

**ANNUAL
REPORT 2022**

SPANISH NATIONAL
CANCER RESEARCH CENTRE, CNIO

ANNUAL
REPORT 2022

CONTENTS

FOREWORD	8	VICE-DIRECTOR	12
ORGANISATION OF RESEARCH	14	BASIC RESEARCH	16
		Molecular Oncology Programme	18
		Telomeres and Telomerase Group - <i>Fundación Humanismo y Ciencia</i>	20
		Experimental Oncology Group	24
		Cell Division and Cancer Group	28
		Genomic Instability Group	32
		Topology and DNA Breaks Group	36
		Chromosome Dynamics Group	40
		DNA Replication Group	44
		Melanoma Group	48
		Epithelial Carcinogenesis Group	52
		Growth Factors, Nutrients and Cancer Group	56
		Transformation and Metastasis Group	60
		Microenvironment & Metastasis Junior Group	64
		Brain Metastasis Junior Group	66
		Metabolism and Cell Signalling Junior Group	68
		Cancer Immunity Junior Group	70
		Structural Biology Programme	72
		Macromolecular Complexes in DNA Damage Response Group	74
		Kinases, Protein Phosphorylation and Cancer Junior Group	78
		Genome Integrity and Structural Biology Junior Group	80
		Computational Cancer Genomics Junior Group	82
		Computational Oncology Junior Group	84
		Spectroscopy and Nuclear Magnetic Resonance Unit	86
		Bioinformatics Unit	88
		Electron Microscopy Unit	90
		Protein Crystallography Unit	92
		Protein Production Unit	94
		TRANSLATIONAL RESEARCH	96
		Human Cancer Genetics Programme	98
		Hereditary Endocrine Cancer Group	100
		Genetic and Molecular Epidemiology Group	104
		Molecular Cytogenetics Unit	108
		Familial Cancer Clinical Unit	110
		Human Genotyping- <i>CEGEN</i> Unit	112
		Clinical Research Programme	114
		Breast Cancer Clinical Research Unit	116
		Molecular Diagnostics Unit	120
		H12O-CNIO Haematological Malignancies Clinical Research Unit	122
		H12O-CNIO Lung Cancer Clinical Research Unit	126
		H12O-CNIO Cancer Immunotherapy Clinical Research Unit	130
		INNOVATION	132
		Biotechnology Programme	136
		Genomics Core Unit	138
		Mouse Genome Editing Core Unit	140
		Monoclonal Antibodies Core Unit	142
		Molecular Imaging Core Unit	144
		Flow Cytometry Core Unit	146

		Confocal Microscopy Core Unit	148
		Proteomics Core Unit	150
		Histopathology Core Unit	152
		Animal Facility	154
		Experimental Therapeutics Programme	156
		Medicinal Chemistry Section	158
		Biology Section	162
		CNIO - Lilly Cell Signalling and Immunometabolism Section	166
		Technology Transfer and Valorisation Office (TTVO)	168
		BIOBANK	170
		Communications	176
		Press Clippings	178
		CNIO Media Impact	181
		2022 Social Network Data	182
		Social Events	183
COMMUNICATIONS	174		
INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY	186		
DEVELOPMENT & PHILANTHROPY	192		
CNIO OFFICES	196	Dean's Office	198
		CNIO Wise Diversity Office	200
FACTS & FIGURES	202	Scientific Management	204
		Competitive Funding	206
		Education and Training Programmes	220
		Scientific Events	226
		Administration	236
		Board of Trustees	236
		Scientific Advisory Board	238
		Management	240
		CNIO Personnel 2022	242
		Other Sponsorships and Partnerships	246
CNIO FRIENDS PHILANTHROPIC DONATIONS	248	Donations to the CNIO	251
		'CNIO Friends' International Predoctoral/Postdoctoral Contracts	252
		'CNIO Friends' Day	255
		World Cancer Day Campaign	256
		Promoting Dialogue Between Art & Science through 'CNIO Arte'	257
		New Major Gift to the CNIO	258
		CNIO Friend Donor Events	259
		Donors to the CNIO 2022	260



“The year 2022 has been a record year in terms of CNIO publications in top journals in our Centre’s history.”

MARIA A. BLASCO
Director

FOREWORD

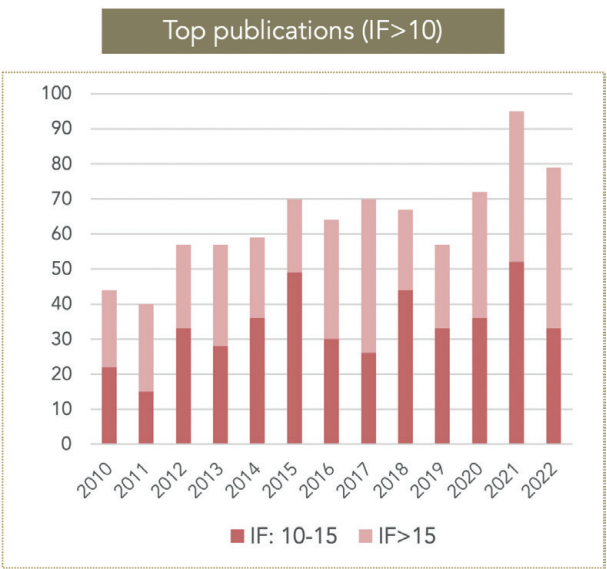
MARIA A. BLASCO Director

I am very glad to say that 2022 was an excellent year for our scientific productivity. We authored a total of 182 papers, 33 of which were published in journals with an impact factor between 10 and 15, and 46 publications in journals with an impact factor greater than 15, the latter representing the maximum of the historical series from the beginning of CNIO operations.

According to *Nature Index*, considering our scientific contributions in the life sciences and healthcare field, the CNIO is ranked second among cancer-focused institutions in Europe and holds the 9th position worldwide. Likewise, the SCImago ranking places CNIO in the 8th position among cancer centres worldwide. These indicators evidence the success of our scientific activity in cancer research and our leadership at the international level.

Additional evidence of CNIO’s excellence is the fact that 10 CNIO researchers are listed in the “World’s Top 2% Scientist List” generated at Stanford University, which ranks the 200,000 most influential researchers worldwide in all scientific fields, based on a score that takes into consideration scientific production across different periods, h-indexes, number of citations and author position.

Adapting the Centre to the constant evolution of science, by incorporating new research groups working in the new emerging fields in cancer research, is key to maintaining our competitive edge. Along these lines, a new operating model for the Clinical Research Programme has been adopted, focusing on the integration of top-notch oncologists from the National Health System by attracting senior clinical oncologists to set up a laboratory at CNIO. To implement this plan, a call was opened for candidates, which concluded in 2022 with the recruitment of 2 renowned oncologists with experience in translational research, working in areas complementary to those covered by the Clinical Research Units already in place. Thus, Luis Álvarez-Vallina, Head of the Cancer Immunotherapy Unit at the Hospital *12 de Octubre*, is now leading a new H12O-



CNIO Cancer Immunotherapy Clinical Research Unit. Dr Vallina is a recognised expert in the design and engineering of monoclonal antibodies for therapeutic use, and co-founder of Leadartis SL, a company working in this field. Likewise, Antonio Pérez, Head of the Service of Paediatric Haemato-oncology at the Hospital *La Paz*, will lead a joint Unit in the paediatric cancer field. Dr 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. The incorporation of Dr Pérez will come into effect in 2023, after closing the agreement with the Hospital *La Paz*.

During the previous year, the Heads of the Proteomics and the Confocal Microscopy Units left the CNIO to pursue other professional opportunities. The selection processes that were launched to identify candidates for those 2 positions culminated with the selection of 2 new Unit Heads. Marta Isasa joined the CNIO in October 2022 as new Head of the Proteomics Unit, after more than 10 years of international postdoctoral experience in proteomics, and formerly Associate Director of the Proteomics and Chemical Biology Group in Odyssey Therapeutics (MA, USA). Likewise, in December 2022, Isabel Peset joined the CNIO as new Head of the Confocal Microscopy Unit. Isabel also has international experience of more than 10 years after obtaining her PhD in the advanced microscopy field, having been Lead Scientist in Advanced Imaging at Medicines Discovery Catapult (Cambridge, UK).

In 2022, the Crystallography and Protein Engineering Unit was expanded to 2 independent Units, each focusing on the 2 distinct fields that were under the scope of the Unit. Thus, the current Protein Crystallography Unit, under the supervision of Inés Muñoz (former Head of the Unit), offers crystallography and SAXS for 3D protein structure determination; whereas a new Protein Production Unit, under the leadership of Jorge L. Martínez-Torrecuadrada, provides access to an array of technologies for heterologous expression and purification of recombinant proteins.

In 2022, the CNIO filed 4 priority patent applications, 12 PCT applications for international extension, and 1 registry of intellectual property to protect our IP assets. Our efforts in promoting collaborations with industry resulted in about €2.4M secured through research agreements with a number of partners from the pharmaceutical and biotech sectors. As much as 65% of CNIO agreements with the private sector were established with international entities. In addition, the income from royalties derived from the licensing of CNIO assets quantified in 2022 (corresponding to the sales in 2021) reached €1.3M, which represents an increase of 47% over the levels achieved in the previous year. This is a clear reflection of the leadership and influence of the CNIO and our researchers worldwide in the innovation field.

Furthermore, we continue supporting and promoting the participation of CNIO researchers in national and international calls from public agencies and private foundations that fund projects with a strong innovation component. Thus, in 2022 there has been very active participation in the new National AEI Innovation calls, co-funded by the EU Reconstruction Funds “NextGenerationEU”. Two public-private collaborative projects have been awarded: a project of the Telomeres and Telomerase Group in collaboration with the spin-off company Telomeres Therapeutics for the development of a novel gene therapy for the treatment of idiopathic fibrosis; and another project of the Histopathology Unit in partnership with MedLumics to develop an effective and safe system for the treatment of atrial fibrillation through irreversible electroporation. Another example is the ERC “Proof-of-Concept” grant awarded to the Transformation and Metastasis Group Leader, Eva González-Suárez, for the project “PLEIO-RANK” focused on targeting RANK receptors as a novel therapeutic strategy in triple negative breast cancer.

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. By the end of 2022, the CNIO reached a new milestone in obtaining the Rare Pediatric Disease Designation (RPDD) by the FDA to a drug candidate developed and licensed by the CNIO. This drug is a potential first-in-class oral kinase inhibitor that targets PI3K, as well as key resistance mechanisms such as PIM and mTOR, for the treatment of neuroblastoma. The product is now being further developed by AUM Biosciences.

Our training programmes are one of the key elements of the Centre’s strategic plan forming an essential part of our mission. Providing 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.

During 2022, Prof. Eva Nogales, from UC Berkeley (USA), and Prof. Gonçalo Bernardes, from University of Cambridge (UK), were beneficiaries of the *Jesús Serra* Foundation’s Visiting Researchers Programme and spent several months in sabbatical stays in our Centre. In addition, Marwa Muhammad Abu-Serie Ali, from GEBRI, SRTA-City, Egypt, was awarded a grant from the “Science by Women” programme of the *Mujeres por Africa* Foundation, as Visiting Scientist, to join the Telomeres and Telomerase Group at the CNIO for a 6-month stay.

In 2022 we were also able to resume the prestigious CNIO-CaixaResearch Frontiers Meetings, which had been put on

hold during the years of the COVID-19 pandemic. These conferences represent a privileged forum for the exchange of new and exciting ideas in oncological research, involving the participation of key leaders in a broad array of fields. Two conferences were held in 2022, focusing on Molecular, Cellular and Organismal Drivers of Aging (May 9-10), and Diet, Nutrition and Cancer Cell Metabolism (October 24-26).

The “Philosophy and Science” symposia series that began in 2019 held its 4th edition this year, with the support of *Banco Sabadell* Foundation. Under the title “The long-term view”, a panel of philosophers and scientists discussed the future of the planet Earth, the future of humanity, and our future in relation to AI, a debate driven by the worrying situation between a very alarming present and the future of the planet and humanity.

The Communications Office has been instrumental across the years in enhancing the CNIO’s impact on society. Nuria Noriega, the Office Head for more than 10 years, left the CNIO in 2022 to pursue her professional career at another research institution. She has been replaced by Mónica González-Salomone, a well-known journalist specialised in the communication of science, with experience in the media at both national and international levels, and a regular collaborator of scientific institutions such as the European Spatial Agency, the *Instituto de Astrofísica de Canarias* (CSIC), the BBVA Foundation, and the CNIO.

During 2022, CNIO news continued attracting the interest of the media, keeping the level of previous years, and marking over 4,600 appearances in press (printed and online). In addition to some of our most relevant research findings, the non-specialised media also covered other CNIO initiatives, as described below.

Thus, on the occasion of World Cancer Day on February 4, we launched a fundraising and awareness campaign under the slogan “The lottery that touches you the most”, together with our logo #CNIOStopCancer, focused on conveying the high frequency of cancer cases and how the only way to mitigate their impact is by investing in research. The success of the campaign brought a significant presence of our Centre in the media, which in turn generated a significant number of new donors for our ‘CNIO Friends’ platform.

The Institutional Image and Outreach Office is leading several projects aimed to generate society’s trust and attention. Thus, in 2022, the 5th 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 sculptor Susana Solano and the epidemiologist Pedro Alonso, Director of the WHO’s Global Malaria Programme. The piece ‘*El mundo de las cosas*’ was



CNIO Arte stand at ARCO, February 23 - 27, 2022. In this photo, from left to right: Borja Baselga, General Manager of the *Banco Santander* Foundation, Susana Solano, CNIO Arte 2022 featured artist, Amparo Garrido, visual artist and CNIO Arte curator, and Maria Blasco, CNIO Director.

exhibited at the CNIO from February 16 throughout the year. For a second year, we had our own space at the contemporary art fair ARCO (February 23-27), where Solano’s work was exhibited, attracting a lot of interest from the public and the media. On top of the social visibility and impact, CNIO Arte generated a donation of €100,000 that contributed directly to our ‘CNIO Friends’ philanthropic initiative.

Derived from the success of this initiative, a proposal was designed jointly with the *Instituto Cervantes* (IC), to exhibit the works of the artists participating in successive CNIO Arte editions in the different IC sites across the world. This initiative was developed through 2022 and materialised on February 3, 2023, when the exhibition “CNIO Arte, Dialogues between Art and Science” was opened at the IC in New York, in a social event that was attended by relevant personalities from the worlds of science and culture. The exhibition, which offers a selection of works from the CNIO Arte programme across the years, including photographs, videos, drawings and collages, will be on display at the IC until April 15 and moved later to other venues, including the Spanish Embassy in Washington and other IC sites.

Aiming to increase the impact of this initiative, we also organised the 3rd 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 5th edition of the CNIO Arte project, under the title “Art, Science and Pandemics”, chaired by Prof. Carlos Jiménez (Emeritus Professor of Aesthetics, historian and art critic).

Another related activity was established in 2022, namely the “CNIO Artistic Residences” initiative, which is financially supported by the Spanish Foundation for Science and

Technology (*FECYT*). After an open call, a young artist, Clara Montoya, was selected to spend several months at the CNIO with the goal of achieving an understanding of the Centre’s research activities and, based on that knowledge, to create one or more art works inspired by or reflecting the work developed at the CNIO. Those works will be exhibited at the CNIO as well as in those entities collaborating in the dissemination of the project, and will be available for sale, the benefits being designated to fund CNIO research activities.

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” initiative, to fully fund the salaries of new post-doctoral research fellows recruited through a highly competitive international call each year. We surpassed an exciting milestone this year, raising over €1 million for the first time. To date, philanthropic donations to the CNIO have made possible the incorporation of 34 new researchers since 2016 (9 during 2022). In an exciting new development, in 2022 we launched a new facet of the philanthropy programme: “CNIO Friends Projects”. In this initiative, major donations to the CNIO (e.g., €250,000+) can support specific research projects, and we were delighted to receive our first major donation through this programme this year to support research in renal fibrosis and telomeres. We are deeply grateful to all of our donors and CNIO Friends for their ongoing support of our research programme. After all, philanthropy is a tool that enables every member of society to have a direct and positive impact on helping us to stop cancer.

Our commitment to gender equality continued during 2022 through the activities spearheaded by our CNIO Women and Science Office (WISE), an effort by CNIO volunteers that is facilitating both cultural changes and institutional improvements. During this year, the WISE Office expanded its name to “Women and Diversity” Office. The Office continued bringing outstanding female leaders in a broad array of fields as speakers to the “WISE Seminars” series, which is open to the general public, aiming to inspire the audience to explore new perspectives and visions on the role of women in society (<https://www.cnio.es/mujeres-y-ciencia/actividades-y-eventos/>). Furthermore, on the occasion of International Working Women’s Day, the WISE Office organised a joint event together with the British Embassy, and with the participation of the Jane Goodall Institute, in which a tribute was paid to the career of Dr Jane Goodall. In our efforts to educate the future generations without gender bias, and for the occasion of International Day of Women and Girls in Science, CNIO released a video in which representatives from all CNIO professional categories of women involved in research tell us why they love research (https://youtu.be/Qhq3se_YFb4).

It is worth mentioning here the selection in November 2022 of CNIO researcher Sara García Alonso as an astronaut for the European Space Agency (ESA). The relevance of her success in the amazingly competitive selection process carried out by the ESA provides further evidence of the level of excellence attained by our scientists, and the impact in the media generated by her appointment has also helped to spread knowledge about the CNIO to society.

It is a pleasure for us to convey to society for another year the report of our activities and achievements during 2022. We 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

“Doing this yearly analysis allows me to get a panoramic view of all the exciting things that are being done by CNIO-ans. My summary is simple: congratulations and, above all, thanks.”

Dear all, 2022 brought developments in many exciting scientific areas, such as the irruption of artificial intelligence or the first images of the James Webb telescope. We at CNIO keep doing our share, and during this last year our scientists made substantial advances in many important fronts. We now have new biomarkers to predict the response to chemotherapy in cancer patients, and new approaches for the early detection of bad prognosis disease. We have a better understanding of how cells respond to radiation and to other DNA damaging chemotherapies, as well as specific strategies to improve the efficacy of these therapies in the clinic. We have obtained atomic information on important cancer drivers that should enable us to develop targeted therapies, and improved our understanding of the mechanisms that limit the efficacy of anti-cancer treatments. Besides cancer, part of our research is focused on fundamental aspects of cell biology that impact many areas. In this regard, we have also made important advances in other diseases such as lung fibrosis, viral infections, or

neurodegeneration. Among our many discoveries, I want to single out one from the Malumbres laboratory, which provided a beautiful example of how much information can be extracted from single-cell sequencing data, when these experiments are coupled with original analyses and well formulated hypotheses. Technology is wonderful, but it only becomes great when coupled with insightful questions. I started this paragraph by quoting the excitement brought by the amazing images coming from the James Webb telescope. Like many others, I also wanted to become an astronaut as a kid. In 2022, one of our colleagues, Sara García Alonso, managed to make this dream true. Contratulations Sara, seeing you succeed is a reminder that, sometimes, if persistent and hardworking, things can work out for us in marvellous ways. Hoping the future still has a few unexpected and positive surprises for all of us, thanks once again for your work, and my best wishes for the coming year. ■

ORGANISATION OF RESEARCH

MARIA A. BLASCO DIRECTOR

ÓSCAR FERNÁNDEZ-CAPETILLO VICE-DIRECTOR

BASIC RESEARCH

MOLECULAR ONCOLOGY PROGRAMME

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

Maria A. Blasco
Telomeres and Telomerase Group
– Fundación Humanismo y Ciencia

Mariano Barbacid
Experimental Oncology Group

Marcos Malumbres
Cell Division and Cancer 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

Héctor Peinado
Microenvironment and Metastasis Junior Group

Manuel Valiente
Brain 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

Núria Malats
Genetic and Molecular Epidemiology Group

Sandra Rodríguez-Perales
Molecular Cytogenetics Unit

Maria Currás
Familial Cancer Clinical Unit

Anna González-Neira
Human Genotyping-CEGEN Unit

CLINICAL RESEARCH PROGRAMME

Miguel Quintela-Fandino Acting Programme Director
María Luisa Anguita Secretary

Miguel Quintela-Fandino
Breast Cancer Clinical Research Unit

Luis J. Lombardía
Molecular Diagnostics Unit

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

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

Luis Álvarez-Vallina (since November)
H12O-CNIO Cancer Immunotherapy Clinical Research Unit

INNOVATION

ROKE I. ORUEZABAL DIRECTOR OF INNOVATION

BIOTECHNOLOGY PROGRAMME

Fernando Peláez Programme Director
Celia María Ramos Secretary

Orlando Domínguez
Genomics Core Unit

Sagrario Ortega
Mouse Genome Editing Core Unit

Giovanna Roncador
Monoclonal Antibodies Core Unit

Francisca Mulero
Molecular Imaging Core Unit

Lola Martínez
Flow Cytometry Core Unit

Isabel Peset (since December)
Confocal Microscopy Core Unit

Marta Isasa (since October)
Proteomics Core Unit

Vacant
Histopathology Core Unit

Isabel Blanco
Animal Facility (Vivoteca Management & Services)

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor Programme Director
Natalia Catalá Secretary

Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
CNIO-Lilly Cell Signalling and Immunometabolism Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Irene Herrera Head

BIOBANK

EVA ORTEGA-PAÍÑO DIRECTOR

Basic Research

Molecular Oncology Programme	18
Telomeres and Telomerase Group – <i>Fundación Humanismo y Ciencia</i>	20
Experimental Oncology Group	24
Cell Division and Cancer Group	28
Genomic Instability Group	32
Topology and DNA Breaks Group	36
Chromosome Dynamics Group	40
DNA Replication Group	44
Melanoma Group	48
Epithelial Carcinogenesis Group	52
Growth Factors, Nutrients and Cancer Group	56
Transformation and Metastasis Group	60
Microenvironment & Metastasis Junior Group	64
Brain Metastasis Junior Group	66
Metabolism and Cell Signalling Junior Group	68
Cancer Immunity Junior Group	70
 Structural Biology Programme	 72
Macromolecular Complexes in DNA Damage Response Group	74
Kinases, Protein Phosphorylation and Cancer Junior Group	78
Genome Integrity and Structural Biology Junior Group	80
Computational Cancer Genomics Junior Group	82
Computational Oncology Junior Group	84
Spectroscopy and Nuclear Magnetic Resonance Unit	86
Bioinformatics Unit	88
Electron Microscopy Unit	90
Protein Crystallography Unit	92
Protein Production Unit	94

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), mitosis (Marcos Malumbres), melanoma (María S. Soengas), epithelial carcinogenesis (Francisco X. Real), metabolism and cell signalling (Nabil Djouder and Alejo Efeyan), immunotherapy (María Casanova), and metastasis (Manuel Valiente, Eva González-Suárez and Héctor Peinado).

During 2022, our scientists reported relevant contributions in many areas, and here I provide a few selected examples of their work. For instance, the Junior Group led by Manuel Valiente discovered biomarkers of resistance to radiotherapy in brain metastasis and developed an innovative platform to test new therapies for this disease *ex vivo*. Such a tool should facilitate the discovery of new treatments for brain tumours and has raised significant attention both by the scientific community and the pharmaceutical industry dedicated to oncology. On a related theme, the Group led by Nabil Djouder identified the cellular interactions that modulate the regeneration of the intestinal epithelia following radiation. In addition, Nabil’s team generated a novel mouse model of liver cirrhosis, which is a nice new tool for preclinical research on this lethal disease. Francisco Real’s team provided further insights into the role of GATA transcription factors that contribute to the changes in cell fate associated with pancreatic cancer, and made additional discoveries that help us to understand the still mysterious role of SA2 mutations in cancer onset. The Group led by Marcos Malumbres further developed our understanding about how mitotic kinases control asymmetric cell division in neural stem cells, which is at the basis of certain hereditary microcephaly disorders. Mariano Barbacid’s Group keeps making progress towards the development of inhibitors of the RAF1 kinase, which they previously identified as an actionable target in pancreatic cancer and, during 2022, they reported the atomic structure of RAF1. The Group led by Maria A. Blasco identified the relevant cell type that contributes to lung fibrosis, which is an important step towards developing targeted therapies against this age-related disease. Finally, in my own Group, we identified a novel mechanism that leads to multi-drug

“During 2022, MOP scientists kept making significant discoveries that help us to understand the molecular bases of cancer and other age-related diseases, and provided new ideas for their treatment.”

resistance in cancer cells, and some initial strategies for how this could be overcome. These examples provide a necessarily incomplete collage of our activities, yet they help to certify that our scientists keep an excellent level of scientific productivity.

I must end by saying that publications are just one of the outcomes of our activities. Our Group Leaders are often a reference in their fields of research and participate in many activities that contribute to raising awareness of the relevance of cancer research. Of note, this is not only done by principal investigators, as our technicians, students, postdocs and staff scientists are also very active in this regard. In addition, I am happy to see that scientists at the MOP are progressively increasing their interactions with clinicians and pharmaceutical environments, with the goal of contributing with their knowledge and discoveries to the development of new therapies, or improving the efficacy of existing ones. Finally, I am also personally glad to observe a progressive trend among our scientists to make their discoveries open as soon as possible in public repositories. I myself am convinced that, while publishing our research in journals of high visibility helps us reach a wider audience, it is also important that our most exciting discoveries are shared with the broader scientific community without extensive delays. If we want a different future for how science is reported, we should contribute to it. ■

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, Buyun Ma, Arpita
Saha (since May), Sarita Saraswati

Graduate Students
José Carlos González, Óscar Laguna,
Jessica Louzame, Amparo Sánchez,
Raúl Sánchez

Technicians
Ana Guío (until Oct.) (TS)*, David

Hernández (since Nov.) (TS)*, Rosa
M. Serrano

**Titulado Superior* (Advanced Degree)

Visiting Students
Sarah Adetchessi (May -July) (PhD
Student, Univ. of Paris, France),
Stavroula Boukoura (Oct.-Dec.) (PhD
Student, Danish Cancer Society
Research Center, Denmark), Robson
Diego Calixto (Aug.-Dec.) (*FAPESP*)

Internship, Univ. of São Paulo, Brazil),
Ana Carolina Cintra (since Nov.)
(Erasmus Fellowship, Tras-os-Montes
e Alto Douro Univ., Portugal),
Mariana Deli (June-Dec.) (Erasmus +
Fellowship, Univ. of Ioannina,
Greece), Julie Klein (June-Aug.) (PhD
Student, *École de Biologie
Industrielle*, France), Neetij Krishnan
(until June) (Fulbright Commission
Fellowship, USA), Cristina Pastor

(Sep.-Dec.) (Traineeship, *Univ.
Autónoma de Madrid*, Spain)

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. The enzyme telomerase is present in more than 95% of all types of human cancers and is absent in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes, essential for chromosome protection and genomic stability. Progressive shortening of telomeres associated with organism ageing leads to ageing. When telomeres are altered, adult stem cells have a maimed regenerative capacity.

Our research focuses on:

- Generating 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 or TERRA.
- Testing telomerase gene therapy in *telomere syndromes* and age-related diseases.
- Role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

“Our potential preclinical mouse model *ki-Pot1a^{R117C}* for Li-Fraumeni-Like syndrome presenting with high angiosarcoma incidence could be a very useful tool in the therapeutics of these tumours.”

RESEARCH HIGHLIGHTS

BRAF^{V600E} in adult mouse models elicits early differential responses

The BRAF gene, which encodes a master kinase of the RAS-pathway, is frequently mutated in human cancers. The most common genetic mutation is a single nucleotide transition that gives rise to a constitutively active BRAF kinase (BRAF^{V600E}), which in turn sustains continuous cell proliferation. The study of BRAF^{V600E} murine models has so far focused mainly on the role played by BRAF^{V600E} in tumour development, so much so that little was known about the early molecular impact of the *in vivo* expression of BRAF^{V600E}. We have now provided the first *in vivo* evidence that acute BRAF^{V600E} expression elicits instant DNA damage in an organ-specific fashion. The senescence marker p21CIP1, which may be activated by p53 upon genotoxic insults and by oncogene activation via pRb/E2F, promotes cell cycle arrest and senescence by inhibiting CDKs. Despite BRAF^{V600E} inducing both DNA damage and p21CIP1 activation *in vitro*, as well as in senescent lung adenomas, we did not find any differences in p21CIP1 levels either in liver or spleen upon BRAF^{V600E} expression. BRAF^{V600E} in lungs provokes an acute inflammatory state with tissue-specific recruitment of neutrophils to alveolar parenchyma and of macrophages to bronchi/bronchioles, as well as bronchial/bronchiolar epithelium transdifferentiation and development of adenomas.

A mouse model for Li-Fraumeni-Like syndrome with cardiac angiosarcomas associated to POT1 mutations

Although the telomeric protein POT1 is mutated in many different familial and sporadic cancers, so far there have been no mouse models to understand the pathobiology of these mutations. We have generated a mouse model for the human *POT1^{R117C}* mutation found in Li-Fraumeni-Like (LFL) families with cases of cardiac angiosarcoma (CAS) by means of introducing this mutation in the *Pot1a* endogenous locus, *knock-in* for *Pot1a^{R117C}*, thus generating *Pot1a^{ki}* mice. While

homozygous *Pot1a^{ki/ki}* are embryonic lethal, heterozygous *Pot1a^{+/-ki}* mice are viable. We also found that both mouse embryonic fibroblasts (MEFs) and tissues from *Pot1a^{+/-ki}* mice harbour longer telomeres than wild-type controls. Like human LFL patients, heterozygous *Pot1a^{+/-ki}* mice spontaneously develop a high incidence of angiosarcomas (FIGURE 1), including CAS, and this is associated with the presence of abnormally long telomeres in endothelial cells as well as in the tumours. The *Pot1a^{+/-R117C}* mouse model therefore constitutes a useful tool to understand human cancers initiated by POT1 mutations.

Impact of telomere dysfunction in fibroblasts, Club and basal cells on the development of lung fibrosis

Telomeric protein TRF1 is an essential component of the telomeric protective complex that prevents telomeric DNA damage, chromosome end-to-end fusions and telomere fragility. We previously showed that induction of telomere dysfunction in alveolar type II (ATII) cells is sufficient to induce progressive and lethal pulmonary fibrosis in mice. The pathological consequences of telomere dysfunction in lung fibroblasts, Club and basal cells remained to be investigated. We have now conditionally deleted *Trf1* in the former mouse tissues. We found that while TRF1 deficiency in fibroblasts does not lead to significant lung pathologies, *Trf1* deletion in Club and basal cells from male mice led to lung inflammation and airway remodelling. While dysfunctional telomeres in ATII cells led to alveolar DNA damage, senescence and apoptosis, as well as to interstitial lung fibrosis, their presence in Club and basal cells increased the presence of neutrophils, lymphocytes and macrophages in the lung, as well as airway collagen deposition and fibroblast abundance, features not observed in female mice upon telomere dysfunction. Depletion of TRF1 in fibroblasts, Club and basal cells did not lead to interstitial fibrosis, underscoring ATII cells as the relevant cell type for the origin of interstitial fibrosis (FIGURE 2). ■

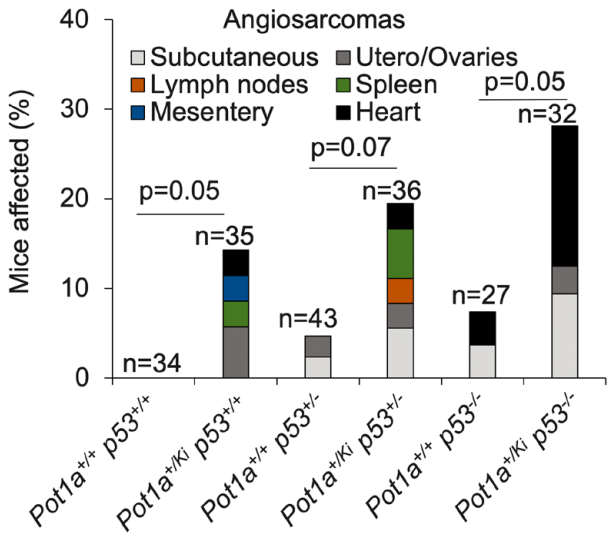


FIGURE 1 Higher incidence of angiosarcomas in *Pot1a^{+/-ki}* mice.

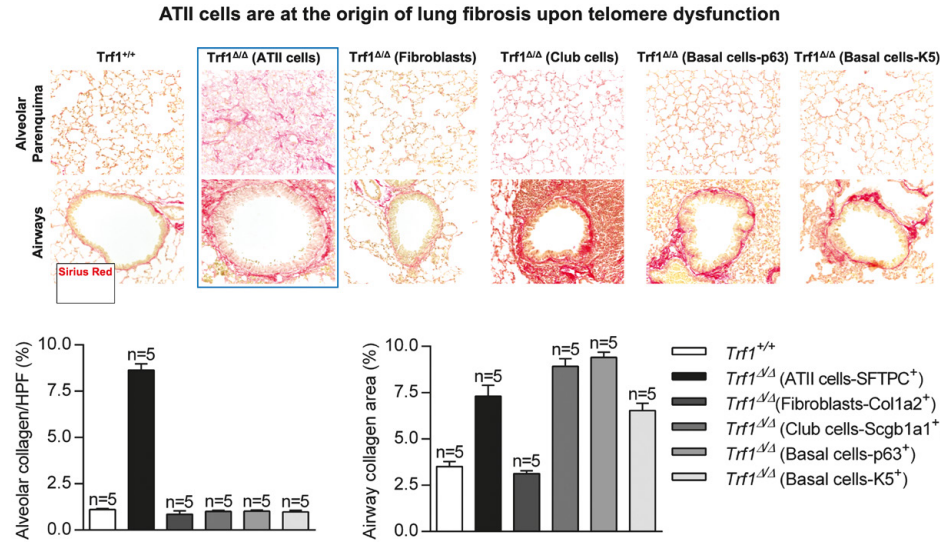


FIGURE 2 Pathological consequences of telomere dysfunction in fibroblasts, Club and basal cells in the lung. Dysfunctional telomeres in ATII cells led to alveolar DNA damage, senescence and apoptosis, and to interstitial lung fibrosis. TRF1 deficiency in Club and basal cells induced telomeric damage and cell cycle arrest, and reduced proliferation. TRF1 deletion in fibroblasts increased telomeric damage, cell cycle arrest, apoptosis, and proliferation. Depletion of TRF1 in fibroblasts, Club and basal cells did not lead to interstitial lung fibrosis.

► PUBLICATIONS

► Piñeiro-Hermida S, Martínez P, Bosso G, Flores JM, Saraswati S, Connor J, Lemaire R, Blasco MA (2022). Consequences of telomere dysfunction in fibroblasts, club and basal cells for lung fibrosis development. *Nat Commun* 13, 5656.

► Bosso G, Lanuza-Gracia P, Piñeiro-Hermida S, Yilmaz M, Serrano R, Blasco MA (2022). Early differential responses elic-

ited by BRAF^{V600E} in adult mouse models. *Cell Death Dis* 13, 142.

► Martínez P, Sánchez-Vázquez R, Ferrara-Romeo I, Serrano R, Flores JM, Blasco MA (2022). A mouse model for Li-Fraumeni-Like Syndrome with cardiac angiosarcomas associated to POT1 mutations. *PLoS Genet* 18, e1010260.

► Montero-Conde C, Leandro-García LJ, Martínez-Montes ÁM, Martínez P, Moya FJ, Letón R, Gil E, Martínez-Puente N, Guadali-

S, Currás-Freixes M, García-Tobar L, Zafon C, Jordà M, Riesco-Eizaguirre G, González-García P, Monteagudo M, Torres-Pérez R, Mancikova V, Ruiz-Llorente S, Pérez-Martínez M, Pita G, Galofré JC, Gonzalez-Neira A, Cascón A, Rodríguez-Antona C, Megías D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.

► Ayora M, Fraguas D, Abregú-Crespo R, Recio S, Blasco MA, Moises A, Derevyanko A, Arango C, Díaz-Caneja CM (2022). Leukocyte telomere length in patients with schizophrenia and related disorders: a meta-analysis of case-control studies. *Mol Psychiatry* 27, 2968-2975.

► Sanz-Ros J, Romero-García N, Mas-Bar-gues C, Monleón D, Gordevicius J, Brooke RT, Dromant M, Díaz A, Derevyanko A, Guío-Carrión A, Román-Domínguez A,

Inglés M, Blasco MA, Horvath S, Viña J, Borrás C (2022). Small extracellular vesicles from young adipose-derived stem cells prevent frailty, improve health span, and decrease epigenetic age in old mice. *Sci Adv* 8, eabq2226.

► PATENTS

► Blasco MA, Saraswati S, Martínez P (2022). Telomerase reverse transcriptase therapy for kidney fibrosis and non-human animals thereof. *PCT/EP2022/051505*.

► Blasco MA, Martínez P, Bosch MF, Jiménez V, García M, Casana E. Recombinant TERT-encoding viral genomes and vectors. PCT application (2022). *PCT/EP2022/062990*. *WO2022238557A1*.

► AWARDS AND RECOGNITION

► *Fundación Eugenio Rodríguez Pascual* 2022 Award, Madrid, Spain.

► *Averroes de Oro Ciudad de Córdoba* 2022 Award in Scientific Research, Córdoba, Spain.

► Full Member (*Académica de número*) of the Royal Spanish Academy of Pharmacy.

► Doctorate *Honoris Causa*, *Universidad Internacional de Valencia*, Valencia, Spain.

► Honorary Academician of *Academia Malagueña de Ciencias*, Málaga, Spain.

► Member of the Board of Trustees of the International Centre for Ageing Research (ICAR) Foundation, Valencia, Spain.

► President of the Scientific Advisory Board of the International Centre for Ageing Research (ICAR), Valencia, Spain.

► Member of the Advisory Board of the Spanish Foundation of Science and Technology (FECYT), Madrid, Spain.

EXPERIMENTAL ONCOLOGY GROUP

Mariano Barbacid
Group Leader

Research Scientists
Carmen Guerra, Sara García-Alonso

Post-Doctoral Fellows
Federico Virga (January-October)

Graduate Students
Gonzalo M. Aizpurua, Sara Barrambana, Oksana Brehey, Laura



de La Puente, Ana María Fernández, Fernando Fernández, Jing Li (until August), Vasiliki Liaki, Lucía Morales, Marina Salmón (until November), Pian Sun, Elena Zamorano (since December; CIBERONC, Madrid)

Bioinformatician
Ruth Álvarez (until November)

Administrative Assistant
Patricia T. Guerra

Undergraduate Students
Lucía Baselga (since Oct.) (Undergraduate trainee, Univ. *Politécnica de Madrid*, Spain), Irene Herruzo (until June) (BS Thesis, *UAM*, Madrid, Spain), Mirto Kostopoulou (since Nov.) (Erasmus + Fellowship, National and Kapodistrian University

of Athens, Greece), Lucía Lomba (since Oct.) (Master's Thesis, *UCM*, Madrid, Spain), Sonsoles Liria (Jan.-Apr.) (Master's Thesis, *Univ. Francisco de Vitoria*, Madrid, Spain), Laura Martínez (Mar.-June) (BS Thesis, *UCM*, Madrid, Spain), Melissa Minic (since Sept.) (Erasmus + Fellowship, *FH Campus Wien*, Austria), Blanca Rosas (since Sept.) (BS Thesis, *UCM*, Madrid, Spain)

External Associates
Alfredo Carrato (*Hosp. Ramón y Cajal*, Madrid, Spain), Matthias Drosten (*Centro de Investigación del Cáncer*, Salamanca, Spain), Monica Musteanu (*UCM*, Madrid, Spain), Bruno Sainz (*UAM*, Madrid, Spain), Juan Velasco (*Eli Lilly*, Alcobendas, Spain)

OVERVIEW

The main area of interest of our Group is to identify therapeutic strategies against KRAS mutant lung and pancreatic tumours. For almost 4 decades, KRAS oncoproteins were thought to be undruggable targets. However, selective KRAS inhibitors, at least against one of the KRAS oncogenic isoforms, KRAS^{G12C}, have been recently approved by the FDA. Yet patients develop drug resistance rather quickly indicating that successful treatment of KRAS mutant tumours will require combination with inhibitors of KRAS signalling pathways, such as the MAP kinase and the PI3 kinase pathways. Unfortunately, all inhibitors tested thus far in the clinic have failed due to excessive toxicities. A potential exception is RAF1. Ablation of this kinase induced significant levels of tumour regression with limited toxicities in experimental models. Ironically, the tumour-inducing effect of RAF1 is not mediated by its kinase activity. Hence, pharmacological targeting of RAF1 will require the use of strategies capable of degrading the protein. To identify such compounds, we have determined the tertiary structure of the full RAF1 protein using Cryo-Electron Microscopy (Cryo-EM) technologies. These results have identified structural vulnerabilities that will make it possible to design selective RAF1 degraders.

“The tertiary structure of RAF1, bound to the Hsp90 and Cdc37 chaperones, has revealed structural vulnerabilities that will make it possible to generate pharmacologically active RAF1 degraders capable of inhibiting KRAS mutant lung tumours.”

RESEARCH HIGHLIGHTS

KSR induces RAS-independent MAPK pathway activation and modulates the efficacy of KRAS inhibitors

KSR1/2 have long been considered scaffolding proteins required for optimal MAPK pathway signalling. However, recent evidence suggests that they play a more complex role within this pathway. We have demonstrated that ectopic expression of KSR1 or KSR2 is sufficient to activate the MAPK pathway and to induce cell proliferation in the absence of RAS proteins. In contrast, ectopic expression of KSR proteins is not sufficient to induce cell proliferation in the absence of either RAF or MEK proteins, indicating that they act upstream of RAF. Indeed, KSR1 requires dimerization with at least 1 member of the RAF family to stimulate proliferation, an event that results in the translocation of the heterodimerized RAF protein to the cell membrane. Mutations in the conserved DFG motif of KSR1 that affect ATP binding impair induction of cell proliferation. We have also shown that increased expression levels of KSR1 decrease the responsiveness to the KRAS^{G12C} inhibitor sotorasib in human cancer cell lines. These results suggest that high KSR1 or KSR2 expression levels in tumours could render strategies aimed at inhibiting RAS largely ineffective. Indeed, we further show that KRAS^{G12C} inhibitors are less effective when KSR1 expression levels are elevated. In conclusion, our data should raise awareness that KSR1 or KSR2 expression levels are direct modulators of the effectiveness of RAS inhibition.

Structure of the RAF1 kinase bound to the HSP90 and CDC37 chaperones: identification of selective RAF1 degrons

We have described the structure of the full-length RAF1 protein in complex with HSP90 and CDC37 obtained by Cryo-Electron Microscopy (FIGURE 1A and B). The reconstruction reveals a RAF1 kinase with an unfolded N-lobe separated from its C-lobe. The hydrophobic core of the N-lobe is trapped in the HSP90 dimer, while CDC37 wraps around the chaperone and interacts with the N- and C-lobes of the kinase. The structure indicates how CDC37 can discriminate between the different members of the RAF family. Our structural analysis also reveals that the folded RAF1 assembles with 14-3-3 dimers, suggesting that after folding follows a similar activation as B-RAF. Finally, disruption of the interaction between CDC37 and the DFG segment of RAF1 unveils potential vulnerabilities to attempt the pharmacological degradation of RAF1 for therapeutic purposes (FIGURE 1C).

Despite the well-conserved sequence amongst members of the RAF family, they contain substantial functional differences.

Whereas RAF1 and A-RAF are client proteins of the HSP90-CDC37 chaperone system, B-RAF is not. Therefore, the HSP90-CDC37 chaperone system adds an extra regulatory layer to this kinase family. The structure of the complex highlights the key interactions of the HSP90 chaperone and its cochaperone CDC37 with RAF1. Moreover, our combined biochemical and functional analysis of the interacting regions indicates that CDC37 can recognise segments of RAF1 that are different from their counterparts in B-RAF.

We propose a model in which RAF1 would be unstable until it becomes associated with CDC37, followed by binding to HSP90. The HSP90-CDC37 chaperone system couples the folding of the client protein with ATP hydrolysis cycles (FIGURE 1B). RAF1 is phosphorylated in residues S259 and S621, thereby, once the HSP90-CDC37 renders this protein folded, the complex is disrupted and RAF1 associates with 14-3-3 in a manner similar to B-RAF. We speculate that the interaction of RAF1 with the HSP90-CDC37 system could control the dynamics of RAF1 heterodimers formed with the 14-3-3 proteins, thus influencing the levels of homo or heterodimers of this signalling module, and thereby controlling cellular proliferation.

Our mutagenesis analysis of the interface between CDC37 and RAF1 highlights the importance of this association for RAF1 stability. Indeed, we observed a reduction in the levels of RAF1 when the mutant isoforms were co-expressed with HSP90 and CDC37 (FIGURE 1D and E). These observations raise the possibility that the interface between RAF1 and CDC37 may represent a vulnerable spot, which could be targeted to induce the degradation of RAF1, reproducing the therapeutic results obtained in experimental models of *Kras*/*Trp53*-induced lung tumours upon ablation of RAF1 expression. ■

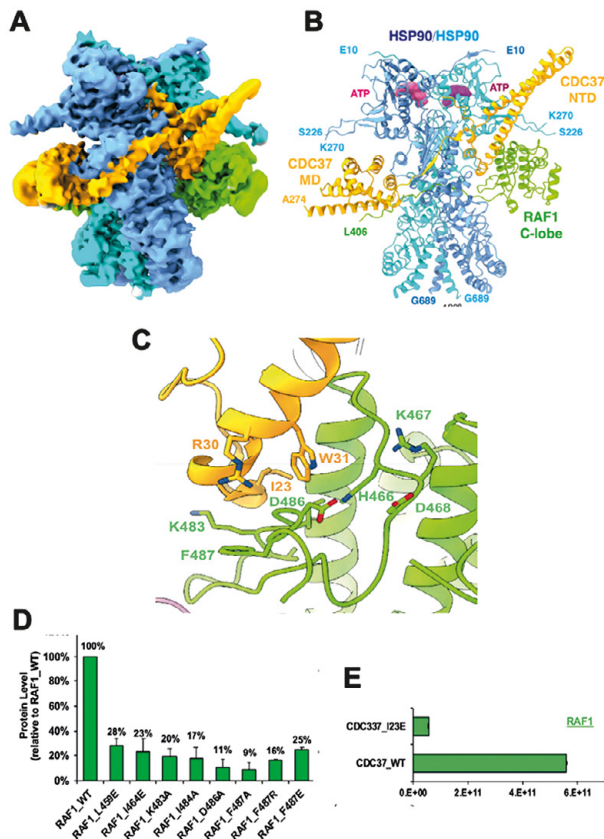


FIGURE 1 Cryo-EM structure of the RAF1-HSP90-CDC37 (RHC) complex. (A) Cryo-EM maps of the RAF1-HSP90-CDC37 complex at 3.67 Å global resolution. Blue and cyan for each monomer of the HSP90 dimer, yellow for CDC37 and green for RAF1. (B) Overview of the RAF1-HSP90-CDC37 assembly in complex with ATP. (C) Detailed view of the main interacting region between CDC37 and RAF1. (D) Levels of wild type and mutant RAF1 proteins co-expressed with HSP90 and CDC37. The levels of each protein were quantified by mass spectrometry, using RAF1 common peptides for all the mutants. (E) Mean levels of RAF1 bound to strep-tagged wild type and mutant CDC37 I23E proteins.

PUBLICATIONS

Drosten M, Barbacid M (2022). KRAS inhibitors: going non-covalent. *Mol Oncol* 16, 3911-3915.

García-Alonso S, Mesa P, Ovejero LP, Aizpurua G, Lechuga CG, Zarzuela E, Santiveri CM, Sanclemente M, Muñoz J, Musteanu M, Campos-Olivas R, Martínez-Torrecuadrada J, Barbacid M*, Montoya G* (2022). Structure of the RAF1-HSP90-CDC37 complex reveals the basis of RAF1 regulation. *Mol Cell* 82, 3438-3452. *Co-senior authors.

Paniagua G, Jacob HKC, Brehey O, García-Alonso S, Lechuga CG, Pons T, Musteanu M, Guerra C, Drosten M, Barbacid M (2022). KSR induces RAS-independent MAPK pathway activation and modulates the efficacy of KRAS inhibitors. *Mol Oncol* 16, 3066-3081.

Drosten M, Barbacid M (2022). Targeting KRAS mutant lung cancer: light at the end of the tunnel. *Mol Oncol* 16, 1057-1071.

Martínez-Bosch N, Cristóbal H, Iglesias M, Gironella M, Barranco L, Visa L, Calafato D, Jiménez-Parrado S, Earl J, Carrato A, Manero-Rupérez N, Moreno M, Morales A, Guerra C, Navarro P, García de Frutos P (2022). Soluble AXL is a novel blood marker for early detection of pancreatic ductal adenocarcinoma and differential diagnosis from chronic pancreatitis. *EBioMedicine*, 75, 103797.

Lazcanoiturburu N, García-Sáez J, González-Corrales C, Roncero C, Sanz J, Martín-Rodríguez C, Valdecantos MP, Martínez-Palacián A, Almalé L, Bragado P, Calero-Pérez S, Fernández A, García-Bravo M, Guerra C, Montoliu L, Segovia JC, Valverde ÁM, Fabregat I, Herrera B, Sánchez A (2022). Lack of EGFR catalytic activity in hepatocytes improves liver regeneration following DDC-induced cholestatic injury by promoting a pro-restorative inflammatory response. *J Pathology* 258, 312-324.

Diego-González L, Fernández-Carrera A, Igea A, Martínez-Pérez A, Real Oliveira MECD, Gomes AC, Guerra C, Barbacid M, González-Fernández Á, Simón-Vázquez R (2022). Combined inhibition of FOSL-1 and YAP using siRNA-lipoplexes reduces the growth of pancreatic tumors. *Cancers (Basel)* 14, 3102-3123.

Casadevall D, Hernández-Prat A, García-Alonso S, Arpi-Llucià O, Menéndez S, Qin M, Guardia C, Moracho B, Sánchez-Martin FJ, Zazo S, Gavilán E, Sabbaghi MA, Eroles P, Cejalvo JM, Lluch A, Rojo F, Pandiella A, Rovira A, Albanell J (2022). mTOR inhibition and T-DM1 in HER2-positive breast cancer. *Mol Cancer Res* 20, 1108-1121.

París-Muñoz A, Aizpurua G, Barber DF (2022). Helios expression is downregulated on CD8+ Treg in two mouse models of lupus during disease progression (2022). *Front Immunol* 13, 922958.

AWARDS AND RECOGNITION

- M. Barbacid:
- “Santiago Ramón y Cajal” National Research Prize 2022, Spain.
 - Honorary Doctorate (“Doctor Honoris Causa”) from the *Universidad Nacional de Educación a Distancia*, Madrid, Spain.
 - “Premio a la Excelencia en la Trayectoria Científica” AstraZeneca Foundation Lifetime Achievement in Science Award, Spain.
 - Member of the *Universidad Internacional Menéndez Pelayo*’s Advisory Board, Madrid, Spain.

Publications at other institutions

- París-Muñoz A, Aizpurua G, Barber DF (2022). Helios expression is downregulated on CD8+ Treg in two mouse models

CELL DIVISION AND CANCER GROUP

Marcos Malumbres
Group Leader

Clinical Investigator
Rodrigo Sánchez

Post-Doctoral Fellows
Begoña Hurtado, Miguel Ruiz, Diana
Vara, Carolina Villarroja



Graduate Students
Gloria Cristina Bonel, Mariona
Cubells (PEJ, CAM)*, Alejandro
García, José González, Fátima
Guerra, Luis Rodrigo López, Enrique
Nogueira (since October), Borja
Pitarch, Agustín Sánchez

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

Technicians
Cristina Aguirre (TS)*, Irene Díaz,
Sandra Díez (since November), Aïcha
El Bakkali

**Titulado Superior (Advanced Degree)*

Students in Practice
Iciar Luna (until June) (UCM, Madrid,
Spain), Sara Mozas (Oct.-Dec.) (Univ.
Francisco de Vitoria, Madrid, Spain),
María Fernanda Pérez (until June)
(UAM, Madrid, Spain), Martina Svedin

Figo (July-Sep.) (University of
Manchester, UK)

Visiting Scientists
Mónica Álvarez (Instituto de
Investigación Sanitaria del Principado
de Asturias, Ovideo, Spain), Senn
Wakahashi (Kobe University, Kobe,
Japan)

OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated in mammalian cells. Our scientific interests are to: i) understand the basic control mechanisms that regulate the cell division cycle; ii) characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) understand self-renewal and pluripotency in stem cell biology and tumour development; and iv) improve the use of old and new targets for cancer therapy. As a final goal, we aim to generate information that will be useful for understanding basic mechanisms of cell function and to improve therapeutic strategies against cancer cell proliferation.

“We have defined the role of mitotic kinases in neural and metabolic disease, and the immune response to chromosomal instability in patients with high tumour susceptibility.”

RESEARCH HIGHLIGHTS

Mitotic kinases in developmental diseases

The cell cycle machinery regulates multiple aspects of cell biology, including the balance between proliferation and differentiation in multiple tissues. Several cell cycle kinases, such as polo-like kinase 1 (PLK1), modulate not only centrosome and chromosome biology but also other cellular processes such as the dynamics of the cytoskeleton, cell movements, etc. Our previous work showed critical implications of PLK1 in vascular biology and tumour development. By using gain- and loss-of-function mouse models of PLK1 function, we recently identified a new role for PLK1 in the control of cell fate in neural progenitors during development. Interestingly, centrosomal alterations are thought to be one of the aetiological reasons for primary microcephaly, a defect in which decreased cortex size is accompanied by mental retardation and other symptoms. By combining *Plk1*-mutant alleles with specific mutations in *Cep135* or *Cdk5rap2*, two genes mutated in microcephaly, we described new genetic interactions that lead to defective asymmetry of centrosomal components during the division of neural progenitors, microcephaly, and defective brain development (González-Martínez et al., 2022). Importantly, these phenotypes are also observed after inhibiting PLK1 with small-molecule inhibitors that are currently under evaluation in clinical trials for cancer therapy, raising a note of caution on the possible secondary effects of inhibiting PLK1 in neural progenitor cells.

Links between cell cycle regulation and metabolism

The serine/threonine kinase MASTL (also known as Greatwall) is a critical regulator of mitosis by inhibiting the PP2A phosphatase (2012). We previously reported that loss of MASTL results in mitotic defects such as defective chromosome condensation and segregation errors in mammalian cells. By using a variety of genetic and biochemical models, we have recently reported a mitotic-independent function of the MASTL-PP2A axis in modulating the response to glucose. In conditions of nutrient excess and high mTOR signalling, a negative feedback loop inhibits AKT activity, thus limiting the continuous activation of the AKT-mTOR pathway. In these conditions, MASTL is activated by phosphorylation and inhibits PP2A activity, thereby preventing the function of this phosphatase in allowing the continuous activation of AKT. These observations identify a new layer of control that interconnects a cell cycle module with the negative feedbacks regulating the AKT-mTOR pathway, and suggest the possible use of MASTL inhibition to specifically improve glucose metabolism in specific pathological conditions such as obesity or diabetes.

Chromosomal instability and cancer

Most human tumours display an abnormal number of chromosomes. A few mutations affecting mitotic regulators

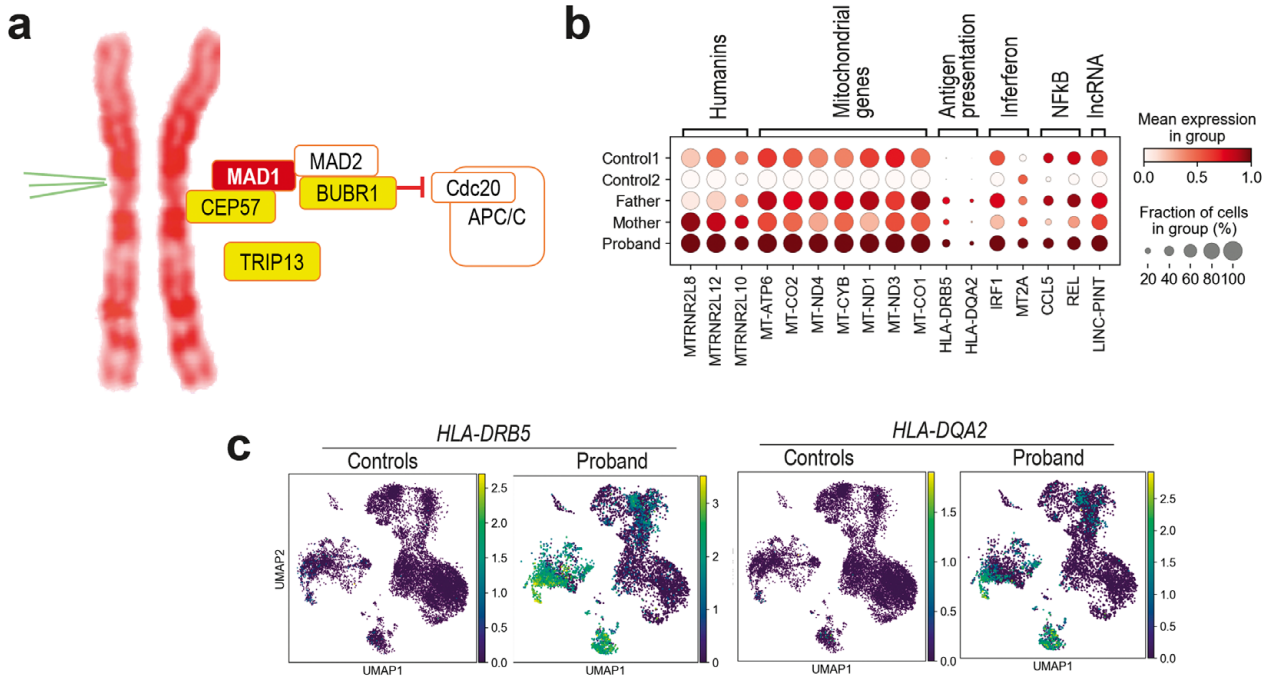


FIGURE 2 MAD1 mutations in a patient with high tumour susceptibility. (a) Critical components of the mitotic checkpoint, with genes previously identified in MVA in yellow. (b) Enrichment in mitochondrial and immune responses in peripheral blood cells from the proband. (c) Single-cell analysis showing upregulation of genes involved in the major histocompatibility complex.

are also detected in familial cancer (Villarroya-Beltri & Malumbres, 2022). In collaboration with the laboratories of Miguel Urioste and Sandra Rodríguez-Perales, we studied the effect of novel mutations in the mitotic checkpoint component MAD1 in a patient with unprecedented levels of tumour susceptibility. Our single-cell data in peripheral blood of the patient suggest that chromosomal instability induced by MAD1 mutations results in an immune response characterised by chronic activation of inflammatory signals (Villarroya-

Beltri *et al.*, 2022). These data suggest a new variant of the Mosaic Variegated Aneuploidy (MVA) syndrome with high tumour susceptibility, and an immune response whose detailed analysis may lead to novel strategies for immunotherapy. ■

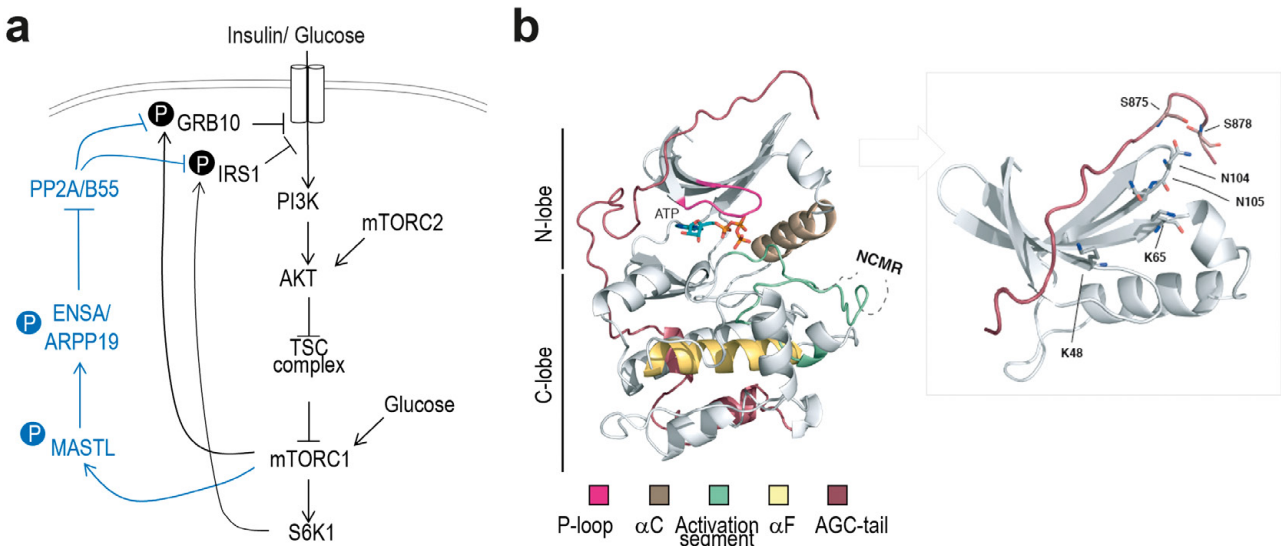


FIGURE 1 A new role for MASTL in the mTOR-AKT feedback loop. (a) A model for the new role (in blue) of MASTL in the negative feedback loop that controls AKT activity. (b) A homology structural model showing the position of the MASTL S875 residue phosphorylated by mTOR in response to high glucose signalling.

PUBLICATIONS

González-Martínez J, Cwetsch AW, Gilabert-Juan J, Gómez J, Garaulet G, Schneider P, de Cárcer G, Mulero F, Caleiras E, Megías D, Porlan E, Malumbres M (2022). Genetic interaction between PLK1 and downstream MCPH proteins in the control of centrosome asymmetry and cell fate during neural progenitor division. *Cell Death Differ* 29, 1474-1485.

Mouron S, Bueno MJ, Lluch A, Manso L, Calvo I, Cortes J, Garcia-Saenz JA, Gil-Gil M, Martínez-Janez N, Apala JV, Caleiras E, Ximénez-Embún P, Muñoz J, González-Cortijo L, Murillo R, Sánchez-Bayona R, Cejalvo JM, Gómez-López G, Fusterro-Torre C, Sabroso-Lasa S, Malats N, Martínez M, Moreno A, Megías D, Malumbres M, Colomer R, Quintela-Fandino M (2002). Phosphoproteomic analysis of neoadjuvant breast cancer suggests that increased sensitivity to paclitaxel is driven by CDK4 and filamin A. *Nat Commun* 13, 7529.

Villarroya-Beltri C, Osorio A, Torres-Ruiz R, Gómez-Sánchez D, Trakala M, Sánchez-Belmonte A, Mercadillo F, Hurtado B, Pitarch B, Hernández-Núñez A, Gómez-Caturia A, Rueda D, Perea J, Rodríguez-Perales S, Malumbres M, Urioste M (2022). Bilallelic germline mutations in *MAD1L* induce a novel syndrome of aneuploidy with high tumor susceptibility. *Sci Adv* 8, eabq5914.

Villarroya-Beltri C, Malumbres M (2022).

Mitotic checkpoint imbalances in familial cancer. *Cancer Res* 82, 3432-3434.

Berenguer I, López-Jiménez P, Mena I, Viera A, Page J, González-Martínez J, Maestre C, Malumbres M, Suja JA, Gómez R (2022). Haspin participates in AURKB recruitment to centromeres and contributes to chromosome congression in male mouse meiosis. *J Cell Sci* 135, jcs259546.

Hidalgo M, Garcia-Carbonero R, Lim KH, Messersmith WA, Garrido-Laguna I, Borazanci E, Lowy AM, Medina Rodriguez L, Laheru D, Salvador-Barbero B, Malumbres M, Shields DJ, Grossman JE, Huang X, Tammam M, Martini JF, Yu Y, Kern K, Macarulla T (2022). A preclinical and phase 1b study of palbociclib plus nab-paclitaxel in patients with metastatic adenocarcinoma of the pancreas. *Cancer Res Commun* 2, 1326-1333.

PATENT

Pastor Fernández J, Martínez González S, Blanco-Aparicio C, García García AB, Rodríguez Aristegui S, Gómez de la Oliva CA, Albarrán Santiaño MI, Cebriá Gómez A, Malumbres Martínez M. Imidazo[1,2-b]pyridazine based tricyclic compounds as inhibitors of HASPIN and therapeutic uses thereof. PCT application (2022). PCT/2022/057636. WO2022200433A1.

GENOMIC INSTABILITY GROUP

Óscar Fernández-Capetillo
Group Leader

Research Scientists
Vanesa Lafarga, Matilde Murga

Post-Doctoral Fellow
Ivó Hernández



Graduate Students
Elena Fueyo (until September), Gema López, Jorge Mota, Belén Navarro, Anabel Sáez, Laura Sánchez (until April), Oleksandra Sirozh (until September), Pablo Valledor

Technicians
Marta E. Antón, Alicia González (TS), Sara Rodrigo
**Titulado Superior (Advanced Degree)*

Student in Practice
Mario López (February-July)
(Bachelor's Degree Final Project, *Universidad de Alcalá de Henares, Spain*)

OVERVIEW

The Genomic Instability Group focuses its research on understanding the molecular mechanisms leading to cancer and other age-related diseases, with the ultimate goal of translating this knowledge into novel therapeutic strategies. Our initial investigations centred on Replicative Stress (RS), a type of DNA damage sensed by the ATR kinase, and that is particularly abundant in some cancer cells. Our work in this area led to the discovery of selective ATR inhibitors that were further improved to enable their clinical development as anticancer agents. Next, we became increasingly interested in understanding the mechanisms of drug resistance to specific agents, such as inhibitors of ATR or USP7. More recently, our group has revealed that one of the most frequent mutations in human cancer, inactivation of the tumour suppressor FBXW7, leads to multidrug resistance (MDR). Importantly, we have also discovered strategies to overcome MDR, which is an important area of our current research.

“We have discovered a new mechanism that drives multidrug resistance. In addition, we have shown that the depletion of PD-L1 expressing cells might be a fruitful approach for cancer therapy.”

RESEARCH HIGHLIGHTS

The Integrated Stress Response as a vulnerability of cancer cells

Last year, we reported that *FBXW7* mutations lead to multidrug resistance (MDR), limiting the efficacy of most available antitumor agents. Importantly, *FBXW7* is one of the 10 most frequently mutated genes in cancer due to either inactivating mutations and/or allelic loss. Furthermore, mutations in this gene are among the most significantly associated with poor survival across all human cancers. Interestingly, we discovered that, despite their MDR phenotype, *FBXW7* deficient cells were preferentially sensitive to therapies targeting mitochondria, such as the antibiotic tigecycline. Subsequently, we identified that the toxicity of tigecycline for cancer cells is mediated by the Integrated Stress Response (ISR). In support of this, nuclear accumulation of ATF4, one of the hallmarks of ISR activity, was induced by tigecycline and reverted by the ISR inhibitor ISRIB. Moreover, and by searching for additional compounds that could target *FBXW7* deficient cells, we found another set of seemingly unrelated compounds that did so, all of which activated the ISR (FIGURE 1). Surprisingly, these compounds were already known to have antitumor effects through very different mechanisms of action, such as inhibition of B-RAF or EGFR. This raises the important question as to what extent the anticancer effects of several clinically used drugs might be partly mediated by a previously

unknown effect of these compounds in activating the ISR. We are currently investigating the basis of these observations, as they suggest the exciting possibility that a targeted activation of the ISR might be able to trigger cell death in cancer cells that are otherwise resistant to other chemotherapies.

Targeting PD-L1 expressing cells in cancer therapy

The latest advances in immunotherapy for the treatment of cancer have incredibly improved the prognosis of a wide range of malignancies. Not surprisingly, the discovery of the immune checkpoint mediated by PD-1 and CTLA-4 receptors and of how targeting these pathways can be exploited for cancer therapy was awarded the Nobel Prize in Medicine in 2018. Antibodies targeting the PD-1/PD-L1 interaction are among the most widely used immunotherapy strategies, but, despite the indisputable success of these treatments, only 20-40% of the patients respond, and even fewer show durable responses. We hypothesised that the elimination of PD-L1 expressing cells, which may display additional checkpoint mediators on their membranes, could have broader antitumoral effects than targeting only the PD-1/PD-L1 interaction. To address this, we generated mice carrying an inducible suicidal reporter allele of PD-L1, which allows the isolation and identification of PD-L1-expressing cells, as well as their selective elimination upon treatment with an otherwise inert compound. Our work with these mice has revealed that the depletion of PD-L1 positive cells potentiates immune responses against different stimuli, such as a septic cytokine storm. In the context of cancer, we found that depletion of PD-L1-expressing cells favoured the clearance of tumour cells in a mouse model of peritoneal metastasis and, consequently, prolonged the survival of the animals (FIGURE 2). This work supports the usefulness of targeting PD-L1⁺ cells in cancer therapy, and provides the immunotherapy research community with a useful genetic tool for further investigations of the PD-1/PD-L1 checkpoint. ■

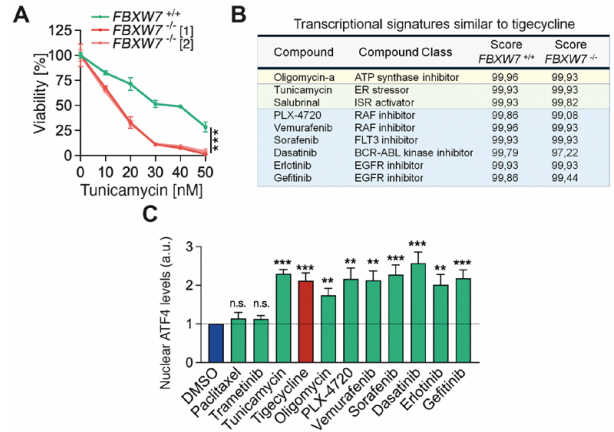
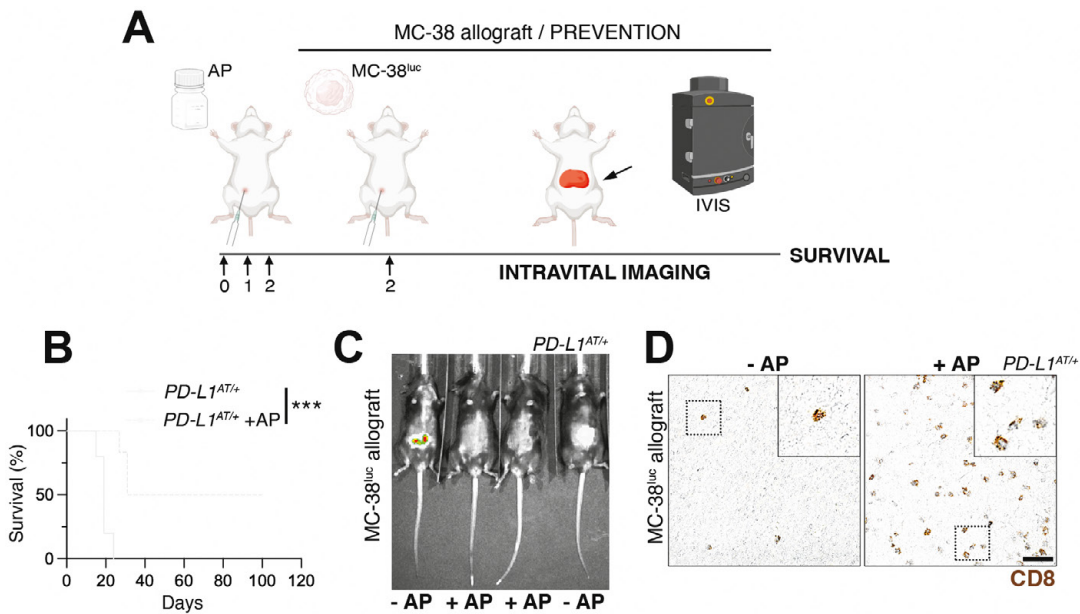


FIGURE 1 Multidrug resistance of *FBXW7*-deficient cells can be overcome by activation of the ISR. (A) Sensitivity of *FBXW7*-deficient DLD-1 to the ISR-activating drug tunicamycin. (B) Drugs that are similar to the antibiotic tigecycline, based on comparison of transcriptional signatures available at the connectivity map (CMAP). Note the presence of several seemingly unrelated tyrosine kinases in this set, all of which trigger a similar transcriptional signature to well established ISR-inducers such as tunicamycin or salubrinal. (C) Nuclear levels of ATF4 as evaluated by High-Content Microscopy in DLD-1 cells. Note that compounds to which *FBXW7*-deficient cells are resistant do not activate the ISR.



PUBLICATIONS

Sanchez-Burgos L, Navarro-González B, García-Martin S, Sirozh O, Mota-Pino J, Fueyo-Marcos E, Tejero H, Antón ME, Murga M, Al-Shahrour F, Fernandez-Capetillo O (2022). Activation of the integrated stress response is a vulnerability for multidrug-resistant *FBXW7*-deficient cells. *EMBO Mol Med* 14, e15855.

Corman A, Sirozh O, Lafarga V, Fernandez-Capetillo O (2022). Targeting the nucleolus as a therapeutic strategy in human disease. *Trends Biochem Sci*. PMID: 36229381.

Hühn D, Martí-Rodrigo P, Mouron S, Hansel C, Tschapalda K, Porebski B, Häggblad M, Lidemalm L, Quintela-Fandino M, Carreras-Puigvert J, Fernandez-Capetillo O (2022). Prolonged estrogen deprivation triggers a broad immunosuppressive phenotype in breast cancer cells. *Mol Oncol* 16, 148-165.

Murga M, Fernandez-Capetillo O (2022). Emerging concepts in drug discovery for cancer therapy. *Mol Oncol* 16, 3757-3760.

Egea J, López-Muñoz F, Fernández-Capetillo O, Reiter RJ, Romero A (2022). Alkylating agent-induced toxicity and melatonin-based therapies. *Front Pharmacol* 13, 873197.

Sanchez-Burgos L, Gómez-López G, Al-Shahrour F, Fernandez-Capetillo O (2022). An in silico analysis identifies drugs potentially modulating the cytokine storm triggered by SARS-CoV-2 infection. *Sci Rep* 12, 1626.

Colicchia V, Häggblad M, Sirozh O, Porebski B, Balan M, Li X, Lidemalm L, Carreras-Puigvert J, Hühn D, Fernandez-Capetillo O (2022). New regulators of the tetracycline-inducible gene expression system identified by chemical and genetic screens. *FEBS Open Bio* 12, 1896-1908.

Galindo-Campos MA, Lutfi N, Bonnin S, Martínez C, Velasco-Hernandez T, García-Hernández V, Martín-Caballero J, Ampurdanés C, Gimeno R, Colomo L, Roué G, Guilbaud G, Dantzer F, Navarro P, Murga M, Fernández-Capetillo O, Bigas A, Menéndez P, Sale JE, Yélamos J (2022). Distinct roles for PARP-1 and PARP-2 in c-Myc-driven B-cell lymphoma in mice. *Blood* 139, 228-239.

Fueyo-Marcos E, Fustero-Torre C, Lopez G, Antón ME, Al-Shahrour F, Fernández-Capetillo O, Murga M (2022). PD-L1

ATTAC mice reveal the potential of targeting PD-L1 expressing cells in cancer therapy. *bioRxiv*. doi: <https://doi.org/10.1101/2022.07.22.501095>.

Book Chapter

Sirozh O, Saez-Mas A, Lafarga V, Fernandez-Capetillo O (2022). Basic concepts and emergent diseases mechanisms of amyotrophic lateral sclerosis. In Bradshaw RA, Hart GW, Stah PD (Eds), *Encyclopedia of Cell Biology Second Edition* (pp. 644-665). Academic Press – Elsevier Inc. <https://doi.org/10.1016/B978-0-12-821618-7.00266-2>.

TOPOLOGY AND DNA BREAKS GROUP

Felipe Cortés Ledesma
Group Leader

Research Scientists
Carlos Gómez (until June), Israel Salguero

Post-Doctoral Fellows
Jonathan Barroso, M. Isabel Espejo (since October), Laura García

Graduate Students
Alicia Avis (PEJ, CAM)*, Alba de Haro



(since December), Ernesto López, Maria del Mar Martínez

Technicians
Marta Muñoz (until August) (TS)**
José Terrón (TS)**

Students in Practice
Francisco J Fernández (June-Aug.) (Summer Traineeship, Univ. Pablo de Olavide, Sevilla, Spain), Sara Kidane (Jan.-May) (Linköping University, Linköping, Sweden), Catalina Sierra (Sept.-Dec.) (Master's Thesis, Univ. Carlos III de Madrid, Spain)

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

**Titulado Superior (Advanced Degree)

Visiting Scientist
Claudia Rodríguez (until Nov.) (Centro de Biología Molecular Severo Ochoa – CSIC, Madrid, Spain)

OVERVIEW

DNA topoisomerases have a dual relationship with the genome. They are essential to solve supercoiling and other topological problems inherent to all DNA transactions, but their intrinsic mechanism of action can result in the formation of DNA breaks, either accidentally during normal cellular metabolism or upon chemotherapy treatment with the so-called topoisomerase poisons. Imbalances in DNA topoisomerase activity can therefore compromise cell survival and genome integrity, entailing serious consequences for human health, such as developmental and degenerative problems and, very importantly, neoplastic transformation processes and their subsequent response to treatment.

We are interested 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.

“We have defined a complete map of the genetic pathways operating in the repair of topoisomerase II-induced DNA breaks, their relationships, and how this affects genome stability and tumorigenesis.”

RESEARCH HIGHLIGHTS

During 2022, we had 2 main areas of interest. The first one is in line with the main research line of the laboratory on the repair of topoisomerase II (TOP2)-induced DNA double-strand breaks (DSBs), while the other one is completely different, and stems from the efforts initiated during the COVID-19 pandemic to develop novel genetic diagnosis methods that could be implemented in a point-of-care setting.

Repair of topoisomerase II-induced DNA breaks

TOP2-induced DSBs are particular DNA lesions in which the ends of the break are blocked by a protein adduct that needs to be removed to allow further repair to take place, and can arise spontaneously or as a consequence of chemotherapeutic regimes including TOP2 poisons. We have used unbiased genetic screening approaches to obtain a comprehensive view of the different factors specifically involved in the repair of these lesions. Our results outline 2 main pathways that operate hierarchically to remove the protein adduct (FIGURE 1). First, cells strongly rely on repair mediated by TDP2, an enzyme that directly removes the adduct without affecting the DNA molecule, thus promoting accurate repair and the maintenance of genome stability. Alternatively, but only if this pathway is overwhelmed or disturbed, cells use nucleolytic activities, such as Artemis or the MRN complex, which eliminate the adduct by trimming off DNA ends, allowing repair, but at the cost of compromising genome integrity. As expected from this model, removal of TDP2 in mouse models leads to increased cancer predisposition. Finally, we found that ATM, a common tumour suppressor and the most relevant kinase controlling the response to DSBs, is important for enforcing the hierarchical preference for the TDP2-mediated pathway, and that ATM deficient tumours specifically accumulate mutations that are compatible with the misrepair of TOP2 breaks. Altogether, these results open new avenues to improve the therapeutic use of TOP2 poisons, demonstrate the spontaneous occurrence of TOP2 lesions *in vivo*, and highlight their oncogenic potential when not appropriately repaired.

Novel nucleic-acid detection method

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 to detect specific nucleic acid sequences based on CRISPR-Cas technology have been recently 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, and 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 the 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 have developed and patented a conceptually novel solution that, instead of amplifying the target nucleic acid, focuses on boosting Cas activation, so the reaction is carried out in a single step at room temperature, providing an ideal setting for point-of-care diagnostics. 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. ■

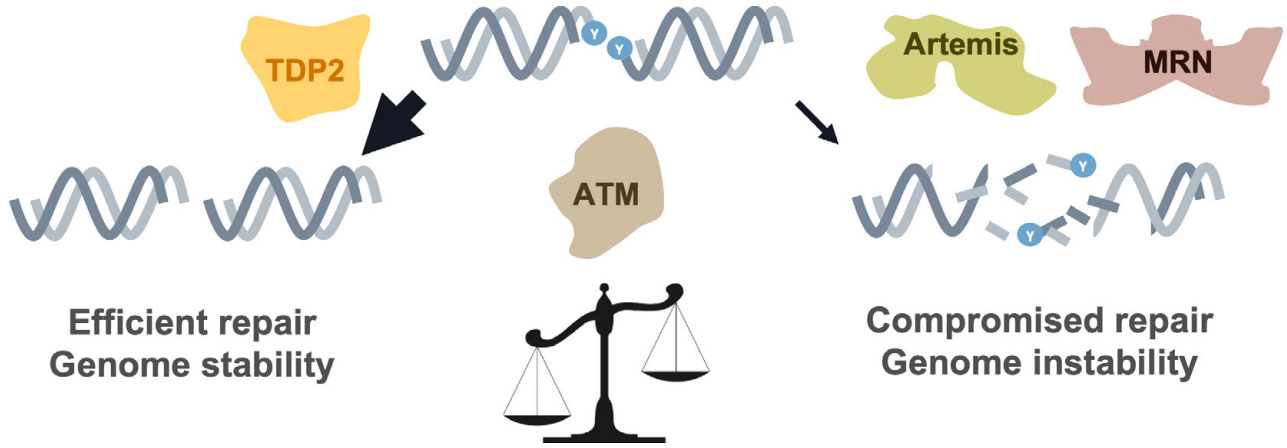


FIGURE 1 Scheme of the different cellular pathways involved in the repair of TOP2-induced DSBs. Protein adducts are removed by either TDP2, which results in accurate repair, or nucleolytic pathways, which compromise genome integrity. ATM establishes a hierarchical preference for the TDP2-dependent pathway.

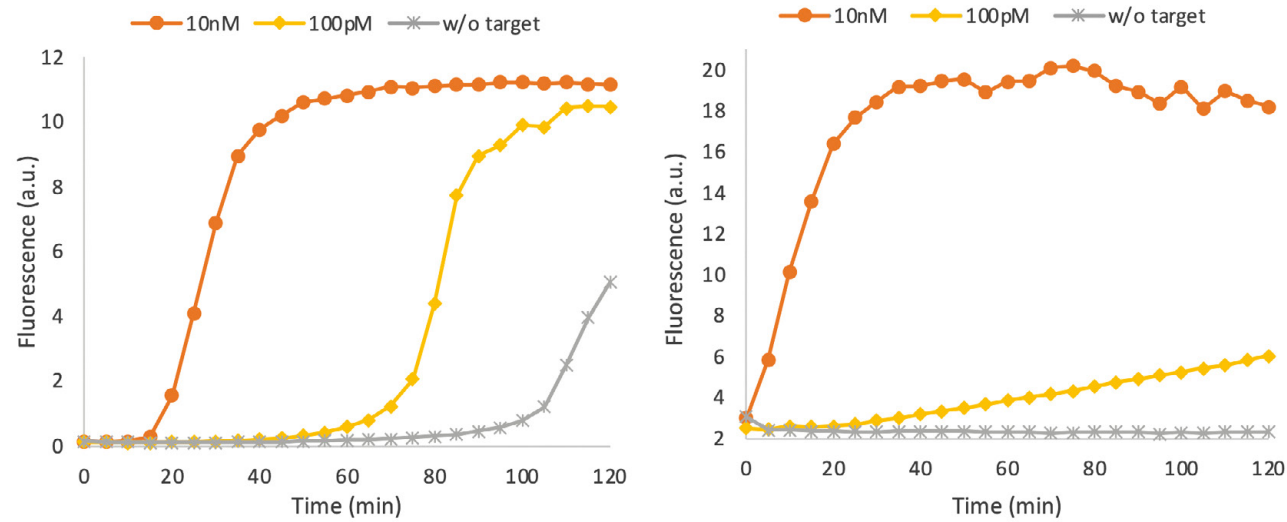


FIGURE 2 CRISPR-Cas12a DNA detection. Our improved method with amplification of Cas activation (left) is compared to direct detection (right).

PUBLICATIONS

Delgado-Chaves FM, Martínez-García PM, Herrero-Ruiz A, Gómez-Vela F, Divina F, Jimeno-González S, Cortés-Ledesma F (2022). Data of transcriptional effects of the merbarone-mediated inhibition of

TOP2. *Data Brief* 44, 108499.
Rodríguez-Cortez VC, Navarrete-Meneses MP, Molina O, Velasco-Hernandez T, Gonzalez J, Romecin P, Gutierrez-Aguera F, Roca-Ho H, Vinyoles M, Kowarz E, Marin P, Rodríguez-Perales S, Gomez-Marín C, Perez-Vera P, Cortes-Ledesma F, Bigas A,

Terron A, Bueno C, Menendez P (2022). The insecticides permethrin and chlorpyrifos show limited genotoxicity and no leukemogenic potential in human and murine hematopoietic stem progenitor cells. *Haematologica* 107, 544-549.

PATENT

Cortés Ledesma F, Gómez Marín C, Muñoz Barrera M, López de Alba E (2022). Nucleic acid detection method. *EP22382608.2*.

CHROMOSOME DYNAMICS GROUP

Ana Losada
Group Leader

Research Scientist
Ana Cuadrado

Post-Doctoral Fellow
María José Andreu



Graduate Students
Dácil del Pilar Alonso, Inmaculada Sanclemente (PEJ, CAM)*, María Solé

Bioinformatician
Daniel Giménez (TS)*

Technicians
Alexandra Mora (since July) (TS)*, Miriam Rodríguez

Visiting Master's Students
Laura Calvo (January-June) (*Universidad Autónoma de Madrid, Spain*), Angela Santos (January-June) (*Universidad de Alcalá de Henares, Spain*)

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

**Titulado Superior (Advanced Degree)*

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. Mutations in cohesin have been found in several tumour types, most prominently in bladder cancer, Ewing sarcoma and acute myeloid leukaemia. Germline mutations in cohesin and its regulatory factors are also at the origin of human developmental syndromes collectively known as cohesinopathies.

Our goal is to understand how cohesin works, how it is regulated, and how its dysfunction contributes to cancer and other human diseases. In particular, we are intrigued by the existence of different versions of the cohesin complex. We use human cells and mouse models carrying *knock out* alleles of genes encoding variant cohesin subunits to investigate their functional specificity.

“We have identified a differential requirement of cohesin-STAG1 and cohesin-STAG2 for NIPBL, a key regulator of cohesin activity and the gene most commonly mutated in cohesinopathy patients.”

RESEARCH HIGHLIGHTS

NIPBL is not required for loading cohesin on chromatin

The spatial organisation of the genome inside the nucleus is critical for transcription, DNA replication and repair. 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). Loop extrusion by cohesin also promotes intermixing of active/inactive chromatin compartments.

There are two versions of the cohesin complex in all somatic vertebrate cells that carry SMC1A, SMC3, RAD21, and either STAG1 or STAG2. Results from our group and others indicate that the two complexes make specific contributions to 3D genome architecture, and further suggest that their different chromatin association dynamics are responsible for these specific functions. In turn, chromatin association is modulated by the interactions of cohesin with its regulators. STAG2 is more often found associated with the unloading factor WAPL, while cohesin acetyltransferase ESCO1 preferentially acts on cohesin-STAG1 at CTCF-bound sites. What it is not known is how the two complexes respond to limited availability of NIPBL.

NIPBL is currently considered the cohesin loader. It activates the cohesin ATPase and is essential for loop extrusion by cohesin *in vitro*. *NIPBL* is an essential gene, and heterozygous mutations have been identified in over 70% of patients with Cornelia de Lange Syndrome (CdLS), the most common developmental syndrome due to cohesin dysfunction. To assess the consequences of NIPBL knock down (KD), we combined a flow cytometry assay that measures chromatin-bound proteins with analyses of genome-wide distribution of cohesin-STAG1 and cohesin-STAG2 by ChIP-seq and of genome contacts by *in situ* Hi-C. Strikingly, we found that cohesin-STAG1 increases on chromatin and further accumulates at CTCF positions after NIPBL knock down, while cohesin-STAG2 diminishes genome-wide. These effects are independent of the presence of the other complex and are epistatic to downregulation of CTCF, ESCO1, or WAPL. Despite the presence of cohesin-STAG1 on chromatin, loop formation is severely impaired. These and additional data support a model in which, contrary to current thinking, NIPBL is not required for association of cohesin with chromatin. However, it is required for loop extrusion, which in turn facilitates stabilisation of cohesin-STAG2 at CTCF positions after being loaded elsewhere (FIGURE 1, right). In contrast, cohesin-STAG1 is loaded and stabilised at CTCF sites even under low

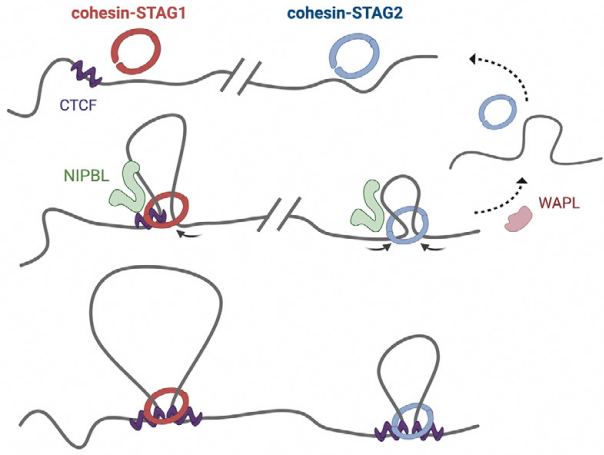


FIGURE 1 Model for the different NIPBL requirement of cohesin-STAG1 and cohesin-STAG2 in the process of DNA loop formation that drives 3D genome organisation. Created with Biorender.com.

NIPBL levels, although in that condition it is unable to form long loops (FIGURE 1, left). 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.

Contribution of STAG2 mutations to 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 alters the gene expression programme of the cell initiating the tumour. It is a highly aggressive cancer with a 5-year survival below 30% in patients that present metastasis. Among the few recurrent mutations identified in EWS, in addition to the oncogenic fusion, are those that inactivate *STAG2*. Importantly, *STAG2* mutations are often present in the most aggressive EWS tumours, suggesting that the loss of cohesin STAG2 may facilitate the acquisition of the aggressive form of EWS.

From the bioinformatic analysis of transcriptomic data from EWS patients and cell lines, we have identified a gene signature dependent on STAG2 loss that correlates with poor survival. We are currently exploring the contribution of these genes to the metastatic phenotype by analysing, both *in vitro* and *in vivo*, the migration and invasion capabilities of EWS cell clones

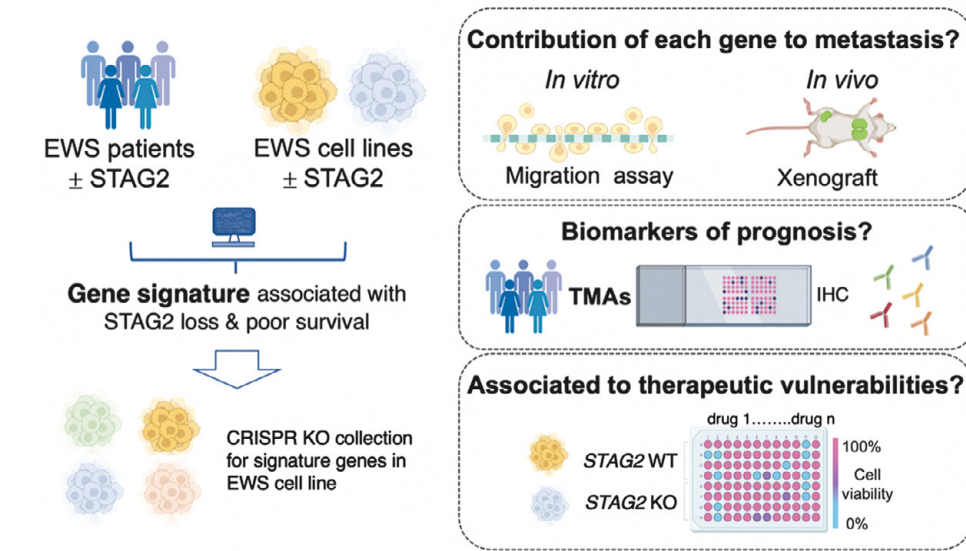


FIGURE 2 Strategy to understand and exploit the contribution of *STAG2* mutations to aggressive Ewing sarcoma.

knocked out for these genes (FIGURE 2). In collaboration with E. de Álava (*Hospital Virgen del Rocío-IBIS*, Sevilla), we are also assessing whether they can serve as biomarkers to predict the presence of metastases before their actual detection using immuno-histochemistry (IHC) in tissue microarrays (TMAs) from patient samples. Finally, with the help of Carmen Blanco (Experimental Therapeutics Programme, CNIO), we are carrying out drug screens to identify vulnerabilities in EWS cells lacking STAG2. ■

PUBLICATIONS

• Cuadrado A, Giménez-Llorente D, De Koninck M, Ruiz-Torres M, Kojic A, Rodríguez-Corsino M, Losada A (2022). Contribution of variant subunits and associated factors to genome-wide distribution and dynamics of cohesin. *Epigenetics Chromatin* 15,37.

• Alonso-Gil A, Cuadrado A, Giménez-Llorente D, Rodríguez-Corsino M, Losada A (2022). Different NIPBL requirements of cohesin-STAG1 and cohesin-STAG2. *BioRxiv*. <https://doi.org/10.1101/2022.11.29.518367>.

• Villarroya-Beltri C, Martins AFB, García A, Giménez D, Zarzuela E, Novo M, Del Álamo C, González-Martínez J, Bonel-Pérez GC, Díaz I, Guillaumot M, Chiesa M, Losada A, Graña-Castro O, Rovira M, Muñoz J, Salazar-Roa M, Malumbres M (2022). Mammalian CDC14 phosphatases control exit from stemness in pluripotent cells. *EMBO J*. PMID: 36326833.

• Andreu MJ, Alvarez-Franco A, Portela M, Giménez-Llorente D, Cuadrado A, Badia-Careaga C, Tiana M, Losada A, Manzanares M (2022). Establishment of 3D chromatin structure after fertilization and the metabolic switch at the morula-to-blastocyst transition require CTCF. *Cell Rep* 41, 111501.

DNA REPLICATION GROUP

Juan Méndez
Group Leader

Research Scientists
Susana Llanos, Sara Rodríguez



Post-Doctoral Fellows
Estrella Guarino, Sergio Muñoz

Graduate Students
Elena Blanco, Roberto Masdemont,
Sergi Roig, Patricia Ubieta

OVERVIEW

Despite the biochemical complexity of the DNA replication process, the molecular machinery that duplicates our genome displays a remarkable capacity to adapt to different cell types, each one with its own transcriptional programme and specific patterns of chromatin organisation. In addition, the replisome proteins react to endogenous and exogenous factors that induce replicative stress (RS) and may cause DNA breaks, recombination events, and genomic instability. Our Group studies the mechanisms that confer operational flexibility to the replicative process, combining molecular and cellular approaches in human and mouse cells. In 2022, we completed two studies describing the cellular responses to specific situations of stress, which involve the regulation of origin activity and the control over replication fork progression. We also continued to study the dynamics of DNA replication and the impact of RS in other cellular contexts, including the acquisition of metastatic capacity by tumour cells.

“We have described how PRIMPOL facilitates DNA synthesis during stress-induced proliferation of haematopoietic stem cells, allowing the haematopoietic system to reconstitute itself after a bone marrow transplantation.”

RESEARCH HIGHLIGHTS

Three-dimensional chromatin organisation underlies the efficiency of replication origins

In earlier work, we had reported that a fraction of mammalian replication origins remains inactive (“dormant”) in S phase but can be activated as a backup mechanism in response to RS. To investigate the regulation of active vs dormant origins, we mapped origin activity in mouse embryonic stem cells (mESCs) undergoing mild RS triggered by aphidicolin, a DNA polymerase inhibitor, or by the ectopic expression of CDC6, an origin licensing factor. The main stress-induced response was an increase in the frequency of activation of existing initiation sites that were used with lower efficiency in unchallenged conditions. This phenotype reflects, at the cell population level, the combined effect of the activation of dormant origins in millions of individual cells. By intersecting origin mapping and Hi-C chromosomal conformation data, we found that origin efficiency is directly proportional to the number of three-dimensional (3D) contacts established between origin-containing chromatin fragments. Origins that cluster in 3D tend to fire with similar efficiencies and share their timing of replication, supporting the organisation of origins in higher-level replication factories (Jodkowska *et al.*, 2022; see FIGURE 1).

PRIMPOL-mediated repriming of DNA synthesis during stress-induced proliferation of haematopoietic stem cells

Since its discovery in 2013, our laboratory has been involved in the characterisation of the PRIMPOL enzyme, a DNA primase specialised in damage tolerance. In a recent study, we described how PRIMPOL mediates the replicative tolerance of DNA inter-strand crosslinks (ICLs; González-Acosta *et al.*, *EMBO J* 2021). Inefficient ICL repair causes Fanconi Anaemia (FA), a rare but severe disease characterised by frequent congenital defects, bone marrow failure, aplastic anaemia and cancer predisposition. In 2022, we completed a study in collaboration with M. Lopes (Institute of Molecular Cancer

Research, University of Zurich), showing that mouse haematopoietic stem cells (HSCs) that are forced to proliferate by a simulated viral infection display accelerated fork progression and accumulate extensive DNA damage. In this critical situation, HSCs rewire their DNA damage response and engage PRIMPOL primase, favouring re-priming of DNA synthesis over fork reversal. Competitive bone marrow transplantations confirmed that PRIMPOL activity is required for HSC amplification and efficient reconstitution of the haematopoietic system (Jacobs *et al.*, 2022; FIGURE 2). This study opens the possibility that in some cases, PRIMPOL-mediated bypass of damaged DNA could also contribute to the onset of leukaemia. In this regard, we are pursuing the identification of small inhibitors of PRIMPOL, in collaboration with the CNIO Experimental Therapeutics Programme.

DNA replication and RS in other cellular contexts

We have participated in two collaborative studies related to the main research topics described above: (a) the characterisation of a protective function of human p38 SAP kinase to maintain genome integrity in response to osmo-stress, mediated by claspin/Mrc1 phosphorylation (Ulsamer *et al.*, 2022); and (b) the analysis of DNA replication in cells harbouring a truncated variant of RAD51B associated with primary ovarian insufficiency (Franca *et al.*, 2022).

Other ongoing projects in the DNA Replication Group include: (i) a genome-wide analysis of the formation of pre-replicative complexes in human and mouse cells, using CUT&RUN with initiator proteins; (ii) a comparative analysis of replisome composition in *naïve* and *primed* mESCs, which could explain the changes in fork speed observed during cell reprogramming; (iii) an investigation of the influence of RS during epithelial-to-mesenchymal transition, a process that underlies the acquisition of resistance to chemotherapy in some tumour cells. ■

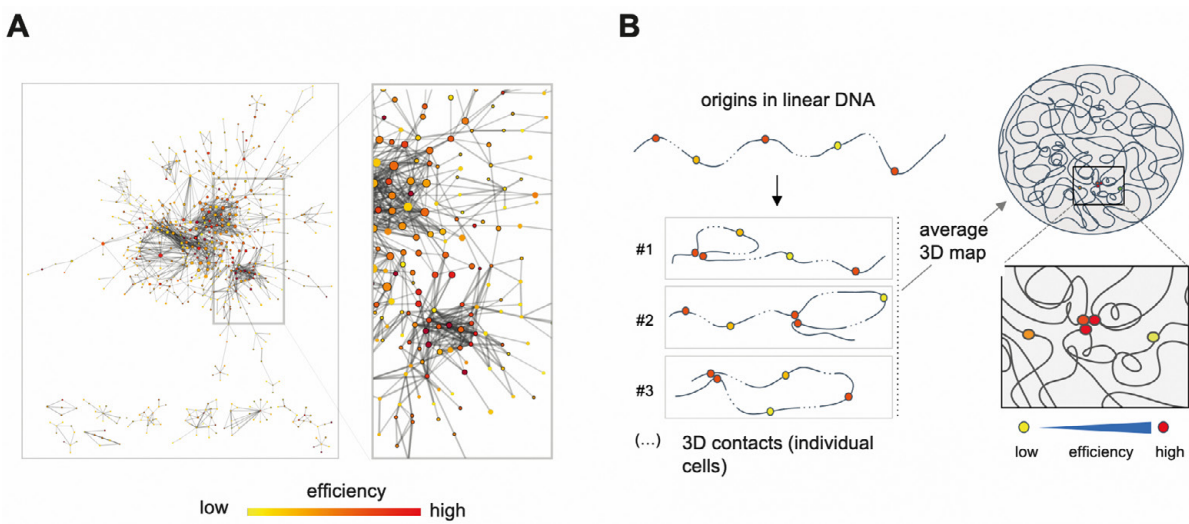


FIGURE 1 Integration of origin maps into 3D chromatin interaction networks. (A) Network of chromatin contacts derived from Hi-C data in mESCs (chrom 1). Origins (coloured circles) located at more connected hubs are activated with higher frequency. (B) Model of a replication factory formed by clustered origins. Adapted from Jodkowska *et al.* (2022).

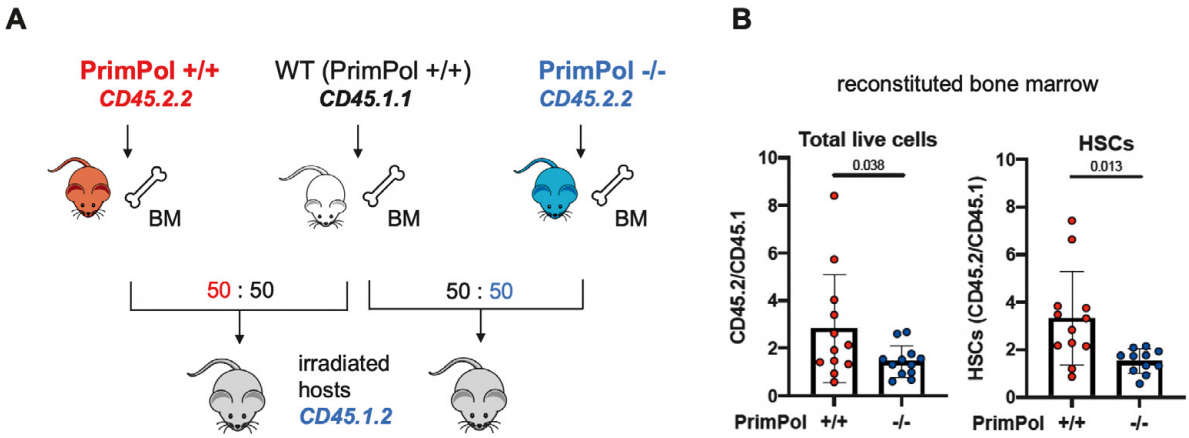


FIGURE 2 Efficient reconstitution of the haematopoietic system requires PRIMPOL activity. (A) Experimental design of a competitive bone marrow (BM) transplantation. (B) Donor chimerism in the reconstituted BM (total live cells and HSCs). Red, PRIMPOL-proficient; blue, PRIMPOL-deficient cells. Adapted from Jacobs *et al.* (2022).

PUBLICATIONS

Jacobs K, Doerdelmann C, Krietsch J, González-Acosta D, Mathis N, Kushinsky S, Guarino E, Gómez-Escobar C, Martínez D, Schmid JA, Leary PJ, Freire R, Ramiro AR, Eischen CM, Méndez J*, Lopes M* (2022). Stress-triggered hematopoietic stem cell proliferation relies on PrimPol-mediated repriming. *Mol Cell* 82, 4176-4188. (*) Co-corresponding authors.

embryonic stem cells. *Nucleic Acids Res* 50, 12149-12165. (*) Co-corresponding authors.

Jodkowska K, Pancaldi V, Rigau M, Almeida R, Fernández-Justel JM, Graña-Castro O, Rodríguez-Acebes S, Rubio-Camarillo M, Carrillo-de Santa Pau E, Pisano D, Al-Shahrour F, Valencia A, Gómez M*, Méndez J* (2022). 3D chromatin connectivity underlies replication origin efficiency in mouse

with primary ovarian insufficiency provides insights into its meiotic and somatic functions. *Cell Death Diff* 29, 2347-2361.

Franca MM, Condezo YB, Elzaiat M, Felipe-Medina N, Sánchez-Sáez F, Muñoz S, Sainz-Urruela R, Martín-Hervás MR, García-Valiente R, Sánchez-Martín MA, Astudillo A, Méndez J, Llano E, Veitia RA, Mendonça BB, Pendás AM (2022). A truncating variant of RAD51B associated

MELANOMA GROUP

María S. Soengas
Group Leader

Research Scientists
Nuria Gago, David Olmeda



Post-Doctoral Fellows
Susana Frago (until May), María Magdalena Leal (until July), Adriana Sanna

Graduate Students
Xavier Catena, Marta Contreras, Guillermo de La Vega (since June) , Naiara Juan-Larrea, Sergio Oterino, Thelma Poluha, José A. Torres (since February)

Technicians
Tonantzin G. Calvo, Cynthia Mucientes (TS)*, Mireia Vallespinós (TS)*

**Titulado Superior* (Advanced Degree)

Students in Practice
Angeliki Christopoulou (until Feb) (University of Patras, Greece), Maria de Rosa (since Sept.) (*Universitat de Lleida*, Spain)

Visiting Scientist
Daniela Cerezo (Dermatology, *Hospital 12 de Octubre*, Madrid, Spain)

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

OVERVIEW

Melanomas are prime examples of aggressive diseases where basic and translational research have significantly improved patient prognosis. Nevertheless, clinical responses are still incomplete. The long-term goals of our Group are to identify new progression biomarkers and therapeutic agents. We are particularly interested in mechanisms of cellular stress that, being selectively deregulated in melanoma, define lineage-specific vulnerabilities (publications in *Nature*, *Cancer Cell*, *Nature Cell Biology*, *Nature Communications*, among others). Our laboratory has also reported first-in-class lymphoreporter (*MetAlert*) mice for non-invasive imaging of pre-metastatic niches in melanoma (*Nature*). These systems have 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. Our ultimate objective is to improve the management of patients with otherwise refractory metastatic melanomas.

“We have visualised and targeted (pre)metastatic niches in melanoma and defined mechanisms of immune suppression with clinical implications for cancer patients.”

RESEARCH HIGHLIGHTS

The long-term goals of our Group are to (see FIGURE 1):

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 tumour drivers that favour melanoma progression

One of the long-term objectives of our Group is to discover novel melanoma drivers. We have previously identified endolysosomal-associated genes (RAB7) and RNA binding proteins (CEFL1, CUGBP1 and IGF2BP1) with lineage-specific protumorigenic functions that are not shared by over 25 cancer types (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, we have pursued melanoma-secreted factors that exert long-range activities, particularly in the generation of premetastatic niches. A prime interest of our Group has been modulators of neolymphangiogenesis, as this is an early stage in melanoma dissemination. Exploiting “lymphoreporter” models generated by Sagrario Ortega’s Group at CNIO, we developed the first “*Melanoma-MetAlert*” mice. These animals have the unique feature of allowing for spatio-temporal analyses of tumour-activated lymphangiogenesis *in vivo* as a way to

define premetastatic niches (Olmeda *et al.*, *Nature* 2017). ‘*MetAlert*’ animals, in combination with human tissue specimens, revealed the growth factor MIDKINE (MDK) as a new melanoma driver with a potent ability to act in a systemic manner to promote neolymphangiogenesis and melanoma metastasis (Olmeda *et al.*, *Nature* 2017). Our expertise in lymphangiogenesis also contributed to collaborative studies to define an unexpected crosstalk of lymphatic genes with lipid metabolism and autophagy (Mece *et al.*, *Nat Commun* 2022). In the course of these studies, we generated computational tools and experimental models that have served to characterise novel dsRNA binding proteins and various immune modulators. In particular, our ability to mine large tumour datasets has helped us to describe immune suppressive roles of IL22 favouring lung metastasis (Briukhovetska *et al.*, *Immunity* 2023).

Impact of the melanoma secretome in the rewiring of the immune system towards tumour-promoting phenotypes

Melanomas are a prime example of tumours quite efficient at bypassing antigen presentation and promoting immunologically “cold” or tolerogenic phenotypes, but the underlying mechanisms are not well understood. Analysing downstream effectors of MDK, we found new immune suppressive roles of this protein, whereby macrophages are recruited to tumours, but instead of attacking the cancer cells, promote dysfunctional CD8+ T cells (Cerezo-Wallis *et al.*, *Nat Medicine* 2020). More recently, we discovered that MDK acts as a multifaceted suppressor of antigen presentation. Mechanistically, MDK was found to repress all main aspects of the differentiation,

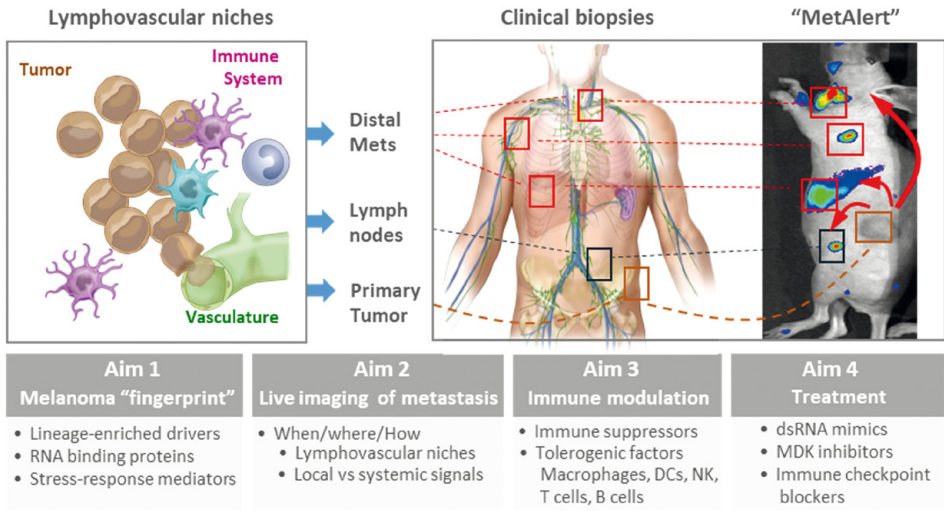


FIGURE 1 Melanoma Group at glance: main aims and experimental models to identify new tumour drivers and therapeutic targets, with a particular emphasis on the crosstalk between lymphatic vasculature and the immune system.

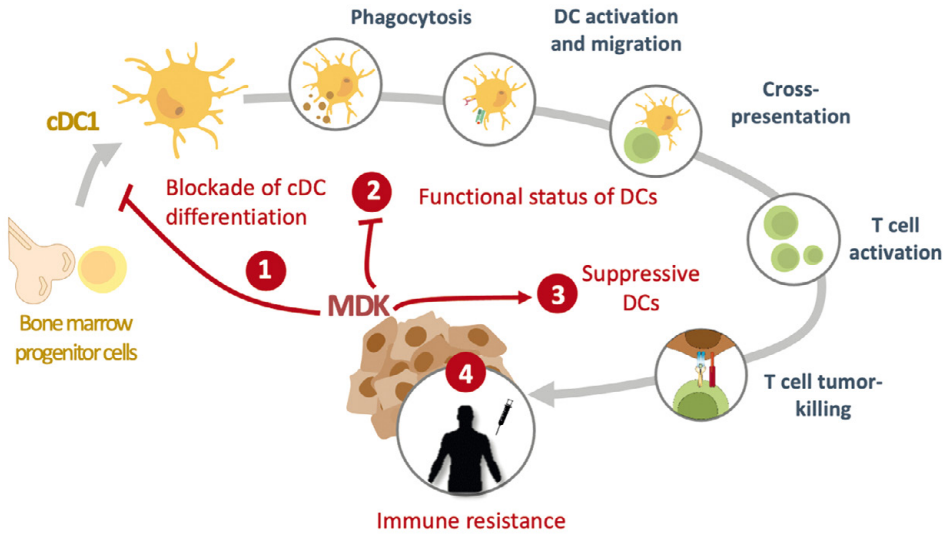


FIGURE 2 Multi-stage rewiring of dendritic cell (DC) differentiation and function by tumour-secreted Midkine (MDK). MDK was found to block DC differentiation and impair all main DC associated functions (phagocytosis, activation, cross-presentation, and T cell activation), shifting DCs into suppressive features. Ultimately, these DC-driven effects reduce the efficacy of immune-based therapies.

activation, and function of dendritic cells (DCs), particularly of conventional type 1 (cDC1). Moreover, we uncovered an MDK-associated signature in DCs that defines bad prognosis and resistance to immune checkpoint blockers actively used in human patients (FIGURE 2). MDK-associated downregulation of cDC1-dependent immune scores were also identified in a variety of other tumour types, further emphasising the translational relevance of MDK as a target to boost antigen presentation in otherwise immune refractory cancers (Catena *et al.*, *BioRxiv* 2022; Catena *et al.*, submitted). In light of the tumour-promoting and immune-suppressive roles of MDK, we are actively pursuing this protein as a therapeutic target. We have 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. ■

PUBLICATIONS

Marine JC, Soengas MS (2022). Cell position matters in tumour development. *Nature* 604, 248-250.

Briukhovetska D, Suarez-Gosalvez J, Voigt C, Markota A, Giannou AD, Schübel M, Jobst J, Zhang T, Dörr J, Märkl F, Majed L, Müller PJ, May P, Gottschlich A, Tokarew N, Lücke J, Oner A, Schwerdtfeger M, Andreu-Sanz D, Grünmeier R, Seifert M, Michaelides S, Hristov M, König LM, Cadilha BL, Mikhaylov O, Anders HJ, Rothenfusser S, Flavell RA, Cerezo-Wallis D, Tejedo C, Soengas MS, Bald T, Huber S,

Endres S, Kobold S (2022). T cell-derived interleukin-22 drives the expression of CD155 by cancer cells to suppress NK cell function and promote metastasis. *Immunity*. PMID: 36630913.

Mece O, Houbaert D, Sassano ML, Durré T, Maes H, Schaaf M, More S, Ganne M, García-Caballero M, Borri M, Verhoeven J, Agrawal M, Jacobs K, Bergers G, Blacher S, Ghesquière B, Dewerchin M, Swinnen JV, Vinckier S, Soengas MS, Carmeliet P, Noël A, Agostinis P (2022). Lipid droplet degradation by autophagy connects mitochondria metabolism to Prox1-driven expression of lymphatic genes and lym-

phangiogenesis. *Nat Commun* 13, 2760.

Catena X, Contreras-Alcalde M, Cerezo-Wallis D, Juan-Larrea D, Olmeda D, Calvo TG, Mucientes C, Oterino S and Soengas MS (2022). Systemic effects of melanoma-secreted MIDKINE in the inhibition of dendritic cell differentiation and function. *BioRxiv*. doi: <https://doi.org/10.1101/2022.12.28.521901>.

AWARDS AND RECOGNITION

Excellence in Science Award by the *Colegio de Biólogos y Biólogas de Galicia*, Spain.

International Award in Oncology, *Ramiro Carregal* Foundation, Spain.

Leadership in Science Award (“*Premio Liderando en Ciencia*”), *Comunidad Autónoma de Madrid*, Spain.

President, Spanish Association for Cancer Research (*Asociación Española de Investigación Contra el Cáncer, ASEICA*).

Elected EMBO Member.

Top 100 Women Leaders in Spain, *Mujeres & Cía*.

EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real
Group Leader

Research Scientist
Miriam Marqués

Post-Doctoral Fellows
Elena del Pilar Andrada, Lavinia
Cabras (until February), Irene Felipe,



Eleonora Lapi, Jaime Martínez de
Villarreal, Cristina Segovia, Sladjana
Zagorac (until September)

Graduate Students
Catalina Berca, Cristina Bodas, Sonia
Corral, Auba Gayà, Irene Millán
(October-December), María Ramal,
Chengsi Wu (since October) (China
Scholarship Council, CSC)

Technicians
Natalia del Pozo, Leticia Rodríguez

Students in Practice
Ester Arroba (May-Dec.) and Olaya de
Dios (until June) (Master's Programme
in Bioinformatics, *ISCIII-ENS*, Madrid,
Spain), Nadine Lebenich (since Sept.)
(IMC Univ. of Applied Sciences Krems,
Austria), Lucía Sancho (until June)
(*UAM*, Madrid, Spain), Francisco
Soriano (until June) (*Universitat Oberta
de Catalunya*, Barcelona, Spain)

Visiting Scientists
Brice Chanez (since Sept.) (*Institut
Paoli-Calmettes*, Marseille, France),

Luis C. Fernández (*Univ. Europea de
Madrid*, Spain), Mark Kalisz (*CIBER*,
Madrid, Spain), Catalina Perello
(Sept.-Dec.) (*JdISBa*, Palma, Spain),
Gabriel Piedrafita (*UCM*, Madrid,
Spain)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial bladder carcinoma (UBC) 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 to dissect the molecular mechanisms involved in very early steps of tumour development, harnessing the excellent genetic mouse models available. 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.

UBC presents with very wide clinical and pathological heterogeneity. We aim to acquire knowledge about the underlying molecular pathways and to apply it for improved prediction of outcome and therapy.

“We have found that antibiotic administration and gut flora depletion rescues a genetic defect present in *Nr5a2* heterozygous mice that sensitises them to acute pancreatic damage and to PDAC.”

RESEARCH HIGHLIGHTS

Pancreatic cancer molecular pathophysiology

In recent years, GWAS have identified a variety of common genetic variants associated with PDAC risk. Several of them are associated with genes involved in acinar cell biology, including *NR5A2* and *HNFI1A*, coding for transcription factors required for full acinar differentiation that we have extensively studied. A few other GWAS hits associate with genes involved in acinar function, such as *XBPI* and *CTRB1/2*. 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 the ER stress response. *Nr5a2* heterozygous mice display more damage and are not able to recover properly upon induction of a mild acute pancreatitis. In addition, they are more susceptible to mutant *KRas*-driven PDAC. Among the modifiable factors that may cooperate with this genetic defect to drive PDAC, we tested diet and the gut microbiome. A high fat diet does not add to the pancreatitis phenotype of *Nr5a2* heterozygous mice. In contrast, antibiotic administration and depletion of the gut microbiota rescues the genetic defect observed upon pancreatitis induction. 16S rDNA analysis does not reveal major differences in the faecal microbiome of wild type and *Nr5a2* heterozygous mice. A variety of experiments fail to support the contribution of heterozygosity at the intestinal level. Transcriptomic changes analysis of the pancreas reveals significant changes both in basal conditions and during pancreatitis. Most notably, in mice that received antibiotics we find an up-regulation of the acinar programme and of mitochondrial pathways and a down-regulation of cell cycle and inflammatory pathways. *Nr5a2* heterozygous mice have higher levels of CD4+ cells in blood, and antibiotic administration reduces their CD4+ cell levels. Gut microbiome reconstitution results in increased CD4+ cell counts (FIGURE 1). Our observations suggest that the gut microbiome induces a basal inflammatory state that contributes to disease, the modulation of which could be exploited therapeutically.

Urothelial bladder carcinoma (UBC) genetics, biology, and clinical translation

We focus on understanding 2 new UBC tumour suppressor genes 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.

Increasing evidence shows that STAG2 acts as a tumour suppressor through rather unique mechanisms, largely unrelated to the canonical role of cohesin in chromosome segregation. *STAG2* alterations occur early during tumorigenesis. Therefore, we are using both normal urothelial cells and tumour cell lines to identify the impact of STAG2 at the genomic and cellular levels. Using RT112 cells, we have integrated ChIP-Seq, HiC chromatin interaction data, and RNA-Seq to assess the impact of STAG2 knockdown. The cohesin-STAG2 complex mediates short- and mid-range interactions that engage genes at higher frequency than cohesin-STAG1. STAG2 knockdown results in the down-regulation of luminal differentiation programmes and up-regulation of basal programmes. These findings are at odds with the fact that *STAG2* mutations are associated with luminal-type bladder cancers, suggesting an intermediate luminal differentiation phenotype. STAG2 knockdown does not affect compartment and domain boundaries, but it rewires intra-TAD DNA interactions and leads to the de-repression of lineage specifying genes (in collaboration with M. Martí-Renom, CRG, Barcelona).

Our translational studies expand several clinical trials with a strong translational component carried out in collaboration with Núria Malats and Spanish uro-oncologists. ■

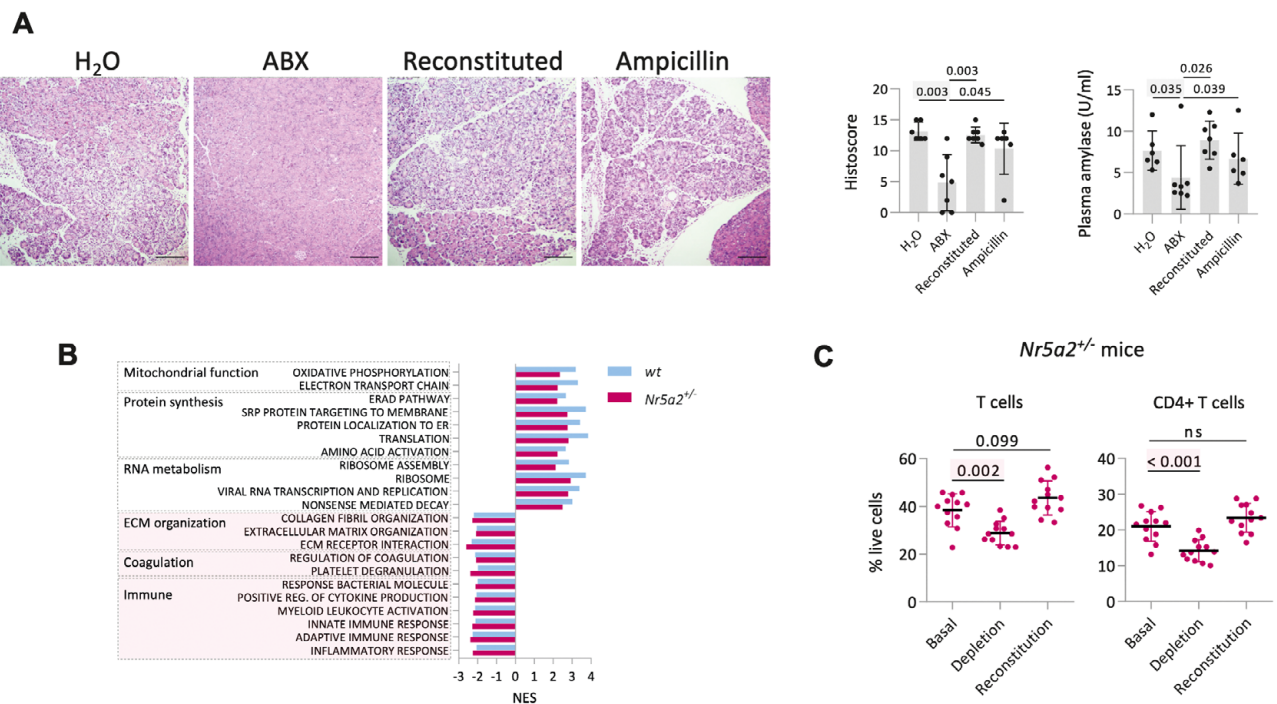


FIGURE 1 Antibiotic-mediated gut flora depletion rescues the genetic defect of *Nr5a2*^{+/-} mice, as shown at the histological level upon induction of acute pancreatitis (**A**). In basal conditions, antibiotic administration has anti-inflammatory effects in the pancreas as shown by RNA-Seq pathway analysis (**B**). Antibiotic administration induces a significant

decrease of CD4+ cells in the blood that is reversed upon gut flora reconstitution by co-housing (**C**).

PUBLICATIONS

Kloesch B, Ionasz V, Paliwal S, Hruschka N, Martínez de Villarreal J, Öllinger R, Mueller S, Dienes HP, Schindl M, Gruber ES, Stift J, Herndler-Brandstetter D, Lomb-erk GA, Seidler B, Saur D, Rad R, Urrutia RA, Real FX, Martinelli P (2022). A GATA6-centred gene regulatory network involving HNFs and ΔNp63 controls plasticity and immune escape in pancreatic cancer. *Gut* 71,766-777.

de Andrés MP, Jackson RJ, Felipe I, Zagorac S, Pilarsky C, Schlitter AM, Martínez de Villarreal 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 (2022). GATA4 and GATA6 loss-of-expression is associated with extinction of the classical programme and poor outcome in pancreatic ductal adenocarcinoma. *Gut*. PMID: 36109153.

Kartal E, Schmidt TSB, Molina-Montes E, Rodríguez-Perales S, Wirbel J, Maistrenko OM, Akanni WA, Alashkar Alhamwe B, Alves RJ, Carrato A, Erasmus HP, Estudillo L, Finkemeier F, Fullam A, Glazek AM, Gómez-Rubio P, Hercog R, Jung F, Kandels S, Kersting S, Langheinrich M, Márquez M, Molero X, Orakov A, Van Rossum T, Torres-Ruiz R, Telzerow A, Zych K; MAG-IC Study investigators; PanGenEU Study investigators; Benes V, Zeller G, Trebicka J, Real FX, Malats N, Bork P (2022). A

faecal microbiota signature with high specificity for pancreatic cancer. *Gut* 71, 1359-1372.

Suarez-Cabrera C, Estudillo L, Ramón-Gil E, Martínez-Fernández M, Peral J, Rubio C, Lodewijk I, Martín de Bernardo Á, García-Escudero R, Villacampa F, Duarte J, de la Rosa F, Castellano D, Guerrero-Ramos F, Real FX, Malats N, Paramio JM, Dueñas M (2022). BlaDimiR: a urine-based miRNA score for accurate bladder cancer diagnosis and follow-up. *Eur Urol* 82, 663-667.

García-Carbonero R, Bazan-Peregrino M, Gil-Martin M, Álvarez R, Macarulla T, Riesco-Martínez MC, Verdaguer H, Guillén-Ponce C, Farrera-Sal M, Moreno R, Mato-Berciano A, Maliandi MV, Torres-Manjon S, Costa M, Del Pozo N, Martínez de Villarreal J, Real FX, Vidal N, Capella G, Alemany R, Blasi E, Blasco C, Cascalló M, Salazar R (2022). Phase I, multicenter, open-label study of intravenous VCN-01 oncolytic adenovirus with or without nab-paclitaxel plus gemcit-

abine in patients with advanced solid tumors. *J Immunother Cancer* 10, e003255.

Chondronasiou D, Martínez de Villarreal J, Melendez E, Lynch CJ, Pozo ND, Kovatcheva M, Aguilera M, Prats N, Real FX, Serrano M (2022). Deciphering the roadmap of in vivo reprogramming toward pluripotency. *Stem Cell Rep* 17, 2501-2517.

Melendez E, Chondronasiou D, Mosteiro L, Martínez de Villarreal J, Fernández-Alfara M, Lynch CJ, Grimm D, Real FX, Alcamí

J, Climent N, Pietrocola F, Serrano M (2022). Natural killer cells act as an extrinsic barrier for in vivo reprogramming. *Development* 149, dev200361.

Martínez-Villarreal J, Kalisz M, Piedrafita G, Graña-Castro O, Chondronasiou D, Serrano M, Real FX (2022). Pseudoalignment tools as an efficient alternative to detect repeated transposable elements in scRNAseq data. *Bioinformatics*. PMID: 36519825.

PATENT

Malats Riera N, Bork P, Kartal E, Molina Montes E, Rodríguez S, Estudillo L, Real FX, Schmidt TSB, Zeller G, Wirbel J, Maistrenko OM. Faecal Microbiota Signature for Pancreatic Cancer. PCT application (2022). *PCT/EP2022/077087*. WO2023052486A1.

GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

Nabil Djouder
Group Leader

Research Scientist
Sladjana Zagorac (since November)

Post-Doctoral Fellows
Albert Harguindey (until September),
Clara Ortigón (since July)



Graduate Students
Mariana Angulo (since October),
Maria Inmaculada Berbel (until June),
Sergio de La Rosa, Rosa Gallo, Irene
Herranz, Carlos Martínez, Maria del
Mar Rigual, Paula Sánchez, Karla

Santos, Fengming Yi (until
November) (China Scholarship
Council, MD oncologist Fellow)

Technicians
Maheva Cruz (September-
December), Ruth Ortego (March-
August)

Student in Practice
Filipa Magalhães Cruz (February-
July) (*Universidade do Porto*,
Portugal)

OVERVIEW

Research over the last 20 years has focused mainly on understanding the functions and roles of newly discovered mutated genes in the development of cancer and associated diseases. However, it remains largely unknown how environmental factors can alter the host’s eukaryotic epithelial cells to cause various pathologies that can progress to cancer. Identifying likely causal links between environmental stresses and diseases that progress to cancer will help to elucidate mechanisms of disease and to identify targets with preventive and therapeutic value for treating frequent lethal human disorders with increased worldwide incidence and unmet medical needs.

In our laboratory, we focus on understanding the mechanisms of diseases associated with the ingestion of toxic diets or nutrient overload that can lead to obesity and associated disorders, including diseases of the digestive system. We have a particular interest in liver disease, including non-alcoholic steatohepatitis and cirrhosis, and their progression to hepatocellular carcinoma (HCC), one of the most aggressive and lethal liver cancers. We also study intestinal disorders that can lead to colorectal cancer. Our ultimate goal is to guide the design of new medicines.

“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

Our research interest is mainly driven by the discovery of two components initially identified in our laboratory to be downstream targets of the growth factor and nutrient signalling cascades: the **URI (Unconventional prefoldin RPB5 Interactor)** and **MCRS1 (Microspherule protein 1)** proteins. URI and MCRS1 are respectively part of 2 independent protein complexes: the **URI prefoldin-like** and the **non-specific lethal (NSL) complexes**. Importantly, URI and MCRS1 expression turned out to be also regulated by environmental factors (nutrients, radiations, bacteria, viruses, etc.), which may compromise their functions and activate pleiotropic circuits supporting complex cell signalling networks, thereby provoking severe outcomes.

Using genetically engineered mouse models generated in our lab for URI and MCRS1 gain- and loss-of-functions, combined with other model systems and cutting-edge technologies and human data, our laboratory has devoted substantial efforts over the last years to determine the molecular, cellular, and pathophysiological mechanisms that link environmental stresses to obesity and disease pathogenesis of the digestive system, with the aim of developing more effective therapeutic strategies. In particular, we have focused on diseases associated to the liver, intestine, and pancreas, as these organs are primarily impacted by environmental stressors, including nutrient overload, but are also physiologically interconnected through their exocrine and/or endocrine functions. In this regard, the following highlights summarise our major achievements during 2022:

- The liver has an exceptional ability to regenerate itself to maintain tissue homeostasis, but this process can be impacted by stress signals, potentially leading to liver cancer. We have reviewed the mechanisms of hepatic regeneration under homeostasis or upon injury (Rigual *et al.*, *Trends Cancer*, 2022).
- Additionally, we have developed a novel murine model that mimics the pathological features of cirrhosis, and uncovered a new function of MCRS1 in regulating histone acetylation, maintaining gene expression and liver homeostasis. The loss of MCRS1 in hepatocytes activates the bile acid/FXR axis in liver fibroblasts, a significant event in cirrhosis development, with important implications for treatment (Garrido *et al.*, *J Hepatol*, 2022).
- We have also determined the mechanisms of regeneration of the intestinal epithelium and demonstrated that URI+ cells play a crucial role in maintaining intestinal homeostasis by controlling R-spondin 1 levels, supporting Lgr5^{high} intestinal stem cell proliferation. These findings highlight the unexpected role of transit-amplifying cells in controlling Lgr5^{high} intestinal stem cell proliferation (Chaves-Peréz *et al.*, *J Exp Med*, 2022).

Future work

Obesity is becoming one of the most increasingly growing risk factors for liver and intestinal disorders, including cancer. By employing multi- and inter-disciplinary approaches, including the use of preclinical mouse models generated in our laboratory combined with human data, we will continue to determine the mechanisms of diseases associated with obesity. In particular, with a special focus on diseases of the digestive system, we aim to: find out what goes wrong in diseased and cancerous tissues; understand how organs can regenerate; potentially engineer new tissues; and, if regeneration goes awry, determine how it contributes to cancer. Our ultimate goal is to help guide the design of new medicines against obesity and its associated disorders (FIGURE 1). ■

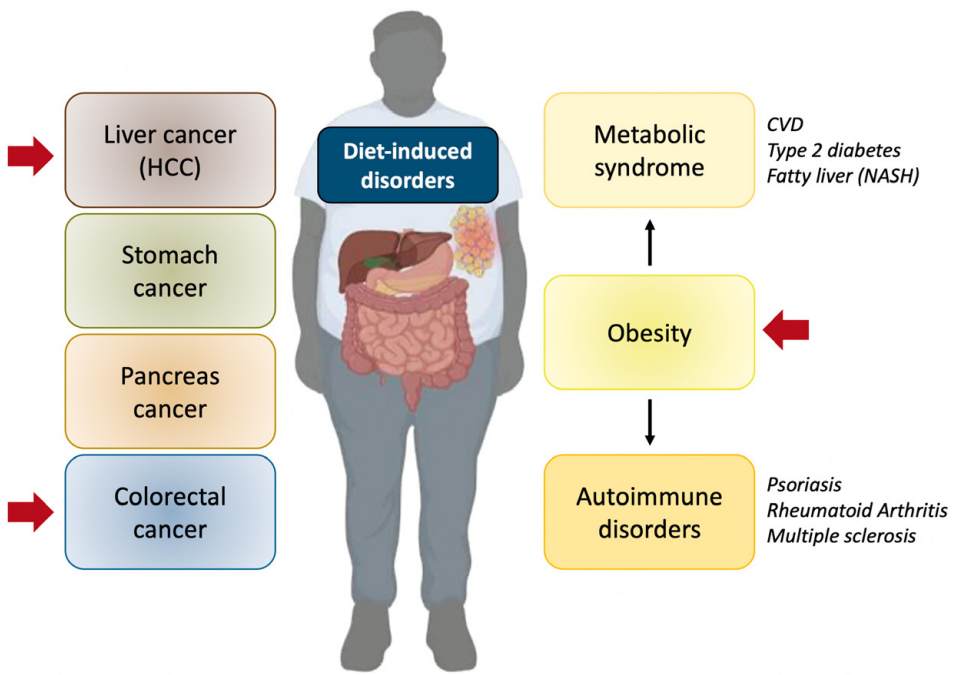


FIGURE 1 Representation of some of our research directions. Obesity is one of the most increasingly growing risk factors for liver and intestinal disorders, including cancer. By employing multi- and inter-disciplinary approaches, including the use of

preclinical mouse models generated in our lab combined with human data, we aim to: find out what goes wrong in diseased tissues; understand how organs can regenerate; potentially engineer new tissues; and, if regeneration goes awry, determine

how it contributes to disorders. Our final goal is to guide the design of new medicines against obesity and its associated disorders, including metabolic, liver and intestinal diseases. CVD: cardiovascular diseases. NASH: Non-alcoholic steatohepatitis.

► PUBLICATIONS

- Chaves-Pérez A, Santos-de-Frutos K, De la Rosa S, Herranz-Montoya I, Perna C, Djouder N (2022). Transit-amplifying cells control R-spondins in the mouse crypt to modulate intestinal stem cell proliferation. *J Exp Med* 219, e20212405.

- Garrido A, Kim E, Teijeiro A, Sánchez Sánchez P, Gallo R, Nair A, Matamala Montoya M, Perna C, Vicent GP, Muñoz J, Campos-Olivas R, Melms JC, Izar B, Schwabe RF, Djouder N (2022). Histone acetylation of bile acid transporter genes plays a critical role in liver cirrhosis. *J*

Hepatol 76, 850-861.

- Rigual MDM, Sánchez Sánchez P, Djouder N (2022). Is liver regeneration key in hepatocellular carcinoma development? *Trends Cancer*. PMID: 36347768.

► AWARDS AND RECOGNITION

- BBVA grant Award in Biomedicine 2022, Spain.
- Spanish national grant from the Ministry of Science and Innovation (*Retos* 2022).
- Member of the European Association for the Study of Diabetes (EASD).

TRANSFORMATION AND METASTASIS GROUP

Eva González Suárez
Group Leader

Research Scientists
Patricia González, María Jiménez,
Gema Pérez



Post-Doctoral Fellows
Sara Lázaro (April-August), Angélica
Santiago (since April)

Graduate Students
Alexandra Barranco, Marina Císcar
(until September), Alejandro Collado,
Jaime Redondo, Andrea Vethencourt
(Clinical Oncologist at *ICO/IDIBELL*,
Barcelona, Spain)

Undergraduate Student
Pedro Luis Echevarria (until
February)

Technicians
Lucía de Andrés (since April), Víctor
López, Sergi Velasco (until
September) (PEJ, CAM)*

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

Students in Practice
Pablo Blanco (February-July)
(Master's Thesis, *UAM*, Madrid,
Spain), Isabel Jiménez (Jan.-July)
(Master's Thesis, *UCM*, Madrid,

Spain), Teresa Martí (Jan.-July)
(*AECC* Traineeship and BS Thesis,
Univ. Francisco de Vitoria, Spain)

Visiting Scientists
Emilia Brizzi (June-July) (Pathology
resident, *Hospital La Paz*, Madrid,
Spain), Marta Matas (Sept.-Nov.)
(*Althaia, Xarxa Assistencial
Universitària de Manresa*, Spain)

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 in order 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.

“Analyses of clinical samples and functional experiments in patient-derived xenografts demonstrate that RANK protein expression in tumour cells is associated with poor survival in ER negative breast cancer, and its inhibition improves chemotherapy response.”

RESEARCH HIGHLIGHTS

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

Despite strong preclinical data, the therapeutic benefit of denosumab in breast cancer, beyond the bone, is unclear. Aiming to select patients who may benefit from denosumab, we analysed RANK and RANKL expression in more than 2000 breast tumours (777 oestrogen receptor-negative, ER⁻) from 4 independent cohorts. RANK expression was more frequent in ER⁻ tumours, where it associated with poor outcome and poor response to chemotherapy. In patient-derived orthoxenografts (PDXs) of ER⁻ breast cancer, RANKL inhibition reduced tumour cell proliferation and stemness, regulated tumour immunity and metabolism, and improved response to chemotherapy.

Intriguingly, RANK expression was associated with poor prognosis in postmenopausal breast cancer patients, activation of NFκB signalling, and modulation of immune and metabolic pathways, suggesting that RANK signalling increases after menopause. Indeed, RANKL inhibition showed greater therapeutic benefit in ER⁻ breast cancer PDXs under postmenopausal conditions. Our results demonstrate that RANK expression is an independent biomarker of poor prognosis in postmenopausal patients with ER⁻ breast cancer and support the therapeutic benefit of RANK pathway inhibitors in breast cancer patients with RANK⁺ ER⁻ tumours after menopause (FIGURE 1).

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

Rank signalling is a known regulator of mammary gland homeostasis, being critical for stem cell maintenance and epithelial cell differentiation. Although the Rank receptor is highly expressed by basal cells and luminal progenitors, its role in each individual cell lineage remains unclear. By combining temporal/lineage specific *Rank* genetic deletion with lineage tracing techniques, we found that loss of luminal Rank leads to aberrant alveolar-like differentiation in virgin mammary glands, reminiscent of pregnancy, and an increase in hormone-sensing luminal population (PR/Rankl-positive cells). During a first pregnancy, Rank-deleted luminal cells are unable to produce milk and expand following successive pregnancies. This results in a “tissue-damage like” scenario in the developing alveoli leading to basal bipotency and the replacement of “unfit luminal cells” by Rank-proficient cells to restore lactation. Transcriptomic analysis and functional assays point to a dual role for luminal Rank signalling in the control of protein translation. In the virgin mammary gland, Rank-depleted luminal cells show aberrant expression of lactogenic genes and increased protein synthesis. This aberrant differentiation exhausts the protein synthesis capability of the parous Rank-depleted luminal cells, making them unable to cope with the high translational demands required for milk production upon pregnancy. Consequently, basal bipotency is awakened through the activation of Rank/NF-κB signalling in basal parous cells of the alveoli in successive pregnancies to restore lactation and tissue homeostasis (FIGURE 2). ■

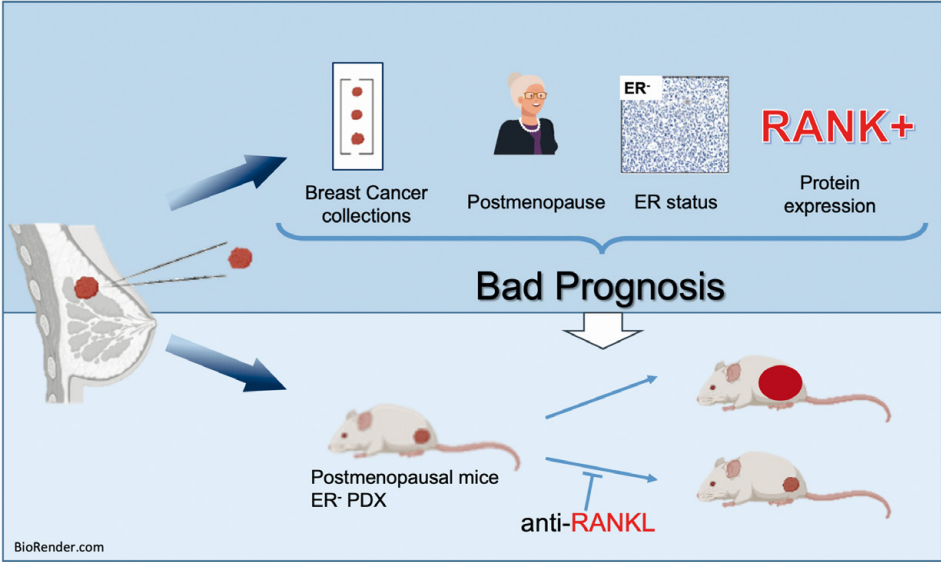


FIGURE 1

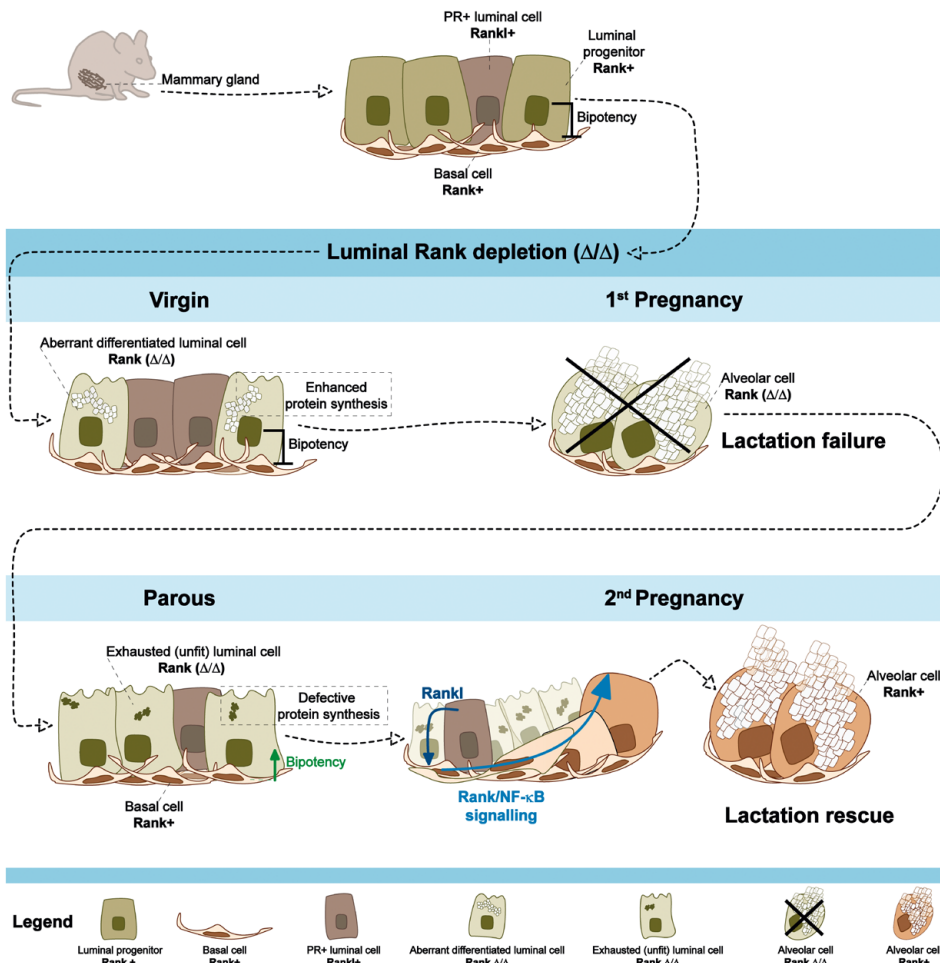


FIGURE 2

► PUBLICATION

- Vethencourt A, Trinidad EM., Petit A., Soler Monso MT, Gomez Aleza C, Urruticochea A, Garcia-Tejedor A, Guma Martinez A, Obadia V, Vazquez S, Villanueva R, Fernandez A, Cejuela M, Penabad R, Stradella A, Gil-Gil M, Pernas S, Gonzalez-Suarez E, Falo C (2022). First results of the randomized window of opportunity clinical trial D-Biomark: immunomodulatory effect of denosumab in early breast cancer. *Cancer Res* 82 (4_Supplement), P2-10-P2-08-10.
- **AWARDS AND RECOGNITION**
 - Eva González Suárez:
 - ERC Proof of Concept Grant 2022, European Research Council.
 - SenesceX-CM Consortium Coordinator, Community of Madrid (CAM).
 - Invited Speaker, Basel Breast Consortium annual meeting on “Personalized breast cancer treatments”, November 2022, Basel, Switzerland.
 - Forum Participant, Forbeck Forum “Aneuploidy in cancer development, prognosis and treatment”, April 2022, Lago Maggiore, Italy.
 - Alejandro Collado:
 - Selected oral presentation, Gordon Conference in Mammary Gland Biology 2022, Barga, Italy.

ulatory effect of denosumab in early breast cancer. *Cancer Res* 82 (4_Supplement), P2-10-P2-08-10.

► AWARDS AND RECOGNITION

- Eva González Suárez:
 - ERC Proof of Concept Grant 2022, European Research Council.
 - SenesceX-CM Consortium Coordinator, Community of Madrid (CAM).

- Invited Speaker, Basel Breast Consortium annual meeting on “Personalized breast cancer treatments”, November 2022, Basel, Switzerland.
- Forum Participant, Forbeck Forum “Aneuploidy in cancer development, prognosis and treatment”, April 2022, Lago Maggiore, Italy.
- Alejandro Collado:
 - Selected oral presentation, Gordon Conference in Mammary Gland Biology 2022, Barga, Italy.

- Barga, Italy.
- Selected oral presentation, Workshop “Fat sensing and the brain control of puberty, 2022, Baeza, Spain.
- Alexandra Barranco:
 - ECI Best Poster Award 2022, The European Congress of Immunology.
- Jaime Redondo:
 - Selected oral presentation, annual meeting of the Spanish Cell Senescence Network, September 2022, Valencia, Spain.

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Research Scientist
Susana García

Post-Doctoral Fellows
Marta Hergueta, Laura Nogués



OVERVIEW

In the Microenvironment and Metastasis laboratory, we are interested in understanding the crosstalk between tumour and stromal cells along metastatic progression. We are interested in how tumour cells can extrinsically influence the evolution of cancer during metastatic spread. For this purpose, we are analysing 1) the role of small extracellular vesicles (sEVs) in primary tumour evolution and pre-metastatic niche formation in melanoma, prostate and pancreatic cancer, and 2) the influence of obesity in breast cancer metastasis, as well as defining 3) the relevance of nerve growth factor receptor (NGFR) in melanoma, oral squamous cell carcinoma, and bladder cancer metastasis, aiming to develop new targeted therapies.

“We analyse the intrinsic and extrinsic mechanisms involved in metastatic dissemination, aiming to develop novel therapeutic targets.”

Graduate Students
Enrique Bastón, Elena Castellano (until December), Juan García-Agulló, Teresa González (until March), Alberto Hernández

Technicians
Sara Sánchez-Redondo, Vanesa Santos

Students in Practice
Eduardo Garvín (until Feb.) (Univ.

Francisco de Vitoria, Madrid, Spain), Sandra López (March-July) (Bachelor's Degree Final Project Student, Univ. Francisco de Vitoria, Madrid, Spain)

Visiting Scientist
Marion Pascale (January-June) (Instituto de Oncología Vall D'Hebron, Barcelona, Spain)

RESEARCH HIGHLIGHTS

Relevance of extracellular vesicles in tumour cell evolution and metastasis. Extracellular vesicles (EVs) contain different biomolecules including DNA and RNA. However, the importance of nucleic acids in EVs and the consequences of its transfer to the tumour microenvironment are poorly understood. We are exploring the influence of tumour-shed EVs in surrounding tumour cells, stroma, and healthy tissue during tumour progression. In addition, we are analysing EV-associated nucleic acids as surrogate markers of tumour progression, developing highly-sensitive methods for detecting minimal residual disease and metastatic risk. Moreover, we are currently investigating the role of extracellular vesicles in prostate cancer premetastatic niche formation through the analysis of their molecular cargo and their influence in the lymph node microenvironment. We aim to define novel biomarkers of early dissemination by liquid biopsy and potentially new anti-metastatic therapies.

Understanding the link between obesity and breast cancer metastasis. Since obesity is linked to hypercoagulability and increased risk of breast cancer, we are evaluating if high-fat diet (HFD) influences breast cancer metastasis. We observed that HFD increases tumour-platelet-endothelial cell interaction favouring tumour cell homing and metastasis. Importantly, our data support that anti-platelet therapies reduce tumour cell homing and metastasis in HFD-fed mice, supporting the observation that anticoagulant agents or caloric intake reduction could modify premetastatic niche formation, decreasing metastasis in obesity models of triple-negative breast cancer (TNBC).

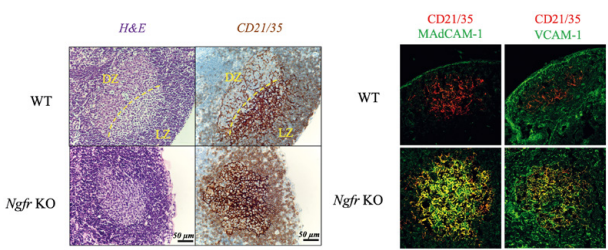


FIGURE 1 *Ngfr* loss leads to lymph node germinal center (GC) hyperproliferation, aberrant structure, and loss of polarisation. Representative H&E and CD21/35 IHC staining (left panels) and immunofluorescence (right panels) of the indicated markers. Immunised wt mice and *Ngfr* KO germinal centres are shown in left panels. Yellow dashed lines divide GC dark zone (DZ) and light zone (LZ). Wt and KO *Ngfr* KO GCs are shown in right panels analysing the expression of the follicular dendritic cell activation markers CD21/35 (red), MadCAM1 or VCAM1 (green); co-expression of markers is shown in yellow. Observe the lack of polarisation and structure in *Ngfr* KO GCs.

Defining the role of NGFR in tumour progression, lymphoproliferative diseases, and autoimmunity. NGFR is emerging as a key gene for metastatic spread and therapy resistance in several tumour types. We are analysing the role of NGFR in tumour metastasis and developing new therapies targeting NGFR to improve immunotherapy treatment in metastatic melanoma and other tumours such as oral squamous cell carcinomas (OSCC) and bladder carcinomas. Moreover, our data support a novel role for NGFR regulating immunity and cell proliferation in lymph nodes, suggesting an important role in follicular lymphoma or autoimmune disorders (FIGURE 1). ■

PUBLICATIONS

- Carretero-González A, Hergueta-Redondo M, Sánchez-Redondo S, Ximénez-Embún P, Manso Sánchez L, Gil EC, Castellano D, de Velasco G, Peinado H (2022). Characterization of plasma circulating small extracellular vesicles in patients with metastatic solid tumors and newly diagnosed brain metastasis. *Oncoimmunology* 11, 2067944.
- González-Muñoz T, Kim A, Ratner N, Peinado H (2022). The need for new treatments targeting MPNST: the potential of strategies combining MEK inhibitors with antiangiogenic agents. *Clin Cancer Res* 28, 3185-3195.
- Leary N, Walser S, He Y, Cousin N, Pereira P, Gallo A, et al. (incl. Peinado H) (2022). Melanoma-derived extracellular vesicles mediate lymphatic remodelling and impair tumour immunity in draining lymph nodes. *J Extracell Vesicles* 11, e12197.
- Almansa D, Peinado H, García-Rodríguez R, Casadomé-Perales Á, Dotti CG, Guix FX (2022). Extracellular vesicles derived from young neural cultures attenuate astrocytic reactivity in vitro. *Int J Mol Sci* 23, 1371.
- Cardeñes B, Clares I, Bezos T, Toribio V, López-Martín S, Rocha A, Peinado H,

- Yáñez-Mó M, Cabañas C (2022). ALCAM/CD166 is involved in the binding and uptake of cancer-derived extracellular vesicles. *Int J Mol Sci* 23, 5753.
- Santos-Coquillat A, González MI, Clemente-Moragón A, González-Arjona M, Albaladejo-García V, Peinado H et al. (2022). Goat milk exosomes as natural nanoparticles for detecting inflammatory processes by optical imaging. *Small* 18, e2105421.

PATENT

- Peinado Selgas H, Saragovi HU, García Silva S, Nogués Vera L, Hernández Bar-

- ranco A. THX-B for treating and preventing cancer and metastasis. PCT Application (2022). *PCT/EP2022/070597. WO2023 002008A1*.

AWARDS AND RECOGNITION

- Héctor Peinado:
- XII National Cancer Research Award “Doctores Diz Pintado”, Spain.
- Listed in the “World Ranking Top 2% Scientists”, 2022 edition of the Stanford University list of World Top 2% scientists.

BRAIN METASTASIS JUNIOR GROUP

Manuel Valiente
Junior Group Leader

Post-doctoral Fellows
Mariam Al-Masmudi, Lluís Cordon,
Neibla Priego



OVERVIEW

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is poor given that local therapies (i.e., surgery and radiation) have limited benefit for patients, and the disease inevitably progresses. The rise in the number of patients with brain metastasis is partially due to the increasing number of systemic therapies that work extra-cranially but are unable to provide the same therapeutic benefit in the brain. Consequently, cancer cells present at this secondary site have additional time to evolve and to grow into clinically detectable lesions. In the laboratory, 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 in order to challenge the current status of this unmet clinical need.

“We reported the first strategy involving a liquid biopsy biomarker and a non-toxic radiosensitizer to personalise the use of radiotherapy in patients with brain metastasis.”

Graduate Students
Laura Adriana Álvaro, Ana de Pablos
Aragoneses, Pedro García, Carolina
Hernández, Irene Salgado

Technicians
Patricia Baena, Virginia García-Calvo
(since April), María Perea (until
November), Diana Patricia Retana,
Olivia Ana Sánchez

Lab Administrative Manager
Jorge Guillermo Ortiz (since March)

Francisco de Vitoria, Madrid, Spain)

Visiting Student
Irene Cornejo (June-December)
(Summer Traineeship, *Universidad*

RESEARCH HIGHLIGHTS

In 2022, we established a novel research line in Cancer Neuroscience, aiming to understand the biology underlying the neurocognitive impact of brain metastasis.

Among other activities, additional single cell approaches (i.e., spatial transcriptomics) were incorporated into our experimental pipeline.

We also consolidated research findings, with an impact on various aspects relevant for brain metastasis, such as novel strategies for immunotherapy, new cellular targets within the pro-metastatic microenvironment, and an unexpected avenue for preventing metastasis.

And, finally, we consolidated our scientific strategy as a productive source of findings to be translated from bench to bedside. The most recent examples are the clinical studies following from the discovery of a biomarker of radiosensitivity compatible with liquid biopsy (now part of a prospective observational multicentric clinical study) and the clinical trial combining a RAGE inhibitor and radiotherapy (now in phase I/II trial). ■



FIGURE (a) The microenvironment enhances the secretion of S100A9 from cancer cells that binds to RAGE, which could be targeted with a specific inhibitor. (b) Targeting of S100A9 blocks brain metastasis radioresistance. (c) S100A9 is a biomarker of radioresistance from liquid biopsy.

PUBLICATIONS

Monteiro C**, Miarka L**, Perea-García M, Priego N, García-Gómez P, Álvaro-Espinosa L, de Pablos-Aragoneses A, Yebra N, Retana D, Baena P, Fustero-Torre C, Graña-Castro O, Troulé K, Caleiras E, Tezanos P, Muela P, Cintado E, Trejo JL, Sepúlveda JM, González-León P, Jiménez-Roldán L, Moreno LM, Esteban O, Pérez-Núñez Á, Hernández-Lain A, Mazarico Gallego J, Ferrer I, Suárez R, Garrido-Martín EM, Paz-Ares L, Dalmasso C, Cohen-Jonathan Moyal E, Siegfried A, Hegarty A, Keelan S, Varešlija D, Young LS, Mohme M, Goy Y, Wikman H, Fernández-Alén J, Blasco G, Alcázar L, Cabañuz C, Grivennikov SI, Ianus A, Shemesh N, Faria CC, Lee R, Lorigan P, Le Rhun E, Weller M, Soffiatti R, Bertero L, Ricardi U, Bosch-Barrera J, Sais E, Teixidor E, Hernández-Martínez A, Calvo A, Aristu J, Martín SM, Gonzalez A, Adler O, Erez N; RENACER, Valiente M*. Stratification of radiosensitive brain metastases based on an actionable S100A9/RAGE resistance mechanism (2022). *Nat Med* 28, 752-756. (**) Shared authorship. (*) Corresponding author.

- A prospective multicentric observational study will be initiated based on these results.
- A phase I/II clinical trial will be initiated based on these results combining a RAGE inhibitor with radiotherapy.

See also:

- *Nature Medicine*. DOI: 10.1038/s41591-022-01776-5 (11 April 2022).
- *Cancer Research*. DOI: 10.1158/0008-5472.CAN-82-11-BI (6 June 2022).

Zhu L, Retana D, García-Gómez P, Alvaro-Espinosa L, Priego N, Masmudi-Martín

PATENT

Zhu L, Graña-Castro O, Valiente M (2022). Signature for the prognosis of brain metastasis relapse after brain surgery. *EP 22382484.8*.

AWARDS AND RECOGNITION

Manuel Valiente:

- Chair of the EANO Scientific Committee, the European Association of Neuro-Oncology.
- Board Member-Elect of the Metastasis Research Society (MRS).
- EACR Reviewers' Panel (panel member), the European Association for Cancer Research.

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, Nerea Deleyto, Elena
Fernández (since October), Ana
Belén Plata, 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, and 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 combine mouse genetics and cell biological tools to gain insight into the genetic and environmental corruptions of nutrient signalling cascades,

“We are beginning to understand how excessive nutrient levels deregulate cellular metabolism as well as cell-to-cell and interorgan communication, contributing to tumour development and the process of ageing.”

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

Technicians
Alba Sanz

**Titulado Superior (Advanced Degree)*

Students in Practice
Elena Fernández (until June)

(Master's Thesis, *UCM*, Madrid, Spain), Paula Seghers (since Oct.) (Undergraduate Student, *Universidad Politécnica de Madrid*, Spain), Andrea Pinilla (since Nov.) (Undergraduate Student *Universidad Industrial de Santander*, Spain),

Camila Silva (until Aug.) (Master's Thesis, *UAM*, Madrid, Spain)

Visiting Scientists
Cristina Lebrero and Ana Ortega (*Centro de Biología Molecular Severo Ochoa, CBMSO*, Madrid, Spain),

Sebastian Thompson (*IMDEA Nanociencia* Institute, Madrid, Spain)

RESEARCH HIGHLIGHTS

Cellular nutrients, such as amino acids and glucose, and systemic metabolic hormones such as insulin, are key mediators of cellular metabolism by control of the mTORC1 kinase, a master switch for most anabolic processes in the cell. We and others have previously dissected the impact of deregulated nutrient signalling (N-ON mice, mimicking a chronic increase in intracellular nutrient levels) and deregulated hormonal signalling (H-ON mice, mimicking chronically high levels of insulin signalling) in the mouse liver. While genetic activation of either input resulted important to unleash the metabolism of the fasted state, chronic nutrient surplus in humans typically causes synchronous, concomitant activation of nutrient and hormonal signalling. Thus, we generated a mouse strain harbouring deregulated nutrient and hormonal signalling to mTORC1 in hepatocytes (N+H-ON). Genetic activation of either nutrient or hormonal signalling on their own resulted in high mTORC1 activity, regardless of the fed/fasted state of the mice. To our surprise, simultaneous activation of both nutrient and hormonal signalling (N+H-ON) resulted in a minimal additional increase in mTORC1 signalling, as compared to either H-ON or N-ON livers (FIGURE 1A). In contrast to this mild increase, the livers of the N+H-ON mice showed multiple evidence of a synergic interaction between nutrient and hormonal signalling. These include a large increase in liver size, accumulation of several markers of liver damage, and aberrant bile acid and bilirubin metabolism (FIGURE 1B, C and D). In addition, N+H-ON mice experience rapid development of liver carcinomas, starting at 15 weeks of age (FIGURE 1E). Pharmacological suppression of mTORC1 by rapamycin (FIGURE 1F) dramatically delays

tumorigenesis and corrects metabolic defects, while chronic restriction in caloric intake (diminishing circulating nutrients and circulating growth factors) fails to correct the phenotype (FIGURE 1G). These findings support a critical role for nutrient and hormonal signalling through mTORC1 in the beneficial effects of dietary restriction. ■

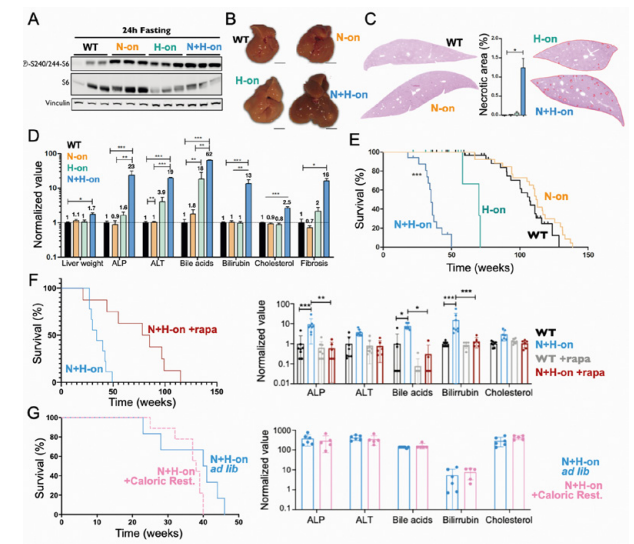


FIGURE 1 (A) Increase in mTORC1 activity (seen by phospho-S6) in livers from fasted N+H-ON mice. (B) Enlarged N+H-ON livers. (C & D) Readouts of liver damage in livers from N+H-ON mice. (E) Premature death of N+H-ON mice due to liver carcinomas. Rapamycin (F) corrects survival and readouts of liver damage in N+HON mice, but caloric restriction (G) does not.

PUBLICATIONS

- Fernández LP*, Deleyto-Seldas N*, Colmenarejo G, Sanz A, Wagner S, Plata-Gómez AB, Gómez-Patiño M, Molina S, Espinosa-Salinas I, Aguilar-Aguilar E, Ortega S, Graña-Castro O, Loria-Kohen V, Fernández-Marcos PJ, Alejo Efeyan A# and Ana Ramírez de Molina* (2022). Foliculin-interacting protein FNIP2 impacts on overweight and obesity through a polymorphism in a conserved 3' untranslated region. (*) Co-first authors. (**) Co-last and Co-corresponding authors. *Genome Biology* 23, 230.
- Vivas-García Y, Efeyan A (2022). The metabolic plasticity of B cells. *Front Mol Biosci* 9, 991188.
- Ong YT, Andrade J, Armbruster M, Shi C, Castro M, Costa ASH, Sugino T, Eelen G, Zimmermann B, Wilhelm K, Lim J, Watanabe S, Guenther S, Schneider A, Zanconato F, Kaulich M, Pan D, Braun T, Gerhardt H, Efeyan A, Carmeliet P, Piccolo S, Grosso AR, Potente M (2022). A YAP/TAZ-TEAD signalling module links endothelial nutrient acquisition to angiogenic growth. *Nat Metab* 4, 672-682.
- Barradas M, Plaza A, Colmenarejo G, Lázaro I, Costa-Machado LF, Martín-Hernández R, Micó V, López-Aceituno JL, Herranz J, Pantoja C, Tejero H, Díaz-Ruiz A, Al-Shahrour F, Daimiel L, Loria-Kohen V, Ramirez de Molina A, Efeyan A, Serrano M, Pozo OJ, Sala-Vila A, Fernandez-Marcos PJ (2022). Fatty acids homeostasis during fasting predicts protection from chemotherapy toxicity. *Nat Commun* 13, 5677.
- Di Lorenzo G, Iavarone F, Maddaluno M, Plata-Gómez AB, Aureli S, Quezada Meza CP, Cinque L, Palma A, Reggio A, Cirillo C, Sacco F, Stolz A, Napolitano G, Marin O, Pinna LA, Ruzzene M, Limongelli V, Efeyan A, Grumati P, Settembre C (2022). Phosphorylation of FAM134C by CK2 controls starvation-induced ER-phagy. *Sci Adv* 8, eabo1215.

AWARDS AND RECOGNITION

- Yurena Vivas was recipient of a *Fundación Domingo Martínez* CNIO Friends Fellowship.
- Ana Belen Plata received an Award for best Selected Short Talk in the EMBO Meeting on Energy Balance in Metabolic Disorders, October 2022, Torremolinos, Spain.
- Nerea Deleyto, excellent *Cum Laude* thesis defence and 'Outstanding PhD Thesis' award in the field of molecular biosciences, Autonomous University of Madrid, Spain.

CANCER IMMUNITY JUNIOR GROUP

María Casanova-Acebes
Junior Group Leader

Graduate Students
Eduardo Garvín (since December),
Federico Lupo (until April), Mariola
Munárriz (since November), Enrique
Nogueira (until October)



OVERVIEW

The Cancer Immunity lab studies myeloid cells in the different tumour microenvironments. By focusing on the remarkable heterogeneity of these cells in a tissue-based manner, we aim to uncover their functional roles in shaping T cell responses.

First, we focus on how myeloid training can impact long-term anti-tumour responses. Next, we study how resident macrophages in the lung and in the ovary shape tumour-associated fibroblasts and metabolic responses, respectively. Lastly, we analyse how circadian biology impacts the initiation, progression and unresponsiveness to current therapies in lung cancer.

“Our laboratory is dissecting novel modulators of tumour immunity by analysing the crosstalk of myeloid cells with the stroma and other physiological cues, such as time-dependency of immune responses and diet-modulatory effects on suppressive and malignant haematopoiesis in solid tumours.”

Technicians
Nines Sanguino Acosta (until October), Sheila Artesero (since April)

Bioinformatician
Gonzalo Soria (since April)

Students in Practice
Sheila Artesero (since May) (Master’s Thesis, *ENS-ISCIII*, Madrid, Spain),

Lucía Córdoba (until April) (Bachelor’s Degree Final Project, *UCM*, Madrid, Spain), Ainhoa Muñoz (until May) (Bachelor’s Degree Final Project, *UAM*, Madrid, Spain)

HIGHLIGHTS

During 2022, we consolidated our laboratory and achieved competitive national and international funding.

In 2023, we aim to expand our team and to continue to fight for cancer cures using innovative myeloid targeting. ■

We also hosted and trained 2 bioinformaticians, 2 medical doctors and 3 undergraduate students.

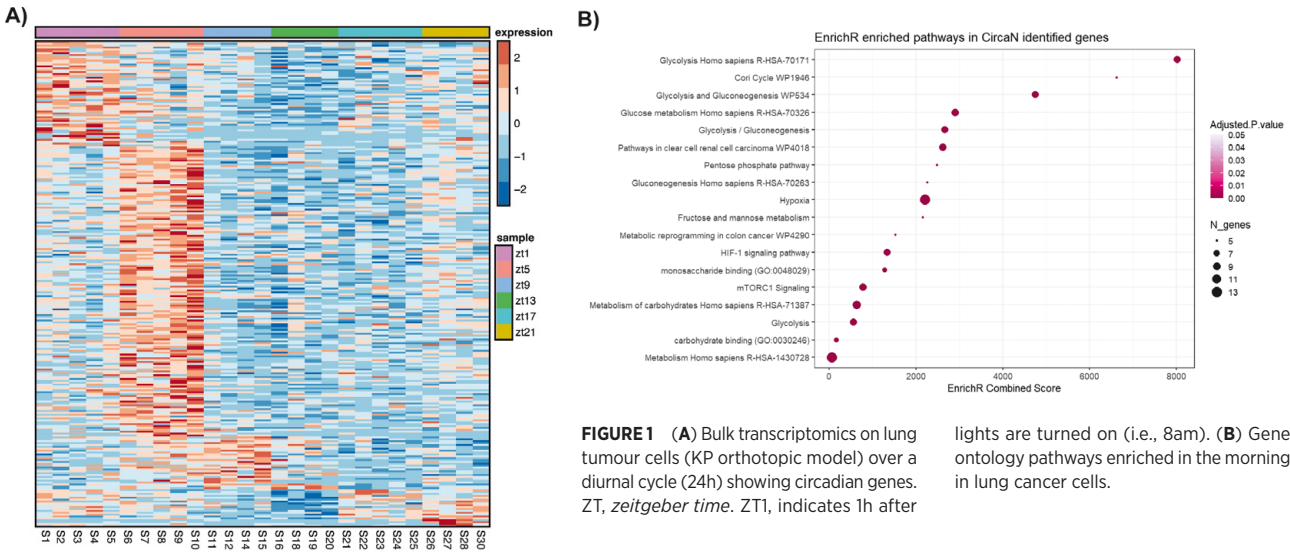


FIGURE 1 (A) Bulk transcriptomics on lung tumour cells (KP orthotopic model) over a diurnal cycle (24h) showing circadian genes. ZT, *zeitgeber* time. ZT1, indicates 1h after lights are turned on (i.e., 8am). (B) Gene ontology pathways enriched in the morning in lung cancer cells.

- **PUBLICATION**
Grout JA, Sirven P, Leader AM, Maskey S, Hector E, Puisieux I, Steffan F, Cheng E, Tung N, Maurin M, Vaineau R, Karpf L, Plaud M, Begue AL, Ganesh K, Mesple J, Casanova-Acebes M, Tabachnikova A, Keerthivasan S, Lansky A, Le Berichel J, Walker L, Rahman AH, Gnjatich S, Girard N, Lefevre M, Damotte D, Adam J, Martin JC, Wolf A, Flores RM, Beasley MB, Pradhan R, Muller S, Marron TU, Turley SJ, Merad M, Kenigsberg E, Salmon H (2022). Spatial positioning and matrix programs of cancer-associated fibroblasts promote T-cell exclusion in human lung tumors. *Cancer Discov* 12, 2606-2625.
- **AWARDS AND RECOGNITION**
XXII Beca FERO 2022 in Translational Oncology Research, FERO Foundation for Oncology Research, Spain.
Education Committee Member, AACR Annual Meeting 2023.

STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA Programme Director



The Programme’s research areas and strategic goals

Scientists at the Structural Biology Programme (SBP) use the enormous opportunities provided by advances in structural and computational biology to improve our understanding of fundamental processes in the origin and progression of cancer, and to generate new knowledge and tools that can ultimately benefit patients. The Programme currently encompasses 5 Groups and 5 Units organised according to 2 major strategic lines: (a) structural biology and (b) computational and cancer genomics. The strategic line in structural biology 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). Their main aim is to determine the structures of proteins and macromolecular complexes relevant in cancer in order to resolve how they work and to support drug discovery efforts. The strategic line in computational and cancer genomics consists of 2 groups (Computational Cancer Genomics and Computational Oncology) and 1 Unit (Bioinformatics). They use bioinformatics, computational biology, and cancer genomics to better understand the complexity of cancer, predict therapy responses, design new therapeutic strategies, and develop new tools. In addition, 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 during 2022

During 2022, scientists at SBP made interesting discoveries in several areas of cancer research and developed new tools. The Computational Oncology Group evaluated chromosomal instability across thousands of tumours, defining “signatures” with predictive value for drug response and possible new drug targets. They also developed a computational tool to help select cell lines for cancer research. The Bioinformatics Unit studied the clinical relevance of tandem exon duplication-derived substitutions in cancer, developed bioinformatics tools to help clinicians to prioritise available drugs for treatment, and assisted several groups across the CNIO. The

“During 2022, research at SBP generated knowledge on fundamental processes in cancer and about the proteins involved, and developed new tools aiming to help cancer research and eventually patients.”

Kinases, Protein Phosphorylation and Cancer Group integrated structural, computational and biochemical data to identify a druggable vulnerability in the RET kinase that could serve to search for new inhibitors useful for patients with RET-driven cancers. The Macromolecular Complexes in DNA Damage Response Group and the Genome Integrity and Structural Biology Group used cryoEM to understand the assembly of the spliceosome, DNA replication, and cytosolic DNA sensing. The Ku70-Ku80 complex is part of an innate immunity system that serves against viral infection and contributes to autoimmune diseases and cancer. The Macromolecular Complexes in DNA Damage Response Group discovered how some viruses inactivate the capacity of Ku70-Ku80 to detect cytosolic DNA. The Electron Microscopy, Protein Production and Protein Crystallography Units made substantial contributions to the research of many groups across the CNIO. The Protein Production Unit also developed nanobodies to be used as probes for triple negative breast cancer imaging. As a final remark, I want to emphasise the involvement of many of the scientists at SBP, especially our junior faculty and the younger members of the groups, in numerous activities to bring science closer to society and make the CNIO a better place to train young scientists. ■

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 Cabezano, María Martínez	Technicians Paloma Escudero (until March) (TS), Ana González	Students in Practice Carmen Arroyo (Feb-June) (Bachelor's Degree Final Project, <i>Univ. Autónoma de Madrid</i> , Spain), Carmen García Martín (March-July) (Master's Thesis, <i>Univ. Complutense de Madrid</i> , Spain)	Visiting Scientists Raquel Arribas (Feb-March) (Univ. of Sussex, UK), Evangelina Nogales (Jan-July) (University of California Berkeley, USA)
Graduate Students Natalia Cuervo, Carmen García (since September), Nayim González	<i>*Titulado Superior</i> (Advanced Degree)		

OVERVIEW

Our Group uses cryo-electron microscopy (cryoEM) to determine the 3D structure of large macromolecular complexes of relevance in cancer at high resolution. 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: the study of i) chaperones essential for the activation of several macromolecular complexes relevant in cancer and ii) 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.

“We have improved our understanding of the molecular mechanisms involved in spliceosome maturation, and cytosolic DNA sensing by the DNA repair protein Ku70/Ku80 and its subversion by some poxviruses.”

RESEARCH HIGHLIGHTS

Understanding the maturation of the spliceosome, a cellular process involved in some types of cancer

Splicing is a cellular mechanism that facilitates the reading of DNA and multiplies the number of potential protein sequences in a cell by allowing the synthesis of several different proteins from a single gene. Alternative splicing is an extraordinarily complex process that requires the coordinated action of multiple proteins, each specialised in very specific functions. These proteins are assembled and matured, forming large macromolecular complexes, a process that is tightly controlled, and any failure can result in genetic diseases (FIGURE 1A). Several types of cancer present failures in the splicing processes, which is an advantage for tumour cells since these failures improve their rate of survival.

We have investigated some of the factors that enable the assembly and maturation of the spliceosome, particularly PRPF8, one of U5 snRNP’s main components. We used biochemistry, interaction mapping, mass spectrometry and cryoEM to study the role of RUVBL1 and RUVBL2 ATPases and the ZNHIT2 protein in the biogenesis of PRPF8. We found that ZNHIT2 forms a network of contacts between several assembly factors required for PRPF8 biogenesis including ECD and AAR2, and that ZNHIT2 connects PRPF8 with the R2TP-HSP90 chaperone machinery, which is required for PRPF8 maturation. In addition, cryoEM showed how ZNHIT2 binds RUVBL1-RUVBL2 and affects the conformation of RUVBL2 (FIGURE 1B), which regulates RUVBL1-RUVBL2 ATPase activity.

Taken together, our results reveal part of the complex mechanisms that regulate the maturation of the splicing machinery, an essential process for the cell that can cause diseases such as cancer when perturbed.

Mechanism that helps some poxviruses to evade our cellular defence system

The Ku70-Ku80 complex is an essential component of the non-homologous end-joining (NHEJ) machinery that repairs DNA double strand breaks. Its structure shows that the protein comprises a preformed ring that can encircle duplex DNA. Ku70-Ku80 is the first protein to detect the presence of a break in the DNA thanks to this capacity to bind DNA like a ring encircles a finger.

Interestingly, Ku70-Ku80 is also present in the cytoplasm of cells, but its role there is not to detect and repair broken DNA but to alert the cell of the presence of viruses and activate

cellular defences. The capacity of Ku70-Ku80 to encircle a linear dsDNA is used in the cytoplasm to detect viral DNA and initiate an inflammatory and innate immunity response. But some of these viruses have evolved countermeasures against these DNA sensors to attempt to block or delay the host immune response and allow the proliferation and spread of the disease. Vaccinia virus (used in the development of the smallpox vaccine and belonging to the poxvirus family) produces 2 proteins, C4 and C16, that bind to Ku70-Ku80 and inactivate its downstream signalling to the cellular immune response; however, the mechanism has not been well understood.

Using cryoEM, we have determined the 3-dimensional structure of C16 and its complex with Ku70-Ku80 (FIGURE 2A). In collaboration with L. H. Pearl’s group (University of Sussex) and the Institute of Cancer Research in UK, we discovered that C16 and C4 proteins produced by the virus act as plugs that insert into the central hole of Ku70-Ku80, which it uses to thread itself into DNA, inhibiting Ku70-Ku80’s ability to recognise viral DNA (FIGURE 2B). The structure of the C16 – Ku70-Ku80 complex was determined at high resolution, which allowed us to identify atomic details of how C16 binds and inactivates Ku70-Ku80, identifying key residues. Interestingly, by comparing the protein sequences of the C4 and C16 homologues in other viruses of the same family, we found that the regions involved in Ku inactivation are conserved in several orthopoxviruses, including smallpox and monkeypox. ■

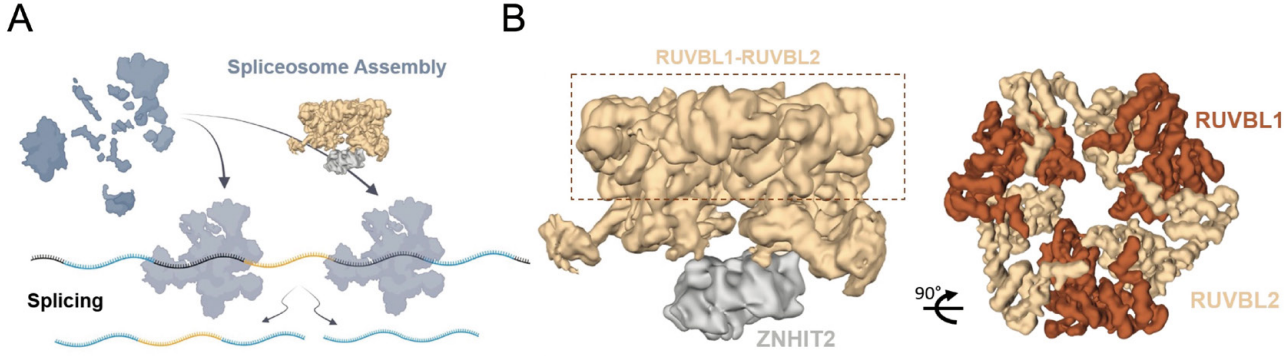
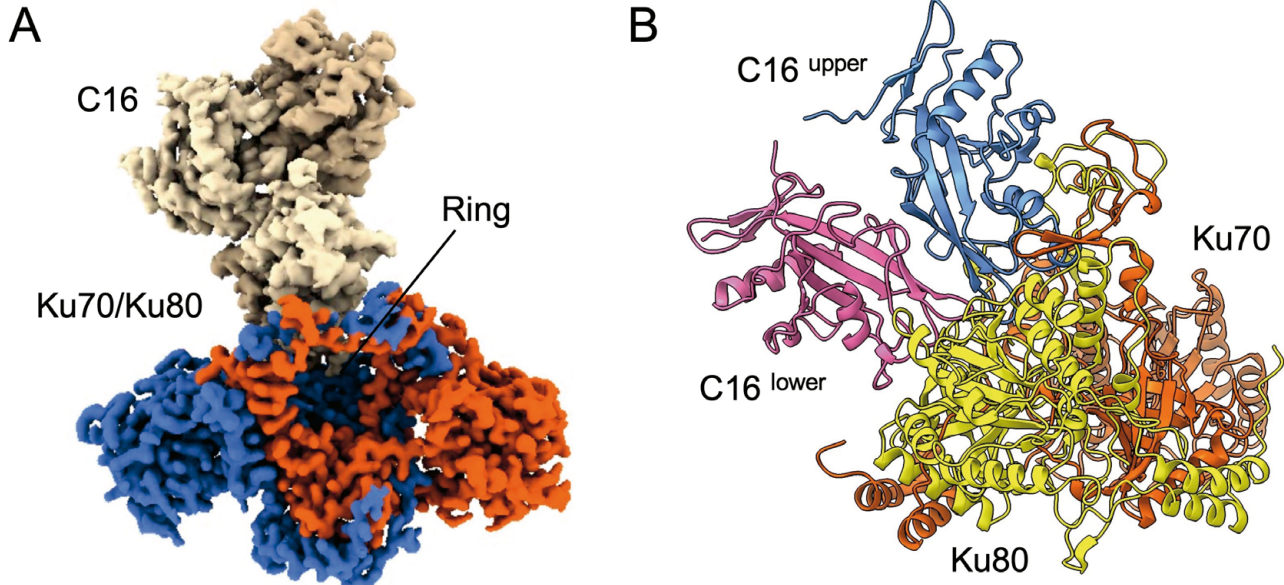


FIGURE 1 RUVBL1, RUVBL2 and ZNHIT2 form a complex required for the maturation of PRPF8 and the spliceosome. (A) Drawing representing the need of several assembly factors during the assembly of the spliceosome. (B) CryoEM map of the RUVBL1-RUVBL2-ZNHIT2 complex. Right panel highlights RUVBL1-RUVBL2 in the complex.



C16 – Ku70/Ku80

FIGURE 2 Structure of C16 – Ku70-Ku80 complex. (A) CryoEM map of the C16 protein from vaccinia bound to Ku70-Ku80. (B) One view of the atomic model of C16 – Ku70-Ku80, showing 2 copies of C16 C-terminal domain binding to Ku70-Ku80.

► **PUBLICATIONS**

► Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver AW, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.

► Rullo-Tubau J, Bartoccioni P, Llorca O, Errasti-Murugarren E, Palacín M (2022). HATs meet structural biology. *Curr Opin Struct Biol* 74, 102389.

► Serna M, González-Corpas A, Cabezudo S, López-Perrote A, Degliesposti G, Zarzuela E, Skehel JM, Muñoz J, Llorca O (2022). CryoEM of RUVBL1-RUVBL2-ZNHIT2, a complex that interacts with

pre-mRNA-processing-splicing factor 8. *Nucleic Acids Res* 50, 1128-1146.

► **AWARDS AND RECOGNITION**

► Marina Serna: *Premio Josep Tormo* Award for Structural Biology 2022, the Spanish Biochemical and Molecular Biology Society (*SEBBM*).

► María Ibarra Dauden: “*Premios Fundación Merck Salud-ASEICA por el Impulso de las Vocaciones Científicas – Investigadoras*”, Merck Salud Foundation and the Spanish Association for Cancer Research (ASEICA).

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellow
Julia M. Contreras



OVERVIEW

Rational and precise targeting of oncogene-driven signalling is a crucial and yet today 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 by combining: molecular biology for the generation of suitable constructs; protein biochemistry and biophysics for protein purification, quality assessment and functional evaluation; mass spectrometry (MS) for the identification and quantification of 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.

Graduate Students
Nicolás Cuesta (until November),
Ana Martín-Hurtado, Moustafa
Ahmed Shehata (until April)

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

Students in Practice
Marina Rodríguez (Bachelor's
Degree Final Project, *Univ. Autónoma
de Madrid*; Master's Thesis, *Univ.
Complutense de Madrid*, Spain),
Alejandro Sánchez (March-

September) (Master's Thesis, *Univ.
de Alcalá de Henares*, 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. In our recent work, we show that c-terminal Tyr 530 is a *de facto* c-Src auto-phosphorylation site with slow time-resolution kinetics and strong intermolecular component. By contrast, activation-loop Tyr 419 undergoes fast kinetics and a cis-to-trans phosphorylation-switch that controls c-terminal Tyr 530 auto-phosphorylation, enzyme specificity, and strikingly, c-Src non-catalytic function as a substrate. In line with this, we visualised by X-ray crystallography a snapshot of Tyr 530 intermolecular phosphorylation in which a c-terminal palindromic phospho-motif flanking Tyr 530 on the substrate molecule engages the G-loop of the active kinase for ready entry prior catalysis. Perturbation of the phospho-motif accounts for c-Src dysfunction as indicated by viral and a colorectal cancer (CRC) associated c-terminal deleted variants. We showed that c-terminal residues 531 to 536 are required for c-Src Tyr 530 and global auto-phosphorylation, and this detrimental effect is caused by the substrate molecule inhibiting allosterically the active kinase. Our work reveals a bi-directional crosstalk between the activation and c-terminal segments that controls the allosteric interplay between substrate and enzyme acting kinases during auto-phosphorylation (Cuesta and Contreras *et al.*, under revision. *BioRxiv*. doi: <https://doi.org/10.1101/2022.10.16.512342>).

2. Structure, function, and pharmacology of protein 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 was established and directed to dissect the functional and structural determinants for two RET oncogenic fusion products, namely CCDC6-RET and KIF5B-RET, which are drivers and therapeutic targets in lung (NSCLC) and thyroid cancers. We have successfully purified these challenging proteins using a baculovirus expression system in different isoforms and length-variants. By applying an integrated approach, we demonstrated that full-length constructs behave like active dimers in solution. Auto-phosphorylation and enzymatic assays demonstrated

fast kinetics compared to wild-type RET, and further phospho-proteomic characterisation by mass spectrometry highlighted important roles for catalytic activity and substrate specificity through unexpected allosteric inputs by distant elements to the catalytic site (Hurtado *et al.*, submitted).

3. Structure-guided drug discovery for next generation protein kinase inhibitors. A third main research line is focused on the exploitation of structural and functional vulnerabilities in RET for the rational design and development of highly specific inhibitors. Our current paradigm is based on the recently developed second generation RET inhibitors LOXO-292 and BLU-667 that showed excellent results in both preclinical models and early clinical trials, resulting in timely FDA approval for the treatment of RET-rearranged or -mutated cancers. 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 functional validation. Following this approach, we identified an allosteric interface in RET with 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 and Contreras *et al.*, *J Adv Res* 2022). This information will be crucial to designing and developing highly specific third generation RET inhibitors able to overcome refractory RET mutations. Based on these results we are optimising chemical scaffolds of second generation RET inhibitors to maximise contacts and interactions with the cryptic pocket, in collaboration with CNIO's Experimental Therapeutics Programme. ■

PUBLICATIONS

- Shehata MA, Contreras J, Martín-Hurtado A, Froux A, Mohamed HT, El-Sherif AA, Plaza-Menacho I (2022). Structural and dynamic determinants for highly selective RET kinase inhibition reveal cryptic drug-gability. *J Adv Res*. PMID: 35595215.
- Cuesta N, Contreras J, Sánchez-Waldermer J, Soriano-Maldonado P, Martín-Hurtado A, Muñoz IG, Llimargas M, Muñoz J, Plaza-Menacho I (2022). An allosteric switch between the activation loop and

a c-terminal palindromic phospho-motif drives c-Src function. *BioRxiv*. doi: <https://doi.org/10.1101/2022.10.16.512342>.

PATENT

- Plaza Menacho I. Identification of a cryptic and druggable pocket in the active site of RET with therapeutic potential: the post-lysine pocket. PCT application (2022). PCT/EP2022/077036. WO2023052462A1.

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno

Graduate 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, when these do not work correctly, genetic information may be damaged or lost, ultimately leading to disease. Deregulation and malfunction of the protein machinery that safeguards our genome are all hallmarks 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 goes wrong with these molecular machines, and when, so 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

“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.”

activities. To understand how they work, we use cryo-electron microscopy (cryo-EM) and biochemistry in an integrative approach. Beyond fundamental research, this structural information provides the necessary detail for drug development.

Technician
Araceli Grande (TS)*

*Titulado Superior (Advanced Degree)

Students in Practice
Emma Areán (Bachelor's Degree Final Project; Master's Thesis, *Univ. Complutense de Madrid, Spain*),
Carlos Chacón (until June) (Bachelor's Degree Final Project,

Univ. Autónoma de Madrid, Spain),
Raquel Cuesta (Bachelor's Degree Final Project; Master's Thesis, *Univ. Complutense de Madrid, Spain*),
Marta Seijo (*Univ. Complutense de 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. Importantly, mtDNA defects have been linked to other prominent diseases, including Parkinson's and Alzheimer's disease, autism spectrum disorders, diabetes, 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 TTAGGG 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 a 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 by 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 will be able to unveil their molecular mechanisms, rationalise pathological mutations and their physiological consequences, and aid in the development of future cancer therapeutic strategies. ■

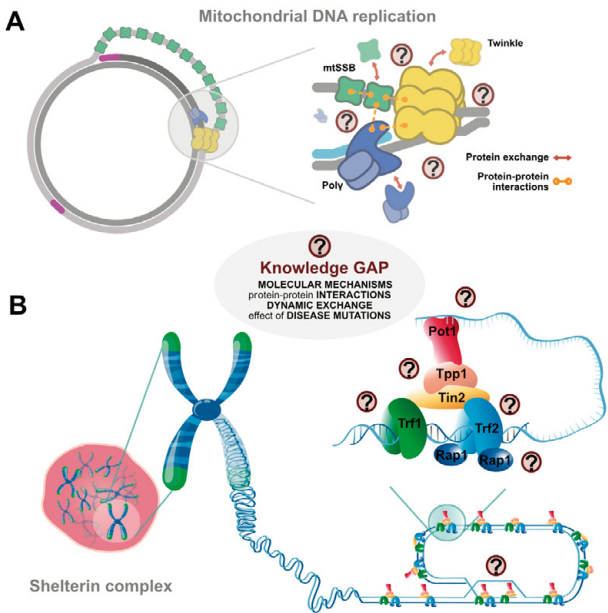


FIGURE 1 (A) Mitochondrial DNA replication machinery. (B) The shelterin complex shapes, protects, and regulates enzymatic activities at telomeres. Several key aspects of their regulation, and the molecular mechanisms through which both protein complexes exert their activities, remain unknown.

PUBLICATION

Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver A.W, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.

PATENT

Lamers MH, Fernández-Leiro R (2022). Device and method for cryogenic electron microscopy sample preparation. *Dutch Patent Application No. 2033291*.

COMPUTATIONAL CANCER GENOMICS JUNIOR GROUP

Solip Park
Junior Group Leader

Post-Doctoral Fellow
Seulki Song (since October)



OVERVIEW

Cancer is a complex disease whereby cells grow and reproduce uncontrollably. One important feature necessary to understand cancer is its heterogeneity, which indicates that the effect of alterations could be different depending on the cellular context. In the Computational Cancer Genomics (CCG) Lab, we aim to understand the context-dependent cancer fitness landscape both by applying a computational approach and by setting up experimental collaborations. For example, we are specifically interested in changing the cancer fitness landscape depending on time, by analysing the associations between germline variants and somatic alterations, or by comparing the differences between the primary tumour and metastasis. In addition, we aim to further pursue how protein-protein interaction networks of cancer driver genes can be perturbed by their somatic or germline variants. We expect that our

“Through large-scale cancer genomics analysis, we aim to understand the complete cancer fitness landscape, analysing both germline variant- and somatic mutation-based perturbation of protein interaction.”

context-dependent cancer fitness landscape will provide a crucial direction for personalised medicine, since we are aiming to address the heterogeneity across patients, conditions, and cellular contexts.

Graduate Students
Pelayo González de Lena, Moustafa
Ahmed Mohamed Shehata (since
May)

Technicians
Luis García (until June), Manuel
Moradiellos (since August), Laia Ollé
(since July)

Visiting Master's Students
Adrián Maqueda (since September)
(Master's Thesis, *Univ. Autónoma de
Madrid, Spain*), Laia Ollé (February-

May) (Master's Thesis, *Univ.
Politécnica de Madrid, Spain*)

RESEARCH HIGHLIGHTS

Context-specific genetic interaction perturbations

Metastasis is the main cause of death in cancer patients. However, most current cancer consortia have focused on primary cancer states. To gain a better understanding of the context-specific cancer fitness landscape across cancer statements, we systematically measured the association between somatic mutations and copy-number changes within the same genes across cancer types and compared their strengths of interaction between cancer statements. We found that several cancer types and cancer genes present significantly different preferences of interaction between mutations and copy-number changes and also proved that these differences are not due to medical treatments or genomic differences (*manuscript in preparation*). We expect that our findings will provide new insights to understand statement-specific perturbations and clues to develop better treatments for cancer patients.

Defining new cancer predisposition genes

Although large-scale cancer genomics data are rapidly accumulating, our understanding of cancer genes is highly biased towards somatic alterations and not germline variants. Germline frequencies are usually low, and there are several technical difficulties associated with their analysis. Since only 130 cancer predisposition genes (CPGs) are currently available, their contributions to cancer risk are underestimated. We hypothesised that germline variants in Mendelian-associated genes (OMIM genes) could contribute to increasing cancer risk. First, we proved that OMIM genes tend to have more pathogenic germline variants in cancer compared to controls (*manuscript under revision*). We then focused on a *PAH* that is associated with phenylketonuria, which presents the strongest enrichment in cancer compared to controls, and this enrichment is reproduced in other cancer data sets. Furthermore, through collaborations in South Korea, we addressed how metabolic dysfunction increases cancer risk experimentally, and we identified the possible contribution of OMIM genes as new CPGs. Currently, we are expanding this concept to predict novel CPGs, not only OMIM genes, by integrating multiple features using a machine learning approach. ■

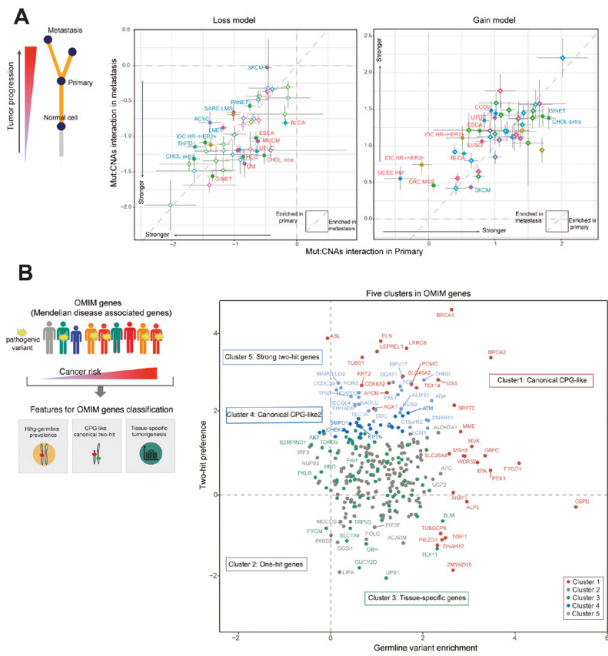


FIGURE 1 Understanding the cancer fitness landscape through both germline and somatic alterations. **(A)** Genetic interaction differences between primary tumours and metastases using 25,000 tumour samples. **(B)** Elucidating the role of Mendelian disease-associated genes as possible new cancer predisposition genes.

COMPUTATIONAL ONCOLOGY JUNIOR GROUP

Geoff Macintyre
Junior Group Leader

Research Scientist
Maria José García (until October)

Post-Doctoral Fellow
Bárbara Hernando



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 have developed a new computational framework to evaluate the extent, diversity and origin of chromosomal instability pan-cancer.”

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.

Graduate Students
Blas Chaves, María Escobar, Ángel Fernández (since May), David Gómez

Technician
Alice Nicole Cádiz (PEJ, CAM)*
*Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Community of Madrid)

Students in Practice
Diego García (since February) (Master's Thesis, *Universidad Politécnica de Madrid*, Spain), Sara Vettorazzo (August-December) (Erasmus + Fellowship, *Università di Trento*, Italy)

RESEARCH HIGHLIGHTS

2022 was a great year for the Computational Oncology Group. We welcomed a new lab member and saw our senior staff scientist, Maria José Garcia, move to CSIC as a PI! Another key highlight was seeing our CIN signature study published in *Nature*. We also secured the front cover artwork with an abstract interpretation of the research painted by Geoff's cousin Julian Aubrey Smith (FIGURE 1). This study was the culmination of years of computational work during the pandemic period in collaboration with the University of Cambridge. We developed a framework to evaluate the diversity and origin of chromosomal instability pan-cancer, identifying 17 genomic signatures of different types of CIN. Barbara was crucial to the success of the project demonstrating that the signatures can predict drug response and identify new drug targets. The work attracted significant press coverage, including appearing on the front page of *El País*. Barbara is now extending this technology to work at single cell resolution to enable a number of other projects in the lab.

Ángel joined the lab as a computational PhD student to understand how CIN contributes to tumour evolution. Working closely with Barbara, they have recently developed an approach to forecast oncogenic amplification in tumours using the CIN signatures. David is combining this approach with his new method to extract robust copy number profiles from targeted gene sequencing data to forecast MET amplification-driven resistance to EGFR inhibitors in lung cancer.

Maria and Blas were busy performing single cell DNA sequencing of organoids and KO cell lines – expect exciting



FIGURE 1 Our CIN signature article on the cover of *Nature*.

results in 2023! Two masters' students also completed their projects: Diego developed a new method for mis-match repair deficiency prediction, and Sara uncovered new patterns of CIN in premalignant oesophageal lesions.

Much of this work will be submitted in 2023 for publication, so hopefully there will be another great year ahead! ■

PUBLICATIONS

► Drews RM, Hernando B, Tarabichi M, Haase K, Lesluyes T, Smith PS, Morrill Gavarró L, Couturier DL, Liu L, Schneider M, Brenton JD, Van Loo P, Macintyre G*, Markowetz F* (2022). A pan-cancer compendium of chromosomal instability. *Nature* 606, 976-983. *Co-corresponding authors.

► Cheng Z, Mirza H, Ennis DP, Smith P, Morrill Gavarró L, Sokota C, Giannone G, Goranova T, Bradley T, Piskorz A, Lockley M; BriTROc-1 Investigators; Kaur B, Singh N, Tookman LA, Krell J, McDermott J, Macintyre G, Markowetz F, Brenton JD, McNeish IA (2022). The genomic landscape of early-stage ovarian high-grade serous carcinoma. *Clin Cancer Res* 28, 2911-2922.

► Chaves-Urbano B, Hernando B, García MJ, Macintyre G (2022). CNpare: matching DNA copy number profiles. *Bioinformatics* 38, 3638-3641.

► Gallego A, Mendiola M, Hernando B, Berjon A, Cadiz A, Chaves-Urbano B, Heredia-Soto V, Spagnolo E, Hernández Gutiérrez A, Hardisson D, Macintyre G, Redondo A, García MJ (2022). Prognostic markers of inflammation in endometrioid and clear cell ovarian cancer. *Int J Gynecol Cancer* 32, 1009-1016.

► Vias M, Gavarró LM, Sauer CM, Sanders D, Piskorz AM, Couturier DL, Ballereau S, Hernando B, Hall J, Correia-Martins F, Markowetz F, Macintyre G, Brenton JD (2022). High-grade serous ovarian carcinoma organoids as models of chromosomal instability. *bioRxiv*. doi: <https://doi.org/10.1101/2022.09.01.506155>.

► Smith PS, Bradley T, Gavarró LM, Goranova T, Ennis D, Mirza H, De Silva D, Piskorz AM, Al-Khalidi S, Sauer C, Funingana IG, Reinus M, Giannone G, Lewsley LA, Stobo J, McQueen J, Bryson G, Eldridge M, Macintyre G, Markowetz F, Brenton JD, McNeish IA, The BriTROc Investigators (2022). The genomic landscape of recurrent ovarian high grade serous carcinoma: the BriTROc-1 study. *medRxiv*. doi: <https://doi.org/10.1101/2022.10.21.22280992>.

► PATENT

► Macintyre G, Drews R, Markowetz F, Hernando B. Method of characterising a DNA sample. PCT application (2022). *PCT/EP2022/077473*. WO2023057392A1.

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 through the Structural Biology Programme. It provides CNIO researchers with equipment and experimental support for biophysical techniques used in studies of molecules involved in cancer. This includes the *in vitro* characterisation of i) the structure and dynamics of proteins using 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. The Unit is also endowed with a state-of-the-

“In 2022, we characterised biophysically 2 nanobodies targeting a matrix metalloproteinase and quantified the affinities and association and dissociation kinetics of both complexes. These results will help to validate the nanobodies as potential tools for breast cancer diagnosis.”

art, multiple-well microplate reader equipped with diverse detectors (absorbance; intensity, polarisation and time-resolved fluorescence; luminescence; and AlphaScreen) for in-solution and adherent cells measurements.

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 and multi-angle static light scattering (MALS) equipments, 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 Group, Metabolism and Cell Signalling Group, Experimental Oncology Group) the Structural Biology Programme used these technologies throughout the year. For example, in collaboration with the Protein Production and Molecular Imaging Core Units, using nanoDSF and MALS, we validated that 2 anti-MT1-MMP nanobodies are well-folded, stable and monomeric proteins (FIGURE 1, panels A and B). In addition, we used SPR to characterise the affinity and kinetics of the interaction of each antibody with human MT1-

MMP protein (FIGURE 1, panels C and D). This research is useful to further develop labelled nanobodies as PET probes for triple negative breast cancer imaging.

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 the screening of small molecule protein binders (together with the Experimental Therapeutics Programme), as well as for metabolite quantification that in 2022 was done in collaboration with the Growth Factors, Nutrients and Cancer, and Transformation and Metastasis Groups (Molecular Oncology Programme) and the Hereditary Endocrine Cancer Group (Human Cancer Genetics 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. ■

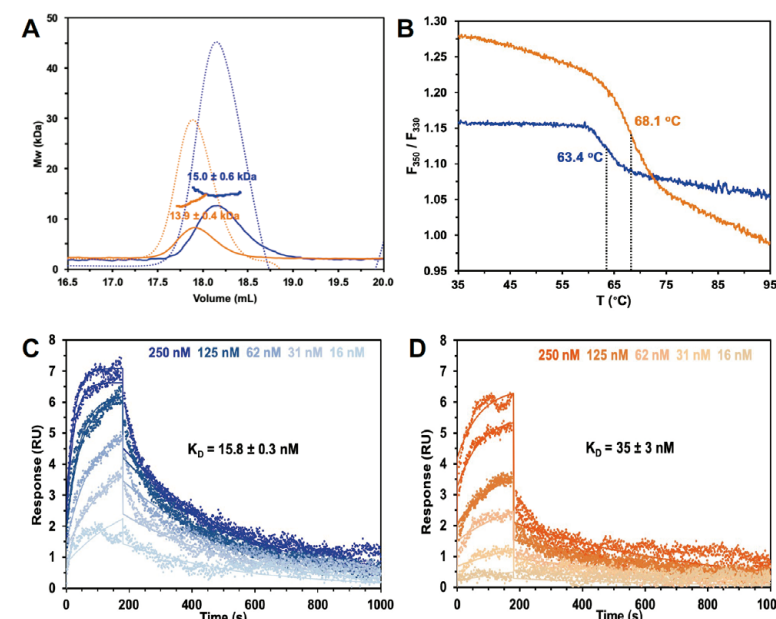


FIGURE 1 Characterisation of 2 nanobodies targeting a matrix metalloproteinase. **(A)** Superimposed MALS chromatographic traces of light scattering (solid lines), refractive index (dashed lines) and the calculated molecular weight from each nanobody. **(B)** NanoDSF thermal unfolding profiles of the 2 nanobodies. Inflection

temperatures (T_i) are shown. **(C, D)** Overlay of the SPR sensorgrams and corresponding kinetic fits to a 1:1 binding model (solid lines) for the interaction of each nanobody with MT1-MMP. Nanobody concentrations and the calculated dissociation constants (K_D) of the complexes are indicated.

PUBLICATIONS

- Garrido A, Kim E, Teijeiro A, Sánchez Sánchez P, Gallo R, Nair A, Matamala Montoya M, Perna C, Vicent GP, Muñoz J, Campos-Olivas R, Melms JC, Izar B, Schwabe RF, Djouder N (2022). Histone acetylation of bile acid transporter genes plays a critical role in cirrhosis. *J Hepatol* 76, 850-861.
- García-Alonso S, Mesa P, Ovejero LP, Aizpurua G, Lechuga CG, Zarzuela E, Santiveri CM, Sanclemente M, Muñoz J, Musteanu M, Campos-Olivas R, Martínez-Torrecuadrada J, Barbacid M, Montoya G (2022). Structure of the RAF1-HSP90-CDC37 complex reveals the basis of RAF1 regulation. *Mol Cell* 82, 3438-3452.
- 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 (2022). The MASTL/PP2A cell cycle kinase-phosphatase module restrains PI3K-Akt activity in an mTORC1-dependent manner. *EMBO J*. PMID: 36354735.
- Chaves-Arquero B, Martínez-Lumbreras S, Camero S, Santiveri CM, Mirassou Y, Campos-Olivas R, Jiménez MA, Calvo O, Pérez-Cañadillas JM (2022). Structural basis of Nrd1-Nab3 heterodimerization. *Life Sci Alliance* 5, e202101252.
- Mulero F, Oteo M, Garaulet G, Magro N, Rebollo L, Medrano G, Santiveri C, Romero E, Sellek RE, Margolles Y, Campos-Olivas R, Arroyo AG, Fernández LA, Morcillo MA, Martínez-Torrecuadrada JL (2022). Development of anti-membrane type-1-matrix metalloproteinase nanobodies as immunoPET probes for triple negative breast cancer imaging. *Front Med* 9, 1058455.

BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Research Scientist
Michael Tress

Graduate Students
Santiago García (PEJ, CAM)*, María
José Jiménez, Laura Martínez,
Fernando Pozo (until September)

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



OVERVIEW

Bioinformatics is a key discipline for understanding the cancer genome and for the future of cancer therapeutics. Bioinformatics-based approaches have the ability to transform the vast amount of biological data into comprehensible models that provide a deep understanding of cancer disease and the complex genotype-phenotype relationships needed to identify molecular cancer-driving alterations and novel therapeutic targets.

The CNIO Bioinformatics Unit (BU) has several objectives: (i) to develop new computational methodologies and bioinformatics tools to enable the integration of biological and clinical data, (ii) to achieve genome analysis in cancer patients’ data to identify new biomarkers and drug response mechanisms, (iii) to provide bioinformatics support with data analysis and interpretation using computational and statistical

“We develop bioinformatics methods to therapeutically characterise differentially drug-responsive tumour cell subpopulations, proposing cell-specific anticancer treatments at single-cell resolution.”

methods, (iv) to maintain the scientific computing facilities at the CNIO, and (v) to provide training in bioinformatics tools and methods.

Bioinformaticians
Ruth Álvarez (since Nov.) (TS)*, Daniel Cerdán (TS)*, Tomas Di Domenico (TS)*, Coral Fustero (until June) (TS)*, Gonzalo Gómez (TS)*, Osvaldo Graña (until Aug.) (TS)*,

Elena Piñeiro (TS)*, Francisco J Soriano (since Oct.) (TS)*

*Titulado Superior (Advanced Degree)

Students in Practice
Lucas Friedman (June-Dec.) (Master’s

in Biocomputing, *ENS-ISCIII*, Madrid, Spain), Paula Gómez (June-Aug.) (Summer Trainee, *Univ. Carlos III de Madrid*, Spain), Adel Samir Saleh (June-Aug.) (Summer Trainee, Nile University, Abuja, Nigeria), Víctor

Sánchez (June-Dec.) (*ENS-ISCIII*, Madrid, Spain)

Visiting Scientist
Carlos Carretero (*Hosp. 12 de Octubre*, Madrid, Spain)

RESEARCH HIGHLIGHTS

In 2022, the Bioinformatics Unit published more than 10 peer-reviewed articles as a result of our ongoing research projects and scientific collaborations (see the full list of our activities in our website: <https://bioinformatics.cnio.es/>). We studied cancer cell biology, inter- and intra-tumour heterogeneity, and drug response, using single-cell and spatial transcriptomics. In particular, we reviewed current approaches for the selection of anticancer therapies based on the type of tumour heterogeneity being targeted and the type of sequencing data available (FIGURE 1, Jiménez-Santos *et al.* 2022).

During 2022, our group participated in whole-genome screenings, identifying N-glycosylations as a genetic and therapeutic vulnerability in calreticulin-mutant myeloproliferative neoplasms, thus advancing the development of clonally selective treatments for this disease (Jutzi *et al.* 2022). We also collaborated in identifying the deficiency in the FBXW7 tumour suppressor gene that leads to multi-drug resistance (Sanchez-Burgos *et al.* 2022). In addition, our laboratory released an updated version of the APPRIS database (Rodríguez *et al.* 2022), which selects principal protein isoforms according to protein structure, function features and conservation.

Beyond the above-mentioned activities, the BU is an active node of the European network ELIXIR (<https://www.eelixir-europe.org/>), leading the ELIXIR Cancer Data Focus Group to provide the framework and expertise for the systematic analysis and interpretation of cancer genomes. BU also co-coordinates the *ISCIII* IMPaCT-Data project (<https://impact-data.bsc.es/>), in which our activity focuses on leading training activities and genomics data management. Our

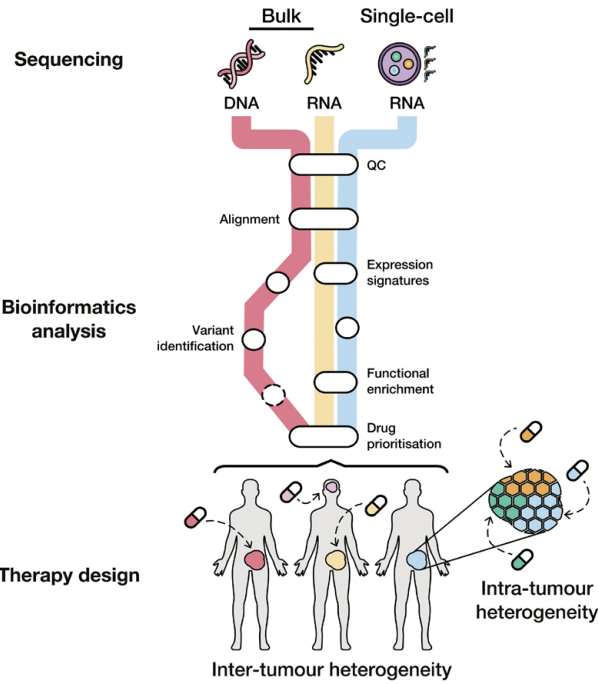


FIGURE 1 Summarised roadmap for drug prioritisation and therapy design to address inter- and intra-tumour heterogeneity using multi-omics data.

training and knowledge-transfer activities include the co-organisation of the MSc in “*Bioinformática aplicada a la medicina personalizada y la salud*” at the *ISCIII*. ■

SELECTED PUBLICATIONS*

- Rodríguez JM, Pozo F, Cerdán-Vélez D, Di Domenico T, Vázquez J, Tress ML (2022). APPRIS: selecting functionally important isoforms. *Nucleic Acids Res* 50, D54-D59.
- Sanchez-Burgos L, Navarro-González B, García-Martin S, Sirozh O, Mota-Pino J, Fueyo-Marcos E, Tejero H, Antón ME, Murga M, Al-Shahrour F, Fernandez-Capetillo O (2022). Activation of the integrated stress response is a vulnerability for multidrug-resistant FBXW7-deficient cells. *EMBO Mol Med* 14, e15855.
- Jiménez-Santos MJ, García-Martín S, Fustero-Torre C, Di Domenico T, Gómez-López G, Al-Shahrour F (2022). Bioinformatics roadmap for therapy selection in cancer genomics. *Mol Oncol* 16, 3881-3908.
- Pozo F, Rodríguez JM, Martínez Gómez L, Vázquez J, Tress ML (2022). APPRIS principal isoforms and MANE Select transcripts define reference splice variants. *Bioinformatics* 38(Supplement_2), ii89-ii94.
- Sanchez-Burgos L, Gómez-López G, Al-Shahrour F, Fernandez-Capetillo O (2022). An in silico analysis identifies drugs potentially modulating the cytokine storm triggered by SARS-CoV-2 infection. *Sci Rep* 12, 1626.
- Rogado J, Pozo F, Troulé K, Sánchez-Torres JM, Romero-Laorden N, Mondejar R,

Donnay O, Ballesteros A, Pacheco-Barcia V, Aspa J, Al-Shahrour F, Alfranca A, Colomer R (2022). Excess weight and anti-PD-1 immune checkpoint inhibitor’s outcomes in non-small cell lung cancer. *Clin Transl Oncol* 24, 2241-2249.

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

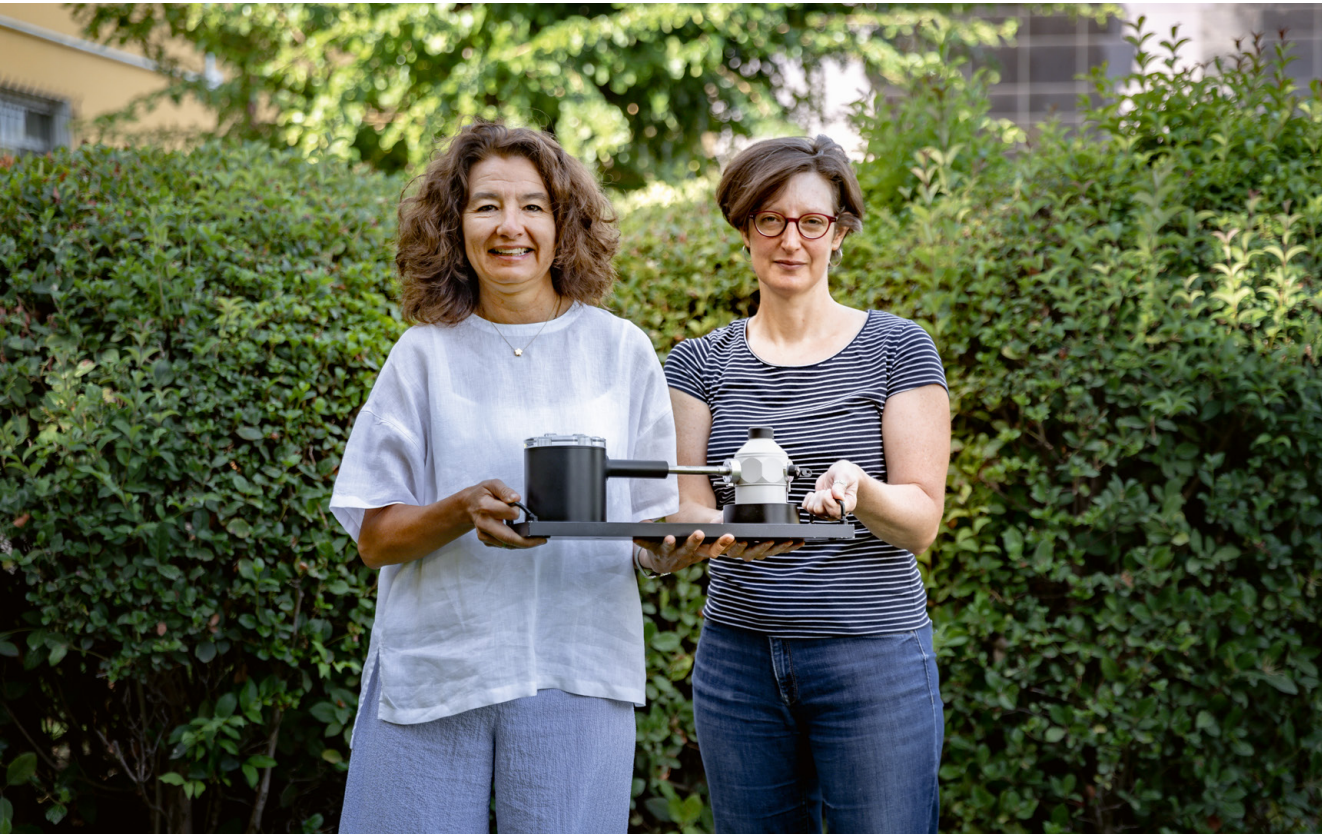
ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Post-Doctoral Fellow
Johanne Le Coq

Technician
Carmen García (until February) (TS)*
(PEJ)**

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



OVERVIEW

The principal goal of the Electron Microscopy (EM) Unit is to offer scientific-technical support to researchers to resolve their scientific questions using different transmission EM techniques. We routinely use cryo-EM and negative staining to prepare samples. We also perform data collection and help in image processing, through 2D analysis and 3D reconstruction. Support is offered in choosing adequate EM techniques and performing sample preparation. Moreover, we manufacture our own sample supports (EM grids) for better quality control and lower cost. In addition, we provide the training necessary for the use of our microscopes and auxiliary equipment. More advanced structural studies are generally carried out through research collaboration.

“In the Electron Microscopy Unit we dedicate our central effort to securing efficient access to all our infrastructure. We also offer the training necessary for the use of our microscopes and auxiliary equipment.”

RESEARCH HIGHLIGHTS

In 2022, we gave support to several CNIO Groups in their research activities. In collaboration with the Transformation and Metastasis Group, we analysed mitochondrial morphology in human breast cancer patient-derived xenografts (PDX). Together with the Microenvironment & Metastasis Group, we studied different types of vesicles, and with the Growth Factors, Nutrients and Cancer Group, we optimised cryoEM grids and collected data for structural studies of the URI complex. We also started a collaboration with the H12O-CNIO Haematological Malignancies Clinical Research Unit to structurally characterise hnRNPK.

We continued collaborating closely with all the groups from the Structural Biology Programme, performing single-particle EM grid preparation, cryo-EM grid screening, data collection, and 2D and 3D analysis of different samples. We collaborated in several projects carried out by the Macromolecular Complexes in DNA Damage Response Group, performing EM grid preparation, data collection, and analysis of different samples: ARN helicase DDX11; RuvBL complex of *Arabidopsis thaliana* (a collaboration with D. Alabadí, *Universitat Politècnica de València*); lncRNA (a collaboration with M. Huarte, *CIMA, Universidad de Navarra*); and different heteromeric amino acid transporters (a collaboration with M. Palacin and J. Fort, *IRB Barcelona*). With the Genome Integrity and Structural Biology Group, we provided cryoEM grid screening and data collection of different samples, and with the Kinases, Protein Phosphorylation and Cancer Group, we performed EM grid optimisation, data collection and processing of PTC1 Kinase, as well as EM grid preparation and imaging of KIF5B-RET kinesin samples. Outside our Centre, together with Rafael Fernández Leiro, we are collaborating with J.A. Costoya Puente (*Universidad de Santiago de Compostela*) on characterising the structure of human hPARP1. Furthermore, together with E. Lara (*CNIC*), we are studying mitochondrial structure in brown adipose tissue (BAT) of KO CnAbeta1 mice. ■

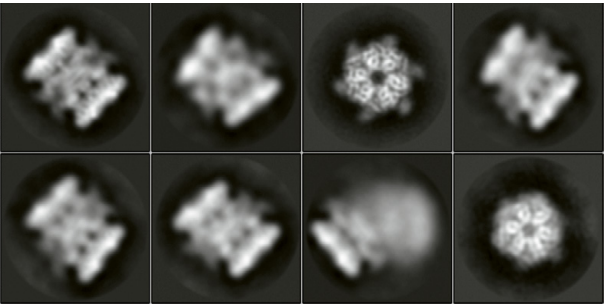


FIGURE 1 Reference-free 2D class averages demonstrating that cryo-electron microscopy is a powerful tool to view the structural flexibility of protein and protein complexes.

- **PUBLICATIONS**
- Le Coq J, Acebrón I, Rodrigo Martin B, López Navajas P, Lietha D (2022). New insights into FAK structure and function in focal adhesions. *J Cell Sci* 135, jcs259089.
- Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver AW, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.

PROTEIN CRYSTALLOGRAPHY UNIT

Inés Muñoz
Unit Head

Postdoctoral Fellow
Yudhi Nugraha



OVERVIEW

The Protein Crystallography Unit is a core facility that provides on-demand services at different levels, from the cloning, expression, and purification of 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 high-quality 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.

“Fragment screening on crystals helps to map new binding sites in the target proteins.”

Technicians

Aida Contreras (until February) (TS)*
(PEJ)*, Lluvia Rebollo (until February (TS)* (PEJ)*, Pilar Redondo

*Titulado Superior (Advanced Degree)

*Plan de Empleo Joven (Youth Employment Plan)

Students in Practice

Daffa Adinegoro (since Dec) (PhD student, Graduate School of Bioagricultural Sciences, Nagoya University, Japan), Eleonora Bado

(Feb-July) (Master's Thesis, Univ. Autónoma de Madrid, Spain), Laura Fernández (July-Aug) (AECC Traineeship, Univ. Rey Juan Carlos, Madrid, Spain)

RESEARCH HIGHLIGHTS

Our Unit works closely with the Experimental Therapeutics Programme on several projects: human TRF1 dimerisation domain; TRF1 DNA binding domain; and kinase domains of human MASTL and HASPIN for biochemical and structural analyses. Furthermore, to support drug discovery projects, we perform several thermal shift assays (thermofluor) in the presence of 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; Metabolism and Cell Signalling; Experimental Oncology; Microenvironment and Metastasis; Topology and DNA Breaks; DNA Replication; Macromolecular Complexes in DNA Damage Response; Kinases, Protein Phosphorylation and Cancer Groups; and

the H12O-CNIO Lung Cancer Clinical Research Unit), providing some of them with recombinant proteins that can be used for protein crystallography, SAXS or thermofluor assays analysis and, in some cases, for other biophysical, biochemical, cell-based functional assays and cryoEM studies.

Throughout 2022, the Unit also continued working on its own scientific project, supported by a grant from the BBVA Foundation. Carried out in collaboration with the Immunoncology and Immunotherapy Unit at the *Hospital 12 de Octubre*, this work 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 also revealed the cryo-EM structure, which shows how the trimerbody (TN^T) binds the trimeric RBD spike ectodomain in a 1:1 equimolar ratio. ■

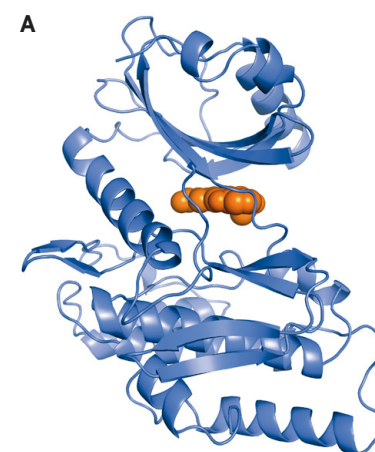
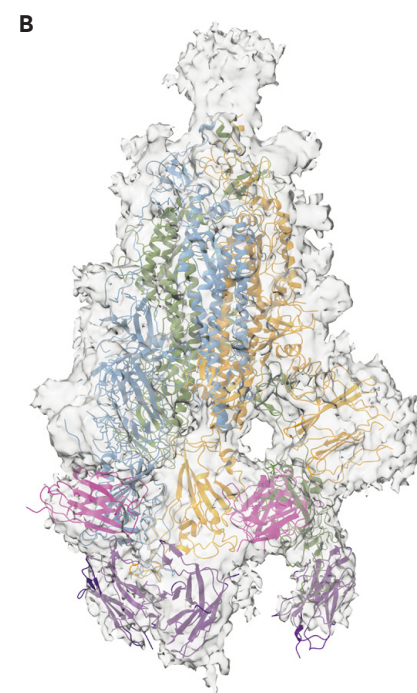


FIGURE 1 (A) Three-dimensional crystal structure of HASPIN kinase (in steel blue) in complex with the drug ETP-53005 (in orange). (B) Side view of the spike protein/TN^T complex model showing TN^T embracing the spike protein in

the 3-up RBD prefusion conformation. The spike protein subunits are coloured in yellow, steel blue, and olive green, while V_{HH}E and V_{HH}V chains from the antibody are in purple and magenta, respectively. The cryo-EM map is coloured in light grey.



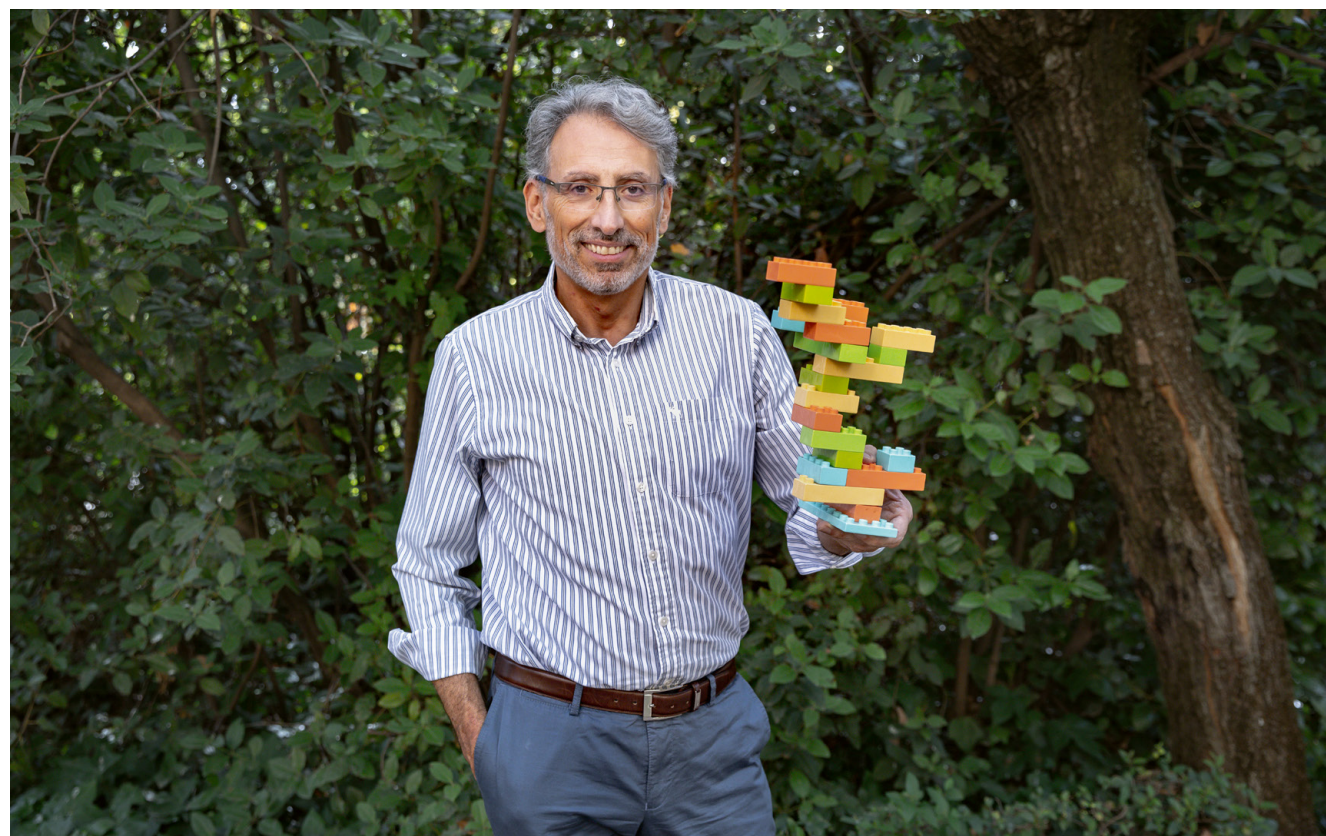
PUBLICATION

► Lama R, Xu C, Galster SL, Querol-García J, Portwood S, Mavis CK, Ruiz FM, Martin D, Wu J, Giorgi MC, Bargonetti J, Wang ES, Hernandez-Ilizaliturri FJ, Koudelka

GB, Chemler SR, Muñoz IG, Wang X (2022). Small molecule MMRI62 targets MDM4 for degradation and induces leukemic cell apoptosis regardless of p53 status. *Front Oncol* 12, 933446.

PROTEIN PRODUCTION UNIT

Jorge L. Martínez-Torrecuadrada
Unit Head



OVERVIEW

The biological functions of thousands of proteins, especially those involved in cancer development, remain unexplored, and understanding their structures, tissue and cellular distributions and functions is critical for biomedical progress. However, researchers often face the challenge of insufficient supply, since proteins of interest identified in a particular process can be very difficult to produce in high quality and in adequate amounts for analysis, causing a bottleneck in how quickly they can be characterised. The Protein Production Unit is a core service lab that aims to address this bottleneck by offering expertise and state-of-the-art technologies to develop effective processes for producing recombinant proteins. These can be used in diverse downstream applications such as the generation of highly-specific antibodies, biophysical, biochemical or functional analyses, or structural studies, with

“The Protein Production Unit provided many high quality recombinant proteins that were essential for drug discovery projects and for cancer diagnosis through the development of specific antibodies.”

the final goal of accelerating cutting-edge cancer research for CNIO and external research groups.

RESEARCH HIGHLIGHTS

The Protein Production Unit was created at the beginning of 2022, with the aim of providing high-quality recombinant proteins to meet the needs of CNIO Research Groups and external collaborators. During the year, the laboratory was refurbished with state-of-the-art technologies for heterologous recombinant protein expression and purification, to implement efficient production protocols for each particular protein. With its portfolio of services ranging from cDNA cloning in expression vectors to purification in milligrams of purified protein, the Unit contributed to the research projects of several CNIO Groups. It is worth mentioning the elucidation of the structure of the RAF1-HSP90-CDC37 complex, in collaboration with the Experimental Oncology Group; the production of active MIDKINE protein for functional assays and the generation of specific monoclonal antibodies for the Melanoma Group; and the production of exosome-secreted micropeptides to develop antibody-based detection tools in collaboration with the Microenvironment and Metastasis Group; among others. In addition, we worked closely with the Monoclonal Antibodies Unit, providing purified proteins to generate highly-specific monoclonal antibodies, such as CSF3R, IL4I1, TACI and PILRA; and with the Biology Section of the

Experimental Therapeutics Programme, producing active full-length human MASTL for functional assays. Other tool proteins for in-house use (i.e. sortases and AG-MNase) were also produced in the Unit.

Apart from providing such services, the Unit carries out research activities focused on the development of specific recombinant antibodies and antibody fragments for diagnostic and therapeutic purposes. In 2022, llama-derived nanobodies against the metalloprotease MT1-MMP were developed and have shown their great potential as radiotracers in PET imaging for the detection of triple-negative breast cancer in mouse models. This project was a joint CNIO-*CIEMAT* collaboration and was supported by a grant from the BBVA Foundation. ■

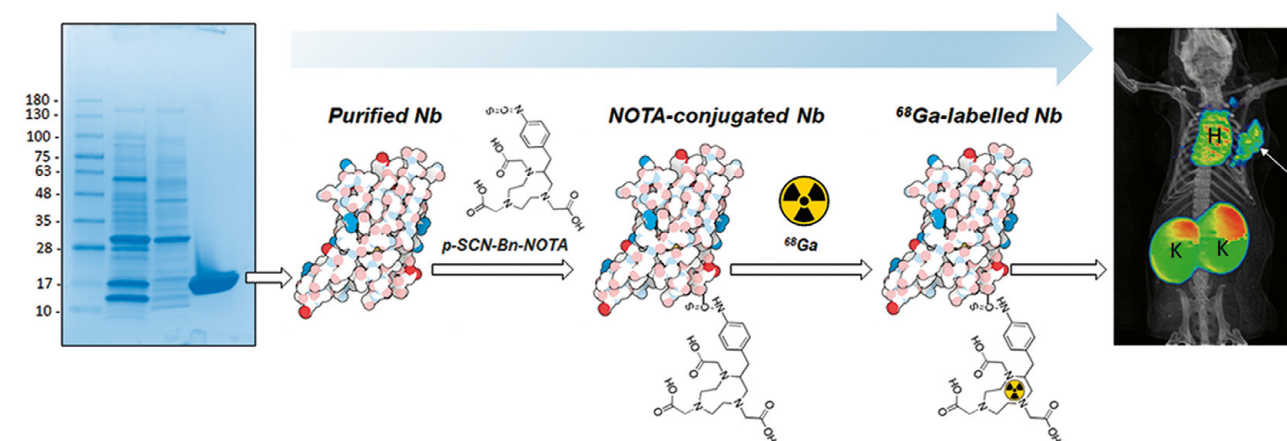


FIGURE 1 Strategy to obtain nanobody-based radiotracers for tumour detection by PET imaging. After nanobody expression and

purification, conjugation with NOTA, and labelling with ^{68}Ga , the biodistribution of the ^{68}Ga -labelled nanobodies was monitored by PET.

Arrow indicates tumour position, K: kidney, H: heart.

PUBLICATIONS

► García-Alonso S, Mesa P, Ovejero LP, Aizpurua G, Lechuga CG, Zarzuela E, Santiveri CM, Sanclemente M, Muñoz J,

Musteanu M, Campos-Olivas R, Martínez-Torrecuadrada J, Barbacid M, Montoya G (2022). Structure of the RAF1-HSP90-CDC37 complex reveals the basis of RAF1 regulation. *Mol Cell* 82, 3438-3452.e8.

► Mulero F, Oteo M, Garaulet G, Magro N, Rebollo L, Medrano G, Santiveri C, Romero E, Sellek RE, Margolles Y, Campos-Olivas R, Arroyo AG, Fernández LA, Morcillo MA, Martínez-Torrecuadrada JL (2022).

Development of anti-membrane type 1-matrix metalloproteinase nanobodies as immunoPET probes for triple negative breast cancer imaging. *Front Med (Lausanne)* 9, 1058455.

Translational Research

Human Cancer Genetics Programme	98
Hereditary Endocrine Cancer Group	100
Genetic and Molecular Epidemiology Group	104
Molecular Cytogenetics Unit	108
Familial Cancer Clinical Unit	110
Human Genotyping- <i>CEGEN</i> Unit	112
 Clinical Research Programme	114
Breast Cancer Clinical Research Unit	116
Molecular Diagnostics Unit	120
H12O-CNIO Haematological Malignancies Clinical Research Unit	122
H12O-CNIO Lung Cancer Clinical Research Unit	126
H12O-CNIO Cancer Immunotherapy Clinical Research Unit	130

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 2022, 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 for the evaluation of families with cancer and the provision of genetic counselling, which is located at the *Hospital Universitario de Fuenlabrada*.

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 2022, 729 patients visited the Familial Cancer Consultancy at the *Hospital de Fuenlabrada*, and the HCGP performed 1,884 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 1-3 months; a total of 27 national visitors and students and 4 medical residents from different Spanish hospitals were hosted in 2022. The Programme’s education activities in 2022 comprised a total of 17 national and 2 international PhD research projects, including the successful defence of 3 doctoral theses.

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 this regard, in 2022, several of the GMEG members, led by Núria Malats, and the European Molecular Biology Laboratory (EMBL) in Heidelberg, led by Peer Bork, in collaboration with the CNIO Epithelial Carcinogenesis Group led by Paco Real, and the CNIO Molecular Cytogenetics Unit led by Sandra Rodríguez-Perales, conducted an international comprehensive study on the influence of the microbiome on pancreatic cancer, published in the prestigious Journal *Gut*.

Also, 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 2022 include:

- Mercedes Robledo: Within the field of personalised precision medicine co-funded by NextGenerationEU, “Development and implementation of a functional genomics validation platform for undiagnosed hereditary cancer.”
- Núria Malats: Chairs ALIPANC, the Alliance of Pancreatic Cancer Research in Spain with 50 scientific groups.
- Núria Malats has contributed to (1) the pancreatic cancer field by proposing a high accuracy faecal metagenomic classifier and (2) the methodological field by proposing a new Mendelian randomization to avoid collider bias.
- Anna González-Neira, Javier Benítez, Ana Osorio: Two multicentre studies in breast cancer as a result of the “Breast Cancer Risk after Diagnostic Gene Sequencing” BRIDGES H2020 Project – “Pathology of tumors associated with pathogenic germline variants in 9 breast cancer susceptibility genes” (*JAMA Oncology*) and “Breast cancer risks associated with missense variants in breast cancer susceptibility genes” (*Genome Medicine*).
- Maria Currás, Ana Osorio: “A large case-control study helps identify a new candidate gene for breast cancer predisposition” (*Cancers*).
- Cristina Rodríguez-Antona: Listed in the “World Ranking Top 2% Scientists”, 2022 edition of the Stanford University list of World Top 2% scientists.

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.

Lastly, I would like to take this opportunity to thank our former Head of the Familial Cancer Clinical Unit, Miguel Urioste, for his dedication and for placing the CNIO as a reference in the field of familial cancer genetic diagnostics. Thank you, Miguel, for having been a part of our CNIO community and we wish you the best on your retirement! I would also like to thank Ana Osorio and Alicia Barroso for their outstanding work on the genetic diagnosis of breast cancer over the last 20 years.

Maria A. Blasco, Director

HEREDITARY ENDOCRINE CANCER GROUP

Group Leader
Mercedes Robledo

Research Scientists
Alberto Cascón, Cristina Rodríguez

Post-Doctoral Fellows
Luis Javier Leandro, Ángel Mario Martínez, Cristina Montero, Alberto Díaz (*CIBERER*, Madrid)



Graduate Students
Javier de Nicolás (since February), Ángel Fernández (until May), Javier Lanillos, Natalia Martínez (*CIBERER*, Madrid), Sara Mellid, Maria Monteagudo, Carlos Valdivia

Technicians
Bruna Calsina (until March) (TS), Eduardo Gil (until December), Rocío Letón, Gabriela Roberta Radu (since December) (PEJ, CAM) ”
”Titulado Superior (Advanced Degree)

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

Student in Practice
Sara Gil (January-September)

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

Visiting Scientist
Noelia Herradón (*Hospital 12 de Octubre*, Madrid, Spain)

OVERVIEW

Our Group is mainly interested in identifying genetic risk factors involved in endocrine tumour susceptibility. Through a comprehensive analysis of tumour genomic features, we have been able to propose diagnostic and prognostic markers, to identify altered pathways that could be 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 identified *PARP1* expression and *PBRM1* mutations as predictive markers of progression free survival in patients with clear cell renal cell carcinoma. In thyroid cancer, telomere shortening leads to a reorganisation of the 5p subtelomeric region, facilitating the accumulation of alterations at the *TERT*-locus.”

RESEARCH HIGHLIGHTS

Massive sequencing technologies to advance pharmacogenomics knowledge

Next generation sequencing technologies have boosted the discovery and clinical implementation of novel markers of drug treatment response. We illustrate this with 2 contributions:

i) Generation of a comprehensive germline landscape of pharmacogenetic actionable variants contained in diagnostic exomes. In this study, we analysed data from 5001 individuals who underwent exome sequencing for genetic diagnosis to provide population frequencies of clinically relevant pharmacogenetic alleles and to estimate the contribution of novel loss-of-function variants.

ii) Identification of *PARP1* expression and *PBRM1* mutation as predictive biomarkers in patients with clear cell renal cell carcinoma. Through analysis of genomic, transcriptomic, and clinical data of the IMmotion151 trial in patients treated with atezolizumab plus bevacizumab or sunitinib, we found that tumour *PARP1* expression was a predictor of progression-free survival regardless of treatment arm, while *PBRM1* mutations exerted an interaction only with sunitinib treatment (FIGURE 1).

Comprehensive molecular analysis of immortalisation hallmarks in thyroid cancer reveals new prognostic markers

Around 1 in 2000 individuals in Spain develop thyroid cancer fatal disease. Comprehensive molecular studies on thyroid tumours are needed to identify prognostic molecular

biomarkers that will allow the early detection, and thus the personalised management and follow-up, of this rare but life-threatening cancer. We extensively characterised cancer immortalisation-related alterations in a series of 106 thyroid tumours enriched with clinically-aggressive carcinomas to define disease prognostic markers. Using a custom-designed RNA-seq panel, we identified 5 telomerase holoenzyme-complex genes over-expressed in clinically-aggressive tumours compared to tumours from long-term disease-free patients, with *TERT* and *TERC* denoted as independent prognostic markers by multivariate regression model analysis. Characterisation of alterations related to *TERT* re-expression revealed that promoter mutations, hypermethylation and/or copy gains exclusively co-occurred in clinically-aggressive tumours. Quantitative-FISH analysis of telomere lengths showed a significant shortening in these carcinomas, which matched with a high proliferative rate measured by Ki-67 immunohistochemistry. RNA-seq data analysis indicated that short-telomere tumours exhibit increased transcriptional activity in the 5 Mb-subtelomeric regions, site of several telomerase-complex genes. Gene upregulation enrichment was significant for specific chromosome-ends such as the 5p, where *TERT* is located. Co-FISH analysis of 5p-end and *TERT* loci showed a more relaxed chromatin configuration in short telomere-length tumours compared to normal telomere-length tumours. Overall, our findings support that telomere shortening leads to a reorganisation of the 5p subtelomeric region, facilitating the transcription and accumulation of alterations at the *TERT*-locus, and unveil a FISH-based assay as a potential cytogenetic tool to predict disease prognosis in thyroid cancer. ■

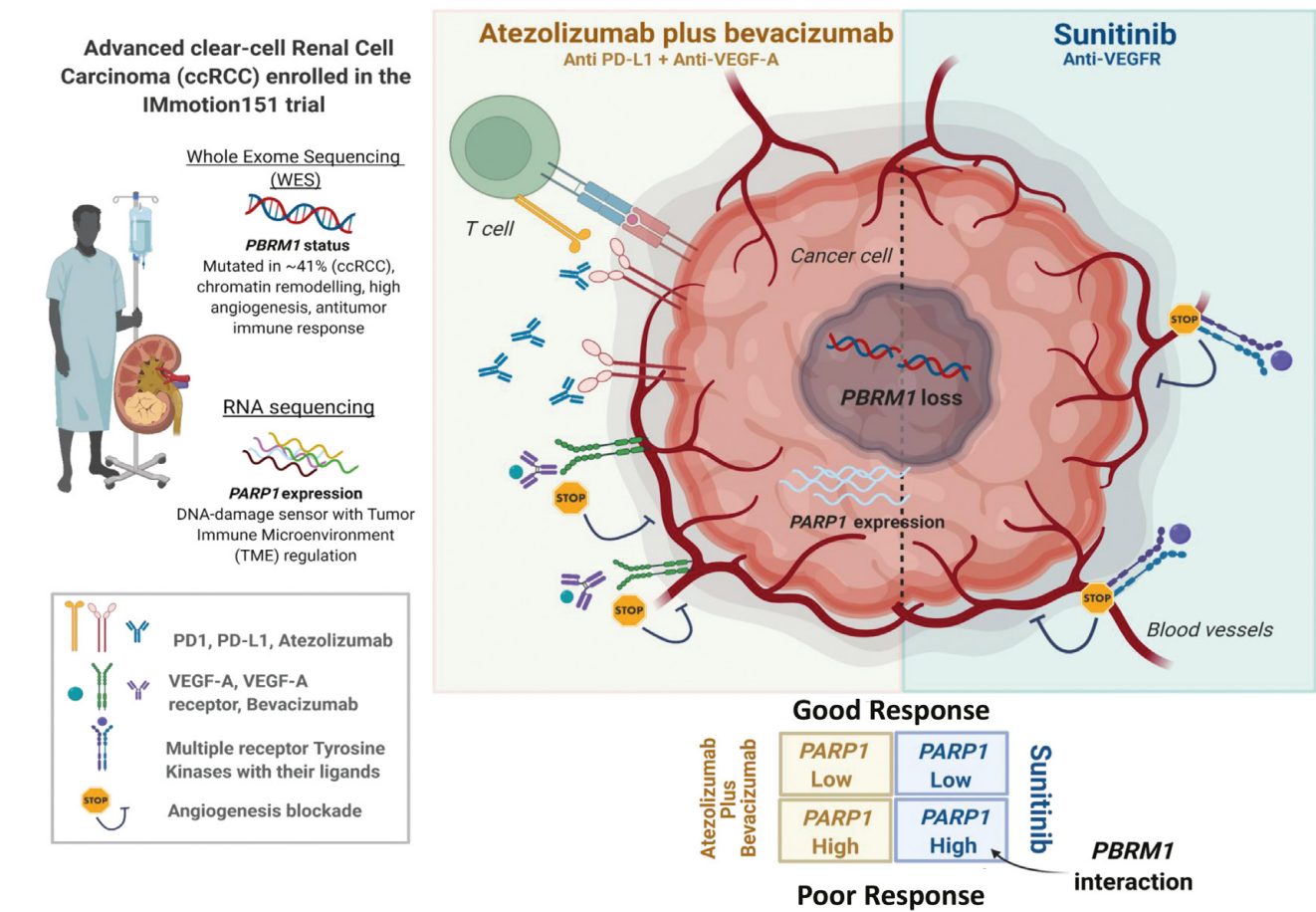


FIGURE 1 Low *PARP1* expression and *PBRM1* loss associate with improved Immunotherapy/antiangiogenic response in clear cell renal cancer patients.

PUBLICATIONS

Montero-Conde C, Leandro-García LJ, Martínez-Montes ÁM, Martínez P, Moya FJ, Letón R, Gil E, Martínez-Puente N, Guadalix S, Currás-Freixes M, García-Tobar L, Zafon C, Jordà M, Riesco-Eizaguirre G, González-García P, Monteagudo M, Torres-Pérez R, Mancikova V, Ruiz-Llorente S, Pérez-Martínez M, Pita G, Galofré JC, Gonzalez-Neira A, Cascón A, Rodríguez-Antona C, Megías D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.

Bliziotis NG *et al.* (incl. Robledo M) (2022). Preanalytical pitfalls in untarget-

ed plasma nuclear magnetic resonance metabolomics of endocrine hypertension. *Metabolites* 12, 679.

Díaz-Talavera A, Montero-Conde C, Leandro-García LJ, Robledo M (2022). PrimPol: a breakthrough among DNA replication enzymes and a potential new target for cancer therapy. *Biomolecules* 12, 693.

Pamporaki C *et al.* (incl. Robledo M) (2022). Determinants of disease-specific survival in patients with and without metastatic pheochromocytoma and paraganglioma. *Eur J Cancer* 169, 32-41.

Pena-Couso L, Ercibengoa M, Mercadillo F, Gómez-Sánchez D, Inglada-Pérez L, Santos M, Lanillos J, Gutiérrez-Abad D, Hernández A, Carbonell P, Letón R, Robledo M, Rodríguez-Antona C, Perea J, Urioste M; PHTS Working Group (2022).

Considerations on diagnosis and surveillance measures of PTEN hamartoma tumor syndrome: clinical and genetic study in a series of Spanish patients. *Orphanet J Rare Dis* 17, 85.

Richter S *et al.* (incl. Robledo M) (2022). Head/neck paragangliomas: focus on tumor location, mutational status and plasma methoxytyramine. *Endocr Relat Cancer* 29, 213-224.

Helm J *et al.* (incl. Robledo M) (2022). Treatment of pheochromocytoma cells with recurrent cycles of hypoxia: a new pseudohypoxic in vitro model. *Cells* 11, 560.

Luque J, Mendes I, Gómez B, Morte B, López de Heredia M, Herreras E, Corrochano V, Bueren J, Gallano P, Artuch R, Fillat C, Pérez-Jurado LA, Montoliu L,

Carracedo Á, Millán JM, Webb SM, Palau F; CIBERER Network; Lapunzina P (2022). CIBERER: Spanish national network for research on rare diseases: a highly productive collaborative initiative. *Clin Genet* 101, 481-493.

Jankovic Miljus J *et al.* (incl. Robledo M) (2022). Circulating microRNA profiles as potential biomarkers for differentiated thyroid cancer recurrence. *J Clin Endocrinol Metab* 107, 1280-1293.

Bliziotis NG *et al.* (incl. Robledo M) (2022). Pre- versus post-operative untargeted plasma nuclear magnetic resonance spectroscopy metabolomics of pheochromocytoma and paraganglioma. *Endocrine* 75, 254-265.

Ben Aim L, Maher ER, Cascon A, Barlier A, Giraud S, Ercolino T, Pigny P, Clift-

on-Bligh RJ, Mirebeau-Prunier D, Mohamed A, Favier J, Gimenez-Roqueplo AP, Schiavi F, Toledo RA, Dahia PL, Robledo M, Bayley JP, Burnichon N (2022). International initiative for a curated *SDHB* variant database improving the diagnosis of hereditary paraganglioma and pheochromocytoma. *J Med Genet* 59, 785-792.

Castellano D, Apolo AB, Porta C, Capdevila J, Viteri S, Rodríguez-Antona C, Martin L, Maroto P (2022). Cabozantinib combination therapy for the treatment of solid tumors: a systematic review. *Ther Adv Med Oncol* 14, 17588359221107112.

Herrero Rivera D, Vacas CG, Kovandzic LM, Vázquez JP, Alonso LA, González BM, Aragón VC, Grande E, Caro RL, Virizueta Echaburu JA, Rodríguez Moreno JF, Etxebarria AA, Rodríguez-Antona C, Durán

I (2022). Single-nucleotide polymorphism associations with efficacy and toxicity in metastatic castration-resistant prostate cancer treated with cabazitaxel. *Pharmacogenomics* 23, 627-638.

Maroto P, Porta C, Capdevila J, Apolo AB, Viteri S, Rodríguez-Antona C, Martin L, Castellano D (2022). Cabozantinib for the treatment of solid tumors: a systematic review. *Ther Adv Med Oncol* 14, 17588359221107112.

Diekstra MHM *et al.* (incl. Rodríguez-Antona C) (2022). Genome-wide meta-analysis identifies variants in *DSCAM* and *PDLIM3* that correlate with efficacy outcomes in metastatic renal cell carcinoma patients treated with sunitinib. *Cancers (Basel)* 14, 2838.

Lanillos J, Santos M, Valdivia C, Mora A,

Rodríguez-Antona C (2022). Interrogating the significance of PARP1 expression and PBRM1 mutation as biomarkers for predicting the response to atezolizumab plus bevacizumab or to sunitinib in patients with clear cell renal cell carcinoma. *Eur Urol* 82, 334-335.

Rodríguez-Antona C, Savieo JL, Lauschke VM, Sangkuhl K, Drögemöller BI, Wang D, van Schaik RHN, Gilep AA, Peter AP, Boone EC, Ramey BE, Klein TE, Whirl-Carrillo M, Pratt VM, Gaedigk A (2022). PharmVar GeneFocus: CYP3A5. *Clin Pharmacol Ther* 112, 1159-1171.

Lanillos J, Carcajona M, Maietta P, Alvarez S, Rodríguez-Antona C (2022). Clinical pharmacogenetic analysis in 5,001 individuals with diagnostic Exome Sequencing data. *NPJ Genom Med* 7, 12.

Dareng EO *et al.* (incl. Osorio A, Rodríguez-Antona) Shan K, Sieh W, Simard J, Singer CF, Sokolenko AP, Song H, Southey MC, Steed H, (2022). Polygenic risk modeling for prediction of epithelial ovarian cancer risk. *Eur J Hum Genet* 30, 349-362.

McDermott JH, Wolf J, Hoshitsuki K, Huddart R, Caudle KE, Whirl-Carrillo M, Steyer PS, Smith RJH, Cody N, Rodríguez-Antona C, Klein TE, Newman WG (2022). Clinical pharmacogenetics implementation consortium guideline for the use of aminoglycosides based on MT-RNR1 genotype. *Clin Pharmacol Ther* 111:366-372.

GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

Group Leader
Núria Malats

Research Scientists
M. Evangelina López de Maturana,
Bárbara Oldrini (until July)

Graduate Students
Raquel Benítez, Claudia Coscia (until April), Jiangchuan He (until December), Francisco J Jurado, Alberto Langtry, Víctor M. Sobrino, Casper Van Eijk (since August), Nannan Xue



Technicians
Lola Alonso (TS)*, Lidia Estudillo, Yaiza Gutiérrez (since April), Ane Moreno (since November): Vanesa Moreno (TS)*, Sergio Sabroso (TS)*, María Olano (since April)

*Titulado Superior (Advanced Degree)

Students in Practice
Sol Aletta (since Aug) (Rabdoud University, Nijmegen, The Netherlands), Tania Chadha (March-July) (ETSIT, Madrid, Spain), Daniel de San Sebastián (until July) (UPM, Madrid, Spain), Soraya Martín (April-June) (IFP Corredor del Henares, Spain), Harold Mena (Aug.-Oct.) (Del Rosario Univ., Bogotá, Colombia), Anaëlle Mescam (June-Aug.) (École de Biologie Industrielle, Cergy, France), Nataly Moreu (March-June) (IES Mirasierra, Spain)

Visiting Master's Students
Miguel Maquedano (until July) (UAM, Madrid, Spain), César Mediavilla (May.-Dec.) and Juana Serrano (since Aug.) (Master's in Bioinformatics, ISCIII-ENS, Madrid, Spain)

Visiting Scientists
Isabel A. Martín (Univ. CEU San Pablo, Madrid, Spain), Esther Molina (July-Sep) (Univ. de Vic, Barcelona, Spain), Ashwag M. Mukhtar (until May) (Al Neelain University, Sudan, Africa) (Science by Women Programme)

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.

“Oral, faecal, and pancreatic microbiome dysbiosis are associated with pancreatic cancer, with stool microbiota-based classifiers that predict pancreatic cancer with high accuracy and specificity.”

RESEARCH HIGHLIGHTS

Research findings

In 2022, GMEG contributed to the pancreatic cancer (PC) field by proposing a faecal metagenomic classifier that identifies PC with an accuracy of 0.84 area under the receiver operating characteristic curve (AUROC) in a Spanish cohort, based on 27 species. The accuracy improves up to 0.94 AUROC when combined with CA19–9 serum marker. The classifier was validated in an independent German PC cohort (0.83 AUROC), and PC disease specificity was confirmed against 25 publicly available metagenomic study populations with various health conditions (n=5792). The presence of marker taxa enriched in faecal samples (*Veillonella*, *Streptococcus*, *Akkermansia*) and also taxa with differential abundance in healthy and tumour pancreatic tissues (*Bacteroides*, *Lactobacillus*, *Bifidobacterium*) was validated by fluorescence *in situ* hybridisation (FIGURE 1). The presented PDAC-specific microbiome signatures, including links between microbial populations across tissues, provide novel microbiome-related hypotheses regarding disease aetiology, prevention, and possible therapeutic intervention. In addition, we also collaborated in elucidating that *GATA4* and *GATA6* cooperate to maintain the classical PC phenotype. We further explored the immune repertoire landscape of 9522 tumour and adjacent non-tumour samples across 28 tumour types in the Cancer Genome Atlas project, and performed diversity and network analysis. We identified differences in diversity and network statistics across tumour types and subtypes and observed a trend towards increased clonality in primary tumours compared to adjacent non-tumour tissues. Regarding **bladder cancer** (BC), GMEG participated in 1 study that delivered suggestive evidence for a multiplicative interaction between the most common class of disinfection by-products, trihalomethanes, and a bladder cancer susceptibility variant (rs907611). Furthermore, we contributed to the validation of BlaDimiR, a urine-based miRNA score for accurate bladder cancer diagnosis and follow-up.

Methodological contributions

We proposed an approach allowing Mendelian randomisation estimation in strata of the population while avoiding collider bias (FIGURE 2). This approach constructs a new variable, the residual collider, as the residual from regression of the collider on the genetic instrument, and then calculates causal estimates in strata defined by quantiles of the residual collider. The new approach generated unbiased estimates in all the simulation settings, and can be used to perform Mendelian randomisation studying heterogeneity among subgroups of the population while avoiding collider bias. Furthermore, GMEG continued exploring the analytic strategies and tools to integrate omics and non-omics data into the cancer risk models, and made progress in the integration of medical image information (radiomics and digital pathology).

Translational activities

GMEG actively supports several clinical trials of 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). 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 Pancreatic Cancer Europe (PCE) multistakeholder platform, and we have moved ahead in increasing awareness of PC. We also contributed to the publication of the UEG position paper on pancreatic cancer. Finally, we joined an initiative of the European Alliance for Personalised Medicine to express concerns that disrupting the current balance of the pharmaceutical legislation to meet objectives that are more precisely targeted could have unintended consequences in the EU, reducing rather than increasing the flow of innovative treatments for rare diseases. ■

PUBLICATIONS

de Andrés MP, Jackson RJ, Felipe I, Zagorac S, Pilarsky C, Schlitter AM, Martínez 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 (2022). GATA4 and GATA6 loss-of-expression is associated with extinction of the classical programme and poor outcome in pancreatic ductal adenocarcinoma. *Gut*. PMID: 36109153.

Kartal E *et al.* (incl. Molina-Montes E, Rodríguez-Perales S, Márquez M, Torres-Ruiz R; MAGIC Study investigators; PanGenEU Study investigators; Real FX, Malats N)P (2022). A faecal microbiota signature with high specificity for pancreatic cancer. *Gut* 71, 1359-1372. **GutTop Paper** 2022.

Nagata N *et al.* (incl. Molina-Montes E, Estudillo L, Malats N) (2022). Metagenomic identification of microbial signatures predicting pancreatic cancer from a multinational study. *Gastroenterology* 163, 222-238.

Yuan C, Kim J, Wang QL, Lee AA, Babic A; PanScan/PanC4 I-III Consortium; Amundadottir LT, Klein AP, Li D, McCullough ML, Petersen GM, Risch HA,

Stolzenberg-Solomon RZ, Perez K, Ng K, Giovannucci EL, Stampfer MJ, Kraft P, Wolpin BM (2022). The age-dependent association of risk factors with pancreatic cancer. *Ann Oncol* 33, 693-701.

Suarez-Cabrera C *et al.* (incl. Estudillo L, Real FX, Malats N) (2022). BlaDimiR: a urine-based miRNA score for accurate bladder cancer diagnosis and follow-up. *Eur Urol* 82, 663-667.

Mouron S, Bueno MJ, Lluch A, Manso L, Calvo I, Cortes J, Garcia-Saenz JA, Gil-Gil M, Martínez-Janez N, Apala JV, Caleiras E, Ximénez-Embún P, Muñoz J, González-Cortijo L, Murillo R, Sánchez-Bayona R, Cejalvo JM, Gómez-López G, Fuster-Torre C, Sabroso-Lasa S, Malats N, Martínez M, Moreno A, Megias D, Malumbres M, Colomer R, Quintela-Fandino M (2022). Phosphoproteomic analysis of neoadjuvant breast cancer suggests that increased sensitivity to paclitaxel is driven by CDK4 and filamin A. *Nat Commun* 13, 7529.

Galesloot TE, Grotenhuis AJ, Kolev D, Aben KK, Bryan RT, Catto JWF, Cheng KK, Conroy S, Dyrskjøt L, Fleschner NE, James ND, Lamy P, Lindskrog SV, Malats N, Mengual L, Verhaegh G, Zeegers MP, Kiemeny LALM, Vermeulen SH (2022). Genome-wide meta-analysis identifies novel genes associated with recurrence

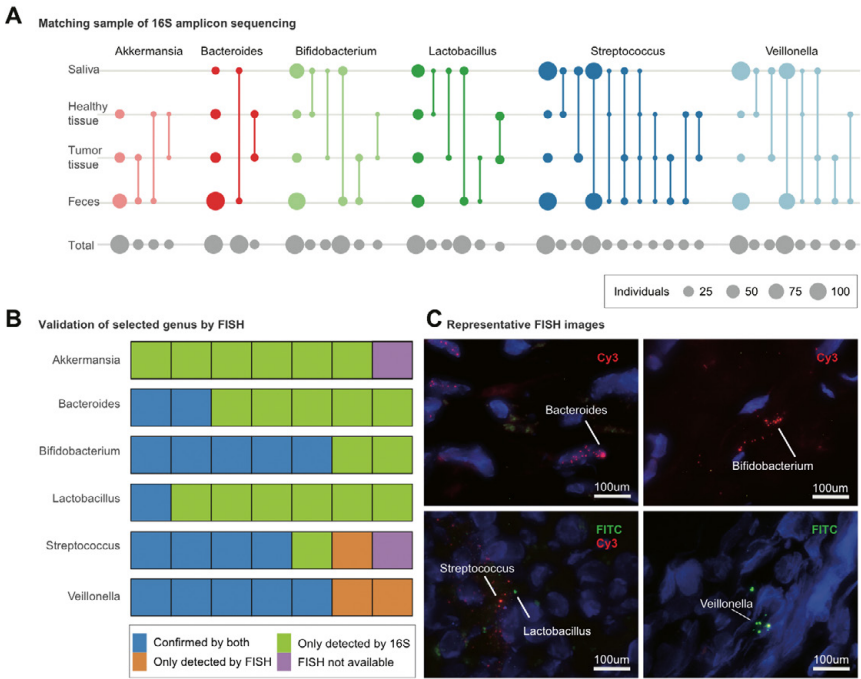


FIGURE 1 Presence of bacteria in 4 different body sites including faecal, saliva, pancreatic tumour and healthy tissue samples (A). Bacterial presence/absence with both 16S amplicon and FISH methods in 7 selected pancreatic tissue samples (B). FISH microscopy images for *Bacteroides* (intranuclear, tumour tissue), *Bifidobacterium* (extranuclear, tumour tissue), *Lactobacillus* (extranuclear, non-tumour tissue), *Streptococcus* (extranuclear, non-tumour tissue), and *Veillonella* (extranuclear, tumour tissue) (C).

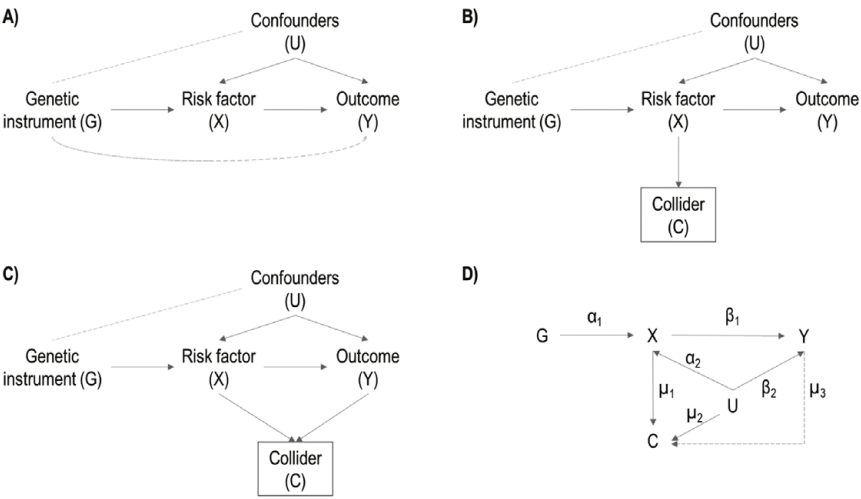


FIGURE 2 Directed Acyclic Graphs (DAGs) illustrating relationships between the variables. (A) Mendelian randomisation causal diagram with the instrumental variable assumptions. (B) DAG considering a collider variable C, being a common child of genetic instrument G and confounders U. (C) DAG considering a collider variable C, being a common child of risk factor X and outcome Y. (D) DAG illustrating the variables and parameters used for the simulation study.

and progression in non-muscle-invasive bladder cancer. *Eur Urol Oncol* 5, 70–83.

Coscia C, Gill D, Benítez R, Pérez T, Malats N, Burgess S (2022). Avoiding collider bias in Mendelian randomization when performing stratified analyses. *Eur J Epidemiol* 37, 671-682.

Beane Freeman LE *et al.* (incl. Malats N) (2022). Disinfection by-products in drinking water and bladder cancer: evaluation of risk modification by common genetic polymorphisms in two case-control studies. *Environ Health Perspect* 130, 57006.

Yu K, Ravoor A, Malats N, Pineda S, Sirota M (2022). A pan-cancer analysis of tumor-infiltrating B cell repertoires (2022). *Front Immunol* 12, 790119.

Daca-Alvarez M, Martí M, Spinelli A, de Miranda NFFC, Palles C, Vivas A, Lachford A, Monahan K, Szczepkowski M, Tarnowski W, Makkai-Popa ST, Vidal R, López I, Hurtado E, Jiménez F, Jiménez-Toscano M, Álvaro E, Sanz G, Ballester A, Melone S, Brandáriz L, Prieto I, García-Olmo D, Ocáña T, Moreira R, Moreno L, Carballal S, Moreira L, Pellisé M, González-Sarmiento R, Holowatyj AN, Perea J, Balaguer F; GEOCODE and SECOC consortia (2022). Familial component of early-onset colorectal cancer: opportunity for prevention. *Br J Surg* 109, 1319-1325.

Serrano MT *et al.* (incl. Sabroso S) (2022).

Mortality and causes of death after liver transplantation: analysis of sex differences in a large nationwide cohort. *Transpl Int* 35, 10263.

Mera-Berriatua L *et al.* (incl. López de Maturana E) (2022). Unravelling the gut microbiota of cow's milk-allergic infants, their mothers, and their grandmothers. *J Invest Allergol Clin Immunol* 32, 395-398.

Horgan D, Koeva-Balabanova J, Capoluongo E, Jagielska B, Cattaneo I, Kozaric M, Tumiene B, El Ahl JP, Lal JA, Kalra D, Malats N (2022). Making sure that orphan incentives tip the right way in Europe. *Healthcare (Basel)* 10, 1600.

PATENT

Malats N, Bork P, Kartal E, Molina-Montes E, Rodríguez S, Estudillo L, Real FX, Schmidt S, Zeller G, Wirbel J, Maistrenko OM. Faecal microbiota signature for pancreatic cancer. PCT application (2022). *PCT/EP2022/077087*. WO2023052486A1.

MOLECULAR
CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Research Scientist
Raúl Torres

Graduate Students
Alejandro Alonso (since September),
Maria Cruz Casado (until August),
Alejandro Nieto (since September),
Pilar Puig



OVERVIEW

Recurrent chromosomal rearrangements, changes in the structure of native chromosomes, are very common and well-known hallmarks of cancer. A better understanding of these cancer-causing mechanisms will lead to novel therapeutic regimens to fight cancer. The research activity of the Molecular Cytogenetics and Genome Editing Unit focuses on increasing our knowledge about the role of chromosomal rearrangements in cancer development and progression and discovering new therapeutic targets. With the combined use of CRISPR genome editing and cytogenetic technologies, we are creating models that recapitulate chromosomal and genetic cancer alterations. The goal of the Unit is to provide CNIO and external researchers with the latest technologies used in the fields of molecular cytogenetics and genome editing. The Unit is continuously implementing and developing new technologies in those fields.

“In 2022, we applied genome engineering approaches to reproduce and eliminate chromosome rearrangements and gene alterations. We also provided access to the latest cytogenetic and CRISPR technologies.”

We also participate in collaborative projects with clinical and basic science investigators across the CNIO and other institutions.

Technicians
M. Carmen Martín, Francisco José Moya (TS) ‘(PEJ)’ ”

“*Titulado Superior* (Advanced Degree)
“*Plan de Empleo Joven* (Youth Employment Plan, until March)

Master’s Students
Alejandro Alonso (Jan.-Aug.) (*Maastricht University*, The Netherlands), Alejandro Nieto (Jan.-Aug.) (*Univ. Autónoma de Madrid*, Spain), Paula M. Ojeda

(Feb.-Sep.) (*Univ. Complutense de Madrid*, Spain)

Visiting Scientists
Beatriz Álvarez and Daniel Lucena (Sep.-Dec.) (*CIB Margarita Salas*,

Madrid, Spain), Carlos Carrasco (July.-Dec.) (*Univ. Complutense de Madrid*, Spain)

RESEARCH HIGHLIGHTS

Molecular cytogenetics. The “Optimization Optical Genome Mapping” (OGM, Bionano) technique, based on the analysis of ultra-high long DNA molecules, provides a genome-wide high-resolution analysis of copy number and structural cytogenetic variations. We are optimising the use of OGM technology in our Unit, comparing it with standard techniques (e.g., karyotyping, fluorescent in situ hybridisation) using hematologic and solid tumour fresh and frozen samples. We think OGM represents a promising complementary approach to existing cytogenetic techniques for the characterisation of cancer cells. OGM enables a time and cost-effective analysis allowing the identification of complex cytogenetic rearrangements, including some that are currently inaccessible to standard techniques.

Technological and translational activities. Approximately 20% of human cancers contain specific fusion oncogenes (FOs). Due to their tumour-specific expression, FOs offer unique advantages such as diagnostic and therapeutic targets.

- **FO targeted diagnosis:** In the clinic, conventional diagnostic techniques like qRT-PCR, FISH, or NGS are routine. But these methods require specialised machinery and personnel, are expensive, time-consuming, and involve multiple steps.
- **FO targeted therapy:** Many currently used treatments are non-selective, leading to severe side effects responsible for prolonged recovery and frequently resulting in relapses.

In this regard, we applied the RNA-targeting Cas13 system to selectively cleave FO transcripts. Contrary to the CRISPR/Cas9

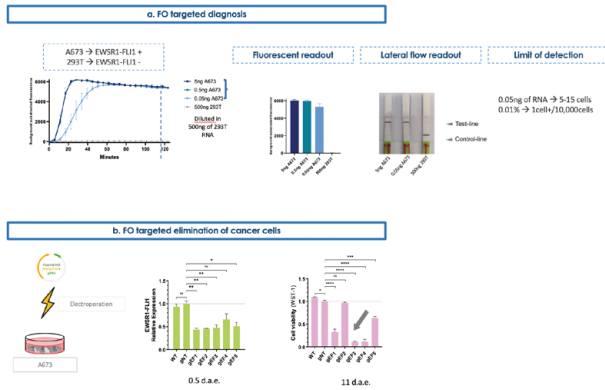


FIGURE 1 We have taken advantage of the versatile CRISPR/Cas13 system to: (a) develop a sensitive, specific, affordable, and instrument-free diagnostic test for FO detection in patient samples; and (b) silence FO RNA inducing efficient and selective elimination of cancer cells.

method, RNA targeting with Cas13 results in reversible and temporally controllable alterations without modifying the DNA. Furthermore, compared to shRNAs, the Cas13 method is associated with high knockdown efficiency and no off-target effects, offering unique advantages when used for therapeutic purposes. Diagnostic methods based on Cas13 provide rapid RNA detection with attomolar sensitivity and single-base mismatch specificity. ■

PUBLICATIONS

Kartal E *et al.* (incl. Rodríguez-Perales S, Torres-Ruiz R, Real FX, Malats N) (2022) A faecal microbiota signature with high specificity for pancreatic cancer. *Gut* 71, 1359-1372.

Zhu L *et al.* (Blanco-Aparicio C, Martínez S, Ortega-Paino E, Torres-Ruiz R, Rodríguez-Perales S, Muñoz J, Caleiras E, Megías D, Quintela-Fandino M, Pastor J, Valiente M) (2022). A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med* 14, e14552.

Villarroya-Beltri C *et al.* (incl. Torres-Ruiz R, Rodríguez-Perales S, Malumbres M, Urioste M) (2022). Biallelic germline mutations in MAD1L1 induce a syndrome of aneuploidy with high tumor susceptibility. *Sci Adv* 8, eabq5914.

Montero-Conde C *et al.* (incl. Moya FJ, Currás-Freixes M, Gonzalez-Neira A, Megías D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M) (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.

Baquero JM *et al.* (incl. Torres-Ruiz R, Blanco-Aparicio C, Rodríguez-Perales S,

Benítez J) (2022). OGG1 inhibition triggers synthetic lethality and enhances the effect of PARP inhibitor olaparib in BRCA1-deficient TNBC cells. *Front Oncol* 12, 888810.

Puig-Serra P, Casado-Rosas MC, Martínez-Lage M, Olalla-Sastre B, Alonso-Yanez A, Torres-Ruiz R, Rodríguez-Perales S (2022). CRISPR approaches for the diagnosis of human diseases. *Int J Mol Sci* 23, 1757. (*) Co-corresponding authors.

PATENT

Malats Riera N, Bork P, Kartal E, Molina Montes E, Rodríguez S, Estudillo L, Real FX, Schmidt TSB, Zeller G, Wirbel J, Mais-trenko OM. Faecal Microbiota Signature for Pancreatic Cancer. PCT application (2022). *PCT/EP2022/077087*. WO2023052486A1.

AWARDS AND RECOGNITION

Raul Torres-Ruiz has received the 2022 ESGCT Young Investigator Award from the European Society for Gene and Cell Therapy (ESGCT).

FAMILIAL CANCER CLINICAL UNIT

Maria Currás
Clinical Unit Head

Research Scientist
Ana Osorio (until September)

Graduate Student
Erik Michel Marchena (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 of cancer patients, especially those at a young age, with multiple tumours or other family members with cancer.* Elucidating hereditary cancer helps the physician to decide on appropriate treatment and, for risk relatives, to initiate preventive strategies if they are carriers. We work mainly with colorectal cancer/Lynch syndrome and breast/ovarian cancer, but we are also a referral unit for rare genetic-based and cancer-related diseases. We provide genetic diagnosis at the Familial Cancer Consultancy (FCC) of the University Hospital of Fuenlabrada (UHF), but also in other hospitals in Madrid and the rest of Spain.
2. *Research work on the elucidation of genetic factors related to familial breast and colorectal cancer.* We focus on

“The Familial Cancer Clinical Unit (FCCU) has confirmed *RECQL5* as a novel breast cancer gene and has been involved in understanding the role of OGG1, *BRCA* carrier modifiers, and the first germline biallelic mutation in *MAD1L1*.”

identifying new driver genes and clarifying their role in patient management. In addition, we are interested in genetic risk factors, prognostic markers, and genetic and molecular factors that could affect therapeutics.

Technicians
Alicia Barroso (until September),
Victoria Fernández (TS)*, Verónica
García (TS)*, Maika González-Neira,
Fátima Mercadillo

Master’s Student
Milton Eduardo Salazar (Jan.-Sep.)
(*Universidad Complutense de Madrid*,
Spain)

*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

Clinical and diagnostic activity. The catalogue of genes has been updated and expanded to tumours that were not previously covered. 729 patients visited our consultancy at the UHF, and 1884 genetic studies were carried out in the FCCU laboratory.

Elucidating new breast cancer (BC) genes. We found a statistically significant association between loss-of-function variants in the *RECQL5* gene and BC risk in almost 2000 index cases of Spanish BC families, supporting its role as a novel moderate-risk BC gene.

Understanding the role of new variants in moderate-risk BC genes. Through a mutational analysis of the *BARD1* gene, in a cohort of 1946 Spanish patients with BC using NGS, we saw that the prevalence and spectrum of *BARD1* mutations could vary between different regions of Spain and highlighted the relevance of analysing copy number variations.

New BC therapeutic approaches. Osorio was involved in the discovery that TH5487, an inhibitor of 8-oxoguanine DNA glycosylase 1 (OGG1), increases sensitivity to the PARP inhibitor Olaparib, especially in the context of BRCA1 deficiency. She was also involved in the description of TH10785, which increases OGG1 recruitment and repair of oxidative DNA damage that may have therapeutic applications.

Contributions to the diagnosis and clinical follow-up of PTEN hamartoma tumour syndrome (PHTS). In the largest study performed in the Spanish population with clinical features of PHTS (n = 145), we concluded that to improve clinical diagnosis we should focus on macrocephaly, mucocutaneous lesions, obesity, and gastrointestinal polyposis. We pointed out

the importance of regular weight control and of considering cancer screening at an earlier age. In addition, we participated in an extensive functional characterisation of variants of unknown significance identified in patients with PHTS.

Mosaic variegated aneuploidy (MVA). Urioste was involved in the description of the first germline biallelic mutation in *MAD1L1* as a novel cause of aneuploidy in an individual with no intellectual disability and an unprecedented number of neoplasias, including 5 malignant tumours before the age of 36. ■

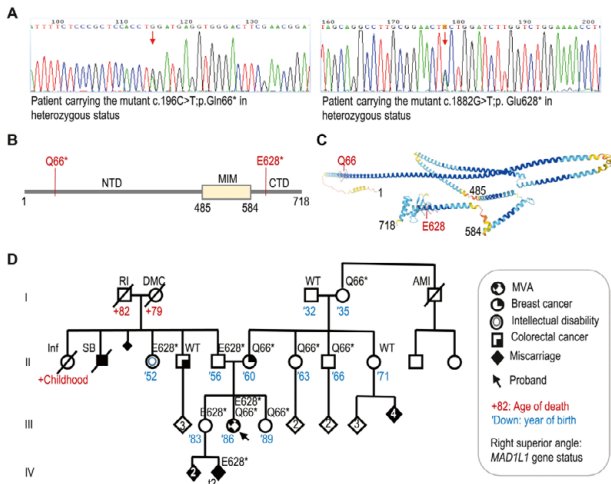


FIGURE 1 Biallelic loss-of-function mutations in *MAD1L1*. (A) *MAD1L1* mutations in the proband. (B) Schematic representation of the MAD1 protein and the mutations found in the proband. NTD, N-terminal domain; MIM, MAD2 interaction motif. (C) Predicted structure of MAD1 and the position of the Q66 and E628 mutated residues. (D) Pedigree of the family. AMI, acute myocardial infarction; DMC, diabetes mellitus complications; Inf, infection; RI, renal insufficiency; SB, stillbirth; t2, trisomy chromosome 2.

SELECTED PUBLICATIONS

- Michel M *et al.* (incl. Osorio A) (2022). Small-molecule activation of OGG1 increases oxidative DNA damage repair by gaining a new function. *Science* 376, 1471-1476.
- Breast Cancer Association Consortium *et al.* (incl. Osorio A) (2022). Pathology of tumors associated with pathogenic germline variants in 9 breast cancer susceptibility genes. *JAMA Oncol* 8, e216744.
- Villarroya-Beltri C *et al.* (incl. Osorio A, Mercadillo F, Urioste M) (2022). Biallelic germline mutations in MAD1L1 induce a syndrome of aneuploidy with hightumor susceptibility. *Sci Adv* 8, eabq5914.
- Marchena-Perea EM *et al.* (incl. Currás-Freixes M, Osorio A) (2022). A large case-control study performed in Spanish population suggests that RECQL5 is the only RECQ

- helicase involved in breast cancer susceptibility. *Cancers (Basel)* 14, 4738.
- Torices L *et al.* (incl. Mercadillo F, Currás M, Urioste M) (2022). Functional analysis of PTEN variants of unknown significance from PHTS patients unveils complex patterns of PTEN biological activity in disease. *Eur J Hum Genet*. PMID: 36543932.
- Grootes I *et al.* (incl. Osorio A) (2022). Incorporating progesterone receptor expres-

sion into the PREDICT breast prognostic model. *Eur J Cancer* 173, 178-193.

- Baquero JM *et al.* (incl. Osorio A) (2022). OGG1 inhibition triggers synthetic lethality and enhances the effect of PARP inhibitor olaparib in BRCA1-deficient TNBC cells. *Front Oncol* 12, 888810.

HUMAN GENOTYPING-CEGEN UNIT

Anna González-Neira
Unit Head

Graduate Students
Hugo Tejera, Alejandro Velasco

Bioinformatician
Guillermo Pita (TS)*



OVERVIEW

In the Unit, we offer researchers access to state-of-the-art methods for high throughput genotyping and sequencing for a wide range of applications. We currently have available different genotyping and sequencing platforms to be used according to the scale of analysis required, and we are continuously developing new techniques to cover all research project needs. The research carried out in the Unit is based on identifying genetic risk factors of breast cancer susceptibility and treatment response. Our main goals are to: i) improve individual breast cancer risk assessment, ii) develop novel strategies for breast cancer early detection, and iii) provide cancer patients more accurate and safe treatment.

“Our research on breast cancer will improve breast cancer risk prediction and guide risk-stratified breast screening strategies.”

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

*Titulado Superior (Advanced Degree)

Student in Practice
Javier Pérez (May.-Dec.) (Master's Programme in Bioinformatics, ENS-ISCIII, Madrid, Spain)

RESEARCH HIGHLIGHTS

Breast cancer risks associated with missense variants in breast cancer susceptibility genes. This study is the result of the European project BRIDGES (Breast Cancer Risk after Diagnostic Gene Sequencing), in which the Unit participates. Protein truncating variants in ATM, BRCA1, BRCA2, CHEK2, and PALB2 are associated with increased breast cancer risk, but risks associated with missense variants in these genes are uncertain. We analysed 59,639 breast cancer cases and 53,165 controls for missense variants in these 5 breast cancer genes, evaluating the risk according to *in silico* prediction-of-deleteriousness algorithms, functional protein domain, and frequency. For ATM, BRCA1, and BRCA2, data were compatible with small subsets (7%, 2%, and 0.6%, respectively) of rare missense variants giving similar risk to those of protein truncating variants in the same gene. For CHEK2, data were more consistent with a large fraction (approximately 60%) of rare missense variants giving a lower risk [OR 1.75, 95% CI (1.47-2.08)] than CHEK2 protein truncating variants. Our results could contribute to the clinical reporting of gene panel testing for breast cancer susceptibility (Dorling L *et al.* 2022).

Pathology of tumours associated with pathogenic germline variants in 9 breast cancer susceptibility genes. The main objective of this study was to determine the distribution of intrinsic subtypes in the 9 confirmed breast cancer genes — ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C, RAD51D, and TP53 — harbouring rare truncating variants and likely pathogenic missense variants associated with increased breast cancer risk. For this purpose, we used data from the BRIDGES project, including 42,680 patients and 46,387 control participants. The results suggested that

variants in the 9 breast cancer risk genes are generally associated with triple-negative and/or high-grade disease. Together, the 9 genes were associated with 27.3% of all triple-negative tumours in women 40 years or younger. (Breast Cancer Association Consortium *et al.* 2022).

Novel genes and sex differences in Covid-19 severity. The study is the result of the Spanish COalition to Unlock Research on host GEnetics on COVID-19 (SCOURGE) consortium, in which the Unit participates. The consortium was launched in May 2020 to find biomarkers of evolution and prognosis that can have an immediate impact on the clinical management and therapeutic decisions in SARS-CoV-2 infections. We conducted a genome-wide study of COVID-19 with patients recruited in Spain from 34 centres in 25 cities. The discovery stage of the study comprised up to 9,371 COVID-19 positive cases and 5,943 population controls. Replication was pursued in an additional 1,598 COVID-19 cases and 1,068 population controls, and in other studies from the Host Genetics Initiative. When we performed sex-disaggregated genome-wide association studies for COVID-19 hospitalisation, genome-wide significance ($P < 5 \times 10^{-8}$) was crossed for variants in 3p21.31 and 21q22.11 loci only among males ($P = 1.3 \times 10^{-22}$ and $P = 8.1 \times 10^{-12}$, respectively), and for variants in 9q21.32 near TLE1 only among females ($P = 4.4 \times 10^{-8}$). The results in the overall analysis revealed 2 novel risk loci in 9p13.3 and 19q13.12, associated with AQP3 ($P = 2.7 \times 10^{-8}$) and ARHGAP33 ($P = 1.3 \times 10^{-8}$), respectively. In summary, new candidate variants for COVID-19 severity and evidence supporting genetic disparities among sexes are provided (Cruz R *et al.* 2022). ■

► PUBLICATIONS

- Breast Cancer Association Consortium *et al.* (incl. González-Neira A) (2022). Pathology of tumors associated with pathogenic germline variants in 9 breast cancer susceptibility genes. *JAMA Oncol* 8, e216744.
- Dorling L *et al.* (incl. González-Neira A) (2022). Breast cancer risks associated with missense variants in breast cancer susceptibility genes. *Genome Med* 14, 51.
- Grootes I *et al.* (incl. González-Neira A) (2022). Incorporating progesterone re-

ceptor expression into the PREDICT breast prognostic model. *Eur J Cancer* 173, 178-193.

- Ahearn TU *et al.* (incl. González-Neira A, Pita G) (2022). Common variants in breast cancer risk loci predispose to distinct tumor subtypes. *Breast Cancer Res* 24, 2.
- Marchena-Perea EM *et al.* (incl. Tejera-Pérez H, Pita G, Núñez-Torres R) (2022). A large case-control study performed in Spanish population suggests that RECQL5 is the only RECQ helicase involved in breast cancer susceptibility. *Cancers (Basel)* 14, 4738.
- Dennis J *et al.* (incl. González-Neira A) (2022). Rare germline copy number var-

iants (CNVs) and breast cancer risk. *Commun Biol* 5, 65.

- Cruz R *et al.* (incl. Pita G, Alvarez N, González-Neira A) (2022). Novel genes and sex differences in COVID-19 severity. *Hum Mol Genet* 31, 3789-3806.
- Hurkmans EGE *et al.* (incl. González-Neira A) (2022). SLC7A8 coding for LAT2 is associated with early disease progression in osteosarcoma and transports doxorubicin. *Front Pharmacol* 13, 1042989.
- Benito-Sánchez B, *et al.* (incl. Núñez-Torres R, Pita G, González-Neira A) (2022). Apparent regional differences in the

spectrum of BARD1 pathogenic variants in Spanish population and importance of copy number variants. *Sci Rep* 12, 8547.

- Santonja Á *et al.* (incl. González-Neira A) (2022). Role of germline variants in the metastasis of breast carcinomas. *Oncotarget* 13, 843-862.
- Vega T *et al.* (incl. González-Neira A) (2022). Genetic, environmental and lifestyle factors associated with longevity. Protocol and response of the LONGECYL Study. *Gac Sanit* 36, 260-264.

CLINICAL RESEARCH PROGRAMME

MIGUEL QUINTELA-FANDINO Acting Programme Director



The Clinical Research Programme (CRP) has 2 main goals: 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) the development of novel agents; 2) the 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 to facilitate interaction between the 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*, *Hospital La Paz*, and *Hospital de Fuenlabrada*). These agreements foster the interaction between clinicians and scientists and enable scientists from all CNIO Programmes to participate in translational research studies. The ongoing collaborations between CRP Units and CNIO Groups from other Programmes now represent 18 projects and 4 coordinated grants, which account for the CNIO's high translational research activity. During 2022, 9 medical oncology residents from different Spanish hospitals completed their optional training visits (3-month stays) at the CNIO.

Although clinical activity was hampered considerably in 2022 due to COVID-19, the clinical groups have managed to produce highly impactful research. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, found the first specific, predictive, and explainable predictive factors for paclitaxel. The 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 Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez López, developed a CAR-T therapy against multiple myeloma, a highly unmet clinical need. The Molecular Diagnostics Unit, headed by Luis Lombardía, continues to provide support to hospitals in the diagnosis of different malignancies, performing >1000 diagnosis this year. Also in 2022, the Junior Prostate Cancer Clinical Research Unit ended its stay with us because of the completion of the Junior Group Leader's career development plan at the CNIO, and we started a process of recruiting additional Groups for the Clinical Research Programme. The selection process is now complete and we are excited to announce the incorporation of 2 new Senior Groups: the *Hospital 12 de Octubre*-CNIO Cancer Immunotherapy Clinical Research Unit, led by Dr Luis Álvarez-

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

Vallina; and the *Hospital La Paz*-CNIO Paediatric Cancer Clinical Research Unit, headed by Dr Antonio Pérez-Martínez. These groups will cover 2 unmet needs at the CNIO: the development of novel cancer immunotherapy agents, as well as research in childhood cancer. ■

BREAST CANCER CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Clinical Research Unit Head

Research Scientists
María José Bueno, Silvana A. Mouron

Post-Doctoral Fellows
Rebeca G. Jimeno, Ana M. Roncero



Graduate Student
José Luis Ruiz

Technicians
Verónica Jiménez, Manuel Muñoz

Student in Practice
Naomí Patricia (March-August)
(Universidad Alfonso X El Sabio,
Madrid, Spain)

Visiting Scientists
Ana Garrido (until October)
(Hospital Universitario de la Princesa,
Madrid, Spain), Elisa I. Gómez (until
September) (Hospital Universitario
de Fuenlabrada, Madrid, Spain),

Cristina Merino (until July) (Hospital
Universitario 12 de Octubre, Madrid,
Spain), Rocío Moreno (Hospital
Universitario 12 de Octubre, Madrid,
Spain), Berta Nassarre (Peaches
Biotech, Madrid, Spain)

OVERVIEW

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

Our current research areas aim to:

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

“At the Breast Cancer Clinical Research Unit, we are focused on individualising therapy for advanced breast cancer.”

RESEARCH HIGHLIGHTS

We have established a collection of 35 patient-derived *tumoroids* from breast cancer patients. We call a *tumoroid* a mix of a patient-derived organoid (a well-established model for cancer research, which perpetuates the tumour material from a given patient, preserving its mutations and general features, and is highly reliable for drug screening and predictive purposes) and the patient’s cells derived from the immune system. This sophisticated model allows us not only to screen conventional drugs, but also to understand their impact on the ability of the immune system to reject the tumour, a feature that is absent in common patient-derived mouse models of cancer. Tumoroids enable us to improve our understanding of immunotherapy and to better understand the impact of other drugs on the immune system, allowing for personalised synergistic treatment combinations. This collection is expanding, and we plan this to be the core of our research in the coming years.

A critical problem in hormone-positive breast cancer is the development of clonal heterogeneity. Tumours, after progression on aromatase plus CDK4/6 inhibitors, develop many different mutations to circumvent drug exposure, impacting the duration of response to subsequent treatment lines. Our preliminary data suggest that different tumour sub-compartments harbour different sets of mutations, and even selecting a “right” therapeutic choice is insufficient for eradicating a whole tumour. We are now undertaking an approach based on mutational signatures that are pervasive across different clones and that may allow for selecting therapies that kill broader tumour compartments than therapies selected according to traditional point mutations. This is being tested in patient tumoroids.

We finalised our work regarding predictive factors of sensitivity to paclitaxel in early breast cancer from the perspective of phosphorproteomics. A CDK4-Filamin A axis that converges in the regulatory machinery of tubulin acetylation is responsible for turning cancer cells sensitive to this drug. This pair of markers is highly accurate in predicting sensitivity in the clinical setting. ■

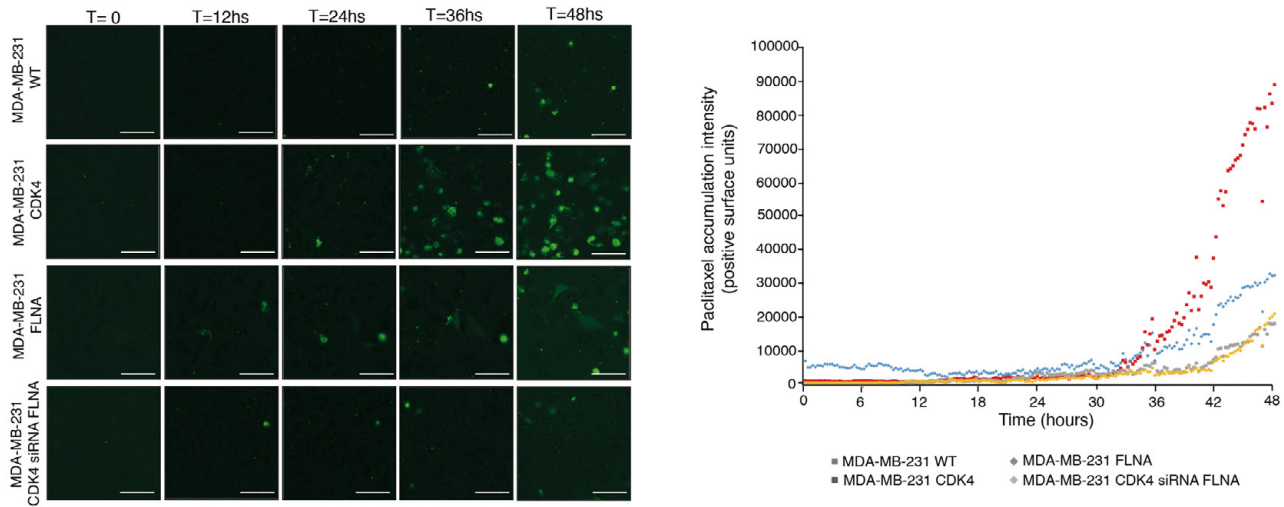


FIGURE 1 Fluorescently labelled paclitaxel was added to live cultures of MDA-MB-231 WT, CDK4 or FLNA cells. MDA-MB-231 CDK4 cells with filamin A knockdown were added to the experiment as well. The greater the green signal, the higher the amount of paclitaxel bound to microtubules. One can appreciate

how both CDK4- and filamin A-overexpressing cell lines display both earlier and higher paclitaxel binding. Scale bar: 75 micrometres. The chart on the right-hand side depicts the signal (in fluorescent surface units) tracing paclitaxel accumulation over a 48-hour time interval, displaying a clear increase in

the 2 overexpressing transfectants (CDK4 and FLNA) compared to the parental cell line, and a reversion of the phenotype by filamin A knockdown in MDA-MB-231 CDK4 cells. General methodology for patient-derived organoid generation.

► PUBLICATIONS

► S. Mouron, M. J. Bueno, A. Lluch, L. Manso, I. Calvo, J. Cortes, J. A. Garcia-Saenz, M. Gil-Gil, N. Martinez-Janez, J. V. Apala, E. Caleiras, Pilar Ximénez-Embún, J. Muñoz, L. Gonzalez-Cortijo, R. Murillo, R. Sánchez-Bayona, J. M. Cejalvo, G. Gómez-López, C. Fustero-Torre, S. Sabroso-Lasa, N. Malats, M. Martinez, A. Moreno, D. Megias, M. Malumbres, R. Colomer, M. Quintela-Fandino (2022). Phosphoproteomic analysis of neoadjuvant breast cancer suggests that increased sensitivity to paclitaxel is driven by CDK4 and filamin A. *Nat Commun* 13, 7529.

► Choudhury AD, Higano CS, de Bono JS, Cook N, Rathkopf DE, Wisinski KB, Martin-Liberal J, Lynch M, Heath EI, Baird RD, García-Carbacho J, Quintela-Fandino M, Barry ST, de Bruin EC, Colebrook S,

Hawkins G, Klinowska T, Maroj B, Moorthy G, Mortimer PG, Moschetta M, Nikolaou M, Sainsbury L, Shapiro GI, Siu LL, Hansen AR (2022). A Phase I study investigating AZD8186, a potent and selective inhibitor of PI3Kβ/δ, in patients with advanced solid tumors. *Clin Cancer Res* 28, 2257-69.

► Zhu L, Retana D, García-Gómez P, Álvaro-Espinosa L, Priego N, Masmudi-Martín M, Yebra N, Miarka L, Hernández-Encinas E, Blanco-Aparicio C, Martínez S, Sobrino C, Ajenjo N, Artiga MJ, Ortega-Paino E, Torres-Ruiz R, Rodríguez-Perales S; RENACER, Soffietti R, Bertero L, Cassoni P, Weiss T, Muñoz J, Sepúlveda JM, González-León P, Jiménez-Roldán L, Moreno LM, Esteban O, Pérez-Núñez A, Hernández-Lain A, Toldos O, Ruano Y, Alcázar L, Blasco G, Fernández-Alén J, Caleiras E, Lafarga M, Megías D, Graña-Castro O, Nör C, Taylor MD, Young LS, Varešlija D, Cos-

grove N, Couch FJ, Cussó L, Desco M, Mouron S, Quintela-Fandino M, Weller M, Pastor J, Valiente M (2022). A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastases. *EMBO Mol Med* 14, e14552.

► Hühn D, Martí-Rodrigo P, Mouron S, Hansel C, Tschapalda K, Porebski B, Häggblad M, Lidemalm L, Quintela-Fandino M, Carreras-Puigvert J, Fernandez-Capetillo O (2022). Prolonged estrogen deprivation triggers a broad immunosuppressive phenotype in breast cancer cells. *Mol Oncol* 16, 148-165.

► **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 application (2022). *PCT/EP2022/051700*. *WO2022161984A1*.

► **INTELLECTUAL PROPERTY REGISTRATION**

► Luis Manso Sanchez, Miguel Quintela-Fandino (2022). LUMICA V.1.0. Algorithm – a proprietary algorithm for precision nutrition allocation for cancer patients. Registered with Safe Creative with registration code 2210252495358.

► **AWARDS AND RECOGNITION**

► Panel Member, *Proyectos de Investigación en Salud, Instituto de Salud Carlos III (ISCIII)*, Spain.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

Students in Practice
Paula Broncano and Giselle Coronel
(March-June) (*Centro Educación M^a*)



OVERVIEW

The Molecular Diagnostics Unit (MDU) is primarily engaged in providing support to oncologists, haematologists and pathologists of our National Health System, by offering quality molecular tests for cancer patients. In this regard, the Unit has developed a catalogue with a broad variety of sensitive and specific assays to determine changes in sequences or expression levels of crucial genes that are involved in cancer, and that help to monitor minimal residual disease in patients showing clinical remission as well as to follow-up on their response to therapy. Consequently, MDU is also committed to implementing novel diagnostic solutions, not only to improve clinical practice but also to resolve periodic inquiries from CNIO’s Research Units and Groups. MDU also forms part of several international and national groups aimed at normalising and improving molecular tests in cancer. Finally,

“The ongoing accumulation and combination of actionable biomarkers included in molecular diagnostics tests is bringing us closer to precision medicine, especially for haematological tumours.”

an essential part of our mission is to contribute to academic programmes by hosting clinical post-residents and pre/post graduate students.

Inmaculada - Ríos Rosas, Madrid, Spain), Silvia Novo (September-October) (*Universidad Autónoma de Madrid, Spain*)

Visiting Scientist
Ana Jambrina (*Hospital General Universitario Gregorio Marañón, Madrid, Spain*)

CORE UNIT HIGHLIGHTS

During 2022, our catalogue grew with the addition of a new assay, which will enable the detection, through bi-directional Sanger sequencing, of structural alterations in exon 3 of the β -catenin gene, *CTNNB1*. High frequencies of CTNNB1 activating mutations and in-frame deletions have been spotted in 3% of all cancers, including melanoma, lung, endometrium, colon, kidney, and ovarian tumours. Since they have been associated with altered sensitivity to specific drugs, their analysis can be useful as a predictive marker by suggesting different therapy options.

We also improved the clinical utility of *KRAS* gene testing by supplementing the detection of the recurrent mutations already implemented in exons 2 and 3 to exon 4. The extended assay is intended to enable clinicians to manage their patients with colorectal, pancreatic, or lung adenocarcinomas, since somatic mutations in exon 4 have been linked to a better prognosis, and they can also be used as an inclusion criterion to enrol patients in active or forthcoming clinical trials.

Additionally, in the context of our partnership with *GBMH* (*Grupo de Biología Molecular y Hematología*), we are participating in the development of comprehensive national guidelines for the management of patients with different haematological cancers. Our initial contribution was to complete a list of diagnostic, prognostic, and predictive markers that should be systematically analysed using Next Gene Sequencing (NGS) in order to manage patients with acute myeloid leukaemia (AML). To evaluate the clinical and analytical utility of this diagnostic tool, the next step will be to design a panel containing at least the markers required for the analysis, and then to establish the feasibility of using RNAseq technology to be able to analyse simultaneously both single and fusion genes (FIGURE 1).

Finally, during 2022, in the framework of our training policy, we hosted a medical resident, an undergraduate student, and 2 future technicians in anatomical pathology. ■

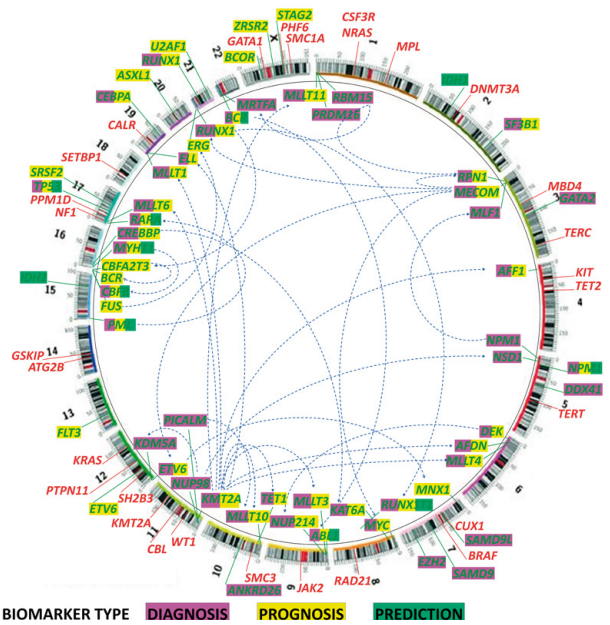


FIGURE 1 The comprehensive massive sequencing of validated markers (green), yet to be validated, are expected to complete the whole panel required for full precision medicine of AMLs. prognosis, and treatment of patients

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head

Research Scientists
Santiago Barrio, Lucía V. Fernández, Miguel Gallardo, María Linares

Clinical Investigators
Rosa Ayala, María Calbacho, Gonzalo Carreño, Pilar Carreras, Teresa Cedena, Francisco Javier de La Serna, Ana Jiménez, Pilar Martínez, Inmaculada Rapado,



Antonia Rodríguez, Ricardo Sánchez

Post-Doctoral Fellows
Almudena García, Larissa A Haertle (since Feb.), Alejandra Leivas, Yanira Ruíz-Heredia, Antonio Valeri, María Velasco-Estévez (MSCA fellow) *

* Marie Skłodowska-Curie Actions (MSCA).

Graduate Students
Pedro Aguilar, Noemí Álvarez, Eva Castellano, Laura Córdoba, Jessica Encinas, Roberto García, Marta Ibáñez, Elena Maroto, Michael Ochieng, Alejandra Ortiz, Álvaro Otero (since Feb.), Alba Rodríguez, Laura Sánchez

Technicians
Raquel Ancos, Andrés Arroyo (since Feb.), Irene Bragado (since Feb.),

Natalia S. Buenache, Sara Dorado, Adrián Fernández, Alicia Giménez, Laura Moreno, Miguel A. Navarro, Juan M. Rosa, Laura Rufián, Daniel Valdés (until Jul.)

Student in Practice
Carmen Cano (Jan.-June) (Bachelor's Degree Final Project) and Andrea Sánchez de La Cruz (until June) (Master's Thesis) (*Univ. Autónoma de Madrid*, Spain)

Visiting Scientists
María Hernández-Sánchez (Jul.-Dec.) (*IBSAL*, Salamanca, Spain), Alfonso Navarro (*FIBHULP*, Madrid, Spain)

OVERVIEW

Haematological clinical research has traditionally focused on haematological malignancies, aplasia and syndromes. Now, with advances in immunotherapy, haematologists play a key role in research on novel immunotherapeutic approaches, the role of the immune response to tumours, or the role of infection and inflammation in cancer.

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

- Traditional haematological neoplasms (leukaemia, myeloma, lymphoma): new diagnostic approaches, biomarkers, and treatments.
- Aplastic haematological malignancies such as bone marrow failures: new drivers and molecular mechanisms.
- Novel diagnosis and tumour burden monitoring: liquid biopsy and minimal residual disease.
- Role of inflammation and infection in haematological neoplasms.
- Novel immunotherapeutic approaches in haematological malignancies: NK-CARs, BITES.
- Traditional immunotherapeutic approaches in haematological malignancies and paediatric cancers: T-CARs and immune checkpoints inhibitors.

“Teclistamab, a bispecific anti-CD3 and anti-BCMA monoclonal antibody, marker of myeloma cells, has demonstrated a high rate of deep and durable response in relapsed multiple myeloma patients.”

RESEARCH HIGHLIGHTS

Teclistamab in relapsed or refractory multiple myeloma

Teclistamab is a bispecific anti-CD3 and anti-BCMA monoclonal antibody. We recently published, in collaboration with other groups in the consortium, a novel clinical trial in multiple myeloma-relapsed patients. Our results showed a high rate of durable and deep response in the patients studied, with toxicity (grade 1 and 2) consistent with T-cell redirection.

Tisagenlecleucel trials in B-cell lymphomas

Tisagenlecleucel is an autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy approved for different B-cell lymphomas. In 2022 we published, in collaboration with other groups in the consortium, an article in the *New England Journal of Medicine* describing the results of second-line tisagenlecleucel in aggressive B-cell lymphoma. Our results showed that tisagenlecleucel was not superior to standard salvage therapy in this trial.

However, in another clinical trial, the ELARA phase 2 multinational trial against follicular lymphoma, we reported its safety and effectiveness in high-risk patients with relapsed follicular lymphoma. This work was recently published in *Nature Medicine*.

Infection prediction in multiple myeloma

Infections are among the most common complications in multiple myeloma, in association with morbidity and mortality. We analysed the clinical variables of 4 clinical trials of the Spanish Myeloma Group with n=1.347 patients. We discovered that an increased risk of severe infection correlates with serum albumin, ECOG, gender, and non-IgA type multiple myeloma. These simple variables led to the stratification into low, intermediate, and high risk of severe infection. Patients with intermediate and high risk could be candidates for prophylactic antibiotic therapy. This work was published in *Blood Cancer Journal*. ■

PUBLICATIONS

- Moreau P *et al.* (incl. Martínez-López J) (2022). Teclistamab in relapsed or refractory multiple myeloma. *N Engl J Med* 387, 495-505.
- Bishop MR *et al.* (incl. Martínez-López J) (2022). Second-line tisagenlecleucel or standard care in aggressive B-cell lym-

- phoma. *N Engl J Med* 386, 629-639.
- Fowler NH *et al.* (incl. Martínez-López J) (2022). Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. *Nat Med* 28, 325-332.
- Termini R *et al.* (incl. Martínez-López J; PETHEMA/GEM and iMMunocell Cooperative Groups) (2022). Circulating tumor and immune cells for minimally invasive

- phoma. *N Engl J Med* 386, 629-639.
- Fowler NH *et al.* (incl. Martínez-López J) (2022). Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. *Nat Med* 28, 325-332.
- Termini R *et al.* (incl. Martínez-López J; PETHEMA/GEM and iMMunocell Cooperative Groups) (2022). Circulating tumor and immune cells for minimally invasive

- phoma. *N Engl J Med* 386, 629-639.
- Fowler NH *et al.* (incl. Martínez-López J) (2022). Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. *Nat Med* 28, 325-332.
- Termini R *et al.* (incl. Martínez-López J; PETHEMA/GEM and iMMunocell Cooperative Groups) (2022). Circulating tumor and immune cells for minimally invasive

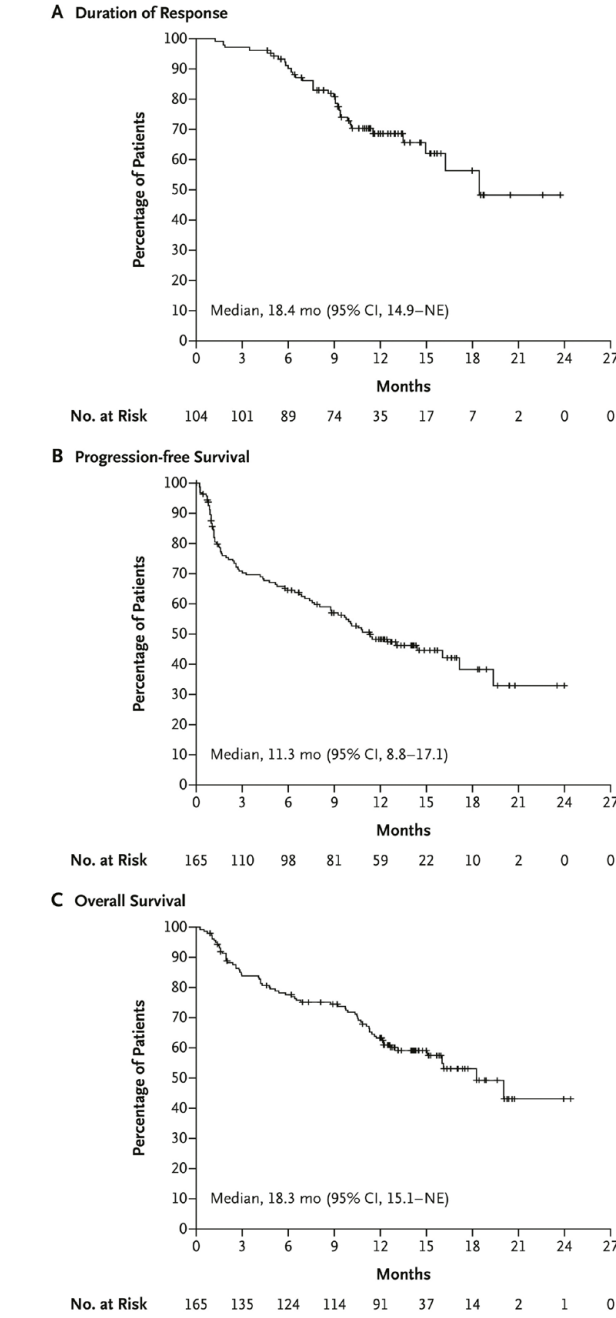


FIGURE 1 Teclistamab trial in multiple myeloma. Kaplan-Meier analysis of response duration and of progression-free and overall survival.

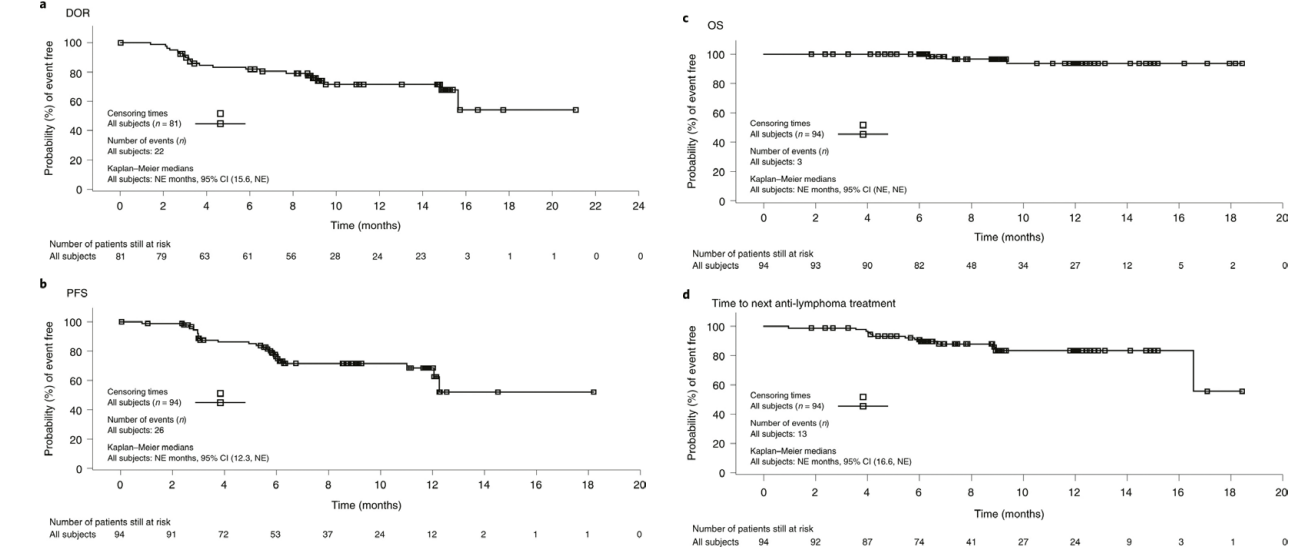


FIGURE 2 Kaplan-Meier curves for patients with relapsed or refractory (r/r) follicular lymphoma who received tisagenlecleucel infusion.

Overcoming tumor resistance mechanisms in CAR-NK cell therapy. *Front Immunol* 13, 953849.

- Salles GA *et al.* (incl. Jiménez-Ubieto A, Martínez-López J) (2022). Efficacy comparison of tisagenlecleucel vs usual care in patients with relapsed or refractory follicular lymphoma. *Blood Adv* 6, 5835-5843.
- Sánchez R, Dorado S, Ruiz-Heredia Y, Martín-Muñoz A, Rosa-Rosa JM, Ribera J, García O, Jiménez-Ubieto A, Carreño-Tarragona G, Linares M, Rufián L, Juárez A, Carrillo J, Espino MJ, Cáceres M, Expósito S, Cuevas B, Vanegas R, Casado LF, Torrent A, Zamora L, Mercadal S, Coll R, Cervera M, Morgades M, Hernández-Rivas JA, Bravo P, Serí C, Anguita E, Barragán E, Sargas C, Ferrer-Marín F, Sánchez-Calero J, Sevilla J, Ruiz E, Villalón L, Del Mar Herráez M, Ríaza R, Magro E, Steegman JL, Wang C, de Toledo P, García-Gutiérrez V, Ayala R, Ribera JM, Barrio S, Martínez-López J (2022). Detection of kinase domain mutations in BCR:ABL1 leukemia by ultra-deep sequencing of genomic DNA. *Sci Rep.* 12, 13057.
- Sanz A, Ayala R, Hernández G, Lopez N, Gil-Alos D, Gil R, Colmenares R, Carreño-Tarragona G, Sánchez-Pina J, Alonso RA, García-Barrio N, Pérez-Rey D, Meloni L, Calbacho M, Cruz-Rojo J, Pedrera-Jiménez M, Serrano-Balazote P, de la Cruz J, Martínez-López J (2022). Outcomes and patterns of treatment in chronic myeloid leukemia, a global perspective based on a real-world data global network. *Blood Cancer J* 12, 94.
- Kayser S, Martínez-Cuadrón D, Hanoun M, Stölzel F, Gil C, Reinhardt HC, Aguiar E, Schäfer-Eckart K, Burgues JMB, Steff-

- en B, Bernal T, Krause SW, Ríaza R, Schliemann C, Cervera J, Kaufmann M, Torres-Miñana L, Hänel M, Acuña-Cruz E, Jost E, Algarra JL, Crysandt M, Fransecky L, Cornago-Navascues J, Kraus S, Martínez-López J, Einsele H, Niemann D, Neubauer A, Seggewiß-Bernhardt R, Scholl S, Klein SA, Schmid C, Schaich M, Schmidt-Hieber M, Zukunft S, Ho AD, Platzbecker U, Baldus CD, Müller-Tidow C, Thiede C, Bornhäuser M, Serve H, Levis M, Montesinos P, Röhlig C, Schlenk RF (2022). Characteristics and outcome of patients with acute myeloid leukemia and trisomy 4. *Haematologica*. PMID: 35678031.
- Garcés JJ *et al.* (incl. Martínez-López J) (2022). Circulating tumor cells for the staging of patients with newly diagnosed transplant-eligible multiple myeloma. *J Clin Oncol* 40, 3151-3161.
- Mosquera Orgueira A *et al.* (incl. Martínez-López J, PETHEMA/GEM Cooperative Group) (2022). Unsupervised machine learning improves risk stratification in newly diagnosed multiple myeloma: an analysis of the Spanish Myeloma Group. *Blood Cancer J* 12, 76.
- Rojas EA *et al.* (incl. Martínez-López J) (2022). Expression of p53 protein isoforms predicts survival in patients with multiple myeloma. *Am J Hematol* 97, 700-710.
- Puig N *et al.* (incl. Martínez-López J) (2022). Mass spectrometry vs immunofixation for treatment monitoring in multiple myeloma. *Blood Adv.* 6, 3234-3239.
- Rodríguez-García A, Linares M, Morales ML, Allain-Maillet S, Mennesson N, Sanchez R, Alonso R, Leivas A, Pérez-Rivilla A, Bigot-Corbel E, Hermouet S, Martínez-López J (2022). Efficacy of antiviral

treatment in hepatitis C virus (HCV)-driven monoclonal gammopathies including myeloma. *Front Immunol* 12, 797209.

- Zozaya N *et al.* (incl. García-Sanz R, Martínez-López J) (2022). A strategic reflection for the management and implementation of CAR-T therapy in Spain: an expert consensus paper. *Clin Transl Oncol* 24, 968-980.

Publications at other institutions

- Ribera JM, García-Calduch O, Ribera J, Montesinos P, Cano-Ferri I, Martínez P, Esteve J, Esteban D, García-Fortes M, Alonso N, González-Campos J, Bermúdez A, Torrent A, Genescà E, Mercadal S, Martínez-López J, García-Sanz R (2022). Ponatinib, chemotherapy, and transplant in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood Adv* 6, 5395-5402.
- Dimopoulos MA, Moreau P, Augustson B, Castro N, Pika T, Delimpasi S, De la Rubia J, Maiolino A, Reiman T, Martínez-López J, Martin T, Mikhael J, Yong K, Risse ML, Asset G, Marion S, Hajek R (2022). Isatuximab plus carfilzomib and dexamethasone in patients with relapsed multiple myeloma based on prior lines of treatment and refractory status: IKEMA subgroup analysis. *Am J Hematol* 98, E15-E19.
- Guerrero C, Puig N, Cedena MT, Goicoechea I, Perez C, Garcés JJ, Botta C, Calasanz MJ, Gutierrez NC, Martín-Ramos ML, Oriol A, Rios R, Hernandez MT, Martínez-Martínez R, Bargay J, de Arriba F, Palomera L, Gonzalez-Rodriguez AP, Mosquera-Orgueira A, Gonzalez-Perez MS, Martínez-López J, Lahuerta JJ, Rosiñol L, Blade J, Mateos MV, San-Miguel JF, Paiva B (2022). A machine learning mod-

el based on tumor and immune biomarkers to predict undetectable MRD and survival outcomes in multiple myeloma. *Clin Cancer Res* 28, 2598-2609.

PATENT

- Martínez López J, Valeri Lozano A, García Ortiz A, Gallardo Delgado M, Encinas Mayoral J, Maroto Martín E, Castellano Esparza E. Combination of CAR-NK cells with NKG2A blocking agents, pharmaceutical composition comprising the same and use thereof. PCT application (2022). *PCT/EP2022/060537. WO2022223684A1*.

AWARDS AND RECOGNITION

- María Velasco-Estevéz: CRIS Cancer Foundation Post-Doc Talent Award, Spain.
- María Linares: Health Research Project (ISCIII); I+D+I RETOS Colaboración Project (MCI), Spain.
- Larissa Haertle: DFG Walter Benjamin Programme Fellowship (German Research Foundation); Poster prize, DGHO congress (German Society for Hematology and Medical Oncology); UNA4CAREER Award, Spain.
- Pedro Aguilar: Young EHA PhD Research Student Award from the European Hematology Association (EHA); FEHH Fellowship from the Spanish Foundation for Hematology and Hemotherapy (FEHH).
- Álvaro Otero: Predoctoral Health Research Training (PFIS) Contract (MCI), Spain.
- Alba Rodríguez: FEHH Fellowship, The Spanish Foundation for Hematology and Hemotherapy.
- Pedro Aguilar and Roberto García: Presidential Symposium of the European Hematology Association.

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. Uceró

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

Post-Doctoral Fellows
M. Magdalena Abraham, M. Cristina



Cirauqui, Juan Manuel Coya, Gorka Ruiz de Garibay

Graduate Students
Carlos Carretero, M. Inés Díaz, M. Carmen Fernández, Santiago García, David Gómez, María Gutiérrez,

Alberto Lens, Ángel Núñez, Javier Ramos, Beatriz Rubio, Joan Salvador Russo (since Sep.), Alba Santos, Patricia Yagüe

Technicians
Eva Álvarez, Nuria Carrizo, Eva M.

Casas (since Mar.), Patricia Cozar, Laura García, Beatriz Gil, Patricia Llamas, Alicia Luengo, Patricia Plaza, Laura Ramírez (since Sep.), Rocio Suárez, César Vélez (since Aug.)

**Titulado Superior (Advanced Degree)*

Students in Practice
Jaime Franco (Jul.-Dec.) (Bachelor's Student, *Univ. de Alcalá de Henares*, Spain), Sara Rico (Mar.-Sep.) (Master's Thesis, *Univ. Complutense de Madrid*, Spain)

OVERVIEW

Lung cancer continues to be 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 are particularly interested in 2 research areas: the identification of new 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 test new therapeutic strategies. Finally, our Unit has extensive experience in taking new drugs to the clinic, as well as in conducting practice-changing phase 2/3 trials in the fields of personalised cancer care and immuno-oncology.

“Our Unit has significantly contributed to the development of novel biomarkers that have impacted the currently available selection of targeted therapies (e.g., EGFR mutation in the clinic) and novel immunotherapeutics (e.g., tumour mutational burden). We have led randomised clinical trials with novel immunotherapies and other agents as monotherapies or in combination (e.g., chemotherapy plus durvalumab in SCLC or chemotherapy plus nivolumab and ipilimumab in NSCLC) in lung cancer that have impacted clinical practice worldwide.”

RESEARCH HIGHLIGHTS

Biomarker discovery and implementation

We own an extensive patient-derived xenograft (PDX) platform of 50 non-small cell lung cancer (NSCLC) and 7 small cell lung cancer (SCLC) models that are comprehensively characterised at the histological, genomic, transcriptomic, and proteomic levels, and that have contributed to the discovery of relevant findings. For example, 2 NSCLC PDX models with high and low expression levels of EGFR contributed to demonstrate that cetuximab-functionalised gold nanoparticles can be used for selective drug delivery in mitochondria-targeted cancer therapy (González-Rubio S *et al.*, *Nanoscale*, 2022). In addition, SCLC PDXs were used to confirm YES1 as a new druggable oncogenic target in SCLC. Pharmacologic blockade with the novel YES1 inhibitor CH6953755 or dasatinib induced marked antitumour activity in organoid models and cell- and patient-derived xenografts (Redin E *et al.*, *J Thorac Oncol*,

2022). Our platforms are expanding in numbers and histologies (NSCLC, SCLC and mesothelioma as well), cell source (tumours but also circulating tumour cells), and include PDX and patient-derived organoids. We have also successfully developed a number of huPDX models.

We have comprehensively characterised the molecular and immune features of a cohort of 18 early-stage, clinically annotated, large cell carcinoma (LCC) cases by genomic and immune-targeted sequencing panels, along with immunohistochemistry of immune cell populations (FIGURE 1). Unbiased clustering defined 2 novel subgroups of LCC that allowed us to identify a set of biomarkers that could potentially predict response to immunotherapy in the least studied form of NSCLC (Ramos-Paradas J, ..., Paz-Ares L, *J Clin Med*, 2022). In addition, we performed a multiparametric characterisation of a cohort composed of 120 resected tumour samples from limited-stage

SCLC patients. Samples were described by immunohistochemistry, RNA-seq targeted panel of immune related-genes, exome sequencing, and spatial transcriptomics. We found a novel classification of early-stage SCLC with potential clinical impact in both prognosis and immunotherapy response (manuscript in preparation).

Early clinical trials

Our Group has significantly expanded its activities regarding the testing of new molecules and combinations in solid tumours, particularly in the field of immune-based approaches and targeted therapies; in 2022, we participated in more than 150 projects in this research area, including 85 new trials. We reported data from a multicenter, international, phase 2 study in which trastuzumab deruxtecan was administered to patients who had metastatic HER2-mutant NSCLC that was refractory to standard treatment. Trastuzumab deruxtecan showed durable anticancer activity, and the observed toxic effects were generally consistent with those in previously reported studies (Li BT, ..., Paz-Ares L, ..., *N Engl J Med*, 2022). We also evaluated the efficacy and safety of pralsetinib in patients with RET fusion-positive solid tumours. Our pan-cancer phase 1/2 clinical trial showed pralsetinib as a potential well-tolerated treatment option with rapid, robust and durable anti-tumour activity in these patients (Subbiah V, ..., Paz-Ares L, ..., *Nat Med*, 2022).

Changing standard-of-care treatments in clinical practice

The Lung Cancer Clinical Research Unit has led phase 3 trials whose results have significantly impacted clinical practice in the context of stage IV lung cancer, such as the combination of first-line nivolumab plus ipilimumab in advanced NSCLC (Paz-Ares L *et al.*, *J Thorac Oncol*, 2022). With the updated results from the randomised, open-label, phase 3 CheckMate 227 Part 1 trial, we showed that at more than 4 years' minimum follow-up, with all the patients off immunotherapy treatment for at least 2 years, first-line nivolumab plus ipilimumab continued to demonstrate durable long-term efficacy. We also assessed pembrolizumab as adjuvant therapy for completely resected stage IB-IIIA NSCLC

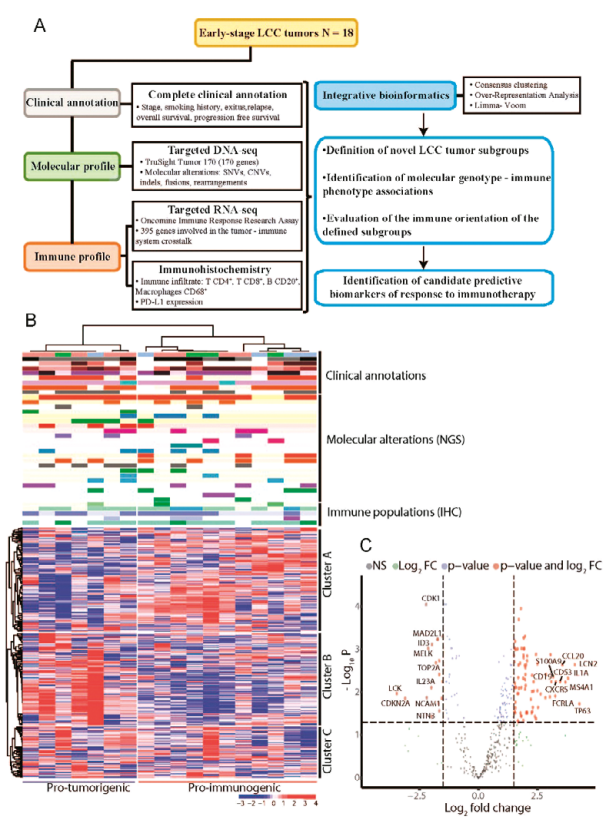


FIGURE 1 Immune profile of large cell carcinoma (LCC) of the lung. **(A)** Experimental design of the study. **(B)** Definition of novel LCC tumour subgroups. Heatmap of expression of genes involved in tumour-immune system communication. Groups of tumours are shown in the horizontal axis and clusters of genes in the vertical axis as defined by consensus clustering. Molecular, immune and clinical annotations are shown above the heatmap. **(C)** Volcano plot of differentially expressed genes between the pro-immunogenic group and the pro-tumorigenic group of LCC tumours. A false discovery rate (FDR) ≤ 0.05 and \log_2 fold change ≥ 1.5 were required to reach statistical significance.

(O'Brien M, Paz-Ares L, *et al.*, *Lancet Oncol*, 2022). In this randomised, triple-blind, phase 3 trial we found that pembrolizumab significantly improved disease-free survival compared with placebo and was not associated with new safety signals (FIGURE 2). Pembrolizumab is potentially a new treatment option for stage IB-IIIA NSCLC after complete resection and, when recommended, adjuvant chemotherapy, regardless of PD-L1 expression. ■

PUBLICATIONS

O'Brien M, Paz-Ares L *et al.* (incl. EORTC-1416-LCG/ETOP 8-15 - PEARLS/KEYNOTE-091 Investigators) (2022). Pembrolizumab versus placebo as adjuvant therapy for completely resected stage IB-IIIA non-small-cell lung cancer (PEARLS/KEYNOTE-091): an interim analysis of a randomised, triple-blind, phase 3 trial. *Lancet Oncol* 23, 1274-1286.

Li BT *et al.* (incl. Paz-Ares L) (2022). Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med* 386, 241-251.

Monteiro C *et al.* (incl. Miarka L, Perea-

García M, Priego N, García-Gómez P, Álvaro-Espinoza L, de Pablos-Aragoneses A, Yebra N, Retana D, Baena P, Fusterro-Torre C, Graña-Castro O, Troulé K, Caleiras E, Ferrer I, Suárez R, Garrido-Martín EM, Paz-Ares L; RENACER, Valiente M (2022). Stratification of radio-sensitive brain metastases based on an actionable S100A9/RAGE resistance mechanism. *Nat Med* 28, 752-765.

Subbiah V *et al.* (incl. Paz-Ares L) (2022). Pan-cancer efficacy of pralsetinib in patients with RET fusion-positive solid tumors from the phase 1/2 ARROW trial. *Nat Med* 28, 1640-1645.

Spigel DR *et al.* (incl. Paz-Ares L) (2022).

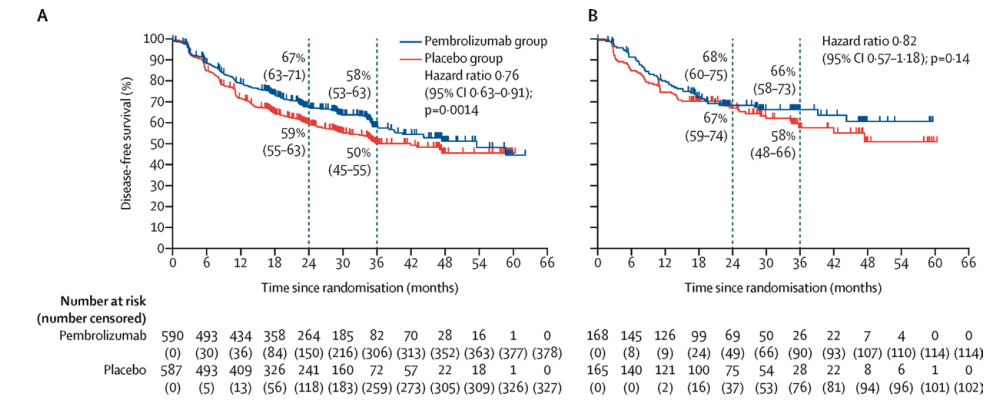


FIGURE 2 PEARL phase 3 trial evaluating adjuvant pembrolizumab versus placebo in patients with resected early-stage NSCLC. Kaplan-Meier estimates of disease-free survival assessed per RECIST version 1.1 for **(A)** the overall population and **(B)** the PD-L1 Tumour Proportion Score (TPS) of 50% or greater population, showing a sustained benefit in the pembrolizumab group (53.6 months) versus the placebo group (42.0 months) (HR 0.76 [95% CI 0.63-0.91], p=0.0014).

Five-year survival outcomes from the PACIFIC trial: durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. *J Clin Oncol* 40, 1301-1311.

Zugazagoitia J, Paz-Ares L (2022). Extensive-stage small-cell lung cancer: first-line and second-line treatment options. *J Clin Oncol* 40, 671-680.

Garassino MC *et al.* (incl. Paz-Ares L) (2022). Durvalumab after sequential chemoradiotherapy in stage III, unresectable NSCLC: the phase 2 PACIFIC-6 trial. *J Thorac Oncol* 17, 1415-1427.

Paz-Ares LG *et al.* (2022). First-line nivolumab plus ipilimumab in advanced NSCLC: 4-year outcomes from the randomized, open-label, phase 3 CheckMate 227 part 1 trial. *J Thorac Oncol* 17, 289-308.

Redin E, Garrido-Martin EM, Valencia K, Redrado M, Solorzano JL, Carías R, Eche- pare M, Exposito F, Serrano D, Ferrer I, Nunez-Buiza A, Garmendia I, García-Ped- rero JM, Gurrpide A, Paz-Ares L, Politi K, Montuenga LM, Calvo A (2022). YES1 is a druggable oncogenic target in SCLC. *J Thorac Oncol* 17, 1387-1403.

García-Carbonero R *et al.* (incl. Ries- co-Martínez MC, Del Pozo N, Martínez de Villarreal J, Real FX) (2022). Phase I, multicenter, open-label study of intrave- nous VCN-01 oncolytic adenovirus with or without nab-paclitaxel plus gemci- tabine in patients with advanced solid tumors. *J Immunother Cancer* 10, e003255.

Moutafi MK, Molero M, Martínez Morilla S, Baena J, Vathiotis IA, Gavrielatou N, Cas- tro-Labrador L, de Garibay GR, Adradas V, Orive D, Valencia K, Calvo A, Montue- ga LM, Ponce Aix S, Schalper KA, Herbst RS, Paz-Ares L, Rimm DL, Zugazagoitia J (2022). Spatially resolved proteomic profiling identifies tumor cell CD44 as a biomarker associated with sensitivity to PD-1 axis blockade in advanced non-small- cell lung cancer. *J Immunother Cancer* 10, e004757.

Peters S, Paz-Ares L, Herbst RS, Reck M (2022). Addressing CPI resistance in NS- CLC: targeting TAM receptors to modulate the tumor microenvironment and future prospects. *J Immunother Cancer* 10, e004863.

Borbath I, García-Carbonero R *et al.* (2022). The European Neuroendocrine Tumour Society registry, a tool to assess the prognosis of neuroendocrine neo- plasms. *Eur J Cancer* 168, 80-90.

González-Rubio S, Salgado C, Manzan- da-González V, Muñoz-Úbeda M, Ahija- do-Guzmán R, Natale P, Almendo-Vedia VG, Junquera E, Barcina JO, Ferrer I, Guerrero-Martínez A, Paz-Ares L, López-Montero I (2022). Tunable gold nanorod/NAO conjugates for selective drug delivery in mitochondria-targeted cancer therapy. *Nanoscale* 14, 8028-8040.

Díaz-Cano I, Paz-Ares L, Otano I (2022). Adoptive tumor infiltrating lymphocyte transfer as personalized immunotherapy. *Int Rev Cell Mol Biol* 370, 163-192.

Jimenez-Fonseca P *et al.* (incl. Riesco MDC, García-Carbonero R) (2022). Ex- ternal validity of somatostatin analogs trials in advanced neuroendocrine neo- plasms: the GETNE-TRASGU study. *Neu- roendocrinology* 112, 88-100.

Senan S *et al.* (incl. Paz-Ares L) (2022). Outcomes with durvalumab after chemo- radiotherapy in stage IIIA-N2 non-small- cell lung cancer: an exploratory analysis from the PACIFIC trial. *ESMO Open* 7, 100410.

Ramos-Paradas J, Gómez-Sánchez D, Rosado A, Utero AC, Ferrer I, García-Lu- ján R, Zugazagoitia J, Carriazo N, Enguita AB, Conde E, Garrido-Martín EM, Paz-Ares L (2022). Comprehensive characterization of human lung large cell carcinoma iden- tifies transcriptomic signatures with po-

tential implications in response to immu- notherapy. *J Clin Med* 11, 1500.

Paz-Ares L, Gondas A, Saldana D, Thom- as M, Mascaux C, Bubendorf L, Barlesi F (2022). Genomic testing among patients with newly diagnosed advanced non-small cell lung cancer in the United States: a contemporary clinical practice patterns study. *Lung Cancer* 167, 41-48.

Paz-Ares L, Spigel DR, Chen Y, Jove M, Juan-Vidal O, Rich P, Hayes T, Calderón VG, Caro RB, Navarro A, Dowlati A, Zhang B, Moore Y, Yao X, Kokhreizde J, Ponce S, Bunn PA (2022). RESILIENT part 1: a phase 2 dose-exploration and dose-ex- pansion study of second-line liposomal irinotecan in adults with small cell lung cancer. *Cancer* 128, 1801-1811.

Bote H, Mesas A, Baena J, Herrera M, Paz- Ares L (2022). Emerging immune check- point inhibitors for the treatment of non- small cell lung cancer. *Expert Opin Emerg Drugs* 27, 289-300.

Suboi M, Goldman JW, Wu YL, Johnson ML, Paz-Ares L, Yang JC, Besse B, Su W, Chao BH, Drilon A (2022). LIBRETTO-432, a phase III study of adjuvant selpercatin- ib or placebo in stage IB-IIIA RET fu- sion-positive non-small-cell lung cancer. *Future Oncol* 18, 3133-3141.

Selected publications at other institutions

Paz-Ares LG *et al.* (2022). Safety of first- line nivolumab plus ipilimumab in patients with metastatic non-small cell lung can- cer: a pooled analysis of CheckMate 227, CheckMate 568, and CheckMate 817. *J Thorac Oncol* 18, 79-92.

Vidal J *et al.* (incl. García-Carbonero R) (2022). Liquid biopsy detects early mo- lecular response and predicts benefit to first-line chemotherapy plus cetuximab in metastatic colorectal cancer: PLAT- FORM-B study. *Clin Cancer Res* 29, 379-388.

Levy B, Barlesi F, Paz-Ares L *et al.* (2022). Phase II study of afatinib plus pembroli- zumab in patients with squamous cell carcinoma of the lung following progres- sion during or after first-line chemother- apy (LUX-Lung-IO). *Lung Cancer* 166, 107-113.

Paredes-Ruiz D *et al.* (incl. Paz-Ares L) (2022). Incidence of venous thrombo- embolism in patients with non-hemato- logical cancer admitted for COVID-19 at a third-level hospital in Madrid. *J Thromb Thrombolysis* 53, 471-478.

Prat A, Paz-Ares L *et al.* (2022). SOL- TI-1904 ACROPOLI TRIAL: efficacy of spartalizumab monotherapy across tu- mor-types expressing high levels of PD1 mRNA. *Future Oncol*. PMID: 36200668.

Chen Y, Paz-Ares L *et al.* (2022). Impact of brain metastases on treatment patterns and outcomes with first-line durvalumab plus platinum-etoposide in exten- sive-stage SCLC (CASPIAN): a brief re- port. *JTO Clin Res Rep* 3, 100330.

AWARDS AND RECOGNITION

International accreditation within the Quality Oncology Practice Initiative (QOPI): to the Medical Oncology Service of the University Hospital 12 de Octubre for achieving quality standard as defined by the American Society of Clinical On- cology (ASCO), in recognition of excel- lence in patient assistance.

José Baselga Prize for Translational Inno- vation in Oncology, 10th annual edition of the Foundation for Excellence and Quality in Oncology (ECO Foundation) - ECO Awards 2022.

H12O-CNIO CANCER IMMUNOTHERAPY CLINICAL RESEARCH UNIT

Luis Álvarez-Vallina
(since November)
Clinical Research Unit Head

Research Scientists
Belén Blanco, Anáis Jiménez

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



OVERVIEW

Our Unit focuses on understanding the molecular and cellular mechanisms of cancer immune escape in order to design next-generation cancer immunotherapies. For example, we have developed a novel strategy based on the secretion of bispecific T cell-engaging antibodies by engineered human T (STAb-T) cells, which has been shown to be effective in solid and haematological malignancies and is currently being tested in clinical trials. The Cancer Immunotherapy Clinical Research Unit has several research areas of interest: 1) reactivation of tumour-specific endogenous T cells; 2) development of tumour-reactive “artificial” T cells; and 3) development of multi-targeting approaches recognising extra- and intracellular tumour antigens. Our group also has a strong interest in the generation of multi-specific antibodies and the use of engineered mRNA-based delivery systems. Finally, our Unit

is firmly committed to introducing new immuno-oncology drugs and adoptive cell therapies in the clinic, to provide high-quality personalised treatments.

Graduate Students
Francisco Javier Arroyo, Laura Díez,
Carmen Domínguez, Marina Gómez,
Laura Rubio, Alejandro Segura,
Miriam Velasco

Technician
María de La Yedra Pacheco

RESEARCH HIGHLIGHTS

The year 2022 saw the consolidation of the “STAb-T” cancer immunotherapy strategy as a viable therapeutic option for many cancer patients. The “STAb-T strategy” is a novel adoptive cell therapy (ACT) designed by our Unit, based on the endogenous Secretion of T-cell engaging (TCE) Antibodies (STAb) by T cells (FIGURE 1). The secreted TCE antibodies recruit and activate T cells against cancer cells expressing a predefined tumour antigen. STAb-T cells offer several potential advantages over current T redirection strategies (FIGURE 1). First, *in vivo* secretion might result in effective concentrations of TCEs. Second, *in vivo* secretion can remove potential concerns regarding the formulation and long-term storage of TCEs in a manner that prevents aggregation and deterioration. Third, in STAb-T strategy, T cell recruitment is not restricted to engineered T cells, as in the case of CAR-T cell approaches. The polyclonal recruitment by TCEs of both engineered and unmodified bystander T cells, present at the tumour site, might lead to a significant boost in antitumour T cell responses (FIGURE 1). During 2022, we demonstrated the remarkable therapeutic impact in preclinical models of haematological cancers (B cell leukaemia, T cell leukaemia and multiple myeloma), with a cell product (STAb-T19) currently in a phase I, first-in-human clinical trial in patients with B cell malignancies. Throughout this period, the implementation of this strategy in solid tumours, as well as the design of dual targeting strategies, has been considerably improved. ■

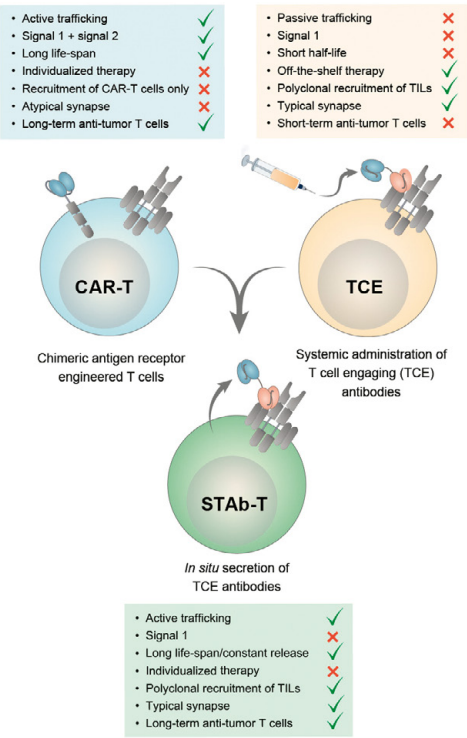


FIGURE 1 Schematic diagram summarising the advantages (green tick) and limitations (red cross) of T cell-redirecting strategies.

PUBLICATIONS

• Jiménez-Reinoso A, Tirado N, Martínez-Moreno A, Díaz VM, García-Peydró M, Hangiu O, Díez-Alonso L, Albitre Á, Penela P, Toribio ML, Menéndez P, Álvarez-Vallina L, Sánchez Martínez D (2022). Efficient preclinical treatment of cortical T cell acute lymphoblastic leukemia with T lymphocytes secreting anti-CD1a T cell engagers. *J Immunother Cancer* 10, e005333.

• Tapia-Galisteo A, Compte M, Álvarez-Vallina L, Sanz L (2022). When three is not a crowd: trispecific antibodies for enhanced cancer immunotherapy. *Theranostics*. doi:10.7150/thno.81494.

Publications at other insitutions

• Blanco B, Ramírez-Fernández Á, Bueno C, Argemí-Muntadas L, Fuentes P, Aguilar-Sopeña Ó, Gutiérrez-Agüera F, Zanetti SR, Tapia-Galisteo A, Díez-Alonso L, Segura-Tudela A, Castellà M, Marzal B, Betriu S, Harwood SL, Compte M, Lykkemark S, Erce-Llamazares A, Rubio-Pérez L, Jiménez-Reinoso A, Domínguez-Alonso C, Neves M, Morales P, Paz-Artal E, Guedan S, Sanz L, Toribio ML, Roda-Navarro P, Juan M, Menéndez P, Álvarez-Vallina L (2022). Overcoming CAR-mediated CD19 downmodulation and leukemia relapse with T lymphocytes secreting anti-CD19 T cell engagers.

Cancer Immunol Res 10, 498-511.

• Ramírez-Fernández Á, Aguilar-Sopeña Ó, Díez-Alonso L, Segura-Tudela A, Domínguez-Alonso C, Roda-Navarro P, Álvarez-Vallina L, Blanco B (2022). Synapse topology and downmodulation events determine the functional outcome of anti-CD19 T cell-redirecting strategies. *Oncoimmunology* 11, 2054106.

• Tapia-Galisteo A, Sánchez Rodríguez Í, Aguilar-Sopeña O, Harwood SL, Narbona J, Ferreras Gutiérrez M, Navarro R, Martín-García L, Corbacho C, Compte M, Lacadena J, Blanco FJ, Chames P, Roda-Navarro P, Álvarez-Vallina L, Sanz L (2022). Trispecific T-cell engagers for dual tumor-targeting of colorectal cancer.

Oncoimmunology 11, 2034355.

• Compte M, Sanz L, Álvarez-Vallina L (2022). Applications of trimerbodies in cancer immunotherapy. *Int Rev Cell Mol Biol* 369, 71-87.

• Hangiu O, Compte M, Dinesen A, Navarro R, Tapia-Galisteo A, Mandrup OA, Erce-Llamazares A, Lázaro-Gorines R, Nehme-Álvarez D, Domínguez-Alonso C, Harwood SL, Alfonso C, Blanco B, Rubio-Pérez L, Jiménez-Reinoso A, Díez-Alonso L, Blanco FJ, Sanz L, Howard KA, Álvarez-Vallina L (2022). Tumor targeted 4-1BB agonist antibody-albumin fusions with high affinity to FcRn induce anti-tumor immunity without toxicity. *iScience* 25, 104958.

Innovation

Biotechnology Programme	136
Genomics Core Unit	138
Mouse Genome Editing Core Unit	140
Monoclonal Antibodies Core Unit	142
Molecular Imaging Core Unit	144
Flow Cytometry Core Unit	146
Confocal Microscopy Core Unit	148
Proteomics Core Unit	150
Histopathology Core Unit	152
Animal Facility	154
 Experimental Therapeutics Programme	156
Medicinal Chemistry Section	158
Biology Section	162
CNIO - Lilly Cell Signalling and Immunometabolism Section	166
 Technology Transfer and Valorisation Office (TTVO)	168



ROKE I. ORUEZABAL
Director of Innovation

CNIO's Research Groups participate in many of the most relevant international scientific societies and research networks. International cooperation with the biotech and pharma sectors increased in 2022 with more agreements signed than ever, deals managed by the Technology Transfer and Valorisation Office. Besides the strengthened collaborations with pharma companies such as Janssen, Loxo Oncology, Boehringer Ingelheim, and Bristol Myers Squibb (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 half of the patents in the 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 2022 represents almost €2.4 million in revenue for CNIO. On

the other hand, in 2022, the net income from CNIO's 2021 asset licensing equalled €1.3 million. Among the new license agreements signed in 2022, in addition to cell lines and antibodies, it is worth highlighting strategic alliances with research centres of excellence, such as the *IRB*, pharmaceutical companies like Loxo Oncology, or biotech companies as Rejuvenon.

Currently, the CNIO's active patent portfolio comprises 48 families of patents. In 2022 a total of 12 patents entered the international phase (PCT). As in previous years, we monitored the possible inventions derived from the work of CNIO scientists.

In 2022, a total of 5 projects were awarded within the framework of the National *AEI* Innovation calls, endowed with over €1.7 million. Of these, 2 were "Strategic Lines" projects in collaboration with the pharmaceutical companies PharmaMar and Lilly: "Manipulation of tumour replication to resensitise against immunotherapy" (Luis Paz-Ares) and "Patient-derived pancreatic tumour organoids: a better

“CNIO's commitment with innovation has continued for another year, hand in hand with private and public partners, in a joint effort to eradicate cancer.”

predictive alternative to animal models" (Mariano Barbacid). Another project corresponds to the "Proof of Concept" call, "Implementation of treatments directed at CDK16-18 for the treatment of cancer" (Marcos Malumbres). Similarly, within the call for "Public-Private Collaboration", 2 collaborations were approved: "An effective and safe system for the treatment of atrial fibrillation through irreversible electroporation" (Fernando Peláez), developed with MedLumics; and "Development of a new gene therapy for the treatment of idiopathic fibrosis" (Maria A. Blasco), coordinated by the CNIO spin-off Telomere Therapeutics.

Within the field of personalised precision medicine, in 2022, 2 projects were awarded by the *ISCIII*, co-funded by "NextGenerationEU": "Integrating longitudinal patient-generated data and multi-omic profiling for comprehensive precision oncology in women's cancers" (Miguel Ángel Quintela), receiving approximately €2.5 million; and "Development and implementation of a functional genomics validation platform for undiagnosed hereditary cancer" (Mercedes Robledo), with nearly €3 million in funding.

At an international level, 2 projects were awarded in the context of Horizon Europe in 2022: the first, a proof-of-concept study entitled "Targeting RANK receptor as a novel therapeutic

strategy in triple negative breast cancer" (Eva González); and the second, encompassed within the Innovative Health Initiative (IHI), entitled "GUIDing multi-moDal thERapies against MRD by liquid biopsies - GUIDE.MRD" (Núria Malats), receiving more than €1.2 million funding.

Among the research contracts with the pharmaceutical industry signed in 2022, those with the CNIO spin-off Telomere Therapeutics, as well as Rejuvenon, Bionam Biotech *AEI*, Loxo Oncology, Altos Labs, and Mirati Therapeutics, stand out. Likewise, strategic alliances were generated with the pharmaceutical companies Sanofi and Ammirall in order to participate in their Open Innovation programmes.

Finally, the Innovation Department, with the aim of fostering the culture of innovation and with the support of the *Fundación Banco de Santander*, selected 3 CNIO researchers to participate in the *Instituto de Empresa* business school course, "Accelerate: Building Business from Science and Technology", which will be held in 2023.

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 protocols. The Programme consists of 9 Core Units covering major areas in Biotechnology, namely Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Mouse Genome Editing, as well as an Animal Facility. Although the Core Units are mainly focused on providing support and collaborating with the CNIO Research Groups, they also collaborate with groups from other research institutions as well as with private companies.

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

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

On the other hand, our technological capabilities continued to be upgraded during 2022. Some examples include the acquisition of an optical imaging IVIS Lumina III system in

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

the Molecular Imaging Unit, used for imaging studies in animal models; several automated platforms for histochemical and immunohistochemical staining (Agilent, Ventana-Roche) for the Histopathology Unit; and a new Chromium iX system for single cell RNA sequencing analysis.

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

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

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Guadalupe Luengo, Ruth Micha
(since October), Jorge Monsech,
Ángeles Rubio

Technicians
Purificación Arribas, Laura Conde,
José Luis Espadas (until April) (PEJ)*,
*Plan de Empleo Joven (Youth Employment Plan)



OVERVIEW

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

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

RESEARCH HIGHLIGHTS

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

The Genomics Unit, with its array of molecular services, contributes to the dissection of molecular processes of biological complexity in research projects conducted by CNIO Research Groups. The genomic-wide level is addressed by NGS-based technologies. NGS constitutes the final readout for a variety of different applications at either the structural or functional levels: on the one hand, genome or exome tumour characterisations, mutation repertoires, location of relevant DNA-bound protein factors, variations in chromatin folding, or on/off functional states; on the other hand, transcriptional profiles reflecting functional choreographies, useful to decipher tumour compositions, uncover therapeutic targets, or predict

disease course. Tissue composition, heterogeneity, and fate can be further explored with single cell resolution, by capturing individual cells in microdroplet emulsions and studying them by the tens of thousands through analysis in the NGS platform.

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

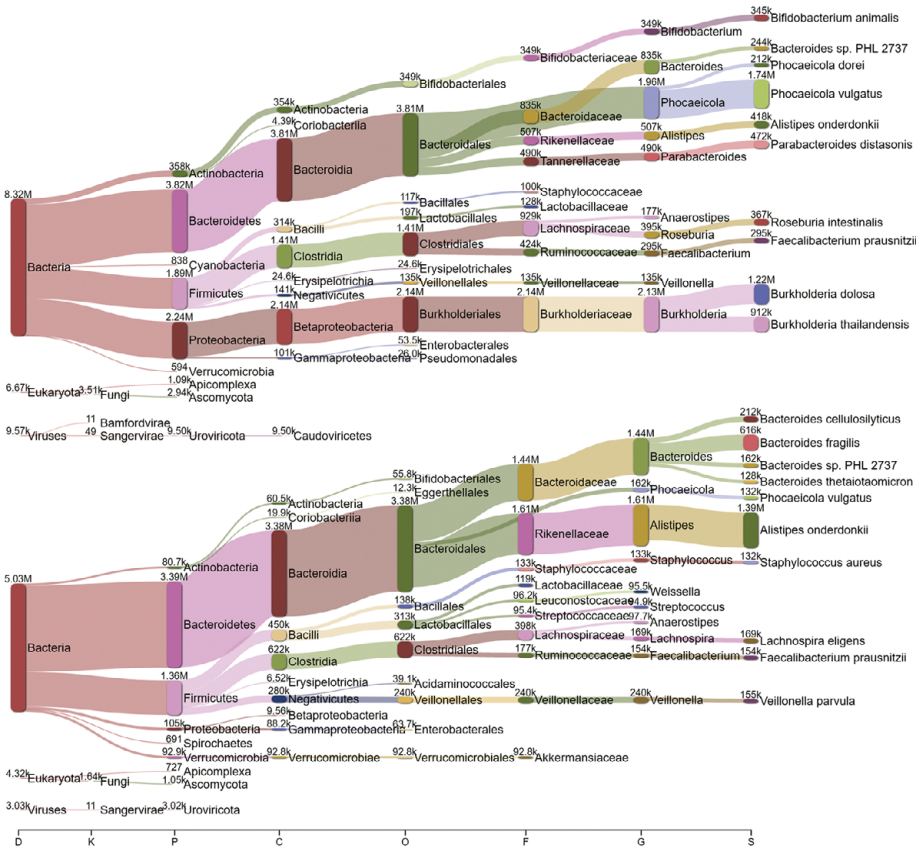


FIGURE 1 In order to ascertain variability factors that might influence each person's oncological process, gut microbiota diversity was explored. The Figure shows metagenomics classifications for 2 samples obtained at different time points from the same patient. Faecal DNA was sequenced (NGS), analysed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

MOUSE GENOME EDITING CORE UNIT

Sagrario Ortega
Core Unit Head

Graduate Student
Aleida Pujol (until April)

Technicians
Estefania Ayala (until July), Marina
Cabrerizo (until March) (TS)* (PEJ)**



OVERVIEW

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

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

models of cancer that are crucial for understanding the molecular basis of tumour development and the preclinical validation of new and more efficient cancer therapies.

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

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

RESEARCH HIGHLIGHTS

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

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

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

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

PUBLICATIONS

- Beucher A, Miguel-Escalada I, Balboa D, De Vas MG, Maestro MA, García-Hurtado J, Bernal A, Gonzalez-Franco R, Vargiu

P, Heyn H, Ravassard P, Ortega S, Ferrer J (2022). The HASTER lncRNA promoter is a cis-acting transcriptional stabilizer of HNF1A. *Nat Cell Biol* 24, 1528-1540.
Fernández LP, Deleyto-Seldas N, Col-

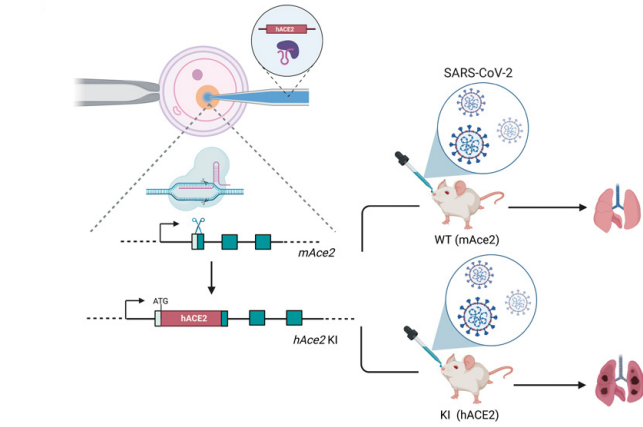


FIGURE 1 Gene editing strategy used to create humanized knockin mouse models to study COVID19. Using CRISPR/Cas9 in embryos, we replaced the mouse *Ace2* gene with its human ACE2 ortholog. The human

receptor is expressed under the transcriptional control of the mouse *Ace2* promoter in the knockin and, simultaneously, the mouse *Ace2* gene is knocked out. *Created with BioRender.*

menarejo G, Sanz A, Wagner S, Plata-Gómez AB, Gómez-Patiño M, Molina S, Espinosa-Salinas I, Aguilar-Aguilar E, Ortega S, Graña-Castro O, Loria-Kohen V, Fernández-Marcos PJ, Efeyan A, Ramírez de Molina A (2022). Folliculin-interacting protein FNIP2 impacts on overweight and obesity through a polymorphism in a conserved 3' untranslated region. *Genome Biol* 23, 230.

MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

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

*Titulado Superior (Advanced Degree)



OVERVIEW

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

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

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

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

resulting in a better classification of cancer and the selection of the most adequate cancer treatment.

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

RESEARCH HIGHLIGHTS

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

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

Research activities:

National and international collaboration. In addition to our collaboration with the CNIO’s Research Groups, during the last 22 years we have also developed many joint projects with groups from other national and international research institutions. In these collaborations, the scientists provide their extensive and profound knowledge of cancer research, generating fresh perspectives, diverse viewpoints, and innovative methodologies, which allow the targeting of proteins that play an important role in tumour transformation. We provide them with access to the generation of reliable tools (mAbs), useful both to confirm the results obtained, as well as to further investigate in their research field. In addition, we can develop and set up novel products that can lead to the generation of diagnostic tools for the prevention and diagnosis of cancer. Some of our most recent (last 2 years) and successful collaborations have been with the Spanish National Centre for Cardiovascular Research, *CNIC* (anti-ALDH14 mAb), the *Hospital Universitario Fundación Jiménez Díaz* (anti-hPIGR mAb), and the Centre for Cooperative Research in Biosciences, *CICbioGUNE* (anti-IL41 mAbs).

TACI (CD267) in lymphomas. In 2022, we produced and characterised a novel mAb against TACI protein (encoded by *TNFRSF13B* gene) that belongs to the tumour necrosis factor

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

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

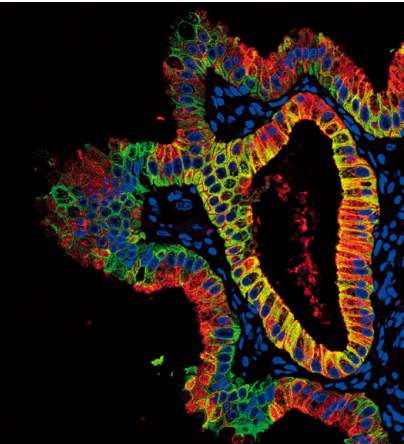


FIGURE 1 Double immunofluorescence staining of PIGR mAb (red) and cytokeratin (green) in paraffin section of human epithelium.

• PUBLICATIONS

- Yamamoto Y *et al.* (incl. Roncador G) (2022). Anti-HBV drug entecavir ameliorates DSS-induced colitis through PD-L1 induction. *Pharmacol Res* 179, 105918.
- Roncador G, Puñet-Ortiz J, Maestre L, Rodríguez-Lobato LG, Jiménez S, Reyes-García AI, García-González Á, García JF, Piris MÁ, Montes-Moreno S, Rodríguez-Justo M, Mena MP, Fernández de Larrea C, Engel P (2022). CD229 (Ly9) a novel biomarker for B-cell malignancies and multiple myeloma. *Cancers (Basel)* 14, 2154.
- Carreras J, Roncador G, Hamoudi R (2022). Artificial intelligence predicted overall survival and classified mature B-cell neoplasms based on immuno-on-

- cology and immune checkpoint panels. *Cancers (Basel)* 14, 5318.
- Peña-Cardelles JF *et al.* (incl. Roncador G) (2022). Prognosis value of immunoregulatory molecules in oral cancer microenvironment: an immunohistochemical study. *Biomedicine* 10, 710.
- Pileri A, Cavicchi M, Bertuzzi C, Righi S, Zengarini C, Sabattini E, Roncador G, Agostinelli C (2022). TOX expression in mycosis fungoides and Sezary Syndrome. *Diagnostics (Basel)* 12, 1582.
- Righi S *et al.* (incl. Roncador G) (2022). Myeloid nuclear differentiation antigen: an aid in differentiating lymphoplasmacytic lymphoma and splenic marginal zone lymphoma in bone marrow biopsies at presentation. *Hum Pathol* 124, 67-75.

MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Tatiana Álvarez, Guillermo Garaulet (TS)^{*}, Guillermo Medrano (TS)^{*}, Jorge Rodríguez (since March), Judit Rey (since November) (TS)^{*}, David



OVERVIEW

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

“Specific imaging of targets will allow a more fundamental understanding of the disease process.”

Sabador (TS)^{*} (PEJ)[†], Gloria Visdomine

^{*}Titulado Superior (Advanced Degree)
[†]Plan de Empleo Joven (Youth Employment Plan, until February)

RESEARCH HIGHLIGHTS

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

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

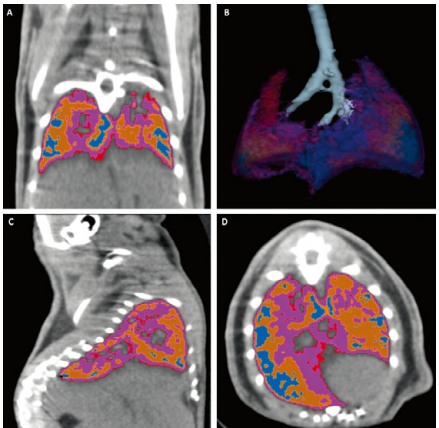


FIGURE 1 Computed Tomography of a mouse with fibrosis in the lungs. Density changes could be segmented and represented in different colours: normal lung (blue), infiltrated fibrotic tissue (orange), and collapsed lung (pink). (A) Coronal projection. (B) 3D rendering. (C) Sagittal projection. (D) Axial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

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

During 2022, and as a result of our increasing expertise in ImmunoPET techniques, we published a special edition

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

PUBLICATIONS

- González-Martínez J, Cwetsch AW, Gilabert-Juan J, Gómez J, Garaulet G, Schneider P, de Cárcer G, Mulero F, Caleiras E, Megías D, Porlan E, Malumbres M (2022). Genetic interaction between PLK1 and downstream MCPH proteins in the control of centrosome asymmetry and cell fate during neural progenitor division. *Cell Death Differ* 29, 1474-1485.
- Gerke C, Zabala Gutierrez I, Méndez-González D, Cruz MCI, Mulero F, Jaque D, Rubio-Retama J (2022). Clickable albumin nanoparticles for pretargeted drug delivery toward PD-L1 overexpressing tumors in combination immunotherapy. *Bioconjug Chem* 33, 821-828.
- Mulero F, Oteo M, Garaulet G, Magro N, Rebollo L, Medrano G, Santiveri C, Rome-

ro E, Sellek RE, Margolles Y, Campos-Olivas R, Arroyo AG, Fernández LA, Morcillo MA, Martínez-Torrecuadrada JL (2022). Development of anti-membrane type 1-matrix metalloproteinase nanobodies as immunoPET probes for triple negative breast cancer imaging. *Front Med* 9, 1058455.

- Francisca Mulero (2022). ImmunoPET in oncology. *Rev Esp Med Nucl Imagen Mol* (Eng Ed) 41, 332-339.

AWARDS & RECOGNITION

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

FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Irene Fernández-Delgado (TS)*
(since October), Julia García-Lestón
(TS)*, Sara García García (until May),

Ana M. Elizabeth Ilie (since July)
*Titulado Superior (Advanced Degree)

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



OVERVIEW

Flow Cytometry is a fast and multiparametric technology of great value in the study of immune responses in the context of cancer. It allows for the identification, quantification and isolation of defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other at the single cell level.

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

We currently have 3 polychromatic flow cytometers and 1 spectral cytometer, plus 3 high-speed cell sorters with different optical configurations to cater our users' needs. We also have an automated magnetic bead separation system and a tissue homogeniser to standardise sample preparation. Users operate

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

the analytical cytometers upon appropriate training, and the Unit staff operate the Unit cell sorters, which can separate up to 4- or 6- defined populations simultaneously, as well as perform single cell cloning and index sorting. We can accept human samples to sort under BSL2 regulations. ■

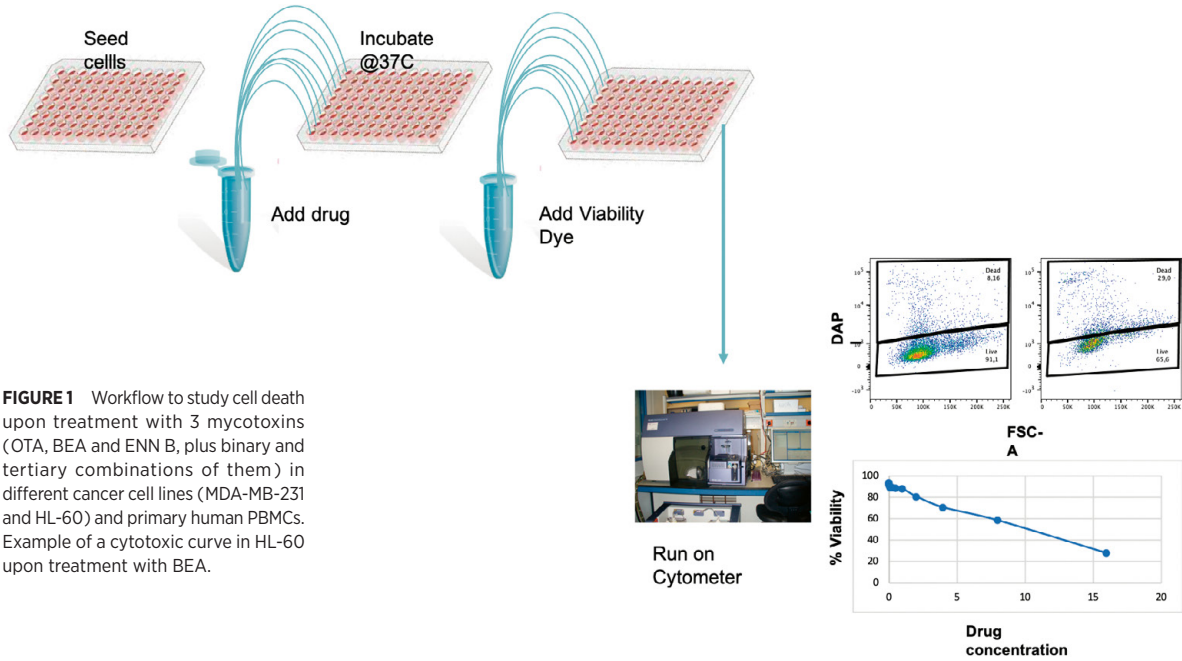
RESEARCH HIGHLIGHTS

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

- Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- Apoptosis studies (Annexin V, Mitochondrial Membrane Potencial, Caspase 3, etc.).
- Multicolour immunophenotyping panels (B and T cell development, Tregs, Inflammation, etc.).
- Functional assays (side population detection, Ca²⁺ flux, intracellular pH, etc.).
- Cytometric bead arrays to measure several cytokines from cell extracts and plasma.
- Platelet studies.

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

In 2022, we further increased our multicolour flow cytometry capabilities for the characterisation of the immune response in various samples, such as haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types, with the incorporation of an AURORA 5L. Single cell deposition using index sorting into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We also expanded our training capacities with many more workshops and small practical analysis sessions in order to provide our users with more tools to successfully perform their flow cytometry experiments. ■



PUBLICATIONS

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

resource laboratory. *Cytometry A* 101, 380-386.

resource laboratory. *Cytometry A* 101, 380-386.

AWARDS AND RECOGNITION

- “Outstanding Poster Award”, 37th Congress of the International Society for the

Advancement of Cytometry (ISAC), CYTO 2022 Philadelphia (USA): García García S, García-Lestón J, and Martínez L. *Benefits of an open-source Similarity Score for multiparametric flow cytometry controls.*

CONFOCAL MICROSCOPY CORE UNIT

Isabel Peset (since December)
Core Unit Head

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

*Titulado Superior (Advanced Degree)



OVERVIEW

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

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

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

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

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

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

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

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

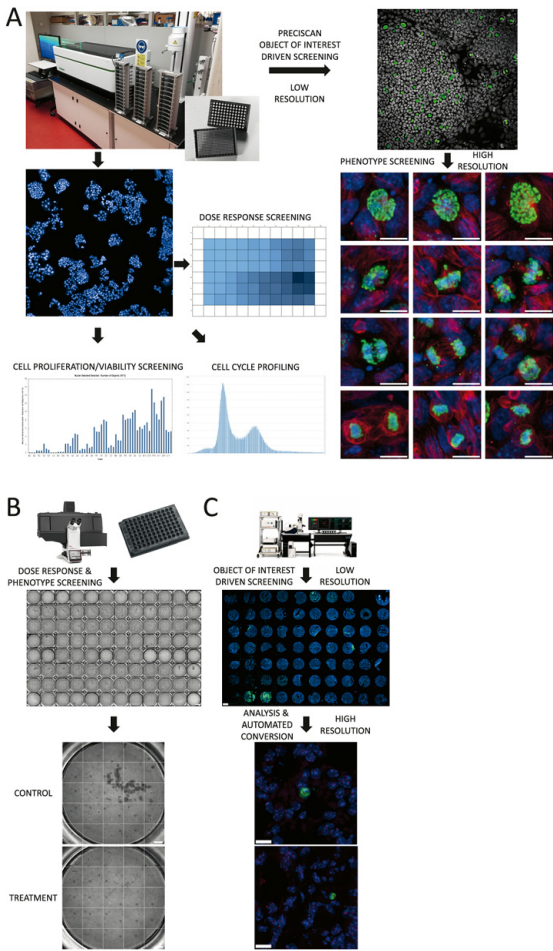


FIGURE 1 Developing automated imaging workflows. (A) Examples of HCS methods. Left. HT nuclei detection enables different cellular analysis. Right. Mitotic phenotypic screening using the PreciScan feature. (B and C) Examples of semi-automated HT feeding. (B) Dose response screening with complete well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

PUBLICATIONS

- Mellor LF *et al.* (incl. Gago-Lopez N, Bakiri L, Jimenez M, Megías D, Oterino-Soto S) (2022). Keratinocyte-derived S100A9 modulates neutrophil infiltration and affects psoriasis-like skin and joint disease. *Ann Rheum Dis* 81, 1400–8.
- González-Martínez J, Cwetsch AW, Gilabert-Juan J, Gómez J, Garaulet G, Schneider P, de Cárcer G, Mulero F, Caleiras E, Megías D, Porlan E, Malumbres M (2022). Genetic interaction between PLK1 and downstream MCPH proteins in the control of centrosome asymmetry and cell fate during neural progenitor division. *Cell Death Differ* 29, 1474–1485.
- Mouron S *et al.* (incl. Caleiras E, Muñoz J, Malats N, Megías D, Malumbres M, Quintela-Fandino M) (2022). Phosphoproteomic analysis of neoadjuvant breast cancer suggests that increased sensitivity to paclitaxel is driven by CDK4 and filamin A. *Nat Commun* 13, 7529.
- Zhu L *et al.* (incl. Blanco-Aparicio C, Martínez S, Ortega-Paino E, Rodríguez-Perales S, Muñoz J, Caleiras E, Megías D, Quintela-Fandino M, Pastor J, Valiente M) (2022). A clinically compatible drug-screening platform based on

- organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med* 14, e14552.
- Montero-Conde C *et al.* (incl. Currás-Freixes M, Gonzalez-Neira A, Megías D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M) (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.
- Jimenez L *et al.* (incl. Megías D) (2022). Multiplexed cellular profiling identifies an organoselenium compound as an inhibitor of CRM1-mediated nuclear export. *Traffic* 23, 587–599.
- Jimenez L *et al.* (incl. Blanco-Aparicio C, Pastor J, Megías D) (2022). Screening health-promoting compounds for their capacity to induce the activity of FOXO3. *J Gerontol A Biol Sci Med Sci* 77, 1485–1493.
- Torra J, Viela F, Megías D, Sot B, Flors C (2022). Versatile near-infrared super-resolution imaging of amyloid fibrils with the fluorogenic probe CRANAD-2. *Chemistry* 28, e202200026.
- Jimenez L *et al.* (incl. Blanco-Aparicio C, Pastor J, Megías D) (2022). mTORC2 is the major second layer kinase negatively regulating FOXO3 activity. *Molecules* 27, 5414.

PROTEOMICS CORE UNIT

Marta Isasa (since October)
Core Unit Head

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

*Titulado Superior (Advanced Degree)



OVERVIEW

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

interrogation of protein expression, modification, and function in cell-based models of human cancer. We aim to provide valuable guidance for experimental strategies, which are critical for cancer research success.

RESEARCH HIGHLIGHTS

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

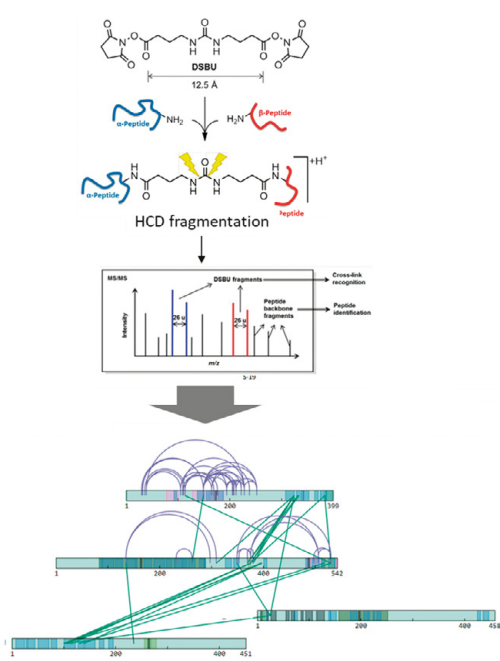


FIGURE 1 Schematic showing the workflow used for cross-linking-based mass spectrometry. Covalently bound peptides derived from cross-linked proteins are identified, providing 3D structure analysis of proteins and protein complexes.

structure and helps reveal novel protein-protein interactions. The protocol, robust and widely applicable, is based on protein cross-linking with MS-cleavable reagents, enzymatic digestion followed by high pH fractionation, and LC-MS/MS analysis. The output allows the identification of cross-links, assessing spatial and morphology constraints for recombinant purified proteins and complexes. ■

SELECTED PUBLICATIONS

- García-Alonso S, Mesa P, Ovejero LP, Aizpuru G, Lechuga CG, Zarzuela E, Santiveri CM, Sanclemente M, Muñoz J, Musteanu M, Campos-Olivas R, Martínez-Torrecedra J, Barbacid M, Montoya G (2022). Structure of the RAF1-HSP90-CDC37 complex reveals the basis of RAF1 regulation. *Mol Cell* 82, 3438-3452.e8.
- Mouron S *et al.* (incl. Bueno MJ, Caleiras E, Ximénez-Embún P, Muñoz J, Gómez-López G, Fustero-Torre C, Sabroso-Lasa S, Malats N, Megias D, Malumbres M, Quintela-Fandino M) (2022). Phosphoproteomic analysis of neoadjuvant breast cancer suggests that increased sensitivity to paclitaxel is driven by CDK4 and filamin A. *Nat Commun* 13, 7529.
- Villarroya-Beltri C, Martins AFB, García A, Giménez D, Zarzuela E, Novo M, Del Álamo C, González-Martínez J, Bonel-Pérez GC, Díaz I, Guillaumot M, Chiesa M, Losada A, Graña-Castro O, Rovira M, Muñoz J, Salazar-Roa M, Malumbres M (2022). Mammalian CDC14 phosphatases control bivalent promoters and exit from stemness in pluripotent cells. *EMBO J*. PMID: 36326833.
- Seidlitz T *et al.* (incl. García F, Muñoz J) (2022). Sensitivity towards HDAC inhibition is associated with RTK/MAPK pathway activation in gastric cancer. *EMBO Mol Med* 4, e15705.
- Kuo SH *et al.* (incl. Ximénez-Embún P, Muñoz J) (2022). Mutant glucocerebrosidase impairs α -synuclein degradation by blockade of chaperone-mediated autophagy. *Sci Adv* 8, eabm6393.
- Carretero-González A *et al.* (incl. Hergueta-Redondo M, Sánchez-Redondo S, Ximénez-Embún P, Peinado H) (2022). Characterization of plasma circulating small extracellular vesicles in patients with metastatic solid tumors and newly diagnosed brain metastasis. *Oncoimmunology* 11, 2067944.
- Marín I *et al.* (incl. Zarzuela E, Muñoz J).

- (2022). Cellular senescence is immunogenic and promotes anti-tumor immunity. *Cancer Discov*. PMID: 36302218.
- Rodríguez-Garzotto A *et al.* (incl. Ximénez-Embún P) (2022). Topical heparin as an effective and safe treatment for patients with capecitabine-induced hand-foot syndrome: results of a phase IIA trial supported by proteomic profiling of skin biopsies. *Ther Adv Med Oncol* 14, 17588359221086911.
- Rovira M *et al.* (incl. García F, Muñoz J) (2022). The lysosomal proteome of senescent cells contributes to the senescence secretome. *Aging Cell* 21, e13707.

HISTOPATHOLOGY CORE UNIT

Vacant
Core Unit Head

Research Scientist
Eduardo José Caleiras



OVERVIEW

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

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

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

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

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

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

RESEARCH HIGHLIGHTS

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

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

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

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

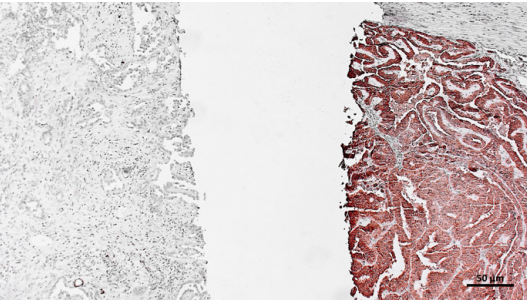


FIGURE 1 Detection of S-(2-Succinyl)-Cysteine (2SC) by immunohistochemistry in papillary carcinoma of the kidney. On the left, another case of the same tumour type without fumarate deficiency. Courtesy of Cristina Rodríguez, Hereditary Endocrine Cancer Group.

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

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

PUBLICATIONS

Monteiro C *et al.* (incl. Caleiras E, Paz-Ares; RENACER; Valiente M) (2022). Stratification of radiosensitive brain metastases based on an actionable S100A9/RAGE resistance mechanism. *Nat Med* 28, 752-765.

Mouron S *et al.* (incl. Bueno MJ, Caleiras E, Ximenénez-Embún P, Muñoz J, Gómez-López G, Fustero-Torre C, Sabroso-Lasa S, Malats N, Megias D, Malumbres M, Quintela-Fandino M) (2022). Phospho-proteomic analysis of neoadjuvant breast cancer suggests that increased sensitivity to paclitaxel is driven by CDK4 and filamin A. *Nat Commun* 13, 7529.

González-Martínez J, Cwetsch AW, Gilabert-Juan J, Gómez J, Garaulet G, Schneider P, de Cárcer G, Mulero F, Caleiras E, Megias D, Porlan E, Malumbres M (2022). Genetic interaction between PLK1 and downstream MCPH proteins in the control of centrosome asymmetry and cell fate during neural progenitor division. *Cell Death Differ* 29, 1474-1485.

Zhu L *et al.* (incl. Blanco-Aparicio C, Martínez S, Ortega-Paino E, Rodríguez-Perales S, Muñoz J, Caleiras E, Megias D, Quintela-Fandino M, Pastor J, Valiente M) (2022). A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med* 14, e14552.

Fenor MD *et al.* (incl. Ruiz-Llorente S, Caleiras E) (2022). MEK inhibitor sensitivity in BRAF fusion-driven prostate cancer. *Clin Transl Oncol* 24, 2432-2440.

Montero-Conde C *et al.* (incl. Currás-Freixes M, Gonzalez-Neira A, Megias D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M) (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.

ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services



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

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

All the work carried out by the Animal Facility complies with both national and EU legislation — RD53/2013 and EU Directive 2010/63/UE — for the protection of animals used

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

for research experimentation and other scientific purposes. Experimental procedures and projects are reviewed by the Research Ethics and Animal Welfare Committee of the *Instituto de Salud Carlos III*, as well as by the Institutional Animal Care and Use Committee (IACUC). The *Orden ECC/566/2015* stipulates that all animal procedures are to be carried out by qualified people with accreditation issued by the competent

authority. The Animal Facility offers CNIO’s new staff a course focused on work with laboratory animals, complementary to the online courses that are a requisite to gain access to the facility.

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

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

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

Daily operations and husbandry procedures are highly automated to safeguard our personnel from any associated risks. Robotic devices perform the potentially hazardous tasks such as the processing of dirty bedding, the washing and filling of cages and bottles, etc. These automated systems maximise productivity and ensure quality standards in our washing and

sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

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

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

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

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

EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director



The following highlights some of the main achievements of the Experimental Therapeutics Programme during 2022:

Mastl inhibitors (MASTL-is). (In collaboration with Marcos Malumbres’ Group). In 2022, we further optimised our MASTL-is and PROTACs, putting special emphasis on their *in vivo* bioavailability. We studied the *in vivo* pharmacokinetic properties of ETP-715, our frontrunner MASTL-i. Unfortunately, the *in vivo* bioavailability (PO route) is still suboptimal. We will continue optimising it in the next stage of our work. By contrast, ETP-184 achieved plasma and tissue levels well above its cellular activity (Nanobret EC50). Furthermore, PROTAC ETP-823 displayed good bioavailability in plasma (IP route), ensuring compound levels above its DC50 in cells. Both compounds are now ready for more advanced *in vivo* PK-PD and efficacy studies to pharmacologically validate MASTL as a therapeutic target.

TRF1. (In collaboration with Maria A. Blasco’s Group). In 2022, we focused our activities on the validation of previously identified hits as potential direct-TRF1 inhibitors. In addition to ETP-631 emerging from a virtual screening campaign, 3 other hits arose from screening a subset of our ETP library. All 4 compounds were active in the TRF1-dsTelDNA proximity assay and inactive in the corresponding counter-screens. Importantly, one of them showed the disruption of the interaction of TRF1 with dsTelDNA in ChIP experiments in cells (M. A. Blasco’s laboratory). We are currently validating these compounds using an orthogonal EMSA assay to ensure that they disrupt the TRF1-dsTelDNA complex, and are testing their direct interaction with TRF1 using the Thermofluor assay. Notably, an international pharmaceutical company has shown interest in TRF1 as a therapeutic target and is now testing these compounds under an MTA agreement.

SETD8 inhibitors. (In collaboration with Óscar Fernández-Capetillo’s Group). After several screening campaigns, we identified covalent and non-covalent high micromolar SETD8 biochemical inhibitors. During 2022, we characterised representative covalent hits by intact protein mass spectrometry. Regarding the non-covalent hits, we carried out an initial chemical exploration with the aim of improving the potency, although with limited success so far. In cells, we tested selected hits and analogues. We also measured the inhibition of the methylation of H4K20, a direct substrate of SETD8, and are currently analysing these results. In the next stage, we will continue optimising current hits and perform

“The Experimental Therapeutics Programme (ETP) continues giving support to Drug Discovery and Chemical Biology projects at the CNIO and collaborates with external partners to discover new therapeutic agents.”

additional hit finding/generation activities to obtain better starting points to develop SETD8 inhibitors.

FOXO activators. (In collaboration with Refoxy Pharmaceuticals GmbH). In 2020, the CNIO established a collaboration with Refoxy Pharma (Berlin, Germany) to discover FOXO activators for potential development in multiple diseases. We identified several FOXO activators after several cell-based screening campaigns, analoging of initial hits, characterisation in mechanistic studies, and preliminary off-target selectivity. Refoxy has expressed its interest in licensing some of these hits (under negotiation).

Others. ETP has worked in the early phases of other internal projects: RANK (Eva González-Suárez, CNIO) / NUDIX5 (*CRG-UIC*). ETP also provided support to several CNIO researchers in exploratory projects or contributed with internally synthesised tool compounds: Felipe Cortés-Ledesma, Juan Méndez, Héctor Peinado, Manuel Valiente, Joaquín Martínez-López, Mariano Barbacid, María S. Soengas, Óscar Fernández-Capetillo, Nabil Djouder, Francisco X. Real. ■

MEDICINAL CHEMISTRY SECTION

Sonia Martínez
Section Head

Research Scientists
Ana Belén García, Cristina Gómez,
Esther González, Sonsoles
Rodríguez, Carmen Varela



Graduate Student
Javier Sánchez (until August)
(PEJ, CAM)*

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

Technicians
Lucía de Andrés (until February)
(TS)* (PEJ)***, Bruno Di Geronimo
(until February) (TS)* (PEJ)**

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

Visiting Scientist
Gonçalo J Lopes Bernardes
(Cambridge University, UK)

OVERVIEW

The Medicinal Chemistry Section is part of the multidisciplinary Experimental Therapeutics Programme (ETP) focused on early drug discovery activities. ETP is integrated into the CNIO's structure, and acts as a bridge between basic research groups in cancer biology and the pharmaceutical industry, with the aim of transferring the results obtained in basic research laboratories to products, potential drugs that help to understand the biology of cancer, or the development of new therapies. The Section deals with the design, synthesis, and optimisation of compounds that are then characterised in the Biology Section of ETP, in order to evaluate their potency in biological targets *in vitro* and *in vivo* and ultimately to demonstrate their efficacy and mechanism of action in animal models (*in vivo* proof-of-concept). As a complementary strategy to the classic inhibitors, we also contemplate the degradation of particular targets using different chemical approaches such as the use of PROTACs. Additionally, we have entered the field of Chemical Biology in order to discover and identify novel drugs and targets from phenotypic screenings. In this regard, we contribute by synthesising high quality chemical tools needed for interrogating the observed phenotype.

“In our MASTL project, we generated the first MASTL PROTAC (ETP-823) that potently degrades MASTL protein via E3 ligase and proteasome recruitment.”

RESEARCH HIGHLIGHTS

Our Section's activities focus mainly on the following projects:

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is led by Maria A. Blasco (Telomeres and Telomerase Group). In previous years, the ETP Biology Section developed an assay to measure the binding of TRF1 to telomeric DNA. After virtual and wet screening campaigns, we identified some disruptors of such binding that do not interfere with the assay system nor with DNA. During 2022, we analytically characterised the hits, resynthesised fresh batches, and synthesised some analogues to establish Structure Activity Relationships (SAR). The compounds are currently being evaluated in orthogonal assays by ETP's Biology Section and by a pharmaceutical company that is interested in the target, under an MTA agreement.

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

This project is being undertaken in collaboration with Marcos Malumbres (Cell Division and Cancer Group). We have been involved in the fine optimisation of ETP-715, a potent cell active, selective compound without cardiotoxic alerts but with low exposure levels after oral administration in pharmacokinetic (PK) studies performed in BALBC mice. Seventy six new analogues have been synthesised so far, and the most promising ones, in terms of potency and *in vitro* ADMET, are being evaluated in PK studies. Additionally, we have started to work on back-up series, to reinforce the intellectual property and to determine the impact of different scaffolds on drug-like properties. Fifty seven new compounds from different chemical series have been synthesised, and we have identified new series with potent compounds that will be characterised in terms of *in vivo* PK. In addition, we continue with our activities developing PROTACs (Proteolysis Targeting Chimeras) to degrade MASTL protein. Previously, we identified ETP-823 as our first MASTL PROTAC that potently degrades MASTL protein via E3 ligase and proteasome recruitment. PK studies showed *in vivo* levels above its DC50 in cells in plasma after IP administration. We performed a fine optimisation of ETP-823, and 62 new PROTACs were synthesised by exploring different linkers, different functional groups in the growing vectors, and different E3 ligase ligands (FIGURE 1). So far, we have identified several new PROTACs with good degradation profiles in different cell lines.

HistoneH4-lysine20 N-methyltransferase (SETD8) inhibitors

In collaboration with Óscar Fernández Capetillo (Genomic Instability Group), we explored one of the initial hits identified in a cellular assay in Capetillo's laboratory, but the chemical exploration of this series was put on hold due to lack of activity in the biochemical assay. After 2 screening campaigns, we identified new hits (covalent and non-covalent), and we are currently working on their validation by re-synthesising the hits and synthesising some analogues to establish SARs.

Foxo activators (collaboration with Refoxy Pharmaceuticals GmbH)

We have been involved in the selection of new analogues of the hits identified in screening campaigns, as well as in the quality control analyses. Several Foxo activators have been identified, and negotiations for licensing the compounds to Refoxy are underway.

NUDT5 inhibition

We are collaborating with GRG-IUC to optimise a hit that inhibits the ATP generating activity of NUDT5 in a biochemical assay. Several analogues have been obtained, and we are currently characterising the compounds.

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

We are collaborating with Eva González-Suárez (CNIO) to develop small molecules that specifically target the RANK receptor. The activities in 2022 focused on acquiring the virtual hits and assessing the quality control to validate them in wet assays (SPR, cells, etc.) and to generate robust data.

Apart from the drug discovery activities, we give support to several Groups by synthesising reference or tool compounds. During 2022, we carried out such work for the following Groups: Brain Metastasis, Genomic Instability, and Telomeres and Telomerase. ■

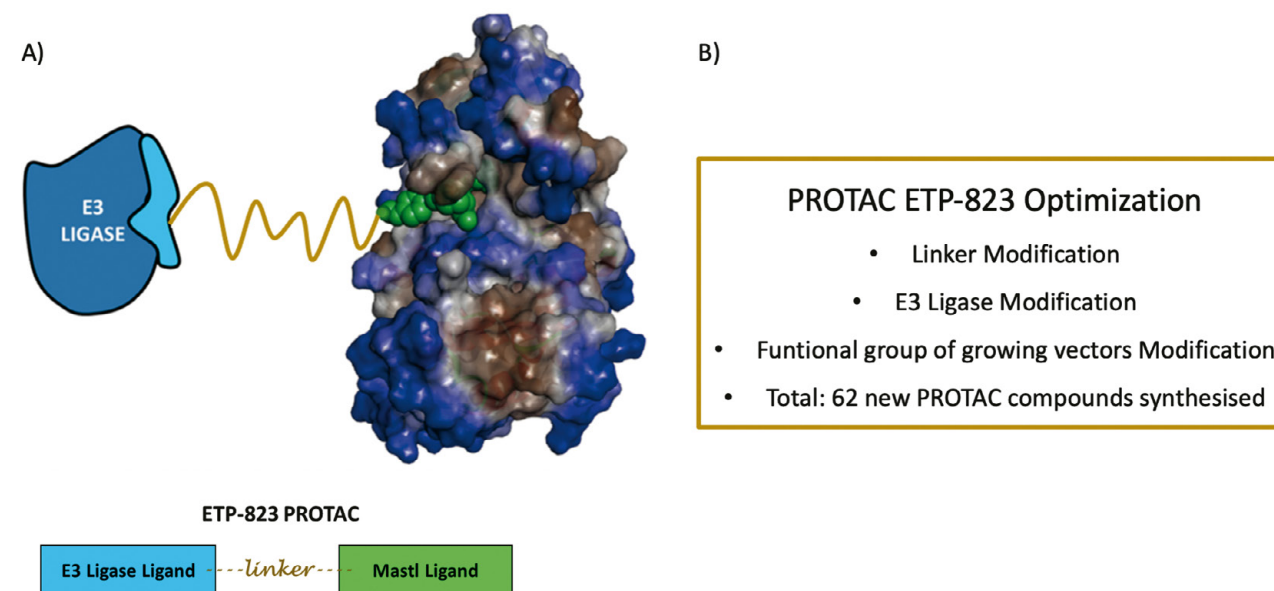


FIGURE 1 (A) Representation of PROTAC ETP-823 binding to the kinase domain of human MASTL protein (PDB 5LOH) through MASTL Ligand, and to E3 Ligase through the corresponding Ligand. (B) Strategies for the optimisation of ETP-823.

PROTAC ETP-823 Optimization

- Linker Modification
- E3 Ligase Modification
- Funtional group of growing vectors Modification
- Total: 62 new PROTAC compounds synthesised

► PUBLICATION

- Zhu L, Retana D, García-Gómez P, Álvaro-Espinosa L, Priego N, Masmudi-Martin M, Yebra N, Miarka L, Hernández-Encinas E, Blanco-Aparicio C, Martínez S, Sobrino C, Ajenjo N, Artiga MJ, Ortega-Paino E, Torres-Ruiz R, Rodríguez-Perales S; RENACER, Soffietti R, Bertero L, Cassoni P, Weiss T, Muñoz J, Sepúlveda JM, González-León P, Jiménez-Roldán L, Moreno LM, Esteban O, Pérez-Núñez Á, Hernández-Lain A, Toldos O, Ruano Y, Alcázar L,

Blasco G, Fernández-Alén J, Caleiras E, Lafarga M, Megías D, Graña-Castro O, Nör C, Taylor MD, Young LS, Varešija D, Cosgrove N, Couch FJ, Cussó L, Desco M, Mouron S, Quintela-Fandino M, Weller M, Pastor J, Valiente M (2022). A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med* 14, e14552.

► PATENTS

- Pastor Fernández J, Martínez González S, Blanco-Aparicio C, García Campos FJ, Cebriá Gómez A. Thiazolopyrimidones as inhibitors of DDR1/2 and therapeutic uses thereof. PCT application (2022). *PCT/EP2022/051064. WO22157166A1*.
- Pastor Fernández J, Martínez González S, Blanco-Aparicio C, González Cantalapiedra E, García García AB, Pastor Fernández M, Hernández Higuera AI, Albarrán Santiaño MI, Cebriá Gómez A.

Imidazo[1,2-a]pyrazines as inhibitors of HASPIN and therapeutic uses thereof. PCT application (2022). *PCT/EP2022/054626. WO22180150A1*.

► Pastor Fernández J, Martínez González S, Blanco-Aparicio C, García García AB, Rodríguez Aristegui S, Gómez de la Oliva CA, Albarrán Santiaño MI, Cebriá Gómez A, Malumbres Martínez M. Imidazo[1,2-b]pyridazine based tricyclic compounds as inhibitors of HASPIN and therapeutic uses thereof. PCT application (2022). *PCT/2022/057636. WO2022200433A1*.

BIOLOGY SECTION

Carmen Blanco
Section Head

Post-Doctoral Fellow
Elena Hernández

Graduate Student
Lucía Cañizares (since December)
(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)**, Javier Klett (until August) (TS)**, José A. Torres (until February) (PEJ)*

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

Students in Practice
Andrea Álvarez (Feb.-July)
(Bachelor's Degree Final Project,

Univ. Autónoma de Madrid, Spain), Jada Li (June - July) (MISTI Internship, USA), Noelia Martín (Feb.-July) (Bachelor's Degree Final Project) and María Cuerda (since Oct.) (Master's Thesis)(Univ. Complutense de Madrid, Spain)

Visiting Scientists
Lucía Jiménez and Wolfgang Link (until November) (IIBm (CSIC-UAM), Madrid, Spain)

OVERVIEW

A high-quality small-molecule probe for target validation has to be cell permeable and demonstrate target engagement and selectivity, as well as pharmacological and phenotypic response. PROTACs (PROteolysis TARgeting Chimeras) have emerged as new promising pharmacological modalities. Moreover, PROTACs represent the chemical equivalent of small interfering RNA (siRNA), albeit allowing removal of a protein at a post-translational level. Parameters such as the maximum level of target degradation (Dmax), confirmation of a proteasome dependent degradation mechanism, and kinetic parameters of POI degradation and selective degradation have to be taken into account to use PROTACs for target validation. In collaboration with Marcos Malumbres, we started an early drug discovery project to develop MASTL inhibitors and PROTACs, as non-advanced inhibitors have already been described. We have been able to develop both types of molecules, generating a set of PROTACS that meet the requirements to be used as chemical tools for target validation and to define their clinical niche.

“We identified selective and potent MASTL PROTACs with *in vivo* levels needed to perform PK/PD and proof of concept studies.”

RESEARCH HIGHLIGHTS

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

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. In 2022, we tested in our biochemical assay using active human full-length MASTL protein around 190 new compounds, both MASTL-i and MASTL PROTAC-like molecules. We measured MASTL engagement in cells (BRET assay). In the case of PROTACs, we also evaluated their MASTL degradation capacity in cells, both in a dose response and time dependent manner. We identified a set of nanomolar MASTL degraders with different linker and E3 ligase ligand that have been used to study their broad degradation capacity with proteomics. In addition, we performed pharmacokinetics studies of several MASTL inhibitors and PROTACs, identifying a MASTL inhibitor and a PROTAC that have achieved enough plasma levels to allow PK/PD studies to be performed (FIGURE 1).

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. We are working to identify disruptors of TRF1 binding to ds telomeric DNA, and so far we have identified several hits from different chemical series after virtual screening and wet assays, and screening of a collection of 1500 molecules selected from our ETP-library and analogue searching. We confirmed the specific disruption of the binding of TRF1 to dsTelDNA with screen and counter screen alpha assays, and a fluorescent displacement assay to discard the binding of the compounds to dsTelDNA. Now we are validating these hits by applying orthogonal assays against TRF1 and the dsTelDNA probe, such as EMSA and thermofluor assay with freshly prepared and/or resynthesised samples. Compounds that disrupt the binding of TRF1 to ds telomeric DNA by binding to TRF1 will be tested in a TRF1 phenotypic assay.

SET domain containing lysine methyltransferase 8 (SETD8)

This project is conducted in collaboration with the CNIO Genomic Instability Group. Our main objective is to generate and optimise novel SET8 inhibitors as new therapeutic agents. After 2 different screening campaigns, we identified both reversible and irreversible possible hits with micromolar activity. The covalent mechanism of action of the hits was validated by time dependent biochemical assays and the formation of adducts by proteomics with purified SETD8. In

order to identify the reactive amino acid in SETD8, we are going to perform biochemical assays with a mutant protein and proteomics studies in cells to evaluate their selectivity. In addition, all possible hits have been tested in a cellular assay that measures monomethylation of H4K20 in order to prioritise chemical serials to improve their biochemical activity.

Collaborations with other CNIO Groups

The ETP-Biology Section performed *in vivo* studies of selected compounds and drugs such as pharmacokinetics and distribution studies in collaboration with the Microenvironment and Metastasis Group, the Brain Metastasis Group, and the Genomic Instability Group. Furthermore, we performed screening campaigns with the Topology and DNA Breaks Group and the H12O - CNIO Haematological Malignancies Clinical Research Unit, identifying several hits that are under validation. Finally, we collaborated with the Experimental Oncology Group, the Melanoma Group, the DNA Replication Group, and the Chromosome Dynamics Group giving support to perform cellular screenings.

Collaborations with other institutions

Refoxy collaboration: We gave logistics and data analysis support.

Collaboration with CRG/UIC: This project is conducted in collaboration with Dr R. Wright. We characterised, in terms of ADME-T and pharmacokinetics, a NUDIX5 inhibitor previously identified by the researcher. ■

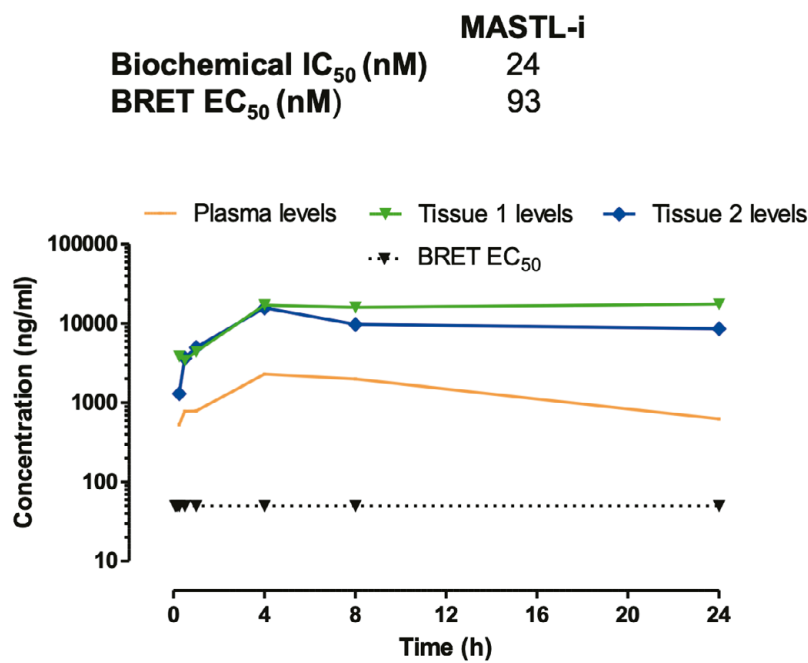


FIGURE 1 Plasma and tissue levels of MASTL inhibitor after oral administration. Nanomolar biochemical and cellular MASTL-I achieves levels in mice clearly above the EC₅₀ to modulate MASTL in cells, which guarantees *in vivo* target modulation.

PUBLICATIONS

► Zhu L, Retana D, García-Gómez P, Álvaro-Espinosa L, Priego N, Masmudi-Martin M, Yebra N, Miarka L, Hernández-Encinas E, Blanco-Aparicio C, Martínez S, Sobrino C, Ajenjo N, Artiga MJ, Ortega-Paino E, Torres-Ruiz R, Rodríguez-Perales S; RE-NACER, Soffietti R, Bertero L, Cassoni P, Weiss T, Muñoz J, Sepúlveda JM, González-León P, Jiménez-Roldán L, Moreno LM, Esteban O, Pérez-Núñez Á, Hernández-Lain A, Toldos O, Ruano Y, Alcázar L, Blasco G, Fernández-Alén J, Caleiras E, Lafarga M, Megías D, Graña-Castro O, Nör C, Taylor MD, Young LS, Varešlija D, Cosgrove N, Couch FJ, Cussó L, Desco M, Mouron S, Quintela-Fandino M, Weller M, Pastor J, Valiente M (2022). A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med* 14, e14552.

► Baquero JM, Marchena-Perea E, Mirabet R, Torres-Ruiz R, Blanco-Aparicio C, Rodríguez-Perales S, Helleday T, Benítez-Buelga C, Benítez J, Osorio A (2022). OGG1 inhibition triggers synthetic lethality and enhances the effect of PARP inhibitor olaparib in BRCA1-deficient TNBC cells. *Front Oncol* 12, 888810.

► Jimenez L, Amenabar C, Mayoral-Varo V, Mackenzie TA, Ramos MC, Silva A, Calissi G, Grenho I, Blanco-Aparicio C, Pastor

J, Megías D, Ferreira BI, Link W (2022). mTORC2 is the major second layer kinase negatively regulating FOXO3 activity. *Molecules* 27, 5414.

PATENTS

► Pastor Fernández J, Martínez González S, Blanco-Aparicio C, García Campos FJ, Cebriá Gómez A. Thiazolopyrimidones as inhibitors of DDR1/2 and therapeutic uses thereof. PCT application (2022). *PCT/EP2022/051064. WO22157166A1*.

► Pastor Fernández J, Martínez González S, Blanco-Aparicio C, González Cantalapiedra E, García García AB, Pastor Fernández M, Hernández Higuera A,

Albarrán Santiño MI, Cebriá Gómez A. Imidazo[1,2-a]pyrazines as inhibitors of HASPIN and therapeutic uses thereof. PCT application (2022). *PCT/EP2022/054626. WO22180150A1*.

► 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. PCT application (2022). *PCT/2022/057636. WO2022200433A1*.

CNIO - LILLY CELL SIGNALLING AND IMMUNOMETABOLISM SECTION

Susana Velasco
Section Head

Research Scientists
Ana Cerezo (until August), Sonia
Hernández Tiedra, Eva P. Lospitao,
Gloria Martínez del Hoyo, Camino
Menéndez (until October)



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

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 that 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.

Technicians
Verónica M. Casquero (until June)
(TS)*, Jenniffer Patricia Condo (since
May), Laura Diezma, Roberto Gómez

(TS)*, Sandra Peregrina (TS)*, Natalia
Riestra
**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 escape. The causes of CIN are diverse, including mitotic errors, replication stress, homologous recombination deficiency (HRD), and telomere crisis. ■

Chromosome instability (CIN) and/or whole genome duplication (WGD) promote tumorigenesis

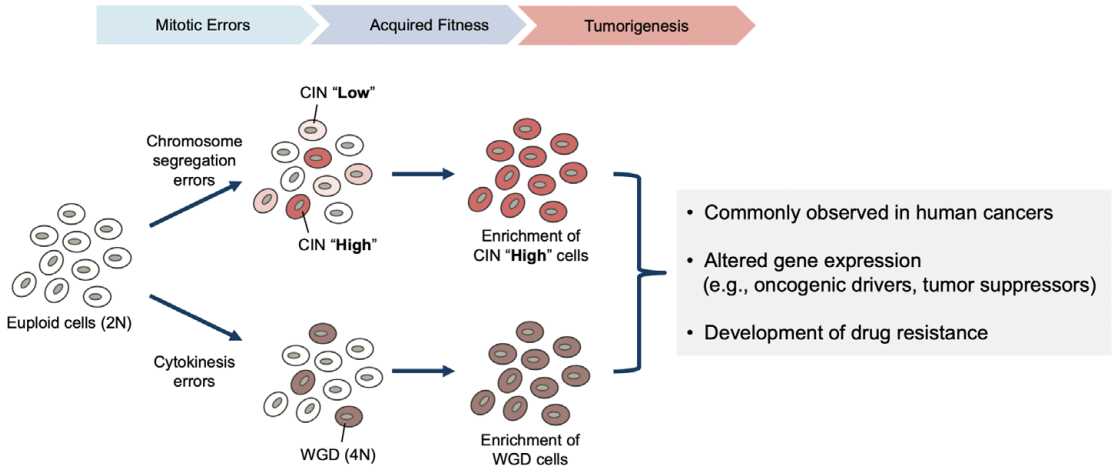


FIGURE 1 Chromosome instability (CIN) and/or whole genome duplication (WGD) promote tumorigenesis.

Lukow and Sheltzer, Trends in Cancer, 2021
Bielski et al., Nature Genetics, 2018
Lopez et al., Nature Genetics, 2020

TECHNOLOGY TRANSFER AND VALORISATION OFFICE (TTVO)

Irene Herrera
Director

Technology Transfer Manager
Irene Pino



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 2022 focused on: monitoring 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 48 active patent families, and provides advice and assistance during the drafting of patent documents, their filing, and the prosecution process. In 2022, 4 priority patent applications were filed, one of them co-owned with *Academisch Ziekenhuis Leiden (LUMC)*. These 4 new patents protect quite varied inventions, including: telomerase gene therapy for kidney fibrosis; a signature for prognosis of brain metastasis relapse; a nucleic acid detection method; and a device and method for cryo-EM sample preparation. Moreover, 12 PCT (Patent Cooperation Treaty) applications for international extension were also filed in 2022.

In addition to the patents, an algorithm for precision nutrition was registered through the Safe Creative intellectual property registry. This will be licensed to a new spin-off in which the CNIO and the Foundation for Biomedical Research of the University Hospital *12 de Octubre (FIBH12O)* will participate.

For yet another year, licensed patents make up a remarkable 50% of the CNIO portfolio. In 2022, the patent family WO2019002581, "Identification and elimination of damaged and/or senescent cells", was licensed to the Swiss company Rejuveron. In addition, the patent PCT/EP2022/051505, "Telomerase reverse transcriptase therapy for kidney fibrosis and non-human animals thereof", was incorporated into the license agreement with Telomere Therapeutics.

In 2022, the TTVO managed 330 agreements (MTAs, CDAs, research collaborations, licenses, etc.). The majority of these agreements (65%) were established with international entities, which is an indicator of the internationalisation of CNIO's research activity. Through collaborations with industry, €2.4 million were secured for research activities.

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

Among the most outstanding agreements signed this year with the private sector is the continuation of the collaboration with Loxo Oncology. This work agreement has been renewed for the period 2022-2023 with a budget of €1.8 million. Other relevant agreements with the private sector include one for €0.24 million with the company Astra Zeneca Ltd. (UK), for a project developed in collaboration with the Brain Metastasis Group; and another agreement for €0.1 million with the company Circle Pharma Inc. (USA), for a project developed in collaboration with the Cell Division and Cancer Group and the Breast Cancer Clinical Research Unit. Also signed was an addendum to the collaboration contract with *CRIS* against Cancer and Bionam Biotech *AIE* for a pancreatic cancer project of the Experimental Oncology Group, with a budget of €0.1 million; and a research agreement with the company Mirati Therapeutics Inc. (USA) of €0.14 million for a project of the same group. Finally, research agreements have been signed with other companies such as MeCo Diagnostics Holdings Inc. (USA) for €0.07 million for a breast cancer project that will be carried out by the Breast Cancer Clinical Research Unit; and an agreement of €0.01 million with Rocket Pharmaceuticals Inc. (USA) for collaboration with the Molecular Cytogenetics Unit.

Patents and unpatented research tools (murine lines, cell lines and antibodies) are licensed to provide financial return to CNIO. The net income generated in 2021 from CNIO asset licenses totalled €1.3 million (about € 1 million from monoclonal antibodies).

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

Biobank

BIOBANK

Eva Ortega-Paíno
Director

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

Artiga (TS)*, Pilar Caro (since
September) (TS)*, Francisco de Luna
(until August) (TS)*, Cecilia Sobrino

**Titulado Superior* (Advanced Degree)

Student in Practice
Patricia Herrero (since September)
(*Universidad Autónoma de Madrid*,
Spain)



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 provides a broad service offer 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.

“The understanding of different diseases depends critically on large-scale analyses of high-quality data to provide effective and personalised health care, while guaranteeing patients’ rights. Making science involves now, more than ever, ethical, legal, and social aspects.”

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 (ISCIII)* (Ref. no: B. 848).

RESEARCH HIGHLIGHTS

Sample and data collections

Currently, CNIO Biobank houses more than 8,500 cases (donors): lymphomas, gynaecologic and digestive neoplasia, mammary carcinomas, non-neoplastic cases, and primary skin cultures. In addition, it houses a collection of patient-derived xenografts (PDX) and tissue microarrays (TMA). As a whole, the biobank has more than 36,000 tissue samples. Furthermore, **Biobank’s Virtual Catalogue** includes 8,064 images from TMAs, histological H&E stains, and IHQ images.

More recently, Biobank has been supporting CNIO’s research groups by creating new collections of samples to meet the needs of their research projects, for example: Covid-19 patients (689 cases); brain metastases (RENACER) from 95 patients (1,980 samples); and a prospective cohort of samples from the Spanish Association of Flight Attendants (AETCP), with a wide set of epidemiological data from 102 cases (5,889 samples available). In order to do that, Biobank has signed agreements with 13 hospitals.

Services to researchers

- **Transfer of samples to research projects:** 450 samples to support 4 research projects and 76 samples to other CNIO technical units for technical validation. The impact of knowledge generation related to this activity resulted in 6 (Q1) publications acknowledging Biobank’s contribution, with a mean impact factor (IF) of 14.
- **Custody of collections:** We offer the service of custody and management of collections for researchers, ensuring the traceability of samples and compliance with GDPR.
- **Ethical and legal advice to researchers:** 28 queries about the ethical and legal aspects of research from both CNIO and external researchers, other biobanks or companies, were answered in 2022. We also helped home-researchers to obtain ethical approval of their projects (6) by the *ISCIII* Research Ethics Committee.

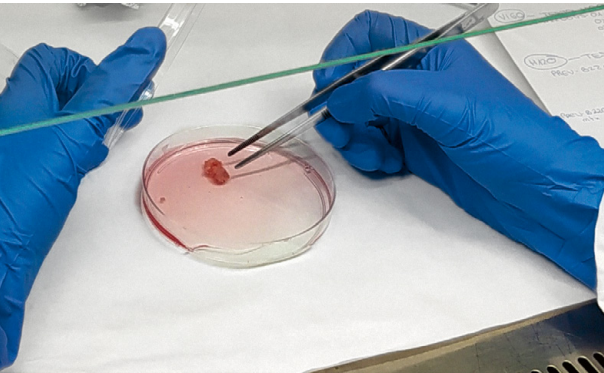


FIGURE 1 Processing of a brain metastasis sample from the RENACER research collection.

Participation in cooperative projects

- *ISCIII* Biobanks and Biomodels Platform, promoted by the *Carlos III* Health Institute.
- National Brain Metastasis Network, the *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 Sweden and South Africa, to investigate viral infections of the lower respiratory tract.

Organisation and participation in training and dissemination activities

- “Biobanking in the era of precision medicine” PhD course, and “Biobanking as the cornerstone for translational research” Symposium, Copenhagen (Denmark) and Lund (Sweden).
- I Conference of the *ISCIII* Biobanks and Biomodels Platform Scientific Committee.
- Participation in national and international congresses, scientific meetings, radio programmes (*RNE*), press and events (e.g., “*Tejedor* Mentoring Programme” - *Univ. Complutense de Madrid*, “*Cursos de Verano*” - *Univ. Autónoma de Madrid*, 4º *ESO+Empresa* Programme, to name a few). ■

PUBLICATIONS

- Monteiro C *et al.* (Caleiras E, Paz-Ares L); RENACER, Valiente M (2022). Stratification of radiosensitive brain metastases based on an actionable S100A9/RAGE resistance mechanism. *Nat Med* 28, 752-765.
- Zhu L *et al.* (incl. Blanco-Aparicio C, Martínez S, Sobrino C, Ajenjo N, Artiga MJ, Ortega-Paíno E, Rodríguez-Perales S; RENACER, Muñoz J, Caleiras E, Megías D, Quintela-Fandino M, Pastor J), Valiente M (2022). A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med* 14, e14552.
- Cruz R *et al.* (incl. Artiga MJ, Ortega-Paíno E) (2022). Novel genes and sex differences in COVID-19 severity. *Hum Mol Genet.* PMID: 35708486.
- Martínez-González S *et al.* (incl. Ajenjo N,

Blanco-Aparicio C, Pastor J). Macrocyclization as a source of desired Ppolypharmacology. Discovery of triple PI3K/mTOR/PIM inhibitors. *ACS Med Chem Lett* 12, 1794-1801.

Ortega-Paíno E, Oliver E (2022). RAICEX: a successful story of the Spanish scientific diaspora. *Front Res Metr Anal* 7, 905765.

AWARDS AND RECOGNITION

- Eva Ortega-Paíno is the Director of Spain’s National Node for the the Biobanking and Biomolecular Resources Research Infrastructure – European Research Infrastructure Consortium.
- CNIO Biobank received €800,000 from the EU REACT Project, led by the *Statens Serum Institut* (Denmark), to investigate viral infections of the lower respiratory tract.

Communications

Communications

- Press Clippings
- CNIO Media Impact
- 2022 Social Network Data
- Social Events

176
178
181
182
183

COMMUNICATIONS

MÓNICA G. SALOMONE (since July)
Director of Communications

Susana Pérez de Pablos
Nuria Noriega (until March)
Head of Communications

Science Communication
and Social Media
Rebeca Santamaría
(until September)



The post-pandemic global communication scenario is different from that prior to 2020. Traditional media are increasingly competing with social media content generators, and audiences have become accustomed to relentless information consumption. In this context, research institutions have the responsibility to nurture social demand with quality information. For the CNIO, which for years has emphasised the need to bring knowledge to society, the new situation is an opportunity to further expand outreach actions in the field of cancer research.

This is why the CNIO's Communication Department had 2 key goals in 2022. The first and immediate one was to keep bringing the outcomes of CNIO researchers to the public, in the clearest and most attractive way. But the CNIO also began in 2022 to broaden its informative offer with more general-public oriented content, specifically on how research to prevent, diagnose and treat cancer is being carried out.

Several of the products generated by the CNIO Communications Department in 2022 fall into this line, such as the interviews with Elisabete Weiderpass, director of the International Agency for Research on Cancer (IARC), who offered high quality information on cancer prevention; and with David Nogués-Bravo, macroecologist, on the impact of the global environmental crisis. Both interviews were covered by national and regional media.

Another novelty that multiplied CNIO's visibility among the non-specialised public has been a collaboration with *Radio Nacional de España*. Twice a month Maria A. Blasco participates in the prime-time programme *Las Mañanas*, with a commentary on the latest issues in culture and science.

All the aforementioned actions build on the CNIO's prestige, which has grown thanks to the dissemination of the excellent work of its research community. In 2022, some of the scientific publications that attracted most interest in the media were the study of a patient who has survived multiple tumours (C. Villarroya-Beltri, M. Malumbres, *Science Advances*); the identification of a mechanism that causes cirrhosis (A. Garrido, N. Djouder, *Journal of Hepatology*); and a stool analysis to detect pancreatic cancer (N. Malats, *Gut*).

The 2 international CNIO-CaixaResearch Frontiers Meetings dealt with topics of great public interest, such as aging and the relationship between cancer and diet, and they were indeed well received by the media.

The campaign launched by the CNIO on the occasion of World Cancer Day, *La lotería que más te toca*, was another milestone in 2022, as was the open event held at CaixaForum (Madrid) to celebrate World Cancer Research Day. During the latter,

“CNIO's prestige has grown thanks to the dissemination of the excellent work of its research community.”

oncologists Luis-Paz Ares and Antonio Pérez explained to a non-specialised audience why cancer immunotherapy has become such a hot topic.

The 5th edition of CNIO Arte, carried out with the *Banco Santander* Foundation, is an excellent opportunity to reach media outlets other than those devoted to health and science. The key players in the 2022 edition were the artist Susana Solano and the epidemiologist Pedro Alonso. Audiovisual media, such as *RTVE*, echoed Solano's trip to Mozambique in search of inspiration from Alonso's work.

The IV Philosophy and Science Congress, organised with the *Banco Sabadell* Foundation, also contributed to the CNIO's presence in media areas beyond science. Two national media devoted space in their printed edition to the congress.

The CNIO can also be a reference for the values that help to build a better society. CNIO's deep commitment to equality and diversity is well known in the media, which not only report on the events of the CNIO Women in Science and Diversity Office, but also regularly seek out CNIO speakers to assess the situation of women in science and how to improve it.

On the whole, CNIO's media presence throughout 2022 has been high both in quality and quantity. The advertising value of these impacts, expressed in economic terms, exceeds €30 million. The influence on social media has also substantially increased – the number of followers on Instagram has doubled, and CNIO's Twitter account has 3,000 followers more than in 2021. In short, the CNIO's reputation in the media is that of a centre of scientific, cultural, and social excellence. ■

PRESS CLIPPINGS



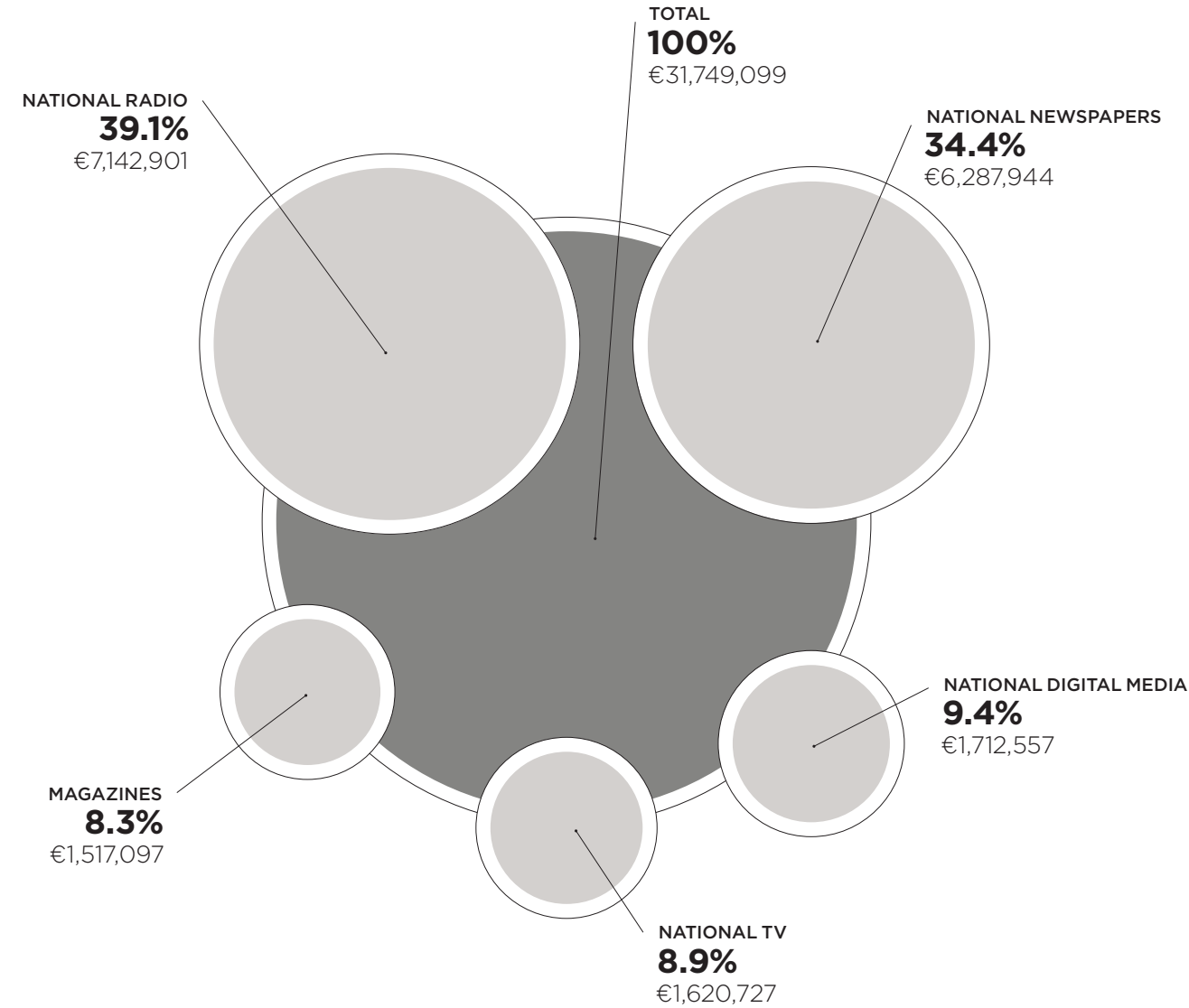
- 1 *El País*, front page, November 3, 2022
- 2 *El País* front page, November 25, 2022
- 3 *El País*, March 3, 2022
- 4 *El Mundo*, November 26, 2022
- 5 *El Mundo*, March 9, 2022
- 6 *ABC*, November 3, 2022
- 7 *La Voz de Galicia*, November 3, 2022
- 8 *Al Jazeera*, December 3, 2022
- 9 *EFE* news agency, June 15, 2022

- 10 *Gaceta Médica*, January 24, 2022
- 11 *El Correo Gallego*, February 3, 2022
- 12 *Diario Menorca*, April 4, 2022
- 13 *TVE 24h*, February 3, 2022
- 14 *TVE, Telediario 2*, January 27, 2022
- 15 *Antena 3, Espejo Público*, February 3, 2022
- 16 *TeleMadrid, Cámara Real*, February 2, 2022
- 17 *Ideal de Granada*, February 4, 2022
- 18 248 media outlets from all over the world covered C. Villarroja-Beltri, et al. *Sci. Adv.* 2022

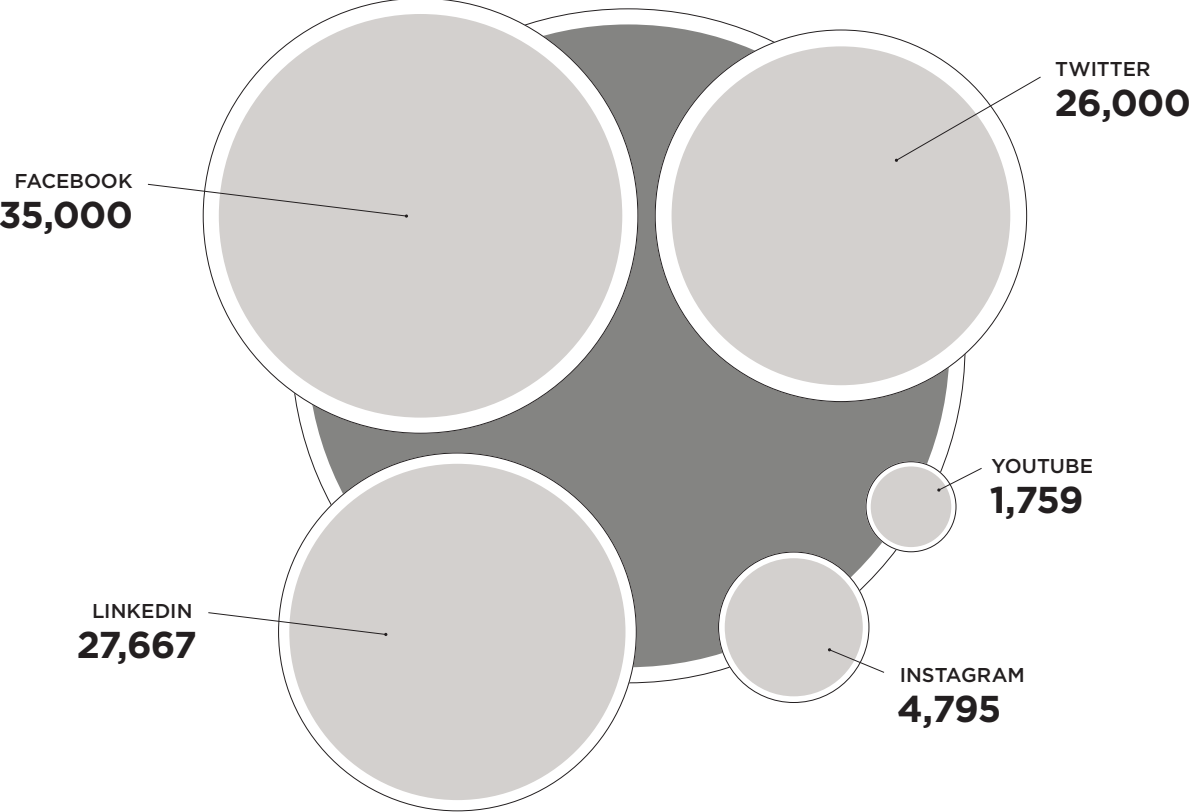


19 *Diario Médico*, October 30, 2022
20 *Canarias 7*, February 11, 2022
21 *TeleMadrid, Informativos Noche*, January 31, 2022
22 *La Vanguardia*, March 9, 2022
23 *Muy Interesante*, December 22, 2022
24 *La Vanguardia*, February 13, 2022
25 *La Vanguardia*, January 30, 2022
26 *Levante EMV*, May 7, 2022
27 *Granada Hoy*, December 30, 2022

CNIO MEDIA IMPACT



2022 SOCIAL NETWORK DATA
FOLLOWERS



LINKEDIN

IMPRESSIONS	980,044
INTERACTIONS	12,575
LINK CLICKS	87,168

FACEBOOK

POST IMPRESSIONS	396,000
REACH	82,428
NEW LIKES	234

INSTAGRAM

REACH	97,327
VISITS TO PROFILE	18,646
NEW FOLLOWERS	683

TWITTER CNIO

IMPRESSIONS	2,200,000
RETWEETS	3,900
LIKES	14,000
MENTIONS	5,000
INTERACTION RATE	3.33%

YOUTUBE

UPLOADED VIDEOS (TOTAL)	206
UPLOADED VIDEOS 2022	50
VIEWS IN 2022	27,542
WATCH TIME (HOURS)	2.8K
AVERAGE VIEW DURATION	6:00

SOCIAL EVENTS

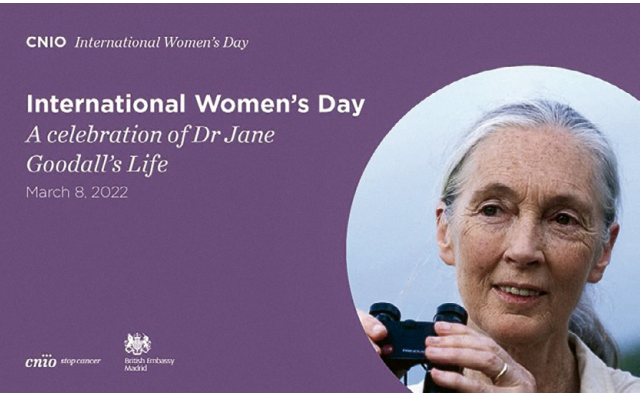


CNIO establishes “lottery” retail store to raise awareness of the importance of cancer research In various information points across the city of Madrid, the CNIO’s World Cancer Day campaign delivered shocking messages like “It may affect your life”; “In 2021, there were 276,239 ‘winners’”; or “One out of 3 people are bound to ‘win’” to catch the attention of passers-by and publicise the work done by the CNIO to reduce these numbers. In an effort to raise awareness of the need for research funds, CNIO established its own lottery retailer to manage this most unfortunate lottery, making this campaign a performance. As summarised by Maria A. Blasco: “Cancer really is like a lottery. Only research can protect us from it”. *February 3, 2022.*



International Women’s Day, a tribute to the career of Dr Jane Goodall On March 8, 2022, International Women’s Day, CNIO held a tribute to the career of Dr Jane Goodall. In addition to the projection of a video that Dr Goodall sent for the occasion, there was a round table moderated by the journalist Lula Gómez and involving Dr Rebeca Atencia (Director of the Jane Goodall Institute

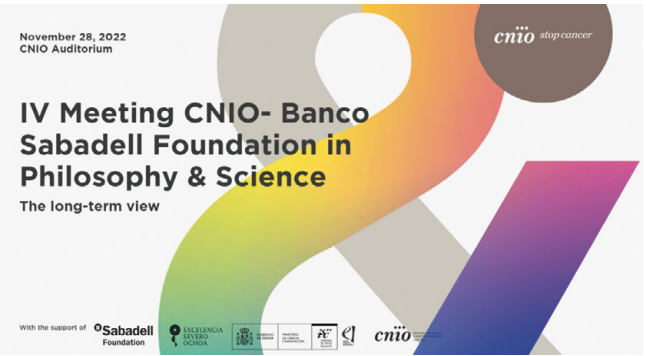
in the Republic of Congo), Federico Bogdanowicz (Director of the Jane Goodall Institute in Spain and Senegal) and Dr. Maria A. Blasco (Director of the CNIO). The act was closed by the British Ambassador Hugh Elliot. This event was organised by the CNIO WISE Diversity Office and the British Embassy in Spain. *March 8, 2022.*





More than 250 people conduct experiments at CNIO's "Festival of Science" held during European Researchers' Night 2022 During our 12th European Researchers' Night (a European Union initiative to publicise the importance of scientific knowledge, held simultaneously in 350 European cities), once again in person after 2 years online, the participants performed DNA extraction, looked at tumour cells under a microscope, and visualised how the three-dimensional structure of proteins is decoded.

This event in Madrid is promoted by the Science, Universities and Innovation Department of the Regional Government of Madrid, and is coordinated by the *Fundación para el Conocimiento madri+d*. The project is funded by the European Union under the Horizon 2020 Research and Innovation Programme – Marie Skłodowska-Curie Actions. At CNIO, the event is part of the Centre's outreach strategy through the Dean's Office, along with the CNIO Training Programme. *September 30, 2022.*



IV CNIO-Banco Sabadell Foundation Workshop on Philosophy & Science: The Long-term View Philosophy creates the critical thinking that is necessary for science and created the scientific method. Today philosophy reflects on the discoveries made by scientists, and science needs to be thought of from a philosophical perspective. Scientists and philosophers need to meet and get to know each other, think together and share ideas, said the speaker explaining the reasons for these meetings.

The fourth such event was entitled "The Long-term View". Since the mid-1980s, we have entered an ecological and social crisis that is now undeniable. We are torn between a very worrying present and the uncertainty of how what we do now will affect the future of the planet and humanity. Science itself may be changing now in the wake of artificial intelligence and big data. Long-termism is a philosophical view concerned with the task of developing ethical thinking that takes into account the beings of the future. *November 28, 2022.*



World Cancer Research Day Event at CaixaForum Madrid CNIO celebrated World Cancer Research Day by holding a seminar entitled "Immunotherapy: Achievements and Challenges in the Latest Breakthrough in the Fight Against Cancer." Oncologists Luis Paz-

Ares and Antonio Pérez-Martínez, along with CNIO Director Maria A. Blasco, other researchers and CNIO Friends, attended the event. The event was organised by the CNIO at CaixaForum (Madrid), with the support of "la Caixa" Foundation. *September 24, 2022.*

Institutional Image & Outreach to Society

INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY

AMPARO GARRIDO (until April) Coordinator



“CNIO Arte connects discovery and creation, bringing together the views that investigate, illuminate, and return to a common path.”

In 2022, the CNIO celebrated the fifth edition of CNIO Arte, carried out with the support of the *Banco Santander* Foundation, as in previous years. Its commissioner was Amparo Garrido, Coordinator of the CNIO Office for Institutional Image and Outreach to Society.

CNIO Arte 2022 featured artist Susana Solano, one of the most internationally renowned Spanish sculptors, and the physician and epidemiologist Pedro Alonso, director of the World Health Organization’s (WHO) Global Malaria Programme in Geneva, Switzerland. In this edition, Solano travelled to Mozambique, accompanied by the Coordinator of the Office and curator of CNIO Arte 2022, Amparo Garrido, to visit the hospital facilities where Dr Alonso works. Thanks to this trip, the artist was able to see his work first-hand, find inspiration, and connect with the subject to create the piece ‘*El mundo de las cosas*’, which has been on display at the CNIO since February 16, 2022.

For the second consecutive year, we have had the honour of having our own exhibition space at the ARCO International Contemporary Art Fair, where Solano’s work was exhibited. The Fair was held between February 23 and 27, and our stand was a great success. There were many visitors, citizens as well as representatives from well-known corporations, foundations, and institutions. These encounters have had a positive impact on disseminating knowledge about our Centre, opening the door to prospective donations and collaborations. CNIO Arte was presented in ARCO on February 25, in the ARCO Presentations Room, where Maria Blasco, CNIO Director and Executive Director of CNIO Arte; Borja Baselga, President of the *Fundación Banco Santander*; and Susana Solano, CNIO Arte 2022 featured artist, participated in a round table discussion moderated by CNIO Arte curator Amparo Garrido.

This year, once again, CNIO Arte 2022 drew the attention of the media. The event made quite an impact on the press and was covered by many different media outlets, including general newspapers, radio stations, and TV channels. Thus, we had the

presence of *El País*, *20 Minutos*, *La Vanguardia*, *RTVE*, *RNE*, *EFE*, *Europa Press*, *Colpisa*, *Diario Siglo XXI*, *Crónica de Cantabria*, *Infosalus.com*, *Con Salud*, *Newtral*, *MSNEspaña*, among others.

As a result of our success at ARCO, the Cervantes Institute proposed to exhibit the artworks of our past CNIO Arte editions in different countries. This led to the initiative “CNIO Arte. Dialogues between art and science”, which begins its cycle of travelling exhibitions of CNIO Arte works at the Cervantes Institute in New York, with the support of the *Fundación Banco Santander*. In addition, the artwork ‘*Fulguraciones*’ by visual artist Daniel Canogar, produced for CNIO Arte 2021, was exhibited at the Centre for Art and Media (ZKM) in Karlsruhe (Germany), as part of the exhibition “Biomedica. The age of media with life-like behaviour”, from December 18, 2021, to August 28, 2022.

Following the success of previous initiatives to promote CNIO Arte, we organised the III Art and Science Symposium, held February 16, 2022, and chaired by Carlos Jiménez, Emeritus Professor of Aesthetics, Historian and Art Critic. In line with



Sculptor Susana Solano, CNIO Arte 2022 featured artist, and Amparo Garrido, visual artist and CNIO Arte curator, at

the CNIO Arte exhibit in ARCOmadrid 2022.



Unveiling of the sculpture ‘*Intra-Venus*’ at the CNIO, on February 2, 2022. Maria Blasco, CNIO Director, and Marina Vargas, artist and author of the sculpture, at the inaugural ceremony.

the dialogue between Susana Solano and Pedro Alonso, the theme chosen for this 3rd edition of the symposium was “Art, Science and Pandemics”. Carlos Jiménez, Director of the Symposium; Luis Enjuanes, Research Professor at the Coronavirus Laboratory of the National Biotechnology Centre (CNB-CSIC); Ana Matey, artist; and Ruth Toledano, opinion columnist at *elDiario.es*, editor, and collaborator for *Fondation Franz Weber*, reflected on the proposed theme from different perspectives and viewpoints. This event, as well as the CNIO Arte Presentation, are available on our YouTube channel.

As part of the initiatives organised by the Institutional Image and Outreach to Society Office, to mark the occasion of World Cancer Day on February 2, 2022, the CNIO celebrated the unveiling of the sculpture ‘*Intra-Venus*’, by the artist Marina

Vargas, and the launch of the “*Intra-Venus*” Association for the visibility and support of female creators with cancer. The sculpture will be on display to the public for one year at the entrance to the CNIO.

On May 4 and 5, the CNIO participated with its own stand represented by the Institutional Image Office at the *Diverciencia* Fair in Algeciras. During those 2 days, educational and science outreach activities were carried out, aimed at primary and secondary school students in the city.

Our Office also participated in the 2022 call for grants to promote Science and Culture, launched by *Fundación Española para la Ciencia y la Tecnología (FECYT)*, with the initiative “CNIO Artistic Residences”. The grant was awarded and the project

was launched in August 2022 with the opening of a selection process for a young or mid-career artist to carry out a 6-month stay at the CNIO. The selected artist will have the opportunity to learn more about the Centre, its facilities and research lines, and to interact with the research staff with the aim of finding convergence points, common interests, and new avenues of inspiration through which he/she will create one or more works of art that reflect the work being carried out at the Centre. These creations will be exhibited at the CNIO and at all the events or institutions engaged in dissemination activities of the project. The artworks may be offered for sale, and the profits will be used in their entirety to fund CNIO research.

In 2022, we further updated the content of our website www.cnio.es, including images, infographics, and news about the Centre and its activities. Our Office collaborated with other CNIO departments to help familiarise them with the latest version of our corporate identity manual. We also participated in the organisation of other cultural and outreach initiatives

held at the CNIO, such as the European Researchers’ Night, the “*La Lotería que más toca*” campaign, the event held at CaixaForum to celebrate World Cancer Research Day, the celebration of the International Day of Women and Girls in Science, where we had Jane Goodall as a guest speaker, and the ‘CNIO’ Friends Day. On all of these occasions, we offered support to the different departments and areas of the Centre, collaborating in the preparation, management, and coordination of the event, internally and with suppliers.

We also continued to update outreach materials (brochures, etc.), coordinating and supervising all design pieces such as banners, posters, campaigns, graphics, etc., in constant dialogue with the designers. In addition, the Image Office supervises the images and design that illustrate this Annual Report, and the subsequent web update of the associated texts and photos. The aim of this work builds on one of the CNIO’s key strategic pillars: to amplify the reach and impact of the CNIO in society and, from there, to strengthen philanthropic support to the institution. ■

Development & Philanthropy

DEVELOPMENT & PHILANTHROPY

JESSICA J. ROSE Director

Development & Philanthropy Officer
Mercedes Antona



2022 was an exciting year of progress for the Office of Philanthropy and Development. For the first time in our history, we raised over €1 million for the CNIO in 2022. Every single euro of this goes directly to research. We are grateful to every one of our donors who generously supported cancer research and contributed to this milestone annual achievement.

We were particularly delighted to close our first major gift for the Centre since the development of the Philanthropy Office 3 years ago. Our existing friend and supporter *Fundación Humanismo y Ciencia* made a generous gift to support renal fibrosis and telomere research at the CNIO. This gift follows from the Foundation’s original gift to fund a CNIO Friends contract, and we are very proud to have continued this collaboration.

In another new step for the office, in conjunction with the Offices of Communication and Institutional Image, we launched a campaign in collaboration with True PR to take our branding and fundraising campaign to a new level. Under the slogan “The lottery that touches you the most” together with our logo #CNIOStopCancer, the main event involved a lottery held on *calle Arenal* in central Madrid. During the event, members of the public and the press received “scratch and win” cards to show the high probability of suffering from cancer and increase the visibility of the CNIO. The successful campaign went on to win a gold medal in the “*Eficacia 2022* Awards” for recognition for the use of communication to promote research.

The CNIO Friends Programme has raised over €3.8 million in donations and pledges since 2015. 100% of these donations go directly to cancer research – the ‘CNIO Friends Contracts’ programme is used to recruit excellent scientists from around the world to conduct 2-year postdoctoral research projects. Major donations to the CNIO go to the ‘CNIO Friends Projects’ programme to support innovative cancer projects within specific Groups or areas of research at the CNIO. These contributions have thus far enabled us to hire 34 new researchers since 2016. In addition, the legacy programme continues to grow. The CNIO has raised a cumulative total of ~€1.5 million since 2015, and we have an additional €1.4m in legacy pledges pending to be executed.

The success of 2022 is thanks in part to the work of the last 3 years. Major gift fundraising in particular is a long, slow process. It takes sustained effort, strategy, and robust systems behind the scenes to enable a major programme to be successful. We hope this is the start of many more innovative and transformative gifts and collaborations to come for the CNIO, and we are excited about the year ahead. We are delighted to be able to enable more of the brilliant work of the CNIO and give the public the opportunity to join us in our mission to stop cancer. ■

“Philanthropy can enable transformative change – every individual can be part of the solution in helping us to stop cancer. 2022 has been record-breaking for our philanthropic programme, and we look forward to continuing to take this exciting work to a new level.”

CNIO Offices

Dean's Office
CNIO Wise Diversity Office

198
200

DEAN’S OFFICE

María S. Soengas
Dean for Academic Affairs

PARTICIPANTS
Personnel in training:
Nicolás Cuesta
Ernesto López
Ana Martín
Ana de Pablos



The main objective of Dean’s Office at the CNIO is to contribute to one of its core missions, that is, to train and promote new generations of scientists. In fact, over 60% of the work force at our institution are young researchers, including undergraduate students, predoctoral and postdoctoral fellows. We also hosted medical residents and a broad spectrum of visiting scientists from different countries and institutions. The CNIO dedicates particular emphasis to career development, supported in part by highly competitive PhD and postdoctoral programmes. 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 postdocs receive feedback on the scientific aspects of their work and on soft

skills that may help further their career. The Dean’s Office also works very closely with the CNIO Student Association (CNIOSA), which has increasingly become a highly active driving force behind an inspiring series of workshops designed to improve soft skills in and beyond academia. These activities are performed in concert with CNIO’s Training Programmes, as well as with the Innovation and Communications Offices, which are deeply committed to providing the best environment for our personnel. We are most grateful to the *Fundación Jesús Serra*, for their continuous support to strengthen career development programmes 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. For example, over 60 CNIO PhD students and postdoctoral fellow volunteers this year took part in the 9th edition of our “*Meet a Scientist, Become a Scientist*” event.

Elena Sánchez
José Luis Sepúlveda

Staff/Faculty:
Ana Cuadrado
Nabil Djouder

Rafael Fernández-Leiro
Eva González-Suárez
Óscar Llorca
Ana Losada
Geoff Macintyre
Juan Méndez

Jorge L Martínez
Héctor Peinado

This is an open doors activity that we hold as part of the *Marie Skłodowska Curie European Researchers’ Night* and through which over 260 participants of all ages learn about daily life at our Centre and have the opportunity to run an experiment side by side with our researchers.

Another of our highlights in 2022 was our Annual CNIO Lab Day. We had over 85 communications, which were evaluated by an *ad hoc* committee to select 14 “elevator pitch presentations” that highlight studies from each of the Centre’s Scientific Programmes at the forefront of research in their fields. We also had external speakers, focusing on different aspects of *Science and Communication* and on *Sustainable Laboratory Environments*. We thus learned from **Arkaitz Carracedo** (*CICbioGUNE*) about tips and tricks for a career in cancer research, and from **Guzmán Sánchez** (*European Climate Foundation*) about options for scientists in consultancy in European institutions. In addition, **Nikoline Borgermann** (*Ava sustain*) and **Iuliia Konko** (*Merck Life Science*) told us about products and solutions for a more nature-friendly laboratory space.

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 2022, the ‘*Antonia Nieto Award*’ went to **Cátia Monteiro** for translationally significant new findings on radiosensitization of brain metastasis, published in the prestigious *Nature Medicine* journal. Additional awards in the PhD category went to **Mónica P de Andrés** (*Gut*), **Amanda Garrido** (*Journal of Hepatology*), **Javier Lanillos** (*European Urology*), **Antonio Galarreta** (*EMBO J*), and **Karolina Jodkowska** (*Nucleic Acids Research*), for their contributions to the understanding of different aspects of tumour initiation, progression and response to therapy.

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

The awardee was **Carolina Villarroya**, for exciting new findings related to different genomic alterations in subtypes of chronic

“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.”

lymphocytic leukaemia (*Science Advances*) and in the control of pluripotent cells in cancer (*EMBO J*).

3. Outstanding Contribution to Outreach and Awareness

The 2022 recipients were *ex aequo*: **Ernesto López**, for his altruistic efforts in reorganising and promoting the CNIO Student Association (CNIOSA), and **Miriam Rodríguez**, for her continued contribution as a volunteer in the various activities related to dissemination of science to society carried out by the CNIO, such as the European Researchers’ Night, among others.

The Lab Day proceeded with additional awards from the Dean’s Office: 4 awards for the Best Oral presentations and 3 for the Best Posters. Beyond these, also presented were the Awards for Scientific Videos (**Anabel Sáez Mas**) and for T-Shirt Design (**Natalia Cuervo-Iturrioz**), both 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 the 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 in society and to help correct imbalances in the career ladder within the CNIO community, especially in leadership positions. The Office is composed of CNIO volunteers from across all the areas represented at the Centre and also includes the Director.

In 2022, the WISE Office expanded its name to “WISE Diversity” Office. In line with CNIO’s commitment to diversity and inclusion, 2 of our invited speakers, the writers and journalists Gabriela Wiener and Paloma Chen, were racialised women (see details below).

We also resumed in-person activities following the pandemic. The WISE Diversity Office dedicated significant efforts into making the CNIO a better place to work and to reconcile work

and private life. We continued our activity as part of the Equality Plan Monitoring Committee (CSPD). The “Workplace Harassment” Protocol was elaborated and submitted to the CNIO Direction for final approval. We also worked on a “Maternity and Pregnant Women” protocol. In addition, the Office was involved in preparing an application for the Human Resources Excellence in Research Award (HRS4R) from the European Commission.

Throughout the year, the WISE Diversity Office continued organising the WISE seminar series, in which we invite numerous top female leaders from different areas to give a talk. The following talks were given during the year:

- María Folguera, artistic director of *Teatro Circo Price*. Title: “*Marta y María han de andar juntas. Vivir entre la acción y las nubes*”. 18/01/2022.
- Fe López, presenter and TV writer in *RTVE*. Title: “*Periodismo, mujer y deporte*”. 17/02/2022.

- Members

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

- Gabriela Wiener, writer and journalist. Title: “*Espejos descoloniales*”. 01/03/2022.
- Paloma Chen, writer and journalist. “*Reflexiones desde una experiencia chino-española sobre la identidad*”. 24/05/2022.
- Eva Orúe, director of the Madrid Book Fair. Title: “*Feria del Libro de Madrid: apocalípticos e integrados se encuentran en El Retiro*”. 20/09/2022.
- Marina Echebarría, professor of commercial law and activist for LGBTI rights. Title: “*Challenges of Law in the face of technology*”. 15/11/2022.
- Cristina Aranda, co-founder of Big Onion. Title: “*Diversity, the main lever for innovation*”. 13/12/2022.

In the spirit of supporting STEM careers among young women and breaking gender stereotypes, on February 11, 2022, International Day of Women and Girls in Science, the CNIO released a video in which representatives from all CNIO professional categories of women involved in research tell us why they love research: https://youtu.be/Qhq3se_YFb4. This video was circulated on social networks under the hashtag #HazteCientífica and had a remarkable impact. In addition, on that day, CNIO scientists participated in several online talks at different organisations.

On March 8, 2022, to mark International Working Women’s Day, the WISE Office organised a joint event together with the British Embassy, and with the participation of the Jane Goodall Institute, in which a tribute was paid to the career of Dr Jane Goodall. In this event, a video sent by Dr Goodall for the occasion was broadcast, and there was a roundtable discussion in which the following speakers took part: Maria A. Blasco, CNIO Director; Federico Bogdanowicz, Director of the Jane Goodall Institute in Spain and Senegal; and Rebeca Atencia, Director of the Jane Goodall Institute in the Republic of Congo. The British Ambassador Hugh Elliot delivered the closing remarks. At the end of October, the WISE Office organised another event, which consisted of an interview with Irene Cano, the General Director of *Meta Iberia*, conducted by our Director Maria A. Blasco. ■

Here at the WISE Diversity Office, we share what the Pakistani activist and Nobel Prize laureate Malala Yousafzai said: “I raise up my voice – not so I can shout but so that those without a voice can be heard... We cannot succeed when half of us are held back.”

Facts & Figures

Scientific Management	204
Competitive Funding	206
Education and Training Programmes	220
Scientific Events	226
Administration	236
Board of Trustees	236
Scientific Advisory Board	238
Management	240
CNIO Personnel 2022	242
Other Sponsorships and Partnerships	246

SCIENTIFIC MANAGEMENT

ISABEL BARTHELEMY Scientific Management Director

M. Dolores Liébanes
Mercedes Moro
Helena Zamora
Sonia Cerdá
Almudena del Codo
Victoria López
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 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.

“Every day we work towards building a strong and flexible framework to support our scientists and to help them achieve excellence.”

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 is used not only to finance the Centre’s outstanding R&D activities, but also strategic actions in innovation together with industry partners. The funding is also used to support other relevant activities related to dissemination and scientific outreach; these activities are aimed at promoting public awareness. In 2022, researchers at the CNIO were involved in 152 projects that received extramural funding.

During this same year, the CNIO actively participated in a total of 66 collaborative projects: 17 were international collaborative projects (4 of which are coordinated by the CNIO), and 49 were collaborative projects conducted at the national level (16 of them are coordinated by the CNIO). The international collaborative projects were 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 US Department of Defense (DoD); 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 that is managed by the Institute of Health *Carlos III (ISCIII)*, the State Research Agency of

the Spanish Ministry of Science and Innovation (*AEI/MCIN*), and the R&D Activities Programmes of the Community of Madrid; most of the 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 2022, 13 of these projects received international funds, while 73 of them received national funding (mainly from the *AEI/MCIN*, the *ISCIII*, and private foundations). The international individual projects are funded by the European Union (7 European Research Council [ERC] grants and 6 Marie Skłodowska-Curie Actions), Worldwide Cancer Research, the American Thyroid Association, and the Mark Foundation for Cancer Research.

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

<div>EUROPEAN UNION RESEARCH & INNOVATION FRAMEWORK PROGRAMME</div> <div></div>	HORIZON EUROPE (2021-2027)	
	RESEARCH INFRASTRUCTURES (PILLAR I)	
	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Al-Shahrour, Fátima	EOSC4Cancer: A European-wide foundation to accelerate Data-driven Cancer Research (Ref.: 101058427)
	HEALTH PROGRAMME (PILLAR II)	
	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Ortega, Eva	REACT: Respiratory Host-Pathogen Interaction (Ref.: 101057129)
	MARIE SKŁODOWSKA-CURIE ACTIONS (PILAR I)	
	PRINCIPAL INVESTIGATOR	PROJECT TITLE
	Soengas, Maria S.	MADRIDNIGHT: Researchers and citizens: facing together the European challenges (Ref.: 101061343)
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
Muñoz, Javier		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
Muñoz, Inés	Targeting Mdm2-MdmX E3 ligase for treatment of drug-resistant lymphoma (Ref.: R01CA208352)
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)

US CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS (CDMRP)/US DEPARTMENT OF DEFENSE



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	Clinical qualification of DNA repair defects as prognostic and predictive biomarker in metastatic prostate cancer using genomics and tissue-based functional assays (Ref.: W81XWH-18-1-0770)

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

WORLDWIDE CANCER RESEARCH (WCR; FORMERLY AICR)



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel (Coordinator)	S100A9-dependent radiation resistance in brain metastasis (Ref.: 19-0177)

LUSTGARTEN FOUNDATION & STAND-UP2CANCER



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	Pancreatic Cancer Collective - Computational Approaches To Identifying High-Risk Pancreatic Cancer Populations: High Risk Cohorts Through Molecular and Genetic Data (Ref.: SU2C #6179)

FUNDACIÓN RAMÓN ARECES/CNIO/ WEIZMANN INSTITUTE OF SCIENCE



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S. (Coordinator)	Heterogeneity in melanoma metastasis and resistance to immune checkpoint blockade
Malats, Núria; Real, Francisco X. (Coordinator)	MIT-BC Study: Tumour Microbiome and Immune profiles as predictors of Treatment response in high-risk Non-Muscle Invasive Bladder Cancer

INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

EUROPEAN COMMISSION



HORIZON 2020 (2014-2020)	
EUROPEAN RESEARCH COUNCIL (ERC)	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ERC Advanced Grant THERACAN: Novel therapeutic strategies to treat pancreatic and lung cancer (Ref.: 695566)
Blasco, Maria	ERC Advanced Grant SHELTERINS: Targeting Shelterin Proteins in Cancer (Ref.: 882385)
Cortés, Felipe	ERC Consolidator Grant TOPOmics: Global dynamics of topoisomerase-induced DNA breaks (Ref.: 647359)
Fernández-Capetillo, Óscar	ERC Proof of Concept TARGETSET: Commercial feasibility of targeting the histone methyltransferase SETD8 in cancer: New chemical entities and biomarkers (Ref.: 963443)
González, Eva	ERC Consolidator Grant PLEIO-RANK: Pleiotropic treatment of cancer: RANK inhibitors targeting cancer stem cells and immunity (Ref.: 682935)
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: Tumour compartment-specific effects of P70S6K in early triple negative breast cancer: regulation of antitumor immune response and therapeutic implications (Ref.: 893597)
Real, Francisco X. Zagorac, Slađana	PDASwItch: Super-enhancer modules controlling plasticity and response to therapy in pancreatic cancer (Ref.: 895943)

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)

AMERICAN THYROID ASSOCIATION (ATA), COFUNDED BY BITE ME CANCER (BMC)



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Montero, Cristina	Discovering novel molecular nodes involved in MTC development and evolution (Ref.: GRANT2020-0000000150)

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 SALUD¹

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Gallardo, Miguel	Deciphering the role of hnRNP K in multiple myeloma (Ref. PI21/00191)
García-Pérez, María José (Coordinator)	Global Instability and Mutation Burden genetic signatures in Clear Cell and Endometrioid Ovarian Carcinomas: Immunogenicity and prognostic and predictive relevance (Ref.: PI19/01730)
González-Neira, Anna (Coordinator)	Role of the mitochondrial genes in cardiotoxicity: identification of predictive biomarkers (Ref.: PI18/01242)
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, Nuria (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)
Osorio, Ana (Coordinator)	Optimising massive sequencing strategies for the identification and clinical translation of new susceptibility genes implicated in familial breast cancer (Ref.: PI19/00640)
Robledo, Mercedes (Coordinator)	Characterization of new drivers associated with development and progression of rare endocrine and neuroendocrine tumours. Predictive markers of sensitivity to treatment (Ref.: PI20/01169)

JOINT INTERNATIONAL COLLABORATIVE ACTIONS: ERA-NETS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ERA PerMed: Personalized multimodal therapies for the treatment of lung cancer (Ref.: AC20/00114)

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 SALUD²

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Eva	Plataforma de Biobancos y Biomodelos (Group, Ref.: PT20/0070)

IMPACT PROJECTS: PRECISION MEDICINE INFRASTRUCTURE³

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

NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION

STRATEGIC LINES PROJECTS (PUBLIC-PRIVATE COLLABORATIVE PROJECTS)⁴

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

EXCELLENCE NETWORKS / REDES DE EXCELENCIA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos (Coordinator)	Research Network iDIFFER: Balancing proliferation and differentiation: mechanisms and relevance in human disease (Ref.: RED2018-102723-T)

PUBLIC-PRIVATE COLLABORATIVE PROJECTS / PROYECTOS DE COLABORACIÓN PÚBLICO-PRIVADA⁵

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peláez, Fernando	An effective and safe system for the treatment of Atrial Fibrillation through Irreversible Electroporation (Ref. CPP2021-008480)
Blasco, María A.	Development of a novel gene therapy for the treatment of Idiopathic Fibrosis (Ref. CPP2021-008483)

1 This Programme is cofunded by the European Regional Development Fund (ERDF) “A Way to Make Europe”



2, 3 This Programme is cofunded by the European Regional Development Fund (ERDF) “A Way to Make Europe”



4, 5 Funded by MCIN/AEI/10.13039/501100011033 and the European Union “NextGenerationEU”/PRTR”



COMMUNITY OF MADRID /
COMUNIDAD AUTÓNOMA DE
MADRID



R&D ACTIVITIES PROGRAMME IN BIOMEDICINE ⁶ :		
PRINCIPAL INVESTIGATOR	PROJECT TITLE	
Al-Shahrour, Fátima; Roncador, Giovanna	<i>Programa LINFOMAS-CM: Linfomas agresivos, análisis clínico y genómico integrado para una medicina de precisión</i> (Ref.: B2017/BMD-3778)	
Blasco, María	<i>Programa RypSE-CM: RNA y proteínas de unión a RNA. Implicaciones en salud y enfermedad</i> (Ref.: B2017/BMD-3770)	
Djouder, Nabil	<i>Programa TomoXliver-CM: Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar</i> (Ref.: B2017/BMD3817)	
Malumbres Marcos (Coordinator); Barbacid, Mariano	<i>Programa iLUNG-CM: Terapias personalizadas y nanotecnología en cáncer de pulmón</i> (Ref.: B2017/BMD-3884)	
Mulero, Francisca	<i>Programa RENIM-CM: Red Madrileña de Nanomedicina en Imagen Molecular</i> (Ref.: B2017/BMD-3867)	
Quintela, Miguel Ángel	<i>Programa IMMUNOTHERCAN-CM: Inmunidad tumoral e inmunoterapia del cáncer</i> (Ref.: B2017/BMD-3733)	
Robledo, Mercedes	<i>Programa TIRONET2-CM: Fisiopatología Tiroidea. Mecanismos implicados en cáncer, autoinmunidad y acción de las hormonas tiroideas</i> (Ref.: B2017/BMD-3724)	
Soengas, María S.	<i>Programa NANODENDMEDII-CM: Nanosistemas dendríticos como agentes y vectores terapéuticos en distintas aplicaciones biomédicas (II)</i> (Ref.: B2017/BMD-3703)	

R&D ACTIVITIES PROGRAMME IN TECHNOLOGIES ⁷ :		
PRINCIPAL INVESTIGATOR	PROJECT TITLE	
Llorca, Óscar	<i>Programa Tec4BioCM: Tecnologías Aplicadas al Estudio de Nanomáquinas Biológicas</i> (Ref.: P2018/NMT4443)	

SYNERGY PROJECTS:		
PRINCIPAL INVESTIGATOR	PROJECT TITLE	
Llorca, Óscar ¹	<i>Programa NanoBioCancer-CM: Nanobiotecnología Estructural y Molecular de Procesos de Reparación de ADN relacionados con Cáncer</i> (Ref.: Y2018/BIO4747)	
Malumbres, Marcos (Coordinator)	<i>Proyecto scCANCER-CM: Convergencia tecnológica para el análisis biofísico y bioquímico de células individuales en la progresión del cáncer de mama</i> (Ref.: Y2020-BIO-6519)	
Ortega, Sagrario (Coordinator); Blasco, María	<i>Proyecto COVID-PREclinical-MODEls: Modelos Animales Para el Estudio de la Covid-19. Desarrollo Pre-Clínico de Estrategias Preventivas y Terapéuticas</i> (Ref.: Y2020/BIO-6576)	

6, 7 This Programme is cofunded by the European Regional Development Fund (ERDF) and European Social Fund (ESF)



SCIENTIFIC FOUNDATION OF
THE SPANISH ASSOCIATION
AGAINST CANCER /
FUNDACIÓN CIENTÍFICA DE
LA ASOCIACIÓN ESPAÑOLA
CONTRA EL CÁNCER (AECC)



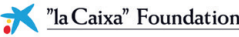
COORDINATED GROUPS		
PRINCIPAL INVESTIGATOR	PROJECT TITLE	
Barbacid, Mariano (Coordinator)	A multifaceted approach to target pancreatic cancer (Ref.: GC16173694BARB)	
Fernández, Lucía; Martínez, Joaquín	A Phase I Trial of Memory T Cells Expressing an ANTI-NKG2D Chimeric Antigen Receptor in Children, Adolescents and Young Adults with Advanced Sarcoma (Ref.: GCAEC19019PERE)	
Peinado, Hector (Coordinator)	Reactivation of anti-tumour immune cell responses by functionalized nanoparticles in melanoma (Ref.: PRYCO223002PEIN)	
Valiente, Manuel	Study of the molecular mechanisms involved in primary (glioblastoma) and secondary (metastasis) brain tumours to identify novel therapeutic targets and anti-cancer agents, biomarkers to select treatments and novel non-invasive methods for molecular diagnosis (Ref.: GCTRA16015SEOA)	

ERA-NETS		
PRINCIPAL INVESTIGATOR	PROJECT TITLE	
Barbacid, Mariano	ERA PerMed: Personalized multimodal therapies for the treatment of lung cancer (Ref.: PERME20707BARB)	
Valiente, Manuel (Coordinator)	ERANET TRANSCAN-3 Reverting immune suppression to elicit brain metastasis control (Ref.: TRNSC213878VALI)	
Casanova, María	ERANET TRANSCAN-3 LipidMac: Exploiting lipid-laden macrophages to overcome resistance to cancer immunotherapy (Ref.: TRNSC213885CASA)	

HEALTH RESEARCH PROJECTS:		
PRINCIPAL INVESTIGATOR	PROJECT TITLE	
Efeyan, Alejo (Coordinator)	NUTRITHELIUM: Decoding the paracrine control of metabolic fitness by endothelial nutrient signalling (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 tumour suppressor: from mechanisms to therapies (Ref.: HR21-01208)	
Soengas, María S.	METSTOP: Exploiting post-transcriptional regulation to uncover novel vulnerabilities of metastatic cells (Ref.: HR17-00232)	
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-Ledesma, Felipe (Coordinator)	Simple and rapid SARS-CoV-2 diagnostic test by phi29 polymerase amplification (Ref.: CF01-00005)	

”LA CAIXA” BANKING
FOUNDATION / FUNDACIÓN
BANCARIA ”LA CAIXA”



BBVA FOUNDATION /
FUNDACIÓN BBVA



LA MARATÓ DE TV3
FOUNDATION / FUNDACIÓ
LA MARATÓ DE TV3



hna FOUNDATION /
FUNDACIÓN hna



COVID-19 SCIENTIFIC RESEARCH TEAMS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Muñoz, Inés	Synthetic immunology to engineer pan-coronavirus immunity (Ref.: Biomed-COVID-19_0155)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González-Suárez, 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)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Cristina	<i>Estudio farmacogenómico de la toxicidad de Trastuzumab-Deruxtecan en pacientes con cáncer de mama</i>

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 HEALTH⁸

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cascón, Alberto	Molecular, OMIC and functional characterisation of mutations in the gene DLST in patients with pheochromocytoma/paraganglioma (Ref.: PI18/00454)
Fernández, Lucía	Exosomes derived from NKG2D CAR T cells (Exo-NKG2D CAR) as therapeutic strategy to treat paediatric CNS tumours (Ref. PI21/01049)
Guerra-González, Carmen	The stroma as a therapeutic target of pancreatic cancer (Ref.: PI19/00514)
Malats, Nuria	Deciphering the complex relationship between asthma/allergy and pancreatic cancer risk (Ref.: PI21/00495)
Olmeda, David	Anti-bacterial stress pathways in melanoma metastasis and response to therapy (Ref. PI21/00641)
Quintela, Miguel Ángel	Longitudinal, single-cell analysis of immunomodulator/antiangiogenic therapies in advanced breast cancer: a refined tool for precision medicine (Ref.: PI19/00454)
Rodríguez, Sandra	Use of CRISPR/Cas13 system for a programmable diagnosis and inhibition of fusion oncogenes (Ref.: PI20/01837)

8 This Programme is cofunded by the European Regional Development Fund (ERDF), “A way of making Europe”



TECHNOLOGICAL DEVELOPMENT PROJECTS / PROYECTOS DE DESARROLLO TECNOLÓGICO⁹

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Sandra	CRISPR-mediated targeting of amplified oncogenes for Neuroblastoma-directed therapy (Ref.: DTS19/00111)
Malumbres, Marcos	A new platform to predict response to CDK4/6 inhibitors in metastatic breast cancer patients (Ref. DTS21/00132)

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	Centre of Excellence “Severo Ochoa” (Ref.: CEX2019-000891-S)

R&D EXCELLENCE PROJECTS / PROYECTOS DE I+D EXCELENCIA¹⁰

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Leiro, Rafael	CRYOTELO: Structural and molecular characterisation of the shelterin complex (Ref.: PID2020-120258GB-I00)
Llorca, Óscar	Structural and molecular mechanisms regulating the PIKK family of kinases, including DNA- PKcs, SMG1 and mTOR (Ref.: SAF2017-82632-P)

CHALLENGES-RESEARCH PROJECTS / PROYECTOS RETOS-INVESTIGACIÓN¹¹

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	CANTHERHET: Computational targeting of cancer heterogeneity: in silico drug prescription for tumour clonal populations (Ref.: RTI2018-097596-B-I00)
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	RAFTING: c-RAF, a key mediator of K-RAS driven cancers: Therapeutic approaches (Ref.: RTI2018-094664-B-I00)
Barbacid, Mariano	PERSCAN: Personalized medicine in pancreatic cancer (Ref.: PID2021-124106OB-I00)
Blasco, Maria	TELOHEALTH: Telomeres and Disease (Ref.: SAF2017-82623-R)
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	HEPATOCAR: Studying the Role and Function of MCRS1 in Hepatocellular Carcinoma Development (Ref.: RTI2018-094834-B-I00)
Djouder, Nabil	MECHANOCIR: From cirrhosis to hepatocellular carcinoma: a mechanobiology perspective (Ref.: PID2021-122695OB-I00)

9, 10, 11 This Programme is cofunded by the European Regional Development Fund (ERDF), “A way of making Europe”



CHALLENGES-RESEARCH PROJECTS / PROYECTOS RETOS-INVESTIGACIÓN ¹²	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	PhysioTOR: The physiological control of the nutrient-mTOR axis and its deregulation in cancer and aging (Ref.: PID2019-104012RB-I00)
Fernández-Capetillo, Óscar	RESCATE: Mechanisms of resistance to anticancer therapies (Ref.: RTI2018-102204-B-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)
Macintyre, Geoffrey J.	TTCIN: Therapeutic targeting of chromosomal instability in cancer (Ref.: PID2019-111356RA-I00)
Malumbres, Marcos	breastCDKs: Therapeutic evaluation of the Cdk14-18 subfamily in advanced breast cancer (Ref.: PID2021-128726OB-I00)
Méndez, Juan	REP&TOL: Mechanisms of DNA replication and damage tolerance (Ref.: PID2019-106707RB-I00)
Park, Solip	CancerFitness: Systematic analysis of the cancer fitness landscape for cancer genes across cancer types (Ref.: PID2019-109571RA-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.	TF-PDAC Transcription factors in pancreatic cancer: from biology to therapy (Ref.: RTI2018-101071-B-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	RCC-MARKER: Improving the clinical management of advanced renal cell carcinoma through genomic technologies (Ref.: RTI2018-095039-B-I00)
Rodríguez, Cristina	kidneyALT: Molecular alterations of metastatic renal cell carcinoma of clinical significance for antitumor drug response (Ref.: PID2021-128312OB-I00)
Soengas, María S.	MEL-STOP Whole-body imaging of melanoma metastasis as a platform for gene discovery and pharmacological testing (Ref.: SAF2017-89533-R)
Soengas, María S.	MEL_IMAGE_TREAT: Imaging and targeting metastatic niches in melanoma (Ref.: PID2020-117621RB-I00)
Squatrito, Massimo	GLIO-TRK: TRKing down oncogenic genetic rearrangements in gliomas (Ref.: RTI2018-102035-B-I00)
Valiente, Manuel	Stat3 ReACTIVE: Biology of Stat3+ reactive astrocytes in brain metastasis (Ref.: SAF2017-89643-R)

12 This Programme is cofunded by the European Regional Development Fund (ERDF), “A way of making Europe”



SPANISH FOUNDATION FOR SCIENCE AND TECHNOLOGY / FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y TECNOLOGÍA (FECYT)



SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)



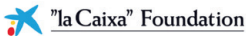
13, 14 Funded by MCIN/AEI/10.13039/501100011033 and the European Union “NextGenerationEU”/PRTR”



”LA CAIXA” BANKING FOUNDATION AND LUZÓN FOUNDATION / FUNDACIÓN BANCARIA ”LA CAIXA” Y FUNDACIÓN LUZÓN



”LA CAIXA” BANKING FOUNDATION / FUNDACIÓN BANCARIA ”LA CAIXA”



CRIS FOUNDATION AGAINST CANCER / FUNDACIÓN CRIS CONTRA EL CÁNCER



SPANISH ASSOCIATION OF PANCREATOLOGY / ASOCIACIÓN ESPAÑOLA DE PANCREATOLOGÍA



GRANTS FOR RESEARCH PROJECTS IN CANCER:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
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)
Real, Francisco X.	STAG2 and FGFR3: cooperation with the DREAM complex in bladder cancer (Ref.: PRYGN223005REAL)

“IDEAS SEMILLA” GRANTS (SEED FUNDING):

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodríguez, Cristina	Bypassing Nonsense Mediated mRNA Decay to enhance immunotherapy response in cancer (Ref.: IDEAS20138RODR)

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)

HEALTH RESEARCH PROGRAMME

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	Targeting Telomeres in Cancer (Ref.: HR18-00023)
Soengas, María S.	Immunomodulatory drivers in melanoma progression and therapy response (Ref.: HR20-00465)

EXCELLENCE GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	Network of myeloid vulnerabilities at metastatic site (Ref.: PR_TPD_2020-09)
Olmos, David	Addressing the biological and clinical role of RB1 loss and DNA repair defects in lethal prostate cancer (Re.: EXCELLENCE 19-26)

CARMEN DELGADO/MIGUEL PÉREZ-MATEO GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Guerra, Carmen	Nueva estrategia terapéutica: estroma e inmunoterapia
Malats, Núria	Marcadores microbianos para el diagnóstico del adenocarcinoma ductal de páncreas

FERO FOUNDATION / FUNDACIÓN FERO



GETNE GROUP / GRUPO GETNE



BBVA FOUNDATION / FUNDACIÓN BBVA



RAMÓN ARECES FOUNDATION / FUNDACIÓN RAMÓN ARECES



GRUPO ESPAÑOL MULTIDISCIPLINAR DE MELANOMA



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	Macrophage-fibroblast cell to cell circuit modulation in NSCLC

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Montero, Cristina	Identificación de marcadores moleculares de respuesta a tratamiento con inhibidores tirosina-quinasa en cáncer (Ref.: G2212)

LEONARDO GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	Nuevos modelos animales de translocaciones a la carta para el desarrollo y estudio linfomas de células B (Ref.: PR[19]_BIO_MET_0029)
Peinado, Héctor	Análisis de la mutación BRAF en exosomas circulantes de pacientes de melanoma (Ref.: IN[21]_BBM_TRA_0050)

SCIENTIFIC RESEARCH TEAMS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Mulero, Francisca	TherAbnostic: Radioimmunotheranostics for metastatic lung cancer with pretargeted clickable Ab fragments (Ref.: PR[19]_BIO_IMG_0096)
Djouder, Nabil	IMMUNO-KETOCAR: The cholinergic immunosurveillance of ketogenic diet in the treatment of hepatocellular carcinoma (Ref.: EIC21-I-243)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Eva	Red Nacional de Metástasis Cerebral: Implantación, Desarrollo y Coordinación (Ref.: CIVP20SI0662)
Peinado, Héctor	Developing a targeted therapy to promote melanoma immune-recognition and suppress metastasis
Valiente, Manuel	Reactive astrocytes as a therapeutic target in brain metastasis

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 are fundamental to guarantee the training of new researchers and the continuity of high-level research projects.

During 2022, 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 and Innovation (*AEI/MCIN*), 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 2022, the CNIO signed several new agreements with Spanish Universities and other institutions, namely with the *Universidad de Santiago de Compostela*, *Erasmus University Medical Center Rotterdam*, *Universidad Camilo José Cela*, *Universidad Pablo de Olavide*, *IES Ribera del Tajo*, *IES Siglo XXI*, *IES Maria Inmaculada*, and *IES Corredor del Henares*.

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2018	2019	2020	2021	2022
Training of PhD students	109	100	109	135	130
Post-doctoral training	50	49	52	62	55
Training for MDs	12	20	7	10	12
Laboratory training for MSc/BSc students	128	150	85	106	136
Laboratory training for technicians	13	15	5	9	17

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 2022, 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, 136 students participated in these programmes, of whom 4 ended up joining the CNIO as pre-doctoral students.

TRAINING OF PHD STUDENTS

The training of PhD students in cutting-edge cancer research is of key importance to the CNIO. The Centre offers many opportunities for bright and dynamic university graduates, of all nationalities, to pursue an ambitious PhD project. To attest this, 16 students obtained their PhD degrees in 2022 and 26 others joined the CNIO in the same year. Over 15% of the students working at the CNIO in 2022 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 2022, 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 2022 was as follows: 68 % of students worked in the Molecular Oncology Programme, 12% in the Structural Biology Programme, 12% in the Human Cancer Genetics Programme, 2% in the Experimental Therapeutics Programme, 2% in the Biotechnology Programme, and 4% in the Clinical Research Programme.

FUNDING OF PHD TRAINING	NO.
SPANISH ORGANISATIONS	106
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science and Innovation / <i>Ministerio de Ciencia e Innovación</i> (Predoctoral Fellowships)	50
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science and Innovation / <i>Ministerio de Ciencia e Innovación</i> (I+D Projects)	8
Spanish Association Against Cancer (<i>AECC</i>) / <i>Fundación Científica de la AECC</i> (I+D Projects)	6
Spanish Association Against Cancer (<i>AECC</i>) / <i>Fundación Científica de la AECC</i> (Fellowships)	4
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i> (Fellowship)	1
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i> (I+D Projects)	2
Comillas University / <i>Univesidad de Comillas</i>	1
<i>FERO</i> Foundation / <i>Fundación FERO</i>	1
<i>Cris</i> Foundation / <i>Fundación Cris</i>	3
Community of Madrid / <i>Comunidad de Madrid</i>	11
Bionam Biotech <i>AIE</i>	3
CNIO	7
“la Caixa” Banking Foundation/ <i>Fundación Bancaria</i> “la Caixa” (I+D Projects)	2
“la Caixa” Banking Foundation/ <i>Fundación Bancaria</i> “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 2022, 55 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 seventh call of the ‘CNIO Friends’ Postdoctoral Contract Programme, launched in 2022, resulted in the recruitment of 9 scientists for a 2-year period each.

FUNDING SOURCES OF POST-DOCTORAL CONTRACTS	NO.
SPANISH ORGANISATIONS	39
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science and Innovation / <i>Ministerio de Ciencia e Innovación</i> (Postdoctoral Fellowships)	3
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science and Innovation / <i>Ministerio de Ciencia e Innovación</i> (I+D Projects)	3
Spanish Association Against Cancer (<i>AECC</i>) / <i>Fundación Científica de la AECC</i> (Fellowships)	4
“la Caixa” Banking Foundation / <i>Fundación Bancaria</i> “la Caixa” (Postdoctoral Junior Leader- INCOMING)	1
“la Caixa” Banking Foundation / <i>Fundación Bancaria</i> “la Caixa” (I+D Projects)	3
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i>	1
<i>Cris</i> Cancer Foundation (<i>CRIS</i>) / <i>Fundación Cris Contra el Cáncer (CRIS)</i>	1
Community of Madrid / <i>Comunidad de Madrid</i>	3
CNIO	18
<i>Banco Santander</i> Foundation / <i>Fundación Banco Santander</i>	1
<i>BBVA</i> Foundation / <i>Fundación BBVA</i>	1
INTERNATIONAL ORGANISATIONS	16
AIRC	1
European Commission	1
European Research Council	7
Janssen	1
Marie Skłodowska-Curie actions of the European Commission	4
ESMO	1
Worldwide Cancer Research UK	1
TOTAL	55

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-ISCHII*).



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*.



Dianas Terapéuticas
en Señalización Celular
Investigación y Desarrollo

Máster Oficial

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. Of the 17 students who participated in this programme in 2022, 1 was hired by 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 2022, 12 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 2022, the *Ramón y Cajal* Programme supported 6 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 and Innovation) 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. Fourteen other scientists are funded by similar programmes, including the *Juan de la Cierva* programme (Spanish Ministry of Science and Innovation, 4 contracts); the *Miguel Servet* programme (1 contract) of the Institute of Health *Carlos III*; and the Spanish Association Against Cancer (*AECC*, 9 contracts).

VISITING RESEARCHERS PROGRAMME

The *Jesús Serra* Foundation, part of the *Catalana Occidente* Group, 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 2022, Eva Nogales, from UC Berkeley (USA), and Gonçalo Bernardes, from the University of Cambridge (UK),



were beneficiaries 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 Alexandria University, Sudan, was awarded a grant to join the CNIO’s Genetic and Molecular Epidemiology Group for a 6-month stay as a visiting scientist.



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 latest development 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.

MOLECULAR, CELLULAR AND ORGANISMAL DRIVERS OF AGING
9-10 MAY 2022

- ORGANISERS:
- Maria A. Blasco, CNIO, Spain
 - Alejo Efeyan, CNIO, Spain
 - Thomas Rando, Stanford University, US

- SESSIONS:
- Senescence and Regeneration
 - Nutrients and Metabolism
 - Epigenetics and Genome Stability



In 2022, we arranged 2 CFM: 1) **Molecular, Cellular and Organismal Drivers of Aging**. Our perception of aging has shifted from an inevitable pace of overall functional decline to a biological process amenable to genetic and pharmacological manipulation, with potential health-promoting interventions against aging-related diseases. The scope of this conference was to get an integrative view of aging, by putting together different fields and disciplines working on different aspects of research on aging. 2) **Diet, Nutrition and Cancer Cell Metabolism**. Recent progress was presented during the conference to provide a forum for discussions on nutrient-sensing pathways and mechanisms, their effect in cellular function and tissue homeostasis, and the impact of their dysregulation in cancer development and metabolic disorders. Both conferences brought together more than 130 cancer experts from the world’s most active groups in the area.

DIET, NUTRITION AND CANCER CELL METABOLISM
24-26 OCTOBER 2022

- ORGANISERS:
- Nabil Djouder, CNIO, Spain
 - Nikla Emambokus, Cell Press, US
 - M. Carmen Fernández-Agüera, Cell Press, Spain
 - Valter Longo, IFOM, Italy
 - Marcos Malumbres, CNIO, Spain

- SESSIONS:
- Metabolic Pathways and Cancer
 - Metabolic Pathways and Disease
 - Nutrition, Epidemiology and Disease
 - Diet, Nutrition and Metabolic Disease



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
17-18 JANUARY 2022
21-22 FEBRUARY 2022
4-5 APRIL 2022
23-24 MAY 2022
27-28 JUNE 2022
12-13 SEPTEMBER 2022
24-25 OCTOBER 2022
28-29 NOVEMBER 2022

- SPEAKERS:
- Sara García García, Flow Cytometry Unit Technician. CNIO
 - Julia García-Lestón, Flow Cytometry Unit Technician. CNIO
 - Lola Martinez, Head of the Flow Cytometry Unit. CNIO
 - Andrea Valle, European Applications Specialist DeNovo Software

MULTICOLOR FLOW CYTOMETRY COURSE
14-15 MARCH 2022
3-4 OCTOBER 2022

- SPEAKERS:
- Sara García García, Flow Cytometry Unit Technician. CNIO
 - Julia García Lestón, Flow Cytometry Unit Staff. CNIO
 - Jose Manuel Ligos, Technical Application Specialist. Cytek Biosciences
 - Lola Martinez, Head of the Flow Cytometry Unit. CNIO
 - Ana Ilie, Flow Cytometry Unit Technician. CNIO
 - Andrea Valle, European Application Specialist. DeNovo Software

FULL SPECTRAL FLOW ANALYSIS TOOLS WITH FCS EXPRESS 7 FOR USERS
21 MARCH 2022

- ORGANISERS:
- Lola Martinez, Head of the Flow Cytometry Unit. CNIO
 - Andrea Valle, European Applications Specialist DeNovo Software

IV CNIO-BANCO SABADELL FOUNDATION WORKSHOP ON PHILOSOPHY AND SCIENCE: THE LONG-TERM VIEW
28 NOVEMBER 2022



WITH THE SUPPORT OF:
Sabadell Foundation

- ORGANISERS:
- **Maria A. Blasco** (CNIO)
 - **Antonio Diéguez** (UMA)
 - **Arantza Etxeberria** (UPV/EHU)

- SPEAKERS:
- **Irene Gómez Franco**, Autonomous University of Barcelona, Spain
 - **Manuel González Bedia**, Biomedical Engineering Information and Communication Technology, University of Zaragoza, Spain
 - **David Nogués Bravo**, Center for Macroecology, Evolution and Climate GLOBE Institute, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
 - **Idoia Salazar García**, Journalist, Social Impact of Artificial Intelligence and Robotics Observatory, CEU Spain
 - **Anders Sandberg**, Future of Humanity Institute, Oxford University, UK

- SESSIONS:
- The Future of Planet Earth
 - The Future of Humanity
 - Our Future with AI

PATHOLOGY OF THE EXOCRINE PANCREAS - OF MEN AND MICE
A 3-DAY “MEET THE EXPERT” INTERNATIONAL COURSE
1-3 DECEMBER 2022

- ORGANISERS:
- **CNIO and CIBERONC**
 - **Nuria Malats**, Genetic and Molecular Epidemiology Group, CNIO
 - **Francisco X. Real**, Epithelial Carcinogenesis Group, CNIO

CNIO - WEIZMANN INSTITUTE OF SCIENCE JOINT SYMPOSIUM
NEW INSIGHTS IN CANCER DISCOVERY
7-8 MARCH 2022

- ORGANISERS:
- **CNIO-Weizmann Institute of Science-*Ramón Areces* Foundation**

- SPEAKERS:
- **Maria A. Blasco**, Spanish National Cancer Research Centre, CNIO
 - **Emilio Bouza**, Chairman of the *Ramón Areces* Foundation scientific committee
 - **Maria Casanova**, Cancer and Immunity Junior Group Leader (CNIO)
 - **Felipe Cortés**, Topology and DNA Breaks Group Leader (CNIO)
 - **Ayelet Erez**, Weizmann Institute of Science (WIS)
 - **Rafael Fernández-Leiro**, Genome Integrity and Structural Biology Junior Group Leader (CNIO)
 - **Tamar Geiger**, Weizmann Institute of Science (WIS)
 - **Leeat Keren**, Weizmann Institute of Science (WIS)
 - **Ana Losada**, Chromosome Dynamics Group Leader (CNIO)
 - **Geoff Macintyre**, Computational Oncology Junior Group Leader (CNIO)
 - **Juan Méndez**, DNA Replication Group Leader (CNIO)
 - **Moshe Oren**, Weizmann Institute of Science (WIS)
 - **Raimundo Pérez-Hernández y Torra**, Director of the *Ramón Areces* Foundation
 - **Ruth Scherz-Shouval**, Weizmann Institute of Science (WIS)
 - **Ofer Shoshani**, Weizmann Institute of Science (WIS)

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 2022.



DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
21/01/2022	Christoph Bock	CeMM Research Center for Molecular Medicine (ÖAW); Institute of Artificial Intelligence, CeMSIIS, Medical University of Vienna, Austria	Looking into the past and future of cells. Single-cell sequencing and computational modeling of epigenetic cell states in immunology and cancer
28/01/2022	Rebecca Fitzgerald	University of Cambridge, UK	Is pre-cancer the key to improving cancer outcomes: challenges, opportunities and lessons from the oesophagus
FEBRUARY			
04/02/2022	Madeline A. Lancaster	MRC Laboratory of Molecular Biology, Cambridge, UK	Exploring human brain size determination in cerebral organoids
25/02/2022	Monica Bettencourt-Dias	The <i>Instituto Gulbenkian de Ciência</i> , Portugal	Centrosomes and Cilia in Development and Disease
MARCH			
04/03/2022	Johanna Joyce	University of Lausanne; Ludwig Institute for Cancer Research, Lausanne, Switzerland	Exploring and Therapeutically Exploiting the Brain Tumor Microenvironment
11/03/2022	Ben Lehner	Centre for Genomic Regulation (CRG), Barcelona, Spain	Mutate Everything
MAY			
06/05/2022	Meritxell Huch	Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany	Adult tissue derived organoid cultures and their application to the understanding of tissue regeneration and cancer
20/05/2022	Julio Aguirre-Ghiso	Cancer Dormancy and Tumor Microenvironment Institute, Gruss Lipper Biophotonics Center, Einstein College of Medicine, New York, US	Disseminated cancer cell dormancy: a homeostatic seed and soil partnership
27/05/2022	John Briggs	Max Planck Institute of Biochemistry, Martinsried, Germany	In situ structural biology of enveloped viruses by cryo-electron tomography
JUNE			
03/06/2022	Gonçalo J. L. Bernardes	Yusuf Hamied Department of Chemistry - University of Cambridge, UK	Translational Chemical Biology
SEPTEMBER			
09/09/2022	Jan Paul Medema	Amsterdam UMC, Cancer Center Amsterdam, The Netherlands	The role of BCL2 family members in intestinal cancer
NOVEMBER			
04/11/2022	Elisabete Weiderpass	International Agency for Research on Cancer (IARC), World Health Organization, Lyon, France	IARC's mission: cancer research for cancer prevention
25/11/2022	Andrea Schietinger	Memorial Sloan Kettering Cancer Center, New York, USA	T cell fate decisions in cancer and autoimmunity

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 26 *ad-hoc* seminars were organised by CNIO researchers in 2022.

DATE	SPEAKER	ORGANISATION	TITLE
FEBRUARY			
08/02/2022	Cristina Viéitez	European Molecular Biology Laboratory (EMBL), Heidelberg, Germany	High-throughput functional characterisation of protein phosphorylations sites: lessons from yeast to human
09/02/2022	Ashley Laughney	Meyer Cancer Center at Weill Cornell Medicine, New York, US	Systems analysis of tumor-microenvironment crosstalk induced by chromosomal instability
11/02/2022	Teresa Guillamón Vivancos	<i>Instituto de Neurociencias de Alicante</i> , Spain	The role of perinatal spontaneous activity in the formation of sensory circuits
22/02/2022	Elena Ortiz-Zapater	University of Valencia, Spain	Epithelial Coxsackievirus Adenovirus Receptor (CAR) promotes house dust mite-induced lung inflammation
MARCH			
10/03/2022	Carlos Pardo Pastor	HFSP & MSCA Research Associate, Jody Rosenblatt Group, Randall Centre for Cell & Molecular Biophysics, King's College London, UK	Piezo1-EGFR interplay: feeling the pressure to grow up
24/03/2022	Margherita Botto	Leiden University Medical Center, The Netherlands	A four-point molecular handover during Okazaki fragments maturation
31/03/2022	José María Pereda	Cellular Biology of Cancer (IBMCC), Salamanca, Spain	Mechanisms of autoregulation and activation of C3G, an activator of the GTPase Rap1
APRIL			
07/04/2022	Daniel Muñoz-Espin	University of Cambridge, Hutchison/MRC Research Centre, Cambridge, UK	Impact of Cellular Senescence in Lung Cancer: Novel Cancer Preventative and Therapeutic Opportunities
19/04/2022	Jorge Moscat	Weill Cornell Medical College, New York, US	Signaling networks and therapeutic vulnerabilities in mesenchymal tumorigenesis
28/04/2022	Christine Moussion	Cancer Immunology Genentech, South San Francisco, US	Deciphering the spatiotemporal control of tumor Immune phenotypes to improve T cell infiltration
MAY			
05/05/2022	Laure Bindels	UCLouvain, Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Brussels, Belgium	Exploring the gut microbiome and its metabolites to tackle cancer cachexia
26/05/2022	Adam Antebi	Max Planck Institute for Biology of Ageing, Cologne, Germany	Nucleolar regulation of longevity

DATE	SPEAKER	ORGANISATION	TITLE
JUNE			
09/06/2022	Maria Robles	LMU Systems Chronobiology Biomedical Center & Institute of Medical Psychology, Munich, Germany	Proteomics approaches to understand circadian biology (EMBO YIP seminar)
14/06/2022	Juan Manuel Povedano Selfa	UT Southwestern Medical Center, Dallas, Texas, USA	Finding the needle in the haystack - target identification of anti-cancer small molecules
27/06/2022	Mao Mao	Founder and Chief Executive Officer SeekIn Inc., Shenzhen, China	NGS and fragmentomics liquid biopsy based-test for multiple cancer early detection
SEPTEMBER			
07/09/2022	Geoff Kraker	Senior Application Scientist, Dotmatics	OMIQ Seminar
20/09/2022	Guillermo Montoya	Protein Structure and Function Programme, Novo Nordisk Foundation Center for Protein Research. University of Copenhagen. Denmark	Understanding protein dynamics and its allosteric mechanisms using cryo-EM: Examples in genome editing
27/09/2022	Justo P. Castaño Fuentes	University of Cordoba (UCO), Spain	Splicing Dysregulation as an Emerging Cancer Hallmark: Insights from Neuroendocrine Tumors and Pancreatic Cancer
29/09/2022	Alessandro Gandelli	Technical Specialist. Antibodies and Immunoassay. Thermo Fisher Scientific	Push the limits of cell analysis - tools to optimise multicolor flow panel design
OCTOBER			
03/10/2022	Leticia Serrano and Raquel Bodoque	National Hospital of Paraplegics at Toledo and General Hospital University of Ciudad Real, Spain	Pancreatic cancer: Study of the effects of Hyperthermic Intraabdominal Chemotherapy (HIPEC) using Gemcitabine and new approaches against pancreatic cancer stem cells (CSCs)
21/10/2022	Josep V. Forment	AstraZeneca, Cambridge, UK	Targeting the DNA-damage response in cancer treatment
27/10/2022	Luisa Escobar Hoyos	Yale School of Medicine, New Haven, US	Altered RNA splicing a driver event in pancreatic cancer
27/10/2022	Nikla Emambokus	Editor of Med (Cell Press), US	Medical publishing at Cell Press
NOVEMBER			
11/11/2022	Raquel Matos	Scientific Support Manager EMEA, Corning Life Sciences, Amsterdam, The Netherlands	3D cell culture, technologies as disease models
30/11/2022	Sara García Alonso	Experimental Oncology Group Molecular Oncology Programme	How to become an astronaut
DECEMBER			
13/12/2022	Rodrigo Bermejo Moreno	DNA replication and Genome Integrity, CIB, Madrid, Spain	Functional architecture of replication fork protection

WOMEN IN SCIENCE SEMINARS

In 2022, the WISE Office invited and welcomed several top female and male leaders from different areas to tell us about their career path experiences.

DATE	SPEAKER	ORGANISATION	TITLE
18/01/2022	María Folguera	Artistic director of the <i>Teatro Circo Price</i> and writer	Marta and María have to walk together. Live between the action and the clouds
17/02/2022	Fe López	Presenter and TV writer of RTVE	Journalism, women and sports
01/03/2022	Gabriela Wiener	Writer and journalist	<i>Espejos descoloniales</i>
08/03/2022	International Women's Day: A celebration of Dr Jane Goodall's Life	CNIO and British Embassy Madrid	A celebration of Dr Jane Goodall's Life
24/05/2022	Paloma Chen	Journalist and writer	Reflections from a Chinese-Spanish experience on identity
20/09/2022	Eva Orúe	Director of the Madrid Book Fair	Madrid Book Fair: apocalyptic and integrated meet at <i>El Retiro</i>
27/10/2022	Irene Cano	<i>Meta Iberia</i> Director	An interview with Irene Cano, General Director of Meta Iberia, carried out by our Director, María A. Blasco
15/11/2022	Marina Echevarría	Professor of Commercial Law and activist for LGBTI Rights	Challenges of Law in the face of technology
13/12/2022	Cristina Aranda Gutiérrez	Co-founder & Chief Business Development Officer, Big Onion	Diversity, the main lever for innovation

SCIENCE DISSEMINATION EVENTS

WORLD CANCER RESEARCH DAY
“**INMUNOTERAPIA: LOGROS Y RETOS DEL ÚLTIMO GRAN AVANCE CONTRA EL CÁNCER**”
22 SEPTEMBER 2022



ORGANISER:
• **The Spanish National Cancer Research Centre (CNIO)**

WITH THE SUPPORT OF:
• **“la Caixa” Foundation**

KEYNOTE LECTURES:
• **“Impacto actual de la inmunoterapia en los pacientes, y vías de mejora”**
Luis Paz-Ares, Head of the H12O-CNIO Lung Cancer Clinical Research Unit
• **“La inmunoterapia en el cáncer pediátrico”**
Antonio Pérez-Martínez, Head of Childhood Blood Cancers at *La Paz* Hospital in Madrid

ROUND TABLE:
• **Maria A. Blasco**, Director of the Spanish National Cancer Research Centre (CNIO)
• **Miguel Calero**, Assistant Director General for Applied Services, Training and Research at the *Carlos III* Health Institute
• **Luis Paz-Ares**, Head of the H12O-CNIO Lung Cancer Clinical Research Unit
• **Antonio Pérez-Martínez**, Head of Childhood Blood Cancers at *La Paz* Hospital in Madrid
• **José Ruiz**, Mayor of the Town of *Torreperogil (Jaén)* – CNIO Friend Institution

MODERATOR
• **Cristina Villanueva**, Journalist

OPEN DOORS DAY: EVERYONE UNRAVELLING CANCER
15 NOVEMBER 2022



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 (XXII *Semana de la Ciencia y de la Innovación*, 7-20 November 2022).

In November 2022, 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 43 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.

EUROPEAN RESEARCHERS’ NIGHT 2022
CNIO: CONOCE A LOS CIENTÍFICOS, SÉ UN CIENTÍFICO
CNIO: MEET THE SCIENTISTS, BECOME A SCIENTIST
30 SEPTEMBER 2022

The Centre opened its doors to the public on September 30 to show its commitment to society and to promote scientific culture.

Between 5 p.m. and 11 p.m., 4 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 212 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 Skłodowska-Curie Action (MSCA) and takes place at hundreds of European institutions across many cities and countries. The 2022 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.



ADMINISTRATION

BOARD OF TRUSTEES

→ Honorary President

- **Diana Morant Ripoli**
Minister of Science and Innovation
Ministra de Ciencia e Innovación

→ President

- **Raquel Yotti Álvarez**
Secretary General for Research of the Spanish Ministry of Science and Innovation
Secretaria General de Investigación del Ministerio de Ciencia e Innovación

→ Vice-President

- **Cristóbal Belda Iniesta**
Director of the National Institute of Health Carlos III
Director del Instituto de Salud Carlos III

→ Appointed Members

- **Silvia Calzón Fernández**
Secretary of State for Health, Spanish Ministry of Health
Secretaria de Estado de Sanidad, Ministerio de Sanidad
- **Eloísa del Pino Matute**
President of the Spanish National Research Council (CSIC)
Presidenta del Consejo Superior de Investigaciones Científicas (CSIC)
- **José Alarcón Hernández**
Director of the Public Policy Department, Cabinet of the Presidency of the Government
Director/a del Departamento de Políticas Públicas del Gabinete de la Presidencia del Gobierno
- **Agustín González González**
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III
Subdirector General de Redes y Centros de Investigación Cooperativa del Instituto de Salud Carlos III
- **Juan Cruz Cigudosa García**
Advisor of University, Innovation and Digital Transformation of the Government of Navarre
Consejero de Universidad, Innovación y Transformación Digital del Gobierno de Navarra
- **Carlos Pesquera González**
Head of Cabinet of the Healthcare Counsellor of the Government of Cantabria
Jefe de Gabinete de la Consejera de Sanidad del Gobierno de Cantabria
- **Sandra García Armesto**
Managing Director of the Aragon Institute of Health Sciences
Directora Gerente del Instituto Aragonés de Ciencias de la Salud

- **Antonio Fernández-Campa García-Bernardo**
Director of the Galician Health Knowledge Agency - ACIS
Director de la Agencia Gallega del Conocimiento en Salud-ACIS
- A Representative of the Science, Technology and Innovation Advisory Council appointed by the plenary session of this Council
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
- **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

→ Secretary

- **Agustín González González**
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III
Subdirector General de Redes y Centros de Investigación Cooperativa del Instituto de Salud Carlos III

→ Legal Advisor

- **Fernando Arenas Escribano**
Chief State’s Attorney of the Spanish Ministry of Health
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 €293,667 in 2022 (€252,708 in 2021). This amount was received as base salary and position salary supplements: €228,938 (€226,896 in 2021); variable remuneration: €56,716 accrued during 2020 (€23,770 in 2021); 3.5% increase: €8,012.
— Members of the CNIO Board of Trustees are not remunerated.

SCIENTIFIC ADVISORY BOARD

- **Mariann Bienz, PhD, FRS, FMedSci (Chair)**
Deputy Director, MRC Laboratory of Molecular Biology
Group Leader, LMB Division of Protein and Nucleic Acid Chemistry
MRC Laboratory of Molecular Biology
Cambridge, United Kingdom
- **Genevieve Almouzni, PhD**
Director of Research Exceptional Class, *Centre National de la Recherche Scientifique*
Honorary Director of the Curie Institute Research Center
Team Leader of Chromatin Dynamics, Nuclear Dynamics Unit
Institut Curie, Paris, France
- **José Costa, MD, FACP**
Professor Emeritus of Pathology and Senior Research Scientist
Yale University School of Medicine
New Haven, USA
- **John Diffley, PhD**
Senior Group Leader - Associate Research Director
The Francis Crick Institute
London, United Kingdom

- **Rosalind Eeles, FMedSci, PhD, FRCP, FRCR,**
Professor of Oncogenetics, the Institute of Cancer Research
Honorary Consultant in Clinical Oncology and Oncogenetics
The Royal Marsden NHS Foundation Trust
Sutton, United Kingdom
- **Denise Galloway, PhD**
Scientific Director, Pathogen-Associated Malignancies Integrated Research Center
Professor, Human Biology & Public Health Sciences Divisions
Paul Stephanus Memorial Endowed Chair
Fred Hutchinson Cancer Research Center
Seattle, USA
- **E. Yvonne Jones, FRS, FMedSci**
Joint Head of the Division of Structural Biology and Deputy Head of the Nuffield Dept. of Medicine
Head of the Cancer Research UK Receptor Structure Research Group
Wellcome Trust Centre for Human Genetics
University of Oxford
Oxford, United Kingdom

- **Scott W. Lowe, PhD**
Chair, Cancer Biology and Genetics Program, SKI
Chair, Geoffrey Beene Cancer Research Center
Investigator, Howard Hughes Medical Institute
Memorial Sloan-Kettering Cancer Center
New York, USA
- **Ángela Nieto, PhD**
Full Professor and Head of Cell Plasticity in Developmental and Disease Group
Neuroscience Institute of Alicante (*CSIC-UMH*)
Alicante, Spain
- **Andre Nussenzweig, PhD**
Chief, Laboratory of Genome Integrity
NIH Distinguished Investigator, Head of the Molecular Recombination Section
Center for Cancer Research, National Cancer Institute
Bethesda, USA
- **Daniela Rhodes, PhD, FRS**
Emeritus Research Leader, MRC Laboratory of Molecular Biology (Cambridge, UK)
Professor, School of Biological Sciences and School of Chemical and Biomedical Engineering
Director Emeritus, NTU Institute of Structural Biology
Nanyang Technological University
Singapore
- **Josep Tabernero, MD PhD**
Director, Vall d’Hebron Institute of Oncology (VHIO)
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

DIRECTOR	Blasco, Maria A.		
	SECRETARIATE	Alcamí, María Jesús	
VICE-DIRECTOR	Fernández-Capetillo, Óscar		
DIRECTOR'S OFFICE	Peláez, Fernando Director		
DEVELOPMENT AND PHILANTHROPY	Rose, Jessica J. Head	Antona, M. Mercedes	
INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY	Garrido, Amparo Director (until March)	Calderón, Paula	
COMMUNICATIONS	Salomone, Mónica Director (since July)	Perez de Pablos, Susana Head Noriega, Nuria Head (until March)	
	SCIENCE COMMUNICATION AND SOCIAL MEDIA	Santamaría, Rebeca Head (until September)	
INNOVATION	Oruezabal, Roke I. Director		
	TECHNOLOGY TRANSFER & VALORISATION OFFICE	Herrera, Irene Head Pino, Irene Head	
SCIENTIFIC MANAGEMENT	Barthelemy, Isabel Director		
	PROJECTS & CONSORTIA	Liébanes, M. Dolores Head	Vergés, Leyre
	EDUCATION & TRAINING PROGRAMMES	Zamora, Helena Head	del Codo, Almudena
	SCIENTIFIC EVENTS	Moro, Mercedes Head	
	SCIENTIFIC PUBLISHING	Cerdá, Sonia	
	LIBRARY & ARCHIVES	López, Victoria	
	SECRETARIATE (COMMUNICATIONS, INNOVATION, SCIENTIFIC MANAGEMENT, DEVELOPMENT AND PHILANTHROPY)	Rodríguez, M. Carmen	

MANAGING DIRECTOR

Arroyo, Juan

SECRETARIATE

Ámez, María del Mar

Muñoz, Laura

MANAGING DIRECTOR'S OFFICE

Fernández, José Ignacio
Attached to the Managing Director's Cabinet & Legal Advisor

SAP

Ferrer, Alfonso Head

Millán, Judith
Tejedor, Ignacio

Pérez, Fernando D.
Guereca, Javier Ignacio

FINANCE & ADMINISTRATION

Fontaneda, Manuela Director

PURCHASING & LOGISTICS

Hernández, Jesús Director
Álamo, Pedro Head (until February)

Baviano, Marta
Novillo, Angélica
Fernández, M. Carmen
Zárate, M. Loreto

LOGISTICS

Corredor, Yimy
Echeverria, Pedro
Larena, Gema

Ortega, Carlos
Sánchez, William
Verde, Begoña

HUMAN RESOURCES

Pérez, José Lorenzo Head

Bardají, Paz
Carbonel, David

ECONOMIC MANAGEMENT

Salido, M. Isabel Head
Galindo, José Antonio

García, Juan J.
Rodríguez, M. José

AUDIT

García-Risco, Silvia Head

Moreno, Ana María Head
Hernando, M. Elena
Fuentes, Claudia

INFRASTRUCTURE MANAGEMENT

de Dios, Luis Javier Director

MAINTENANCE

Aguilera, Alejandro
Alonso, Antonio
Alonso, Jesús M
Copado, José Antonio
Cristobal, Herman
Damián, Emilio

López, Jesús
Moreno, José
Paucar, Edgar
Pedrajas, Miguel
Pop, Andrei
Reviejo, Mariano

Rivas, José

Romero, Vicent
Sánchez, Alejandro
Serrano, Alberto
Vázquez, Manuel
Yague del Ejido, Alberto

PREVENTION & BIOSECURITY

Cespón, Constantino Head

Bertol, Narciso
Giménez, Andrés J.
Gómez, Víctor

Mingo, Emilio
Oset, Francisco J.
Tendero, María

INFORMATION TECHNOLOGIES

Fernández, José Luis Head

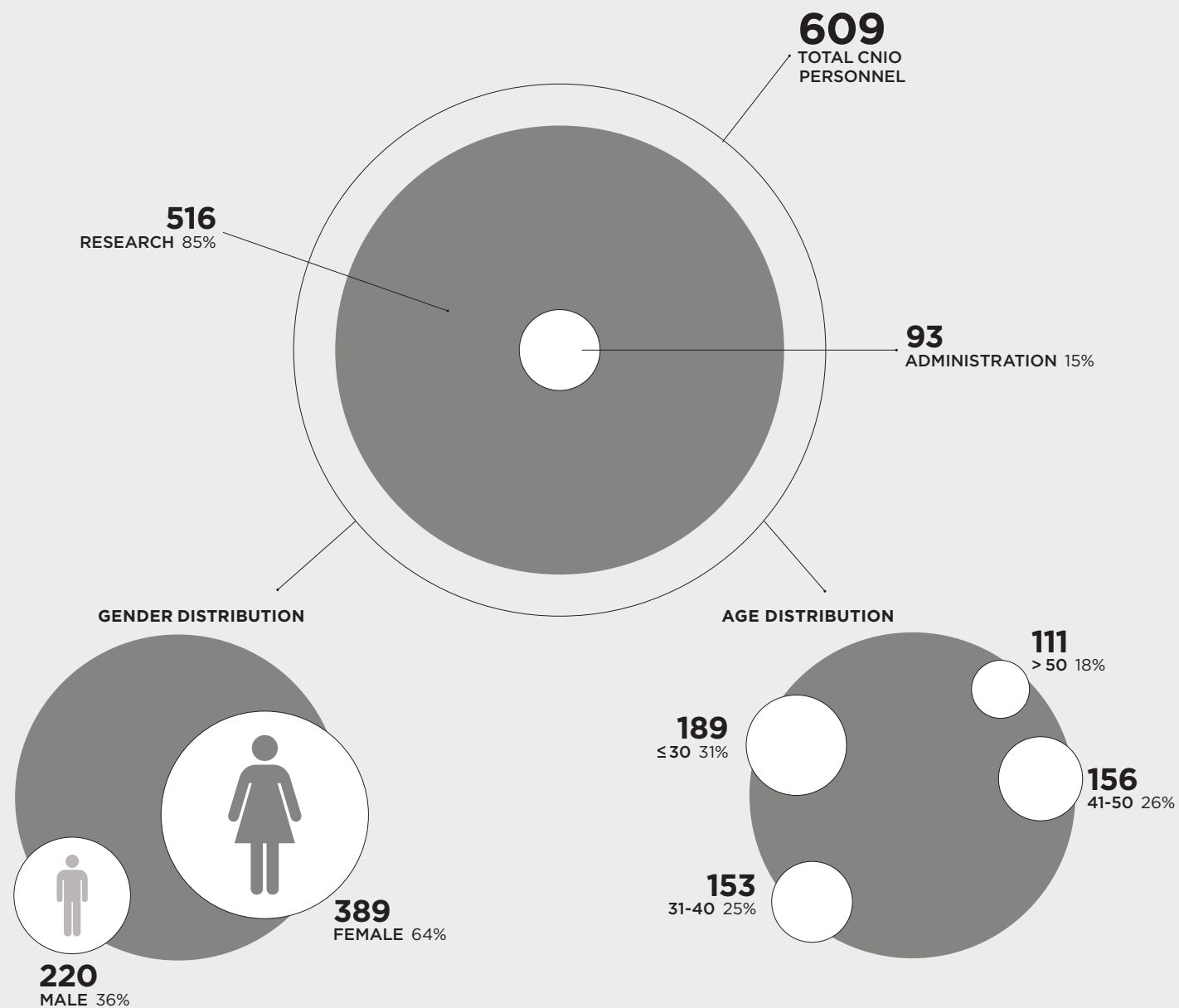
de Miguel, Marcos
García, Jose Manuel
Hernández, Julio

Martín, Félix
Martínez, Rubén
Reviriego, Pablo







EXTRAMURAL CLINICAL RESEARCH

López, Antonio Director

CNIO PERSONNEL 2022



GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

Category	Gender	Percentage	Count	Visual Representation
GROUP LEADERS, HEADS OF UNIT/SECTION	FEMALE	53%	25	
	MALE	47%	22	
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	FEMALE	60%	9	
	MALE	40%	6	
MANAGEMENT: DIRECTORS, HEADS OF AREA	FEMALE	31%	4	
	MALE	69%	9	




SCIENTIFIC PERSONNEL 2022

TOTAL SCIENTIFIC PERSONNEL **516**

DISTRIBUTION BY PROGRAMMES

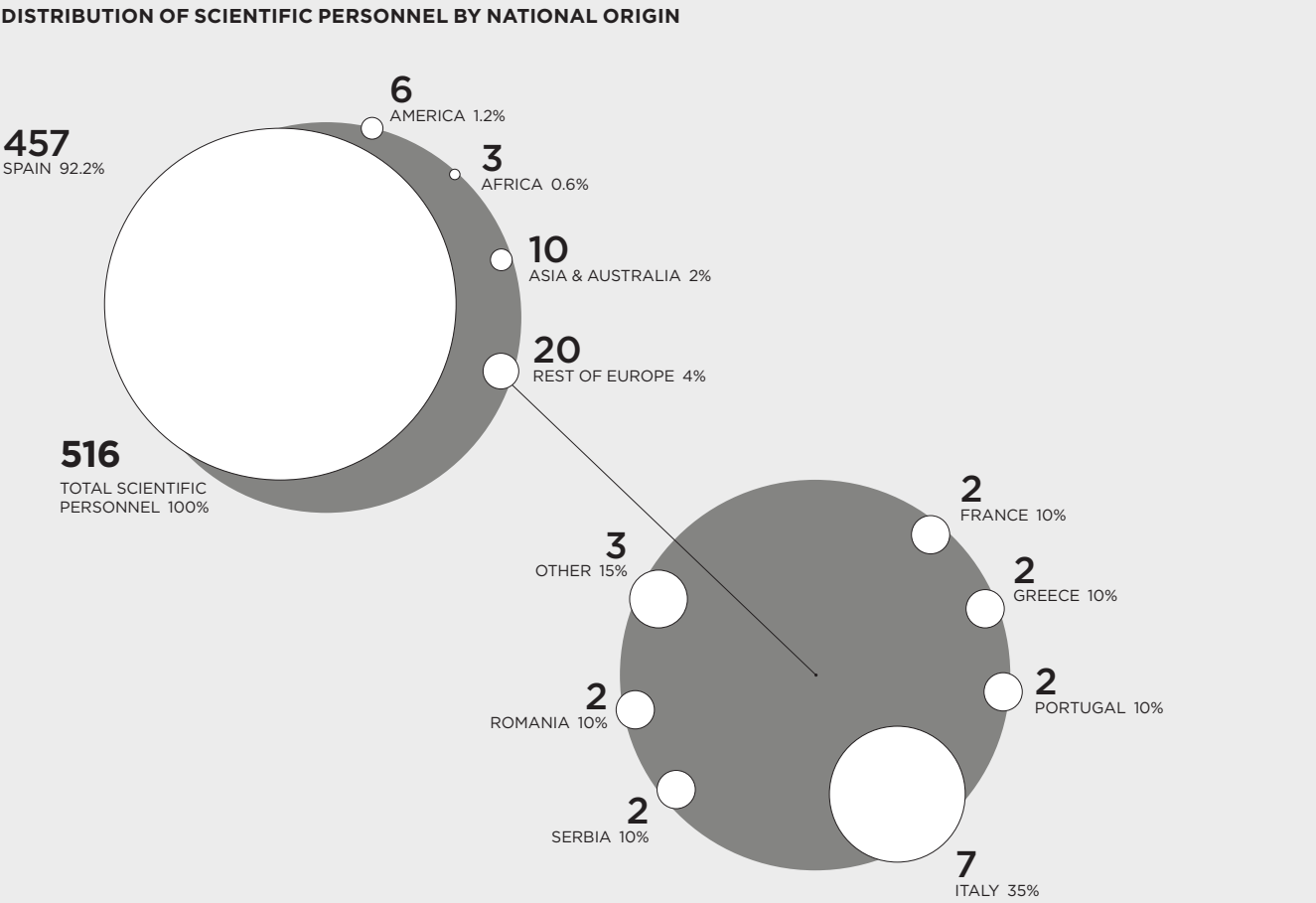
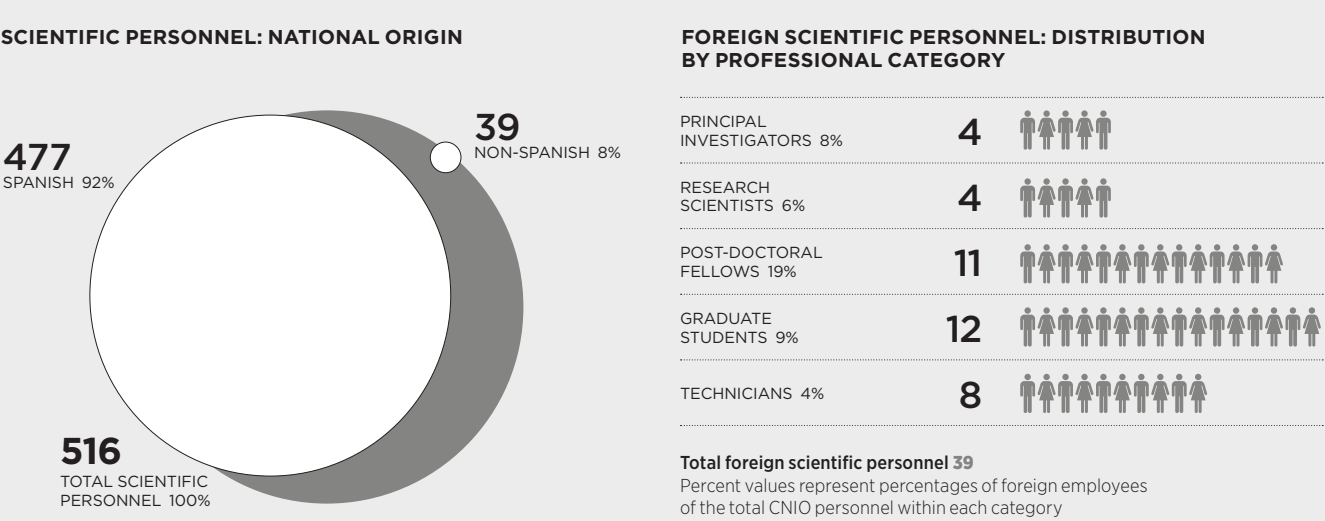
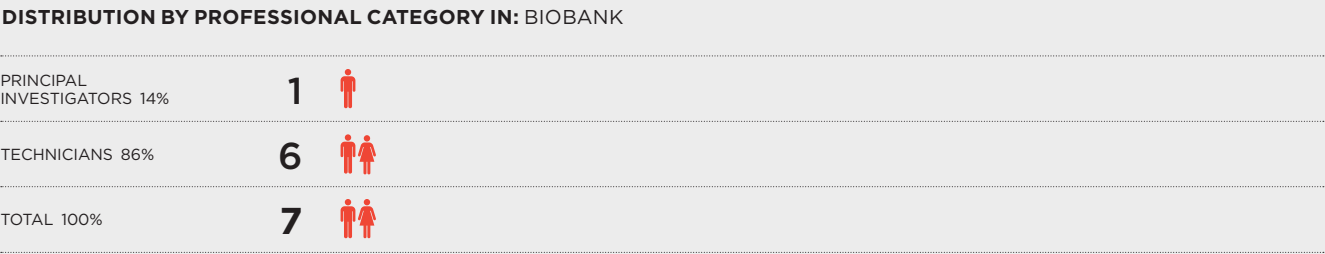
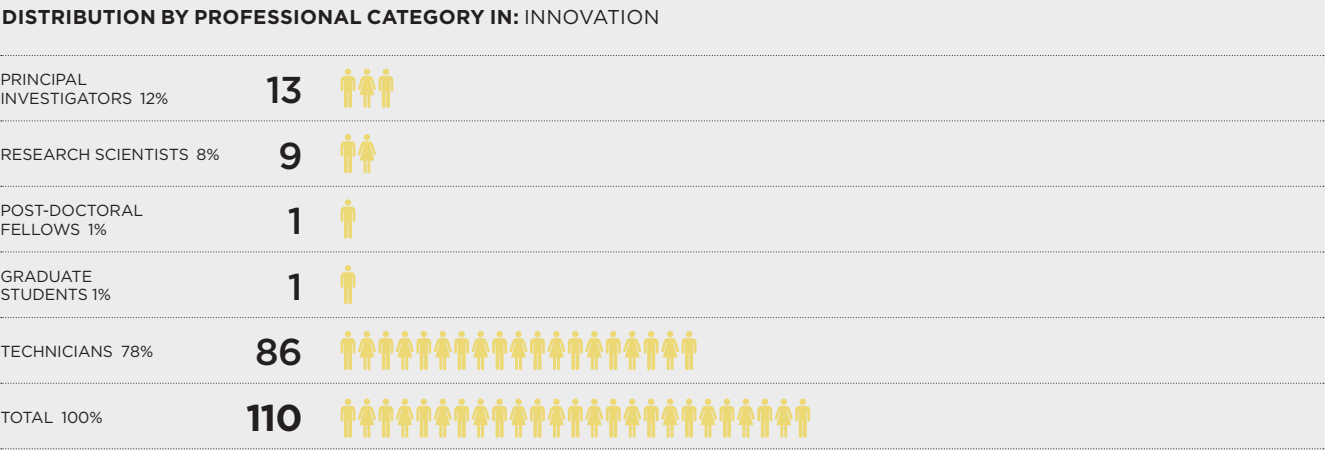
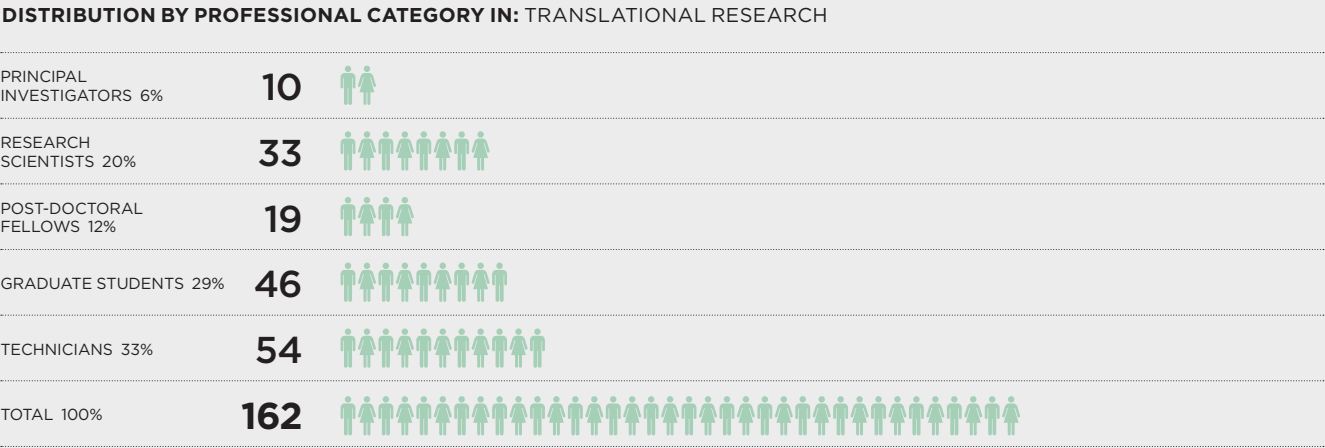
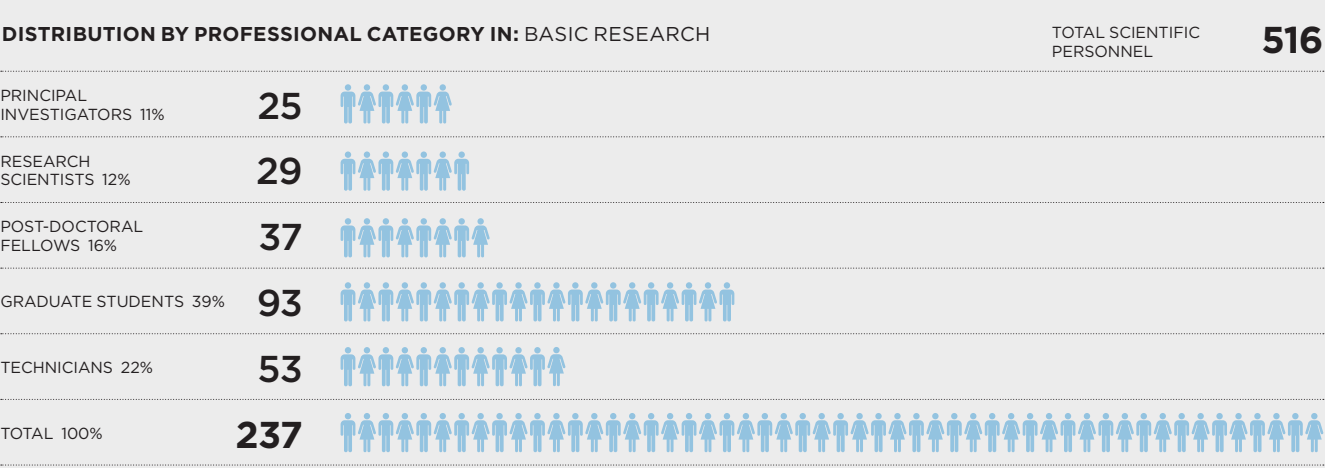
MOLECULAR ONCOLOGY 35.5%	183	
STRUCTURAL BIOLOGY 10.5%	54	
HUMAN CANCER GENETICS 9%	47	
CLINICAL RESEARCH 22.3%	115	
BIOTECHNOLOGY 17%	88	
EXPERIMENTAL THERAPEUTICS 4.3%	22	
BIOBANK 1.4%	7	

DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS 9.5%	49	
RESEARCH SCIENTISTS 14%	71	
POST-DOCTORAL FELLOWS 11%	57	
GRADUATE STUDENTS 27%	140	
TECHNICIANS 38.5%	199	

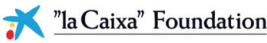
GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

Category	Gender	Percentage	Count	Visual Representation
Principal Investigators	Female	51%	25	25 female icons
	Male	49%	24	24 male icons
Research Scientists	Female	76%	54	54 female icons
	Male	24%	17	17 male icons
Post-Doctoral Fellows	Female	68%	39	39 female icons
	Male	32%	18	18 male icons
Graduate Students	Female	57%	80	80 female icons
	Male	43%	60	60 male icons
Technicians	Female	76%	152	152 female icons
	Male	24%	47	47 male icons
TOTAL SCIENTIFIC PERSONNEL	Female		350	350 female icons
	Male		166	166 male icons



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 2022. 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 2022, 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, Prostate Cancer, Haematological Malignancies and Lung Cancer 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 will receive 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-Catalana Occidente* continues to fund the Visiting Researchers' Programme that was established to support prestigious international professors for short stays at the CNIO. The recipients of the *Jesús Serra* Foundation's Visiting Researchers' Award in 2022 were Eva Nogales, from UC Berkeley (USA), and 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 2022, our research activities and seminars were also supported, among others, by *Fundación Investigación Biomédica Hospital Universitario 12 de Octubre*, *Fundación Investigación Oncológica*, *Fundacio Centre de Regulacio Genomica*, *Fundación Española de Hematología y Hemoterapia*, *Fundación Banco Sabadell*, and the *British Embassy*.

CNIO Friends Philanthropic Donations

Donations to the CNIO	251
‘CNIO Friends’ International Predoctoral/Postdoctoral Contracts	252
‘CNIO Friends’ Day	255
World Cancer Day Campaign	256
Promoting Dialogue Between Art & Science through ‘CNIO Arte’	257
New Major Gift to the CNIO	258
CNIO Friend Donor Events	259
Donors to the CNIO 2022	260

The CNIO Friends Programme enjoyed a record-breaking year in which we raised over €1 million in donations and pledges for the first time. We also raised our first major gift since the inception of the Office of Philanthropy and Development, with an innovative and generous donation from *Fundación Humanismo y Ciencia*.

In all, the CNIO Friends Programme raised €1.017 million in 2022, which is particularly generous given the financial effects of the last few years due to the pandemic and other economic global challenges.

For the third year, CNIO Friends messaging was supported by a multichannel branding and fundraising World Cancer Day campaign in February, this time in collaboration with award-winning PR agency True PR. The lottery campaign “The lottery that touches you the most” encouraged members of the public, companies and foundations to support CNIO with philanthropy, by highlighting the chances of developing different types of cancer in Spain. The campaign was supported by the generosity of Exterior Plus and JCDecaux *España* to spread the message around Spain.

The 5th annual CNIO Arte initiative showcased a collaboration between artist Susana Solano and scientist Pedro Alonso, director of the World Health Organization’s (WHO) Global Malaria Programme. Sculptor Solano travelled to Mozambique to visit the research centre founded by Alonso, who is also an epidemiologist. On Solano’s return, she created the piece entitled “The world of things”. Any profits from the sale of artworks from this initiative go directly to the CNIO Friends Programme.

In 2022, we strengthened our partnership with a number of our closest donors and Friends. We were delighted to receive a generous donation once again from Brother Iberia, and were honoured to receive €100k from an anonymous donor in support of CNIO Arte. We strengthened our alliance with *Santa Lucia Seguros* this year and were grateful to receive donations from cancer patients’ associations such as Rosae, L@s Fuertes, Marea Rosa, Bandera Rosa, and *El Arbol de la Vida*. New collaborations were formed with *Corporación RTVE*, and the *Ayuntamiento de Torreperogil* (Jaén), among others.

After working together for a number of years on joint communication efforts, we were delighted to raise a funded CNIO Friends contract from L’Oréal *España* in the name of La Roche - Posay. In addition, we continued to grow our alliance with our trusted friend *Fundación Domingo Martínez*.

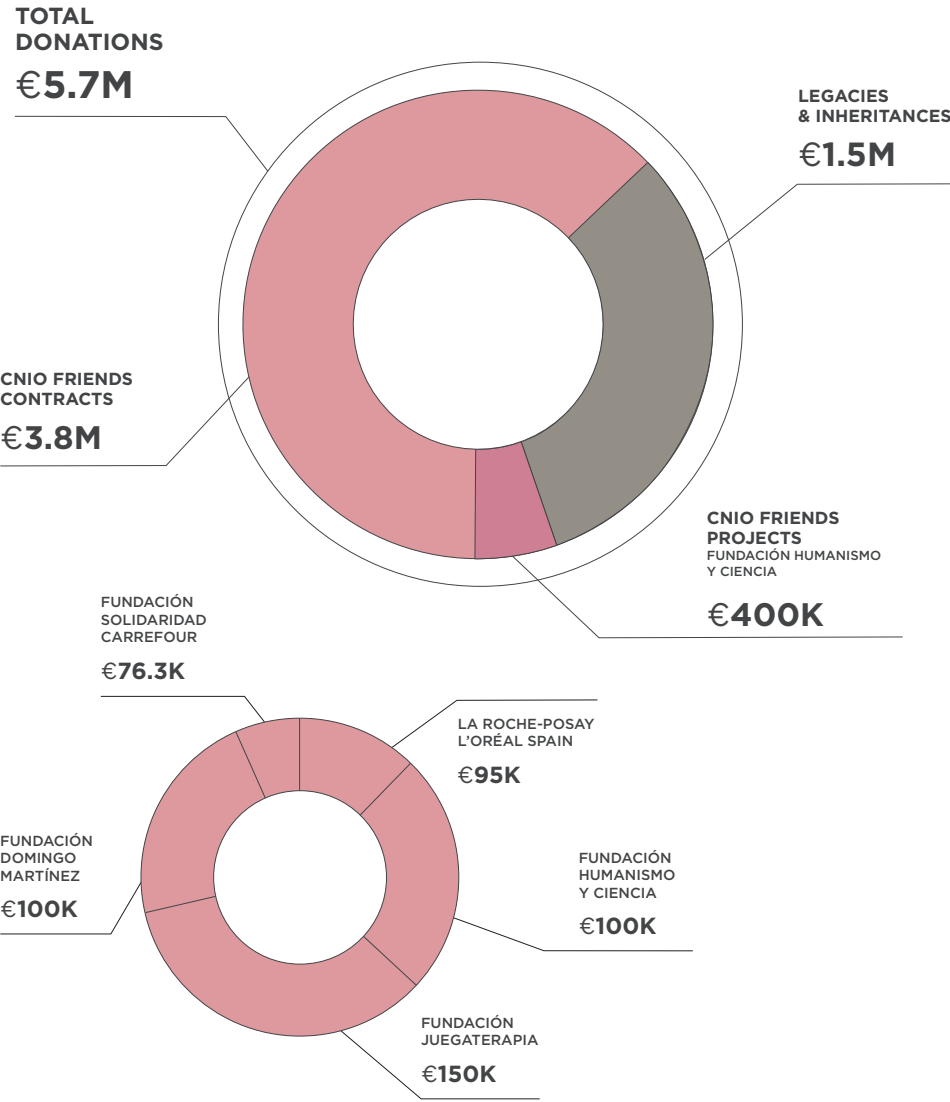
In July we celebrated the “CNIO Friends” Day, in person for the first time since the start of the pandemic. We enjoyed a very emotional event that brought together more than 90

“CNIO Friends moves to a new level in 2022.”

Friends to have the opportunity to meet first-hand the postdoctoral researchers hired thanks to their contributions. After an overview of the work of the CNIO by Maria Blasco, 7 researchers presented their projects and then we concluded the event with a visit to our laboratories to see first-hand the innovative work of our scientists. It was a lovely opportunity for our funded Fellows to see the impact of their work on their supporters and vice versa.

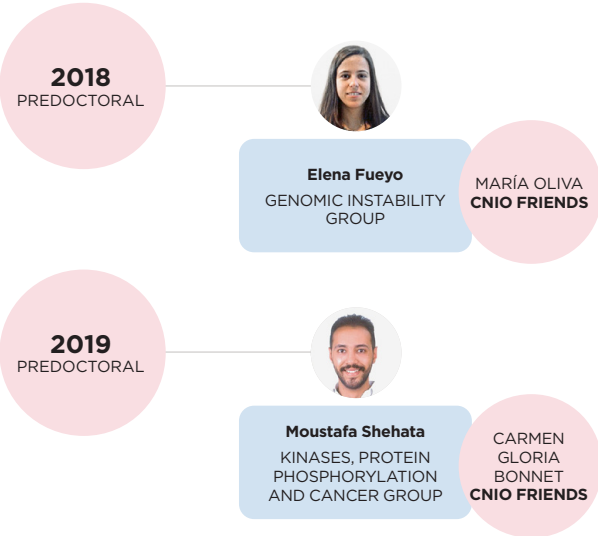
After a couple of unusual years, it was exciting to get back to a more “normal” fundraising model this year, despite the current volatile situation, which has been reflected in our success. It has been both challenging and rewarding to have the opportunity to create new alliances and collaborations via events and networking opportunities, and we have been humbled by the generosity of many of our new and existing Friends. In 2023, we will continue to develop and grow our philanthropic programme. Most importantly, for 2022 we extend a heartfelt message to our donors – thank you. ■

DONATIONS TO THE CNIO

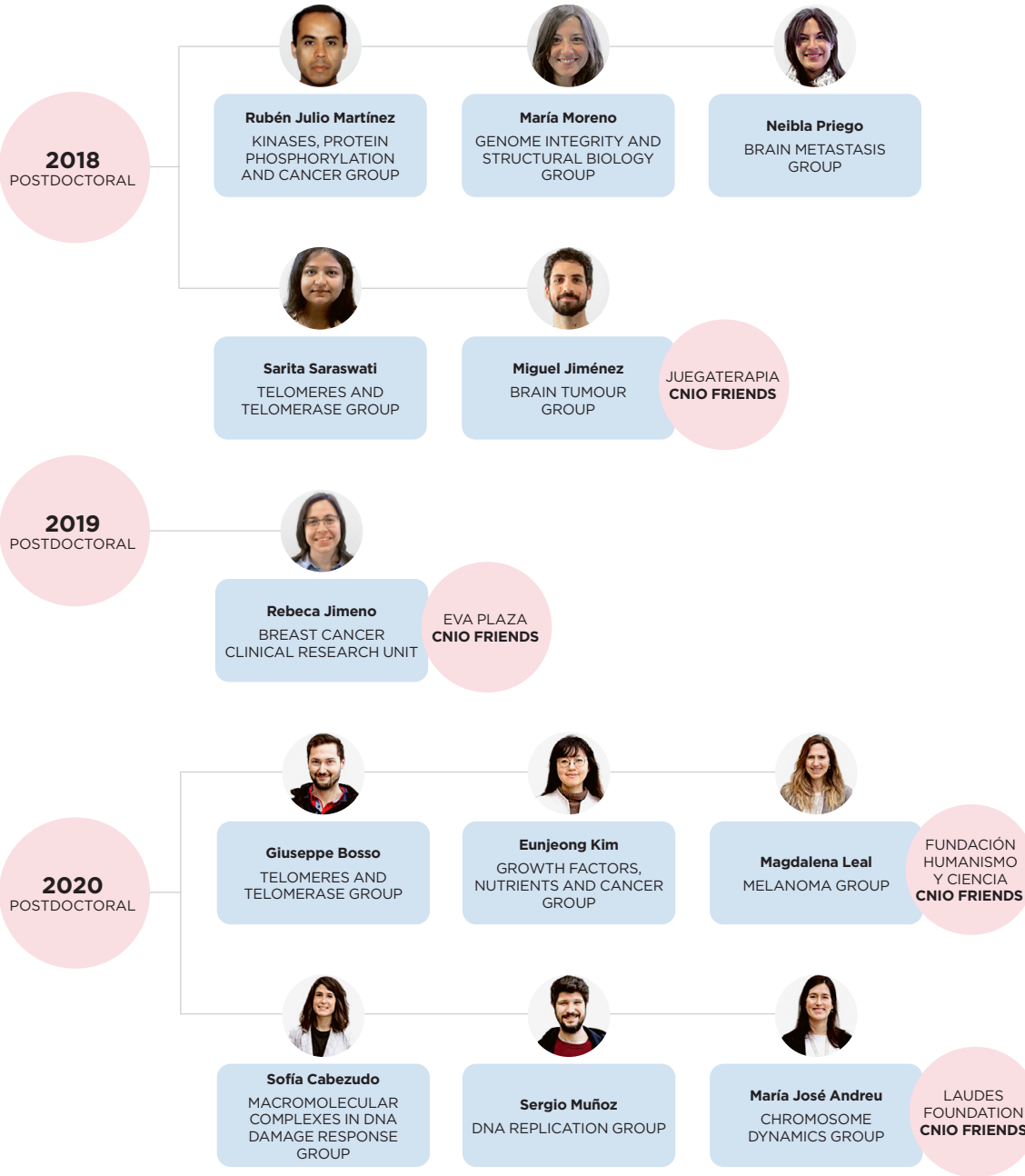
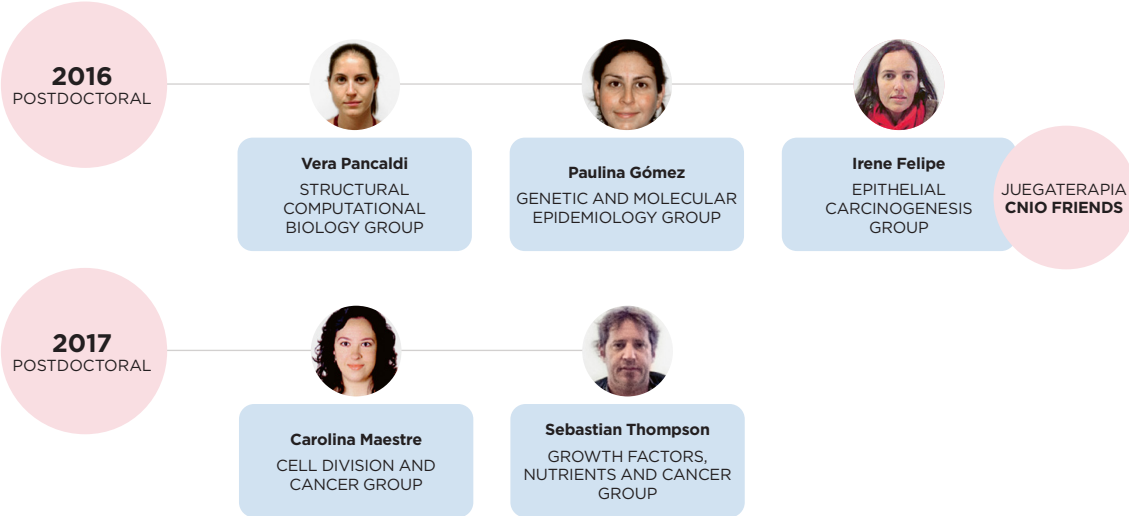


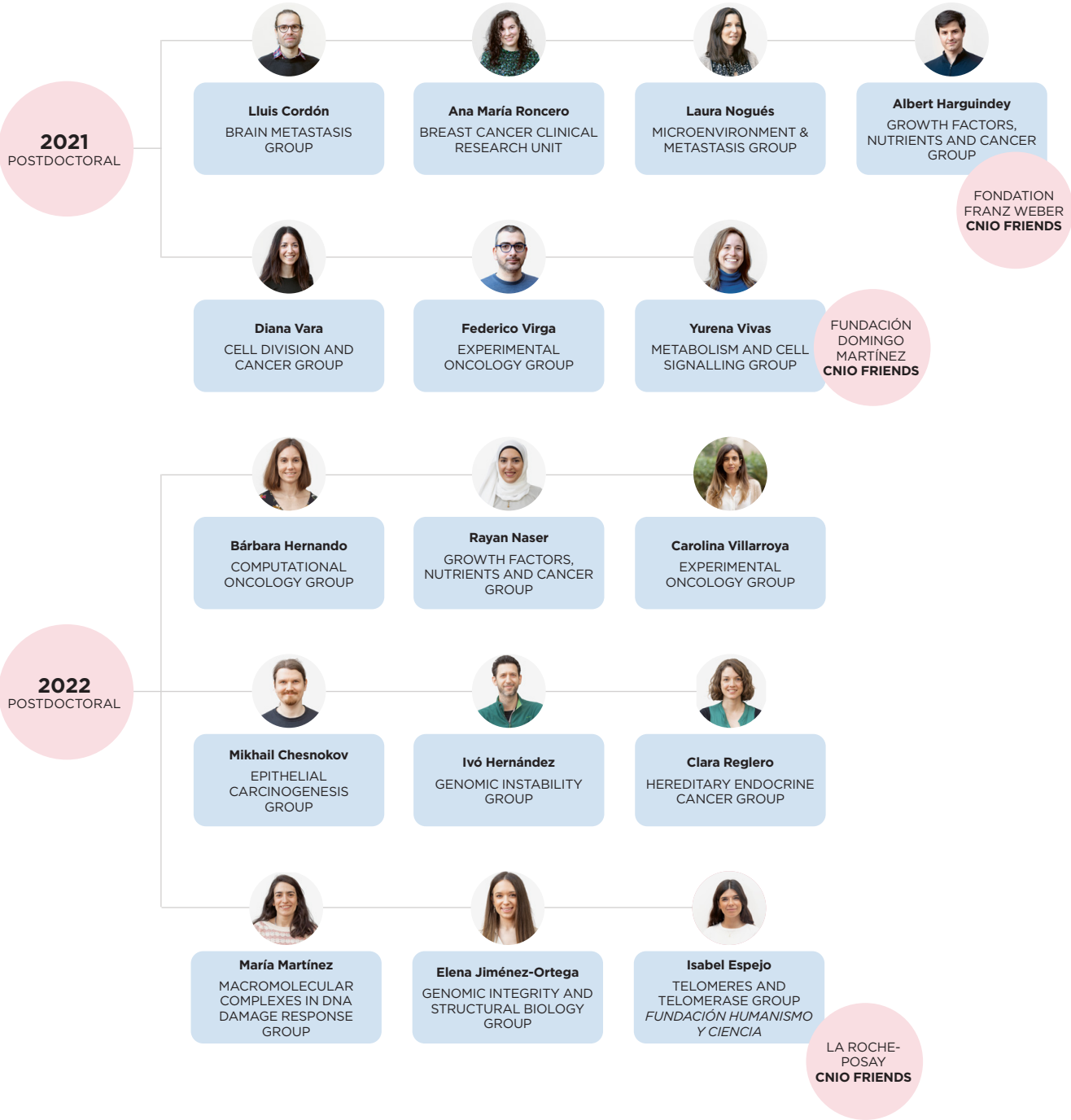
‘CNIO FRIENDS’ INTERNATIONAL PREDOCTORAL/
POSTDOCTORAL CONTRACTS

PREDOCTORAL CONTRACTS



POSTDOCTORAL CONTRACTS





‘CNIO FRIENDS’ DAY

In July we celebrated the ‘CNIO Friends’ Day. We enjoyed a very emotional event that brought together more than 80 Friends for the first time since the pandemic. Our Friends had the opportunity to meet first-hand the postdoctoral researchers

hired thanks to their contributions, and hear about their research projects. We concluded the event with a visit to our laboratories to see first-hand the exciting work of our scientists.



WORLD CANCER DAY CAMPAIGN



For the 3rd consecutive year, on February 4, we launched a joint fundraising and communication campaign, created and led by the communication agency True, under the slogan “The lottery that touches you the most” together with our logo #CNIOStopCancer. The main event involved a lottery held on *Calle Arenal* in central Madrid in which members of the public received “scratch and win” cards to show the high probability of suffering from cancer and increase the visibility of the CNIO. The campaign was strengthened by exterior billboards across bus stops, train stations and shopping centres in Spain thanks to the support of JCDecaux and ExteriorPlus.



PROMOTING DIALOGUE BETWEEN ART & SCIENCE THROUGH ‘CNIO ARTE’



The 5th edition of CNIO Arte this year was created through conversations between artist Susana Solano and scientist Pedro Alonso, director of the World Health Organization’s (WHO) Global Malaria Programme. Sculptor Susana Solano travelled to Mozambique to visit the research centre founded by the epidemiologist Pedro Alonso. On her return, she created the piece entitled “The world of things”. Any profits from the sale of artworks from this initiative go directly to the CNIO Friends programme.

NEW MAJOR GIFT TO THE CNIO

Fundación Humanismo y Ciencia, on the occasion of their 25th Anniversary and in recognition of one of the founding patrons Carlos Zapata, generously supported the CNIO with a major gift to support a research project on renal fibrosis and telomeres at the CNIO. This generous donation was recognised in a moving ceremony, culminating in the installation of a plaque on the 3rd floor.



CNIO FRIEND DONOR EVENTS

Fundación Domingo Martínez had the opportunity to meet their funded CNIO Friends Postdoctoral Research Fellow and visit her lab in person for the first time.



Brother Iberia continued their support of our cancer research with another generous donation in 2022.



DONORS TO THE CNIO 2022

BENEFACTOR FRIENDS

· Alberto Heras Hermida	· Julita Bermejo Alonso
· Alejandro Mendoza Plaza	· Lucía Martínez Chicano
· Alfonso Carrobles Romero	· Luís Carlos Nuñez Arias
· Antonio Segura Baeza	· Luís Grau Orts
· Dr Clara & Twitch Friends and María Sol Vallejo Prieto	· Manuel Lopez Perez
· Cruz Díaz Beltrán	· Manuela Caballero Del Pozo
· Eneko Novo Sukia	· María Carmen Merino Rodríguez
· Esther Valdivia Carrión	· María Dolores Díaz Almagro
· Estrella Delgado Domínguez	· María Dolores Florinda Antelo Reino
· Fernando Pascual Carreras	· María Lourdes Murillo Álvarez
· Francisco José Franco Sánchez	· María Natividad Alonso Mayor
· Gema Rubio González	· María Pilar Castro Carvajal
· Ignacio Gómez Arraiz	· María Teresa Palermo
· Inés Lamela Trobat	· María Virtudes Morales Contreras
· Íñigo Paredes Camuñas	· María Rodríguez López
· Jesús Labrador Fernández	· Mercedes Cáceres Alonso
· Jesús Miguel Iglesias Retuerto	· Roberto García Mancebo
· Jorge J. Parrado Nicolás	· Robert Milner
· Jorge Manuel Rodríguez Cabrera	· Santiago Rodríguez Uriel
· Juan José Lozano Jurado	· Vicente Belenguer Tarín

BENEFACTOR ASSOCIATIONS

· <i>Asociación Cultural Marea Rosa Montemolín, Pallares, Santa María de Nava</i>	· <i>Marcha Contra el Cáncer Puebla de Maestre</i>
· <i>Asociación de Mujeres Progresistas de Hornachos</i>	· <i>Ofrenca Floral en Honor a la Mare de Deu, l'Assumpció de Valafamés</i>

BENEFACTOR COMPANIES AND INSTITUTIONS

· <i>I.E.S. Airén</i>	· iZanda Portable Machine Tools <i>SL</i>
· <i>I.E.S. Tierra de Campos</i>	· <i>Qué te Cuentas, C. B.</i>
· Irtecon <i>S. L.</i>	

SPONSOR FRIENDS

· Alfonso Agüera Nieto	· Guillermo Alonso Borrego
· Álvaro Gil Conejo	· José Limiñana Valero
· Andres Sánchez Arranz	· María Josefa Azcona Peribáñez
· Anunciación De Los Milagros García Calvo	· Nemesio Carro Carro
· Fernando Inglés Musoles	

SPONSOR ASSOCIATIONS

· <i>Asociación de Pacientes y Familiares de CG “Bandera Rosa”</i>	· <i>Grupo Freesia</i>
· <i>Asociación Esperanza Burela</i>	· <i>El Árbol de la Vida</i>
· <i>Asociación Social Los Fuertes</i>	· <i>“Rosae” Asociación De Mujeres Afectadas De Cáncer De Mama</i>
· <i>Colectivo de Mujeres Afectadas de Cáncer “Las Supernenas”</i>	

SPONSOR COMPANIES & FOUNDATIONS

· <i>Ayuntamiento de Torreperogil</i>	· <i>Grupo Santa Lucía Seguros, S.A.</i>
· Brother Iberia	· JCDecaux <i>España</i>
· Exterior Plus	· José Borrell, <i>S.A.</i>
· <i>Fundación Domingo Martínez</i>	· L’Oreal <i>España</i> / La Roche - Posay
· <i>Fundación Humanismo y Ciencia</i>	

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.

Centro Nacional de Investigaciones Oncológicas (CNIO)
Melchor Fernández Almagro, 3
28029 Madrid, Spain
www.cnio.es

Coordination **Sonia Cerdá**
Edition **Sonia Cerdá and Carolyn Straehle**
Direction **Scientific Management**
Text, data and figures **CNIO Faculty and Management**
Publication database **Victoria López**
Photo Shoot Coordination **M. Carmen Rodríguez**
Photography **Laura M. Lombardía**
Design **underbau**
Typesetting **Nicolás García Marque**
Prepress **La Troupe**
Printing **Artes Gráficas Palermo**

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reproduction on microfilms or in any other way, and storage in data banks.

© Fundación del Sector Público Estatal Centro Nacional de Investigaciones Oncológicas Carlos III (F.S.P. CNIO), 2023

National book catalogue number M-12010-2023
ISSN 2529-9514

cnio *stop cancer*