular Epidemiology Group (GMEG) ranges from the identification of aetiological agents and genetic pathways to the translation of the findings into the clinical and public health domains, focusing on bladder, pancreatic, and breast cancers.

We employ a wide variety of biomarkers, including omics data, to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. While omics data provide a unique opportunity in this regard, their integration with non-omics data poses important challenges, and GMEG explores this methodological field in epidemiologic studies.

The strategic goals of the Group are to:

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

"Epidemiological studies aim to analyse the causal relationship between an exposure and an outcome. We have applied causal inference approaches to assess bias generated by factors that may distort the real effect of the exposure."

RESEARCH HIGHLIGHTS

Research findings

In 2023, GMEG contributed to the **pancreatic cancer** (PC) field by undertaking a study that showed that PDAC tumours of women are more sensitive to gemcitabine-based neoadjuvant chemoradiotherapy (nCRT), resulting in longer survival after resection compared with men. The tumour microenvironment (TME) of women contained fewer protumoural macrophages after nCRT, highlighting the importance of considering sex disparities for PDAC treatment. GMEG also contributed to showing that GATA4 and GATA6 cooperate to maintain the classical phenotype. Reduced expression of both proteins in tumours was associated with the worst patient survival. *GATA4* and *GATA6* expression was significantly decreased in metastases and negatively correlated with basal markers. On **bladder cancer** (BC), GMEG conducted a study to characterise the muscle-invasive bladder cancer (MIBC) microenvironment by analysing the tumour-infiltrating B and T cell repertoire according to the taxonomic molecular subtypes. We used RNAseq data from 396 MIBC samples included in TCGA. We found different patterns of tumour-infiltrating immune repertoire among the different MIBC subtypes. In addition, we observed that the Ba/Sq and Stroma-rich tumours were more clonally expanded than the Luminal subtypes (FIGURE 1). We also found that higher TCR richness and diversity were significantly associated with better survival in the Stroma-rich and Ba/Sq subtypes. Importantly, GMEG contributed to an international meta-analysis of genome-wide genotype data led by the NCI (USA) to identify new susceptibility variants for bladder cancer, summing up 24 independent BC susceptibility markers at genome-wide significance. Combined with smoking, they may inform screening interventions for BC. Also in collaboration with the NCI, we demonstrated that smoking and BK polyoma virus infection contribute to bladder tumorigenesis through distinct molecular mechanisms involving different *FGFR3* and *PIK3CA* mutations. Finally, we
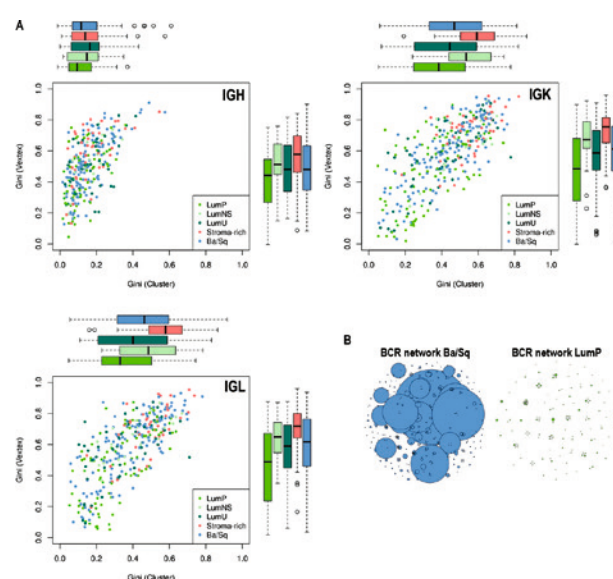
FIGURE 1 BCR clonal expansion and diversification by muscle invasive bladder cancer subtypes. (**A**) Clonal expansion by using the Gini Index (Y axis) and clonal diversification displayed by Gini Vertex (X axis). (**B**) B-cell repertoire networks from 2 samples representing one Ba/Sq (blue) and one LumP (green).

identified a predictive signature for response to neoadjuvant chemotherapy in MIBC patients that integrates the expression of 3 genes with clinicopathological characteristics and taxonomic subtypes.

Methodological contributions

We further applied causal inference approaches such as mediation analysis (CMA), which consider the mediation effect of a third variable, and proposed an extension of CMA,

▸ **PUBLICATIONS**

▸ Van Eijck CWF, Mustafa DAM, Vadgama D, de Miranda NFCC, Groot Koerkamp B, van Tienhoven G, van der Burg SH, Malats N*, van Eijck CHJ*; Dutch Pancreatic Cancer Group (DPCG) (2023). Enhanced antitumour immunity following neoadjuvant chemoradiotherapy mediates a favourable prognosis in women with resected pancreatic cancer. *Gut*. PMID: 37709493. (*) Co-corresponding authors.

▸ de Andrés MP, Jackson RJ, Felipe I, Zagorac S, Pilarsky C, Schlitter AM, Martinez de Villareal J, Jang GH, Costello E, Gallinger S, Ghaneh P, Greenhalf W, Knösel T, Palmer DH, Ruemmele P, Weichert W, Buechler M, Hackert T, Neoptolemos JP, Notta F, Malats N, Martinelli P, Real FX (2023). GATA4 and GATA6 loss-of-expression is associated with extinction of the classical programme and poor outcome in pancreatic ductal adenocarcinoma. *Gut* 72, 535-548.

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▸ Filip I, Wang A, Kravets O, Orenbuch R, Zhao J, Perea-Chamblee TE, Manji GA, López de Maturana E, Malats N, Olive KP, Rabadan R (2023). Pervasiveness of HLA allele-specific expression loss across tumor types. *Genome Med* 15, 8.

▸ Benítez R, Yu K, Sirota M, Malats N, Pineda S (2023). Characterization of the tumor-infiltrating immune repertoire in muscle invasive bladder cancer. *Front Immunol* 14, 986598.

▸ King SD *et al.* (incl. Malats N, Real FX) (2023). Genetic susceptibility to non-alcoholic fatty liver disease and risk for pancreatic cancer: Mendelian random-ization. *Cancer Epidemiol Biomarkers Prev* 32, 1265-1269.

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▸ Rao N, Starrett, GJ, Piaskowski ML, Butler KE, Golubeva Y, Yan W, Lawrence SM, Dean M, Garcia-Closas M, Baris D, Johnson A,
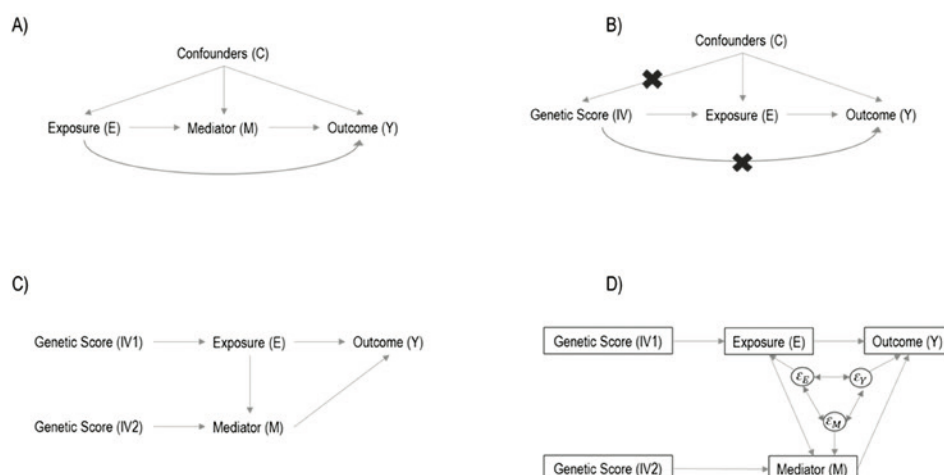
FIGURE 2 Causal diagrams. (**A**) Causal mediation analysis (CMA) graph. (**B**) Mendelian randomisation graph. (**C**) Mediation analysis with instrumental variables (IVs). (**D**) Structural equation model (SEM), where directed arrows represent causal effect, bidirectional arrows indicate a correlation between variables.

combining it with Mendelian randomisation (MRinCMA) to address the limitations resulting from fitting strong assumptions on confounding bias (FIGURE 2). We applied the new approach to analyse the causal effect of obesity and diabetes on pancreatic cancer, considering each factor as potential mediator. By applying MRinCMA, we did not find any evidence of causality of obesity or diabetes on pancreatic cancer. With this new methodology, researchers would be able to address CMA hypotheses by appropriately accounting for the confounding bias assumption, regardless of the conditions used in their studies in different settings. Furthermore, we performed a benchmarking analysis of 5 tools for microbe sequence detection using transcriptomics data (Kraken2, MetaPhlAn2, PathSeq, DRAC, and Pandora). To this end, we built a synthetic database mimicking real-world structure with tuned conditions accounting for microbe species prevalence, base calling quality, and sequence length. Results from this study supported the use of Kraken2 for routine microbiome profiling based on its competitive sensitivity and runtime performance. Nonetheless, we strongly endorse to complement it by combining with MetaPhlAn2 for thorough taxonomic analyses.

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 we have advanced in increasing awareness about PC. We also contributed to the European Alliance for Personalised Medicine (EAPM) series of expert interviews to ascertain the current state of the uptake of advanced molecular diagnostics/NGS for quick and efficient genetic profiles of tumour cells across member states. ∎

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▸ Coscia C, Molina-Montes E, Benítez R, López de Maturana E, Muriel A, Malats N, Pérez T (2023). New proposal to address mediation analysis interrogations by using genetic variants as instrumental variables. *Genet Epidemiol* 47, 287-300.
▸ Rodríguez-García Y, Martínez-Moreno M, Alonso L, Sánchez-Vencells A, Arranz A, Dagà-Millán R, Sevilla-Movilla S, Valeri A, Martínez-López J, Teixidó J (2023). Regulation of miRNA expression by α4β1 integrin-dependent multiple myeloma cell adhesion. *EJHaem* 4, 631-638.
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Book Chapter

▸ López de Maturana E, Zagorac S, Malats N, Real FX (2023). Vitamin D and pancreatic cancer. In M. Hewison, R. Bouillon, E. Giovannucci, D. Goltzman, M. Meyer, J. Welsh (Eds.), *Feldman and Pike's Vitamin D Volume II: Health, Disease and Therapeutics* (5th ed., pp. 937-966). Elsevier Academic Press. ISBN: 9780323913386.

▸ **AWARDS AND RECOGNITION**

▸ GMEG was awarded a European Innovative Health Initiative project "Guiding Multi-Modal Therapies Against Minimal Residual Disease by Liquid Biopsies" (GUIDE.MRD Study), PI: K. Pantel (UKE Hamburg), with the participation of both academic and private partners.

MOLECULAR CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Research Scientist
Raúl Torres

Gradutate Students
Alejandro Alonso, Alejandro Nieto, Pilar Puig

OVERVIEW

The Molecular Cytogenetics and Genome Editing Unit is dedicated to understanding the role of chromosomal rearrangements in cancer progression and identifying new therapeutic targets. These rearrangements, which alter the structure of chromosomes, are frequent and recognised characteristics of cancer. By using a combination of CRISPR genome editing and cytogenetic technologies, the Unit creates models that mimic chromosomal and genetic alterations found in cancer. The aim is to enhance our understanding of cancer-causing mechanisms, which could lead to the development of innovative cancer treatments. The Unit also provides researchers at the CNIO and other institutions with the latest molecular cytogenetics and genome editing technologies, and actively collaborates on projects with clinical and basic science investigators. The Unit continually implements and develops new technologies in these fields.

"We use gene editing to mimic and eliminate chromosomal or gene alterations, enhancing our understanding of cancer and developing new treatment tools. We offer cutting-edge cytogenetic and CRISPR technologies."

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

Titulado Superior (Advanced Degree)

Students in Practice
Cristina Benito (Jan.-Sept.) (Master's Thesis, *UAM,* Madrid, Spain), Ana M. Calleja and Chaimaa El Morabit (March-June) (*IES José Luis Sampedro,* Madrid, Spain), Sara Carpintero

(Jan.-July) (*Univ. de Alcalá de Henares*, Spain), Pauline de Sousa (May-Aug.) (*Univ. Clermont Auvergne*, France), María Fernández (since Oct.) (Bachelor's Degree Final Project, *Univ. CEU San Pablo*, Madrid, Spain)

Visiting Scientist
Paula Díaz (since Oct.) (*Instituto i+12*, Madrid, Spain)

RESEARCH HIGHLIGHTS

Around 20% of human cancers have specific fusion oncogenes (FOs), which are unique diagnostic and therapeutic targets due to their tumour-specific expression. Conventional diagnostic techniques like qRT-PCR, FISH, or NGS are routine but require specialised machinery and personnel, are expensive, time-consuming, and involve multiple steps. Many current treatments are non-selective, leading to severe side effects. We have applied the RNA-targeting Cas13 system for selective cleavage of FO transcripts. Unlike the CRISPR/Cas9 method, RNA targeting with Cas13 results in reversible and temporally controllable alterations without DNA modification. Compared to shRNAs, the Cas13 method has high knockdown efficiency and no off-target effects, offering unique advantages for therapeutic purposes. Diagnostic methods based on Cas13 provide rapid RNA detection with attomolar sensitivity and single-base mismatch specificity.

Our Unit has been enhancing the Optical Genome Mapping (OGM) technique, a method that analyses ultra-long DNA molecules for high-resolution, genome-wide analysis of cytogenetic variations. We are comparing OGM to standard techniques like karyotyping and fluorescent *in situ* hybridisation using various tumour samples. OGM is a promising complementary approach to cytogenetic techniques for cancer cell characterisation, offering a cost-effective analysis and identification of complex cytogenetic rearrangements. ■
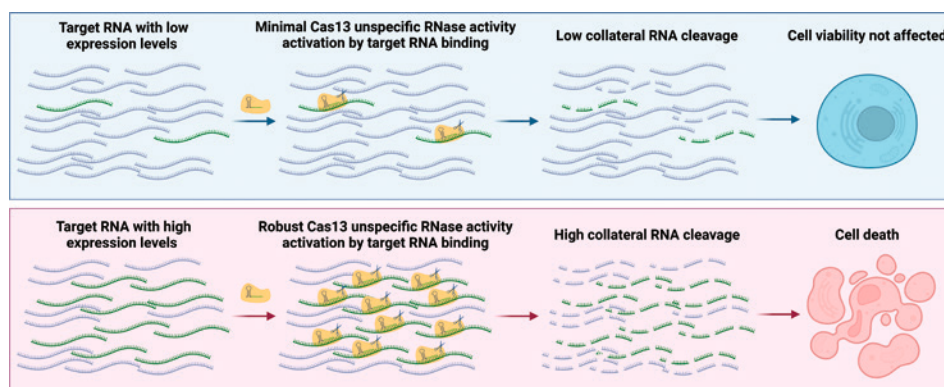
FIGURE 1 CRISPR/Cas13 targets RNA, not DNA. Once activated by target RNA, it cleaves nearby RNA molecules indiscriminately, known as the collateral effect. The extent of this effect is linked to the target transcript's expression level and varies with the cell type, potentially leading to cell death.

▸ **PUBLICATIONS**

▸ Bueno C, Torres-Ruiz R *et al.* (incl. Rodriguez-Perales S) (2023). A human genome editing-based MLL::AF4 ALL model recapitulates key cellular and molecular leukemogenic features. *Blood* 142, 1752-1756.
▸ Salmón M *et al.* (incl. Martín-Guijarro MC, Rodríguez-Perales S, Mulero F, Al-Shahrour F, Martínez L, Domínguez O, Caleiras E, Ortega S, Barbacid M) (2023). Kras oncogene ablation prevents resistance in advanced lung adenocarcinoma. *J Clin Invest* 133, e164413.
▸ Calsina B *et al.* (incl. Caleiras E, Martin MC, Rodríguez-Perales S, Domínguez O, Macintyre G, Currás-Freixes M, Roncador G, Al-Shahrour F, Robledo M) (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.
▸ Morgado-Palacin L *et al.* (incl. Rodriguez-Perales S) (2023). The TINCR ubiquitin-like microprotein is a tumor suppressor in squamous cell carcinoma. *Nat Commun* 14, 1328.
▸ Zheng K *et al.* (incl. Rodríguez-Perales S, Torres-Ruiz R) (2023). Neuroblastoma RAS viral oncogene homolog (N-RAS) deficiency aggravates liver injury and fibrosis. *Cell Death Dis* 14, 514.
▸ Martín-Adrados B *et al.* (incl. Torres-Ruiz R, Rodriguez-Perales S) (2023). Expression of HMGCS2 in intestinal epithelial cells is downregulated in inflammatory bowel disease associated with endoplasmic reticulum stress. *Front Inmmunol* 14, 1185517.
▸ Lahera A *et al.* (incl. Rodríguez-Perales S, Torres-Ruiz R) (2023). Comprehensive characterization of a novel, oncogenic and targetable SEPTIN6::ABL2 fusion in T-ALL. *Br J Haematol* 202, 693-698.
▸ Ugalde L *et al.* (incl. Torres R) (2023). CRISPR/Cas9-mediated gene editing. A promising strategy in hematological disorders. *Cytotherapy* 25, 277-285.

FAMILIAL CANCER CLINICAL UNIT

Maria Currás
Clinical Unit Head

Post-Doctoral Fellow
Bruna Calsina

Gradutate Student
Erik Michel Marchena (until March)
(PEJ, CAM) *

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

OVERVIEW

The Unit's activity is divided into 2 main areas:

1. *Genetic diagnosis in patients with suspected hereditary cancer using next-generation sequencing.* 52% of our activity is dedicated to genetic diagnosis in the Familial Cancer Clinic of the University Hospital of Fuenlabrada (FCC-UHF), and 48% to providing this service to other hospitals in Madrid and the rest of Spain. 59% of the genetic diagnoses were carried out in index cases, while 41% were predictive studies in relatives to determine if they are carriers of a variant. We completed 203 urgent case studies to guide surgery and/or treatment, with results reported in less than 6-8 weeks. We also performed up to 50 extensions of previous studies (gene updates/tumour) and 67 variant reclassification reports (genetic information updates).

2. *Research work.* We participated in the identification of

"The FCCU performed genetic diagnoses on more than 2000 patients, and more than 1000 were evaluated at the FCC-UHF. We played an important role in selecting families for the execution of the IMPaCT-GENóMICA project."

genetic factors and the interpretation of genetic variants of unknown significance (VUS) in the PTEN Hamartoma Tumour Syndrome (PHTS). We are involved in the IMPaCT-GENóMICA and IMPACT-VUSCan projects, having been one of the main participants in the Spanish Hereditary Cancer Variants Database (SpadaHC).

Technicians
Ana I. Corao, Victoria Fernández (TS)*, Verónica García (TS)*, Eduardo Gil, Miguel Á. Grillo (since March), Maika González-Neira (until May), Fátima Mercadillo,

Pilar Redondo (since May), Milton E. Salazar (since August) (TS)*

*Titulado Superior (Advanced Degree)

Undergraduate Visiting Student
Katelyn Lee (June-July) (Massachusetts Institute of Technology, USA)

RESEARCH HIGHLIGHTS

Clinical and diagnostic activity

1068 patients were evaluated at the FCC-UHF and 2016 genetic studies were performed at the Familial Cancer Clinical Unit (FCCU) during 2023. A total of 355 pathogenic or probable pathogenic variants were identified, allowing families and patients to benefit from appropriate clinical follow-up, early detection in unaffected patients, and/or personalised medicine. We implemented the diagnosis of MLH1 methylation in endometrial and colorectal tumours and the genetic diagnosis of healthy individuals with deceased relatives affected by cancer. In addition, we updated the genes to be studied per tumour and expanded the range of tumours in which the presence of germline mutations is excluded (including bladder and genitourinary tract tumours, among others).

IMPaCT-GENóMICA

This project aims to identify genetic variants that could explain cancer predisposition. The FCCU participated, among other centres in Spain, in the clinical decision-making committee to choose the most appropriate unsolved familial cases. A total of 22 families from Madrid and 169 Spanish families were recruited. Our Unit contributed 6 families with cases related to prostate (3), testicular (1), pancreatic (1), and breast (1) cancers.

We will also be involved in the analysis and integration of data for candidate variant prioritisation and functional evaluation, among other CNIO Groups and Units.

SpadaHC

SpadaHC is a database for sharing genetic variants identified in hereditary cancer genes, which currently includes data from 15 Spanish genetic diagnostic reference laboratories. The database also provides frequencies of these variants in the Spanish population and is a useful resource for research and clinical genetic laboratories to improve knowledge of the genetic basis of hereditary cancer.

The current version of the database contains 1.16M genetic variants from 4294 individuals and 14776 variant classifications. A total of 2469 variants had different pathogenicity classifications, and we participated in their homogenisation. For this purpose, we took into account the criteria of the American College of Medical Genetics and the results of our laboratory, and resolved the discrepancies in 84 clinically relevant variants. For most of the variants, a consensus on the best classification was reached to provide better clinical decision-making support.

PTEN variants

We participated in the comprehensive functional characterisation of 6 novel variants of unknown significance identified in the *PTEN* gene and also initiated a collaboration with the PTEN Research Foundation on premalignant lesions and vascular malformations in patients with PHTS. ∎

‣ **PUBLICATIONS**

‣ Pairo-Castineira E *et al.* (incl. Mercadillo F as collaborator) (2023). GWAS and meta-analysis identifies 49 genetic variants underlying critical COVID-19. *Nature* 617, 764-768.
‣ Calsina B *et al.* (incl. Currás-Freixes M, Gil E) (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.
‣ Mellid S *et al* (incl. Calsina B, Gil E) (2023). DLST mutations in pheochromocytoma and paraganglioma cause proteome hyposuccinylation and metabolic remodeling. *Cancer Commun (Lond)* 43, 838-843.
‣ Castro-Santos P *et al.* (incl. Mercadillo F as collaborator) (2023). HLA-A*11:01 and HLA-C*04:01 are associated with severe COVID-19. *HLA* 102, 731-739.
‣ Roldán-Romero JM *et al* (incl. Calsina B) (2023). Deubiquitinase USP9X loss sensitizes renal cancer cells to mTOR inhibition. *Int J Cancer* 153, 1300-1312.
‣ Mellid S *et al.* (incl. Gil E) (2023) Co-occurrence of mutations in NF1 and other suceptibility genes in pheochromocytoma and paraganglioma. *Front Endocrinol (Lausanne)* 13, 1070074.
‣ Torices L *et al.* (incl. Mercadillo F, Currás M). (2023). Functional analysis of PTEN variants of unknown significance from PHTS patients unveils complex patterns of PTEN biological activity in disease. *Eur J Hum Genet* 31, 568-577.
‣ Cascón A *et al.* (incl. Calsina B, Currás-Freixes M) (2023). Genetic bases of pheochromocytoma and paraganglioma. *J Mol Endocrinol* 70, e220167.
‣ Li M *et al* (incl. Calsina B) (2023). Regulation of epinephrine biosynthesis in HRAS-mutant paragangliomas. *Endocr Relat Cancer* 30, e230230.

‣ **AWARDS AND RECOGNITION**

Bruna Calsina :
‣ ENSAT Scientific Award, The European Network for the Study of Adrenal Tumors (ENSAT).
‣ Award for PhD-Authored Publications, CNIO Lab day.

HUMAN GENOTYPING-*CEGEN* UNIT

Anna González-Neira
Unit Head

Gradutate Students
Hugo Tejera, Alejandro Velasco (until May)

Bioinformatician
Guillermo Pita (TS)*

OVERVIEW

Our Unit offers cutting-edge genotyping and sequencing services tailored to diverse research needs, continuously innovating to meet evolving requirements. In 2023, the Centre has boosted its research capabilities with the acquisition of a cutting-edge sequencing platform (*NovaSeq X Plus System*). This advanced technology has propelled the Centre to the forefront of scientific innovation, enabling the provision of new sequencing services at reduced cost.

Our research focuses on identifying genetic factors that influence breast cancer susceptibility and treatment efficacy and toxicity. Through our research, we aim to refine personalised risk assessment for breast cancer, pioneer innovative strategies for early detection, and improve the precision and safety of cancer treatment for patients.

> "Our research on breast cancer aims to enhance breast cancer risk prediction, guide risk-stratified breast screening strategies, and improve the safety of treatment approaches."

Additionally, we strive to implement this information in the clinical setting. By translating our research findings into practice, we aim to improve patient outcomes by providing more individualised and effective approaches to cancer prevention, detection, and treatment.

Technicians
Charo Alonso, Núria Álvarez,
Belén Herráez, Rocío Núñez (TS)*

Titulado Superior (Advanced Degree)

Students in Practice
Susana González (June-Oct.)
(Master's Programme in
Bioinformatics, *ENS-ISCIII*, Madrid,
Spain), Courtney Saqueton (since

Sep.) (Fulbright Predoctoral
Researcher, USA)

RESEARCH HIGHLIGHTS

Identification of novel breast cancer susceptibility genes by exome sequencing

To comprehensively assess the role of rare coding variants, we conducted a meta-analysis using 3 extensive whole-exome sequencing datasets, comprising 26,368 female cases and 217,673 female controls. Significant associations between protein-truncating variants and breast cancer were discovered for 6 genes at an exome-wide significance level (P < 2.5 × 10-6): ATM, BRCA1, BRCA2, CHEK2, PALB2, and MAP3K1. Additionally, associations were found for LZTR1, ATR, and BARD1 with P < 1 × 10-4 (Wilcox *et al.* 2023).

Genetic characterisation and clinical impact of 21 actionable pharmacogenes in the Spanish population

We analysed genetic data from 3,006 Spanish individuals to determine the allele frequencies for 21 actionable pharmacogenes. Our findings show that 98% of the Spanish population carries at least 1 allele linked to a therapeutic change. We translated this genetic data into clinical recommendations, suggesting an average need for therapeutic changes in 3.31 out of 64 associated drugs (https://csvs.clinbioinfosspa.es/) (Nuñez-Torres *et al.* 2023).

Novel genetic variants associated to susceptibility to SARS-CoV-2 infection and disease severity

We participated in the second updated genome-wide association study (GWAS) on COVID-19 severity and infection susceptibility to SARS-CoV-2 from the COVID-19 Host Genetic Initiative. A meta-analysis of up to 219,692 cases and over 3 million controls identified 51 distinct genome-wide significant loci – adding 28 loci from the previous data release (Pairo-Castineira E *et al.* and Castro-Santos P *et al.* 2023).

Identification of anthracycline-induced cardiotoxicity (AIC) genetic risk markers by intermediate molecular phenotypes (IMPs)

AIC affects cancer patients, but we cannot predict who may suffer from this complication. We propose that levels of IMPs in the myocardium associated with histopathological damage could explain AIC susceptibility, so variants of genes encoding these IMPs could identify patients susceptible to this complication. We found genetic variants linked to these markers, and 2 genetic risk scores for paediatric and breast cancer patients were constructed (Gómez-Vecino A *et al.* 2023). ∎

▸ **PUBLICATIONS**

▸ Pairo-Castineira E *et al.* (incl. González-Neira A as collaborator) (2023). GWAS and meta-analysis identifies 49 genetic variants underlying critical COVID-19. *Nature* 617, 764-768.

▸ COVID-19 Host Genetics Initiative (incl. González-Neira A as collaborator) (2023). A second update on mapping the human genetic architecture of COVID-19. *Nature* 621, E7-E26.

▸ Lopes Cardozo JMN *et al.* (incl. González-Neira A as collaborator) (2023). Associations of a breast cancer polygenic risk score with tumor characteristics and survival. *J Clin Oncol* 41, 1849-1863.

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CLINICAL RESEARCH PROGRAMME

MIGUEL QUINTELA-FANDINO Acting Programme Director

The Clinical Research Programme (CRP) has 2 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) development of novel agents; 2) study of mechanisms of action of novel compounds and tackling drug resistance; and 3) moving forward in the field of biomarkers, functional taxonomy, and precision medicine. Currently, 2 functional objectives summarise the new operating model: A) generating synergies with ongoing research lines in the basic research programmes; and B) constituting a bi-directional bridge closing interactions between CNIO and tertiary cancer hospitals.

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 4 coordinated grants, which translate into the high translational research activity of the Centre. During the past year, 11 medical oncology residents from different Spanish hospitals completed their 3-month optional visiting stays at CNIO.

For 2023 we are pleased to mention the following research highlights. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, launched a large multi-centric, high-definition oncology project aiming to design the first cancer "Patient Digital Twin". 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.

Of note, during 2023 we incorporated 2 new senior groups into the CRP: the H12O-CNIO Cancer Immunotherapy Clinical Research Unit, led by Luis Álvarez-Vallina; and the IdiPaz-

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

CNIO Pediatric Onco-Haematology Clinical Research Unit, led by Antonio Pérez-Martínez. These 2 groups will cover 2 unmet needs at the CNIO: the development of novel cancer immunotherapy agents, and research in children's cancers. ∎

BREAST CANCER CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Clinical Research Unit Head

Research Scientists
María José Bueno, Leonardo Garma
(since April), Silvana A. Mouron

Clinical Research Fellow
Desirée Jiménez (since February)

Post-Doctoral Fellows
Rebeca G. Jimeno (until March),
Ana M. Roncero

Gradutate Student
José Luis Ruiz (until November)

Technicians
Verónica Jiménez, Manuel Muñoz, Ángela Sánchez (since February) (TS)*

Titulado Superior (Advanced Degree)

Students in Practice
Pien Debets (until August) (Master's Thesis, University of Amsterdam, The Netherlands), William Murray (March-June) (*Universidad Carlos Iii*, Madrid, Spain)

Visiting Scientists
Andrea Gutiérrez (until November) (*Hospital Universitario de Fuenlabrada*, Madrid, Spain), Rocío Moreno (*Hospital Universitario 12 de Octubre*, Madrid, Spain), Berta Nassarre (Peaches Biotech,

Madrid, Spain), Mai Tolba (February-May) (Ain Shams University, Egypt) (Science by Women Programmme)

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.

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.

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

RESEARCH HIGHLIGHTS

In 2023, we started a new major line of research – High-Definition Oncology. Currently, precision oncology is largely based on genomics and performs poorly outside the context of matching targeted agents with oncogene addiction driving alterations. Outside 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 interpatient heterogeneity beyond genomics. These factors include, for example, the patient's exposome (explained by factors such as diet or the environment, and translated 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 points in time, obtaining only snapshots of what it is in reality a disease trajectory. To take into account these 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 on 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".

Still in the area of precision medicine, we published a high-impact manuscript regarding the real-world impact of NGS panels outside the context of oncogenic addiction. We established a set of indications according to clinical criteria for these genetic panels and compared the efficacy in terms of patient overall survival according to the currently accepted ESCAT criteria. We found that the most important factor determining overall survival in patients with advanced cancer was the adhesion to these clinical guidelines (shown in FIGURE 1).
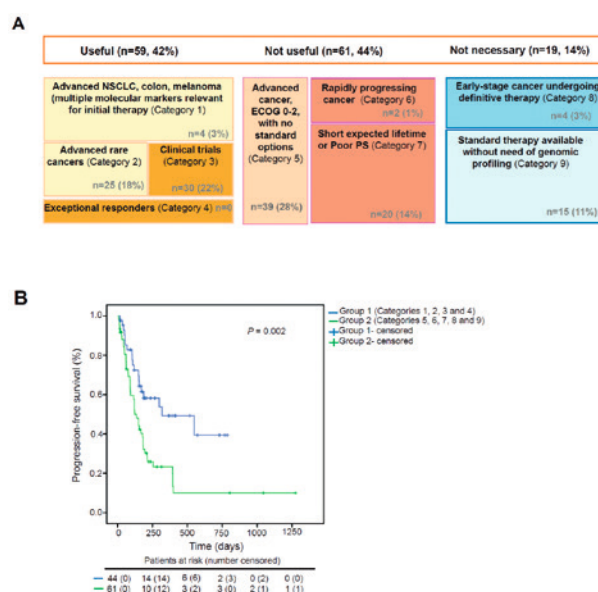
FIGURE 1 Clinical indications for NGS panels in oncology. (**A**) Among all common situations in which clinicians order NGS panels, only a few have demonstrated clinical utility: advanced tumours in which several targets need to be determined, such as lung, melanoma or colorectal cancer; rare cancers; patients screened for clinical trials; or exceptional responders. In most other situations, NGS panels are simply not justified. (**B**) Kaplan-Meier curves showing the progression-free survival of patients in which an NGS panel was ordered in the indicated categories (blue chart) versus patients in which the panel was ordered in other situations (green).

Another area of relevance within the field of treatment personalisation is the role of diet as an adjunct 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. Because 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

▸ **PUBLICATIONS**

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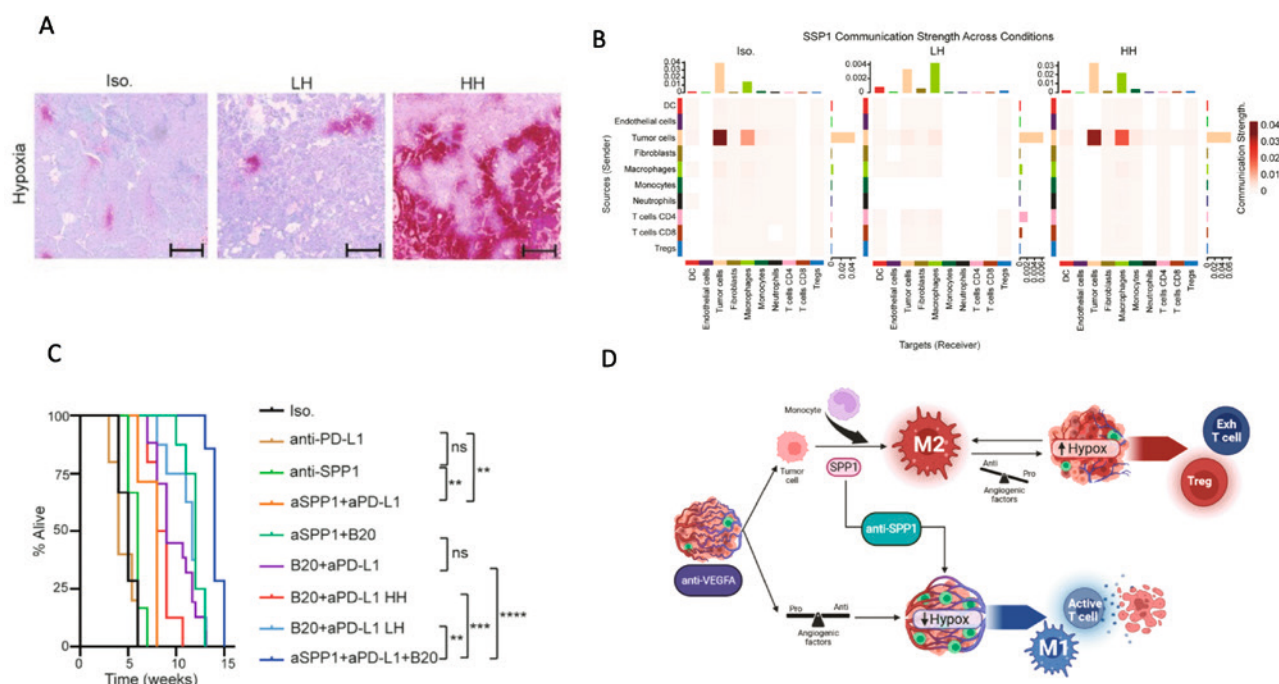
FIGURE 2 Acquired resistance against hypoxia-inducing antiangiogenics. (**A**) Murine breast tumour treated with isotype control (left), anti-SPP1 plus anti-angiogenic (middle), or antiangiogenic alone (right), stained with anti-pimonidazole, which labels hypoxic areas (red). It is notable how blocking SPP1 abrogates the development of hypoxia. (**B**) Single-cell RNA-seq CellChat analysis showing how in hypoxic conditions (right) there is increased communication through the SPP1 pathway between tumour cells and monocytes, compared with untreated tumours (left) or tumours that do not develop hypoxia (middle). (**C**) Kaplan-Meier mouse survival curves showing synergy between antiangiogenics and anti-PD-L1 when SPP1 is targeted, triplicating overall survival. (**D**) Mechanistic model: when SPP1 is high, monocytes are attracted to the tumour and differentiated into M2 macrophages, which initiate the immunosuppressive loop, segregate large quantities of VEGF, and perpetuate the hypoxic cycle.

microbiota parameters, and even patient dietary preferences, and that 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*.

Finally, in the field of resistance against antiangiogenics, we completed our comprehensive model. In the past, we described that when tumours experience vascular normalisation in response to such treatment, they become sensitive to mitochondrial inhibitors. We have now finalised our study investigating the circumstances under which tumours experience increased vascular abnormality: Osteopontin (Spp1) starts an immunosuppressive loop that elicits tumour escape and refractoriness to immunotherapy. Clearing Spp1 avoids the development of hypoxia and induces synergy between antiangiogenics and immunotherapy in breast cancer (FIGURE 2). ∎

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‣ **PATENT**

‣ Quintela Fandiño MA. P27 single-nucleotide polymorphism as a predictor of benefit of hormonal therapy alone or in combination with CDK inhibitors in breast cancer. *PCT/EP2022/051700*.

WO2022/161984. National Phase Entry (2023).

‣ **LICENSED PRODUCT**

‣ Luis Manso Sánchez, Miguel Quintela-Fandino (2023). LUMICA V. 1.0. Algorithm – a proprietary algorithm for precision nutrition allocation for cancer patients. Licensed to *TNC Nutrición Terapéutica S.L.*

‣ **AWARDS AND RECOGNITION**

‣ Creation of the CNIO Spin-Off *TNC Nutrición Terapéutica*, Spain.
‣ Personalised Precision Medicine Grant from the *Carlos III* Institute of Health (*IS-CIII*), awarded to Miguel Quintela-Fandino (CNIO), €2.5 million in funding over 3 years (2023-2025). *Integrating longitudinal patient-generated data and multi-omic profiling for comprehensive precision oncology in women's cancers*; this project is led by CNIO, in partnership with

9 hospitals, 2 universities and the *CRIS* Cancer Foundation, Spain.
‣ *AECC* Project Award from the Scientific Foundation of the Spanish Association Against Cancer (AECC), Spain.
‣ Advisory Board Member, Flagship Centers OHU-SDU, Odense University Hospital and South Denmark University, Denmark.
‣ Clinical Advisor, Kaertor Foundation, Spain.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

OVERVIEW

The Molecular Diagnostics Unit's (MDU) commitment to quality molecular testing, in both clinical and laboratory settings, ensures comprehensive support for cancer patient care and cancer research efforts by aligning with the growing needs of healthcare professionals and researchers. Thus, MDU plays a role in the Spanish healthcare system by offering a range of molecular diagnostic tests that aid clinicians in early cancer diagnosis, the detection of relapses, and monitoring therapy responses. These assays are constantly revised, integrating the latest diagnostic tests and upgrading the established ones. Likewise, the Unit provides support to CNIO's Research Groups by analysing their samples for specific biomarker alterations or by providing specialised technical assistance. MDU is at the forefront of molecular diagnostics standardisation, collaborating with international and national

"IVDR implementation will improve the accuracy, reliability and efficiency of cancer diagnostics testing, thus ensuring improved patient care." (FIGURE 1)

organisations. Finally, through laboratory training, the Unit coaches biomedical students, technicians, and residents.

Student in Practice
Génesis Belén Chérrez (February-
April) (Bachelor's Student,
Universidad Rey Juan Carlos, Madrid,
Spain)

CORE UNIT HIGHLIGHTS

During 2023, our catalogue grew with the addition of a new assay. This assay will enable the detection, through direct sequencing, of p.C515S substitution in exon 15 of the *BTK* (Bruton tyrosine kinase) gene that mediates resistance to a BTK inhibitor, ibrutinib, by affecting its covalent binding to BTK. As a result, the detection of this mutation will help haematologists to switch the treatment of patients with chronic lymphocytic leukaemia by using second line non-covalent BTK inhibitors.

We have also improved the clinical utility of 2 assays listed in our catalogue. Firstly, to amend the diagnosis, prognosis and/ or personalised therapy of cancer patients, especially those with acute myeloid leukaemia, we have extended the mutation detection scope in the *TP53* gene - previously limited to exons 5, 6, 7 and 8 - to its whole sequence. Likewise, the detection of recurrent mutations already done in exons 9, 11, 13 and 17 of the *KIT* gene has been extended to exon 18. This upgrade will allow oncologists to offer new therapeutic options to their patients with gynaecological melanomas that harbour mutations in the *KIT* gene, not previously detected in cutaneous melanomas.

Additionally, last summer, aiming to replace the existing European *In Vitro* Diagnostic Directive (IVDD), we launched a key development that will lead us to implement a new *In Vitro* Diagnostic Regulation (IVDR) that is mandatory for all CNIO's diagnostics support units in the midterm (FIGURE 1). To comply with IVDR requirements, our efforts address the upgrading and validation of the current assays to guarantee their safety and their analytical and clinical performance. With the aid of experts in Quality Management Systems, we are already beginning to establish robust quality control and assurance procedures, as well as documentation schemes that will warrant firm compliance with IVDR guidelines.

Finally, during 2023, in the framework of our training policy, we hosted an undergraduate student in biomedical engineering. ∎
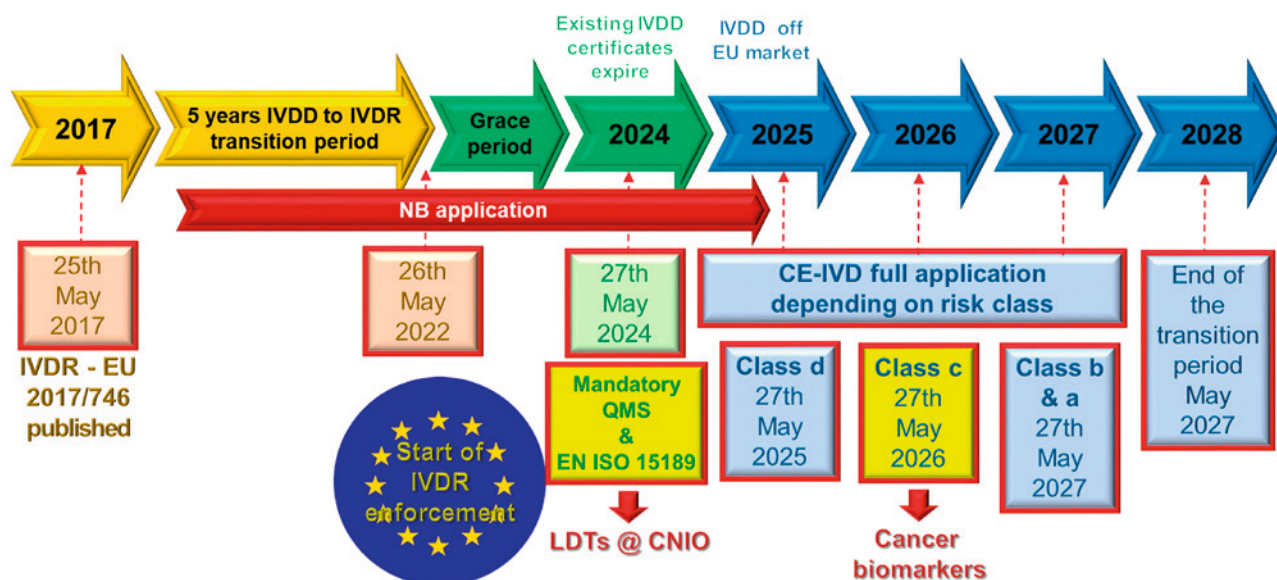
FIGURE 1 Transition timeline from IVDD to IVDR that emphasises extended deadlines for regulatory compliance, ensuring a smooth shift to IVDR standards, and highlighting revised milestones and the commitments of stakeholders, laboratories, and authorities to align with the new regulations. (NB: Notify Body; LDTs: Laboratory Developed Tests).

H12O-CNIO CANCER IMMUNOTHERAPY CLINICAL RESEARCH UNIT

Luis Álvarez-Vallina
Clinical Research Unit Head

Research Scientists
Belén Blanco, Anáis Jiménez

Post-Doctoral Fellows
Rodrigo Lázaro, Antonio Tapia, Ivana Zagorac

Gradutate Students
Francisco Javier Arroyo, Carmen Domínguez (until September), Eva García (since March), Marina Gómez, Susana Luengo (since June),

Lucía Rivas (since November), Laura Rubio, Alejandro Segura, Miriam Velasco

Technician
María de la Yedra Pacheco

Students in Practice
Maria I. López (Feb.-June) (Bachelor's Degree Final Project, *Univ. de Alcalá de Henares*, Spain), Iris Rioseras (Feb.-June) (*Univ. Francisco de Vitoria*, Madrid, Spain)

Visiting Student
Diego Pilati (March-June) (Aarhus Univ., Denmark)

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:

→ Reactivation of tumour-specific endogenous T cell repertoires through the design of multi-specific antibodies against a combination of immunomodulatory targets. Preclinical and early clinical data show that this is a promising approach to enhance the clinical benefit of conventional checkpoint blockers.
→ Generation of 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).
→ Development of multi-target approaches that simultaneously recognise extracellular and intracellular tumour antigens.
→ Rational design of mRNA-based therapeutics.
→ 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 T cell immunity against cancer."

RESEARCH HIGHLIGHTS

STAb-T cancer immunotherapy

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 T cells (FIGURE 1). The secreted TCE antibodies redirect T cells against cancer cells expressing a predefined TAA. STAb-T cells offer several potential advantages over current T redirection strategies (FIGURE 1): *in vivo* endogenous secretion could result in effective concentrations of TCEs, 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). In 2023, we confirmed the remarkable therapeutic impact of single-targeted STAb-T cells in haematological cancers, B cell leukaemia, T cell leukaemia and multiple myeloma (Diez-Alonso L,....,Álvarez-Vallina L. *Sci Transl Med,* in press), and showed that dual-targeted STAb-T therapies (FIGURE 1) have superior control of leukaemia progression than dual-targeted CAR-T cells. We also showed that TCE-secreting tumour-infiltrating lymphocytes (STAb-TIL), but not conventional TIL, induce responses in solid tumours (non-small cell lung cancer) when administered intratumorally and systemically.

Dendritic cell-mediated cross-priming by bispecific antibodies

Dendritic cells (DCs) are professional antigen-presenting cells that play a central role in the induction of antigen-specific adaptive immune responses. DC natural killer lectin group receptor-1 (DNGR-1) is a C-type lectin receptor (CLR) selectively expressed at high levels by mouse CD8α^+ and CD103$^+$ DCs, and by their human equivalents. In this DC subset, defined as conventional type 1 DCs (cDC1s), DNGR-1 promotes cross-priming of cytotoxic CD8$^+$ T cell (CTL) responses by diverting of necrotic cell cargo into a recycling endosomal compartment, resulting in preferential major histocompatibility complex (MHC) class I cross-presentation to CTLs. Our group developed a bispecific anti-RBD x anti-DNGR-1 antibody (FIGURE 2a) to target neutralised SARS-CoV-2 virions to cDC1 and promote T cell cross-priming. Therapeutic administration of the bispecific antibody protected transgenic K18-hACE2 mice from lethal SARS-CoV-2 infection (Lázaro-Gorines R,...., Álvarez-Vallina L. Adv *Sci,* 2023). A strategy combining immediate virus neutralisation with specific DC targeting to accelerate and enhance adaptive immune responses during infection has never been explored. Given the critical activity of DCs in most successful cancer immunotherapies, we are currently exploring strategies to enhance their ability to prime more potent anti-tumour CTLs. Early research suggests that this dual modulatory approach could lead to the unleashing and boosting of immune responses against tumours and protective immunity (Rubio-Pérez L,...., Álvarez-Vallina L. *Oncoimmunology,* 2023) (FIGURE 2b).

Early clinical trials

Our Unit, in collaboration with the Haematology and Medical Oncology Departments of the *Hospital Universitario 12 de Octubre*, has launched 2 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; and a phase I, first-in-human clinical trial to evaluate the safety of intratumoral administration of P32-specific Chimeric Antigen Receptor (CAR)-engineered T cells for progressive high-grade gliomas. A Phase I trial of a novel bispecific immunomodulating antibody for patients with EGFR-overexpressing cancer is also underway. ■
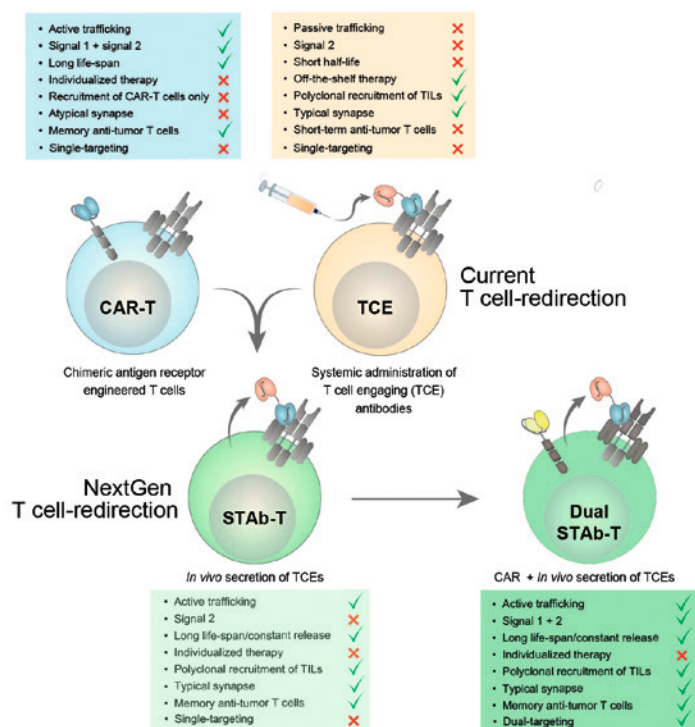
FIGURE 1 Schematic diagram summarising the advantages (green tick) and limitations (red cross) of current and next-generation T cell-redirecting strategies.
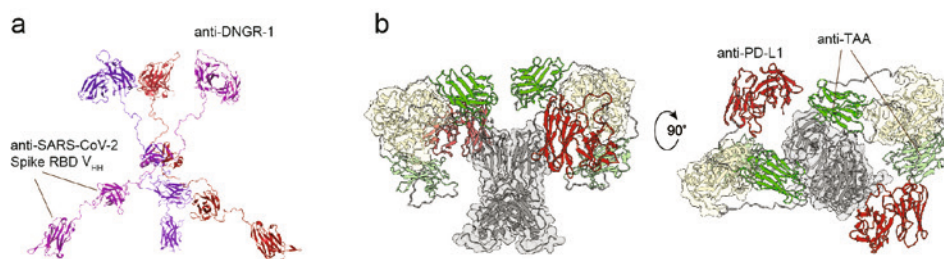
FIGURE 2 Hypothetical model of the Fc-less bispecific anti-RBD x anti-DNGR-1 antibody designed to promote cross-priming and boost adaptive responses against SARS-CoV-2 (**a**). Each polypeptide chain is shown in a different colour (blue, red and purple). Hypothetical model of the Fc bearing bispecific anti-PD-L1 x anti-TAA antibody designed to prime anti-tumour CTLs (**b**).

▸ **PUBLICATIONS**

▸ Lázaro-Gorines R, Pérez P, Heras-Murillo I, Adán-Barrientos I, Albericio G, Astorgano D, Flores S, Luczkowiak J, Labiod N, Harwood SL, Segura-Tudela A, Rubio-Pérez L, Nugraha Y, Shang X, Li Y, Alfonso C, Adipietro KA, Abeyawardhane DL, Navarro R, Compte M, Yu W, MacKerell AD Jr, Sanz L, Weber DJ, Blanco FJ, Esteban M, Pozharski E, Godoy-Ruiz R, Muñoz IG, Delgado R, Sancho D, García-Arriaza J, Álvarez-Vallina L (2023). Dendritic cell-mediated cross-priming by a bispecific neutralizing antibody boosts cytotoxic T cell responses and protects mice against SARS-CoV-2. *Adv Sci* 10, e2304818.

▸ Tapia-Galisteo A, Álvarez-Vallina L*, Sanz L* (2023). Bi- and trispecific immune cell engagers for immunotherapy of hematological malignancies. *J Hematol Oncol* 16, 83. *Co-corresponding authors.

▸ Tapia-Galisteo A, Compte M, Álvarez-Vallina L*, Sanz L* (2023). When three is not a crowd: trispecific antibodies for enhanced cancer immunotherapy. *Theranostics* 13, 1028-1041. *Co-corresponding authors.

▸ Rubio-Pérez L, Lázaro-Gorines R, Harwood SL, Compte M, Navarro R, Tapia-Galisteo A, Bonet J, Blanco B, Lykkemark S, Ramírez-Fernández Á, Ferreras-Gutiérrez M, Domínguez-Alonso C, Díez-Alonso L, Segura-Tudela A, Hangiu O, Erce-Llamazares A, Blanco FJ, Santos C, Rodríguez-Peralto JL, Sanz L, Álvarez-Vallina L (2023). A PD-L1/EGFR bispecific antibody combines immune checkpoint blockade and direct anti-cancer action for an enhanced anti-tumor response. *Oncoimmunology* 12, 2205336.

▸ Antunes A, Alvarez-Vallina L, Bertoglio F, Bouquin N, Cornen S, Duffieux F, Ferré P, Gillet R, Jorgensen C, Leick MB, Maillère B, Negre H, Pelegrin M, Poirier N, Reusch D, Robert B, Serre G, Vicari A, Villalba M, Volpers C, Vuddamalay G, Watier H, Wurch T, Zabeau L, Zielonka S, Zhang B, Beck A, Martineau P (2023). 10th antibody industrial symposium: new developments in antibody and adoptive cell therapies. *MAbs* 15, 2211692.

▸ **PATENT**

▸ Álvarez-Vallina L, Díez-Alonso L, Martín Antonio AB, Juan Otero M, Urbano Izpisua A. T cells expressing Anti-BCMA/Anti-CD3 antibodies and Uses Thereof. PCT Application (2023). *PCT/EP2023/383410*.

▸ **AWARDS AND RECOGNITION**

▸ Ivana Zagorac and Anaïs Jiménez shared 'Best Poster' Award at the CNIO Lab Day.

▸ Francisco Javier Arroyo received an Award for 'Best Oral Communication' in the Spanish Society of Immunology III Alicante-Winter Immunology Symposium in Health (A-WISH 2023), Alicante, Spain.

▸ Alejandro Segura was recipient of a '*Rio Hortega*' Fellowship from the *Carlos III* Health Institute.

▸ Eva García was recipient of a PhD Fellowship from the *Comunidad de Madrid*.

H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis G. Paz-Ares
Clinical Research Unit Head

Research Scientists
Teresa Agulló, Irene Ferrer,

Itziar Otano, Beatriz Soldevilla,
Álvaro C. Ucero

Clinical Investigators
Rocio García-Carbonero, José Luis
Solórzano, Jon Zugazagoitia

Post-Doctoral Fellows
Magdalena Abraham,
Cristina Cirauqui, Juan Manuel Coya,
Jose Garrido (since September),
Arantza Lamas, Miguel Ruiz (since
February), Gorka Ruiz de Garibay

Gradutate Students
Carlos Carretero, Inés Díaz, M.

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Wajahat Nadeem (since November),
Ángel Núñez, Alba Santos,
Beatriz Rubio, Joan Salvador Russo,
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Technicians
Vera Adradas, Eva Álvarez,
Nuria Carrizo, Eva M. Casas,
Patricia Cozar, Laura García,
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Patricia Llamas, Patricia Plaza,
Laura Ramírez (until April),
Belén Revuelta (since February),

Jacinto Sarmentero, Rocío Suárez,
César Vélez

Students in Practice
Daniel Parra (until July) (Master's
Thesis, *Univ. Complutense de Madrid*,
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(Bachelor's Thesis, *Univ. de Alcalá de
Henares*, 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 2 research areas: the identification of novel molecular biomarkers for diagnostic, prognostic, and predictive purposes; and the development of novel treatment strategies, 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.

"We have provided proof of concept that T cell engagers (TCEs) provide an efficacious strategy, at the cost of reasonable side effects, in cold solid tumours such as SCLC, including those lacking canonical T cell activation through antigen presentation (*JCO* 2023; *NEJM* 2023)."

RESEARCH HIGHLIGHTS

Tarlatamab showed promising antitumour activity in patients with small cell lung cancer (SCLC)

Two early trials led by our group demonstrated the efficacy of tarlatamab, a bispecific T-cell engager targeting DLL3 and CD3, in patients with SCLC. In the first-in-human (FIH) clinical trial, 107 patients treated with tarlatamab revealed a 23.4% objective response rate, a median response duration of 12.3 months and median survival of 13.2 months, with encouraging safety outcomes, despite treatment-related adverse events in 90.7%, mainly cytokine release syndrome (52%). In the second phase 2 clinical trial, involving 220 patients with previously treated SCLC, the administration of tarlatamab at dosages of 10 mg or 100 mg every 2 weeks demonstrated antitumour activity with durable responses, showing 40% and 32% objective response rates, respectively. Median progression-free survival was 4.9 months and 3.9 months, with estimated 9-month overall survival rates of 68% and 66%, respectively. Cytokine-release syndrome was the most common adverse event, but a low percentage of patients discontinued treatment due to adverse events in both arms. These findings underscore tarlatamab's potential in pretreated SCLC, warranting further evaluation.

Efficacy of sotorasib compared to docetaxel for the treatment of non-small cell lung cancer (NSCLC)

In this clinical trial, we evaluated the efficacy and safety of sotorasib compared to docetaxel in the treatment of NSCLC with $KRAS^{G12C}$ mutation. Patients treated with sotorasib showed significant improvement in progression-free survival (HR 0.65) and overall response rate compared to patients receiving docetaxel; in addition, sotorasib-treated patients presented better toxicity profiles, providing better quality of life. As a result of this investigation, the EMA recently approved the therapeutic use of tarlatamab in pretreated patients with NSCLC having underlying $KRAS^{G12C}$ mutations.

ONCOS-102 combined with pemetrexed and platinum chemotherapy for the treatment of malignant pleural mesothelioma (MPM)

In this early clinical trial, we showed the synergistic effect of combining a new immunotherapy agent, ONCOS-102 (an oncolytic adenovirus), with the conventional pemetrexec plus platinum chemotherapy regimen for the treatment of MPM. We evaluated both the clinical outcome of patients plus the impact of the novel strategy on the tumour microenvironment. The treatment resulted in a relevant median overall survival (up to 30 months), and the results were particularly promising in chemotherapy-naïve patients. We observed increased T-cell infiltration in ONCOS-102-treated patients, and differences in the transcriptome of several genes, including those associated with complement activation, humoral immune response, and regulation of acute inflammatory response. The results might shed light on new ways to tackle this very challenging disease. ∎

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Onset and Duration of Response
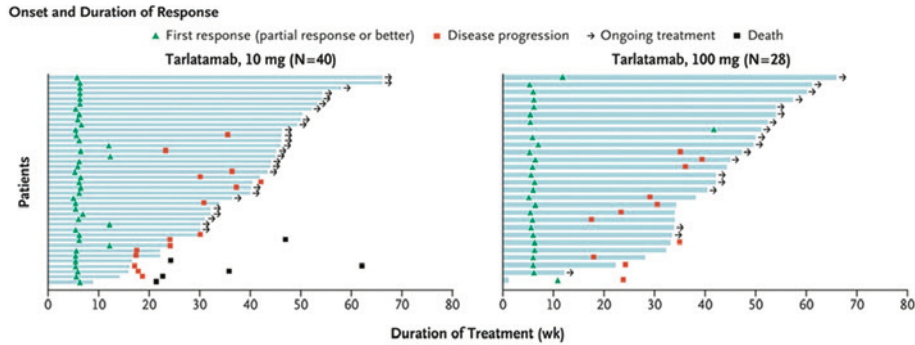
FIGURE 1 The panel shows the time to response, the duration of response, and patient status as of the data cut-off date for all the patients who were assessed as having an objective response (complete or partial response; primary end point) to 10 mg or 100 mg of tarlatamab.
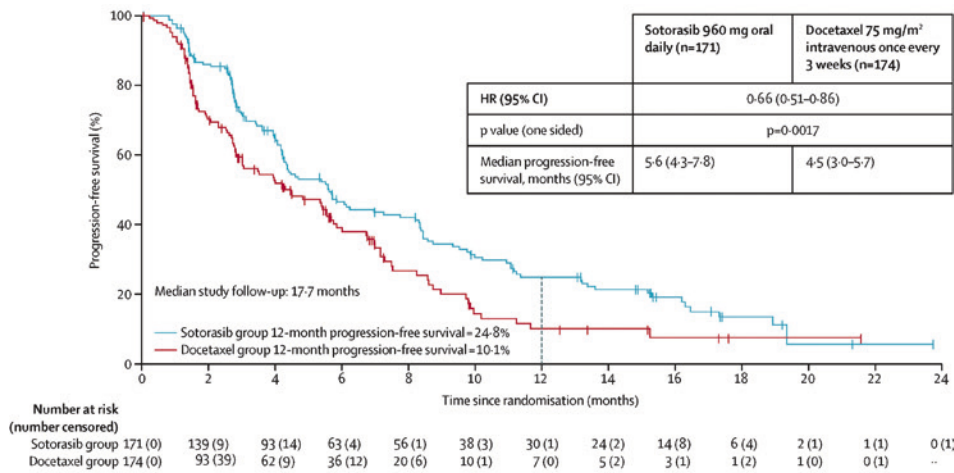
FIGURE 2 The Kaplan-Meier curve of progression-free survival among patients in the sotorasib group and patients in the docetaxel group who could be assessed for a response according to blinded independent central review.

patients with ECOG performance status 2 and other special populations: CheckMate 817. *J Immunother Cancer* 11, e006127.

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‣ Herrera-Juárez M, Serrano-Gómez C, Bote-de-Cabo H, Paz-Ares L (2023). Targeted therapy for lung cancer: beyond EGFR and ALK. *Cancer* 129, 1803-1820.

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‣ **PATENT**

‣ Macintyre G, Hernando B, Fernández Sanromán A, Paz-Ares L, Zugazagoitia J, Gómez-Sánchez D, Goya JM (2023). Prediction of gene amplification. *EP 23383180.9*.

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

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Clinical Research Unit Head

Research Scientists
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Miguel Gallardo, María Linares

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Ana Rodriguez (since March), Antonio Valeri, María Velasco-Estévez

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Technicians
Raquel Ancos, Andrés Arroyo, Irene Bragado, Natalia Sofía Buenache, Sara Dorado (until September), Adrián Fernández, Alicia Giménez, Laura Moreno, Miguel Ángel Navarro, Alejandra Ortíz, Juan Manuel Rosa (until September), Laura Rufián, Laura Sánchez (until June)

Studenst in Practice
Irene Ortega (until June) (Bachelor's Degree Final Project, *Universidad Complutense de Madrid*, Spain), Miguel Ángel Marugal (until June) (Master's Thesis, *Universidad Complutense de Madrid*, Spain), Alba Garrote (until June) (Master's Thesis, *Universidad Complutense de Madrid*, Spain), David Ortega (until June) (Master's Thesis, *Universidad Complutense de Madrid*, Spain)

OVERVIEW

The Haematological Malignancies Clinical Research Unit focuses on the identification of new molecular biomarkers and drivers of diseases and the development of novel therapeutic approaches. Moreover, we are developing novel strategies and cutting-edge technology tools to better characterise and monitor minimal residual disease to anticipate cancer outcomes. We have contributed to elucidating the molecular determinants of critical molecular processes in haematological malignancies such as splicing, ribosome biogenesis, nucleolus biology, and mechanoreception.

In the Haematological Malignancies Clinical Research Unit at CNIO we investigate:

→ Splicing: Traditional haematological neoplasms (leukaemia, myeloma, lymphoma): new diagnostic approaches, biomarkers, and treatments.
→ Nucleolus biology and ribosome biogenesis: Novel drivers of haematological malignancies such as bone marrow failures.
→ Mechanoreception: Novel drivers of haematological neoplasms.
→ Ultra-deep sequencing: Minimal residual disease (MRD) monitoring (LiqBio-MRD).
→ Immunotherapy: NK-CARs, BITES, T-CARs and immune checkpoints inhibitors.

"Our results establish that RNA splicing inhibition, alone or combined with venetoclax, could be useful for the treatment of newly diagnosed or relapsed/refractory AML."

RESEARCH HIGHLIGHTS

Post-translational splicing modifications as a key mechanism in cytarabine resistance in acute myeloid leukaemia (AML)

Despite the approval of several drugs for AML, cytarabine is still widely used as a therapeutic approach. However, 85% of patients show resistance and only 10% overcome the disease. Using RNA-seq and phosphoproteomics, we show that RNA splicing and serine-arginine-rich (SR) protein phosphorylation were altered during cytarabine resistance. Moreover, phosphorylation of SR proteins at diagnosis was significantly lower in responder than in non-responder patients, pointing to their utility to predict response. These changes correlated with altered transcriptomic profiles of SR protein target genes. Notably, splicing inhibitors were therapeutically effective in treating sensitive and resistant AML cells as monotherapy or in combination with other approved drugs. The H3B-8800 and venetoclax combination showed the best efficacy *in vitro,* demonstrating synergistic effects in patient samples and no toxicity in healthy haematopoietic progenitors. Our results establish that RNA splicing inhibition, alone or combined with venetoclax, could be useful for the treatment of newly diagnosed or relapsed/refractory AML.

Real-life disease monitoring in follicular lymphoma patients using liquid biopsy ultra-deep sequencing and PET/CT

In this study, we screened 84 patients with follicular lymphoma (FL) for somatic mutations suitable as liquid biopsy MRD biomarkers using a targeted next-generation sequencing (NGS) panel. We found trackable mutations in 95% of the lymph node samples and 80% of the liquid biopsy baseline samples. We then used an ultra-deep sequencing approach with 2×10^{-4} sensitivity (LiqBio-MRD) to track those mutations on 151 follow-up liquid biopsy samples from 54 treated patients. The results demonstrated that LiqBio-MRD is a robust and non-invasive approach, complementary to metabolic imaging, for identifying patients with FL at high risk of having their treatment fail, and should be considered in future response-adapted clinical trials. ∎
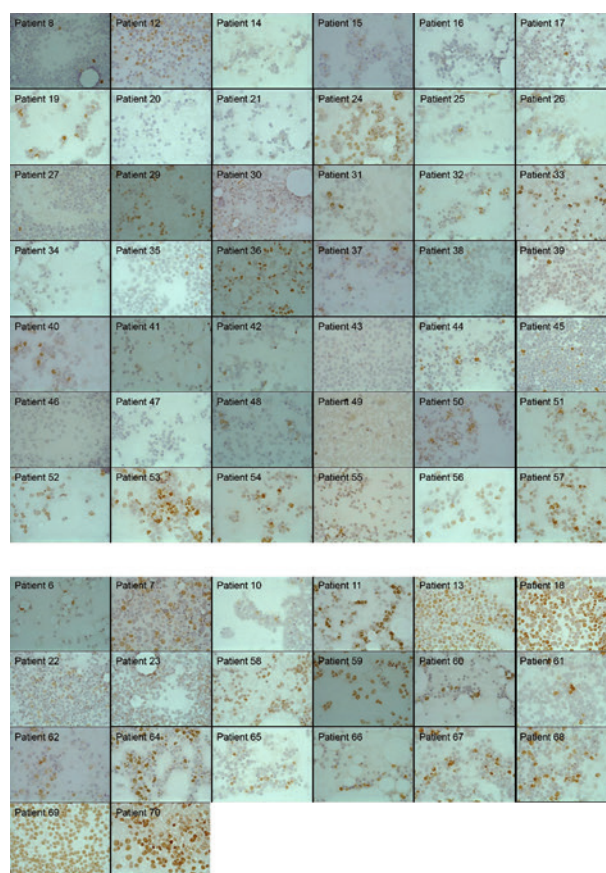
FIGURE 1 Phosphorylation of SR proteins could predict cytarabine response and is related to an aberrant splicing process.

▸ **PUBLICATIONS**

▸ Lopez-Muñoz N, Hernández-Ibarburu G, Alonso R, Sanchez-Pina JM, Ayala R, Calbacho M, Cuellar C, Cedena MT, Jiménez-Ubieto A, Iñiguez R, Pedrera M, Cruz J, Meloni L, Pérez-Rey D, Serrano P, de la Cruz J, Martinez-Lopez J (2023). Large-scale real-life analysis of survival and usage of therapies in multiple myeloma. *J Hematol Oncol* 16, 76.

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▸ Morales ML, García-Vicente R, Rodríguez-García A, Reyes-Palomares A, Vincelle-Nieto Á, Álvarez N, Ortiz-Ruiz A, Garrido-García V, Giménez A, Carreño-Tarragona G, Sánchez R, Ayala R, Martínez-López J*, Linares M* (2023). Posttranslational splicing modifications as a key mechanism in cytarabine resistance in acute myeloid leukemia. *Leukemia* 37, 1649-1659.

▸ Rodríguez-García A, Mennesson N, Hernandez-Ibarburu G, Morales ML, Garderet L, Bouchereau L, Allain-Maillet S, Piver E, Marbán I, Rubio D, Bigot-Corbel E, Martínez-López J, Linares M, Hermouet S (2023). Impact of viral hepatitis therapy in multiple myeloma and other monoclonal gammopathies linked to hepatitis B or C viruses. *Haematologica*. PMID: 37199121.

▸ Jiménez-Ubieto A, Martín-Muñoz A, Poza M, Dorado S, García-Ortiz A, Revilla E, Sarandeses P, Ruiz-Heredia Y, Baumann T, Rodríguez A, Calbacho M, Sánchez PM, Pina JMS, García-Sancho AM, Figaredo G, Rufián L, Rodríguez M, Carneros L, Martínez-Laperche C, Bastos-Oreiro M, Wang C, Cedena MT, Rapado I, de Toledo P, Gallardo M, Valeri A, Ayala R, Martínez-López J, Barrio S (2023). Personalized monitoring of circulating tumor DNA with a specific signature of trackable mutations after chimeric antigen receptor T-cell
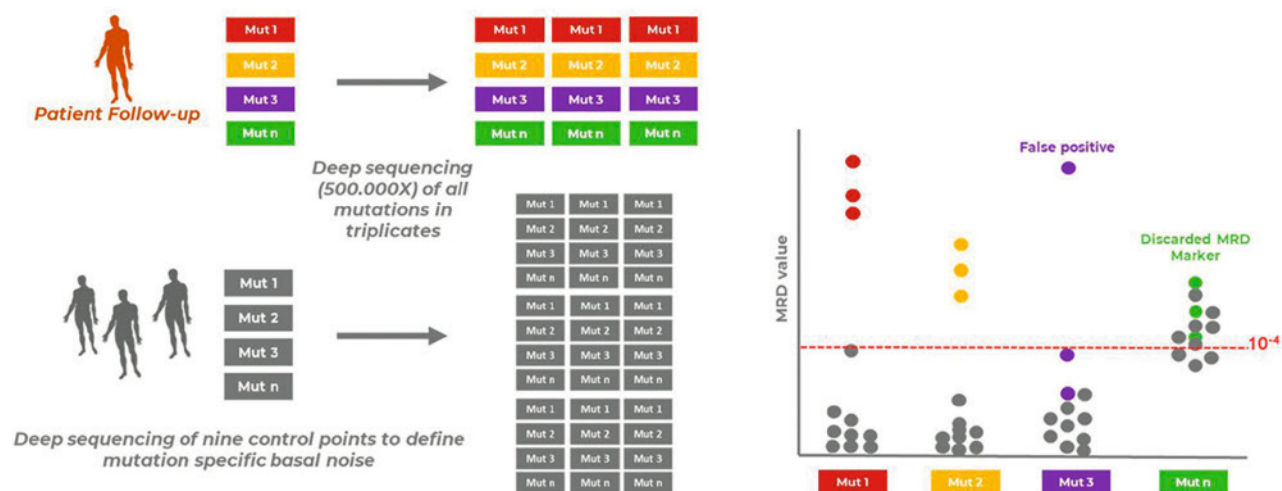
FIGURE 2 Visual summary of the LiqBio-MRD monitoring method.

therapy in follicular lymphoma patients. *Front Immunol* 14, 1188818.

‣ Buenache N, Sánchez-de laCruz A, Cuenca I, Giménez A, Moreno L, Martínez-López J, Rosa-Rosa JM (2023). Identification of immunoglobulin gene rearrangement biomarkers in multiple myeloma through cfDNA-based liquid biopsy using tchDNAseq. *Cancers (Basel)* 15, 2911.

‣ Haertle L, Buenache N, Cuesta Hernández HN, Simicek M, Snaurova R, Rapado I, Martinez N, López-Muñoz N, Sánchez-Pina JM, Munawar U, Han S, Ruiz-Heredia Y, Colmenares R, Gallardo M, Sanchez-Beato M, Piris MA, Samur MK, Munshi NC, Ayala R, Kortüm KM, Barrio S, Martínez-López J (2023). Genetic alterations in members of the proteasome 26S subunit, AAA-ATPase (*PSMC*) gene family in the light of proteasome inhibitor resistance in multiple myeloma. *Cancers (Basel)* 15, 532.

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Selected publications at other institutions

‣ Richardson PG *et al.* (incl. Martínez-López J) (2023). Mezigdomide plus dexamethasone in relapsed and refractory multiple myeloma. *N Engl J Med* 389, 1009-1022.

‣ San-Miguel J *et al.* (incl. Martinez-Lopez J) (2023). Cilta-cel or standard care in lenalidomide-refractory multiple myeloma. *N Engl J Med* 389, 335-347.

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‣ Burgos L *et al.* (incl. Cedena MT, Martinez-Lopez J) (2023). Definition and clinical significance of the monoclonal gammopathy of undetermined significance-like phenotype in patients with monoclonal gammopathies. *J Clin Oncol* 41, 3019-3031.

‣ Rosiñol L *et al.* (incl. Martinez-López J) (2023). Lenalidomide and dexamethasone with or without ixazomib maintenance tailored by residual disease status in myeloma. *Blood*. PMID: 37506339.

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‣ Sargas C *et al.* (incl. Martínez-López J) (2023). Comparison of the 2022 and 2017 European LeukemiaNet risk classifications in a real-life cohort of the PETHEMA group. *Blood Cancer J* 13, 77.

‣ **AWARDS AND RECOGNITION**

‣ Joaquín Martínez-López: SenesCAR Research Project, *FIBH12O,* Spain.

‣ María Linares and Joaquín Martínez-López (coordinator): ALMA Research Project, *ISCIII-CDTI,* Spain.

‣ Rosa Ayala: *AES* 2023 Health Research Project; PMP22/00069 Platform (Research Collaborator), Spain.

‣ Santiago Barrio: *Miguel Servet* Research Contract, Spain.

‣ Ricardo Sánchez: *Juan Rodés* Grant, Spain.

‣ Pablo Blanco: Pre-doctoral Fellowship, *Tatiana Pérez de Guzmán el Bueno* Foundation, Spain.

‣ María Linares: Scientific Image (*Pinacoteca de la Ciencia*) and Article of the Month, Spanish Society for Biochemistry and Molecular Biology; Best Presentation Award, *XV Congreso de Investigación Estudiantes Grado Ciencias de la* Salud. *XIX Congreso de Ciencias Veterinarias y Biomédicas*, Spain.

‣ Laura Córdoba: Fulbright Pre-doctoral Research Grant.

‣ Jessica Encinas: Young Investigator Award, XX International Myeloma Workshop, Athens, Greece.

IDIPAZ-CNIO PEDIATRIC ONCO-HEMATOLOGY CLINICAL RESEARCH UNIT

Antonio Pérez-Martínez
(since September)
Clinical Research Unit Head

Clinical Investigators
Dolores Corral, Víctor Galán, Pilar Guerra, Isabel Martínez Romera, Antonio Muñoz

OVERVIEW

The Pediatric Onco-Hematology Clinical Research Unit, headed by Antonio Pérez-Martínez, comprises a multidisciplinary team of physicians, geneticists, biologists, biochemists, and bioinformaticians, who, for the past decade, have carried out projects ranging from knowledge generation to direct clinical application through the development of clinical trials. Our research, which is mostly funded by *Fundación Cris Contra el Cáncer*, focuses on the design of therapies applied to paediatric oncology, infectious diseases, and paediatric transplantation. Briefly, our research interests include: 1) the use of haematopoietic stem cell transplantation as a platform for cell therapy; 2) the improvement of human stem cell progenitors transplantation in children; 3) cell therapy with memory T lymphocytes as adoptive immunotherapy; and 4) the use of both activated and memory-like NK cells and CART-T cells in paediatric oncology. Together with this, we led several projects aimed at studying the efficacy and feasibility of cell therapy against infectious diseases. Finally, we are also working on the induction of immunological tolerance in solid organ transplantation by inducing mixed haematopoietic chimera through cell therapy. We maintain a strong commitment to patients and to improving their quality of life, having a direct impact on society's well-being. In this sense, we have several active academic clinical trials and collaborations with industry, with the objective of developing new and more effective therapies with fewer side effects.

Post-Doctoral Fellows
Cristina Aguirre, Halin Bareke, Adela Escudero, Lucía Fernández, Elisa Izquierdo, Odelaisy León, Adriana Mañas, Jordi Minguillón, Andrés París

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

*Titulado Superior (Advanced Degree)

Student in Practice
Mahdieh Naghavi (October-December) (Universita' degli Studi di Perugia, Italy)

RESEARCH HIGHLIGHTS

Sarcomas represent approximately 10% of paediatric cancers. The survival rate for patients with high-risk disease, with metastasis at the time of diagnosis, or experiencing relapse, does not exceed 30%. In these cases, current treatments remain ineffective, and their administration is associated with acute and chronic effects that compromise the survival and quality of life of patients. There is an urgent need to find new therapeutic alternatives to improve the prognosis of patients with sarcoma.

In November 2023, a clinical trial for paediatric, adolescent, and young adult patients with advanced sarcoma, coordinated by Antonio Pérez Martínez and the Advanced Therapy Medicines Production Unit of *La Paz* University Hospital, received authorisation from the Spanish Association of Medicines and Medical Devices (Eudra CT 2019-004310-33, No. PEI 20-091). Under the acronym CAR4SAR, this is the world's first clinical trial with an allogeneic academic CART designed for paediatric patients with advanced sarcoma. It is an open-label, prospective, single-centre (HULP), non-randomised, dose-escalation trial aimed at determining the dose-limiting toxicity and maximum tolerated dose of systemically infused NKG2D-CAR memory T cells derived from donors (arm A) and a dual treatment involving both systemic and local infusions of NKG2D-CAR memory T cells derived from donors (arm B). This trial is the result of a collaboration between the *Hospital La Paz* Institute for Health Research, the CNIO, the *Carlos III* Health Institute, and the Central Hospital of Asturias, along with *CRIS* Cancer Foundation, which has been supporting this project for the past 6 years. The clinical trial initiation with the first patient will start in February 2024. In addition to its evident scientific impact, this trial will have a significant social impact. Over time it will enhance therapeutic options not only for paediatric patients with recurrent/refractory sarcoma but could also extend to other paediatric solid tumours with a poor success rate using conventional therapies. Study Details | A Phase I Trial of Memory T Cells Expressing an NKG2D Chimeric Antigen Receptor in Children, Adolescents and Young Adults With Advanced Sarcoma | ClinicalTrials.gov. ■

▸ **PUBLICATIONS**

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▸ Ibáñez-Navarro M, Fernández A, Escudero A, Esteso G, Campos-Silva C, Navarro-Aguadero MÁ, Leivas A, Caracuel BR, Rodríguez-Antolín C, Ortiz A, Navarro-Zapata A, Mestre-Durán C, Izquierdo M, Balaguer-Pérez M, Ferreras C, Martínez-López J, Valés-Gómez M, Pérez-Martínez A, Fernández L (2023). 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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▸ García-Solís B *et al.* (incl. Navarro-Zapata A, Mestre-Durán C, Ferreras C, Pérez-Martínez A) (2023). Inherited human ezrin deficiency impairs adaptive immunity. *J Allergy Clin Immunol* 152, 997-1009.
▸ Marin-Bejar O *et al.* (incl. Pérez-Martínez A) (2023). Epigenome profiling reveals aberrant DNA methylation signature in GATA2 deficiency. *Haematologica* 108, 2551-2557.
▸ Stringa P *et al.* (incl. Pérez-Martínez A) (2023). Native spleen preservation during visceral transplantation inhibits graft-versus-host-disease development: clinical and experimental study. *Ann Surg* 277, e235-e244.
▸ Valle-Simón P, Borobia AM, Pérez-Martínez A (2023). Clinical research with targeted drugs in paediatric oncology. *Drug Discov Today* 28, 103672.
▸ Águeda Molinos-Quintana A *et al.* (incl. Pérez-Martínez A) (2023). Impact of disease burden and late loss of B cell aplasia on the risk of relapse after CD19 Chimeric Antigen Receptor T Cell (Tisagenlecleucel) infusion in pediatric and young adult patients with relapse/refractory acute lymphoblastic leukemia: role of B-cell monitoring. *Front Immunol*. PMID: 38292483**.**
▸ Gonzaga A *et al.* (incl. Al-Akioui-Sanz K, Ferreras C, Pérez-Martínez A) (2023). Rationale for combined therapies in severe-to-critical COVID-19 patients. *Front Immunol* 14, 1232472.
▸ Bareke H *et al.* (incl. Ibáñez-Navarro A, Guerra-García P, Pérez-Martínez A) (2023). Prospects and advances in adoptive natural killer cell therapy for unmet therapeutic needs in pediatric bone sarcomas. *Int J Mol Sci* 24, 8324.

Innovation

ROKE I. ORUEZABAL
Director of Innovation

Lola Pérez (since October)
Innovation Manager

Carlos Alonso (since November)
Innovation Facilitator

CNIO's Research Groups share a strong commitment to 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. The commitment in this field is highlighted by the consolidation of a number of staff positions in these programmes, providing new capabilities and skills to the entire Centre.

International cooperation with the biotech and pharma industry is shown by the 213 agreements managed by the Technology Transfer and Valorisation Office. Besides the strengthened collaborations with pharma companies such as Janssen, Loxo Oncology and AstraZeneca (among others), the CNIO, with the Experimental Therapeutics Programme in the lead, was also successful in generating and stepping up alliances with foreign biotech companies and public research institutions, giving added value to the CNIO's small-compound library. This reflects the fact that 36% 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 for an amount above €1.5 million, especially in the monoclonal antibodies field.

The volume of collaborative research agreements signed in 2023 represents almost €4 million in revenue for CNIO, a 66% increase compared to the previous year. Similarly, the net income from CNIO's 2022 asset licensing equalled €1.6 million (23% increase). Among the new license agreements signed in 2023, in addition to cell lines and antibodies, it is worth highlighting the continuation of strategic alliances with research centres of excellence, such as the *IRB* and *CRG*, as well as companies such as L'Oreal, Mysthera Therapeutics AG, Verastem or Tailor Bio. Among the most outstanding agreements signed this year with the private sector is the 7-figure license made from compounds generated by ETP to the biotech company Refoxy Pharmaceuticals GmbH.

Currently, the CNIO's active patent portfolio comprises 44 families of patents. In 2023, 2 new patents entered the

"CNIO's impact on society has continued for another year, together with our public and private partners in a joint effort to eradicate cancer."

international phase (PCT), and 7 new priority patent applications were filed as well. 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 pharma companies in order to find potential matches. In this regard, and specifically in Sanofi's Open Innovation Programme, the project "Novel strategies to target KRAS in lung and pancreatic cancer" (Mariano Barbacid) was awarded a grant.

In 2023, a total of 3 projects were awarded funding within the framework of Public-Private Partnerships calls, endowed with over €2.4 million. Of these, 2 were projects in collaboration with the biotech company Leadartis: "CONDICOS: Conditional 4-1BB costimulation exploiting Crosspriming in CANCER" and "EFESO: Immune check point-conditional 4-1BB co-stimulation for effective and safe cancer immunotherapy" (Luis Álvarez Vallina). The third project, in collaboration with the pharmaceutical company Lilly, corresponds to "Genetic, pharmacological and computational approaches to identify precision therapies in pancreatic cancer" (Mariano Barbacid).

In 2023, the CNIO achieved an alliance with the platform for the dynamization and innovation of the industrial capacities of the National Public Healthcare System and their effective transfer to the productive sector (ITEMAS platform), which implies participation in the platform as a collaborating entity. CNIO was also recognised as a "key innovator" in the development of "A novel plasmonic theranostic probe" through Manuel Valiente's participation in the EU-funded Research & Innovation project NanoBRIGHT. This acknowledgment brings with it a number of privileges for the institution:

-A dedicated CNIO webpage was set up (within the Innovation Radar's public website), with all the details about the organisation, its innovations and activities.

-The CNIO will have access to a range of services offered by the European Commission; in particular it will benefit from "go to market" training and support from Dealflow.eu; Horizon Results Booster support which include "Portfolio Dissemination and Exploitation Strategy", "Business Plan Development" and "Go to Market" services; and free, expert-led standardisation support services from the EU Standardisation Booster.

As a research institution of excellence, the CNIO has developed a strong commitment to innovation and public-private collaboration, commitment that will have an impact on 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 9 Core Units covering major areas in Biotechnology, namely Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Mouse Genome Editing, as well as an Animal Facility. Although the Core Units are mainly focused on providing support and collaborating with the CNIO Research Groups, they also collaborate with groups from other research institutions, as well as with private companies.

2023 brought the good news of the consolidation of a number of staff positions in some of our Core Units, which contributed to provide better stability and to improve professional perspectives. Indeed, ensuring adequate working conditions for the staff is a key element to guarantee their motivation and compromise with the objectives and goals of the Programme, and the CNIO as a whole.

The constant upgrade of the CNIO's technological capabilities continues being one of the main areas where we focus our efforts. For instance, the Genomics Unit can now offer deep sequencing services using the most recent technology in the field provided by the Illumina NovaSeq X-Plus, which was acquired this year by the Centre. We have also worked extensively on exploring the technological options available in the market to improve our infrastructures, particularly in the fields of mass spectrometry for proteomics analysis, confocal microscopy, and cell sorting by flow cytometry. This analysis has led us plan a number of equipment acquisitions that will be implemented throughout 2024 and 2025, and that are expected to represent a game changer in those fields for the CNIO.

Regarding the attraction of external resources by the Core Units, it is worth mentioning the grant awarded to the Mouse Genome Editing Unit from the call "*Plataformas ISCiii de apoyo a la I+D+I en Biomedicina y Ciencias de la Salud de la AES 2021-2023*" for a joint proposal together with the CNIO Biobank. The objective is to develop a biomodels and biobanks platform with the financial support of over €640k for the CNIO.

"The access to state-of-the-art technologies, assisted by expert technical staff provided by the Biotechnology Programme, continues being a key element in achieving the scientific goals of the CNIO Research Groups."

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

Last but not least, 2023 was once again a very productive year scientifically for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in nearly 20 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)*, Ileana B. González (TS)* (April-August),

Julia I. Morales (until Feb.) (TS)*,
Laura C. Woods (since April) (TS)*,
Pilar Ximénez de Embún (TS)*,
Eduardo Zarzuela (TS)*

*Titulado Superior (Advanced Degree)

OVERVIEW

Proteomics is acquiring a critical role in the comprehensive understanding of human biology. The rapid development of mass spectrometry-based proteomics instrumentation and data analysis pipelines has enabled the scientific community to delve deeper into the proteome than ever before. In the last decade, the output of proteomics studies has evolved from long lists of proteins to the generation of full statistically robust analyses, allowing proteomics to become truly functional. For example, cancer proteomics has unravelled key data on the mechanisms underlying tumour growth and metastasis, contributing to the identification of clinical biomarkers and novel therapeutic targets. The CNIO Proteomics Core Unit develops and applies state-of-the-art proteomics, bioinformatics and related technologies for the direct interrogation of proteome expression, modification, and

"...the goal is to transform data into information and information into insight" - Carly Fiorina.

function in cell-based models of human cancer. In collaboration with CNIO investigators, the Unit aims to provide valuable guidance for experimental strategies, which are critical for cancer research success.

RESEARCH HIGHLIGHTS

Here we describe some applications developed by our Unit:

Global proteome and post translational modifications (PTMs) profiling. In collaboration with the Melanoma Group, the Unit benefitted from the multiplicity of Tandem Mass Tag (TMT) isobaric reagents to perform both deep proteome and phosphoproteome analyses on multiple shDDX46 human and mouse disease-model cell lines. The data indicated dysregulated pathways common to both species, validating the role of DDX46 in tumour progression and immune suppression. With the CNIO-Lilly Cell Signalling and Immunometabolism Section, the Unit used a similar multiplexed quantitative strategy to characterise the mechanism of action and proteome selectivity of 3 different kinase inhibitors (FIGURE 1) in both human cell lines and mouse xenografts. *In vitro* phosphoproteomics data revealed significant differences across the 3 drugs, which were validated by the xenograft models.

Targeted proteomics. The Unit teamed up with the Epithelial Carcinogenesis Group to evaluate the expression levels of two RB1 phosphorylation sites under osmotic stress in mouse cell lines. Due to the low abundance of the RB1 diphosphorylated peptide, the Unit used a targeted proteomics approach called parallel reaction monitoring (PRM). PRM allows the quantification of peptides of interest with high sensitivity and accuracy. First, the corresponding stable isotope-labelled synthetic peptide was spiked into the samples to account for chromatographic variation and enhance precision. Second, PRM analysis validated the downregulation of both phosphorylation sites after inducing osmotic stress.

Interactomics. Mass spectrometry-based proteomics is an ideal tool to better understand the complete set of protein-protein interactions and the conditions in which they occur. Together with the Epithelial Carcinogenesis Group, the Unit validated the majority of the DREAM complex proteins as clear LIN9 interactors in both quiescent and proliferative mouse cell lines. In collaboration with the Transformation
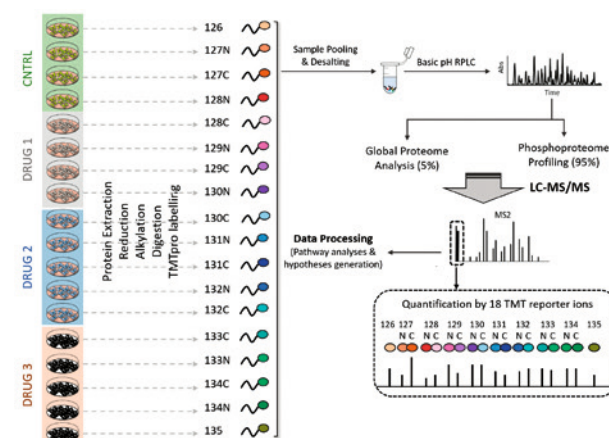
FIGURE 1 Schematic illustration of the multiplexed isobaric-labelling quantitative proteome and phosphoproteome profiling workflow.

and Metastasis group, the Unit is working to elucidate the interactome of both RANKL1 and RANKL3, 2 isoforms of the receptor activator of nuclear factor-kappaB–ligand. On this specific project, TurboID proximity-based proteomics will be used to further validate the hits initially identified with the traditional antibody-based pulldown immunoprecipitation.

Cross-linking. In collaboration with several groups of the Structural Biology Programme, the Unit has setup a new cross-linking mass spectrometry-based workflow. This technology interrogates protein structure and helps reveal novel protein-protein interactions. The Unit has used this approach to assess the different spatial and morphology constraints when comparing the phosphorylated to the non-modified form of the oncogenic fusion PCT1. ■

▸ **PUBLICATIONS**

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▸ Cuesta-Hernández HN *et al.* (incl. Muñoz J) (2023). An allosteric switch between the activation loop and a c-terminal palindromic phospho-motif controls c-Src function. *Nat Commun* 14, 6548.

▸ Sayago C, Sánchez-Wandelmer J, García F, Hurtado B, Lafarga V, Prieto P, Zarzuela E, Ximénez-Embún P, Ortega S, Megías D, Fernández-Capetillo O, Malumbres M, Munoz J (2023) Decoding protein methylation function with thermal stability analysis. *Nat Commun* 14, 3016.

▸ Mellid S *et al.* (incl. García F, Campos-Olivas R, Caleiras E, Muñoz J, Robledo M) (2023). DLST mutations in pheochromocytoma and paraganglioma cause proteome hyposuccinylation and metabolic remodelling. *Cancer Commun* 43, 838-843.

▸ Sanz-Castillo B *et al.* (incl. Campos-Olivas R, Ximénez-Embún P, Muñoz J, Malumbres M) (2023). The MASTL/PP2A cell cycle kinase-phosphatase module restrains PI3K-Akt activity in an mTORC1-dependent manner. *EMBO J* 42, e110833.

▸ Xia Q *et al.* (incl. Zarzuela E, Muñoz J) (2023). Peroxiredoxin 2 is required for the redox mediated adaptation to exercise. *Redox Biol* 60, 102631.

▸ Ibars E *et al.* (incl. Ximénez-Embún P, Muñoz J) (2023). Ubiquitin proteomics identifies RNA polymerase I as a target of the Smc5/6 complex. *Cell Rep* 42, 112463.

▸ Benito-Martin A *et al.* (incl. Ximénez-Embún P, Muñoz J, Peinado H) (2023). Mast cells impair melanoma cell homing and metastasis by inhibiting HMGA1 secretion. *Immunology* 168, 362-373.

▸ Peinado-Izaguerri J *et al.* (incl. Zarzuela E, Muñoz J) (2023) A novel dynamic proteomics approach for the measurement of broiler chicken protein fractional synthesis rate. *Rapid Commun Mass Spectrom* 37, e9497.

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Laura Conde,
Rodrigo Gómez (since May),
Guadalupe Luengo, Jorge Monsech,
Ángeles Rubio

OVERVIEW

The Genomics Unit provides centralised research services and expert consultation in the expansive fields of genomics and genetics. These services encompass a broad range of applications, from traditional to the latest genomic technologies, and contribute to uncovering biological complexity, therapeutic targets, and prognostic biomarkers. Leveraging their capacity to scrutinise entire genomes and their activities, these technologies can reveal the whole spectrum of structural and functional features. These include 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 staple among these technologies, while more traditional methodologies like Sanger capillary DNA

"Our service portfolio is customised to meet the needs of CNIO scientists in genomic and genetic technologies. It provides a flexible response for both generic and boutique services, ranging from basic housekeeping activities to advanced explorations of biological complexity."

sequencing are also provided. Additionally, the Unit manages a genotyping service for genetic models.

RESEARCH HIGHLIGHTS

With its array of molecular services, the Genomics Unit contributes to the dissection of biological complexity conducted by CNIO Research Groups. The Unit employs next-generation sequencing-based (NGS) technologies for different purposes, both at the structural and functional levels. On the one hand, genome or exome tumour characterisations, mutation repertoires, location of binding sites in DNA of relevant protein factors, variations in chromatin folding, and determination of on/off functional states are performed. On the other hand, transcriptional profiles reflecting functional choreographies are interrogated to decipher tumour compositions, uncover therapeutic targets and response to treatment, or to predict disease course. Moreover, the Unit can explore tissue composition, heterogeneity, and fate at single-cell resolution by capturing tens of thousands of cells in individual micro droplets and analysing them with the NGS platform. At the single locus level, other services are provided as well. A traditional DNA capillary sequencing service is still used to find and confirm mutations in candidate genes or for the verification of cloned genes or DNA segments. A cell authentication service, based on individual STR marker profiles, provides confidence in 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 a quick and efficient turnaround time.

Some of our contributions are reflected in the following 2 publications, with some of the Unit's members as authors. Bruna Calsina *et al*. describe specific genetic alterations associated with metastatic PPGL neuroendocrine tumours, including signalling networks involved in the metastatic competence as revealed by transcriptomic analyses. The impact of the immune ecosystem of the tumour microenvironment on clinical outcomes is highlighted. In another report, Marina Salmón *et al*. show how genetic ablation of mutant KRAS prevents the emergence of resistant cells in lung adenocarcinomas, while treatment with KRAS inhibitors leads to the rapid onset of resistance. The article provides insights into the mechanisms of resistance and how they can be targeted for treatment. ∎
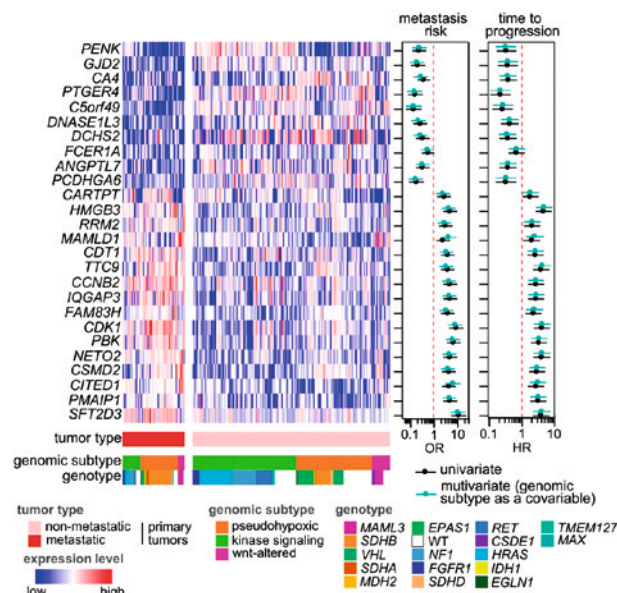
FIGURE 1 26-gene signature obtained by transcriptional profiling (RNA-seq) of pheochromocytoma/paraganglioma (PPGL) tumours, found to be associated with their clinical behaviour. The different analysed tumours are depicted in columns. Data kindly provided by B. Calsina and M. Robledo, from the CNIO Hereditary Endocrine Cancer Group.

▸ **PUBLICATIONS**

▸ Calsina B, Piñeiro-Yáñez E, Martínez-Montes ÁM, Caleiras E, Fernández-Sanromán Á, Monteagudo M, Torres-Pérez R, Fustero-Torre C, Pulgarín-Alfaro M, Gil E, Letón R, Jiménez S, García-Martín S, Martin MC, Roldán-Romero JM, Lanillos J, Mellid S, Santos M, Díaz-Talavera A, Rubio Á, González P, Hernando B, ..., Rodríguez-Perales S, Domínguez O, Macintyre G, Currás-Freixes M, Rodríguez-Antona C, Cascón A, Leandro-García LJ, Montero-Conde C, Roncador G, García-García JF, Pacak K, Al-Shahrour F, Robledo M (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.

▸ Salmón M, Álvarez-Díaz R, Fustero-Torre C, Brehey O, Lechuga CG, Sanclemente M, Fernández-García F, López-García A, Martín-Guijarro MC, Rodríguez-Perales S, Bousquet-Mur E, Morales-Cacho L, Mulero F, Al-Shahrour F, Martínez L, Domínguez O, Caleiras E, Ortega S, Guerra C, Musteanu M, Drosten M, Barbacid M (2023). Kras oncogene ablation prevents resistance in advanced lung adenocarcinomas. *J Clin Invest* 133, e164413.

CONFOCAL MICROSCOPY CORE UNIT

Isabel Peset
Core Unit Head

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

*Titulado Superior (Advanced Degree)

OVERVIEW

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

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

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

courses, talks and visits, always with the aim of increasing our understanding of the cellular and molecular disorders that lead to cancer and the study of potential treatments.

RESEARCH HIGHLIGHTS

The Confocal Microscopy Unit remains dedicated to providing imaging solutions to support research across the CNIO. The team plays a pivotal role in exploring various scientific questions, contributing with valuable insights to a range of projects.

In 2023, the Unit focused on enhancing tissue analysis by implementing immunofluorescence multiplex techniques for simultaneous visualisation and analysis of multiple markers. Together with the Histopathology Unit, the Unit has optimised an automate and reliable multiplex staining protocol for immune panels of 6 cellular markers. Semi-automated acquisition of whole tissue sections was also implemented using the Thunder widefield system to enable spatial analysis and understanding of tissue complexity. These images can be also imported and aligned in the SP8 confocal system for the automated acquisition of highly resolved tissue information. Furthermore, the Unit also developed advanced image analysis algorithms to extract quantitative and spatial information from complex immunofluorescence multiplex images, using deep learning tools for automated cell segmentation, classification, and feature extraction, reducing analysis time, and improving accuracy. Altogether, the Unit's efforts have contributed to improving the CNIO immunofluorescence multiplex tissue analysis platform, facilitating efficient analysis of diverse cellular markers simultaneously in different tissue samples.

To boost the screening capacity at the CNIO, the Unit started to develop Cell Painting high-content methods for cellular morphological profiling. Up to 6 fluorescence dyes are used to label different compartments of the cell to form their unique phenotypic profile by extracting features with automated image analysis. Biological perturbation upon drug treatments can be described by changes in the phenotypic profile providing the possibility to screen for unknown targets.

In June, Ana Cayuela joined the Confocal Microscopy Unit as a bioimage analyst, bringing signinficant experience in implementing image analysis tools, including deep learning for subtracting quantitative information from optical microscopy images. ∎
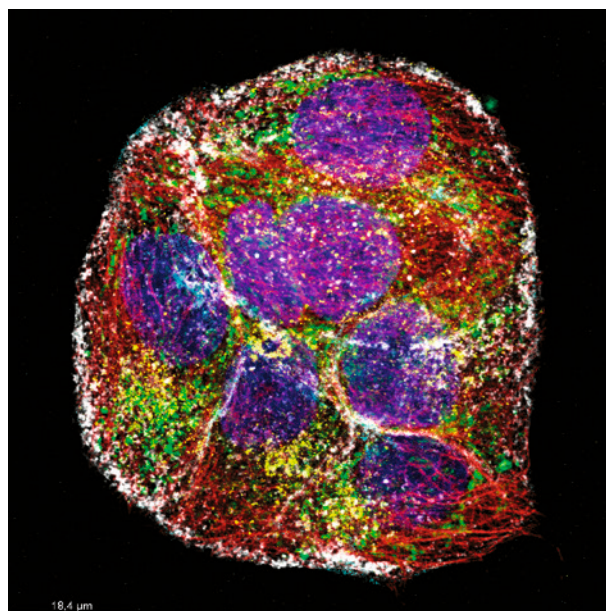
FIGURE 1 Developing immunofluorescence multiplex workflows. Cell Painting image. Nuclei are in blue, microtubules are in red, cell membrane is in white, actin is blue, and lysosomes are in yellow. Image courtesy of Manuel Pérez, Confocal Microscopy Unit.

‣ **PUBLICATIONS**

‣ Costa-Machado LF, Garcia-Dominguez E, McIntyre RL, Lopez-Aceituno JL, Ballesteros-Gonzalez Á, Tapia-Gonzalez A, Fabregat-Safont D, Eisenberg T, Gomez J, Plaza A, Sierra-Ramirez A, Perez M, Villanueva-Bermejo D, Fornari T, Loza MI, Herradon G, Hofer SJ, Magnes C, Madeo F, Duerr JS, Pozo OJ, Galindo MI, Del Pino I, Houtkooper RH, Megias D, Viña J, Gomez-Cabrera MC, Fernandez-Marcos PJ (2023). Peripheral modulation of antidepressant targets MAO-B and GABAAR by harmol induces mitohormesis and delays aging in preclinical models. *Nat Commun* 14, 2779.

‣ Sayago C, Sánchez-Wandelmer J, García F, Hurtado B, Lafarga V, Prieto P, Zarzuela E, Ximénez-Embún P, Ortega S, Megías D, Fernández-Capetillo O, Malumbres M, Munoz J (2023). Decoding protein methylation function with thermal stability analysis. *Nat Commun*, 14, 3016.

MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Tatiana Álvarez, Guillermo Garaulet (TS)*, Lourdes Huertas,

Guillermo Medrano (TS)*,
Luis Ordoñez (since March),
Judith Rey (TS)*, Jorge Rodríguez (until May), Gloria Visdomine

*Titulado Superior (Advanced Degree)

OVERVIEW

Molecular imaging is a medical imaging modality that enables the visualisation, characterisation, and measurement of biological processes at the molecular and cellular levels within living organisms. This field of medical imaging combines principles from various disciplines, including chemistry, biology, physics, and imaging technology, to provide detailed information about the biochemical and physiological processes occurring in tissues and organs. Preclinical molecular imaging techniques are non-invasive, meaning they do not require the sacrifice or dissection of animals. This allows for longitudinal studies where researchers can monitor disease progression and treatment effects over time in the same animals.

The Molecular Imaging Unit provides the CNIO Research Groups with a range of advanced equipment and technologies

"Molecular imaging is a powerful tool in biomedical research that allows scientists to gain insights into the molecular and cellular processes."

that can be instrumental in early disease detection, monitoring disease progression, assessing the effectiveness of treatments, and gaining a deeper understanding of disease mechanisms at the molecular level. This support is crucial for advancing medical research and improving patient care.

Student in Practice
Irene Bravo (February-June) (*E.T.S.I. Agronómica, Alimentaria y de Biosistemas*, Madrid, Spain)

Visiting Student
M. Carmen Ávila (June-August) (Massachusetts Institute of Technology, USA)

RESEARCH HIGHLIGHTS

The Molecular Imaging Unit is at the forefront of cutting-edge imaging technologies. Our core mission is to assist CNIO Research Groups in their endeavours through advanced molecular imaging techniques.

State-of-the-Art Equipment: A micro PET-CT system (eXplore Vista) from GE. A CT device (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 Perkin Elmer.

Expert Team: Our highly trained staff, dedicated to advancing the field, offers imaging analysis ranging from the fundamental to the most complex. Additionally, we provide optical imaging training to our users.

Research Advancements: Our Unit actively supports ground-breaking research and is committed to developing new imaging tools through funded research projects. We remain devoted to maintaining our technological leadership and pushing the boundaries of molecular imaging.

Our ongoing projects include:

→ BBVA Foundation Project ("Theragnosis using 177 Lu labelling and antibodies for cancer treatment"): This project stands out for its innovative approach to cancer treatment. The combination of ^{7}Lu labelling and antibodies for targeted cancer therapy is a promising avenue. The integration of ^{8}Ga PET imaging for primary tumour and metastasis localisation adds a valuable diagnostic element to treatment (FIGURE 1).
→ Renewed support from the *Comunidad de Madrid* grant (RENIM 2): The Spanish Network for Nanoparticles in Molecular Imaging, in a collaboration with the top centres in Molecular Imaging and Nanomedicine. The focus on developing and optimising molecular imaging probes, especially in the context of PET and optical imaging, aligns well with the current trends in medical research.
→ Installation of a 3T MRI System: The acquisition of a 3T MRI system through a Next Generation EU infrastructures grant is a substantial investment in CNIO research capabilities.

Overall, our ongoing projects showcase a strong emphasis on innovation and collaboration in the field of medical imaging and cancer treatment. The combination of ImmunoPET, collaborative efforts with top research centres, and the acquisition of advanced equipment positions our Unit as a leading force in the field. ■

FIGURE 1 PET Imaging with ^{68}Ga-LEM2/15 nanobody (Nb): 3D rendering projection showing tumour uptake in a breast cancer model. Abdominal uptake is a consequence of kidney and liver radioisotope elimination.

▸ **PUBLICATIONS**

▸ Salmón M, Álvarez-Díaz R, Fustero-Torre C, Brehey O, Lechuga CG, Sanclemente M, Fernández-García F, López-García A, Martín-Guijarro MC, Rodríguez-Perales S, Bousquet-Mur E, Morales-Cacho L, Mulero F, Al-Shahrour F, Martínez L, Domínguez O, Caleiras E, Ortega S, Guerra C, Musteanu M, Drosten M, Barbacid M (2023). Kras oncogene ablation prevents resistance in advanced lung adenocarcinoma. *J Clin Invest* 133, e164413.

▸ Martínez-Illescas NG, Leal S, González P, Graña-Castro O, Muñoz-Oliveira JJ, Cortés-Peña A, Gómez-Gil M, Vega Z, Neva V, Romero A, Quintela-Fandino M, Ciruelos E, Sanz C, Aragón S, Sotolongo L, Jiménez S, Caleiras E, Mulero F, Sánchez C, Malumbres M, Salazar-Roa M (2023). miR-203 drives breast cancer cell differentiation. *Breast Cancer Res* 25, 91.

▸ Mulero F (2023). Editorial: ImmunoPET imaging in disease diagnosis and therapy assessment. *Front Med* 10, 1231525.

▸ **AWARDS & RECOGNITION**

▸ Scientific Advisor of the Distributed Network of Biomedical Imaging (*REDIB ICTS*), Spain.
▸ Faculty and Mentor of *IDEA2* and *Catalyst* 2023 Programmes with MIT (Massachusetts Institute of Technology), USA.
▸ Editorial Board Member, *PLoS One*.

MOUSE GENOME EDITING CORE UNIT

Sagrario Ortega
Core Unit Head

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

*Titulado Superior (Advanced Degree)

OVERVIEW

Cancer encompasses an extensive range of highly intricate diseases. Alterations in the genetic and epigenetic makeup of tumour cells result in the acquisition of "malignant" characteristics, allowing them to elude normal physiological regulation. Techniques involving genome editing and transgenesis are employed to faithfully replicate these changes in mice, creating indispensable animal models essential for comprehending and improving cancer treatment. Tumour cells engage with various bodily systems, including the immune, cardiovascular, and lymphatic systems, influencing tumour growth, invasion, and expansion. Behavioural elements such as diet or smoking also influence cancer development. To unravel this intricate complexity, dependable *in vivo* models are imperative, replicating cancer features in a comprehensive "whole body" setting. The precise, targeted, and controlled manipulation of the mouse genome, utilising cutting-

"The Unit has more than 2 decades of expertise in designing, creating, and validating genetically modified mouse models through cutting-edge genome editing methods. Additionally, it houses a cryo-archive of numerous genetically modified mouse lines created at the CNIO."

edge genome editing tools, facilitates the development of genetic mouse models crucial for deciphering the molecular mechanisms of tumour formation and for preclinically validating advanced and more effective cancer therapies.

Student in Practice
Estefania Ayala (until April)
(Bachelor's Degree Final Project,
Universidad Autónoma de Madrid,
Spain)

RESEARCH HIGHLIGHTS

For over 2 decades, the Unit has been dedicated to the creation of genetically modified mice for disease modelling and preclinical drug testing. For a large fraction of this time, the only technology available to introduce targeted mutations in the germ line of the mouse was "gene targeting". Developed in the late 1990´s, gene targeting is based on the precise modification of the genome of pluripotent mouse embryonic stem cells by DNA homologous recombination of complex targeting vectors, selection of recombinant clones *in vitro*, and reconstitution of viable chimeric mice by reintroducing these modified cells into a host mouse embryo. The Unit established this technology in 2001 and, since then, has used it to create hundreds of knockouts, knockin and conditional mutant mice.

With the development of the CRISPR/Cas9 genome editing system in 2012, many limitations of gene targeting were overcome. A DNA double strand break (DSB) introduced at a precise location in the genome by the Cas9/gRNA complex turns on the cell's DNA repair machinery to fix the otherwise lethal break. If a proper DNA template is provided, the cell will repair the lesion by DNA homologous recombination orders of magnitude more efficiently than without the previous introduction of a DSB. With this system, targeted mutations can be efficiently introduced directly into mouse zygotes, making the use of embryonic stem cells unnecessary for the creation of many different types of targeted mutations.

The advantage of the CRISPR/Cas9 system over other targeted nucleases previously developed, such as Zinc Fingers or TALENs, which never replaced gene targeting in the mouse, is that it is easy to use, accessible, reliable, and efficient. Thanks to CRISPR/Cas9 gene editing tools, now we can create complex mouse mutants as fast as we can create transgenic mice by delivering the reagents directly into mouse zygotes, either by pronuclear injection or by electroporation (FIGURE 1).
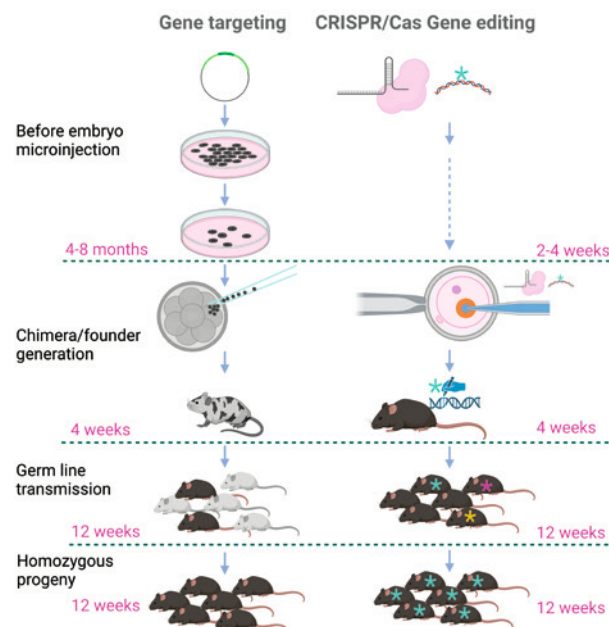
FIGURE 1 The use of CRISPR/Cas9 genome editing tools results in a significant reduction in the total time required to generate genetically modified mice, when compared to classical gene-targeting. Additionally, both labour and costs are substantially reduced, particularly in the initial stages.

During the last years, the Unit has optimised protocols for creating large knockin integrations and conditional (floxed) alleles directly in mouse zygotes. ∎

▸ **PUBLICATIONS**

▸ Maus M, López-Polo V, Mateo L, Lafarga M, Aguilera M, De Lama E, Meyer K, Sola A, Lopez-Martinez C, López-Alonso I, Guasch-Piqueras M, Hernandez-Gonzalez F, Chaib S, Rovira M, Sanchez M, Faner R, Agusti A, Diéguez-Hurtado R, Ortega S, Manonelles A, Engelhardt S, Monteiro F, Stephan-Otto Attolini C, Prats N, Albaiceta G, Cruzado JM, Serrano M (2023). Iron accumulation drives fibrosis, senescence and the senescence-associated secretory phenotype. *Nat Metab* 5, 2111-2130.

▸ Sayago C, Sánchez-Wandelmer J, García F, Hurtado B, Lafarga V, Prieto P, Zarzuela E, Ximénez-Embún P, Ortega S, Megías D, Fernández-Capetillo O, Malumbres M, Munoz J (2023). Decoding protein methylation function with thermal stability analysis. *Nat Commun* 14, 3016.

▸ Salmón M, Álvarez-Díaz R, Fustero-Torre C, Brehey O, Lechuga CG, Sanclemente M, Fernández-García F, López-García A, Martín-Guijarro MC, Rodríguez-Perales S, Bousquet-Mur E, Morales-Cacho L, Mulero F, Al-Shahrour F, Martínez L, Domínguez O, Caleiras E, Ortega S, Guerra C, Musteanu M, Drosten M, Barbacid M (2023). Kras oncogene ablation prevents resistance in advanced lung adenocarcinomas. *J Clin Invest* 133, e164413.

MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
Clara E. Gavira (since May) (TS)*, Scherezade Jiménez-Villa, Lorena Maestre (TS)*, Ana I. Reyes, María Villa (since October)

*Titulado Superior (Advanced Degree)

OVERVIEW

Monoclonal antibodies (mAbs) have become indispensable in biomedicine, revolutionising our approach to understanding and addressing complex biological processes. By providing researchers with a reliable and precise tool, mAbs contribute significantly to the advancement of our knowledge about the mechanisms involved in tumour transformation and development. Beyond their fundamental role in laboratory research, mAbs play a crucial part in cancer detection and therapy, offering practical solutions for its diagnosis and treatment.

The Monoclonal Antibodies Unit provides CNIO Research Groups with the "à la carte" generation of mAbs using hybridoma technology. We are highly specialised in the production of mouse and rat monoclonal antibodies. The

"The Monoclonal Antibodies Unit is highly specialised in the production and characterisation of mAbs, offering CNIO researchers reliable and well-validated reagents that significantly enhance the value of their research projects."

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

Students in Practice
Nuria Belén Caghago (March-June) (*IES Antonio* Machado, Alcalá de Henares, Spain), Álvaro García (until April) (Bachelor's Degree Final

Project, *Universidad Autónoma de* Madrid, Spain)

RESEARCH HIGHLIGHTS

During the last 23 years, the Monoclonal Antibodies Unit has generated a large number of mAbs, directed against more than 200 different antigens, mostly targeting molecules for which mAbs are not commercially available. Many of those 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 more than 100 thoroughly validated, high-quality mAbs (accessible at http://www.cnio.es/ing/servicios/anticuerpos/default.aspx). This catalogue is offered to specialised companies looking for licensing opportunities.

FIGURE 1 Schematic illustration outlines the key stages involved in the production of monoclonal antibodies.

Research activities:

The diagnostic value of TOX2 mAb in lymphomas. TOX2, a member of the "Thymocyte selection-related HMG box" protein family, shares structural similarities with TOX, TOX3, and TOX4. While TOX is linked to T cell exhaustion, TOX2 exhibits diverse functions, playing a critical role in human NK cell maturation by positively regulating TBX21 expression. Additionally, TOX2 influences invariant natural killer T cells (iNKT1) and modulates T follicular helper cell (Tfh) differentiation, by suppressing inflammation-related gene pathways.

Despite the importance of TOX2 in immune regulation, its distribution in reactive and neoplastic lymphoid tissues remains largely unexplored. To address this gap, our study employed a novel rat monoclonal antibody (TOM924D) specifically designed to detect TOX2 in paraffin-embedded tissue sections. Our study presents a comprehensive analysis of a large series of normal tissues and B and T-cell lymphomas, utilising both whole sections and tissue, contributing to a deeper understanding of TOX2 involvement in immune responses. Furthermore, by unravelling its expression patterns, this study not only expands our knowledge of the roles of TOX2 in health and disease, but also lays the foundations for potential diagnostic and therapeutic applications, especially in the context of lymphomas originating from NK cells.

CNIO recombinant mAbs. In the near future, the Monoclonal Antibodies Unit will enhance its portfolio by introducing the capability to convert hybridoma-based antibodies into a recombinant format. This innovative approach promises improved production quality, reduced variability, and the establishment of a secure and consistent supply of mAbs. This strategic addition not only represents a technological advantage, but also emphasises our commitment to implementing and providing cutting-edge solutions in every aspect of antibody development. ■

▸ **PUBLICATIONS**

▸ Larrayoz M *et al.* (incl. Roncador G, Gonzalez P) (2023). Preclinical models for prediction of immunotherapy outcomes and immune evasion mechanisms in genetically heterogeneous multiple myeloma. *Nat Med* 29, 632-645.
▸ Calsina B *et al.* (incl. Caleiras E, Jiménez S, Rodríguez-Perales S, Domínguez O, Macintyre G, Currás-Freixes M, Roncador G, Al-Shahrour F, Robledo M) (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.
▸ Fernández S *et al.* (incl. Maestre L, Roncador G) (2023). JAK/STAT blockade reverses the malignant phenotype of Hodgkin and Reed-Sternberg cells. *Blood Adv* 7, 4135-4147.
▸ Roldán-Romero JM *et al.* (incl. Roncador G, Robledo M, Rodriguez-Antona C) (2023). Deubiquitinase USP9X loss sensitizes renal cancer cells to mTOR inhibition. *Int J Cancer* 153, 1300-1312.
▸ Del Rio ML *et al.* (incl. Roncador G, Caleiras E) (2023). Genetic deletion of HVEM in a leukemia B cell line promotes a preferential increase of PD-1- stem cell-like T cells over PD-1+ T cells curbing tumor progression. *Front Immunol* 14, 1113858.
▸ Carreras J *et al.* (incl. Roncador G) (2023). Mutational profile and pathological features of a case of interleukin-10 and RGS1-positive spindle cell variant diffuse large B-cell lymphoma. *Hematol Rep* 15, 188-200.

HISTOPATHOLOGY CORE UNIT

Vacant
Core Unit Head

Research Scientist
Eduardo José Caleiras

Technicians
Nuria Cabrera, María Gómez,
Patricia González, Steffanny Annais
Mejía (since July), Verónica Neva,
Andrea Romero, Zaira Vega

OVERVIEW

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

"Progress in cancer research relies heavily on the access to state-of-the-art technologies and know-how facilitating the histopathological study of mouse models and human samples."

services to other institutions, including hospitals, research centres and private companies.

Student in Practice
Estefanía Mena (March-June) (*IES Antonio Machado*, Alcalá de Henares, Spain)

RESEARCH HIGHLIGHTS

In 2023, the Unit increased its workload, with the number of histological techniques performed (more than 12,000) 40% higher than in 2022. In addition, around 23,000 immunohistochemistry (IHC) techniques were performed (20% of them double and triple immunostaining), an increase of approximately 10%. Beyond this, 80 new antibodies have been optimised and made available to users, so the Unit currently has a portfolio of more than 920 antibodies available for IHC staining, including mice, humans, and xenograft tissues. Furthermore, a total of 19,300 slides were digitised, which is 10% above 2022. About 15% of this digitised material has been analysed with the support of the Unit, training researchers in the use of several image analysis software platforms.

We also consolidated the application of *in situ* hybridisation technology focusing on mRNA detection to paraffin-embedded tissue sections using RNAScope technology. Along the same lines, we incorporated BaseScope, which allows the detection of smaller RNA molecules, such as microRNAs. As many as 383 cases were analysed, some of them with double staining, using the Ventana-Roche's automated platform for IHC staining.

We continue our collaboration in the project focused on developing a system for the treatment of atrial fibrillation by irreversible electroporation, together with the company MedLumics and the *Universitat Pompeu Fabra*. The Unit is involved in the analysis of the pathological characteristics and mechanisms that mediate cell death in cardiac tissue after ablation of atrial fibrillation.
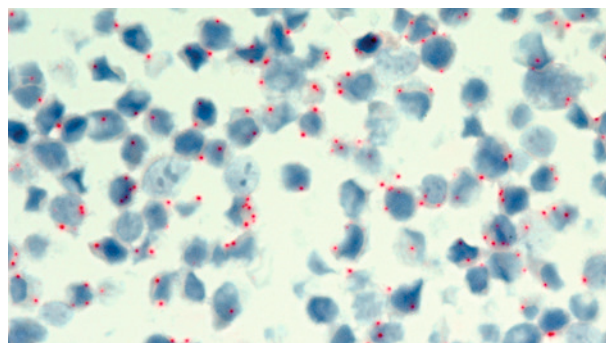
FIGURE 1 HeLa cells labelled with a probe for human peptidylprolyl isomerase B (PPIB) using BaseScope on a Ventana Ultra system. The red dots indicate the presence of the mRNA corresponding to PPIB. Courtesy of Cristina Montero (CNIO Hereditary Endocrine Cancer Group).

The high quality of the techniques run by the Unit continues being endorsed by External Quality Assessment Schemes. For example, our histochemical techniques were evaluated by UK NEQAS. On the other hand, NordiQC and *SEAP* (*Sociedad Española de Anatomía Patológica*) evaluated a subset of our IHC techniques under different modules, obtaining very good scores.

Training and outreach activities are also a key component of the Unit's activities. We had 1 student of *FP Grado Superior en Anatomía Patológica* in the lab for 3 months, developing a practical module. Finally, the Unit staff also participated in 2 master's courses on oncology research. ∎

▸ **PUBLICATIONS**

▸ Calsina B *et al.* (incl. Caleiras E, González P, Rodríguez-Perales S, Domínguez O, Macintyre G, Currás-Freixes M, Roncador G, Al-Shahrour F, Robledo M) (2023). Genomic and immune landscape of metastatic pheochromocytoma and paraganglioma. *Nat Commun* 14, 1122.

▸ Salmón M *et al.* (incl. Rodríguez-Perales S, Mulero F, Al-Shahrour F, Martínez L, Domínguez O, Caleiras E, Ortega S, Barbacid M) (2023). Kras oncogene ablation prevents resistance in advanced lung adenocarcinoma. *J Clin Invest* 133, e164413.

▸ Mellid S *et al.* (incl. Campos-Olivas R, Caleiras E, Muñoz J, Robledo M, Cascón A) (2023). DLST mutations in pheochromocytoma and paraganglioma cause proteome hyposuccinylation and metabolic remodeling. *Cancer Commun (Lond)* 43, 838-843.

▸ Martín A *et al.* (incl. Caleiras E, Velasco-Miguel S) (2023). Mitochondrial RNA methyltransferase TRMT61B is a new, potential biomarker and therapeutic target for highly aneuploid cancers. *Cell Death Differ* 30, 37-53.

▸ Del Rio ML *et al.* (incl. Roncador G, Caleiras E) (2023). Genetic deletion of HVEM in a leukemia B cell line promotes a preferential increase of PD-1- stem cell-like T cells over PD-1+ T cells curbing tumor progression. *Front Immunol* 14, 1113858.

▸ Martínez-Illescas NG *et al.* (incl. González P, Gómez-Gil M, Vega Z, Neva V, Romero A, Quintela-Fandino M, Caleiras E, Mulero F, Malumbres M) (2023). miR-203 drives breast cancer cell differentiation. *Breast Cancer Res* 25, 91.

▸ Mellid S, Gil E, Letón R, Caleiras E, ... Robledo M, Cascón A (2023). Co-occurrence of mutations in *NF1* and other susceptibility genes in pheochromocytoma and paraganglioma. *Front Endocrinol (Lausanne)* 13, 1070074.

▸ Santos M, Lanillos J, Caleiras E, Valdivia C, Roldan-Romero JM, ... Robledo M, Rodriguez-Antona C (2023). *PBRM1* and *KDM5C* cooperate to define high-angiogenesis tumors and increased antiangiogenic response in renal cancer. *Am J Cancer Res* 13, 2116-2125.

▸ Bueno MJ, Mouron S, Caleiras E, Martínez M, Manso L, Colomer R, Quintela-Fandino M (2023). Distribution of PD-L1, TROP2 and HER2-"lowness" in early triple-negative breast cancer: an opportunity for treatment de-escalation. *Clin Transl Oncol*. PMID: 37851244.

FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
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Julia García-Lestón (TS)*, Ana M.
Elizabeth Ilie

*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 the quantitative measurement of 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

"We keep an eye out for new technologies and developments potentially useful to the CNIO community. This year we tested several cell sorters and analysers with spectral as well as imaging capabilities to push CNIO capabilities further."

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 as well as perform single cell cloning and index sorting. We can accept human samples to sort under BSL2 regulations.

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in setting up and optimising flow cytometry techniques relevant to their research projects. Some applications developed and validated at our Unit include the following:

→ Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
→ Apoptosis studies (Annexin V, Mitochondrial Membrane Potencial, Caspase 3, etc.).
→ Multicolour immunophenotyping panels (B and T cell development, Tregs, inflammation, etc.).
→ Functional assays (side population detection, Ca^{2+} flux, intracellular pH, etc.).
→ Cytometric bead arrays to measure several cytokines from cell extracts and plasma.

→ Platelet studies.
→ Extracellular vesicles detection (microvesicles and exosomes).
→ CTC detection and isolation.
→ Single cell sorting for OMICs analysis.

We have further optimised our multicolour flow cytometry panels to characterise immune response in various samples from haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. 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 keep expanding 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. ■
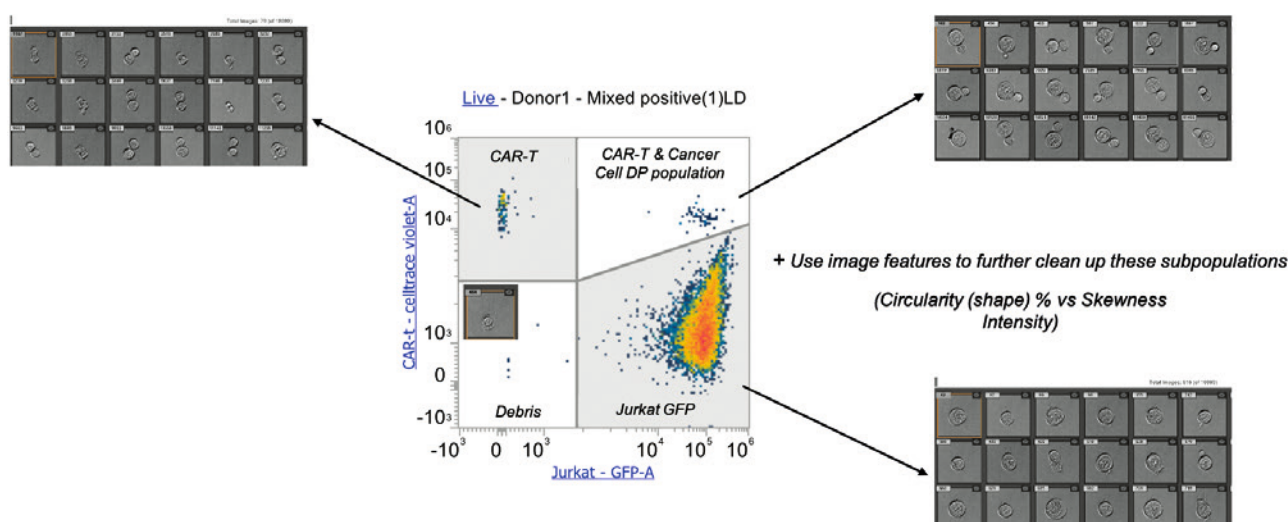
FIGURE 1 Example of applying imaging-derived parameters available at the CytPix (ThermoFisher) cytometer to further elucidate the CAR-T–Jurkat synapse. CAR-T cells (CellTraceTM Violet) co-cultured with Jurkat cells expressing GFP. Samples provided by the IdiPaz-CNIO Pediatric Onco-Hematology Clinical Research Unit.

▸ **PUBLICATION**

▸ Salmón M, Álvarez-Díaz R, Fustero-Torre C, Brehey O, Lechuga CG, Sanclemente M, Fernández-García F, López-García A, Martín-Guijarro MC, Rodríguez-Perales S, Bousquet-Mur E, Morales-Cacho L, Mulero F, Al-Shahrour F, Martínez L, Domínguez O, Caleiras E, Ortega S, Guerra C, Musteanu M, Drosten M, Barbacid M (2023). Kras oncogene ablation prevents resistance in advanced adenocarcinomas. *J Clin Invest* 133, e164413.

ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services

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

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

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

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 offers CNIO's new staff a course focused on work with laboratory animals, complementary to the online courses that are a legal requisite (*Orden ECC/566/2015*) to gain access to the facility. In 2023, a course was established in collaboration with the *ISCIII* and the *CNIC*, aimed to provide sustained training for users, ensuring improved management of animals and compliance with the regulation.

The high standards achieved by the CNIO regarding 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, considered one of the top international recognitions in this field, was first obtained in October 2016. Since 2022, the Animal Facility's Head has served as an AAALAC AdHoc Consultant, providing assistance to members of the Council on Accreditation in evaluating animal care and use programmes.

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

In 2023, the Head of the Animal Facility was elected Coordinator of the Spanish Animal Welfare Body Network (*ROEBA*) and appointed as the Spanish contact person for the European Network of Animal Welfare Bodies (ENAWB). This European network, endorsed by FELASA and receiving practical support from the European Commission, is dedicated to promoting a culture of care with the overarching goal of fostering ethical and humane treatment of animals. Also, the Assistant Veterinarian was recently elected Treasurer of *SECAL*, the most prominent scientific society in the field of laboratory animals in Spain.

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

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

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

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

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

EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director

The following highlights summarise some of the main achievements of the Experimental Therapeutics Programme (ETP) during 2023:

FOXO Activators (in collaboration with Refoxy Pharmaceuticals GmbH)*.* In 2020, CNIO established a collaboration with Refoxy Pharma (Berlin, Germany) to discover FOXO activators for potential development in multiple diseases. We identified several FOXO activators as the result of drug discovery related activities carried out in our Programme. Importantly, Refoxy has in-licensed several compounds of this project under a seven-figure license agreement signed in 2023. The license contemplated upfront and milestone-related payments.

PIM Inhibitors (sublicense from Inflection Biosciences to Mysthera Therapeutics). In 2013, Inflection Biosciences licensed a series of PIM selective inhibitors developed by ETP-CNIO. In 2023, Mysthera Therapeutics acquired the rights to continue the development of these molecules in the clinic. As result of this six-figure sublicense, the CNIO will receive the corresponding payments.

Mastl Inhibitors (MASTL-is) (in collaboration with Marcos Malumbres' Group). In 2023, we advanced in optimising our MASTL-is and PROTACs. We focused our attention on characterising and selecting molecules with the best inhibition / degradation potency and proteome-wide selectivity. We are currently selecting MASTL-dependent cancer cells to test their efficacy. In addition, we characterised several MASTL-is and PROTACs in ADME-T and pharmacokinetics studies. This will allow us to select lead compounds to perform future PK/PD studies in MASTL-dependent xenografts.

TRF1 (in collaboration with Maria A. Blasco's Group). In 2023, we focused our activities on validating previously identified hits as direct-TRF1 inhibitors. Several molecules were active in the TRF1-dsTelDNA Alpha-assay and inactive in the corresponding counter-screens. Ultimately, we demonstrated the functionality of several qualified hits by using non-radioactive EMSA experiments. Selected TRF1 inhibitors will be tested in the Telomeres and Telomerase Laboratory in a TRF1-dependent cell phenotypic assay.

SETD8 inhibitors (in collaboration with Óscar Fernández-Capetillo's Group)*.* After several screening campaigns, and using an H4 peptide as substrate, we identified potential SETD8 biochemical inhibitors of FL-SETD8 activity. In 2023,

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

we set up an assay using nucleosomes (NCPs) as substrate (NCPs were provided by the Macromolecular Complexes in DNA Damage Response Group). Unfortunately, none of the previous hits had been validated using NCPs. This prompted us to develop a more physiologically relevant cellular assay that measures H4K20 monomethylation. Currently, we are adapting this assay to the high-throughput screening format in order to perform future screening campaigns.

Others. ETP has worked in the early phases of other projects incorporated in the Programme: 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 Scientist
Ana Belén García, Cristina Gómez,
Esther González,
Sonsoles Rodríguez, Terry Pascal
Tomakinian (since April),
Carmen Varela

Visiting Scientists
Gonçalo J Lopes Bernardes (until August) (Cambridge University, UK), Justina Nwodo Ngozi (May-November) (University of Nigeria, Nusukka, Nigeria) (Science by Women Programme)

OVERVIEW

Medicinal Chemistry (MedChem) is a scientific discipline concerned with the design and synthesis of bioactive molecules to address unmet medical needs or to improve existing drugs. The discipline combines expertise in organic chemistry with knowledge of ligand-receptor interactions and pharmacology to design and modify the structure and properties of molecules, to improve potency and drug-like properties.

The Medicinal Chemistry Section is part of the multidisciplinary Experimental Therapeutics Programme (ETP), which focuses on early drug discovery activities. The ETP is integrated into the CNIO's structure, and acts as a bridge between basic research groups in cancer biology, which identify innovative targets that play a relevant role in cancer, and the pharmaceutical industry. Our goal is to generate molecules against these cancer targets and to demonstrate their efficacy and mechanism of action in animal models (*in vivo* proof of concept). This allows us to translate the results obtained in basic research laboratories into potential drugs that contribute to the understanding of cancer biology, as well as to increase the interest of the pharmaceutical industry to develop new therapies (FIGURE 1).

"We have licensed several hit compounds (Foxo activators) to Refoxy Pharmaceuticals GmbH as a result of a collaboration with them."

RESEARCH HIGHLIGHTS

Our MedChem activities focus mainly on the following projects:

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is carried out in collaboration with Maria A. Blasco (CNIO Telomeres and Telomerase Group). The ETP Biology Section previously developed an assay to measure the binding of TRF1 to telomeric DNA. This assay allowed us to identify several inhibitors that did not interfere with the assay system and did not bind to ds-TelDNA. After validating some of the hits, in 2023 we focused on generating structure-activity relationships (SARs) by synthesising analogues. These compounds were further characterised in a non-radioactive EMSA assay and will be tested in additional assays to ultimately demonstrate their efficacy against TRF1.

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

This project is undertaken in collaboration with Marcos Malumbres (CNIO Cell Division and Cancer Group). We envisaged 2 different approaches in this project: the search for small molecule inhibitors and for degraders. For the inhibitors, we continued to refine and characterise our lead compounds and generated back-up series to strengthen intellectual property and to determine the impact of different scaffolds on drug-like properties. In terms of degraders, we focused our activities on optimising our lead degraders by exploring different linkers, linking functional groups and several E3 ligase ligands. So far, we have identified several PROTACS with good degradation profiles in different cell lines. The final goal of the project is to achieve *in vivo* proof of concept (PoC) with the small molecule inhibitors and degraders.

Histone H4-Lysine 20 N-methyltransferase (SETD8) inhibitors

The search for SETD8 inhibitors is the result of a collaboration with Óscar Fernández Capetillo (CNIO Genomic Instability Group) and the Macromolecular Complexes in DNA Damage Response Group. Several assays were set up in the Biology Section (i.e., a biochemical assay with full-length SETD8 in the absence or presence of nucleosomes, and a cellular assay based on the determination of H4K20 monomethylation) to identify and characterise hit compounds. To date, the hits identified in initial biochemical screening campaigns have not yet been validated in the entire panel of assays. Moreover, we conducted a chemical exploration around the hits without significant success.

RANK antagonists as a novel therapeutic approach for the treatment of TNBC patients

We are collaborating with Eva González-Suarez (CNIO Transformation and Metastasis Group CNIO) to develop small molecules that specifically target the RANK receptor. The group of X. Barril (*Universitat de Barcelona*) conducted a second virtual screening of our ETP library of compounds. We supported the hit validation activities (i.e., SPR by the CNIO Spectroscopy and NMR Unit, and cell experiments by the Transformation and Metastasis Group), supervising the acquisition of hits and assessing their quality.

NUDT5 inhibition

Project under a strategic alliance with the *CRG/UIC* (R. Wright) 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. Currently, an initial SAR has been generated.

Synthesis of reference or tool compounds

Apart from the drug discovery activities in the above-mentioned projects, we give support to several groups by synthesising reference or tool compounds. During 2023, we carried out this work for the following CNIO Groups: Brain Metastasis, Genomic Instability, Metabolism and Cell Signalling, Telomeres and Telomerase, Topology and DNA Breaks, and Growth Factors, Nutrients and Cancer. ∎
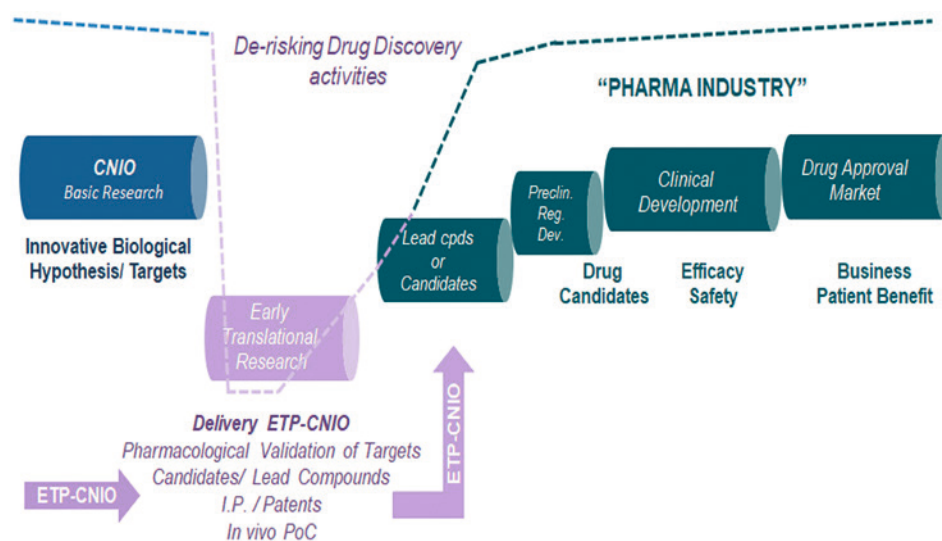
FIGURE 1 ETP-CNIO contributes to the pharmacological validation of innovative cancer targets discovered by the CNIO's Basic Research Groups through the generation of advanced lead compounds, thereby reducing the risks related to drug discovery around these targets and increasing the interest of industry in them.

▸ **PUBLICATION**

▸ Sanchez-Aguilera A, Masmudi-Martín M, Navas-Olive A, Baena P, Hernández-Oliver C, Priego N, Cordón-Barris L, Alvaro-Espinosa L, García S, Martínez S, Lafarga M; RENACER; Lin MZ, Al-Shahrour F, Menendez de la Prida L, Valiente M (2023). Machine learning identifies experimental brain metastasis subtypes based on their influence on neural circuits. *Cancer Cell* 41, 1637-1649.e11.

▸ **PATENTS**

▸ Pastor Fernández J, Martínez González S, Blanco-Aparicio C, García García AB, Rodríguez Aristegui S, Gómez de la Oliva CA, Albarrán Santiño MI, Cebriá Gómez A, Malumbres Martínez M. Imidazo[1,2-b] pyridazine based tricyclic compounds as inhibitors of HASPIN and therapeutic uses thereof. *WO2022200433*. National Phase Entry (2023).

▸ Pastor Fernández J, Blanco-Aparicio C, Martínez González S, Albarrán Santiño MI, Alvarez Escobar RM, Cebriá Gómez A, Cebrián Muñoz DA, García García AB, Gómez de la Oliva CA, González Cantalapiedra E, Hernández Encinas E, Martín Hernando JI, Ramos Lima FJ, Riesco Fagundo RC. Compounds. *PCT/EP2022/078724. WO2023099072*.

BIOLOGY SECTION

Carmen Blanco
Section Head

Research Scientist
Pablo Aparicio (since June)

Post-Doctoral Fellow
Elena Hernández

Gradutate Student
Lucía Cañizares (PEJ, CAM)*

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

Technicians
M. Isabel Albarrán (TS)**,
Antonio Cebriá (TS)**· Elena Gómez-Casero (TS)**

**Titulado Superior* (Advanced Degree)

Students in Practice
María Cuerda (until June) (Master´s Thesis, *Universidad Complutense de* Madrid, Spain)
Lucía González (since September) (Master´s Thesis, *Universidad Complutense de* Madrid, Spain)

Adriana Gorgen (February-May) (Bachelor's Degree Final Project, *Universidad Autónoma de* Madrid, Spain), María Villoro (March-June) (Bachelor's Degree Final Project, *Universidad Autónoma de* Madrid, Spain)

OVERVIEW

The Biology Section works in early drug discovery projects with the final aim of achieving *in vivo* proof of concept (PoC) with the lead molecules developed by the Programme. There is a long road before we can select the best or even the first candidate to perform PoC experiments in mice. These molecules have to be novel, potent at both the biochemical and cellular levels, selective, and with good ADME (absorption, distribution, metabolism, and excretion) properties to predict good plasma/tumour levels in mice.

Pharmacokinetic studies allow us to determine if the compounds have achieved plasma levels after intravenous, intraperitoneal, or oral administration, the oral being the preferred route. *In vitro* and cellular ADME studies help us to identify the liabilities behind the poor oral bioavailability of compounds (i.e., solubility, permeability/efflux or metabolic stability).

"We have out-licenced several hit compounds (Foxo activators) to Refoxy Pharmaceuticals GmbH as result of our collaboration."

RESEARCH HIGHLIGHTS

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

This project is carried out in collaboration with the CNIO Cell Division and Cancer Group. In 2023, we characterised around 130 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 the proliferation of MASTL-dependent cancer cells. In addition, in ADME-T and pharmacokinetics studies, we characterised several MASTL-is and PROTACs to facilitate the selection of lead compounds to perform future PK/PD studies in MASTL-dependent xenografts.

Telomeric repeat binding factor 1 (TRF1)

This project is undertaken in collaboration with the CNIO Telomeres and Telomerase Group. We are focused on identifying inhibitors of TRF1 binding to ds-Telomeric DNA. We have validated a series of inhibitors by using our AlphaScreen and readout counter-screen assays, as well as a SYBR-Green fluorescence displacement assay to discard potential ds-TelDNA binders. Ultimately, the functionality of qualified hits has been demonstrated by using non-radioactive EMSA experiments. All these experiments were carried out with freshly prepared and/or resynthesized samples. We have characterised around 80 analogues. Another structurally distinct hit, pending validation, has been identified from a screening campaign with an alternative ETP library. Selected TRF1 inhibitors will be tested in the Telomeres and Telomerase Laboratory in a TRF1-dependent cell phenotypic assay.

SET domain containing lysine methyltransferase 8 (SETD8)

This is a collaborative project 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 set up a biochemical assay with purified SETD8 f.l. and nucleosomes (NCPs), its natural substrate, to validate hits identified from 2 different screening campaigns using an H4 peptide as substrate. Unfortunately, none of the hits were validated with NCPs. This prompted us to move to a more physiologically relevant cellular assay to perform new screening campaigns. Currently, we are setting up a new cellular assay to measure monomethylation of H4K20 in an HTS format.

Collaborations with other CNIO Groups

ETP-Biology provided support in biochemical screening campaigns and hit validation activities for the Topology and DNA Breaks and Melanoma Groups. Moreover, we collaborated with the Experimental Oncology, DNA Replication, Chromosome Dynamics and Melanoma Groups by giving support in phenotypic cellular screening campaigns. Finally, we helped the Transformation and Metastasis Group with the cellular validation of hits previously identified from a virtual screening and with the establishment of a new cellular assay for screening.

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

→ Collaboration with the *CRG/UIC* (R. Wright): We characterised, in terms of ADME-T and pharmacokinetics, a series of analogues selected to generate SAR information for a NUDIX5 inhibitor previously identified by the researcher.
→ Collaboration with the *IRB* (C. Mayor-Ruiz): We supported the project by submitting our ETP-5K library and the analogues of current hits for screening. ∎
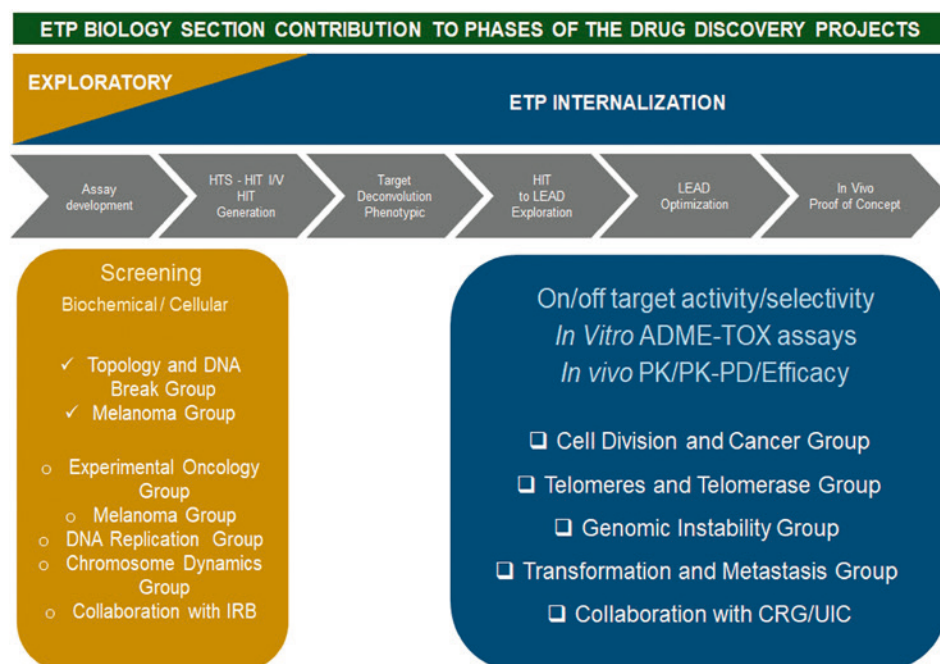
FIGURE 1 ETP Biology Section contributions to phases of the drug discovery projects. Exploratory projects: the ETP Biology Section contributes to cellular and biochemical campaigns for hit identification. Internalised projects: the ETP Biology section contributes to all the phases of drug discovery projects generating biochemical/cellular on- and off-target data, *in vitro* ADME-T data, and *in vivo* pharmacological data.

▸ **PUBLICATION**

▸ González-Muñoz T, Di Giannatale A, García-Silva S, Santos V, Sánchez-Redondo S, Savini C, Graña-Castro O, Blanco-Aparicio C, Fischer S, De Wever O, Creus-Bachiller E, Ortega-Bertran S, Pisapia DJ, Rodríguez-Peralto JL, Fernández-Rodríguez J, Pérez-Portabella CR, Alaggio R, Benassi MS, Pazzaglia L, Scotlandi K, Ratner N, Yohay K, Theuer CP, Peinado H (2023). Endoglin, a novel biomarker and therapeutical target to prevent malignant peripheral nerve sheath tumor growth and metastasis. *Clin Cancer Res* 29, 3744-3758.

▸ **PATENTS**

▸ Pastor Fernández J, Martínez González S, Blanco-Aparicio C, García García AB, Rodríguez Aristegui S, Gómez de la Oliva CA, Albarrán Santiño MI, Cebriá Gómez A, Malumbres Martínez M. Imidazo[1,2-b] pyridazine based tricyclic compounds as inhibitors of HASPIN and therapeutic uses thereof. *WO2022200433*. National Phase Entry (2023).

▸ Pastor Fernández J, Blanco-Aparicio C, Martínez González S, Albarrán Santiño MI, Alvarez Escobar RM, Cebriá Gómez A, Cebrián Muñoz DA, García García AB, Gómez de la Oliva CA, González Cantalapiedra E, Hernández Encinas E, Martín Hernando JI, Ramos Lima FJ, Riesco Fagundo RC. Compounds. *PCT/EP2022/078724. WO2023099072*.

CNIO - LILLY CELL SIGNALLING AND IMMUNOMETABOLISM SECTION

Susana Velasco
Section Head

OVERVIEW

Our laboratory, in collaboration with Loxo@Lilly Oncology, is working on the identification and validation of novel molecular targets engaged in the induction of Chromosomal instability (CIN). We aim to uncover novel vulnerabilities missed in previous studies using deep functional genomic screens. 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 and personalised therapeutic options.

A combination of *in vitro* and *in vivo* approaches is being utilised to obtain a complete understanding of the role of CIN in tumour development and anti-tumour response. Each target goes through an *in vivo* validation process using xenografts, allografts, and mouse models developed at the CNIO; this process includes the use of non-invasive *in vivo* imaging technologies, and immune histochemical characterisation of tumours for different metabolic, immune, and 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 (since March), Sandra Peregrina (TS)*

*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

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). 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 micronuclei 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 scape. The causes of CIN are diverse, including mitotic errors, replication stress, homologous recombination deficiency (HRD), and telomere crisis. ∎
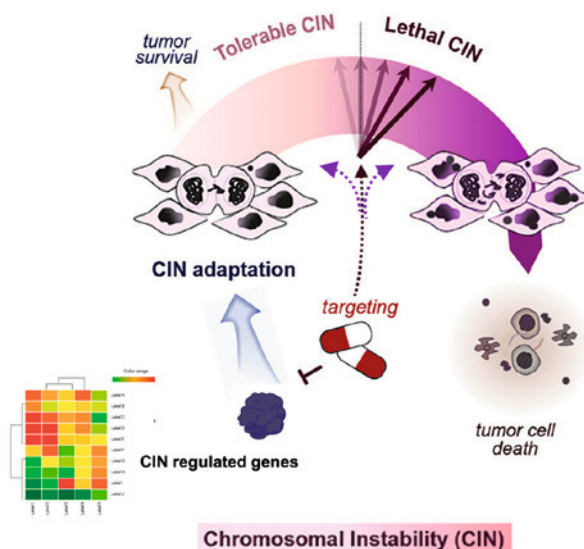
Modified from Dhital *et al.*, 2023, *Cell Reports Medicine* 4.

FIGURE 1 Chromosome instability (CIN): Identify vulnerabilities in cancer cell line models with various forms of genomic instability. 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 (TTVO)

Irene Herrera
Director

Technology Transfer Manager
Irene Pino

Administrative Technician
Rocío Manzano (since August)

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 to develop them further and thereby impact society.

The activities of the TTVO during 2023 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 impact on the biomedical industrial sector and society, both through exploitation licenses and through the creation of spin-off companies.

TTVO manages a portfolio of 44 active patent families, and provides advice and assistance during the drafting of patent documents, their filing, and the prosecution process.

"Our Office is fully aligned with the CNIO objective of translating new discoveries into cancer prevention, diagnosis and treatment for the benefit of society."

In 2023, 7 priority patent applications were filed, one of them co-owned with Seoul National University, another co-owned with *Fundación de Investigación Biomédica Hospital 12 de Octubre* (*FIBH12O*), and one more co-owned with *FIBH12O* and the company Tailor Bio Ltd. These 7 new patents protect quite varied inventions, including a combination therapy for PDAC; a signature for the prognosis of brain metastasis relapse; amplification and detection methods using Hyperbranched Rolling Circle Amplification; a method for analysing genomic sequencing data from targeted sequencing; a method of

characterising chromosomal instability in a DNA sample obtained from a tumour; a computer-implemented method of predicting whether a tumour is likely to acquire a gene amplification; and a method for detecting pathogenic germline variants in Mendelian disease-associated genes from samples of cancer patients. Moreover, 2 PCT (Patent Cooperation Treaty) applications for international extension were also filed in 2023.

Licensed patents make up 36% of the CNIO portfolio. In 2023, pharmaceutical industry interest in compounds identified and characterised by the Experimental Therapeutics Programme (ETP) resulted in 2 agreements. First, the German company Refoxy Pharmaceuticals GmbH and CNIO signed an exclusive license agreement for the intellectual property pertaining to several FOXO activators identified by ETP in collaboration with Refoxy. Second, the Irish company Inflection Biosciences Ltd entered into a license agreement with the Swiss-based biotech Mysthera Therapeutics AG for its PIM kinase inhibitor programme licensed from CNIO, with the aim to treat major unmet needs in autoimmune diseases. In addition, Tailor Bio signed an exclusive option to negotiate a commercial license for the patents EP23383179.1 "Detection of chromosomal instability" and EP23383180.9 "Prediction of gene amplification" from Geoff Macintyre's Group.

Over the course of the year, the TTVO managed 213 agreements (MTAs, CDAs, Research Collaborations, licenses, etc.). The majority of these agreements (63%) were established with international entities, which is an indicator of the internationalisation of CNIO's research activity. Through collaborations with industry, €4 million were secured for research activities.

Among the most outstanding agreements signed with the private sector is for the continuation of the collaboration with *CRIS* S.L. and THOT BIOTECH A.I.E. for the second phase of the "Kras Cancer" project directed by Mariano Barbacid (CNIO Experimental Oncology Group), with a budget of €1.5 million for 3 years.

Other relevant agreements with the private sector include the collaboration with Loxo Oncology Inc. The Signalling and Immunometabolism Section headed by Susana Velasco is working to identify and validate novel molecular targets involved in the induction of chromosomal instability (CIN). Loxo Oncology and CNIO renewed their agreement until the completion of the research activities, with a budget of €1.8 million per year.

Finally, several other research agreements were signed, such as one with L'Oreal *España* for a project with the Telomeres and Telomerase Group directed by Maria A. Blasco; a collaboration agreement between Janssen Pharmaceutica NV, the Genetic & Molecular Epidemiology Group (Núria Malats) and the Epithelial Carcinogenesis Group (Francisco X. Real); and a sponsored research agreement between Verastem Inc. and the Experimental Oncology Group (Mariano Barbacid).

Patents and unpatented research tools (murine lines, cell lines and antibodies) are licensed to provide financial return to CNIO. In 2023, the net income generated in 2022 from CNIO asset licenses was posted, which amounted to €1.6 million (about €1.1 million from monoclonal antibodies alone).

The creation of spin-off companies is one of the technology transfer mechanisms through which knowledge is translated into commercial products and/or services. A new spin-off in the field of precision therapeutic nutrition "*TNC Nutrición Terapéutica*", in which the CNIO and the *FIBH12O* participate, provides nutritional and dietary advice for patients with cancer and other diseases. CNIO and *FIBH12O* granted a license to *TNC* for the commercial exploitation of a proprietary algorithm for precision nutrition developed by Miguel Quintela (CNIO) and Luis Manso (*FIBH12O*).

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

Biobank

BIOBANK

Eva Ortega-Paíno
Director

Technicians
Nuria Ajenjo (TS)*, Daniel Alba (TS)*, Inmaculada Almenara, M. Jesús

Artiga (TS)*, Pilar Caro (TS)*, Cecilia Sobrino, José M. Escolano (since March) (TS)*

*Titulado Superior (Advanced Degree)

Visiting Researcher
Inmaculada de Melo Martín (Weill Cornell Medicine, Medical College, Cornell University, New York, USA)

OVERVIEW

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

CNIO Biobank is a cross-service platform for CNIO researchers and the general scientific community that offers broad service covering all stages in research project management requiring the use of human samples. CNIO Biobank offers: sample processing; collection management; quality, ethical and legal consultancy; acquisition and design of valuable research collections; negotiation with different stakeholders to find suitable samples; and/or help to obtain the ethical approval for research projects.

"Modern translational research cannot happen without collaboration, biobanks, and their networks. Biobanks are a trademark of high quality, both for the samples and their associated data."

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

RESEARCH HIGHLIGHTS

Collections of sample and data

CNIO Biobank houses more than 9,000 cases (donors) amounting to almost 45,000 samples. These samples correspond to the historical archive collections (preserved tissue of lymphomas, gynaecologic and digestive neoplasia, mammary carcinomas, non-neoplastic cases and tissue microarrays [TMAs]); primary skin cultures; patient-derived xenograft (PDX) collection (with 243 cases); the Covid collection (700 cases); the brain metastasis (RENACER) collection, which has grown to 170 cases thanks to the agreements signed with 19 hospitals; and a prospective cohort of samples from the Association of Flight Attendants (*AETCP*), with a wide set of epidemiological data of 120 cases. Furthermore, the Biobank's Virtual Catalogue includes 8,064 images from TMAs, histological H&E stains, and IHQ images.

Services to researchers

→ Transfer of samples to research projects: 730 samples have been transferred to support research projects and 9 samples for technical validations. The knowledge generation impact related to this activity has resulted in 7 publications acknowledging the Biobank's contribution, 5 of them in Q1 journals, with a mean impact factor (IF) of 31.06.

→ Custody of collections: we offer the service of custody and management of collections for researchers, ensuring traceability of the samples and compliance with GDPR.

→ Ethical-legal advice to researchers: 35 queries about ethical and legal aspects of research were received and answered from both CNIO and external researchers, and other biobanks or companies. We have also helped home-researchers to obtain ethical approval of their projects (6) by the *ISCIII* Research Ethics Committee.

FIGURE 1 Biobank resources: image of the archive of paraffin-embedded specimens.

Participation in cooperative projects

→ *ISCIII* Biobanks and Biomodels Platform, promoted by the *Carlos III* Health Institute.

→ National Brain Metastasis Network. *Ramón Areces* Foundation.

→ COST Action CA20122-Harmonizing clinical care and research on adrenal tumours in European countries.

→ REACT (Respiratory Host Pathogen Interaction), led by the *Statens Serum Institut* (SSI, Denmark) in collaboration with other institutions in Spain, Denmark, Sweden and South Africa, to investigate viral lower respiratory tract infections.

→ Establishment of a precision medicine pipeline to personalise the treatment of brain metastasis – HR23-00051 *"la Caixa"* (2023).

→ RENACER, a national network approach as a strategy to challenge brain metastasis-associated lethality, therapeutic resistance, and impact on quality of life. *AECC* (2023).

Organisation and participation in formation and dissemination activities

The CNIO Biobank actively contributes to and participates in the organisation of training and dissemination activities. ∎

PUBLICATIONS

▸ Pairo-Castineira E, *et al.* (incl. Ortega-Paino E as collaborator) (2023). GWAS and meta-analysis identifies 49 genetic variants underlying critical COVID-19. *Nature* 617, 764-768.

▸ COVID-19 Host Genetics Initiative (2023). A second update on mapping the human genetic architecture of COVID-19. *Nature* 621, E7-E26.

▸ Sanchez-Aguilera A, Masmudi-Martín M, Navas-Olive A, Baena P, Hernández-Oliver C, Priego N, Cordón-Barris L, Alvaro-Espinosa L, García S, Martínez S, Lafarga M; RENACER; Lin MZ, Al-Shahrour F, Menendez de la Prida L, Valiente M (2023). Machine learning identifies experimental brain metastasis subtypes based on their influence on neural circuits. *Cancer Cell* 41, 1637-1649.

▸ Valiente M, Ortega-Paino E (2023). Updating cancer research with patient-focused networks. *Trends Cancer*. PMID: 37802739.

▸ Castro-Santos P, Rojas-Martinez A, Riancho JA, Lapunzina P, Flores C, Carracedo Á, Díaz-Peña R; Scourge Cohort Group (2023). HLA-A*11:01 and HLA-C*04:01 are associated with severe COVID-19. *HLA* 102, 731-739.

AWARDS AND RECOGNITION

Eva Ortega-Paíno:
▸ Appointed new Secretary General for Research (December 27, 2023), Ministry of Science, Innovation and Universities, Spain.
▸ XI edition *GEPAC* Award in the category "Social and Scientific Research in the Oncological Field" for the collaborative project RENACER (creation of the National Network of Brain Metastases), Spain.

Eva Ortega-Paíno and Nuria Ajenjo:
▸ Panel Members of the Ethics Board on "Advising Personalised Oncology" of the canSERV Consortium (2023). CanSERV - providing cutting-edge cancer research services across Europe.

Communications

COMMUNICATIONS
MÓNICA G. SALOMONE Director

Communications
Pilar Gil (since June)

Science Communication and Social Media
Esther Sánchez (since March)

CNIO is a leading cancer research institution on a global scale, and the Communications team must ensure that the general public identifies it as such. To do so we need to engage in a productive dialogue with society: as a knowledge-generating institution, CNIO must meet the citizens' demand for information, while enriching the public conversation by sharing its discoveries in an attractive and comprehensible way.

Our strategy is based on producing very high-quality media content; strengthening CNIO's own channels, such as social media; encouraging dialogue with the media; and creating new opportunities for effective communication between CNIO and the public.

Support from the whole CNIO community is therefore crucial. In 2023 CNIO researchers flagged up new results and topics of interest, and showed generosity in their dealings with the media. The number of such requests increased significantly, in parallel with substantial growth in the generation of our own content and in the activities organised by CNIO Communications.

In 2023, we sent to the media and published on the website a total of 117 press releases and interviews, mostly about results, projects, and initiatives of the CNIO community. It is a 41% increase compared with 2022. We also more than duplicated the posts on CNIO social media: 1,114 on X (formerly Twitter); 340 on Instagram; and 175 on LinkedIn.

Qualitative changes were also made. We produced more social media shorts in which researchers get the message across in a clear and concise way —see the videos of speakers from the CNIO-*Caixa*Research Frontiers Meeting on Metastasis, and the V Philosophy and Science Seminar organised with the support of *Fundación Banco de Sabadell*.

2023 gave us more opportunity for direct interaction between CNIO scientists and members of the general public. We organised the webinar *Pregunta a @Astro_SaraG*, in which schools from all over Spain interacted by videoconference with CNIO researcher and astronaut from the European Space Agency (ESA) Sara García Alonso. Over a hundred schools joined in —including some from outside Spain—, each with groups of more than 20 students. For the occasion, we created several videos about Sara's research, which could also be used subsequently to raise the profile of CNIO in other forums.

CNIO participated in the *Madrid es Ciencia* fair, thanks to volunteer researchers who organised experiments and answered questions from hundreds of visitors. And on the occasion of European Researchers' Night, we celebrated the first 'live stream' on CNIO's Instagram, a pioneering event for a scientific institution which saw hundreds of followers tuning in.

"We strive to produce high-quality media content, strengthen CNIO's own channels, encourage dialogue with the media, and create new opportunities for effective communication between CNIO and the public."

Along with these new outreach actions we strengthened initiatives established in previous years. These include World Cancer Research Day at *Caixa*Forum, dedicated to cancer prevention in 2023. It was attended by the director of the International Cancer Agency, Elisabete Weiderpass; Marina Pollán, former director of the National Epidemiology Centre and current director of the *Carlos III* Health Institute (ISCIII); and CNIO director, Maria A. Blasco. The event was a hit with the public and the media.

Also providing great visibility to the CNIO is Maria A. Blasco's regular collaboration with *RNE*, which opens up a window to scientific topics in prime time.

We worked closely with the Institutional Image & Outreach Office to communicate the VI edition of CNIO Arte, with visual artist Amparo Garrido and Nobel Prize winner Elizabeth Blackburn. The event was covered by key mainstream media such as *TVE, RNE* and *SER*, in addition to art media and supplements such as *El Cultural*. Several of these media services also covered the route of the travelling exhibition *Dialogues between Art and Science*, at the headquarters of the Cervantes Institute in New York and Chicago, and at the Spanish Embassy in Washington.

Our collaboration with the Philanthropy Office was particularly strong in CNIO's campaign on World Cancer Day, to communicate CNIO Friends Day, and on the production of the CNIO Friends newsletter.

On the whole, our efforts brought a substantial increase in CNIO followers on social media, as well as in the impact through traditional media. This has been possible because two experienced new members joined CNIO Communications in 2023: Esther Sánchez, an expert in social media and scientific information, and Pilar Gil, a veteran science journalist. Thanks to the support from the entire CNIO community, we will continue working to make CNIO known as the leading Spanish centre in cancer research. ∎

PRESS CLIPPINGS

1   *El Español*, February 2, 2023
2   *RTVE*, April 13, 2023
3   *El Correo Bizkaia*, May 19, 2023
4   *El Diario*, June 13, 2023
5   *La Sexta*, June 28, 2023
6   *El Mundo*, October 1, 2023
7   *RTVE*, October 5, 2023
8   *20 Minutos*, October 11, 2023
9   *20 Minutos*, October 18, 2023
10 *La Sexta*, October 30, 2023
11 *El País*, October 31, 2023
12 Bioengineer, October 31, 2023

CNIO MEDIA IMPACT

TRADITIONAL MEDIA

VALUE OF MEDIA APPEARENCES DURING 2023

DIGITAL
43.6%
€16,696,000

TOTAL
100%
€36,000,000*

PRINT
40.0%
€14,400,000

OTHER
3%
€1,080,000

AUDIOVISUALS
13.4%
€4,824,000
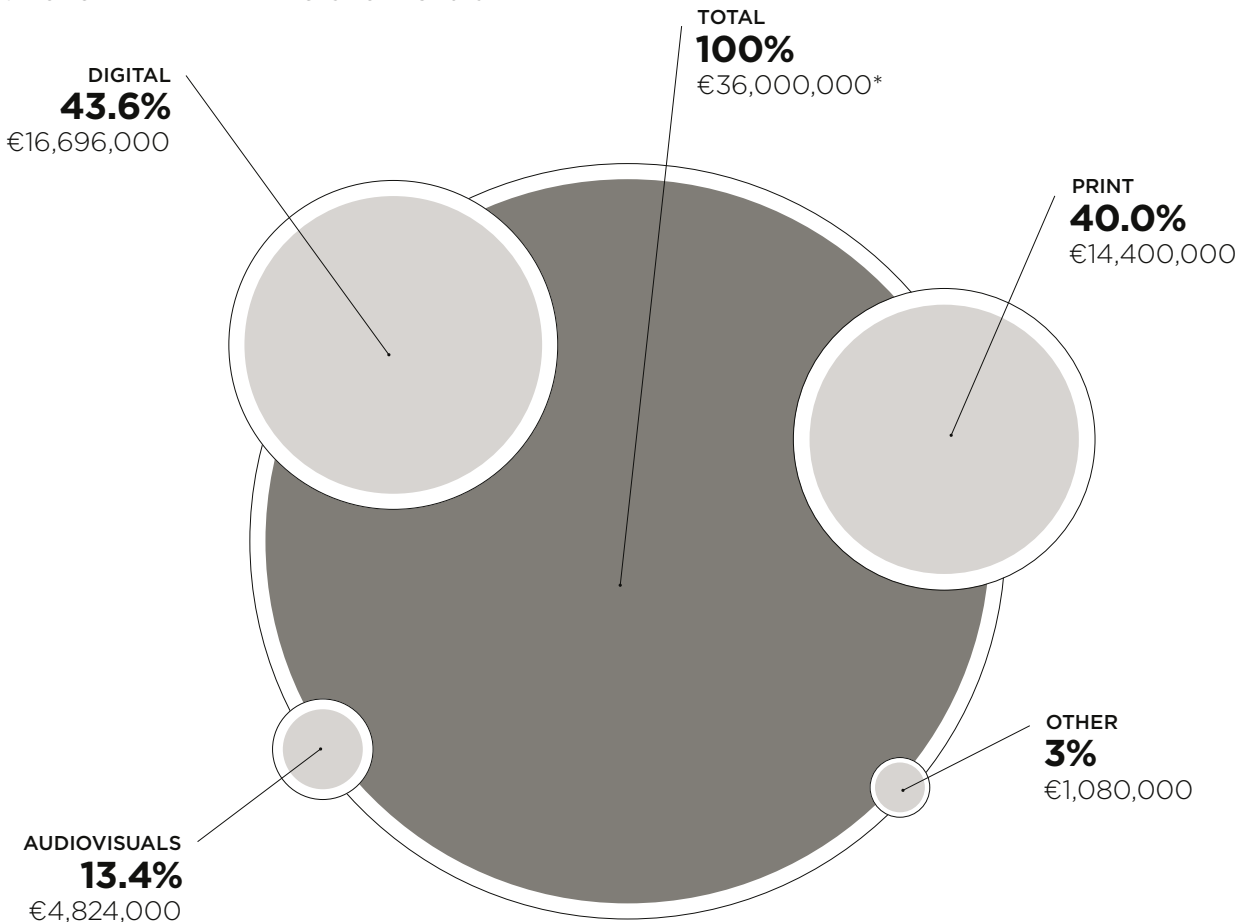
*4 million increase compared to 2022

VALUE OF MEDIA APPEARENCES (HISTORIC)

2021	**€ 25 MILLION**	
2022	**€ 32 MILLION**	
2023	**€ 36 MILLION**	
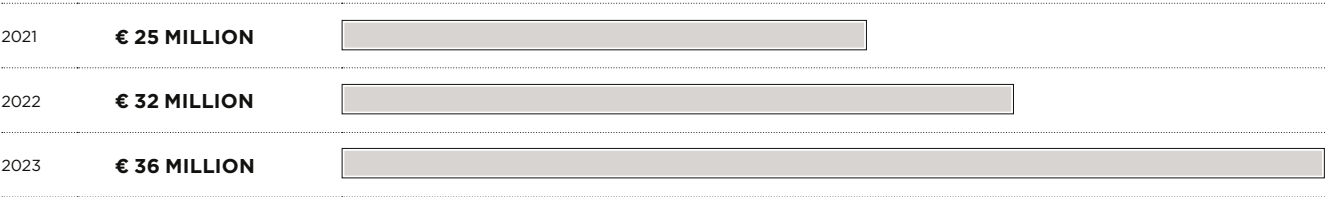
SOCIAL MEDIA

X (TWITTER)

	2022	2023	Δ
FOLLOWERS	25,352	**26,707**	+1,355
POSTS	1,497	**1,114**	-383
AUDIENCE	2,204,111	**1,434,049**	-770,062

INSTAGRAM

	2022	2023	Δ
FOLLOWERS	4,795	**6,002**	+1,207
POSTS	248	**340**	+92
AUDIENCE	97,327	**279,053**	+181,726

LINKEDIN

	2022	2023	Δ
FOLLOWERS	27,667	**32,611**	+4,944
POSTS	51	**175**	+124
AUDIENCE	471,870	**914,865**	+442,995

FACEBOOK

	2022	2023	Δ
FOLLOWERS	34,803	**34,811**	+8
POSTS	129	**128**	-1
AUDIENCE	384,729	**133,511**	-251,218

YOUTUBE

	2022	2023	Δ
FOLLOWERS	1,759	**1,911**	+152
POSTS	50	**24**	-26
AUDIENCE	27,500	**31,500**	+4,000

CNIO SOCIAL MEDIA CONTENT
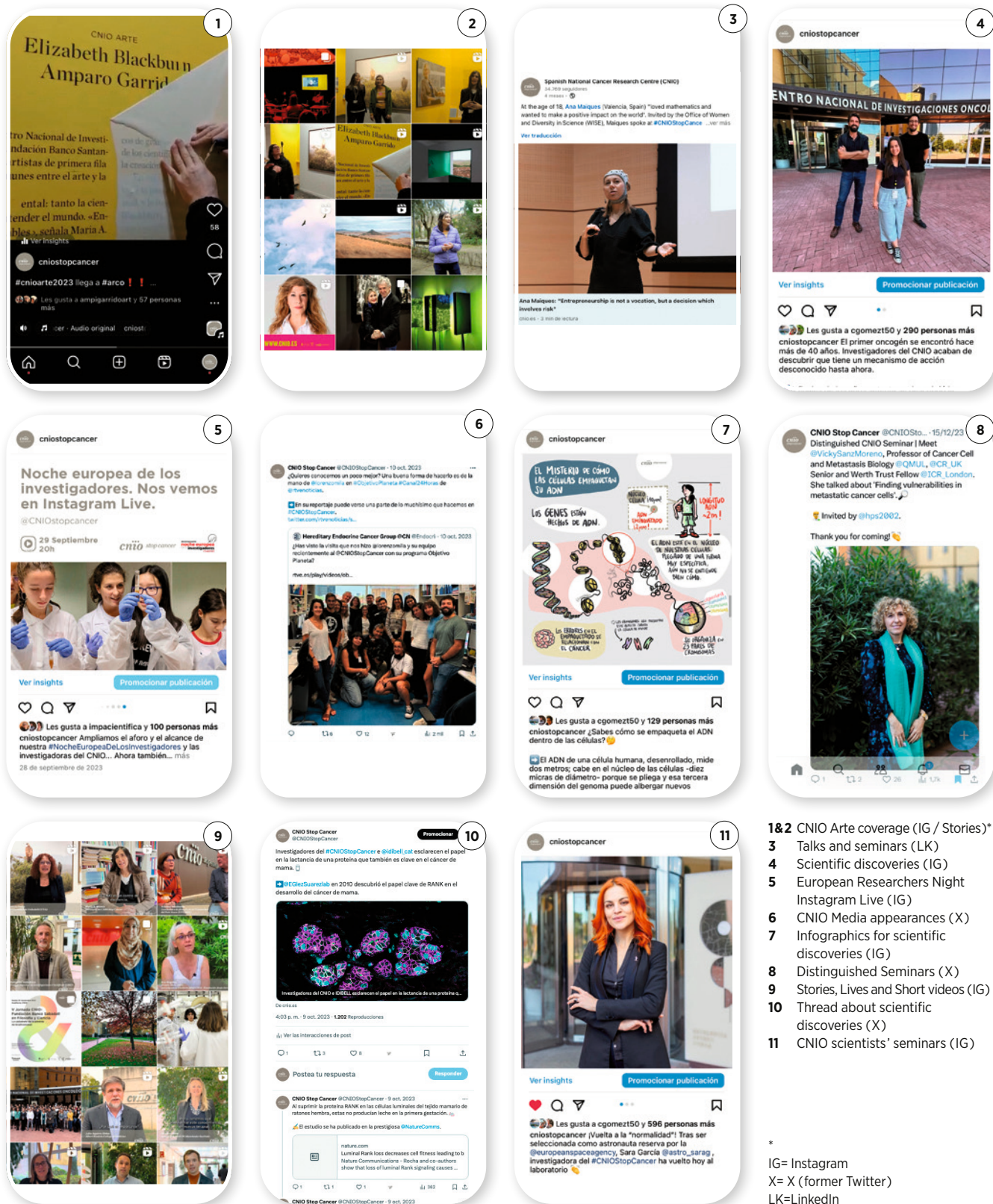
1&2 CNIO Arte coverage (IG / Stories)*
3 Talks and seminars (LK)
4 Scientific discoveries (IG)
5 European Researchers Night Instagram Live (IG)
6 CNIO Media appearances (X)
7 Infographics for scientific discoveries (IG)
8 Distinguished Seminars (X)
9 Stories, Lives and Short videos (IG)
10 Thread about scientific discoveries (X)
11 CNIO scientists' seminars (IG)

*
IG= Instagram
X= X (former Twitter)
LK=LinkedIn

SOCIAL EVENTS

Awareness about the impact of pollution and processed meat on cancer. On World Cancer Day CNIO alerted the public about the influence of environmental and life-style factors on our health. "Taking care of your health is also taking care of your planet, and vice versa," Maria A. Blasco said. "This message has not yet caught on with the general public, but the evidence is overwhelming, and emphasising it seems to us a matter of social responsibility". In an altruistic collaboration, actress Nathalie Seseña starred in the campaign, which focused particularly on the risks posed by pollution on lung cancer and processed meat consumption on colon cancer. The message also encouraged contributions to cancer research by joining the CNIO Friends philanthropic initiative. *February 4, 2023.*

Video Capture

Sara García shares her passion for research with school children. "The beauty of working in science is that every single day is different from the day before", CNIO researcher Sara García Alonso said. CNIO celebrated International Women and Girls in Science Day, releasing two short videos about her research at CNIO, as well as the recording of her virtual meeting with boys and girls from more than a hundred primary and secondary schools. "To cure cancer, we have to understand its origin first, that is one of our goals at CNIO", she told the young audience. Sara is also a European Space Agency (ESA) astronaut. *February 11, 2023.*

A fair to experience lab work and talk to scientists. "We do research to prevent, diagnose and treat cancer". This was the slogan of the CNIO stand at "Madrid is Science", an outreach fair organised by the Community of Madrid and aimed especially at high schools. From March 23 to Saturday March 25, visitors had the opportunity to observe samples of healthy and diseased tissue (melanoma, pancreatic cancer...), to experience how skin lesions are studied in dermatology, and —the most successful part— to meet engaged and inspiring researchers. *March 23-25, 2023.*

Prevention, the first step to fight cancer. At the conference *How to reduce the risk of cancer? Prevention through research*, organised by CNIO on World Cancer Research Day, Elisabete Weiderpass, director of the International Agency for Research on Cancer (IARC), pointed out that half of cancer cases are due to preventable causes, such as smoking, alcohol or a poor diet, among other factors. Marina Pollán, director of the Epidemiology and Public Health Networking Biomedical Research Centre (*CIBERESP*), stressed that in Spain 20% of adults smoke, and two-thirds of men and half of women are overweight. The abandonment of the Mediterranean diet (vegetables, legumes, fruit, olive oil, nuts, little meat) is already noticeable in clinical practice. This problem is greater in more disadvantaged areas. *September 18, 2023.*

Sharing the awe of lab work with the youngest public. On September 29th, CNIO joined the European Researchers' Night celebrations by offering the public a series of hands-on experiments where they could extract DNA from a tomato, observe tissue samples through different microscopes, and learn about lab work. A handful of young researchers talked about their career paths at the Margarita Salas Auditorium, and several units of the centre showed their particular areas of work. For those who could not attend the event, there was an Instagram-Live session that allowed followers to ask young researchers about their tasks, interests, and careers. *September 29, 2023.*

Philosophers warn our life-style could lead to a sixth mass extinction. The *V Philosophy and Science Seminar* held at CNIO with the support of *Fundación Banco Sabadell* brought together thinkers on major issues that impact our current way of life. Ecologists Anna Traveset and Fernado Valladares shared facts that show biodiversity loss is irrecoverable. With impacts ranging from food security to mental illness, they mentioned the necessity of economical degrowth to avoid a sixth mass extinction. Philosophers Antonio Diéguez, Arantza Etxeberria, and Alicia Puleo underlined the dialogue between philosophy and science as essential to confront the ecological crisis, which co-exists with a "crisis of truth", according to Puleo. Ecofeminism, cooperation, and escape from extreme individualism were presented as valuable attitudes to recover the ecological balance. *December 4, 2023.*

Institutional Image & Outreach to Society

INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY

Institutional Image & Outreach Officer
Paula Calderón

JUAN DE NIEVES (since April) Director

"We were not alone in successfully accomplishing all these programmes and had the immense fortune of being able to count on, yet again, the support of *Fundación Banco de Santander*, but also of our new partners, *Instituto Cervantes, Museo Nacional Thyssen-Bornemisza,* and *FECYT.*"

In terms of its goals to engage with society, 2023 was a passionate and enormously productive year for CNIO. This is particularly true of the programmes we pursued from our Institutional Image Office, which led to the 6th edition of CNIO Arte, the 4th Art and Science Symposium, the 1st CNIO Artistic Residences, and the organisation of an exhibition entitled "CNIO Arte: Dialogues between Art and Science" touring various US cities. With all these activities, as well as some others we will mention later, CNIO made great advances in its engagement with civil society, largely thanks to the vast potential of art and the bonds forged with the field of science.

However, we were not alone in successfully accomplishing all these programmes and had the immense fortune of being able to count on, yet again, the support of *Fundación Banco de Santander,* as well as our new partners, *Instituto Cervantes, Museo Nacional Thyssen-Bornemisza,* and *FECYT* (Spanish Foundation for Science and Technology). We must also add to the

CNIO Arte stand at ARCO 2023. From left to right: Marina Vargas, Rocío de la Villa, Amparo Garrido, Maria A. Blasco, and Borja Baselga.

list the collaboration with the Spanish Embassy in Washington, DC, and the Guggenheim Museum in Bilbao. We are extremely satisfied that such highly reputable institutions placed their trust in us to develop a whole series of programmes that are unquestionably helping to make CNIO an increasingly more recognised institution within broader society in Spain. This, of course, owes both to its scientific achievements and the cultural and artistic programmes we are undertaking and offering society.

Without a doubt, we should start with our flagship programme, CNIO Arte, which, predicated on the hugely important field of science, wishes to build bridges between artists and scientists in order to generate new artistic ideas and works. In 2023, we addressed the passionate subject matter of life from a poetic focus in *The Thread of Life in the Arts and Sciences,* the title of the 4th Art and Science Symposium held in February 2023, which came on the back of a dialogue between 2 extraordinary women we had introduced to each other. The first, Liz Blackburn (Hobart, Tasmania, 1948), is a recognised molecular biologist and 2009 Nobel laureate in Medicine or

Physiology for her work on telomeres, structures of our genetic material that condition the life of cells and of organisms. She was teamed up with visual artist Amparo Garrido, who had been working as coordinator of CNIO's Institutional Image Office up until that moment but who took leave of the institution to continue pursuing her career as an artist. The 2 women began discussions and exchanged their respective learnings with a view to stimulating the creation of a series of artworks to be undertaken by Garrido.

Liz Blackburn's research had shown that severe chronic stress shortens telomeres and, in addition, demonstrated that meditation therapies are able to reverse these effects. Given the evidence of Blackburn's research, Amparo Garrido decided to undergo a profound immersion in one of Spain's most beautiful areas: the Monfragüe National Park in the northeast of Cáceres, a special reserve for the protection of birds. There she filmed a visual poem called *Meditation* and made a series of photographs of birds, more specifically black vultures.

Opening ceremony of the exhibition at the *Instituto Cervantes* in New York. From left to right: Luis Rojas-Marcos, Juan Luis Arsuaga, Rodrigo Echenique Gordillo, Richard Bueno Hudson, Maria A. Blasco, María José Magaña, and Borja Baselga.

Blackburn and Garrido took part in the aforementioned symposium, alongside the symposium director Carlos Jiménez, CNIO director Maria A. Blasco, and the theologist María Gelpí.

Amparo Garrido's visual poem, as well as 2 wonderful large-format photographs, were presented at CNIO in mid-February to coincide with the Art and Science symposium, and 1 week later, between 22 and 26 February, we attended ARCOmadrid, the annual international art fair, with Garrido's excellent works. The project was curated by the artist Marina Vargas, a friend and colleague at CNIO ever since she presented the *Intra-Venus* sculpture in 2022.

Without a doubt, ARCOmadrid marks a special moment in the annual calendar for our programmes at CNIO, given that the art fair provides enormous visibility and guarantees diffusion among the general public and specialised audiences from Spain, and indeed from other countries, who visit the fair every year. We can confidently claim that CNIO Arte is the only initiative at the fair specifically focused on the exhibition and diffusion of artworks and programmes connecting art and science. And thus, on Friday 24 February, CNIO Arte officially opened its booth at ARCOmadrid, in a ceremony attended by Maria A. Blasco, CNIO director and executive director of CNIO Arte; Borja Baselga, managing director of *Fundación Banco de Santander*; Amparo Garrido, artist for CNIO Arte 2023, and Marina Vargas, curator of CNIO Arte.

As a result of the growing projection of our activities within Spain's art scene, *Instituto Cervantes* steadfastly committed to working with our initiative and jointly to begin organising a number of exhibitions in several of its centres in the USA, more specifically in New York (February-April 2023) and Chicago (October 2023-January 2024), all with the support of *Fundación Banco de Santander*. The excellent response to the exhibition in New York encouraged the Spanish Embassy in Washington, DC, to invite the exhibition to its cultural centre in the former ambassador's residence in the city, where it was held between April and June. The exhibition, entitled "Dialogues between Art and Science", brought together works by all the

CNIO Arte 2023 encounter at the Guggenheim Museum in Bilbao. From left to rightx: Juan Ignacio Vidarte, Amparo Garrido, Juan de Nieves, Maria Blasco, María Fernández Sabau.

artists who have taken part in the CNIO Arte programme since it was first set up: Eva Lootz, Chema Madoz, Carmen Calvo, Daniel Canogar, and Susana Solano.

Also worth underscoring is the collaboration between CNIO and the Guggenheim Museum in Bilbao. Given the theme of CNIO Arte in 2023 and its obvious connection with Wellbeing, the Guggenheim's programme of activities exploring issues concerning physical and emotional wellbeing, the museum contacted our institution to invite us to hold a conversation on 5 July between our director Maria A. Blasco with Liz Blackburn and Amparo Garrido.

One of the greatest new developments in our programmes to foster a rapprochement between art and science consisted in setting up the first Artistic Residences at CNIO. This was possible with the invaluable support of *FECYT*, which decided, as part of its mission, to catalyse the relationship between science and society by supporting the transfer of knowledge through our CNIO Artistic Residences initiative. A

jury comprising Maria A. Blasco, Amparo Garrido, Semíramis González, Marina Vargas, and Carlos Jiménez chose the artist Clara Montoya (Madrid, 1974) from among 40 candidates.

In May 2023 we decided the subject matter for the 7th edition of CNIO Arte 2024. In view of current circumstances, climate change presented itself almost as a given. The chosen duo is the macroecologist David Nogués Bravo and the recognised Spanish artist Dora García. In their first conversation, Nogués, a professor at the University of Copenhagen, suggested to García, who lives and teaches at the University of Oslo, that they meet in the Svalbard archipelago to start talking and exchanging impressions that would form the basis for Dora García's future artwork. Over the course of just a few months, García organised the crew necessary to shoot a film in different locations in Svalbard during the month of August. ∎

Development & Philanthropy

DEVELOPMENT & PHILANTHROPY

JESSICA J. ROSE   Director

Development & Philanthropy Officer
Mercedes Antona

For many fundraising organisations, a large percentage of the gifts received must go towards supporting the administrative structures around giving. This can be up to 80% in some cases, or in more sobering terms, enabling just 20 cents of every euro to go to the actual cause. At the CNIO, we have a model in which *every single euro* goes directly to research; nothing else. This is the benefit of giving directly to the research centre. The CNIO Friends Contracts programme provides 100% of the funding of the salaries of the CNIO Friends post-doctoral researchers. This means that no matter at what capacity a person can donate, every single contribution directly funds the salaries of our research Fellows, be it €1 or €100,000.

Donations to the CNIO Friends Projects programme also go directly and fully to research. These larger 6- and 7-figure gifts can fund specific projects, research areas, equipment, or scientific collaborations at the CNIO. We are in the early days of this new programme at the CNIO, but we are excited about the funding opportunities and potential partnerships that are starting to emerge.

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, in a nod to the World Health Organisation's "environmental impact of cancer", the campaign claim highlighted some of the environmental risk factors such as diet and pollution that are associated with increased incidences of cancer.

The CNIO Friends Programme has raised over €4.45 million in donations and pledges since its inception. We opened the new call for another 7 researchers in 2023. In total, philanthropic donations to the CNIO have thus far enabled us to hire 41 new researchers since 2016. In parallel, legacies and inheritances to the CNIO provide funding for existing research groups at the CNIO, and we have cumulatively raised ~€1.58 million since 2015. We have an additional €2.46m in legacy pledges pending to be executed.

Philanthropy can be such rewarding work, and it can also be slow and frustrating. Great success can seem like it arrives overnight, but it has always been the effort of sustained, strategic, and effective work that has been built over many years by the fundraising team. My heartfelt thanks to the fantastic work of my colleague Mercedes Antona, who puts her whole heart into the role. Together, we are delighted to be enabling some of the brilliant cancer research at the CNIO. ■

"Philanthropy opens doors to innovation, scientific exploration, and ground-breaking discoveries. It is a pleasure to provide the Spanish public and beyond with the means to get directly involved in our wonderful work here at the CNIO."

CNIO Offices

DEAN'S OFFICE

María S. Soengas
Dean for Academic Affairs

PARTICIPANTS
Personnel in training:
Pedro Aguilar
Cristina Bodas
Blas Chaves
Guillermo de la Vega
Ana De Pablos

The main objective of Dean's Office at the CNIO is to support a key mission of the Centre, namely to support career development of new generations of scientists. In fact, over 60% of the work force at our institution comprises young researchers, including undergraduate students, predoctoral and postdoctoral fellows. We also host medical residents and a broad spectrum of visiting scientists from different countries and institutions. Agreements are also in place with multiple universities and medical centres to ultimately bridge the gap between academic and clinical environments.

Central to the activities of the CNIO Dean's Office is to ensure the **efficient training** of our research personnel. We oversee PhD committee meetings and coordinate weekly Progress Report seminars, where PhD students and postdoctoral fellows receive feedback on technical aspects of their work and on soft skills that may improve their ability to succeed in and beyond academia. The Dean's Office also works very closely with the CNIO Student Association (CNIOSA), which has increasingly become a highly active driving force at the Centre. In particular, this year, CNIOSA organised highly impactful **workshops** on "Communicating Science to Peers", as well as on "Scientific Writing", with very dynamic, interactive feedback sessions. They also organise different mentoring and networking activities, which we intend to further expand in the future. These events for career development are supported in part by the *Fundación Jesús Serra/Fundación Occident,* which we sincerely thank for the continuous support to strengthen career development initiatives at the CNIO.

We believe that an informed society is better prepared to understand (and if needed, face) the diseases that constitute human cancer. Therefore, we are actively involved in **knowledge dissemination**. One example is our participation at the annual *Marie Sklodowska Curie European Researchers' Night.* This year, being the 10th edition for the CNIO, over 70

		Staff/Faculty:	
Nayim Gonzalez	María Ramal	Ana Cuadrado	Ana Losada
Oscar Laguía	Luis Rodrigo	Nabil Djouder	Geoff Macintyre
Ernesto López	José Luis Ruiz	Rafael Fernández-Leiro	Jorge L. Martínez
Ana Martín	Elena Sánchez	Eva González-Suárez	Juan Méndez
Belén Navarro	Anabel Sáez	Óscar Llorca	Héctor Peinado
Sergio Oterino	María Solé		

volunteers organised talks and hands-on experiments so that visitors could "Meet a scientist and become a scientist" for a day. We hosted over 260 participants of all ages, but also organised live streaming events in social media to showcase research and our laboratory life to a broad audience.

Another of our highlights in 2023 was our XIII CNIO Lab Day. We had over 100 communications, which were evaluated by an *ad hoc* committee to select 14 short talks and 8 "elevator pitch presentations" that highlighted studies from each of the Centre's Scientific Programmes at the forefront of research in their fields. We were fortunate to count on **Nicholas McGranahan** (UCL Cancer Institute, UK) to learn about cancer genome evolution, and on **Iris Uribesalgo** (EU-Life) for insights on science policy and grants consultancy at the European level.

We also enjoyed the "*Director"s List Awards*", which recognise outstanding contributions made by our personnel in 3 categories:

1. Awards for Excellence in Research by Predoctoral Fellows

We are grateful to the Agüera-Nieto family for a generous donation in the name of their mother Antonia Nieto to support an award to acknowledge the PhD student authoring the article with the highest impact in a scientific journal. In 2023, the "*Antonia Nieto Award*" went to **Bruna Calsina** for new insights on endocrine tumours, published in the prestigious *Nat Commun* journal. Additional awards in the PhD category went to **Dácil Alonso Gil**, **Isidoro Cobo**, **Alejandro Collado-Solé**, and **Nicolás Cuesta-Hernández**, each first authors of publications in *Nat Commun*. **Sara Mellid**, **Nayim González-Rodríguez**, and **Teresa González-Muñoz** also received awards for articles in *Cancer Commun*, *Nucl Acids Res* and *Clin Cancer Res*, respectively.

2. *Award for Excellence in Research by Postdoctoral/Senior Researchers*

The awardee was **Mariam Al-Masmudi Martín**, for exciting new findings related to machine learning and brain metastasis subtypes (*Cancer Cell*).

3. Award for Outreach and Volunteering Actions

The 2023 recipients were CNIO scientists for their volunteer activities in science fairs and other events organised by the Centre for the general public: **Inmaculada Sanclemente**,

"At the CNIO we aim high: to carry out the most innovative basic and translational research, and to prepare our trainees "to think outside the box" so that they can best fulfil their potential as influential leaders."

Sergi Roig, **Diana Patricia Retana**, **Neibla Priego**, **Jorge Luis Martínez Torrecuadrada**, **Vanesa Lafarga**, **Elena Blanco**, **Susana Llanos**, **Maria Solé**, **Gema Pérez Chacón**, **Francisco Jurado**, and **Ana Cuadrado**.

The Lab Day proceeded with additional awards from the Dean's Office: 3 awards for the Best Oral presentations, 3 for the Best Posters, and 1 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. We thank all those public and private contributors that have helped fuel their efforts, and we will strive in our commitment to being useful to other investigators and to 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 across all the areas represented at the Centre, including the Director.

In 2023, 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 WISE 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:

→ Sara García, staff scientist and ESA reserve astronaut. Title: "*Sara García, la científica del CNIO que está a un paso de la luna*". 23/02/2023.
→ Maria A. Blasco, Núria Malats, Ana Losada, and Francisca Mulero, CNIO Group Leaders. Colloquium "*Mujeres referentes en ciencia*". 16/03/2023.
→ Chus Guitiérrez, film director, screenwriter and actress. Title: "*Gestión de Equipos desde una Mirada Feminista*". 11/07/2023.
→ Victoria Cirlot, professor of romance philology and director of the University Institute of Culture at *Pompeu i Fabra* University. Title: "*Místicas en la Edad Media*". 19/09/2023.
→ Carme Riera, professor of literature and Academician of the Royal Spanish Academy. Title: "*Cómo se escribe una novela*". 03/10/2023.
→ Ana Maiques, CEO Neuroelectrics. Title: "*Revolucionando las terapias cerebrales de Barcelona a Boston - una historia de emprendeduría en el sector salud*". 20/11/2023.

Members
Maria A. Blasco
Maria Jesús Alcamí
Isabel López de Silanes
Lola Martínez
Francisca Mulero

Eva Ortega
Fernando Peláez
Sandra Rodríguez-Perales

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

In the spirit of supporting STEM careers among young women and breaking gender stereotypes, on the occasion of February 11, 2023, International Day of Women and Girls in Science, the WISE Office organised an event for the general public that consisted of a talk given by Sara Garcia, a CNIO scientist who was recently selected as a reserve astronaut by the European Space Agency. After her inspiring talk, the audience (mainly 12 to 15-year-olds), had the chance to listen to short talks from other female CNIO scientists and enjoyed a guided tour through the CNIO facilities.

For International Women's Day, the WISE Office organised an internal event entitled "Women leaders in science" that consisted of a colloquium among 4 CNIO women Group Leaders, Maria A. Blasco, Ana Losada, Núria Malats, and Francisca Mulero. The colloquium was moderated by Mónica Salomone, Head of our Communications Department. The speakers told us about what motivated them to become Group Leaders, how they deal with having more responsibility, and how they reconcile their scientific and family lives. ■

Here at the WISE Diversity Office, we share what Virginia Woolf said: "No need to hurry. No need to sparkle. No need to be anybody but oneself." *(A Room of One's Own)*

Facts
& Figures

SCIENTIFIC MANAGEMENT

ISABEL BARTHELEMY Scientific Management Director

M. Dolores Liébanes
Mercedes Moro
Helena Zamora
Irene Castaño (Since June)
Sonia Cerdá

Almudena del Codo
(until September)
Victoria López
Ana Pérez (since October)
Leyre Vergés

The Scientific Management Department at the CNIO is committed to assisting with the facilitation of all those key areas that help our scientists to better focus their efforts on their research. The Department encompasses various Offices: Projects and Consortia, Education and Training Programmes, Scientific Events, Scientific Publishing, and Library and Archives. It also manages agreements with different institutions, mainly with Universities.

The mission of the Projects′ Office is to guide CNIO scientists through all stages related to the application and management processes of externally-funded projects, whether they be financed through either public and/or private institutions, or stem from either national or international funding bodies. The Office coordinates the internal call alerts, gives advice about the ethical certification for projects involving animal experimentation or human samples together with the Biobank and the IACUC, supports scientists with the preparation of the project proposals, manages the ongoing projects, and contacts the funding agencies to resolve any issues or deal with questions.

The Training Office is the central point for training at the CNIO; it aids the recruitment process, serves as an advocate for all fellows, provides administrative support, and creates educational and learning opportunities. It is responsible for helping PhD students, postdoctoral scientists, and post-resident MDs by announcing call alerts and providing the relevant key information; helping foreign students with their paperwork at the foreign office; organising the summer training call; and, in general, in collaboration with the Personnel Department, managing students' grants.

CNIO′s events are a reference in the scientific field. The quality of our lectures as well as the topics we deal with make our Centre an extraordinary place to achieve interaction with scientists and exchange knowledge on scientific achievements. The Events Office organises CNIO meetings, such as the CNIO - CaixaResearch Frontiers Meetings, the Distinguished Seminars series, the external Scientific Advisory Board meeting, CNIO Progress Reports, as well as Faculty Retreats, among others. The Office also helps scientists by providing advice for the organisation of specific events, including science outreach events.

The Library administers the electronic subscriptions of over 300 scientific journals at the CNIO and manages journal article

"Every day we work towards building a strong and flexible framework to support our scientists and to help them achieve excellence."

requests for journals to which the CNIO is not subscribed to. The Library also provides information regarding reference management software, manages the open-access repository *REPISALUD*, and organises the CNIO Progress Reports seminars and the CNIO guided visits.

The Scientific Publications Office is responsible for the preparation of institutional publications, including the CNIO Annual Report, the CNIO′s Action and Strategic Plans, booklets of the Scientific Advisory Board (SAB) meeting and those of other symposia, as well as scientific dissemination leaflets. The Office also provides support for the scientific editing of press notes and other publications of scientific divulgation to a non-specialised audience. ■

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 it is used not only to finance the Centre's outstanding R&D, 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; these activities are aimed at promoting public awareness. In 2023, researchers at the CNIO were involved in 138 projects that received extramural funding.

During this same year, the CNIO is actively participating in 63 collaborative projects: 17 were international collaborative projects (3 of which are coordinated by the CNIO), and 46 were 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 its research & innovation framework programmes Horizon 2020 (2014-2020) and Horizon Europe (2021-2027); the US National Institutes of Health (NIH); the Paradifference Foundation; Worldwide Cancer Research; and the Lustgarten Foundation - Stand Up To Cancer Initiative. At national level, collaborative projects received important public grants from the Strategic Health Action, managed by the Institute of Health *Carlos III* (*ISCIII*), the State Research Agency of the Spanish Ministry of Science, Innovation and Universities

(*AEI/MCIU*), and the R&D Activities Programmes of the Community of Madrid; most of these projects were co-funded by European Structural and Investment Funds (European Regional Development Fund and European Social Fund). The CNIO also obtained a significant number of grants funded by NextGeneration EU funds to develop strategic projects to foster recovery from the COVID-19 crisis, in accordance with the Spanish Recovery, Transformation and Resilience Plan (*PRTR*). Private funders and charities also recognised the excellence of our scientific projects, among them, the Scientific Foundation of the Spanish Association Against Cancer (*FC AECC*), the *Ramón Areces* Foundation, *La Marató de TV3* Foundation, the *BBVA* Foundation, or "la Caixa" Banking Foundation.

In addition to these collaborative projects, researchers at the CNIO have attracted funding for projects carried out by individual groups. In 2023, 9 of these projects received international funds, while 66 of them received national funding (mainly from the *AEI/MCIU*, the *ISCIII* and private foundations). The international individual projects are funded by the European Union (5 European Research Council [ERC] grants and 2 Marie Skłodowska-Curie Actions), Worldwide Cancer Research, and the Mark Foundation for Cancer Research.

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

EUROPEAN UNION RESEARCH & INNOVATION FRAMEWORK PROGRAMME

HORIZON EUROPE (2021-2027)

INNOVATIVE HEALTH INITIATIVE (IHI) (PILLAR III)

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

RESEARCH INFRASTRUCTURES (PILLAR I)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	EOSC4CAncer: A European-wide foundation to accelerate Data-driven Cancer Research (Ref.: 101058427)

MARIE SKŁODOWSKA-CURIE ACTIONS (PILAR I)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	MADRIDNIGHT: Researchers and citizens: facing together the European challenges (Ref.: 101061343)
Losada, Ana	MSCA Doctoral Network Cohesinet: Cohesin and its regulators: from chromosome dynamics and nuclear architecture to human diseases (Ref.: 101072505)

HEALTH PROGRAMME (PILLAR II)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Eva (until 27 December 2023), Ajenjo, Nuria (from 28 December 2023)	REACT: Respiratory Host-Pathogen Interaction (Ref.: 101057129)

MISSION CANCER (PILLAR II)

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

HORIZON 2020 (2014-2020)

FET OPEN – NOVEL IDEAS FOR RADICALLY NEW TECHNOLOGIES

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

INTEGRATING AND OPENING RESEARCH INFRASTRUCTURES OF EUROPEAN INTEREST

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peláez, Fernando	EPIC-XS: European Proteomics Infrastructure Consortium providing Access (Ref.: 823839)

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)

MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	ITN proEVLifeCycle: The life cycle of extracellular vesicles in prostate cancer: from biogenesis and homing, to functional relevance (Ref.: 860303)
Real, Francisco X.	ITN TranSYS: Translational SYStemics: Personalised Medicine at the Interface of Translational Research and Systems Medicine (Ref.: 860895)

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.: U41 HG007234)

THE PARADIFFERENCE FOUNDATION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima Robledo, Mercedes (Coordinator)	Integration of multi-omics profiling and immune contexture in metastatic PPGL patients

FUNDACIÓN RAMÓN ARECES/CNIO/ WEIZMANN INSTITUTE OF SCIENCE

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria; Real, Francisco X. (Coordinator)	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

INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

EUROPEAN COMMISSION

HORIZON 2020 (2014-2020)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	ERC Advanced Grant SHELTERINS: Targeting Shelterin Proteins in Cancer (Ref.: 882385)
González, Eva	ERC Consolidator Grant PLEIO-RANK: Pleiotropic treatment of cancer: RANK inhibitors targeting cancer stem cells and immunity (Ref.: 682935)
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, INDIVIDUAL FELLOWSHIPS (MSCA-IF)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Martínez, Joaquín; Velasco, María	MAtChing: Mechanically Activated Channels in Glioma: the role of mechanoreceptor Piezo1 and hnRNP K in cancer as novel oncoregulators (Ref.: 101027864)
Quintela, Miguel A.; Jimeno, Rebeca	P70-IMMUNEBREAST: Tumor compartment-specific effects of P70S6K in early triple negative breast cancer: regulation of antitumor immune response and therapeutic implications (Ref.: 893597)

WORLDWIDE CANCER RESEARCH (WCR) AND *FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (FC AECC)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	Exploring the use of CDK4/6 inhibitors in combination with classical chemotherapy (Ref.: 20-0155)

MARK FOUNDATION FOR CANCER RESEARCH

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	ASPIRE project: Deconstructing the biology of local relapse post-surgery to develop novel preventive strategies in brain metastasis

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
Gallardo, Miguel (Coordinator; until 31 January 2023)	Deciphering the role of hnRNP K in multiple myeloma (Ref. PI21/00191)
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)
Malats, Núria (Coordinator)	Study of the environmental and genetic risk profiles and clinical behaviour of the basal-like phenotype of pancreatic cancer. Comparison with bladder, breast and endometrial cancers (Ref.: PI18/01347)
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)

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 TRANSCAN3 LipidMac: Exploiting lipid-laden macrophages to overcome resistance to cancer immunotherapy (Ref.: AC22/00044)
Valiente, Manuel (Coordinator)	ERANET TRANSCAN3 RISEBrain: Reverting immune suppression to elicit brain metastasis control (Ref.: AC22/00019)

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*[3]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Eva (until 27 December 2023), Ajenjo, Nuria (from 28 December 2023)	*Plataforma de Biobancos y Biomodelos (Group, Ref.: PT20/0070)*

IMPACT PROJECTS: PRECISION MEDICINE INFRASTRUCTURE ASSOCIATED WITH SCIENCE AND TECHNOLOGY[4]

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

PRECISION PERSONALIZED MEDICINE RESEARCH PROJECTS (STRATEGIC HEALTH ACTION)[5]

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

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)[6]

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 (Coordinator)	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[7]*

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)
Barbacid, Mariano	Genetic, pharmacological and computational approaches to identify Precision therapies in pancreatic cancer (Ref.: CPP2022-009609)
Blasco, Maria	Development of a novel gene therapy for the treatment of Idiopathic Fibrosis (Ref. CPP2021-008483)
Peláez, Fernando	An effective and safe system for the treatment of Atrial Fibrillation through Irreversible Electroporation (Ref. CPP2021-008480)

EXCELLENCE NETWORKS / *REDES DE EXCELENCIA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cortés, 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 iLUNG 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)

R&D ACTIVITIES PROGRAMME IN TECHNOLOGIES[8]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Llorca, Óscar	*Programa Tec4BioCM: Tecnologías Aplicadas al Estudio de Nanomáquinas Biológicas* (Ref.: P2018/NMT4443)

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; Ortega, Eva (until 27 December 2023); Artiga, María Jesús (from 28 December 2023)	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)

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8   This Programme is cofunded by the European Regional Development Fund (ERDF) and European Social Fund (ESF)

Funded by the European Union

ERA-NETS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ERA PerMed: Personalized multimodal therapies for the treatment of lung cancer (Ref.: PERME20707BARB)
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"*
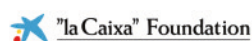
HEALTH RESEARCH PROJECTS

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)
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)
Peinado, Héctor	OncoExoPeptides: Defining the Role of Exosome-Secreted Micropeptides in Pancreatic Cancer (Ref.: HR18-00256)
Real, Francisco X.	RBM10across: RBM10, a novel splicing regulator and tumor suppressor: from mechanisms to therapies (Ref.: HR21-01208)
Zugazagoitia, Jon	IL7R_LungCan: IL7R in lung cancer development, metastasis and resistance to immune checkpoint inhibitor therapy (Ref.: HR21-00761)

CAIXAIMPULSE COVID-19

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cortés, Felipe (Coordinator)	Simple and rapid SARS-CoV-2 diagnostic test by phi29 polymerase amplification (Ref.: CF01-00005)

BBVA FOUNDATION / *FUNDACIÓN BBVA*

COVID-19 SCIENTIFIC RESEARCH TEAMS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Muñoz, Inés	Synthetic immunology to engineer pan-coronavirus immunity (Ref.: Biomed-COVID-19_0155)

LA MARATÓ DE TV3* FOUNDATION / *FUNDACIÓ LA MARATÓ DE TV3

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González, Eva	Identification of mechanisms of response to CDK4/6 inhibition in hormone receptor-positive breast cancer (Ref.: 43/C/2019)
Valiente, Manuel	Organ-specific biomarkers and therapies to improve the management of brain metastasis (Ref.: 141/C/2019)

hna* FOUNDATION / *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)*

RESEARCH PROJECTS IN HEALTH[9]

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, Núria	Deciphering the complex relationship between asthma/allergy and pancreatic cancer risk (Ref.: PI21/00495)
Olmeda, David	Anti-bacterial stress pathways in melanoma metastasis and response to therapy (Ref. PI21/00641)
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)

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

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*[10]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Leiro, Rafael	CRYOTELO: Structural and molecular characterisation of the shelterin complex (Ref.: PID2020-120258GB-I00)

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9   This Programme is cofunded by the European Regional Development Fund (ERDF) "A Way to Make Europe"

10   This Programme is cofunded by the European Regional Development Fund (ERDF), "A way of making Europe" from year 2020 on

CHALLENGES-RESEARCH PROJECTS OR KNOWLEDGE GENERATION / *PROYECTOS RETOS-INVESTIGACIÓN O DE GENERACIÓN DE CONOCIMIENTO*[11]

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)
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: Physiopathological implications of DNA supercoiling and topoisomerase function as master regulators of genome dynamics (Ref.: PID2020-119570RB-I00)
Djouder, Nabil	MECHANOCIR: From cirrhosis to hepatocellular carcinoma: a mechanobiology perspective (Ref.: PID2021-122695OB-I00)
Efeyan, Alejo	PhysioTOR: The physiological control of the nutrient-mTOR axis and its deregulation in cancer and aging (Ref.: PID2019-104012RB-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)
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)
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)
Macintyre, Geoffrey J.	TTCIN: Therapeutic targeting of chromosomal instability in cancer (Ref.: PID2019-111356RA-I00)
Méndez., Juan R.	FLEXI-REP: Flexibility in the DNA replication program in mammalian cells (Ref.: PID2022-142771NB-I00)
Méndez, Juan R.	REP&TOL: Mechanisms of DNA replication and damage tolerance (Ref.: PID2019-106707RB-I00)
Otano, Itziar	Immune evasion in oncogene-addicted NSCLC subgroups (Ref.: PID2022-143169OB-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)
Peinado, Héctor	OUTANERVE: Role of NGFR regulating the immunoevasive phenotype of melanoma metastasis initiating cells (Ref.: PID2020-118558RB-I00)
Plaza, Iván	ESFRRET: Functional and structural characterization of KIF5B-RET rearrangements (Ref.: PID2020-117580RB-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)
Rodríguez, Cristina (until 3 October 2023)	kidneyALT: Molecular alterations of metastatic renal cell carcinoma of clinical significance for antitumor drug response (Ref.: PID2021-128312OB-I00)
Soengas, María S.	MEL_IMAGE_TREAT: Imaging and targeting metastatic niches in melanoma (Ref.: PID2020-117621RB-I00)
Valiente, Manuel	METneural: Crosstalk between brain metastasis and neural circuits (Ref.: PID2021-124582OB-I00)

EUROPEAN EXCELLENCE INITIATIVE/ *PROYECTOS EUROPA EXCELENCIA*[12]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	INN-TIME: Co-option of host circadian rhythms in cancer (Ref.: EUR2023-143451)

SCIENTIFIC INFRASTRUCTURES / *INFRAESTRUCTURAS CIENTÍFICO-TECNOLÓGICAS*[13]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Mulero, Francisca	High Resolution Magnetic resonance 3T based in superconducting magnet wihtout helium as criogenizer (Ref.: EQC2021-006797-P)
Peláez, Fernando	Implementation of automated microscopy platform for high-performance screening (High Content Screening) (Ref.: EQC2021-007743-P)

PROOF OF CONCEPT[14]

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	THankX: Development of THX-B as a novel anti-metastatic agent (Ref.: PDC2021-121102-I00)
Soengas, María S.	MDK-INHIBITORS: rewiring tumor-immune system crosstalk by targeting MIDKINE (Ref.: PDC2021-121831-I00)

SPANISH FOUNDATION FOR SCIENCE AND TECHNOLOGY / *FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y TECNOLOGÍA (FECYT)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	CNIO Artistic Residences (Ref. FCT-21-17623)
Barthelemy, Isabel	Access to bibliografic data bases: web of Science (Ref.: MDG-23-11372)

SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / *FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (FC AECC)*

GRANTS FOR EMERGING GROUPS (*AECC* LAB)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Defining the mutational landscape in plasma and lymphatic fluid-derived exosomes in melanoma patients (Ref.: LABAE19027PEIN)
Rodríguez, Sandra	Programmable detection and inhibition of fusion oncogenes using CRISPR/Cas13 (Ref.: LABAE20049RODR)
Valiente, Manuel	New treatments for brain metastasis based on the study of their biology (Ref.: LABAE19002VALI)

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)
Fernández-Capetillo, Óscar	Targeting the histone methyltransferase SETD8 in cancer: from biomarker identification to drug development and mechanisms of resistance (Ref.: PROYE20101FERN)
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)

"LA CAIXA" BANKING FOUNDATION AND *LUZÓN* FOUNDATION / *FUNDACIÓN BANCARIA "LA CAIXA" Y FUNDACIÓN LUZÓN*
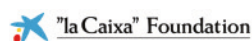
HEALTH RESEARCH PROGRAMME

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Targeting Telomeres in Cancer (Ref.: HR18-00023)
González, Eva	Role of RANK in myeloid cells and tumor development (Ref.: HR23-00361)
Soengas, María S.	Immunomodulatory drivers in melanoma progression and therapy response (Ref.: HR20-00465)
Valiente, Manuel; Al-Shahrour, Fátima; Ortega, Eva (until 27 December 2023); Artiga, María Jesús (from 28 December 2023)	Establishment of a precision medicine pipeline to personalize the treatment of brain metastasis (Ref.: HR23-00051)

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 (PR_TPD_2022-21)

SPANISH ASSOCIATION OF PANCREATOLOGY/ *ASOCIACIÓN ESPAÑOLA DE PANCREATOLOGÍA*

CARMEN DELGADO/MIGUEL PÉREZ-MATEO GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Guerra, Carmen	*Nueva estrategia terapéutica: estroma e inmunoterapia* (ACP2021 MC Guerra)
Malats, Núria	*Marcadores microbianos para el diagnóstico del adenocarcinoma ductal de páncreas* (ACP2018 Malats)

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

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)
Mulero, Francisca	TherAbnostic: Radioinmunotheragnostics for metastatic lung cancer with pretargeted clickable Ab fragments (Ref.: PR[19]_BIO_IMG_0096)

RAMÓN ARECES FOUNDATION / FUNDACIÓN RAMÓN ARECES

frA FUNDACIÓN RAMÓN ARECES

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Eva (until 27 December 2023); Artiga, María Jesús (from 28 December 2023)	*Red Nacional de Metástasis Cerebral: Implantación, Desarrollo y Coordinación* (Ref.: CIVP20S10662)

GRUPO ESPAÑOL MULTIDISCIPLINAR DE MELANOMA

GEM 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

EDUCATION AND TRAINING PROGRAMMES

One of the principal goals of the CNIO 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 to train 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 2023, 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*, China Scholarship Council, and the European Research 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 addition, in 2023, the CNIO signed several new agreements with Spanish Universities and other institutions, namely with the *Universidad de Jaén, IES José Luis Sanpedro, IES Rosa Chacel, IES Leonardo Da Vinci, Centro Educativo ILERNA,* and *Centro Superior CEAC de Formación Profesional.*

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2019	**2020**	**2021**	**2022**	**2023**
Training of PhD students	100	109	135	130	**130**
Post-doctoral training	49	52	62	55	**57**
Training for MDs	20	7	10	12	**9**
Laboratory training for MSc/BSc students	150	85	106	136	**115**
Laboratory training for technicians	15	5	9	17	**14**

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 obtain hands-on practical laboratory experience by working on ongoing research projects in one of the CNIO Groups. The CNIO offers 2 types of short-term laboratory training:

→ An annual Summer Training Programme for undergraduate students, from any country, who are in their last years of study in the biomedical field. 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 2023, 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, 115 students participated in these programmes, of whom 5 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, 16 students obtained their PhD degrees in 2023 and 27 others joined the CNIO in the same year. Over 15% of the students working at the CNIO in 2023 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

Since 2008, the "la Caixa" Foundation offers 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. During 2023, 3 pre-doctoral students received a doctoral fellowship from the INPhINIT programme of the "la Caixa" Foundation to join the CNIO.

The distribution of students across the CNIO's Research Programmes in 2023 was as follows: 46 % of students worked in the Molecular Oncology Programme, 9% in the Structural Biology Programme, 12% in the Human Cancer Genetics Programme, 6% in the Experimental Therapeutics Programme, 16% in the Biotechnology Programme, and 11% in the Clinical Research Programme.

FUNDING OF PHD TRAINING	NO.
SPANISH ORGANISATIONS	106
State Research Agency / *Agencia Estatal de Investigación* (*AEI*). Ministry of Science, Innovation and Universities / *Ministerio de Ciencia, Innovación y Universidades* (Predoctoral Fellowships)	50
State Research Agency / *Agencia Estatal de Investigación* (*AEI*). Ministry of Science, Innovation and Universities / *Ministerio de Ciencia, Innovación y Universidades* (I+D Projects)	8
Spanish Association Against Cancer (*AECC*) / *Fundación Científica de la AECC* (I+D Projects)	6
Spanish Association Against Cancer (*AECC*) / *Fundación Científica de la AECC* (Fellowship)	4
Institute of Health Carlos III / *Instituto de Salud Carlos III* (*ISCIII*) (Fellowship)	1
Institute of Health Carlos III / *Instituto de Salud Carlos III* (*ISCIII*) (I+D Projects)	2
Comillas University / *Univesidad de Comillas*	1
FERO Foundation / *Fundación FERO*	1
Cris Foundation */ Fundación Cris*	3
Community of Madrid / *Comunidad de Madrid*	11
Bionam Biotech AIE	3
CNIO	7
"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	24
AXA	2
China Scholarship Council (CSC)	3
European Research Council	11
GENCODE	2
ESMO	1
MERCK	1
Marie Skłodowska-Curie actions of the European Commission	2
VCN	1
Pfizer	1
TOTAL	**130**

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 to offer them highly attractive research projects at the forefront of cancer research.

In 2023, 57 postdoctoral fellows worked at the CNIO. Notably, about one fourth of these fellows were from outside of Spain, many coming from very prestigious international institutions.

Thanks to the donations received through the 'CNIO Friends' platform, the 8th call of the 'CNIO Friends' Postdoctoral Contract Programme, launched in 2023, resulted in the recruitment of 3 scientists for a 2-year period each.

FUNDING SOURCES OF POST-DOCTORAL CONTRACTS	NO.
SPANISH ORGANISATIONS	40
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* (Fellowship)	4
"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	**57**

POSTGRADUATE PROGRAMMES

In addition, the CNIO – in collaboration with academic institutions across Spain – provides 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 the CNIO are developed in collaboration with the Faculty of Medicine and Faculty of Sciences at the Autonomous University of Madrid (*UAM*). These trainings fall under 4 official Postgraduate Programmes, namely, the Doctorate in Molecular Biosciences, Master's in Biomolecules & Cell Dynamics, Master's in Molecular Biomedicine, and Master's in Biotechnology. CNIO also collaborates with the *UAM* as a partner institution of *UAM*'s Doctoral School (*EDUAM*) and is a member of the Management Committee.

Master's Degree in Biocomputing Applied to Personalised Medicine and Health

The Master's in *Bioinformática Aplicada a la Medicina Personalizada y la Salud* 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 Clinical and Applied Cancer Research

The CNIO and the *CEU-San Pablo* University in Madrid (*USP-CEU*) co-organise a Postgraduate Training Programme in Clinical and Applied Cancer Research: the *Máster Universitario en Investigación Clínica y Aplicada en Oncología*.

Official Master's Degree in Therapeutic Targets of Cell Signalling: Research and Development

The CNIO collaborates with the Biochemistry and Molecular Biology Department 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 in Anatomical Pathology, Clinical Diagnostic Laboratory, and Archiving/Recording; it is organised through agreements with 19 institutions that provide secondary education for laboratory technicians in Spain. It provides students with hands-on knowledge in cellular and molecular biology techniques. The programme consists of 14 weeks (370-400 hours) of laboratory training. In 2023, 14 students participated in this programme at the CNIO.

TRAINING FOR MDS

In line with CNIO's commitment to bridge the 'bench to bedside' gap, the Centre offers 3 training opportunity programmes to MDs and other health care professionals. Training usually consists of a 3-month period during residency. In 2023, 9 medical residents from 5 different hospitals enjoyed the benefits of rotations within the different Groups and Units at the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2023, the *Ramón y Cajal* Programme supported 5 scientists. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (currently the State Research Agency of the Spanish Ministry of Science, Innovation and Universities) aims to encourage Spanish or foreign scientists working abroad to return to or relocate to Spain. Successful candidates are selected on the basis of their potential capacity to lead independent projects and groups, or to contribute successfully to the ongoing research in the existing groups.

VISITING RESEARCHERS PROGRAMME

The *Fundación Jesús Serra*, now *Fundación Occident*, 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 2023, Gonçalo Bernardes, from the University of Cambridge (UK), was beneficiary of the *Jesús Serra* Foundation's Visiting Researchers Programme.

'SCIENCE BY WOMEN' PROGRAMME

Thanks to the 'Science by Women' Programme, launched by the Spanish "*Fundación Mujeres por África*", Marwa Muhammad Abu-Serie Ali, from GEBRI, SRTA-City (Alexandria, Egypt), was awarded a grant to join the CNIO's Genetic and Molecular Epidemiology Group for a 6-month stay as a visiting scientist. During 2023, thanks to this Programme, we also had the pleasure of hosting Ngozi Justina Nwodo, from the University of Nigeria, and Mai Fathy Bayomi Tolba, from Ain Shams University (Cairo, Egypt), for a 6-month stay as visiting scientists in the CNIO's Medical Chemistry Section and the Breast Cancer Clinical Research Unit, respectively.

SCIENTIFIC EVENTS

MEETINGS & CONFERENCES

CNIO-CaixaResearch Frontiers Meetings (CFM)

CNIO- CaixaResearch Frontiers Meetings are the main international conferences that are organised by the CNIO and "la Caixa" Foundation. They focus on specific, cutting-edge aspects of cancer research, thus providing a unique platform for an intensive and dynamic exchange and debate on scientific ideas. The invited speakers – around 20 internationally renowned leaders in oncology – present their latest findings during 2 and a half days. The learning environment encourages delegates to exchange experiences, ideas, and practices upheld at their companies; network and create connections with researchers with similar interests; listen to and meet the keynote speakers; enjoy the extra-curricular conference programme; and hear about the latest developments in the research field. Up to 100 additional participants are selected – via a widely publicised call for applications – based on their potential to make relevant contributions to the conference by presenting hot topics as posters or short talks.

In 2023, we arranged 2 CFMs: 1) **Genome Organisation and Stability.** The three-dimensional (3D) folding of chromatin within the nucleus determines the framework in which all genome dynamics processes occur. This has attracted extraordinary attention in the last few years, fundamentally, with the development of genome-wide chromosome-conformation-capture techniques, which have radically changed our understanding of how the genome is spatially organised in a dynamic fashion. The most illustrative example for this is perhaps the regulation of gene expression, which is now inconceivable to understand without a 3D context of controlled chromatin interactions. The connections between 3D genome organisation and the processes that signal and repair DNA damage to maintain genome integrity are, however, only now starting to emerge, and are proving fundamental to understand the endogenous sources of DNA breaks and chromosomal rearrangement events that drive cancer onset and progression. In this meeting, we aimed to bring together world-leading researchers in both fields, 3D genome organisation and the DNA damage response, in order to provide an ideal setting to bridge the gap between these 2 fundamental aspects of genome dynamics. 2) **Metastasis.** Metastasis is the main driving force for cancer-associated lethality, as cancer metastases are more difficult to treat than primary tumours. The exponential increase in heterogeneity throughout the metastatic cascade, and the underlying biology that involves the generation of novel ecosystems incorporating resident and peripheral components of the microenvironment, might explain the increased ability to resist therapies. The CNIO-CaixaResearch Frontiers Meeting on Metastasis provides the most up-to-date perspective on metastasis, including the best ways to model metastasis, embrace its heterogeneity, prevent metastases from happening, and exploit organ-specific survival mechanisms of cancer cells, among other key topics. These novel research findings were presented by leaders in the field, which made the CNIO-CaixaResearch Frontier Meeting on Metastasis the most relevant cancer research forums of the year.

GENOME ORGANISATION AND STABILITY
22-23 MAY 2023

ORGANISERS:
- **Felipe Cortés**, Spanish National Cancer Research Centre – CNIO, Spain
- **Oscar Fernández-Capetillo**, Spanish National Cancer Research Centre – CNIO, Spain
- **Ana Losada**, Spanish National Cancer Research Centre – CNIO, Spain
- **Andre Nussenzweig**, National Institutes of Health – NIH, US

SESSIONS:
- The 3D Genome I / II
- The Genome in Health and Disease I / II
- Genome Dynamics and Stability I / II

METASTASIS
6-8 NOVEMBER 2023

ORGANISERS:
- **Julio Aguirre-Ghiso**, Albert Einstein Cancer Center, US
- **Caroline Dive**, CRUK Manchester Institute, UK
- **Eva González**, Spanish National Cancer Research Centre – CNIO, Spain
- **Héctor Peinado**, Spanish National Cancer Research Centre – CNIO, Spain
- **Manuel Valiente**, Spanish National Cancer Research Centre – CNIO, Spain

SESSIONS:
- Modelling Evolution of Metastasis
- Non-Genetic Adaptation in Metastasis (Metabolism, Epigenetics and Stress)
- Microorganismal Influence on Metastasis
- Anti-Metastasis Therapies and Clinical
- Influence of Microenvironment in Metastasis
- CTCS/ DTC/ CTDNA: New Technologies and Implications
- Neurobiology of Metastasis

PANCREATIC CANCER AI FOR GENOMICS AND PERSONALIZED MEDICINE
19-20 JANUARY 2023

EAPM Stakeholder Conference 'Building an Open Innovation ecosystem for healthcare in Europe: Access & Diagnostics for All & Public Health Genomics'

ACCESS, INNOVATION AND INCENTIVES: POWER FOR CIVILIZATION TO TACKLE CANCER - AND FOR BETTER HEALTHCARE FOR ALL
19-20 OCTOBER 2023

ORGANISERS:
· **European Alliance for Personalised Medicine**   ·
· **Núria Malats,** Head of the Genetic and Molecular Epidemiology Group at the Spanish National Cancer Research Centre (CNIO)

V JORNADA CNIO-FUNDACIÓN BANCO SABADELL EN FILOSOFÍA Y CIENCIA: LA CATÁSTROFE DE LA PÉRDIDA DE BIODIVERSIDAD
29 NOVEMBER 2023

WITH THE SUPPORT OF:
· **Sabadell Foundation**

ORGANISERS:
· **Maria A. Blasco (CNIO)**
· **Antonio Diéguez (UMA)**
· **Arantza Etxeberria (UPV/EHU)**

SPEAKERS:
· **Alicia Puleo**, *Universidad de Valladolid*
· **Fernando Valladares**, *Consejo Superior de Investigaciones Científicas* (*CSIC*)
· **Anna Traveset**, *Instituto Mediterráneo de Estudios Avanzados* (*CSIC-UIB*)
· **José María Rey Benayas**, *Universidad de Alcalá* (*UAH*)
· **Laura Menatti**, *Instituto Konrad Lorenz para la Evolución y la Cognición* (*KLI*)
· **Cristian Moyano**, *Universidad Autónoma de Barcelona* (*UAB*)

SESSIONS:
· *El valor de la biodiversidad y su pérdida*
· *Iniciativas para revertir la pérdida de biodiversidad*

Madrid 29 Noviembre 2023
Auditorio CNIO

V Jornada CNIO-
Fundación Banco Sabadell
en Filosofía y Ciencia
La catástrofe de la pérdida
de biodiversidad

cnïo *stop cancer*

TRAINING COURSES AND WORKSHOPS

The CNIO is committed to disseminating the results of state-of-the-art cancer research to the wider community, including medical professional and junior scientists, thereby enabling them to stay abreast of recent developments in specialised techniques. This is achieved through training courses and hands-on workshops organised by CNIO scientists and technologists.

FLOW CYTOMETRY INDUCTION COURSE: FUNDAMENTALS, APPLICATIONS, DATA ANALYSIS AND DATA PRESENTATION
16-17 JANUARY 2023
20-21 FEBRUARY 2023
27-28 MARCH 2023
8-9 MAY 2023
12-13 JUNE 2023
4-5 SEPTEMBER 2023
9-10 OCTOBER 2023
15-16 NOVEMBER 2023

SPEAKERS:
· **Julia García-Lestón,** Flow Cytometry Unit Technician. CNIO
· **Lola Martínez,** Head of the Flow Cytometry Unit. CNIO
· **Jamie McCarthy**, European Application Specialist. DeNovo Software
· **Andrea Valle,** European Applications Specialist DeNovo Software

MULTICOLOR FLOW CYTOMETRY COURSE
1-2 FEBRUARY 2023
31 MAY-1 JUNE 2023
24-25 OCTOBER 2023

SPEAKERS:
· **Serena Di Cecilia**, Senior European Application Scientist, FlowJo. BD
· **Lola Martínez**, Head of the Flow Cytometry Unit. CNIO
· **Jamie McCarthy**, European Application Specialist. DeNovo Software
· **Laura Ferrer**, High Dimensional Spectral Cytometry Specialist. Malaghan Institute. Wellington, NZ
· **Andrea Valle**, European Application Specialist. DeNovo Software
· **Ana Vieira**, European Technical Application Specialist. Cytek Biosciences

PAN-PROSTATE CANCER GROUP TECHNICAL WORKING GROUP MEETING
19-20-21 APRIL 2023

Meeting members of the international Pan Prostate Cancer Group

WORKSHOP: "TOWARDS ADVANCED NANO-IMMUNOTHERAPIES OF CANCER"
27-28 NOVEMBER 2023

ORGANISERS:
· **Marisol Soengas**, Spanish National Cancer Research Centre. CNIO
· **Luis Álvarez-Vallina**, Spanish National Cancer Research Centre. CNIO
· **Fernando Torres Andón**, Biomedical Research Institute of *La Coruña*
· **María José Alonso**, CIMUS Research Institute, University of Santiago de Compostela (USC)

EUROPEAN NETWORK OF BREAST DEVELOPMENT AND CANCER ENBDC THINK TANK MEETING
14 DECEMBER 2023

CNIO DISTINGUISHED SEMINARS

The purpose of the Distinguished Seminars Series is to invite outstanding and internationally renowned scientists to give a seminar and to meet with researchers at the CNIO. Distinguished Seminars are recurrent events that are open to the public and are held throughout the year, usually on Fridays at noon in the CNIO Auditorium. Each Distinguished Seminar Series includes world-leading scientists who address topics that are of general interest to the CNIO faculty.

In total, the CNIO hosted 13 distinguished speakers in 2023.

DATE	SPEAKER	ORGANISATION	TITLE
FEBRUARY			
10/02/2023	**Montserrat García- Closas**	National Cancer Institute, Rockville, USA	Breast cancer risk stratification for precision prevention
MARCH			
17/03/2023	**Tak Mak**	University Health Network (UHN), Toronto, Canada	Metabolic Regulation of Immune Responses and Cancer
24/03/2023	Claudio Joazeiro	Center for Molecular Biology of Heidelberg University (ZMBH), Heidelberg, Germany	Translational surveillance: mechanisms, evolution, and roles in disease
APRIL			
14/04/2023	**Patrick Hsu**	University of California, Berkeley, USA	Systematic discovery of recombinases for integrating large DNA sequences into the human genome
JUNE			
23/06/2023	**Tony Hunter**	Molecular and Cell Biology Laboratory, Salk Institute, La Jolla, USA	A journey from phosphotyrosine to phosphohistidine and what it has revealed about cancer mechanisms
SEPTEMBER			
22/09/2023	**Jody Rosenblatt**	School of Basic & Medical Biosciences School of Cancer and Pharmaceutical Sciences, London, UK	Misregulation of cell extrusion in cancer and how to reverse it
OCTOBER			
06/10/2023	**Sergio A. Quezada**	UCL Cancer Institute University College London, UK	Targeting Regulatory T cells in Cancer: informing mechanisms and novel therapies
20/10/2023	**Luis A. Diaz**	Memorial Sloan Kettering Cancer Center, NY, USA	Immunotherapy and mismatch repair deficiency
27/10/2023	**Marta María Alonso Roldán**	*CIMA* University of Navarra, Spain	Locking TIM3 as a therapeutic strategy for pediatric diffuse midline Glioma
NOVEMBER			
03/11/2023	**Walid Khaled**	Jeffrey Cheah Biomedical Centre, University of Cambridge, UK	Tumour Initiation Through Aberrant Differentiation: Lessons from Single Cell Genomics
17/11/2023	**Joseph Takahashi**	UT Southwestern Medical Center, Dallas, USA	Role of Circadian Clocks in Healthspan and Lifespan in Mice
DECEMBER			
01/12/2023	**Stefan Knapp**	Goethe University Frankfurt (SGC Frankfurt), Germany	Targeting strategies for the development of highly selective allosteric and orthosteric kinase inhibitors
15/12/2023	**Victoria Sanz-Moreno**	Cancer Research UK Queen Mary University of London, UK	Finding vulnerabilities in metastatic cancer cells

AD-HOC SEMINARS

In addition to the CNIO Distinguished Seminar Series, the CNIO also hosts numerous *ad-hoc* seminars throughout the year. *Ad hoc* seminars are organised for the purpose of academic interactions, academic elevation and enrichment, and academic *vis-a-vis* social networking; in addition, they facilitate socialising with colleagues from other institutions. A total of 32 *ad-hoc* seminars were organised by CNIO researchers in 2023.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
10/01/2023	**Thomas Wilkie**	UT Southwestern Medical Center, Pharmacology Department, Dallas, USA	Targeting GPCR-Gq-RGS signaling in pancreatic cancer
17/01/2023	**Sara García Alonso**	Experimental Oncology Group, Molecular Oncology Programme	Pregunta a @Astro_SaraG!
23/01/2023	**Carlos Benítez-Buelga**	"Alberto Sols" Biomedical Research Institute (IIBM), Department of Experimental Models of Human Disease, Madrid, Spain	Exploiting oxidative DNA damage response in cancer using small molecules modulating OGG1 activity
27/01/2023	**Maria Alieva**	"Alberto Sols" Biomedical Research Institute (IIBM), Madrid, Spain	Dynamic image analysis to uncover drivers of tumor and immune cell behavior and function
FEBRUARY			
02/02/2023	**Judit Jiménez-Sainz**	Yale University Medical School, USA	The BRCA2 landscape: demystifying domains, mutations and Variants of Uncertain Significance
02/02/2023	**Pasi A. Jänne**	Dana Farber Cancer Institute, Boston, USA	New strategies for the treatment of EGFR mutant Non-Small Cell Lung Cancer
MARCH			
09/03/2023	**Richard G. Vile**	Mayo Clinic Cancer Center, Rochester, USA	Using viruses to drive T cells, and T cells to drive viruses, for cancer immunotherapy
14/03/2023	**Marc Martí-Renom**	*CNAG-CRG*, Barcelona, Spain	Sex-determining 3D regulatory hubs revealed by genome spatial auto-correlation analysis
16/03/2023	**Kader Thiam**	GenOway, Lyon, France	Precise and translational preclinical models for assessment of immune-targeting agents
28/03/2023	**María José Alonso**	Professor of Pharmaceutical Technology at the University of Santiago de Compostela, Spain	Nanotechnology as a tool for the development of personalized oncological therapies
APRIL			
21/04/2023	**Thales Papagiannakopoulos**	New York University Grossman School of Medicine, USA	Dissecting the mechanisms of progression in genetic subtypes of lung cancer
26/04/2023	**Kamini Singh**	Albert Einstein School of Medicine, New York, USA	mRNA Translation Control in Cancer
27/04/2023	**Roger Gomis**	Institute for Research in Biomedicine (*IRB* Barcelona), Spain	ER+ Breast Cancer metastasis cell fate
MAY			
18/05/2023	**Gerd Müller**	UCSC, Santa Cruz, USA	Mechanisms of cell-cycle gene regulation by MuvB and E2F:RB complexes

DATE	SPEAKER	ORGANISATION	TITLE
JUNE			
07/06/2023	**Ernesto Arias Palomo**	CIB-Margarita Salas, Madrid, Spain	Making ends meet: molecular and structural insights into DNA transposition
13/06/2023	**Luis Mariano Esteban Escaño**	Polytechnic University School of La Almunia, University of Zaragoza, La Almunia, Zaragoza, Spain	Validation of Predictive Models
15/06/2023	**Inmaculada de Melo-Martín**	Division of Medical Ethics, Weill Cornell Medicine Medical College-Cornell University, New York, USA	Ethics: An Obstacle to Scientific Progress?
15/06/2023	**Laura E. Maglio**	Modulation of Neural Excitability and Synaptic Transmission Lab, Department of Anatomy, Histology, and Neuroscience School of Medicine, Autonomous University of Madrid, Spain	Role of IFG-1 in conditioned fear
16/06/2023	**Alejandro Bertolet**	Department of Radiation Oncology Massachusetts General Hospital and Harvard Medical School, USA	Radiobiology of Radiopharmaceutical Therapy for Metastatic Cancer
JULY			
03/07/2023	**Guadalupe Sabio**	Myocardial Pathophysiology Area, *CNIC*, Madrid, Spain	Metabolic Dysfunction as a Driver of Cancer Development
12/07/2023	**Juan Sastre**	University of Valencia, Spain	Inflammation and cancer in the pancreas
SEPTEMBER			
11/09/2023	**Pau Castel**	Department of Biochemistry and Molecular Pharmacology New York University Grossman School of Medicine, NY, USA	Oncoprotein duality: lessons learned from RAS GTPases
12/09/2023	**Oscar Martínez**	Sarcoma Research Group, *IDIBELL*, Barcelona, Spain	Multi-omic approach to decipher the molecular mechanisms associated to Ewing Sarcoma metastasis using a spontaneous metastasis mouse model
21/09/2023	**Tuomas Tammela**	MSKCC, New York, USA	Functional interrogation of intra-tumoral heterogeneity in lung and pancreas cancer
OCTOBER			
03/10/2023	**David Herreros**	Department of Macromolecular Structures, CNB, Madrid, Spain	Approaches to approximate molecular states from CryoEM data
05/10/2023	**Eugenia Pastushenko**	Faculty of Medicine -ULB, Belgium	Mechanisms regulating tumor transition states
09/10/2023	**Julio Sáez-Rodríguez**	Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Germany	Knowledge-based machine learning on multi-omics to dissect cancer mechanisms
10/10/2023	**Faiyaz Notta**	Princess Margaret Cancer Research Centre, University of Toronto, Canada	Phenotypes of pancreatic cancer
23/10/2023	**Claus Jorgensen**	CRUK Manchester, UK	Dichotomous effects of the pancreatic cancer tumour microenvironment
NOVEMBER			
13/11/2023	**David Vilchez**	CECAD – Cluster of Excellence for Aging Research - University of Cologne, Germany	Cold temperature delays aging and proteostasis collapse
30/11/2023	**Iban Ubarretxena Belandia**	Biophysics Institute (*CSIC*, *EHU/UPV*) and Bizkaia Biophysics Foundation, Spain	Cryo-EM studies on DNA polymerases and Protein Chaperonins
DECEMBER			
19/12/2023	**Roger Castells-Graells**	UCLA-DOE Institute for Genomics and Proteomics, University of California Los Angeles, USA	Engineered Protein Cages for Imaging of Small Proteins by Cryo-EM

WOMEN IN SCIENCE SEMINARS

In 2023, the WISE Diversity 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
23/02/2023	Sara García Alonso	Experimental Oncology Group, Molecular Oncology Programme	*Sara García, la científica del CNIO que está a un paso de la luna*
16/03/2023	Ana Losada, Núria Malats, Francisca Mulero and Maria A. Blasco	CNIO	Colloquium 'Women leaders in science'
11/07/2023	Chus Gutiérrez	Film director, screenwriter and actress	Team Management from a Feminist Perspective
19/09/2023	Victoria Cirlot	Professor of Romance Philology at the University Pompeu i Fabra, Barcelona, Spain	*Místicas en la Edad Media*
03/10/2023	Carme Riera	*Escritora, catedrática de Literatura y académica de la Real Academia Española*	*Cómo se escribe una novela*
20/11/2023	Ana Maiques	Co-Founder & Chief Executive Officer of Neuroelectrics, Spain	Revolutionizing brain therapies from Barcelona to Boston - a story of entrepreneurship in the healthcare sector

SCIENCE DISSEMINATION EVENTS

WORLD CANCER RESEARCH DAY
"¿CÓMO REDUCIR EL RIESGO DE CÁNCER? PARA PREVENIR, INVESTIGACIÓN"
18 SEPTEMBER 2023

ORGANISER:
· *Centro Nacional de Investigaciones Oncológicas (CNIO)*

WITH THE SUPPORT OF:
· "la Caixa" Foundation

KEYNOTE LECTURES:
· *Investigación para descubrir sustancias o hábitos carcinógenos*
  Elisabete Weiderpass, director of the International Agency for Research on Cancer (IARC)
· *Cómo podemos prevenir el cáncer en España*
  Marina Pollán, director of the National Centre of Epidemiology (*CNE-ISCIII*)

ROUND TABLE:
· **Maria A. Blasco**, Director of the Spanish National Cancer Research Centre-CNIO
· **César López-Palop**, President of the Domingo Martínez Foundation, a member institution of CNIO Friends
· **Marina Pollán**, director of the National Centre of Epidemiology *(CNE-ISCIII)*
· **Elisabete Weiderpass**, director of the International Agency for Research on Cancer (IARC)

CHAIR:
· **Cristina Villanueva**, Journalist

EUROPEAN RESEARCHERS' NIGHT 2023
CNIO: CONOCE A LOS CIENTÍFICOS, SÉ UN CIENTÍFICO
CNIO: MEET THE SCIENTISTS, BECOME A SCIENTIST
29 SEPTEMBER 2023

The Centre opened its doors to the public on September 29 to show its commitment to society and to promote scientific culture.

Between 5 p.m. and 11 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 doubts. Altogether, more than 213 people and 60 volunteers signed up for the day.

The activity aims to inspire a scientific vocation in very young people, to break down stereotypes associated with people involved in research, and to show what CNIO cancer research is all about.

Our "Meet the scientists, become a scientist" event consisted of the following activities:

→ A welcome talk with the showing of an inspirational video, and a presentation by students/postdocs on "Why I chose science".
→ Horizon Europe corner to show the impact of CNIO research beyond our borders/ interactions with EU missions.
→ Hands-on-experiments.
→ "Lab stands" to show "real" laboratory tools and small equipment.
→ "Speed-dating" with scientists.

European Researchers' Night is funded by the Marie Sklodowska-Curie Action (MSCA) and takes place at hundreds of European institutions across many cities and countries. The 2023 edition became even more important as Cancer is one of the 5 EU "missions" that defines the greatest challenges that we face as a society.

OPEN DOORS DAY: EVERYONE UNRAVELLING CANCER
16 NOVEMBER 2023

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 (XXIII *Semana de la Ciencia y de la Innovación*, 6-19 November 2023).

In November 2023, 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 35 attendees, who took the opportunity to learn more about a top research institution like the CNIO.

The Science and Innovation Week in Madrid encompasses a series of events organised by the *madri+d* Foundation to familiarise citizens with aspects of science, technology and innovation that they may not be fully aware of, nor understand how they impact on everyone's life. Above all, it is a programme aimed at reaching out to young people and demonstrating that research is very definitely a career in which girls are welcome to study from an early age without having to worry about gender barriers. At the CNIO we are delighted to have the opportunity to throw open the laboratory doors and demonstrate to everyone where, what, how and especially why we have chosen this line of work.

ADMINISTRATION

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Director General de Universidades e Investigación de la Región de Murcia

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Un representante del Consejo Asesor de Ciencia, Tecnología e Innovación designado por el Pleno de este Consejo

→ **Elected Members**

· *Fundación BBVA*
Represented by Rafael Pardo Avellaneda, Director
First Alternative Representative: Laura Poderoso Velasco, Deputy Director
Second Alternative Representative: Silvia Churruca Zarasqueta, Director of Communications and Institutional Relations

· *Fundación Bancaria Caixa d' Estalvis i Pensions de Barcelona, "la Caixa"*
Represented by Antonio Vila Bertrán, General Manager
Alternative Representative: Ignacio López Verdeguer, Director of Relations with Research and Health Institutions

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Abogado del Estado-Jefe en el Ministerio de Sanidad

*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 €363,640.92 in 2023 (€293,667.16 in 2022). This amount was received as base salary and position salary supplements: €236,914.56 (€228,938.28 in 2022); variable remuneration: €118,433.52 accrued during 2021 and 2022 (€56,716 in 2022, accrued in 2020); 3.5% increase: €8,292.84.
— Members of the CNIO Board of Trustees are not remunerated.

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  Head, Medical Oncology Department of Vall d'Hebron
  University Hospital
  Co-Director, VHIO's Research Unit for Molecular
  Therapy of Cancer (UITM) – CaixaResearch
  Barcelona, Spain

MANAGEMENT
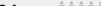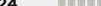
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CNIO PERSONNEL 2023

674
TOTAL CNIO
PERSONNEL

562
RESEARCH 84%

112
ADMINISTRATION 16%

GENDER DISTRIBUTION

425
FEMALE 63%

249
MALE 37%

AGE DISTRIBUTION

126
> 50 18.7%

182
< 30 27%

192
30-40 28.5%

174
41-50 25.8%
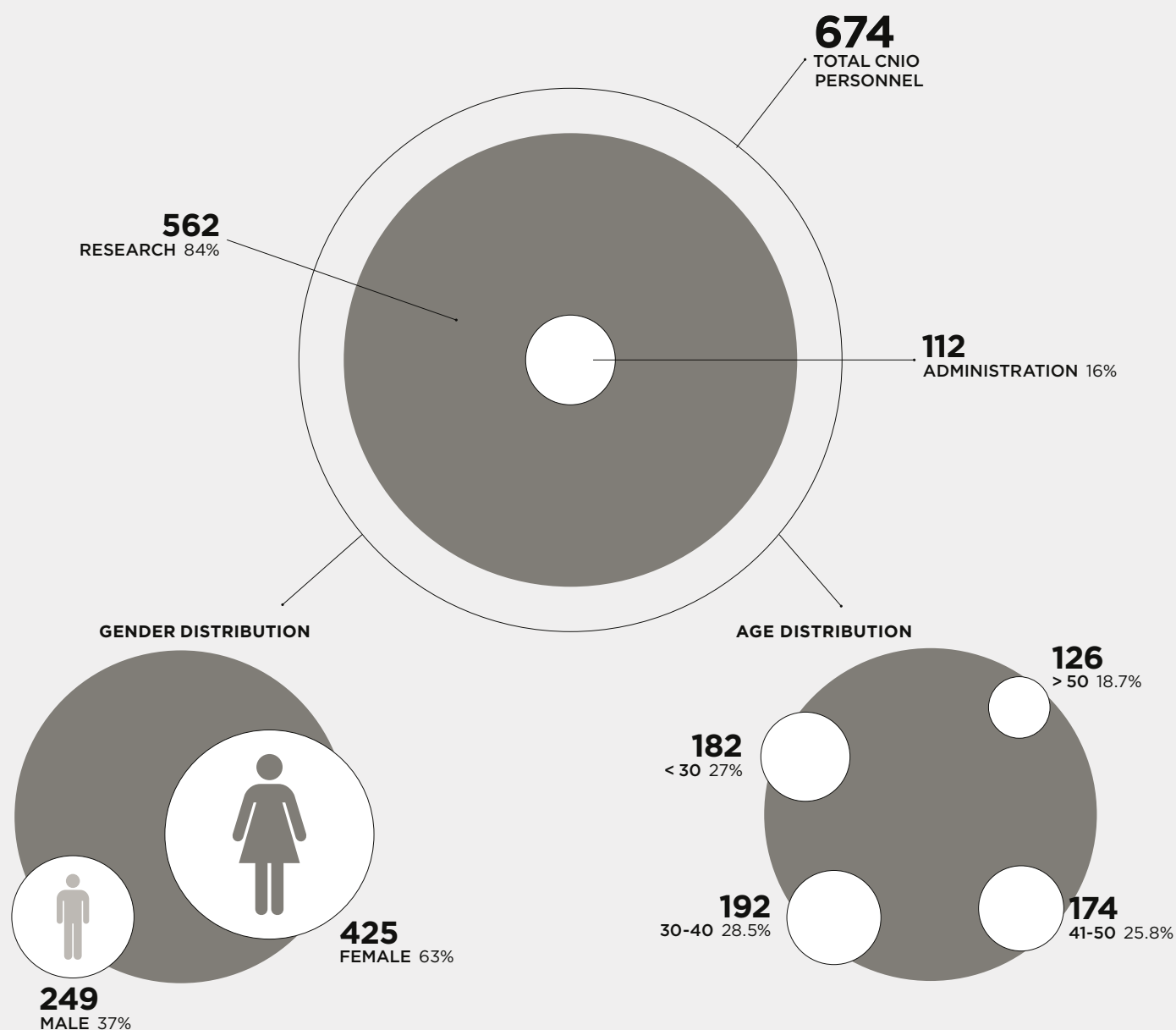
GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

PRINCIPAL INVESTIGATORS (GROUP LEADERS, HEADS OF UNIT/SECTION)	FEMALE	51%	**25**
	MALE	49%	**24**
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	FEMALE	60%	**9**
	MALE	40%	**6**
MANAGEMENT: DIRECTORS, HEADS OF AREA	FEMALE	27%	**4**
	MALE	73%	**11**

SCIENTIFIC PERSONNEL 2023

TOTAL SCIENTIFIC PERSONNEL **562**

DISTRIBUTION BY PROGRAMMES

MOLECULAR ONCOLOGY 32.7%	**184**	
STRUCTURAL BIOLOGY 9.6%	**54**	
HUMAN CANCER GENETICS 10%	**56**	
CLINICAL RESEARCH 25.4%	**143**	
BIOTECHNOLOGY 16.4%	**92**	
EXPERIMENTAL THERAPEUTICS 4.6%	**26**	
BIOBANK 1.3%	**7**	

DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS 8.7%	**49**	
RESEARCH SCIENTISTS 13.5%	**76**	
POST-DOCTORAL FELLOWS 12%	**67**	
GRADUATE STUDENTS 26%	**146**	
TECHNICIANS 39.8%	**224**	

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS	FEMALE	51%	**25**	
	MALE	49%	**24**	
RESEARCH SCIENTISTS	FEMALE	74%	**56**	
	MALE	26%	**20**	
POST-DOCTORAL FELLOWS	FEMALE	64%	**43**	
	MALE	36%	**24**	
GRADUATE STUDENTS	FEMALE	58%	**85**	
	MALE	42%	**61**	
TECHNICIANS	FEMALE	75%	**168**	
	MALE	25%	**56**	
TOTAL SCIENTIFIC PERSONNEL	FEMALE		**377**	
	MALE		**185**	

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC PERSONNEL **562**

PRINCIPAL INVESTIGATORS 10.5%	**25**	
RESEARCH SCIENTISTS 12.6%	**30**	
POST-DOCTORAL FELLOWS 15.1%	**36**	
GRADUATE STUDENTS 37.8%	**90**	
TECHNICIANS 24%	**57**	
TOTAL 100%	**238**	
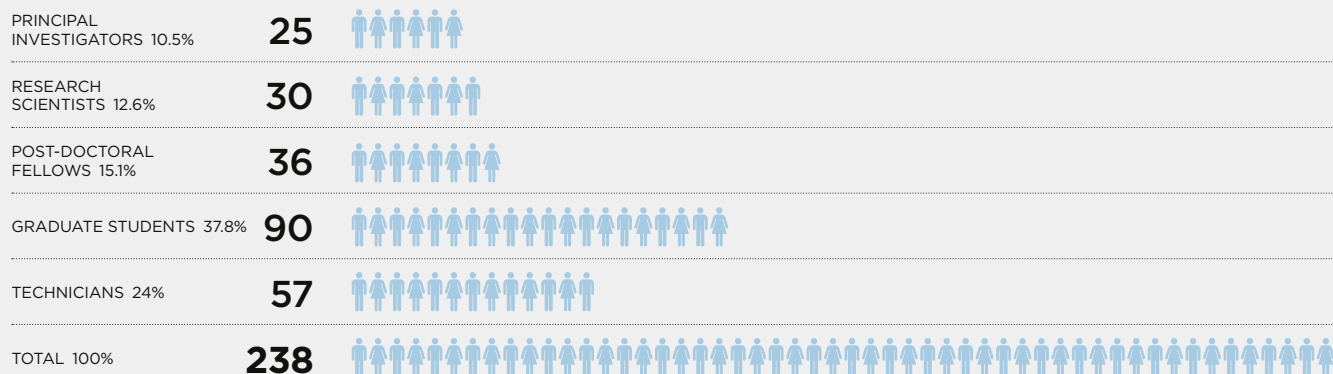
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH

PRINCIPAL INVESTIGATORS 5.5%	**11**	
RESEARCH SCIENTISTS 17.1%	**34**	
POST-DOCTORAL FELLOWS 15.1%	**30**	
GRADUATE STUDENTS 27.6%	**55**	
TECHNICIANS 34.7%	**69**	
TOTAL 100%	**199**	

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION

PRINCIPAL INVESTIGATORS 11%	**13**	
RESEARCH SCIENTISTS 10%	**12**	
POST-DOCTORAL FELLOWS 1%	**1**	
GRADUATE STUDENTS 1%	**1**	
TECHNICIANS 77%	**91**	
TOTAL 100%	**118**	
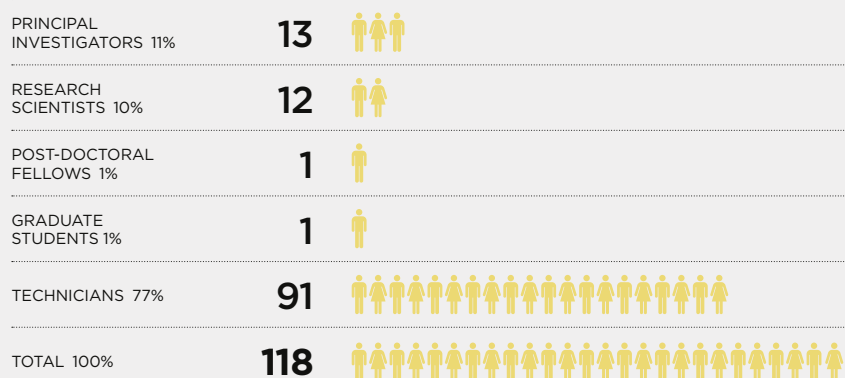
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BIOBANK

TECHNICIANS 100%	**7**	
TOTAL 100%	**7**	
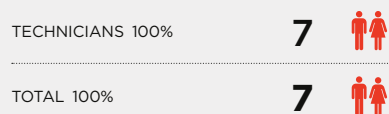
SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

508
SPANISH 90%

54
NON-SPANISH 10%

562
TOTAL SCIENTIFIC
PERSONNEL 100%
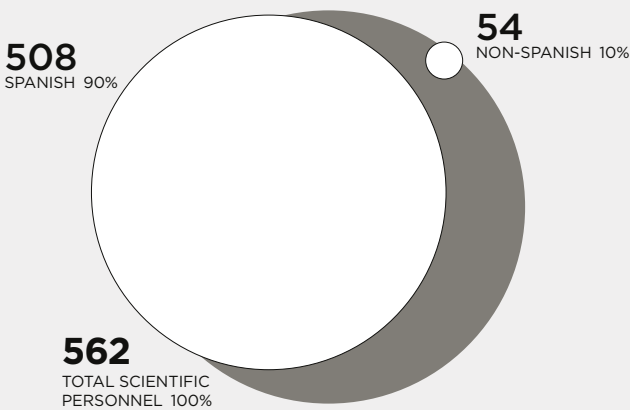
FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

Category	%	Count
PRINCIPAL INVESTIGATORS	8.2%	4
RESEARCH SCIENTISTS	8%	6
POST-DOCTORAL FELLOWS	19.4%	13
GRADUATE STUDENTS	12.3%	18
TECHNICIANS	5.8%	13

Total foreign scientific personnel 54
Percent values represent percentages of foreign employees
of the total CNIO personnel within each category

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN

508
SPAIN 90.3%

10
AMERICA 1.8%

2
AFRICA 0.4%

14
ASIA & AUSTRALIA 2.5%

28
REST OF EUROPE 5%

562
TOTAL SCIENTIFIC
PERSONNEL 100%

4 FRANCE 14.3%
3 GREECE 10.7%
3 PORTUGAL 10.7%
7 ITALY 25%
3 ROMANIA 10.7%
8 OTHER 28.6%
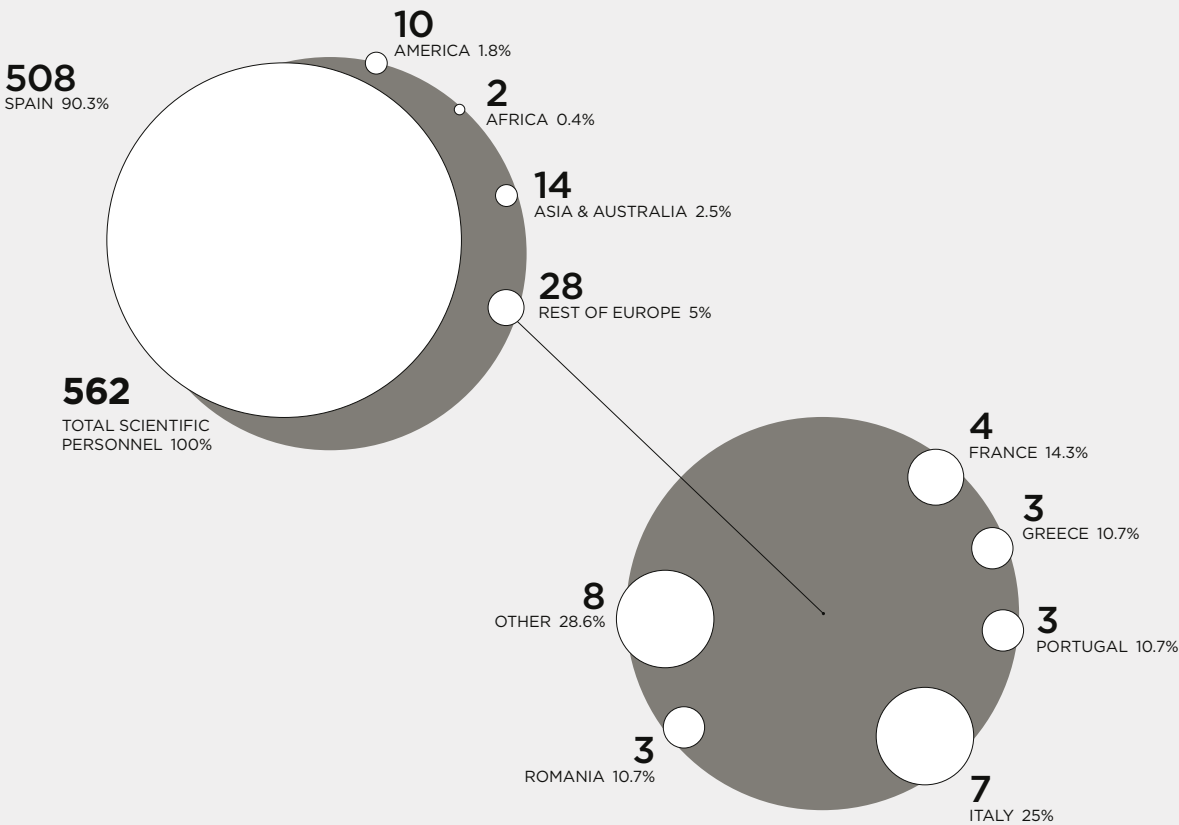
OTHER SPONSORSHIPS AND PARTNERSHIPS

"We take this opportunity to express our thanks and appreciation to all our sponsors for the generous support that we received from them in 2023. They play an inherent role in our present and future successes."

The *Fundación "la Caixa"* helps finance our most prominent international conferences, the CNIO - CaixaResearch Frontiers Meetings. Another main goal of the "la Caixa" Foundation is to support an innovative programme aimed at fostering international fellowships to attract the most outstanding students from the international arena to obtain their doctoral degrees at accredited *"Severo Ochoa"* Centres of Excellence. Since 2017, the CNIO participates in the doctoral INPhINIT fellowship programme of the "la Caixa" Foundation. The aim of this programme is to attract outstanding international students to carry out doctorates at top Spanish research centres. During 2023, 3 pre-doctoral students received one of these internationally recognised fellowships.

Fundación CRIS is dedicated to the promotion and development of research with the aim of eliminating the serious health threat of cancer. *Fundación CRIS* generously supports several research groups at the CNIO: the Experimental Oncology Group; the Breast Cancer, Haematological Malignancies, Lung Cancer, and Pediatric Onco-Hematology Clinical Research Units; and the Cancer Immunity Group. These Groups focus on the translation of advances in cancer research into novel therapeutics and improvements in patient care.

Thanks to the support of the *Fundación Banco Santander*, a group of 3 researchers received training on managerial and entrepreneurial skills in 2023, in collaboration with the *IE* Business School. *Banco Santander* Foundation also supports our successful outreach project, CNIO Arte.

The ***Fundación Jesús Serra***, now ***Fundación Occident***, continues to fund the Visiting Researchers Programme that was established to support prestigious international professors for short stays at the CNIO. The recipient of the *Jesús Serra* Foundation's Visiting Researchers' Award in 2023 was Gonçalo Bernardes, from the University of Cambridge (UK).

The AXA Research Fund (ARF) — a global initiative of scientific philanthropy run by the insurance group AXA — awarded an AXA-CNIO Endowed Permanent Chair position in Molecular Oncology to Mariano Barbacid as part of its 2011 call.

During 2023, our research activities and seminars were also supported, among others, by *Fundación Investigación Biomédica Hospital Universitario 12 de Octubre, Fundación Española de Hematología y Hemoterapia,* and *Fundación Banco Sabadell.* ∎

CNIO Friends Philanthropic Donations

The CNIO Friends Programme has been growing steadily over the years and once again enjoyed a profitable year, raising almost €650k from individuals, associations, and businesses.

The World Cancer Day campaign in February returned to the previous format encouraging members of the public to stop cancer by donating. This year, actress Nathalie Seseña was the face of the campaign, and we once again had the generous support of *JCDecaux España* to spread the message around Spain in bus stops, commercial shopping centres, and in *MetroMadrid*.

The CNIO was invited to be part of a bizum campaign titled "Numbers that matter". Graphic artists designed creatives that demonstrate the numeric bizum code of 7 social entities to be displayed on posters around Spain. The CNIO image was selected to be one of the front covers of Yorokobu magazine during the campaign.

In 2023, we were once again supported by several of our collaborating partners such as *Brother Iberia, Santa Lucía Seguros, L@s Fuertes, Rosae, Marea Rosa, AFECC* and *Bandera Rosa,* among others. Following our direct grant from *Ayuntamiento Torreperogil,* we have developed an exciting new collaboration with *the Federación Española de Municipios y Provincias* (*FEMP*) with the aim of raising awareness of the importance of cancer research and spread our work at the CNIO to all the councils of Spain to create a new network: *Red de Ayuntamientos Amigos/as del CNIO.*
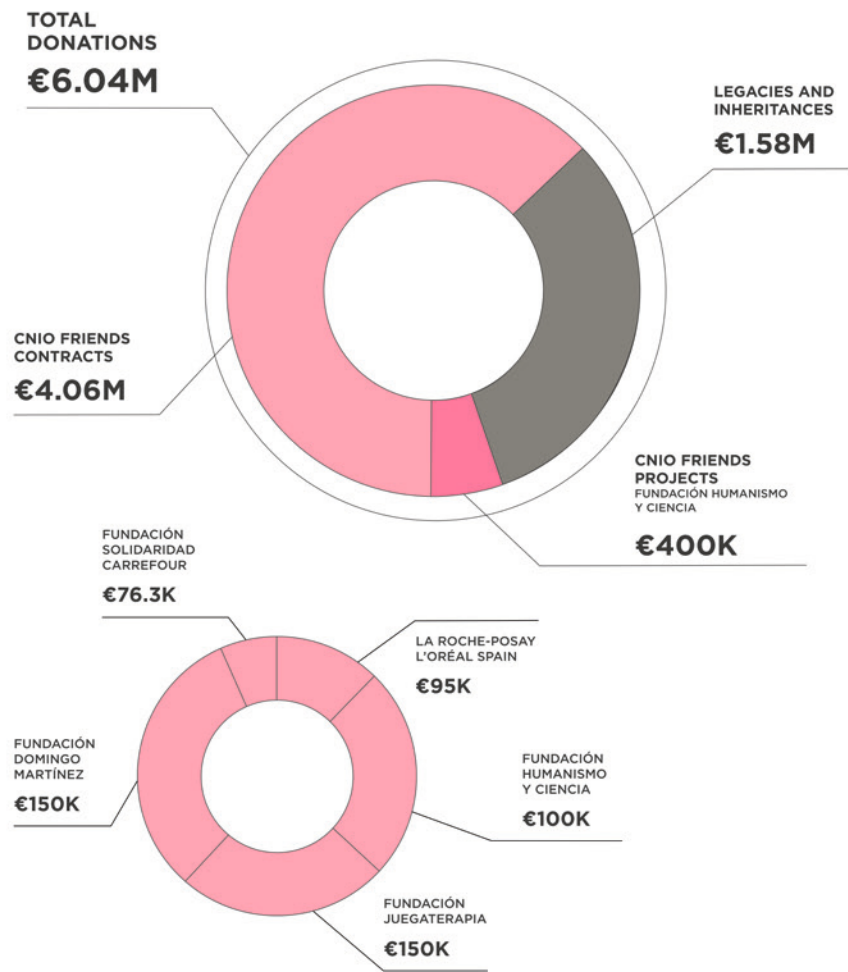
In addition, we developed new opportunities with corporate partners or foundations such as *Corporación RTVE,* and associations such as *Alicante Para la Lucha Contra El Cáncer.* Special thanks to our trusted friend *Fundación Domingo Martinez* for their continued support of our research at the CNIO. We are also delighted to be continuing the collaboration with *L'Oréal* in the name of *La Roche-Posay.*

As usual, we invited our donors to join us in person to celebrate "CNIO Friends" Day in July. The event brings together around 90 Friends, some familiar faces and some new ones each year. It is an opportunity for the CNIO Friends funded postdoctoral researchers to share their projects directly with donors to the CNIO. It is motivating for our CNIO Friends Fellows to see the impact of their research acknowledged by their supporters, and it is of course very rewarding for the donors to see exactly how we are utilising their generous support.

"CNIO Friends continues to support innovative research at the CNIO."

Finally, in 2023 we commenced work on our new major gifts programme in which we are working on attracting bigger gifts for specific projects, groups, and aligned areas of research at the CNIO. This is a slow process; major gifts can take more than 2 years to close. But the rewards can be great, and we are excited for the possibilities emerging. As ever, we extend a heartfelt message to our donors – thank you. You all play a part in helping us to stop cancer. ■

DONATIONS TO THE CNIO

TOTAL
DONATIONS
€6.04M

LEGACIES AND
INHERITANCES
€1.58M

CNIO FRIENDS
CONTRACTS
€4.06M

CNIO FRIENDS
PROJECTS
FUNDACIÓN HUMANISMO
Y CIENCIA
€400K

FUNDACIÓN
SOLIDARIDAD
CARREFOUR
€76.3K

LA ROCHE-POSAY
L'ORÉAL SPAIN
€95K

FUNDACIÓN
DOMINGO
MARTÍNEZ
€150K

FUNDACIÓN
HUMANISMO
Y CIENCIA
€100K

FUNDACIÓN
JUEGATERAPIA
€150K

'CNIO FRIENDS' INTERNATIONAL PREDOCTORAL/ POSTDOCTORAL CONTRACTS

PREDOCTORAL CONTRACTS

2019
PREDOCTORAL

Elena Fueyo
GENOMIC INSTABILITY GROUP

MARÍA OLIVA
CNIO FRIENDS

2020
PREDOCTORAL

Moustafa Shehata
KINASES, PROTEIN PHOSPHORYLATION AND CANCER GROUP

CARMEN GLORIA BONNET
CNIO FRIENDS

POSTDOCTORAL CONTRACTS

2017
POSTDOCTORAL

Vera Pancaldi
STRUCTURAL COMPUTATIONAL BIOLOGY GROUP

Paulina Gómez
GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

Irene Felipe
EPITHELIAL CARCINOGENESIS GROUP

JUEGATERAPIA
CNIO FRIENDS

2018
POSTDOCTORAL

Carolina Maestre
CELL DIVISION AND CANCER GROUP

Sebastian Thompson
GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

2019
POSTDOCTORAL

Rubén Julio Martínez
KINASES, PROTEIN PHOSPHORYLATION AND CANCER GROUP

María Moreno
GENOME INTEGRITY AND STRUCTURAL BIOLOGY GROUP

Neibla Priego
BRAIN METASTASIS GROUP

Sarita Saraswati
TELOMERES AND TELOMERASE GROUP

Miguel Jiménez
BRAIN TUMOUR GROUP

JUEGATERAPIA
CNIO FRIENDS

2020
POSTDOCTORAL

Rebeca Jimeno
BREAST CANCER CLINICAL RESEARCH UNIT

EVA PLAZA
CNIO FRIENDS

2021
POSTDOCTORAL

Giuseppe Bosso
TELOMERES AND TELOMERASE GROUP

Eunjeong Kim
GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

Magdalena Leal
MELANOMA GROUP

FUNDACIÓN HUMANISMO Y CIENCIA
CNIO FRIENDS

Sofía Cabezudo
MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Sergio Muñoz
DNA REPLICATION GROUP

María José Andreu
CHROMOSOME DYNAMICS GROUP

LAUDES FOUNDATION
CNIO FRIENDS

2022
POSTDOCTORAL

Lluis Cordón
BRAIN METASTASIS
GROUP

Ana María Roncero
BREAST CANCER CLINICAL
RESEARCH UNIT

Laura Nogués
MICROENVIRONMENT &
METASTASIS GROUP

Albert Harguindey
GROWTH FACTORS,
NUTRIENTS AND CANCER
GROUP

FONDATION
FRANZ WEBER
CNIO FRIENDS

Diana Vara
CELL DIVISION AND
CANCER GROUP

Federico Virga
EXPERIMENTAL
ONCOLOGY GROUP

Yurena Vivas
METABOLISM AND CELL
SIGNALLING GROUP

FUNDACIÓN
DOMINGO
MARTÍNEZ
CNIO FRIENDS

Bárbara Hernando
COMPUTATIONAL
ONCOLOGY GROUP

Rayan Naser
GROWTH FACTORS,
NUTRIENTS AND CANCER
GROUP

Carolina Villarroya
EXPERIMENTAL
ONCOLOGY GROUP

2023
POSTDOCTORAL

Mikhail Chesnokov
EPITHELIAL
CARCINOGENESIS
GROUP

Ivó Hernández
GENOMIC INSTABILITY
GROUP

Clara Reglero
HEREDITARY ENDOCRINE
CANCER GROUP

María Martínez
MACROMOLECULAR
COMPLEXES IN DNA
DAMAGE RESPONSE
GROUP

Elena Jiménez-Ortega
GENOMIC INTEGRITY AND
STRUCTURAL BIOLOGY
GROUP

Isabel Espejo
TELOMERES AND
TELOMERASE GROUP
*FUNDACIÓN HUMANISMO
Y CIENCIA*

LA ROCHE-
POSAY
CNIO FRIENDS

'CNIO FRIENDS' DAY

Our CNIO Friends Day was held in June. The event brings together around 90 Friends and gives our CNIO Friends funded Fellows the opportunity to present their research projects directly with donors to the CNIO. It is always a lovely opportunity for our funded Fellows to see the impact of their work on their supporters and vice versa.

WORLD CANCER DAY CAMPAIGN

We launched our multichannel branding and fundraising campaign on World Cancer Day to build awareness of our #CNIOStopCancer initiatives and to invite new donations for cancer research. Many thanks to *JCDecaux* for the continued support of our campaigns.

CNIO STEWARDSHIP ACTIVITIES

Following our direct grant from *Ayuntamiento Torreperogil*, we have developed an exciting new collaboration with the *Federación Española de Municipios y Provincias* (*FEMP*) with the aim of raising awareness of the importance of cancer research and spread our work at the CNIO to all the councils of Spain to create a new network: *Red de Ayuntamientos Amigos/as del CNIO*.

We had the opportunity to introduce *L'Oréal España* (*La Roche-Posay*) to their funded CNIO Friends Fellow.

We also continue to receive generous annual gifts from patient associations, including *Rosae, L@s Fuertes, SuperNenas, Bandera Rosa,* and our researchers, other CNIO staff, and their families continue to make the effort to participate in their fundraising events.

DONORS TO THE CNIO 2023

BENEFACTOR FRIENDS

- Alberto Rus Martínez
- Alejandra Vélez Bernal
- Alejandro Casamayón Antón
- Alejandro Mendoza Plaza
- Alicia Fernández Ojosnegros
- Ana Elisa González López
- Ana Esmeralda Pomareta Pastor
- Ana María De Lucas Gómez
- Ángel Fernández Bonache
- Antonio Bravo López
- Carlos Cilleruelo Rodríguez
- Carlos Rey Astray
- Carmen Fernández Martín de la Torre
- Carolina Riesco Fernández
- Christian López Carretero
- Concha De Pablo Rico
- Cruz Díaz Beltrán en memoria de Mario Leonardo Heras
- Daniel Gutiérrez Contreras
- Dr. Clara & Twitch Friends y María Sol Vallejo Prieto
- Enriqueta Sesmero Cutanda
- Esther Valdivia Carrión
- Familia Doncel Elena, en memoria de José María
- Fernando Pascual Carreras
- Francisco José Franco Sánchez
- Gema Mazón Gutiérrez y Familia Mazón Gutiérrez
- Gema Rubio González
- Gonzalo Pérez Cano
- Inés Lamela Trobat
- Irene Quiroga Hernández
- Iván Torres Sanz
- Javier Casado López

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- Jesús Miguel Iglesias Retuerto
- Joaquín Ángel Martínez Andújar
- Jorge Baz López
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- José Antonio Martinsanz Aguado
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- José Ignacio Soriano Campos
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- Luís Carlos Nuñez Arias
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- Luis Grau Orts
- Luis Tortosa Pardo de Santayana
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- Luisa Vázquez Bejarano
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- Manuela Caballero del Pozo
- Margarita Casado Alegre
- Margarita de Lucas Gómez
- María Cruz Matías Caviedes
- María del Carmen Castaño Hidalgo
- María de Los Ángeles Santiago Gordillo
- María Jesús Ramírez Guadalix
- María José Lucas Soriano
- María José Rosillo Ortega
- María Paz Emma Díaz Díaz
- María Rodríguez López
- María Rosario Muñoz López
- María Teresa Sainz de la Maza Escobal

· Micaela Lugones Sánchez

· Natalia Judith Laso Martínez

· Pablo Redondo Aranda

· Paula García Santiago

· Paulina Vicente Armengod

· Pedro Ángel López Méndez

· Rafael Eugenio Méndez Devesa

· Rafaela Vidal Romero, en memoria de José Amadio

· Santiago de la Fuente de Castro

· Santiago Rodríguez Uriel

· Silvia Durán Porro

· Sonia Arrabal Espejo

· Vicente González del Pozo

· Víctor Fuentetaja Muñoz

· Yolanda Casado Santamarta

BENEFACTOR ASSOCIATIONS

· *Amigos Bowling Leagues Arboleas*

· *Asociación Alicante para la lucha contra el cáncer*

· *Asociación Círculo Artístico San Lucas de las Pedroñeras*

· *Asociación de Mujeres Progresistas de Hornacho*

· *Marcha Contra el Cáncer Puebla de Maestre*

· *Plataforma de Enfermos y Solidaridad*

· *Zambomba Solidaria. Coro Parroquial San Juan Bautista*

BENEFACTOR COMPANIES AND INSTITUTIONS

· *Act One Producciones Audiovisuales*

· *Alúa Cid S.L.U.*

· *IES San Vicente*

· *Import Interbalear S.L.*

· *Minerva Project*

· *Qué Te Cuentas, C.B.*

· *Sansor Viajes*

SPONSOR FRIENDS

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· Antonio Segura Baeza

· Anunciación Calvo Prieto

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· Francisca Nogueroles Galiana

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· María del Pilar Castro Carvajal

· María Victoria Jiménez Quevedo

· Mercedes Cáceres Alonso

· Vicente Belenguer Tarín

· Victoria Caturla Vicente

SPONSOR ASSOCIATIONS

- *Asociación para la Atención Integral de Afectados de Cáncer Miranda*
- *Asociación cultural Marea Rosa Montemolín, Pallarés, Santa María de Nava*
- *"Bandera Rosa" Asociación de pacientes y familiares de cáncer de mama*
- *Asociación Recreativo-Cultural de apoyo a personas afectadas de cáncer Esperanza Burela*
- *Asociación Social "L@s Fuertes", El Espinar*
- *Colectivo de Mujeres Afectadas de Cáncer "Las Supernenas"*
- *Freesia Group*
- *"Rosae" Asociación de mujeres afectadas de cáncer de mama*

SPONSOR COMPANIES & FOUNDATIONS

- *Ayuntamiento de Torreperogil*
- *Brother Iberia S.L.U.*
- *Fundación Domingo Martínez*
- *Fundación Humanismo y Ciencia*
- *IES Airén*
- *International Road Technology Consulting S.L.*
- *iZanda Portable Machine Tools S.L.*
- *JCDecaux España S.L.U.*
- *José Borrel S.A.*
- *L'Oréal España/ La Roche – Posay*
- *Santa Lucía Seguros, S.A.*

We extend our most heartfelt thanks to all the anonymous donors and benefactors who have made philanthropic gifts or left legacies to the CNIO to support cancer research; in doing so they have contributed to society for generations to come.

cnïo *stop cancer*