

**ANNUAL
REPORT 2021**

ANNUAL REPORT 2021

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“Surely 2021 will be remembered as the year of the vaccines against COVID-19, powerful tools that have set a new scenario in the evolution of the pandemic, bringing profound changes in the way we are dealing with the disease at every level.”

MARIA A. BLASCO
Director

FOREWORD

Maria A. Blasco Director

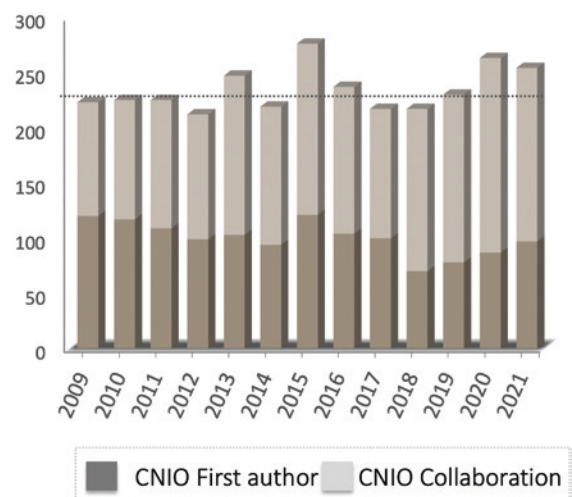
Surely 2021 will be remembered as the year of the vaccines against COVID-19, powerful tools that have set a new scenario in the evolution of the pandemic, bringing profound changes in the way we are dealing with the disease at every level, in the workplace as well as in our daily life.

In 2021, CNIO researchers authored a total of 254 papers, 52 of which were published in journals with an impact factor between 10 and 15, and 43 publications in journals with an impact factor greater than 15. Therefore, this year was very productive in terms of the number of high-quality publications, which increased by 32% with respect to 2020. According to the *Nature Index*, considering our scientific contributions in the life sciences and healthcare field, the CNIO ranked second among cancer-focused institutions in Europe and holds the eighth position worldwide. These indicators provide a clear evidence of the success of our scientific efforts in basic and translational cancer research.

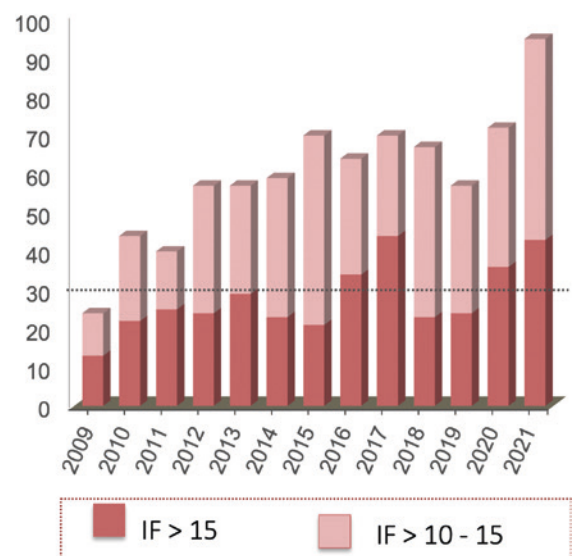
Our contribution to the fight against the COVID-19 pandemic continued this year through several Groups and Units at the Centre who are involved in projects focusing on different aspects, including the generation of humanised mouse models susceptible to SARS-CoV-2 infection; study of the role of short telomeres in the severity and sequelae of the Covid-19 disease; the identification of genetic variants correlated with disease severity; the development of a new diagnostic method using phi29 polymerase amplification; and the evaluation of drugs that can modulate the cytokine storm associated to SARS-CoV-2 infection. Obviously, the expertise in the study of the molecular and cellular mechanisms of biology can be useful not only to address cancer related issues, but other diseases as well.

Adapting the Centre to the constant evolution of science and the new emerging fields in cancer research, by recruiting new groups working at the forefront of science, is key to maintaining our competitive edge. Thus, in January 2021 a new Research Group joined the Molecular Oncology Programme, the Cancer Immunity Group, led by Maria Casanova, coming from the

TOTAL NUMBER OF PUBLICATIONS



TOP PUBLICATIONS



Icahn School of Medicine at Mount Sinai Hospital, New York. This new Junior Group will contribute to boosting the activity of the CNIO in the immuno-oncology area.

On the other hand, as a consequence of the application of the internal policy related to the career development of the Junior Group Leaders at CNIO, which sets a limit of 8 years for their stay at the CNIO, Massimo Squatrito, Leader of the CNIO Seve-Ballesteros Foundation Brain Tumours Group, left the CNIO in October 2021 to join AstraZeneca in Cambridge (UK) as Associate Director Translational Medicine. After his successful stay at the CNIO where he made important contributions to the understanding of the biology of brain tumours and published in high impact journals, Massimo will continue his scientific career with an executive position in a multinational pharmaceutical company. We wish him the best of luck for his professional career in the future.

Upon the retirement of Javier Benitez, the Director of the Human Cancer Genetics Programme in 2020, an international search was launched for a new director to lead a new Cancer Genomics Programme. The new programme will incorporate the Groups and Units from the former programme, together with other Groups that use biocomputational tools for the study of cancer, currently under the Structural Biology Programme. This area has the support of Raúl Rabadán, Professor in the Department of Systems Biology and Biomedical Informatics and Director of the Center for Topology of Cancer Evolution and Heterogeneity of Columbia University (New York, USA). In July 2021, Raúl was appointed as Adjunct Professor at the CNIO, an honorary appointment that allows him to maintain an official link with the Centre, reinforcing the presence of the CNIO in the field of computational biology approaches to cancer research.

In 2021, a new Head of the Familial Cancer Clinical Unit joined the CNIO, after the retirement of Miguel Urioste. We thank him for his dedication and placing the CNIO as a reference in the field of familial cancer genetic diagnostics. The new Head, Maria Currás, is an M.D./Ph.D. with a strong background in clinical genetics, who moved from the *Clínica Universitaria de Navarra* in Madrid to the CNIO in January 2022.

The relevance and broad scope of our innovation activities represent another meaningful indicator of the social impact of our scientific activities. In June 2021, a new Director of the Innovation Area joined the CNIO, after an international search put in place upon the departure of the previous director in 2020. The new Director, Roke Iñaki Oruezabal, was previously Director of Development and Innovation of the “*Red Andaluza de Diseño y Traslación de Terapias Avanzadas*” (RADyT_{TA}), an organisation of the Andalusian Regional Government, and he brings broad experience in the private sector at the level of biotechnological companies and consultancy.

In 2021, the CNIO filed as many as 12 priority patent applications, 3 requests of divisional patents, and 2 PCT applications for international extension, protecting our intellectual property assets. Also, our efforts in promoting collaborations with industry resulted in about €3M secured through research agreements (an amount 20% higher than in 2020). As much as 65% of CNIO agreements with the private sector were established with international entities. This is a clear reflection of the leadership and influence of the CNIO and our researchers worldwide.

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 2021 there has been a very active participation in the new National *AEI* Innovation calls, cofunded by the EU Reconstruction Funds “NextGenerationEU”. Two “Proof of Concept” projects have been awarded: a project lead by Héctor Peinado to develop THX-B as a novel anti-metastatic agent, and another project lead by Maria S. Soengas to study the reactivation of the immune system in cancer via MIDKINE. Furthermore, two “Strategic Lines” projects have been awarded involving Marcos Malumbres: a collaboration with Pharmamar to generate a micrometastases-on-chip platform, and a partnership with *CSIC, Hospital Universitario 12 de Octubre* and MECWINS SA to give rise to an optoplasmonic immunoassay platform for early detection of breast cancer. Besides that, 4 CNIO *CaixaImpulse* projects are ongoing.

Our drug discovery programme (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 2021 the CNIO signed an agreement with the US-based company TOTUS Medicines, by which we licensed them a family of patented PI3K inhibitors developed by the Programme, which has represented a significant upfront payment, plus potential additional income for milestones and royalties.

Our training programmes are one of the key features of the Centre, as an essential part of our mission. To provide training to the new generation of highly skilled researchers is a priority for the CNIO, and we warmly thank our sponsors as they contribute to supporting all our training initiatives.

During 2021, Raúl Rabadán, from Columbia University in New York (USA), was beneficiary of the *Jesús Serra* Foundation’s Visiting Researchers Programme. In addition, Ashwag Mukhtar, from Alneelain University, Sudan, was awarded a grant from the “Science by Women” programme of the *Mujeres por Africa* Foundation, as Visiting Scientist, to

join the Genetic and Molecular Epidemiology Group at the CNIO for a 6-month stay.

In a year marked by COVID-19 in the media, CNIO news continued attracting the interest of the media, keeping the good pace of previous years and marking over 3,033 appearances in press (printed and online) and over 200 audio-visual hits. Not only did our cancer research news attract the attention of journalists, but initiatives such as the #CNIOStopCancer campaign by CNIO Friends, or the 4th edition of CNIO Arte (see below), were also covered by general interest media, which science does not usually reach.

On the occasion of World Cancer Research Day on September 24, we organised the 5th edition of the event focused on giving visibility to our research activities. After the 2020 edition, which was held online due to the pandemic crisis, in 2021 the event was held in a hybrid format, online and presential at *CaixaForum* Madrid, under the title “Vaccines against COVID-19 and cancer control”. Mariano Esteban, Director of the Poxvirus and Vaccines Group of the National Centre of Biotechnology (CNB-CSIC), was the keynote speaker. His keynote address was followed by a round table focusing on the issue of how cancer research will benefit from what we have learnt during the last two years of the COVID-19 pandemic. Initiatives like this help us to fulfill our goal of bringing science closer to society.

The Institutional Image and Outreach Office is leading several projects that aim to open up new avenues to gain society's trust and attention, as well as to emphasise the value of science. Thus, in 2021, the 4th 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 computational biologist, leader of the Human Cell Atlas, Sarah Teichmann and the visual artist Daniel Canogar. The work “*Fulguraciones*” was exhibited at the CNIO from June 14 to September 11. The funds raised from this initiative totalled €100,000, which contributed directly to our ‘CNIO Friends’ philanthropic initiative. Also, for the first time in Spain, a scientific research institution has been given its own space at the contemporary art fair ARCO (July 7-11). In our stand we exhibited all the works of the different artists who participated in the four editions of CNIO Arte to date, i.e., Eva Lootz, Chema Madoz, Carmen Calvo and Daniel Canogar. The initiative was extremely successful and generated a large number of visits and interactions with citizens, companies, foundations and institutions, which favour the promotion of our Centre and open new venues for potential donations and collaborations. The work “*Fulguraciones*” is being 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*, which will be open from December 18, 2021, to August 28, 2022.



CNIO Arte 2021. “*Fulguraciones*” by visual artist Daniel Canogar.

Also, thanks to the media impact achieved with the CNIO Arte project over the last four years, the work that Eva Lootz designed for the first edition of this initiative, which was based on the figure of Margarita Salas, was selected to participate, together with other Spanish artists, in the exhibition “*Synapsis Art and Science in Spain from Cajal to S. XXI*” at the Nobel Museum in Stockholm, Sweden (November 24-December 5, 2021).

To increase the impact of this initiative, in 2021 we organised the second edition of a new series of “*Art and Science Symposia*”, which aims to bring those two areas closer by inviting renowned professionals from both fields to facilitate in an open and enriching discussion on a selected topic. The symposium was held jointly with the presentation of the 4th edition of the CNIO Arte project, under the title “*Cartography in the Digital Age*”, chaired by Prof. Carlos Jiménez (Emeritus Professor of Aesthetics, historian and art critic).

Also in 2021, the third event in the series “*CNIO Workshop on Philosophy, Science and Medicine: The use of animals in science, ethical and epistemic problems and alternatives*” was held at CNIO, with the support of the *Banco Sabadell* Foundation. Discussion topics reviewed the convenience of science continuing to use animals for its tests and clinical trials, both from the point of view of ethical and epistemic values. In addition, the panel of experts discussed possible alternatives to animal experimentation that current science is opening up, and their advantages or disadvantages.

The CNIO Philanthropy and Development Office has been implementing a new fundraising strategy for the CNIO, to build upon the existing and very successful ‘CNIO Friends’ crowdfunding programme. In addition to donations from the public, the team has been working on identifying and providing new philanthropic funding sources, in particular from the

foundation and corporate sectors that will contribute to hiring new research talent for the CNIO. Every donation to the CNIO goes to the 'CNIO Friends' initiative, which covers the salaries of new post-doctoral research fellows. Our philanthropic programme actively seeks to build collaborative partnerships with corporate partners, individuals and philanthropic foundations. In 2021, CNIO Friends raised €669,000 in philanthropic donations and received €80,000 from legacies and bequests. We were delighted to welcome 6 new post-doctoral researchers to the CNIO this year, thanks to the funding from our generous donors. To date, philanthropic donations to the CNIO have made possible the incorporation of 26 new researchers since 2016 (24 postdocs and 2 PhD students). We would like to thank all of our donors and CNIO Friends for their continued support of our research programme. Philanthropy enables everyone to contribute to our cancer research, providing support that will have a positive impact on society for generations to come.

Our commitment to gender equality was consolidated in 2021 through the activities spearheaded by our CNIO Women and Science Office (WISE). This is an effort from CNIO volunteers that has contributed to impactful changes that are facilitating both cultural changes and institutional improvements. During this year and thanks to the Zoom platform, our WISE office was able to keep bringing outstanding female speakers, who inspire and encourage us to explore new perspectives and visions on the role of women in society <https://www.cnio.es/en/women-in-science/activities-events/>. In our efforts to educate the future generations without gender bias, and for the occasion of International Day of Women and Girls in Science (February 11th), CNIO released a video featuring 6 of its scientists who list the reasons why women should become scientists and invite audiences to join the campaign in social media using the hashtag #HazteCientífica.

With great pleasure and pride, we provide our society once again with a story of our achievements in 2021 and convey our commitment to continue making 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

“CNIO scientists keep making important discoveries that advance our understanding of cancer and bring hope to patients.”

Dear all, 2021 has been yet-another Covid year. While we are slowly gaining back our lives, the CNIO experience (and life-experience as a whole) is still only halfway through. Nevertheless, our scientists have once again provided numerous examples of the excellent science being done in the Centre. With AlphaFold knocking on our doors, our structural biologists demonstrated that rigorous bench work is still fundamental for deciphering the molecular mechanisms of key enzymatic regulators of growth, DNA repair or gene expression. Computational work has nevertheless helped us better understand the genetic circuitry behind tumour suppressors. 2021 also saw an important contribution from the now retired group of our previous Director of the Human Genetics Programme, Javier Benitez, another example of collaborative international work to clarify the

mutational landscape of breast cancer. Our clinical associated groups contributed to the development of immunotherapies based on checkpoint inhibitors or CAR-T technologies. Scientists at the Molecular Oncology Programme have made important advances in many independent areas of cancer research such as identifying new drivers of metastasis, revealing how nutrient signalling might affect carcinogenesis or providing a mechanistic understanding of how cancer cells respond to genotoxic chemotherapy and might become resistant to it. Besides cancer, our work also sheds light on other areas such as ageing, obesity, hereditary microcephalies, neurodegeneration and Covid-19. Progress might seem small when analysed on a daily basis but is impressive when analysing our output as a whole. Be proud of being part of CNIO. Keep safe, keep strong, keep pushing.

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

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

Massimo Squatrito (until September)
Seve-Ballesteros Foundation-CNIO Brain Tumour 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

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

Rafael Fernández Leiro
Genome Integrity and Structural Biology Junior Group

Solip Park
Computational Cancer Genomics Group

Geoff Macintyre
Computational Oncology Group

Ramón Campos-Olivas
Spectroscopy and Nuclear Magnetic Resonance Unit

Fátima Al-Shahrour
Bioinformatics Unit

Jasminka Boskovic
Electron Microscopy Unit

Inés Muñoz
Crystallography and Protein Engineering 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

Miguel Urioste
Maria Curras (since December)
Familial Cancer Clinical Unit

Sandra Rodríguez-Perales
Molecular Cytogenetics 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 Junior Clinical Research Unit

David Olmos (until December)
Prostate Cancer Junior 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

INNOVATION

ROKE I. ORUEZABAL DIRECTOR OF INNOVATION (since June)

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

Diego Megías (until December)
Confocal Microscopy Core Unit

Javier Muñoz (until August)
Proteomics Core Unit

Vacant
Histopathology Core Unit

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

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor Programme Director
Natalia Catalá Secretary

Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
CNIO-Lilly Cell Signalling and
Immunometabolism Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Irene Herrera Head

BIOBANK

EVA ORTEGA-PAÍNO DIRECTOR

Basic Research

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MOLECULAR ONCOLOGY PROGRAMME

ÓSCAR FERNÁNDEZ-CAPETILLO Programme Director



Scientists at the Molecular Oncology Programme (MOP) aim to discover new molecular mechanisms that drive cancer onset, mediate its progression, or influence the response to therapy. The ultimate goal is to generate knowledge that can be translated into applications that are beneficial to cancer patients and that can help in the diagnosis and treatment of their disease. To do so, we integrate leading groups that cover a wide range of complimentary expertises of relevance in oncology, including 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); and metastasis (Manuel Valiente, Eva González-Suárez and Héctor Peinado). During 2021, we recruited a new Group, led by Maria Casanova, that will work on cancer immunity. This is an important area that was previously less represented at CNIO, which will benefit from its incorporation. Welcome Maria, looking forward to seeing your science flourish with us! Also in 2021, as a consequence of the application of the internal policy related to the career development of the Junior Group Leaders at CNIO, Massimo Squatrito's Group working on brain tumours ended its stay with us, and Massimo took up a new job in the pharmaceutical industry. During his stay at CNIO Massimo made exciting contributions to glioblastoma research and was a very nice colleague to have around. We wish him the very best in this new step in his career.

2021 brought to light many interesting discoveries made by MOP members. The Brain Tumour Group made important contributions to the genetics of glioblastoma and to potential treatments for this fatal disease. We now also know that a specialised polymerase (PrimPol) might modify the response to one of the most frequent chemotherapies and how the response of another novel therapy (USP7 inhibitors) might be influenced by CDK activity. We made progress in our understanding of how RAS variant isoforms or mutations in STAG2 might promote carcinogenesis and revealed new connections between senescence and stemness in breast cancer. The Telomeres and Telomerase Group keeps adding evidence that supports the usefulness of a telomerase-based therapy for the treatment of age-related diseases such as kidney fibrosis and illustrated the impact that short telomeres might have on the severity of covid-19. The Melanoma Group further supported the value of studying lymphangiogenesis

“During 2021 MOP scientists made important insights into basic aspects of cancer research, which can hopefully provide ideas for novel cancer therapies in the longer term.”

as a surrogate of metastasis, and additional insights into the influence of the environmental signals during the initiation of meiosis were provided by the Transformation and Metastasis Group. Our works also revealed how inflammatory processes might contribute to obesity, and the role of nutrient signalling in cancer and overall lifespan.

During 2021 scientists at the MOP were very active in activities that go beyond publications and that are important to bringing science to society and to defending the importance of research. To end, I particularly want to congratulate our junior faculty at the MOP. These have not been the easiest years to start up a laboratory, and yet, your work shows that it was possible to do so and to make important discoveries. Congratulations to you all for a nice start to your independent scientific careers and looking forward to seeing your next discoveries. ■

TELOMERES AND TELOMERASE GROUP

Maria A. Blasco
Group Leader

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

Post-Doctoral Fellows
Giuseppe Bosso, João Tiago
Carvalho Jordão (until June), Buyun
Ma, Sergio Piñeiro (until December),
Sarita Saraswati



Graduate Students

José Carlos González, Óscar Lagua,
Jessica Louzame, Amparo Sánchez,
Raúl Sánchez

Technicians

Ana Guío (TS)¹, Rosa M. Serrano

¹*Titulado Superior* (Advanced Degree)

Visiting Students

Buse Kantekin (July-August)
(Erasmus Fellowship, Üsküdar
University, Turkey), Neetij Krishnan
(since September) (Fulbright

Commission Fellowship, USA), Pablo
Lanuza (March-June) (Bachelor's
Student, *Universitat de Lleida*, Spain),
Irene Sánchez (until September)
(Bachelor's Student, *University of
Huddersfield*, UK), Büsra Sertkaya
(July-September) (Erasmus +

Fellowship, Üsküdar University,
Turkey), Merve Yılmaz (September-
November) (The Federation of
European Biochemical Societies
International Fellowship, Turkey)

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 and are 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 finding that signals outside the cell, which induce cell proliferation and are implicated in tumorigenesis, regulate telomeres opens the door to new therapeutic avenues targeting telomeres to help treat cancer.”

RESEARCH HIGHLIGHTS

AKT-mediated response of telomeres to external signals impacts on tumorigenesis

We have progressed in our quest to unravel the role that telomeres — the chromosome ends responsible for cellular ageing as they shorten — play in cancer. Telomeres are bound by shelterin, a multi-protein complex essential for the protection of chromosome ends and genomic stability. Knowledge is scarce on the regulation of shelterin components by extracellular signals including developmental and environmental cues.

Using the gene-editing tool CRISPR/Cas9, we generated *knock-in* human cell lines carrying non-phosphorylatable mutant forms of the AKT-dependent phosphorylation sites that are present in the shelterin protein TRF1 and showed that TRF1 is subjected to AKT-dependent regulation. TRF1 mutant cells had decreased TRF1 binding to telomeres as well as increased global and telomeric DNA damage. Human cells bearing non-phosphorylatable mutant TRF1 alleles exhibited accelerated telomere shortening (FIGURE 1), demonstrating that AKT-dependent TRF1 phosphorylation regulates telomere maintenance *in vivo*. TRF1 mutant cells showed an impaired response to proliferative extracellular signals as well as a decreased tumorigenesis potential. Telomere protection and telomere length can thus be regulated by extracellular signals upstream of PI3K/AKT pathway activation, such as growth factors, nutrients, or immune regulators, and can interfere with immortality of cancer cells.

Short and dysfunctional telomeres contribute to renal fibrosis. A connection between short telomeres and the epithelial-to-mesenchymal transition programme

Telomere shortening or telomere dysfunctionality are responsible for the human pathologies known as *telomere*

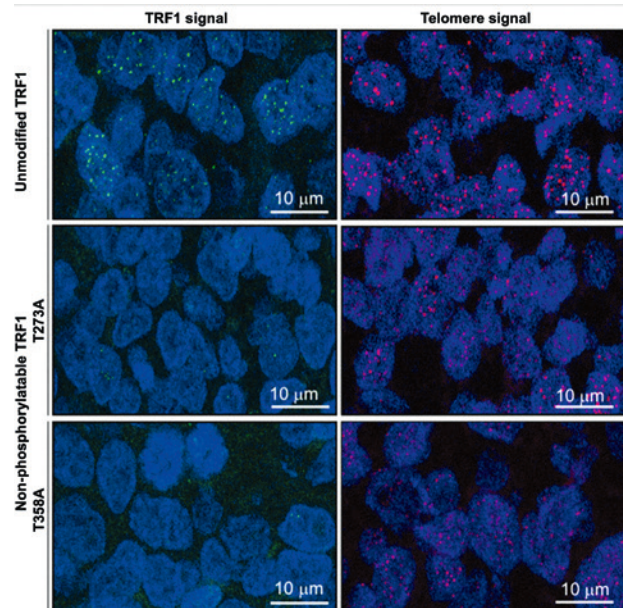


FIGURE 1 Telomere length is normal upon AKT-phosphorylation of TRF1 (top) and shorter in AKT-phosphorylation unresponsive TRF1 cell lines (bottom).

syndromes characterised by loss of the regenerative capacity of tissues and fibrotic pathologies. We generated two different mouse models of kidney fibrosis: one that combines telomerase deficiency to induce telomere shortening and a low dose of folic acid (a kidney toxin), and another in which *Trf1* is conditionally deleted from the kidneys.

We found that short telomeres make the kidneys sensitive to developing fibrosis in response to folic acid and exacerbate the epithelial-to-mesenchymal transition (EMT) programme. Short telomeres play an important role in the development

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- ▶ Martínez P, Blasco MA (2021). An enzyme to cure age-related diseases. *Nature Catalysis* 4, 738-739.
- ▶ Sánchez-Vázquez R, Martínez P, Blasco MA (2021). AKT-dependent signaling of extracellular cues through telomeres impact on tumorigenesis. *PLoS Genet* 17, e1009410.
- ▶ Piñeiro-Hermida S, Martínez P, Blasco MA (2021). Short and dysfunctional telomeres protect from allergen-induced airway inflammation. *Aging Cell* 20, e13352.
- ▶ Sánchez-Vázquez R, Guío-Carrión A, Zapatero-Gaviria A, Martínez P, Blasco MA (2021). Shorter telomere lengths in patients with severe COVID-19 disease. *Aging (Albany NY)* 13, 1-15.
- ▶ Monteagudo M, Martínez P, Leandro-García LJ, Martínez-Montes AM, Calsina B, Pulgarín-Alfaro M, Díaz-Talavera A, Mellid S, Letón R, Gil E, Pérez-Martínez M, Megías D, Torres-Ruiz R, Rodríguez-Perales S, González P, Caleiras E, Jiménez-Villa S, Roncador G, Álvarez-Escolá C, Regajo RM, Calatayud M, Guadaliix S, Currás-Freixes M, Rapizzi E, Canu L, Nölting S, Remde H, Fassnacht M, Bechmann N, Eisenhofer G, Mannelli M, Beuschlein F, Quinkler M, Rodríguez-Antona C, Cascón A, Blasco MA, Montoro-Conde C, Robledo M (2021). Analysis of telomere maintenance related genes reveals NOP10 as a new metastatic-risk marker in pheochromocytoma/paraganglioma. *Cancers (Basel)* 13, 4758.
- ▶ Vincure OS, Whittemore K, Kushwah D, Blasco MA, Kulshreshtha R (2021). miR-490 suppresses telomere maintenance program and associated hallmarks in glioblastoma. *Cell Mol Life Sci* 8, 2299-2314.
- ▶ Bacalini MG, Reale A, Malavolta M, Ciccarone F, Moreno-Villanueva M, Dollé MET, Jansen E, Grune T, Gonos ES, Schön C, Bernhardt J, Grubeck-Loebenstein B, Sikora E, Toussaint O, Debacq-Chainiaux F, Capri M, Hervonen A, Hurme M, Slagboom PE, Breusing N, Aversano V, Tagliatesta S, Franceschi C, Blasco MA, Bürkle A, Caiafa P, Zampieri M (2021). Ageing affects subtelomeric DNA methylation in blood cells from a large European population enrolled in the MARK-AGE study. *Geroscience* 43, 1283-1302.
- ▶ Alvarez-Palomo AB, Requena-Osete J, Delgado-Morales R, Moreno-Manzano V, Grau-Bove C, Tejera AM, Otero MJ, Barrot C, Santos-Barriopedro I, Vaquero A, Mezquita-Pla J, Moran S, Naya CH, García-Martín

of renal fibrosis, a finding that could be of use to devise new treatments of this pathology.

Trf1 deletion in kidneys resulted in fibrosis and EMT activation. This is the first time that short telomeres have been linked to EMT, a connection of relevance since EMT, and the genes that regulate this programme, are also involved in cancer.

Telomere shortening or dysfunction may therefore contribute to pathological, age-associated renal fibrosis by influencing the EMT programme.

Shorter telomere lengths in patients with severe COVID-19 disease

Incidence of severe manifestations of COVID-19 increases along with age, with older patients showing the highest mortality rate suggestive of the contribution of the molecular pathways underlying ageing to the severity of COVID-19. The progressive shortening of telomeres (FIGURE 2) is one of the mechanisms of ageing. Critically short telomeres impair the regenerative capacity of tissues and trigger loss of tissue homeostasis and disease. The SARS-CoV-2 virus infects many different cell types, forcing cell turnover and regeneration to maintain tissue homeostasis.

Our starting hypothesis was that short telomeres in older patients limit the tissue response to SARS-CoV-2 infection. We measured telomere length in peripheral blood lymphocytes from COVID-19 patients aged 29-85 years and found that patients who manifested severe forms of COVID-19 had significantly shorter telomeres.

We postulate that telomere shortening arising from the viral infection impedes tissue regeneration and that is the reason why a significant number of patients suffer long COVID-19. In this context, telomerase-based gene therapy may be a tool in the treatment of post-COVID-19 pulmonary injury. ■

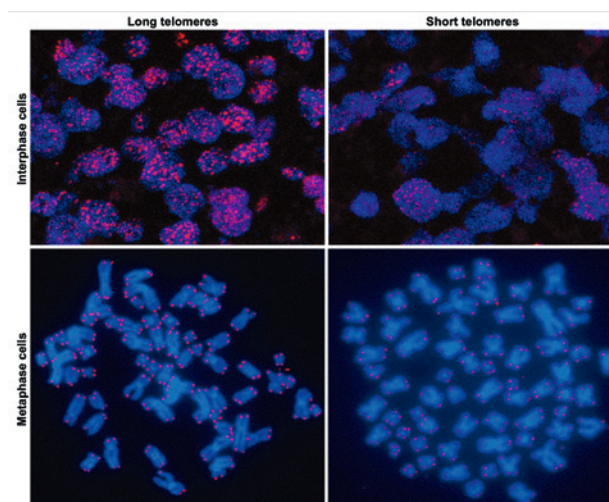


FIGURE 2 Representative immunofluorescence images of human cells bearing either long or short telomeres at the indicated cell cycle phases.

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► Sanz-Ros J, Mas-Bargues C, Monleón D, Gordevicius J, Brooke RT, Dromant M, Derevyanko A, Guio-Carrión A, Román-Domínguez A, Romero-García N, Inglés M, Blasco MA, Horvath S, Viña J, Borrás C (2021). Small extracellular vesicles from young mice prevent frailty, improve healthspan and decrease epigenetic age in old mice. *bioRxiv*. doi: <https://doi.org/10.1101/2021.07.29.454302>.

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- Bobadilla M, Formentini I, Blasco MA, Baer C, Bosch F (2021). Telomerase reverse transcriptase-based therapies. Divisional application. *US20210260169*.
- Bobadilla M, Formentini I, Blasco MA, Baer C, Bosch F (2021). Telomerase reverse transcriptase-based therapies. Divisional application. *EP3848056*.
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transcriptase-based therapies. Divisional application. *EP21198809.2*.

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► AWARDS AND RECOGNITION

- Chair of SOMMa ("Severo Ochoa" Centres and "María de Maeztu" Units of Excellence Alliance), Spain.
- Merck Health Foundation Award in recognition of Professional Excellence in

Science and Research, Spain.

- Zenda Award in recognition of lifetime achievements in the life sciences and the role of women in this field, II International Zenda Awards, Spain.
- "Premio FIDEM" in the Technology category, International Foundation for Women Entrepreneurs, Spain.
- "Premio Influentials" Lifetime Achievement Award in Science and Health, *El Confidencial* Daily, Spain.
- Medal in recognition of female talent endowed by Naturgy Foundation on World Breast Cancer Day 2021, Spain.

EXPERIMENTAL ONCOLOGY GROUP

Mariano Barbacid
Group Leader

Staff Scientists
Matthias Drosten (until September),
Raquel García-Medina (until
November), Carmen Guerra

Post-Doctoral Fellows
Sara García-Alonso, Guillem
Paniagua (until February)

Graduate Students
Gonzalo M. Aizpurua (since March),
Sara Barrambana, Oksana Brehey,

Laura de la Puente, Ana María Fernández (since October), Fernando Fernández, Jing Li, Vasiliki Liaki, Lucía Morales, Marina Salmón, Pian Sun (since October)	<i>Titulado Superior</i> (Advanced Degree) Bioinformatician Ruth Álvarez (since March) Administrative Assistant Patricia T. Guerra	Undergraduate Students Lucia Fuentes (February-June) (Master's Thesis, <i>Universitat Rovira i Virgili</i> , Tarragona, Spain), Irene Herruzo (since October) (BS Thesis, <i>Universidad Autónoma de Madrid</i> , Spain), Blanca Rosas (since October) (Undergraduate trainee, <i>Universidad Complutense de Madrid</i> , Spain)	External Associates Alfredo Carrato (<i>Hospital Ramón y Cajal</i> , Madrid, Spain), Monica Musteanu (<i>Universidad Complutense de Madrid</i> , Spain), Bruno Sainz (<i>Universidad Autónoma de Madrid</i> , Spain), Juan Velasco (<i>Eli Lilly</i> , Alcobendas, Spain)
Technicians M. Carmen González (TS), Silvia Jiménez, Marta San Román, Raquel Villar			

OVERVIEW

We recently demonstrated that ablation of RAF1 induces significant levels of tumour regression in mice bearing lung adenocarcinomas induced by KRas/Tp53 mutations. We observed similar results in mice bearing pancreatic tumours providing that RAF1 ablation is combined with elimination of the EGF Receptor. We are now attempting to translate these observations to a pharmacological scenario. Unfortunately, none of the available putative RAF1 inhibitors has shown antitumour activity in the clinic. Therefore, we decided to interrogate by genetic means the best strategy to block RAF1 activity. Unexpectedly, expression of 2 kinase dead isoforms of RAF1 failed to exhibit therapeutic activity, indicating that RAF1 does not contribute to tumour development via its kinase activity. Hence, pharmacological targeting of RAF1 will require the use of other strategies such as the use of degrons, small chemotypes capable of inducing the degradation of their target proteins.

The *KRAS* locus encodes 2 protein isoforms, KRAS4A and KRAS4B, which differ in intracellular trafficking and location in the plasma membrane. *KRAS* mutations in human cancer affect both protein isoforms. Efforts to selectively target the KRAS4B isoform are under development. We have observed that expression of the endogenous KRAS4A mutant oncoprotein is sufficient to induce lung adenocarcinomas. Hence, effective therapeutic strategies against KRAS mutant tumours must take into account inhibition of both protein isoforms.

"The protein kinase activity of RAF1 is dispensable for KRAS induced tumour development."

"The oncogenic form of the KRAS4A protein isoform is sufficient to induce lung adenocarcinomas that undergo proximal metastasis."

RESEARCH HIGHLIGHTS

RAF1 kinase activity is not required for KRAS/p53-driven tumour progression

We generated mouse strains that express conditional knocked-in alleles that encode 2 independent RAF1 kinase dead isoforms, RAF1^{D468A} and RAF1^{K375M} (FIGURE 1A). Surprisingly, systemic expression of these kinase dead isoforms under the control of the endogenous *Raf1* locus in mice bearing advanced *Kras/Trp53* tumours failed to induce tumour regression (FIGURE 1B). Previous studies indicated that, in addition to its role in MAPK signalling, RAF1 has anti-apoptotic activity. This effect is mediated, at least in part, by its ability to inhibit the pro-apoptotic kinases ASK1 and MST2. Moreover, *in vitro* studies suggested that this anti-apoptotic activity is likely to be kinase-independent. To interrogate whether the anti-proliferative effect of ablating RAF1 is mediated by these kinases, we blocked proliferation of human A549 lung adenocarcinoma cells as well as of cells obtained from a patient-derived xenograft (PDX) model, PDX-dc1, with lentiviral vectors expressing 2 independent shRNAs against *RAF1* (FIGURE 1C). Co-infection of these *RAF1* shRNAs with two independent shRNAs against *ASK1* or *MST2* restored the proliferative properties of these human lung tumour cells (FIGURE 1D). Re-expression of a cDNA encoding the murine RAF1 protein, whose sequences could not be recognised by the human *RAF1* shRNAs, restored proliferation of the A549 and PDX-dc1 cells to levels similar to those observed upon co-infection with *ASK1* or *MST2* shRNAs. These results demonstrate that the anti-proliferative effect of silencing RAF1 expression in human lung adenocarcinoma cells is mediated by the pro-apoptotic properties of ASK1 and MST2. These results, taken together, have important implications for the design of effective therapeutic strategies to block progression of *KRAS* mutant human cancers. They also help to explain, at least in part, the poor results obtained so far in the clinic with RAF inhibitors that either block their kinase activity or other features that regulate their involvement in MAPK signalling. In summary, these studies strongly suggest that current therapeutic strategies based on inhibition of RAF1 kinase activity are unlikely to produce anti-tumour results in the clinic. Instead, pharmacological targeting of RAF1 will require novel strategies that prevent the anti-apoptotic activity of RAF1, either by blocking its interaction with the ASK1 or MST2 kinases or, more directly, by inhibiting RAF1 expression with selective RAF1 degraders.

KRAS4A induces metastatic lung adenocarcinomas *in vivo* in the absence of the KRAS4B isoform

In mammals, the *KRAS* locus encodes 2 protein isoforms, KRAS4A and KRAS4B, which differ only in their extreme C-terminus via alternative splicing of distinct fourth exons. Previous studies have shown that whereas *KRAS* expression is essential for mouse development, the KRAS4A isoform is expendable. To unveil the unique properties of the KRAS4A isoform, we generated a mouse strain that carries a point mutation in exon 4B that causes the selective degradation of KRAS4B while leaving KRAS4A expression unaffected. Mice selectively lacking KRAS4B developed to term but died perinatally due to hypertrabeculation of the ventricular wall, a defect reminiscent of that observed in midgestation embryos lacking the *Kras* locus. Introduction of an oncogenic mutation (G12V) into the *Kras*^{FSFG12V} allele allowed expression of an endogenous KRAS4A^{G12V} oncogenic isoform in the absence of KRAS4B. Exposure of *Kras*^{+/FSF4AG12V4B-} mice to Adeno-FLPo induced lung tumour formation with complete penetrance, albeit with increased latencies than control *Kras*^{+/FSFG12V} animals that expressed both oncogenic isoforms. Interestingly, a significant percentage of these mice developed proximal metastasis, a feature seldom observed in mice expressing both mutant isoforms, probably due to their shorter tumour latencies. These results illustrate that expression of the KRAS4A^{G12V} mutant isoform is sufficient to induce lung tumours, thus indicating that effective anti-tumour strategies against *KRAS* mutant tumours must take into account inhibition of both protein isoforms. ■

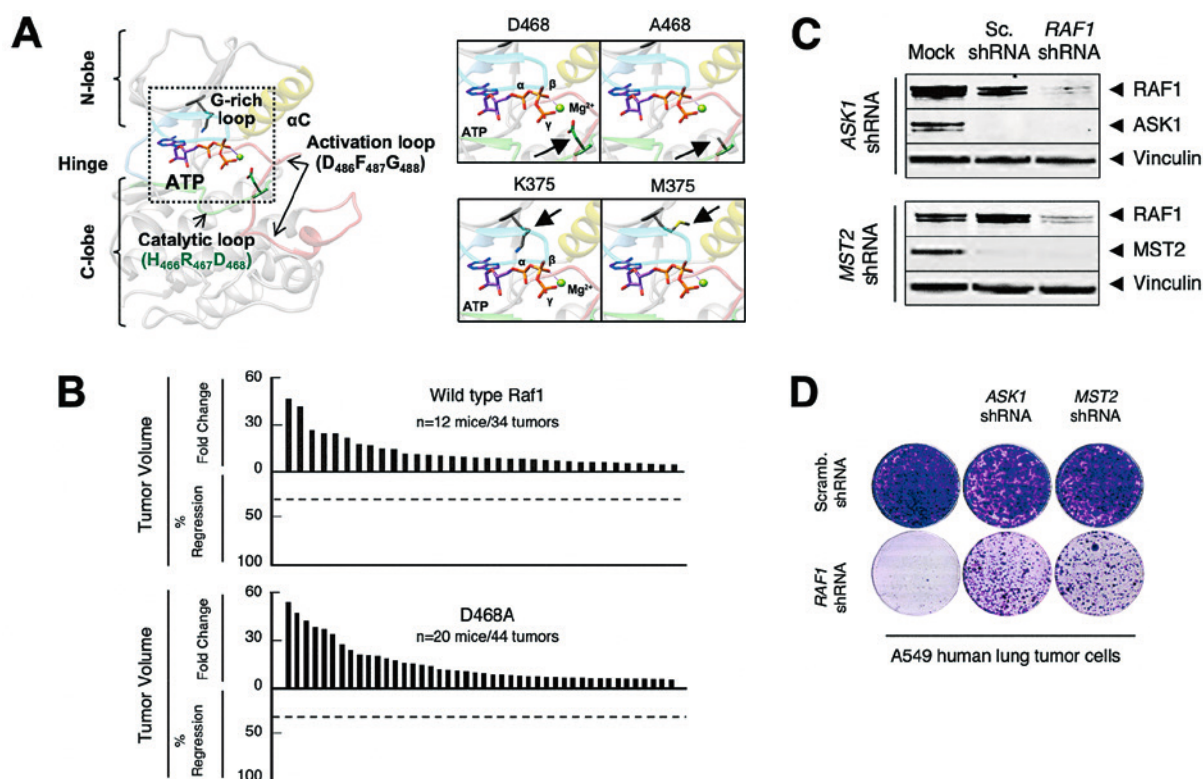


FIGURE 1 RAF1 kinase activity is dispensable for KRAS/p53-driven tumour progression. (A) Human RAF1 kinase domain structure highlighting the position of the D468 residue along with its respective mutations A468. (B) Waterfall plots

representing the change in tumour volume of individual tumours expressing the wild type RAF1 or the mutant RAF1D468A proteins in mice exposed to a tamoxifen (TMX) containing diet for 2 months. (C) Western blot analysis of RAF1, ASK1

and MST2 expression in lysates obtained from A549 human lung tumour cells either not infected or infected with representative shRNAs against ASK1 (top panels) or MST2 (bottom panels) in combination with either a scrambled shRNA or a

representative shRNA against RAF1. Vinculin was used as loading control. (D) Colony formation in A549 human lung tumour cells infected with a scrambled shRNA and representative shRNAs against RAF1, ASK1 and MST2 sequences.

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- Assi M, Achouri Y, Lorient A, Dauguet N, Dahou H, Baldan J, Libert M, Fain JS, Guerra C, Bouwens L, Barbacid M, Le-maigre FP, Jacquemin P (2021). Dynamic regulation of the expression of KRAS and its effectors determines the ability of pancreatic acinar cells to initiate tumorigenesis. *Cancer Res* 81, 2679-2689.

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- García-Alonso S, Romero-Pérez I, Gándullo-Sánchez L, Chinchilla L, Ocaña A, Montero JC, Pandiella A (2021). Altered proTGFα/cleaved TGFα ratios offer new

therapeutic strategies in renal carcinoma. *J Exp Clin Cancer Res* 40, 256.

PATENT

- Barbacid M, Guerra C, Blasco MT, Navas C (2018). Combined therapy against cancer. WO 2020/020942.A1. National phase entry (2021).

AWARDS AND RECOGNITION

- Premio Fundación CRIS contra el Cáncer, CRIS Foundation, Spain.
- Inaugural Conference, SEMERGEN National Congress, Zaragoza, Spain.
- Keynote Lecture, IV International Workshop on Genomic Testing in Cancer, Pamplona, Spain.

CELL DIVISION AND CANCER GROUP

Marcos Malumbres
Group Leader

Post-Doctoral Fellows
Begoña Hurtado, Miguel Ruiz (since
July), Carolina Villarroja

Graduate Students
Gloria Cristina Bonel (since Sept.),
Mariona Cubells (since March) (PEJ,
CAM) ; Alejandro García (since Nov.),
José González, Fátima Guerra (since
Dec.), Luis Rodrigo López, Diego



Martínez (until Dec.), Borja Pitarch <i>'Plan de Empleo Joven de la CAM</i> (Youth Employment Plan, Community of Madrid) Technicians Cristina Aguirre (TS)" (since June),	Irene Díaz (since Feb.), Aicha El Bakkali <i>"Titulado Superior</i> (Advanced Degree) Students in Practice María Franco (Feb.-Aug.) (<i>Univ. Carlos III de Madrid</i> , Spain), Mónica Novo (March-July) (<i>Univ. Rovira i</i>	<i>Virgili</i> , Tarragona, Spain), Anna Melati (Feb.-Sept.) (ERASMUS + Fellow, <i>Università di Pavia</i> , Italy), María Fernanda Pérez (since Oct.) (<i>Univ. Autónoma de Madrid</i> , Spain), Iciar Luna (since Oct.) (<i>Univ. Complutense de Madrid</i> , Spain)	Visiting Scientists Ana Sánchez (since February) (<i>Hospital Universitario 12 de Octubre</i> , Madrid, Spain), Senn Wakahashi (Kobe University, Kobe, Japan)
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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: i) to understand the basic control mechanisms that regulate the cell division cycle; ii) to characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) understanding self-renewal and pluripotency in stem cell biology and tumour development; and iv) improving 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 generated a new platform of breast cancer patient-derived samples and studied new therapeutic uses of inhibiting CDKs in breast, lung and liver cancer."

RESEARCH HIGHLIGHTS

Cell cycle inhibition in cancer therapy

Cell cycle deregulation is a common feature of cancer cells. Many tumour-associated mutations lead to dysregulation of the machinery that controls cell division and, in particular, of cyclin-dependent kinases (CDKs), considered to be the engines of cell proliferation. Inhibition of some CDKs, such as CDK4 and CDK6, is currently part of the standard-of-care for the treatment of hormone receptor-positive, metastatic breast cancer. We recently generated data suggesting, both *in vitro* and *in vivo*, that applying CDK4/6 inhibitors right after classical chemotherapy strongly cooperates in preventing tumour cell proliferation (Salvador-Barbero *et al.*, 2020) and are now analysing the mechanism behind these observations both in breast and lung cancer cells. Furthermore, we described that CDK4/6 activity is required for homologous recombination and DNA repair, as well as for the recovery from chromosomal damage imposed by DNA damaging agents. We are currently testing various cellular pathways involved in sensing DNA and chromosomal damage, as well as possible new combinations between CDK4/6 inhibitors and inhibitors of these pathways.

A new platform of breast cancer patient-derived models

Despite the success of CDK4/6 inhibitors in breast cancer treatment, many patients with metastatic tumours develop resistance to these therapies. The lack of biomarkers to predict sensitivity to them makes it difficult to select patients. In addition, the molecular basis of resistance to CDK4/6 inhibitors is still under debate. To study these questions in detail, we started a collaboration with the team led by E. Ciruelos at the *Hospital 12 de Octubre* to generate a platform of cellular models derived from breast cancer patients who are candidates for, or are subjected to, cell cycle therapies. In addition to the primary biopsy of the metastatic tumour, we are isolating circulating tumour cells from the blood at different time points before or after starting CDK4/6-targeted therapies (FIGURE 1). Generating these temporal series of models will be critical to understanding the molecular changes during treatment with these inhibitors and the origin of the resistant clones. Our ultimate goal is to predict the resistance events with the appropriate biomarkers, and/or to propose combinatorial treatments effective against the resistant clones.

Understanding the effect of cell cycle dysregulation 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 were able to describe new genetic interactions leading to defective asymmetry of centrosomal components during the division of neural progenitors, microcephaly and defective brain development (FIGURE 2; González-Martínez *et al.*, 2021). Importantly, these phenotypes are also observed after inhibiting Plk1 with small-molecule inhibitors 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

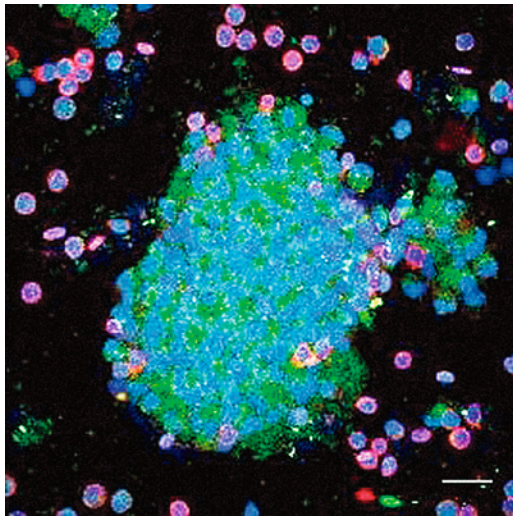


FIGURE 1 Three-dimensional structures generated *in vitro* after the culture of circulating tumour cells derived from breast cancer patients. Circulating tumour cells (in blue and green) were isolated from blood and cultured in the presence of other blood mononucleated cells (blue and red).

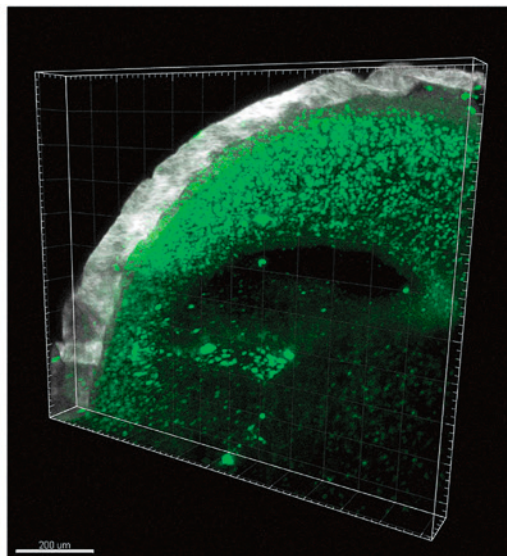
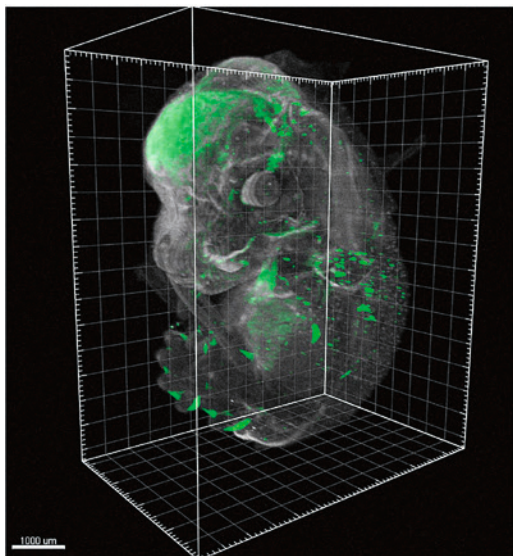


FIGURE 2 Detection of apoptotic cell death (green) in mouse embryos (left) and brain cortex (right) with specific mutations in *Plk1*, *Cep135* or *Cdk5rap2*. A collaboration with Jorge Almagro and Axel Behrens (Crick Institute, London). See González-Martínez *et al.*, *JCI Insights* 2021, for details.

► PUBLICATIONS

- Alfaro E, López-Jiménez P, González-Martínez J, Malumbres M, Suja JA, Gómez R (2021). PLK1 regulates centrosome migration and spindle dynamics in male mouse meiosis. *EMBO Rep* 22, e51030.
- Galarreta A, Valledor P, Ubieto-Capella P, Lafarga V, Zarzuela E, Muñoz J, Malumbres M, Lecona E, Fernandez-Capetillo O (2021). USP7 limits CDK1 activity through-

out the cell cycle *EMBO J* 40, e99692.

- González-Martínez J, Cwetsch AW, Martínez-Alonso D, López-Sainz LR, Almagro J, Melati A, Gómez J, Pérez-Martínez M, Megías D, Boskovic J, Gilbert-Juan J, Graña-Castro O, Pierani A, Behrens A, Ortega S, Malumbres M (2021). Deficient adaptation to centrosome duplication defects in neural progenitors causes microcephaly and subcortical heterotopias. *JCI Insight* 6, e146364.

- González-Martínez J, Malumbres M (2021) Expanding the differentiation potential of already-established pluripotent stem cells. *Methods Mol Biol.* PMID: 34128208.

► PATENT

- Malumbres M, Álvarez-Fernández M, Zapatero E, Salazar-Roa M, Sanchez J (2020). Pyrido[2,3-d]pyrimidin-7(8h)-ones as

CDK inhibitors. *PCT/US2021/022328*. PCT application (2021).

► AWARDS AND RECOGNITION

- Member of the Scientific Advisory Board of EU-TRANSCAN-3 (<http://transcan.eu/>).

GENOMIC INSTABILITY GROUP

Óscar Fernández-Capetillo
Group Leader

Staff Scientists
Vanesa Lafarga, Matilde Murga

Post-Doctoral Fellow
Ivó Hernández (since May)

Graduate Students
Elena Fueyo, Gema López, Jorge
Mota (since October), Belén Navarro



(since October), Anabel Sáez (since September), Laura Sánchez, Oleksandra Sirozh, Pablo Valledor

Technicians

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

**Titulado Superior (Advanced Degree)*

Visiting Students

Jorge Mota (until July) and Belén Navarro (until June) (Master's Students, *Universidad Autónoma de Madrid*, Spain)

Visiting Scientist

Raúl Rabadán (until June) (Columbia University Medical Centre, New York, USA)

OVERVIEW

The main goal the Genomic Instability Group is to understand the molecular mechanisms underlying cancer and other age-associated diseases, with the ultimate objective of translating this knowledge into effective treatments for patients. To this end, we have developed over the years several molecular tools and *in vivo* models, which have led us to make important progress in basic as well as in translational research. Among other achievements, we have extensively studied the molecular mechanisms by which cells duplicate and repair their genomes, developed new inhibitors that can be used for targeted cancer therapy, and created mouse models that revealed the physiological consequences of genomic instability. More recently, we have developed an interest in exploring the mechanisms of drug resistance in cancer therapy and how to overcome this problem in cancer, as well as in other age-related diseases lacking a cure, such as neurodegeneration.

“In 2021 we discovered that one of the most frequent mutations in cancer drives multidrug resistance and advanced in our understanding of how replisomes are dissolved once DNA replication ends.”

RESEARCH HIGHLIGHTS

Overcoming multi-drug resistance associated to FBXW7 deficiency

Drug resistance is a huge medical challenge. In cancer, it is estimated that resistance to therapy accounts for almost 90% of treatment failures. Thus, there is an urgent need to obtain a better understanding of the mechanisms behind drug resistance, which are still rather fragmented. Over the last few years, our laboratory has developed strong expertise in genome-wide CRISPR screens to identify mutations related to resistance to different cancer treatments. Throughout the course of these studies, we found sgRNAs targeting *FBXW7* as recurrent hits that provided resistance to many independent agents. We later confirmed that *FBXW7* deficiency indeed confers resistance to the vast majority of currently used antitumour agents. These observations were supported by informatic analyses indicating that *FBXW7* deficiency is among the most significant multidrug resistance mutations

that can be detected. Of note, *FBXW7* is among the top 10 most mutated genes in cancer, highlighting the relevance of our discovery. Importantly, in our study we also identified that, despite their multidrug resistance, *FBXW7* deficient cells were preferentially vulnerable to treatment with therapies that target mitochondrial activity, such as the antibiotic tigecycline (FIGURE 1). In summary, this work has revealed that one of the most frequent mutations in cancer is associated to multidrug resistance and provided some initial ideas about how this resistance might be overcome.

Extracting replisome components from chromatin when DNA replication ends

DNA replication, carried out by a large protein complex known as the replisome, requires finely-tuned regulatory mechanisms that often involve post-translational modifications such as

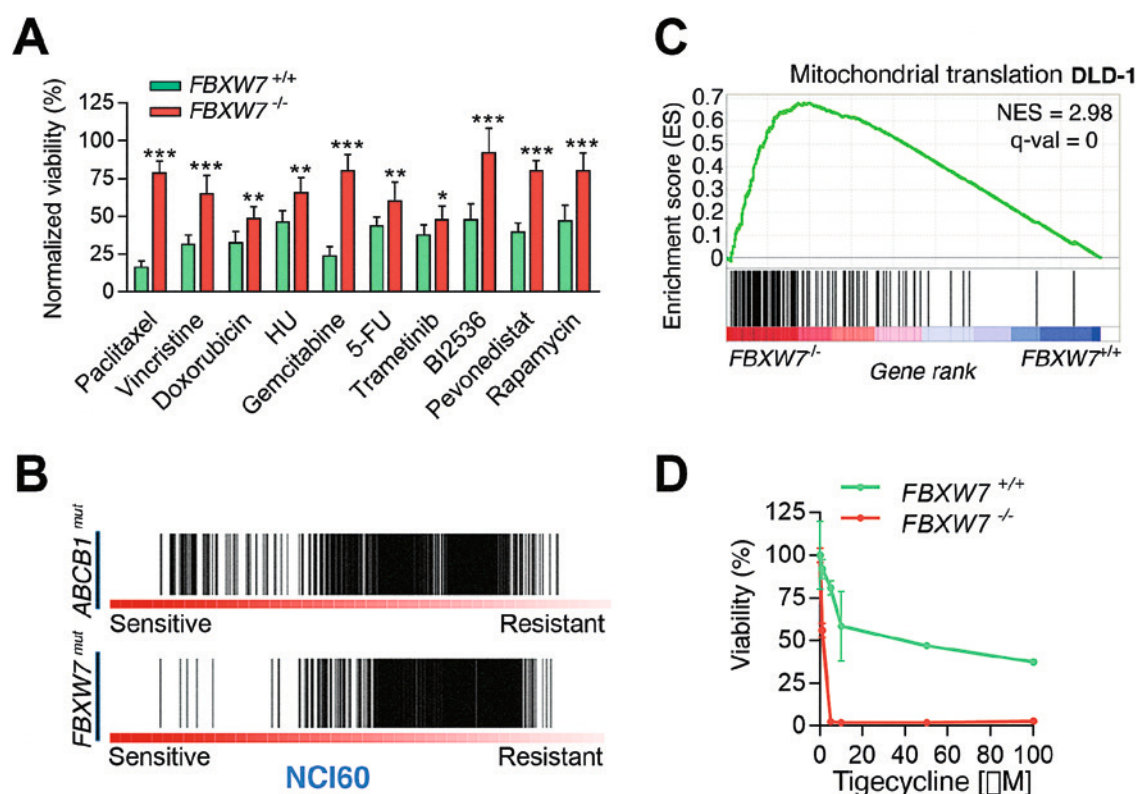


FIGURE 1 Overcoming multidrug resistance. (A) Effects of the indicated drugs on the viability of *FBXW7* wild type and knockout DLD-1 human colon adenocarcinoma-derived cell lines. (B) Bioinformatic analysis of drug

responses associated to mutations in the drug efflux pump *ABCB1* or in *FBXW7*. (C) GSEA analysis of the “Mitochondrial translation” hallmark, done with proteomics data from *FBXW7*^{+/+} and *FBXW7*^{-/-} DLD-1 cells.

(D) Viability of *FBXW7*^{+/+} and *FBXW7*^{-/-} DLD-1 cells exposed to increased doses of the antibiotic tigecycline at the indicated doses.

SUMOylation or ubiquitination. A few years ago, while performing one of the first proteomic characterisations of the human replisome, our group found an intriguing feature: whereas replisomes are SUMO-high/Ubiquitin-low environments, mature non-replicating chromatin displays the opposite trend. Later, we discovered that this balance is maintained in part by the action of the SUMO deubiquitinase USP7 that maintains this ubiquitin-low environment and is essential for DNA replication. We also discovered that USP7 inhibition mimics the end of DNA replication and simultaneously drives CDK1 activation in such a manner that is toxic and that can help to understand the antitumour effects of USP7 inhibitors. During 2021, we resolved what happens

to the replisome components that become ubiquitinated upon USP7 inhibition. We now know that these factors become extracted from chromatin by the segregase VCP, which uses FAF1 as an adaptor to bind SUMOylated and ubiquitylated factors (FIGURE 2). Noteworthy, equivalent conclusions were drawn from a genetic screen completed in *C. elegans* by our collaborator Thorsten Hoppe, highlighting the evolutionary conservation of this pathway. In this line of research, we are now particularly intrigued by the mechanisms that drive CDK1 activation upon the completion of DNA termination, as we believe this is still one of the key missing pieces from our basic understanding of the cell cycle. ■

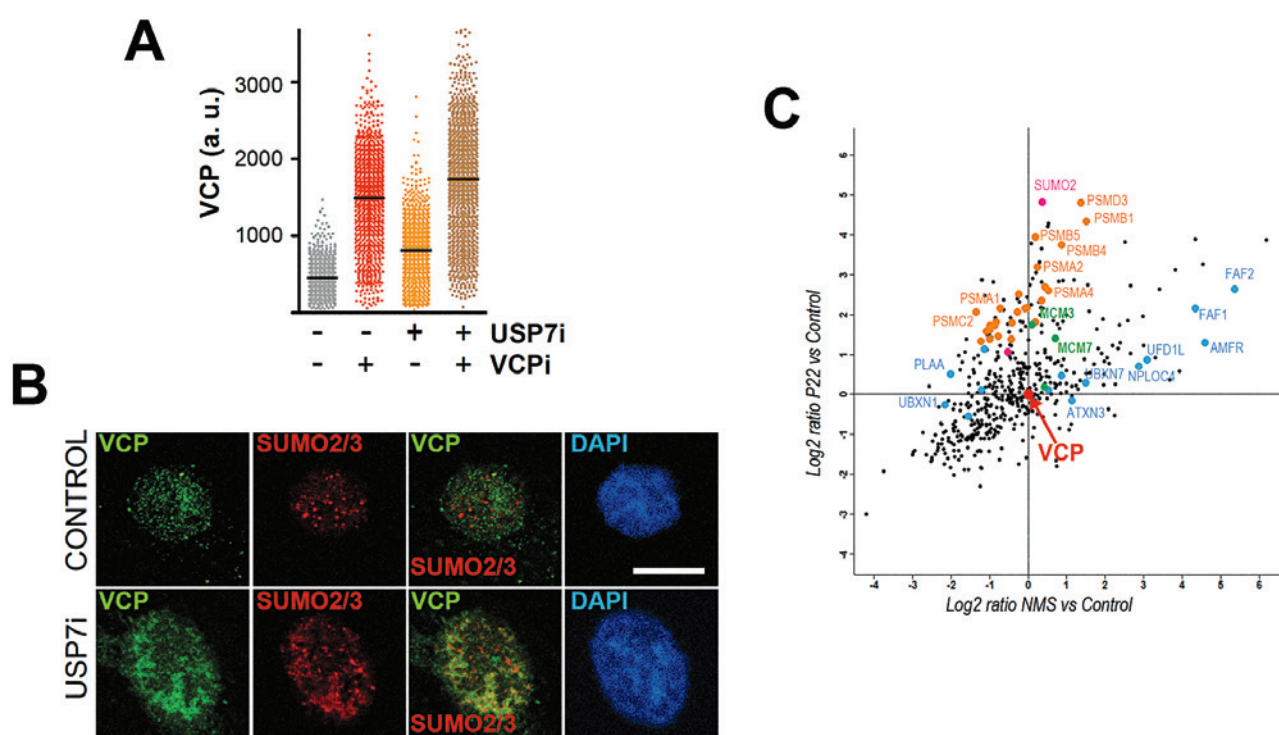


FIGURE 2 FAF1 recruits VCP to SUMO/ubiquitylated chromatin. (A) High-throughput microscopy illustrating the increase in chromatin-bound levels of VCP upon USP7 inhibition. (B) Distribution of

chromatin-bound SUMO2/3 and VCP upon USP7 inhibition in U2OS cells. Note the accumulation of VCP in areas that become enriched in SUMOylated factors. (C) Proteomics of the VCP-interactome upon inhibition of USP7

(P22) or VCP (NMS). The analyses revealed enrichment of the VCP adaptors FAF1 and FAF2 upon either form of inhibition, as well as a selective increase in the binding of VCP to SUMO2 upon USP7 inhibition.

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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 uncovered a novel mechanism of transcriptional regulation that allows quick changes in gene expression and has implications in the control of cellular proliferation and cancer progression.”

RESEARCH HIGHLIGHTS

Accumulation of topological stress in the form of DNA supercoiling is inherent to the advance of RNA polymerase II (Pol II) and needs to be resolved by DNA topoisomerases to sustain productive transcriptional elongation, and therefore the correct expression of genes. Topoisomerases are therefore traditionally considered general positive facilitators of gene expression, especially for long genes in which the load of DNA supercoiling can become particularly burdensome. However, work in our laboratory and others has shown that topoisomerases accumulate at genomic regulatory regions such as enhancers and promoters, suggesting a potential regulatory function for DNA topoisomerases, beyond being mere topological “problem-solvers”.

We unexpectedly found that catalytically inhibiting one of the main cellular DNA topoisomerases (TOP2A) caused a dramatic and acute upregulation of immediate early response genes (FIGURE 1). These genes (IEGs) are normally characterised by quickly responding to different types of cellular stimuli and triggering the subsequent transcriptional waves that control important functions such as neuronal activation or cellular proliferation. Interestingly, the response observed was directly caused by the absence of TOP2A function and not by a response to some kind of topological stress or DNA damage being generated.

Intrigued by these surprising results, pointing to repressive regulatory roles of TOP2A, we developed novel methods to measure topoisomerase activity at specific genomic locations in cells, and combined them with the analysis of supercoiling and genome-wide transcription in cell lines deficient for different DNA topoisomerases engineered with CRISPR-Cas9 technology.

The results obtained allowed us to propose a novel model for transcription regulation based on the control of DNA supercoiling at promoter regions (FIGURE 2). By removing transcription-associated negative supercoiling from promoters, TOP2A ensures the resetting of the topological status after each transcriptional cycle, so transcription occurs in a regulated and controlled manner. When TOP2A activity is limiting or overwhelmed, the accumulated negative supercoiling at the promoters facilitates transcription of subsequent cycles, in a positive feedback loop that results in the typical transcriptional bursts that characterise IEG expression.

These results open up the possibility of modulating topoisomerase activity and DNA supercoiling to regulate IEG expression, and therefore to control cellular proliferation and responses to different types of stimuli, and will need to be taken into account in chemotherapeutic regimens that currently use topoisomerase inhibitors. ■

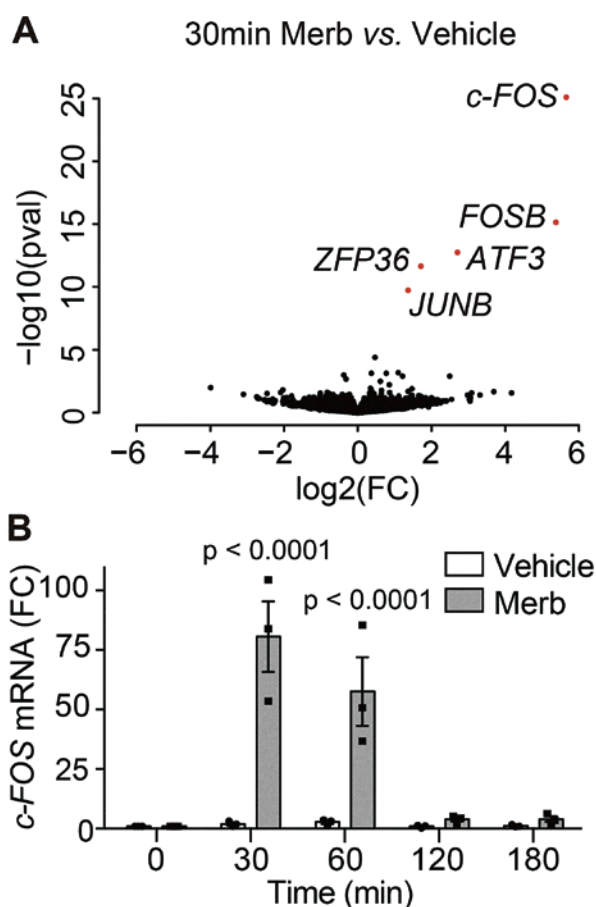


FIGURE 1 Immediate early genes (IEGs) are upregulated upon TOP2 inhibition. **(A)** Global transcriptional changes upon merbarone treatment. **(B)** Transcription of the IEG *c-FOS* at the indicated times following treatment with merbarone.

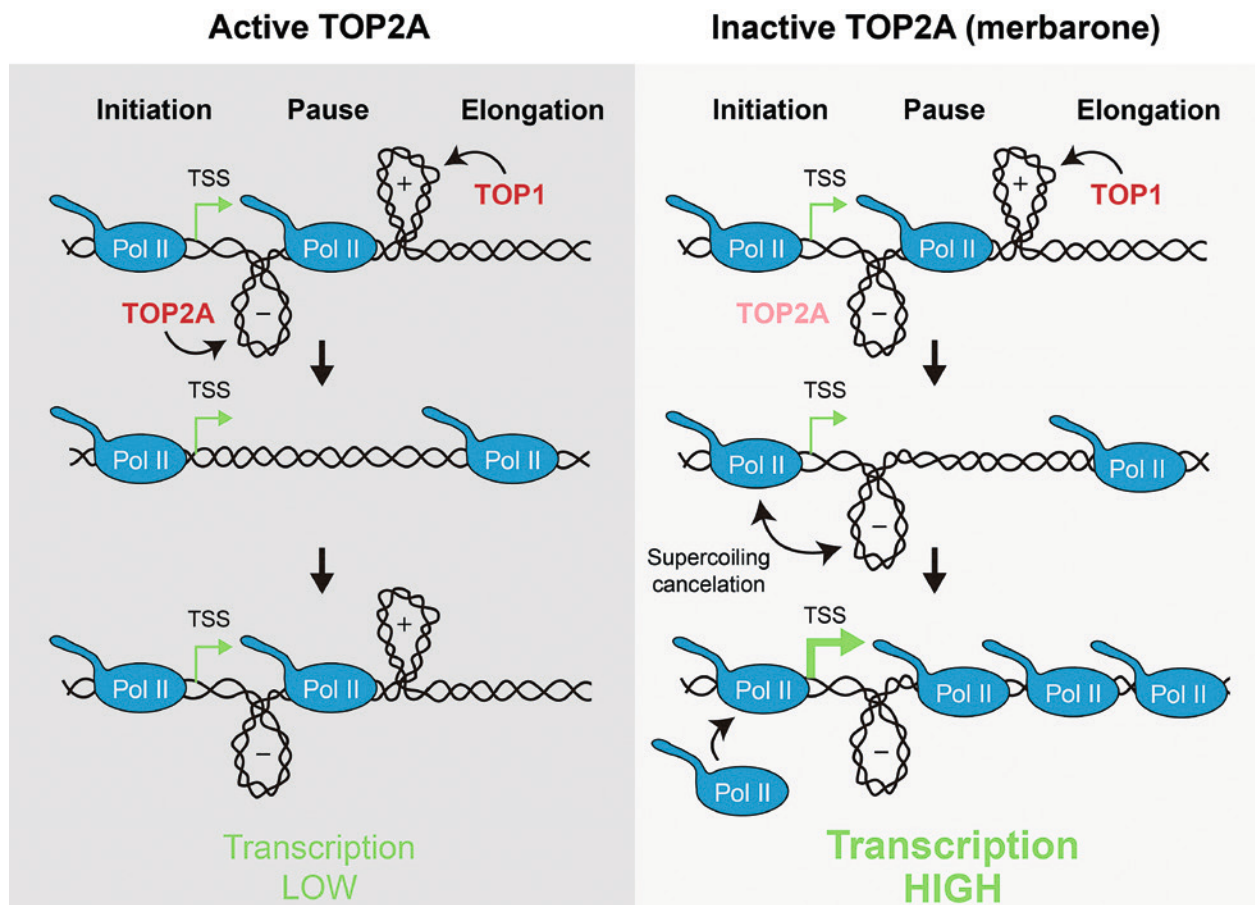


FIGURE 2 Model of supercoiling-mediated transcriptional regulation. TOP2A activity resets the topological context at promoters, maintaining transcription under controlled conditions (left). Upon TOP2A inhibition (right), (-) DNA supercoiling accumulates at promoters, facilitating the advance of the following Pol II complexes, and resulting in a transcriptional burst.

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OVERVIEW

Our research focuses on a protein complex named cohesin that embraces DNA to mediate sister chromatid cohesion, a process essential for chromosome segregation and faithful DNA repair by homologous recombination. Cohesin also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation. 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 are investigating the contribution of mutations in cohesin *STAG2* to the aggressive phenotype of Ewing sarcoma, the second most common bone cancer in children, in order to improve diagnosis and treatment of these tumours.”

RESEARCH HIGHLIGHTS

Distinct contribution of variant subunits and regulators to genome-wide distribution and dynamics of cohesin

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 CTCF bound sites (FIGURE 1A). 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 variants of the cohesin complex in all somatic vertebrate cells that carry SMC1A, SMC3, RAD21 and either STAG1 or STAG2. The association of these complexes to chromatin is modulated by additional proteins: NIPBL, PDS5A/B, WAPL and ESCO1/2 acetyltransferases. Our studies in human and mouse cells deficient for STAG1 or STAG2 have identified differential contributions of the two complexes to genome architecture and transcriptional regulation. Cohesin-STAG1 plays a more important role in the demarcation of TADs, together with CTCF, and in counteracting compartmentalisation. Cohesin-STAG2 promotes more local chromatin contacts that are relevant for tissue-specific transcription independently of CTCF. Consistent with this, STAG1 is found almost exclusively at CTCF-bound sites while a fraction of STAG2 can be also detected at non-CTCF, NIPBL-bound cohesin positions along the genome (FIGURE 1B). Salt extraction of chromatin fractions and fluorescence recovery after photobleaching (FRAP) experiments show that binding of cohesin-STAG2 to chromatin is more salt sensitive and more dynamic, respectively, than binding of cohesin-STAG1. One factor contributing to this behaviour is the preferential association of STAG2 with cohesin releasing factor WAPL. We continue to explore the molecular determinants underlying these preferences and how they contribute to shape chromatin architecture.

Cohesin and disease

We address the role of cohesin in disease in two different lines of research. In the first one, we are interested in the consequences of cohesin dysregulation during development. Our analyses of murine embryos lacking STAG1 or STAG2 have revealed their differential requirements during embryonic development, which lead to lethality by mid-gestation. We plan to complete these studies by examining early developmental stages *in vivo* (FIGURE 2). *Ex vivo*, we are dissecting the contribution of each cohesin variant to mouse embryonic stem cell differentiation using the auxin-dependent degron technology. We have also collaborated with the group of Miguel Manzanares (CBMSO) to show that CTCF is required to establish proper chromatin structure in early embryos. Finally, we are investigating the pathophysiology of Cornelia de Lange Syndrome (CdLS), the most prevalent cohesinopathy, in collaboration with the group of Ethel Queralt (IDIBELL). Consistent with our previous analyses in mouse cells deficient for the cohesin loader NIPBL, studies in fibroblasts from CdLS patients show altered distribution of cohesin and transcriptional dysregulation.

The second line of research addresses how mutations in *STAG2* promote metastasis in Ewing sarcoma (EWS). This is the second most frequent type of bone cancer in children, and it is driven by a fusion protein that 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 cohesin *STAG2*. Importantly, *STAG2* mutations are often present in the most aggressive EWS tumours. From the bioinformatic analysis of transcriptomic data from EWS patients and cell lines, we have identified a gene signature dependent on *STAG2* mutation that correlates with poor survival. We are currently exploring the contribution of these genes to the metastatic phenotype of EWS cells and its potential use as a diagnostic tool. ■

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Lange Syndrome provokes cohesin genome-wide redistribution with an impact on the transcriptome. *Nat Commun* 12, 4551.

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FIGURE 1 (A) Loop extrusion by cohesin and its associated factors. (B) Distribution of these proteins in a region of the murine genome. CTCF and non-CTCF cohesin positions are indicated by white and black arrows, respectively. Data come from ChIP-seq experiments in mouse embryo fibroblasts.

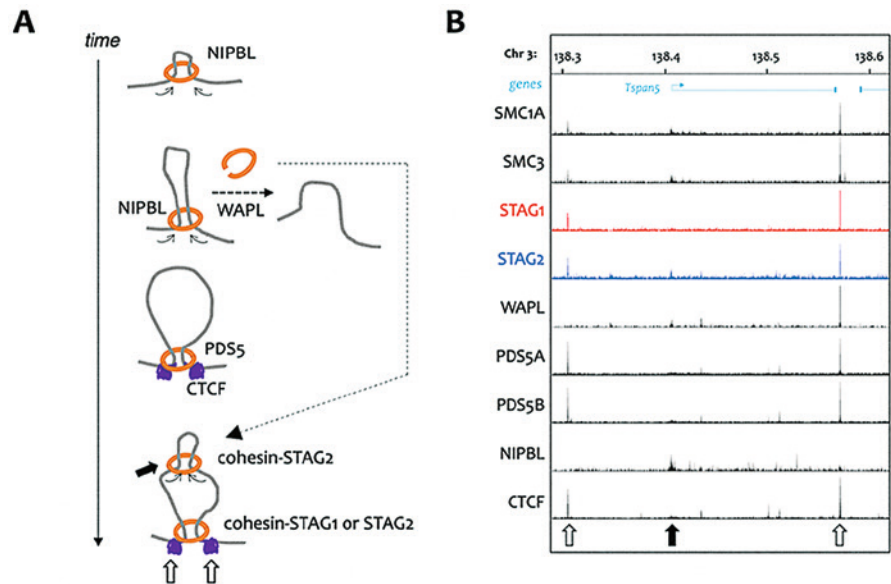
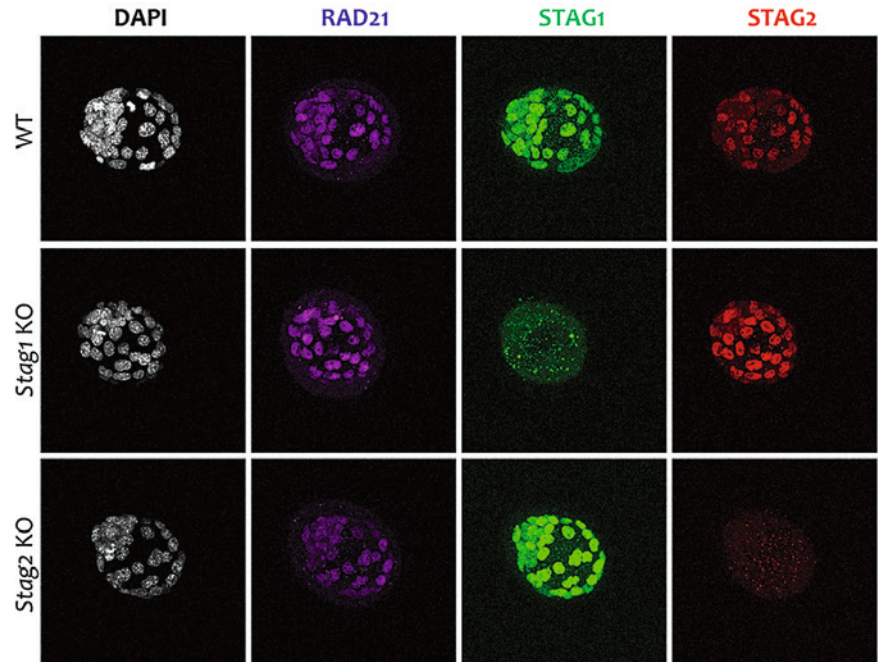


FIGURE 2 Images of preimplantation embryos (E3.5) either wildtype (WT) or knock out (KO) for cohesin *Stag1* or *Stag2* stained with antibodies against a common cohesin subunit (RAD21), the two STAG subunits and DAPI (for DNA).



DNA REPLICATION GROUP

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OVERVIEW

DNA replication is an essential feature of life but it entails molecular risks ranging from the introduction of mutations to the generation of breaks and chromosomal re-arrangements that promote tumorigenesis. “Replicative stress”, defined as the temporary difficulty of replisome proteins to make a copy of the original DNA, is exacerbated by environmental agents that modify the DNA chemical structure, including UV light, ionising radiation, chemicals in tobacco, and other pollutants. The efficacy of cisplatin, mitomycin C, and other chemotherapy drugs actually relies on the extensive introduction of DNA lesions that cannot be replicated or repaired. In recent years we have focused on the cellular responses to DNA lesions induced by chemotherapy, such as intra-strand and inter-strand crosslinks. In 2021 we continued to investigate the fundamental mechanisms that govern DNA replication in normal and cancer cells, and completed 2 studies about the “re-priming” mechanism activated in response to many DNA lesions.

“We have identified the function of PrimPol and RPA proteins in the replicative tolerance and repair of DNA inter-strand crosslinks.”

RESEARCH HIGHLIGHTS

Replicative tolerance mediated by repriming of DNA synthesis

Replicative DNA polymerases and their associated factors (the “replisome”) are challenged by different obstacles including G-quadruplexes, stable DNA-RNA hybrids (R-loops) and DNA alterations caused by endogenous and exogenous agents. In response, cells activate “replicative tolerance” or “DNA damage tolerance” (DDT) mechanisms that facilitate DNA synthesis through these hard-to-replicate structures. Classical DDT responses include error-prone polymerases capable of trans-lesion synthesis and the recombination-related “template-switch” mechanism. In recent years, our Group has focused on another DDT pathway that involves the bypass of the lesion/obstacle by re-initiating DNA synthesis from a downstream point. At the molecular level, re-initiation strictly depends on repriming, i.e., the synthesis of new primer molecules by PrimPol, the second primase encoded by the human genome. Through our own studies, and in collaboration with other groups, we have reported that PrimPol-dependent repriming is responsible for the bypass of UV-generated photo-adducts (Mourón *et al.*, *Nat Struct Mol Biol* 2013), cisplatin-induced intra-strand crosslinks (Quinet *et al.*, *Mol Cell* 2020), and bulky DNA adducts induced by benzopyrenes (Piberger *et al.*, *Nat Commun* 2020). In 2021 we completed a new study showing that PrimPol-mediated repriming plays a key role in the tolerance and subsequent repair of inter-strand crosslinks (ICLs). We also gained new insights into the biochemical characteristics of the primase reaction mediated by PrimPol.

Repriming at DNA inter-strand crosslinks

Previous studies about the repriming mechanism had focused on DNA obstacles and lesions affecting one of the 2 DNA strands. In our most recent study (Gonzalez-Acosta *et al.*, 2021) we describe a new function for PrimPol at ICLs, in which both strands of the double helix are covalently linked. ICL repair is coupled to DNA replication and involves a large group of proteins called the Fanconi Anaemia (FA) pathway, because their inactivation causes this severe disease: FA patients suffer from bone marrow failure and are predisposed to leukaemia and other tumours.

Until recently, ICLs were considered absolute blocks for replication proteins, and the signal to initiate their repair was believed to rely on the convergence of 2 replication forks coming from both sides of the lesion. In 2013, M. Seidman’s group (NIH, Bethesda, MD, USA) identified individual forks that managed to “traverse” ICLs, a finding that was

controversial because the crosslink prevents DNA unwinding. We have now reported that fork traverse is mediated by PrimPol-mediated repriming, acting in cooperation with ssDNA binding protein RPA and the FANCM translocase complex. The structure generated around the ICL by the repriming mechanism is similar to the structure generated by the convergence of 2 forks, and also triggers the recruitment of ICL repair factors. The choice between fork traverse or convergence mechanisms depends on the availability of dormant replication origins in the vicinity of each ICL. The replicative pathways leading to the initiation of ICL repair are summarised in FIGURE 1.

Because PrimPol counteracts the cytotoxic effect of DNA crosslinks, its inactivation might enhance the effect of cisplatin in chemotherapy. We continue to screen for PrimPol small molecule inhibitors in collaboration with the CNIO Experimental Therapeutics Programme.

An amino acid motif in PrimPol essential for its primase activity

We are also interested in structure-function studies of PrimPol, in a collaborative effort with L. Blanco’s group (*Centro de Biología Molecular “Severo Ochoa”*, Madrid). In 2021 we completed a study showing that Trp87 and Tyr90, 2 invariant amino acids within conserved motif WYFF, are required to stabilise the incoming nucleotides at the active site. Consequently, PrimPol mutant versions W87G and Y90D display deficient primase activity *in vitro* and repriming activity *in vivo* (Calvo *et al.*, 2021).

Other projects in the DNA Replication Group

Besides the topics described above, our Group is pursuing other lines of research: (i) the study of replication origins in human cells through genome-wide SNS-Seq and CUT&RUN techniques; (ii) the analysis of replicative dynamics in embryonic stem cells with different levels of pluripotency; and (iii) the identification of genes that restrict DNA over-replication in the event of aberrant reactivation of replication origins. ■

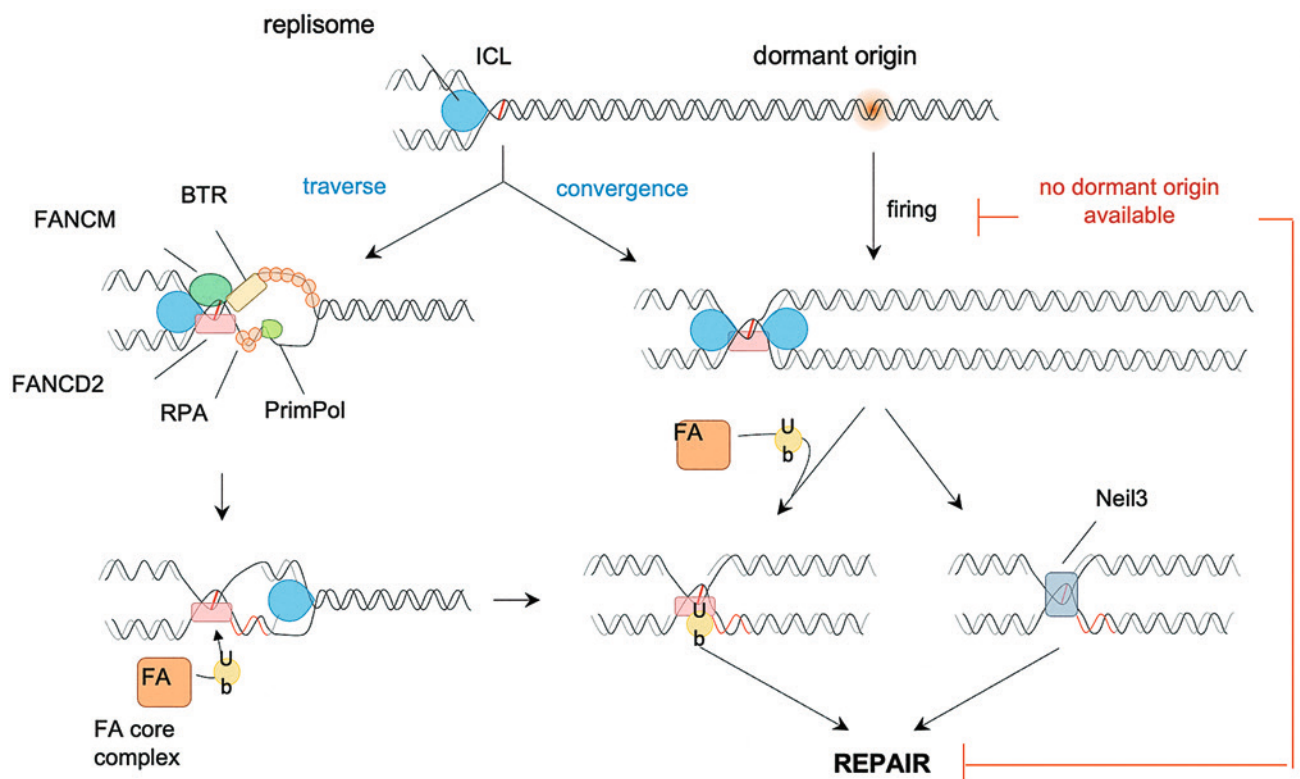


FIGURE 1 PrimPol-mediated ICL traverse and fork convergence mechanisms to initiate ICL repair. See text for details. Adapted from González-Acosta *et al.* (2021).

PUBLICATIONS

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OVERVIEW

Melanomas are inherently aggressive cancers for which 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 tumour biomarkers and to validate more effective 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 the first-in-class lymphoreporter (*MetAlert*) mice for non-invasive imaging of pre-metastatic niches in melanoma (*Nature*). These systems led to the identification of new mechanisms of immune resistance (*Nature Medicine*) and the generation of nanoparticle-based treatments (*Cancer Cell*, *EMBO Mol. Med*), with derivatives now being tested in clinical trials. 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

An increasing number of (epi)genetic alterations and mechanisms of immune evasion have been identified in malignant melanomas. Nevertheless, no molecular biomarker has been approved as a *bona fide* prognostic indicator. The field is also in need of improved treatments, as a significant fraction of patients is resistant to targeted and immune-based therapies. The long-term goal of our Group is to define and target drivers of this aggressive behaviour. Our main aims are:

- To define when and how melanomas act “at a distance” before tumour cell dissemination.
- To determine how melanoma cells evade the immune system, and whether distinct mechanisms may be activated at different anatomical sites.
- To develop anticancer agents to prevent and eliminate metastatic sites.

New immune suppressors that favour melanoma progression

One of the long-term objectives of the Melanoma Group is to discover **new melanoma drivers**. We previously identified a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Curbelo *et al.*, *Cancer Cell* 2014). Further analyses of lysosomal-dependent pathways also revealed distinctive features of autophagy genes (ATG5) and RNA binding proteins (CPEB4,

CELF1 and IGF2BP1) with selective roles in melanoma (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 could exert long-range activities at visceral organs, particularly in the generation of premetastatic niches. Our Group pioneered the analysis of such systemic effects *in vivo* by exploiting melanoma **MetAlert** mice, which have the unique feature of visualising tumour-activated lymphatic vasculature (Olmeda *et al.*, *Nature* 2017).

MetAlert animals, in combination with human tissue specimens, have revealed the growth factor MIDKINE (MDK) as a new driver of melanoma metastasis. Further functional studies in animal models and expression analyses in large patient cohorts have shown yet another function of MDK in immune suppression. Specifically, we found that MDK promoted an immunotolerant microenvironment whereby macrophages are recruited to tumours, but instead of attacking the cancer cells, promote a dysfunctional state in CD8⁺ T cells, ultimately favouring immune escape (Wallis *et al.*, *Nature Medicine* 2020). More recently, we have demonstrated a broader impact of MDK on the immune system in other immune cell types, with global implications for antigen presentation. **MetAlert** mice is part of large collaborative efforts in the melanoma community to develop better models for gene discovery and testing of pharmacological agents (Patton *et al.*, *Cancer Cell* 2021).

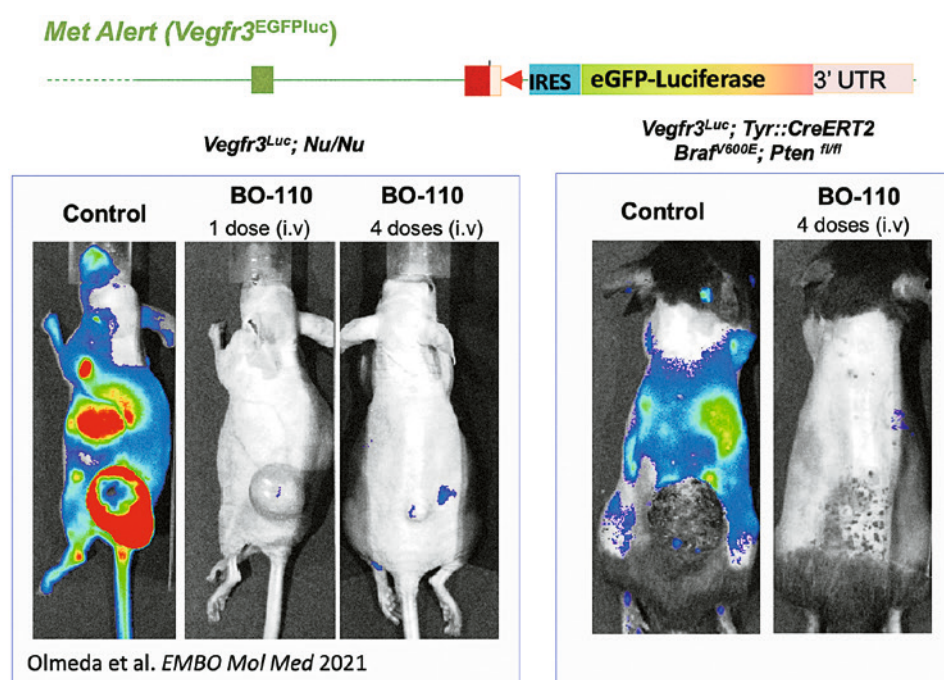


FIGURE 1 Pharmacological analyses in the **MetAlert** lymphoreporter model. The upper schematic summarises the knock-in model used, which reports neolymphangiogenesis by means of Vegfr3-coupled emission of bioluminescence. Shown are images pre- and post-treatment with BO-110 in melanomas generated in immunosuppressed (left) or in immune competent strains (right).

MetAlert mice for the discovery of drivers of (pre) metastatic sites

We have now exploited the *MetAlert* mice to screen for anticancer agents. First, we demonstrated that these *MetAlert* mice could recapitulate partial antitumoural effects of compounds in clinical testing in patients (i.e., inhibitors of BRAF and of the immune checkpoint blocker PD-L1). We then used these mice to identify more potent therapeutic agents. Specifically, we tested **nanoplexes of dsRNA (BO-110)**, which we had previously found to exert long-term antimelanoma effects *in vivo* (Tormo *et al.*, *Cancer Cell* 2009). Using the *MetAlert* mice, we uncovered potent systemic anti-lymphangiogenic blockers, with activity observed just after a single administration. Mechanistically, dsRNA nanoplexes were found to exert a rapid dual action in tumour cells and in their associated lymphatic vasculature, involving the transcriptional repression of the lymphatic drivers Midkine and Vegfr3, respectively (FIGURE 2). This suppressive function was mediated by cell-autonomous type I interferon signalling and was not shared by FDA-approved anti-melanoma treatments. These results reveal an alternative strategy for targeting tumour cell-lymphatic crosstalk and underscore the power of Vegfr3-lymphoreporters for pharmacological testing in otherwise aggressive cancers (Olmeda *et al.*, *EMBO Mol Med.* 2021). The results of these studies contributed to María Soengas being nominated to the *Real Academia Nacional de Farmacia* and the *Real Academia de Galicia de las Ciencias*. She also received the prestigious *Pezcoller-Marina Larcher Fogazzaro-EACR Women in Cancer Research Award*. ■

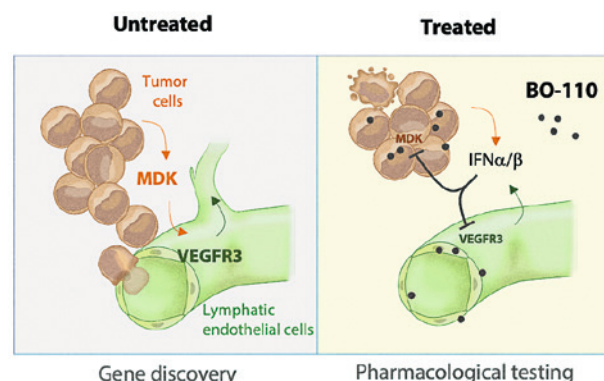


FIGURE 2 *Vegfr3^{Luc}* “MetAlert” lymphoreporter mice for gene discovery and pharmacological testing of anticancer agents. Analyses in untreated mice had revealed the growth factor Midkine (MDK) as a

new prolymphangiogenic and prometastatic factor. Using these mice, BO-110 is now found as a new blocker of neolymphangiogenesis, acting by an IFN-associated dual repression of MDK and VEGFR3.

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► AWARDS AND RECOGNITION

- *Carmen y Severo Ochoa Award* for Research in Molecular Biology, *Fundación Carmen y Severo Ochoa*, Spain.
- *Pezcoller-Marina Larcher Fogazzaro-EACR Women in Cancer Research Award* 2022, *Fondazione Pezcoller* and the European Association for Cancer Research (EACR).
- Academician of the Royal National Academy of Pharmacy, Spain.
- Top500 Influential Women in Spain, *Correo Gallego*.
- Nominated Top100 Women Leaders in Spain, *Mujeres & Cía*.
- Elected Member, *Real Academia de Galicia de Ciencia*, Spain.

EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real
Group Leader

Staff Scientist
Miriam Marqués

Post-Doctoral Fellows
Elena Del Pilar Andrada, Lavinia
Cabras, Irene Felipe, Eleonora Lapi,
Jaime Martínez De Villarreal, Gabriel
Piedrafita (until Dec.), Cristina
Segovia, Sladjana Zagorac



Graduate Students

Catalina Berca, Cristina Bodas, Sonia Corral, Auba Gayà, Ana Margarita Maldonado (until Feb.), Irene Millán (until Nov.), Mónica Pérez (until Nov.), María Ramal

Technicians

Miguel Deblas (until Aug.), Natalia del Pozo, Leticia Rodríguez (since Aug.)

Visiting Students

Olaya De Dios (July-Sept.) (*Master's Programme in Bioinformatics,*

ISCI-ENS, Madrid, Spain), Jacob E González (until March) (Dartmouth College, USA), Pablo Pérez (until June) (*UAM, Madrid, Spain*), Francisco J Soriano (since Sept.) (*Univ. Oberta de Catalunya, Spain*)

Visiting Scientists

Luis C. Fernández (*Univ. Europea de Madrid, Spain*), Mark Kalisz (*CIBER, Madrid, Spain*), Cintia L Massillo (Jan.-April) (*IBYME-CONICET, Buenos Aires, Argentina*)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial bladder carcinoma (UBC) using 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. Our aim is to acquire knowledge about the underlying molecular pathways and to apply it for improved prediction of outcome and therapy.

“We have shown that, *in vitro*, STAG2 is involved in the control of urothelial cell differentiation, in agreement with the fact that STAG2 mutations are associated with luminal-type bladder cancer.”

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

The genetic/genomic changes associated with PDAC have been extensively described by genome consortia, and there is increasing interest in defining the molecular changes that precede tumour development. Our laboratory has pioneered the notion that cell differentiation is the first tumour suppressor mechanism in the pancreas. Focusing on acinar cells, we have identified several novel transcriptional factors (TFs) involved - including GATA6, GATA4, NR5A2, HNF1A, and NFIC. Dysregulation of these transcriptional programmes is associated with a scenario of pre-inflammation or inflammation, providing the basis for the pharmacological and genetic manipulation of acinar differentiation as a tumour preventative strategy. We have generated super-Nr5a2 mice where the concept that inducible differentiation has a tumour protective role is currently being tested (with Sagrario Ortega, CNIO).

GATA6 and GATA4 are critically involved in the maintenance of the “classical” phenotype in PDAC but their distinctive roles in normal acinar cells are not well established. *Gata4* deletion in pancreatic progenitors results in a histologically adult normal pancreas with reduced expression of digestive enzyme transcripts, in sharp contrast with the requirement of *Gata6* for acinar cell maintenance in adult mice. We and others have shown that activation of mutant *KRas* in the pancreas leads to increased activity of inflammatory and cell cycle pathways. Deletion of *Gata4* or *Gata6* has opposite effects on the activation of inflammatory pathways in this context, but both genes act as tumour suppressors, indicating the existence of shared and unique roles for them in pancreatic homeostasis and carcinogenesis (FIGURE 1A-C).

To better understand how these TFs cooperate in normal pancreas and in early steps of tumorigenesis, we have built a gene regulatory network integrating public ATAC-Seq data

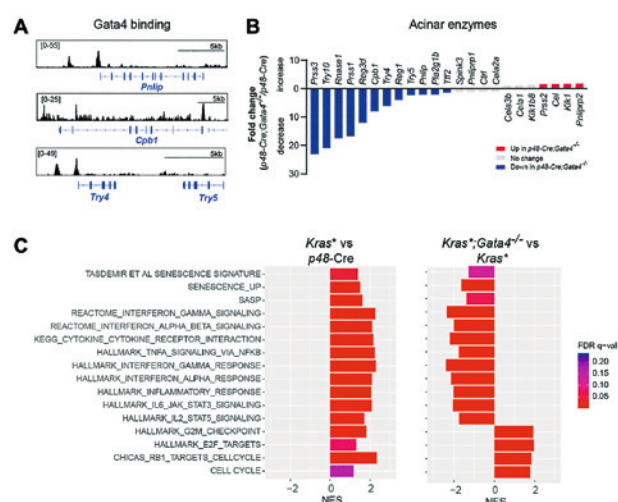


FIGURE 1 Understanding the role of TF in mouse pancreatic carcinogenesis. (A) ChIP-seq shows GATA4 binding to acinar gene loci. (B) *Gata4* deletion in the pancreas leads to reduced expression of multiple digestive enzyme transcripts.

(C) Mutant *Kras* expression in the pancreas results in increased activity of inflammatory and cell cycle-related pathways; *Gata4* deletion in the pancreas reverts the activity of the former but not the latter.

with our own – and public – ChIP-Seq and RNA-Seq data. This network reveals dramatic changes in TF hierarchies upon perturbation through induction of pancreatitis, *KRas* activation, TF deletion, or a combination thereof. Our overarching goal is to establish the rules governing the control of acinar differentiation and their contribution to preneoplasia and cancer.

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

We focus on understanding two 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 two genes and are exploring their role in development and in 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 (FIGURE 2) (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. ■

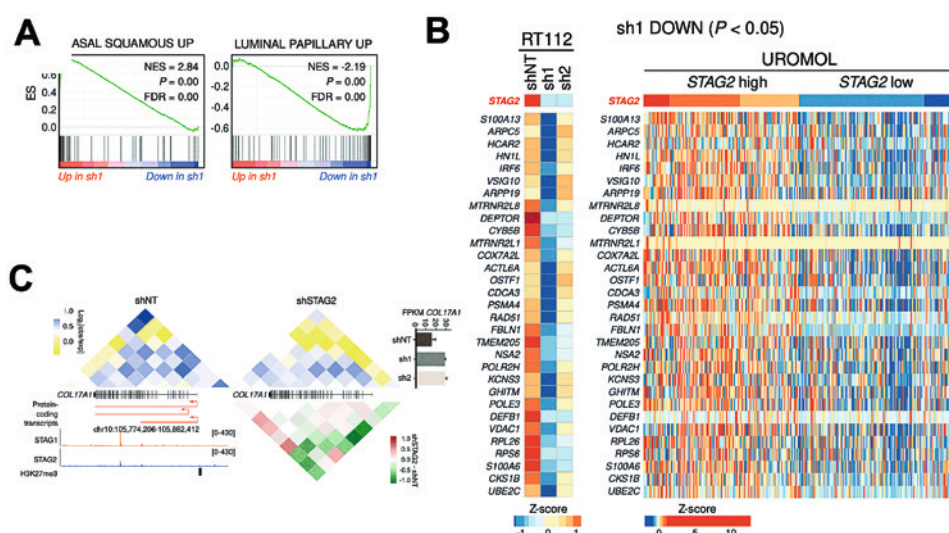


FIGURE 2 *STAG2* depletion in RT112 cells is associated with down-regulation of the luminal programme and rewiring of chromatin looping. (A) Up-regulation of a basal signature and down-regulation of a luminal signature (GSEA). (B) Down-regulated genes are expressed at lower levels in *STAG2*-low bladder tumours. (C) HiC contact matrices, ChIP-Seq tracks, differential contact matrices and expression of the *COL17A1* locus (basal gene) (Richart *et al.*, 2021).

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AWARDS AND RECOGNITION

- "la Caixa" Foundation Health Research Project 2021 (Francisco X. Real co-PI; coordinator, Juan Valcárcel); *RBM10*, a novel splicing regulator and tumor suppressor: from mechanisms to therapies, Fundación "la Caixa", Spain.

GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

Nabil Djouder
Group Leader

Post-Doctoral Fellows
Albert Harguindey (since February),
Eunjeong Kim (until November)



Graduate Students

Maria Inmaculada Berbel (since October), Sergio De La Rosa, Rosa Gallo (since February), Amanda Garrido (until July), Irene Herranz, Carlos Martínez (since November),

Maria Del Mar Rigual, Paula Sánchez, Karla Santos, Ana Isaura Teijeiro (until June), Fengming Yi (since November) (China Scholarship Council, MD oncologist Fellow)

Medical Residency – Visiting Scientist

Edurne Muruzábal (since August) (*Hospital Universitario de Navarra*, Pamplona, Spain)

OVERVIEW

Our laboratory focuses on understanding mechanisms of diseases associated to the digestive system, with a special focus on liver and intestinal diseases. By employing multi- and inter-disciplinary approaches, including the use of mouse models mimicking human disease combined with human data, 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 interest is mainly driven by the discovery of 2 components initially identified in our laboratory to be downstream targets of the growth factor and nutrient signalling cascades: **URI (Unconventional prefoldin RPB5 Interactor)** and **MCRS1 (Microspherule protein 1)** proteins. URI and MCRS1 expression turned out to be regulated in response to various environmental factors (radiation, nutrients, bacteria, viruses, etc.), compromising their functions and activating pleiotropic circuits to support complex cell signalling networks, provoking severe outcomes. Importantly, URI and MCRS1 are respectively part of 2 independent protein complexes: the **URI prefoldin-like** and the **non-specific lethal (NSL) complexes**. While URI might have some co-chaperone activities to maintain proteostasis, we identified MCRS1 to be a new regulator of histone acetylation and, therefore, a central component of the chromatin modifier complex NSL, whose loss in hepatocytes leads to cirrhosis development.

“As part of our research direction to understand mechanisms of human diseases associated to the digestive tract, we have generated the first genetic mouse model of liver cirrhosis that exhibits many attributes of the human liver disease. This mouse will help us to determine the role of cirrhosis in liver cancer development.”

RESEARCH HIGHLIGHTS

Using various **genetically engineered mouse models**, including the ones generated in our laboratory for **URI and MCRS1 gain- and loss-of-function**, combined with other model systems and cutting-edge technologies (including cell biology with organoid culture and quantitative imaging, biochemistry, and functional genomic methodologies) and human data, our laboratory has devoted substantial effort over the last 5 years to understanding the molecular, cellular, and pathophysiological mechanisms linking environmental stresses to disease pathogenesis affecting organs of the digestive system; in particular, we have focused on the liver, intestine, and pancreas, as these organs are physiologically interconnected and influenced through their exocrine and/or endocrine functions.

We intend in the near future to continue deconstructing the mechanisms of pathologies associated to the digestive system in response to environmental stressors. Special effort will be put on studying **the mechanobiology of liver tissue** in healthy and diseased contexts from the physical and mechanical perspectives at the molecular, cellular, and tissue levels. This will enable us to determine how mechanical forces exerted within, as well as between, cells and their interactions with the surrounding microenvironments establish precise contributions at both micro- and macroscopic levels leading to liver disease. The goal is also to understand how an injured and diseased liver progresses to becoming cancerous tissue (FIGURE 1). In this context, mechanobiology-dependent immune mechanisms will also be genetically manipulated *in vivo* to better understand their impact on the diseased liver. Additionally, applying mathematical models to quantitatively study and analyse mechanical forces and cellular plasticity is an important focus of our collaborations with other research groups. Bioinformatics analysis of various datasets will also be used to complement our studies.

Moreover, the use of nanotechnology-based theranostics combined with the latest imaging technologies and *in vivo* liver disease models generated in our laboratory might provide additional opportunities to complement our work and impact the field of medicine in diagnosis and treatment.

Additionally, recent data from our lab indicate that URI and MCRS1 are essential for early stages of development. Therefore, we are currently putting efforts into elucidating the role and functions of the URI prefoldin-like complex and the NSL complex during embryonic development, where both complexes might play a role in cellular plasticity. We will pay special attention to deciphering the molecular and cellular mechanisms implicated in these processes.

Finally, one of our future goals is also to understand the functions of URI prefoldin-like and NSL complexes by deciphering their structural organisation via electron microscopy or crystallography. These long-term perspective projects have just been started in our lab. ■

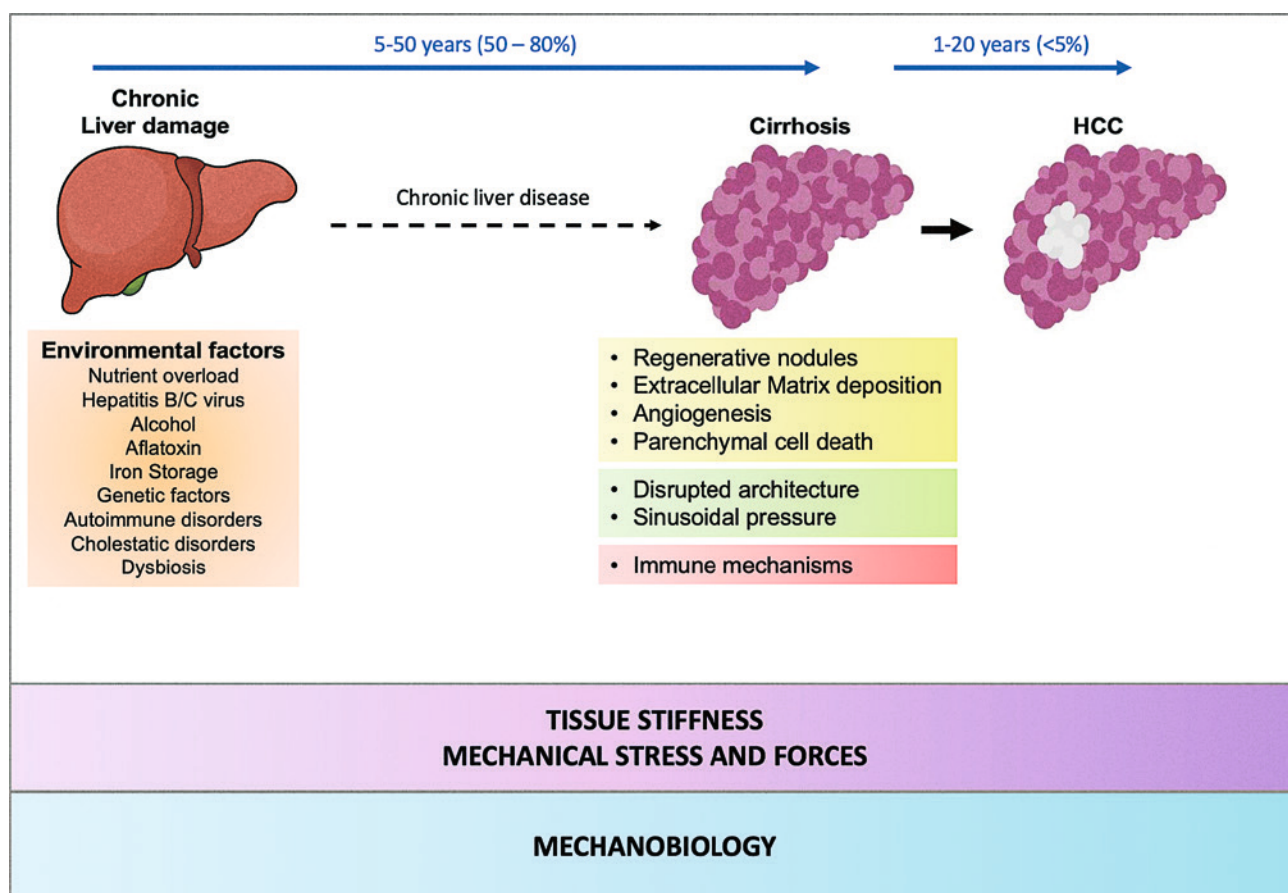


FIGURE 1 Representation of some of our research directions. We aim to determine and target the mechano-

transduction pathways in the progression of liver cirrhosis to hepatocellular carcinoma.

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► AWARDS AND RECOGNITION

- AECC Grant "Ayuda Proyectos Generales AECC 2021", the Spanish Association Against Cancer (AECC).
- Member of the European Association for the Study of Diabetes (EASD).

TRANSFORMATION AND METASTASIS GROUP

Eva González Suárez
Group Leader

Staff Scientists
Patricia González, María Jiménez,
Gema Pérez



Graduate Students Alexandra Barranco, Marina Císcar, Alejandro Collado, Jaime Redondo, Sergi Velasco (since Aug.) (PEJ, CAM), Andrea Vethencourt (clinical oncologist at ICO/IDIBELL, Barcelona, Spain)	<i>Plan de Empleo Joven de la Comunidad de Madrid</i> (Youth Employment Plan, Community of Madrid) Undergraduate Student Pedro Luis Echevarria (since April)	Technician Víctor López (since March) Student in Practice Teresa Martí (August-December) (AECC Traineeship and BS Thesis,	<i>Universidad Francisco de Vitoria, Pozuelo, Spain)</i>
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OVERVIEW

Research in the Transformation and Metastasis Group aims to identify novel therapeutic targets for epithelial cancer treatment and to elucidate resistance mechanisms to drugs currently available. Tumours exploit and manipulate for their benefit the same mechanisms that regulate homeostasis in healthy tissue. Thus, we first aim to understand normal development and then to identify the key events that lead to tumour initiation, progression, and metastasis in order to avoid and combat them. Complementary tools, including primary cell cultures and organoids, mouse models, and clinical samples, are used with the final goal of translating basic knowledge into clinically relevant findings.

“Our findings demonstrate that RANK overexpression induces senescence in luminal mammary epithelial cells through p16/p19, and that Rank-induced senescence enhances stemness.”

RESEARCH HIGHLIGHTS

RANK links senescence to stemness in the mammary epithelia, delaying tumour onset but increasing tumour aggressiveness

Our previous results indicate that Rank signalling enhances stemness in mouse and human mammary epithelial cells (MECs) and mediates mammary tumour initiation. Mammary tumours initiated by oncogenes or carcinogen exposure display high levels of Rank, and Rank pathway inhibitors have emerged as a new strategy for breast cancer prevention and treatment. Here we show that ectopic Rank expression in the mammary epithelia unexpectedly delays tumour onset and reduces tumour incidence in the oncogene-driven Neu and PyMT models. Mechanistically, we have found that ectopic expression of Rank or exposure to Rankl induces senescence, even in the absence of other oncogenic mutations. Rank leads to DNA damage and senescence through p16/p19. Moreover, RANK-induced senescence is essential for Rank-driven stemness and, although it initially translates into delayed tumour growth, eventually promotes tumour progression and metastasis. We have uncovered a dual role for Rank in the mammary epithelia: Rank induces senescence and stemness, delaying tumour initiation but increasing tumour aggressiveness.

Immunomodulatory effect of denosumab in early breast cancer: preliminary results of a randomised window-opportunity clinical trial D-Biomark (NCT03691311)

Most breast cancers (BC) exhibit low immune infiltration and are unresponsive to immunotherapy. Hence, the urgency to find new mechanisms of immune activation, postulating receptor activator of nuclear factor kappa-B ligand (RANKL) and its receptor RANK as potential immunomodulator. Our previous data demonstrated that RANK pathway inhibitors, such as denosumab used for the treatment of bone metastasis, could also prevent and/or treat BC and regulate the tumour immune crosstalk. The D-Biomark clinical trial aims to identify denosumab-driven changes in breast cancer cells and to identify the population of breast cancer patients who may benefit from denosumab.

Patients with early-stage HER2-negative BC, candidates to tumour excision as first therapeutic approach are included. Patients are randomised 2:1 to denosumab: control (no treatment); experimental arm received 2 doses of 120 mg subcutaneous denosumab (once per week) before surgery (2-4 weeks later). Putative changes in tumour cell proliferation by Ki67 immunohistochemistry (IHC), cell survival by cleaved caspase 3 IHC (primary endpoints), and stromal tumour infiltrating lymphocytes (TILs) quantified by haematoxylin and eosin staining between baseline (biopsy sample) and surgery are evaluated.

We present the results of the first 36 patients enrolled out of 60. Clinical and tumour characteristics were well balanced between the groups. No relevant toxicities were reported. No clinically significant differences in Ki67 and cleaved caspase-3 were observed after denosumab treatment. Interestingly, a statistically significant increase in TILs was observed in the denosumab-treated group ($p=0.03$, Paired t test) but not in the control group ($p=0.80$). 33% of patients treated denosumab showed a $\geq 10\%$ increase in TILs vs 0% in the control group ($p=0.05$). ■

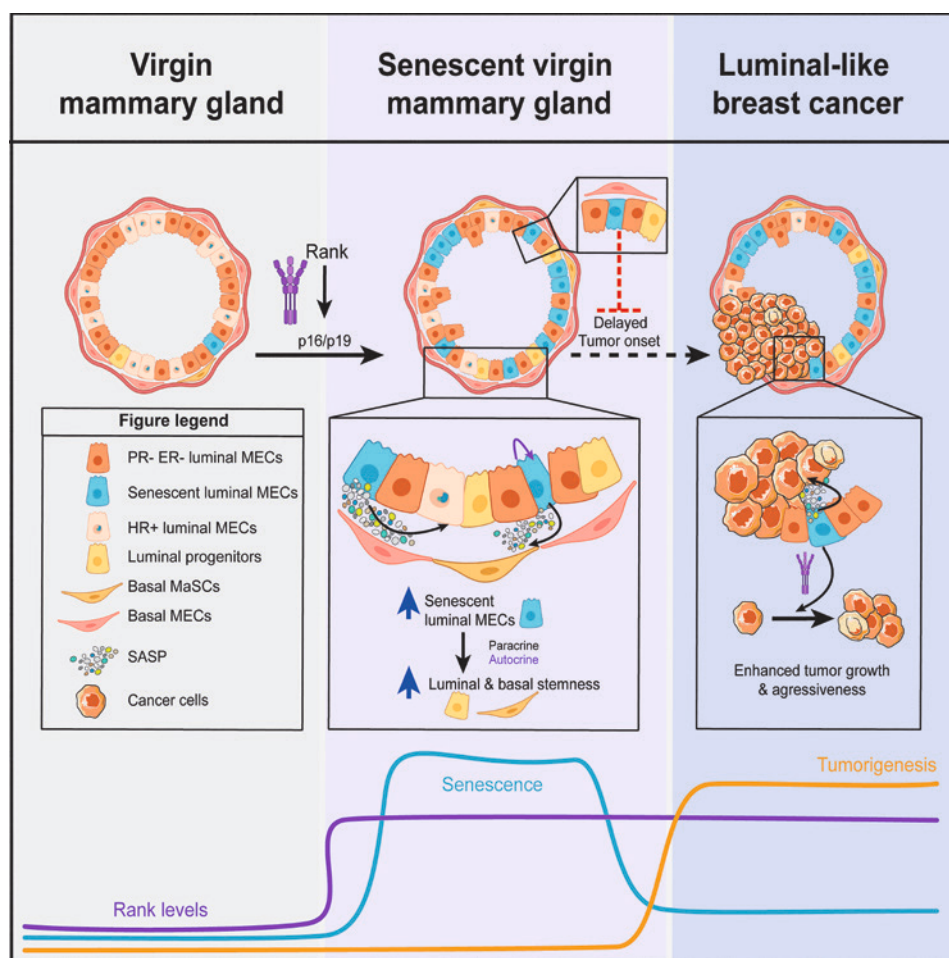


FIGURE RANK links senescence to stemness in the mammary epithelia, delaying tumour onset but increasing tumour aggressiveness.

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RANK is an independent biomarker of poor prognosis in estrogen receptor-negative breast cancer and a therapeutic target in patient-derived xenografts. *bioRxiv*. doi:https://doi.org/10.1101/2021.12.13.47091.

PUBLICATIONS AT OTHER INSTITUTIONS

- Vethencourt Casado A, Trinidad EM, Gómez Aleza C, Pernas Simon S, Petit A, Soler T, Urruticoechea A, Gumà Martínez A, García-Tejedor A, Pla MJ, Capó C, Gil Gil M, Stradella A, Fernández A, Recalde S, Cejuela M, Vazquez S, Iserte A, Falo C, Gonzalez-Suarez E (2021). Immunomodulatory effect of denosumab in early breast cancer: preliminary results of a randomized window-opportunity clinical trial D-Biomark (NCT03691311). *Ann Oncol* 32 (suppl_2), S21-S36. 10.1016/annonc/annonc503.

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Staff 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 especially interested in analysing the role of small extracellular vesicles (sEVs) in premetastatic niche formation in melanoma, pancreatic and prostate cancer. These particles reinforce tumour cell homing and metastasis in organs. Besides the role of sEVs, we are interested in understanding the influence of obesity and platelets in triple negative breast cancer and tumour metastasis in rare diseases such as follicular lymphoma and malignant peripheral nerve sheath tumours. Finally, we are defining new approaches to use circulating sEVs in liquid biopsies, analysing the use of mutations as indicators of minimal residual disease in melanoma and breast cancer.

“Our Group aims to understand the extrinsic mechanisms influencing metastatic dissemination.”

Graduate Students

Elena Castellano, Juan García-Agulló (since November), Teresa González, Alberto Hernández

Technicians

Enrique Bastón (since September), Sara Sánchez-Redondo, Vanesa Santos

Students in Practice

Eduardo Garvín (since September) (*Universidad Francisco de Vitoria, Madrid, Spain*), Ane Rubio (until July) (*Universidad Complutense de Madrid, Madrid, Spain*)

Visiting Scientist

Alberto Carretero (until September) (*Hospital Universitario 12 de Octubre, Madrid, Spain*)

RESEARCH HIGHLIGHTS

Development of EV-based liquid biopsy tests. We are developing state-of-the-art technologies to implement EV-based tests in the prognosis of patients with melanoma and breast cancer. We are working on developing a new method for residual disease detection based on the combination of circulating nucleic acids and EVs that will facilitate the stratification of patients for the use of adjuvant therapies after surgery.

Novel mechanisms driving local and distal metastasis in melanoma and prostate cancer. We have discovered that nerve growth factor receptor (NGFR) is shuttled in sEVs, reinforcing lymph node pre-metastatic niche formation and metastasis. NGFR is secreted in sEVs, orchestrating the activation of lymphatic endothelial cells, which favours lymph node metastasis. Moreover, we have found that therapeutic targeting of NGFR reduces both local and distal metastasis and can be efficiently combined with immunotherapy. We are currently developing the use of NGFR inhibitors as novel anti-metastatic agents in melanoma and other cancer types. In addition, we aim to understand how sEVs influence lymph node metastasis in prostate cancer (PCa) and are analysing the proteomic signatures in PCa-secreted sEVs in order to define novel biomarkers of early dissemination.

Obesity and breast cancer metastasis: the tumour-platelet connection. In this project, we hypothesised that obesity influences systemic changes that pre-condition future organs of metastasis, generating a specialised microenvironment that we have termed “obese premetastatic niche”. We found that obesity reshapes metastatic organ composition, enhancing platelet activation, tumour cell homing and metastasis. Importantly, we identified that anti-platelet therapies reduced tumour cell homing and metastasis in obese mice, supporting the hypothesis that anti-coagulant agents could be used as anti-metastatic therapy in obesity models of breast cancer.

Relevance of tumour microenvironment in metastasis. In order to understand the relevance of the microenvironment in metastatic progression of rare cancers, we are analysing 1) the role of NGFR in follicular lymphoma progression, and 2) therapeutic strategies against Endoglin and MEK inhibitors in malignant peripheral nerve sheath tumours (MPNSTs). ■

▶ SELECTED PUBLICATIONS*

- ▶ García-Silva S, Benito-Martín A, Nogués L, Hernández-Barranco A, Mazariegos MS, Santos V, Hergueta-Redondo M, Ximénez-Embún P, Kataru RP, Lopez AA, Merino C, Sánchez-Redondo S, Graña-Castro O, Matei I, Nicolás-Avila JA, Torres-Ruiz R, Rodríguez-Perales S, Martínez L, Pérez-Martínez M, Mata G, Szumera-Cieckiewicz A, Kalinowska I, Saltari A, Martínez-Gómez JM, Hogan SA, Saragovi HU, Ortega S, García-Martín C, Boskovic J, Levesque MP, Rutkowski P, Hidalgo A, Muñoz J, Megías D, Mehrara BJ, Lyden D, Peinado H (2021). Melanoma-derived small extracellular vesicles induce lymphangiogenesis and metastasis through an NGFR-dependent mechanism. *Nat Cancer* 2, 1387-1405.
- ▶ Verweij FJ *et al.* (incl. Peinado H) (2021). The power of imaging to understand extracellular vesicle biology in vivo. *Nat Methods* 18, 1013-1026.
- ▶ Olmeda D, Cerezo-Wallis D, Castellano-Sanz E, García-Silva S, Peinado H, Soengas MS (2021). Physiological models for in vivo imaging and targeting the lymphatic system: nanoparticles and extracellular vesicles. *Adv Drug Deliv Rev* 175, 113833.
- ▶ Saltari A *et al.* (incl. Peinado H) (2021). Specific activation of the CD271 intracellular domain in combination with chemotherapy or targeted therapy inhibits melanoma progression. *Cancer Res* 81, 6044-6057.
- ▶ Hernández-Barranco A, Nogués L, Peinado H (2021). Could extracellular vesicles contribute to generation or awakening of “sleepy” metastatic niches? *Front Cell Dev Biol* 9, 625221.
- ▶ García-Silva S, Gallardo M, Peinado H (2021). DNA-loaded extracellular vesicles

in liquid biopsy: tiny players with big potential? *Front Cell Dev Biol* 8, 622579.

- ▶ Senís E *et al.* (incl. Peinado H). TUNAR lncRNA encodes a microprotein that regulates neural differentiation and neurite formation by modulating calcium dynamics (2021). *Front Cell Dev Biol* 9, 747667.
- ▶ Amor López A, Mazariegos MS, Capuano A, Ximénez-Embún P, Hergueta-Redondo M, Recio JA, Muñoz E, Al-Shahrour F, Muñoz J, Megías D, Doliana R, Spessotto P, Peinado H (2021). Inactivation of EMI-LIN-1 by proteolysis and secretion in small extracellular vesicles favors melanoma progression and metastasis. *Int J Mol Sci* 22, 7406.
- ▶ González Muñoz T, Amaral AT, Puerto-Camacho P, Peinado H, de Álava E (2021). Endoglin in the spotlight to treat cancer. *Int J Mol Sci* 22, 3186.
- ▶ García-Silva S, Ximénez-Embún P, Muñoz J, Peinado H (2021). Postlymphadenec-

tomy analysis of exosomes from lymphatic exudate/exudative seroma of melanoma patients. *Methods Mol Biol* 2265, 345-359.

* Please see the Group's web site for a list of all publications (19 publications in 2021).

▶ PATENT

- ▶ Peinado Selgas H, Saragovi HU, García Silva S, Nogues Vera L, Hernández Barranco A (2021). THX-B for treating and preventing cancer and metastasis. *EP21382669*.

▶ AWARDS AND RECOGNITION

- ▶ Hector Peinado was awarded the *Leonardo Grant* by the *BBVA* Foundation for Researchers and Cultural Creators in 2021, Spain.

BRAIN METASTASIS JUNIOR GROUP

Manuel Valiente
Junior Group Leader

Post-doctoral Fellows
Mariam Al-Masmudi, Lluís
Cordón (since May), Neibla Priego

Graduate Students
Laura Adriana Álvaro, Ana De Pablos
Aragoneses, Pedro García, Carolina
Hernández, Lucía Zhu (until
September)



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 in order to challenge the current status of this unmet clinical need.

“We have established a nation-wide network of hospitals (*RENACER*) to generate a large repository of brain metastases and patient-derived organotypic cultures, which we have used in our projects.”

Visiting Graduate Student
Lauritz Miarka (until September)
(Boehringer Ingelheim Fonds MD
Fellowship) (*Christian-Albrechts-
University Kiel, Kiel, Germany*)

Technicians
Patricia Baena (since July), María
Isabel García (until July), María
Perea, Diana Patricia Retana, Oliva A.
Sánchez (since November)

Visiting Master's Students
Irene Salgado (since November)
(*Universidad Autónoma de Madrid,
Spain*), Paniz Rahimi (until August)
(*Universidad Complutense de Madrid,
Spain*)

RESEARCH HIGHLIGHTS

We have applied single cell technology (scRNAseq) both within the cancer cell and the non-cancer cell (microenvironment) compartments of brain metastasis in our experimental models, identifying previously unknown subpopulations that we are currently evaluating functionally.

We have confirmed that our drug-screening platform (METPlatform) could be exploited clinically as a patient

“avatar”, being potentially transformative for the future design of clinical trials.

We have initiated a novel research line to evaluate the influence of metastases in neural circuits and brain function in order to elucidate the molecular mechanisms underlying neurocognitive deterioration in patients. ■

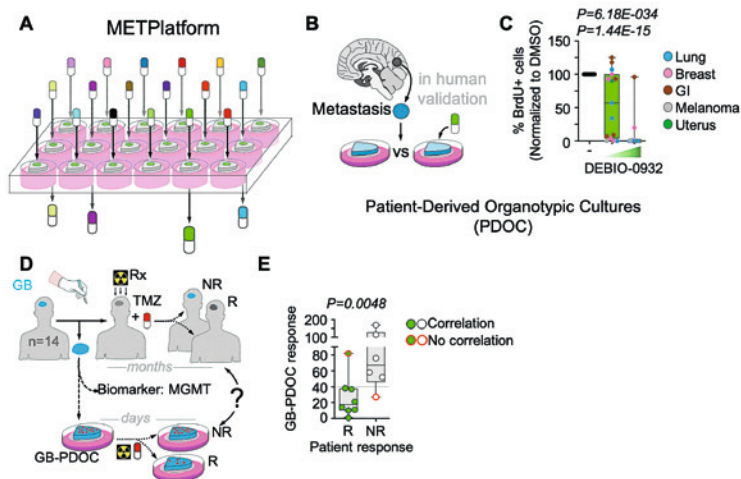


FIGURE (A) METPlatform is a novel drug-screening strategy using live organs with metastases. (B, C) Selected drugs can be translated to

patient-derived organotypic cultures. (D, E) METPlatform has the potential to predict within days the response of a patient to a given therapy.

PUBLICATIONS

- ▶ Masmedi-Martín M*, Zhu L*, Sanchez-Navarro M, Priego N, Casanova-Acebes M, Ruiz-Rodado V, Giralt E, Valiente M** (2021). Brain metastasis models: what should we aim to achieve better treatments. *Adv Drug Deliv Rev* 169, 79-99. (*) Equal contribution. (**) Corresponding author.
- ▶ Zhu L, Retana D, García-Gómez P, Álvaro-Espinosa L, Priego N, Masmedi-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 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* (2021). A clinically compatible drug-screening platform based on organotypic cultures identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med*. PMID: 35174975. (*) Corresponding author.
- ▶ Pisano F, Kashif MF, Balena A, Pisanello M, De Angelis F, de la Prida LM, Valiente M, D'Orazio A, De Vittorio M, Grande M, Pisanello F (2021). Plasmonics on a neural implant: engineering light-matter interactions on the nonplanar surface of tapered optical fibers. *Adv Opt Mater*. doi: 10.1002/adom.202101649.
- ▶ Álvaro-Espinosa L, de Pablos-Aragoneses A, Valiente M, Priego N* (2021). Brain

microenvironment heterogeneity: potential value for brain tumors. *Front Oncol* 11, 714428. (*) Corresponding author, postdoc in the lab.

- ▶ Jablonska PA, Bosch-Barrera J, Serrano D, Valiente M, Calvo A, Aristu J (2021). Challenges and novel opportunities of radiation therapy for brain metastases in non-small cell lung cancer. *Cancers (Basel)* 13, 2141.
- ▶ Miarka L, Valiente M* (2021). Animal models of brain metastasis. *Neurooncol Adv* 3, 144-156. (*) Corresponding author.

AWARDS AND RECOGNITION

- ▶ Manuel Valiente:
 - Co-founder of the National Network of Brain Metastasis (RENACER), Spain.
 - Finalist, Dr Josef Steiner Cancer Research Award, Switzerland.

- ASPIRE Award (Accelerating Scientific Platforms and Innovative Research), The Mark Foundation for Cancer Research, USA.

- ▶ Lluís Cordón was awarded a “CNIO Friends” Postdoctoral Contract.
- ▶ Lucía Zhu: “Best Talk Award”, UAM PhD Research Symposium in Health Sciences and Biomedicine; selected oral presentations at the 16th EANO Meeting (European Association of Neuro-Oncology) and the 3rd Annual SNO Conference on Brain Metastases (Society for Neuro-Oncology).
- ▶ Neibla Priego: Invited talk at the 19th National Congress of the Italian Society for Neuroscience.
- ▶ Lauritz Miarka: Selected oral presentation at the 3rd Annual SNO Conference on Brain Metastases (Society for Neuro-Oncology).

METABOLISM AND CELL SIGNALLING JUNIOR GROUP

Alejo Efeyan
Junior Group Leader

Staff Scientists
Bárbara Martínez, Ana Ortega (until
August)



OVERVIEW

In the Metabolism & Cell Signalling Lab we study the links between nutrients, cancer and aging. 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 why and how excessive nutrient levels deregulate cellular metabolism, pinpointing actionable links between nutrients, cancer and the process of ageing.”

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

Graduate Students

Celia de la Calle (until March), Lucía De Prado, Nerea Deleyto, Ana Belén Plata, Elena Sánchez (since March)

Technicians

Cristina Lebrero (until Oct.) (TS), Ana Sagrera, Alba Sanz

**Título Superior* (Advanced Degree)

Student in Practice

Elena Fernández (since November) (*Universidad Complutense de Madrid*, Madrid, Spain)

Visiting Scientist

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 suppression of either input resulted important to unleash the metabolism of the fasted state, chronic nutrient surplus in humans typically causes synchronous activation of both nutrient and hormonal signalling. Thus, we generated a mouse strain harbouring deregulated nutrient and hormonal signalling to mTORC1 in hepatocytes. 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) minimally increased mTORC1 signalling (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). We are currently determining whether such

synergism, which failed to be explained exclusively by the modest increase in the activation of mTORC1, is caused by a) nutrients and hormonal signalling differentially activating downstream targets of mTORC1, or b) mTORC1-dependent and -independent functions of nutrient and/or hormonal signalling. ■

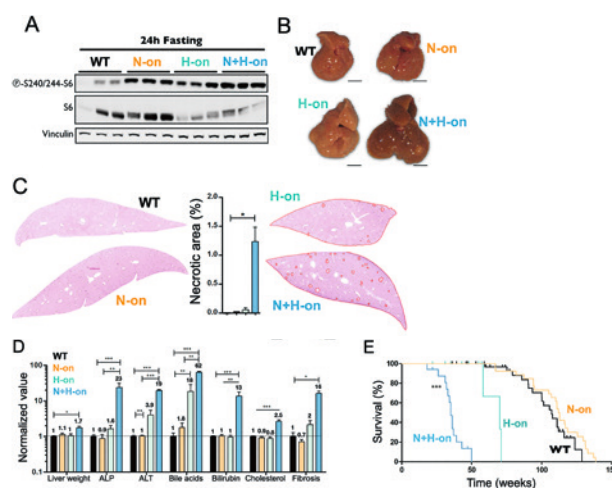


FIGURE 1 (A) Modest increase in mTORC1 activity (compared with N-on and H-on, seen by phospho-S6) in livers from fasted N+H-ON mice. (B) Micrographs of enlarged N+H-ON

livers. (C & D) Multiple readouts of liver damage in livers from N+H-ON mice. (E) Premature death of N+H-ON mice due to liver carcinomas.

PUBLICATIONS

- de la Calle Arregui C, Plata-Gómez AB, Deleyto-Seldas N, García F, Ortega-Molina A, Abril-Garrido J, Rodríguez E, Nemazany I, Tribouillard L, de Martino A, Caleiras E, Campos-Olivas R, Mulero F, Laplante M, Muñoz J, Pende M, Sabio G, Sabatini DM, Efeyan A (2021). Limited survival and impaired hepatic fasting metabolism in mice with constitutive Rag GTPase signalling. *Nat Commun* 12, 3660.
- Pae J, Ersching J, Castro TBR, Schips M, Mesin L, Allon SJ, Ordovas-Montanes J, Mlynarczyk C, Melnick A, Efeyan A, Shalek AK, Meyer-Hermann M, Vitorica GD (2021). Cyclin D3 drives inertial cell cycling in dark zone germinal center B cells. *J Exp Med* 218, e20201699.
- Ortega-Molina A, Lebrero-Fernández C, Sanz A, Deleyto-Seldas N, Plata-Gómez AB, Menéndez C, Graña-Castro O, Caleiras E, Efeyan A (2021). Inhibition of Rag GTPase signaling in mice suppresses B cell responses and lymphomagenesis with minimal detrimental trade-offs. *Cell Rep* 36, 109372.
- Martinez-Pastor B, Silveira GG, Clarke TL, Chung D, Gu Y, Cosentino C, Davidow LS, Mata G, Hassanieh S, Salsman J, Ciccio A, Bae N, Bedford MT, Megias D, Rubin LL, Efeyan A, Dellaire G, Mostoslavsky R (2021). Assessing kinetics and recruitment of DNA repair factors using high content

screens. *Cell Rep* 37, 110176.

- Deleyto-Seldas N, Efeyan A (2021). The mTOR-autophagy axis and the control of metabolism. *Front Cell Dev Biol* 9, 655731.
- Spicer G, Gutierrez-Erlandsson S, Mate-sanz R, Bernard H, Adam AP, Efeyan A, Thompson S (2021). Harnessing DNA for nanothermometry. *J Biothronics* 14, e202000341.
- Plata-Gómez AB, Crespo M, de Prado Rivas L, de la Calle Arregui C, de Prado-Rivas L, Sabio G, Efeyan A (2021). Protocol for the assessment of mTOR activity in mouse primary hepatocytes. *STAR Protoc* 2, 100918.
- Ortega-Molina A, Efeyan A (2021). From mouse genetics to targeting the Rag

GTPase pathway. *Mol Cell Oncol* 8, 1979370.

AWARDS AND RECOGNITION

- Alejo Efeyan:
 - EMBO Young Investigator, European Molecular Biology Organization.
 - Leonardo Grant for Researchers and Cultural Creators, BBVA Foundation, Spain.
 - Keynote Speaker, Keynote Lecture, 2nd "TOR de France" 2021 meeting, France.
- Elena Sanchez Garcia was selected for an FPI PhD Student Fellowship (MCI), Spain.

SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito (until
September)
Junior Group Leader

Staff Scientist
Bárbara Oldrini

Post-Doctoral Fellow
Miguel Jiménez (until July)

Graduate Students
María Del Mar Gardeazabal, Verónica
Matía



OVERVIEW

A decade of studies has underlined the complexity of the genetic events that characterise the genomic landscapes of common forms of human cancer, including gliomas. While a few cancer genes are mutated at high frequencies (>20%), the greatest number of cancer genes in most patients appear at intermediate frequencies (2–20%) or lower. Strikingly, the functional significance of the vast majority of these alterations remains elusive. A current high priority in glioma research is to functionally validate candidate genetic alterations in order to identify those that are significant for cancer progression and treatment response.

In our laboratory, we use a combination of genomic analyses, mouse models, and primary tumour cell cultures, with the main goal of identifying the molecular mechanisms that could provide the basis for novel treatments for glioma patients.

“The main focus of our group is to uncover the genetic alterations present in glioma patients that are responsible for the aggressiveness and the poor treatment response of these tumours.”

Technicians
Alicia Marie Gaëlle Leblond (until
September)

**Titulado Superior (Advanced Degree)*

Visiting Scientist
Ernesto Mejías (until March) (Ludwig
Maximilian University of Munich,
Munich, Germany)

RESEARCH HIGHLIGHTS

Point mutations accumulate in the cells of multicellular organisms over cycles of cell divisions. The majority of point mutations that occur in somatic cells are innocuous to the organism. However, some somatic mutations are capable of driving the tumorigenic transformation of cells. To properly recapitulate the high genetic heterogeneity observed in cancer patients, we need flexible and informative genetic models able to recreate not just a handful of genetic alterations, but potentially dozens.

Base editing is a genome editing method that directly generates precise point mutations in genomic DNA or in cellular RNA without directly generating DNA double-strand breaks (DSBs) or requiring a donor DNA template. DNA base editors (BEs) comprise fusions between a catalytically impaired Cas nuclease and a base-modification enzyme that operates on single-stranded DNA (ssDNA) but not double-stranded DNA (dsDNA). To faithfully model *in vivo* a variety of brain tumour-associated mutations, we have combined CRISPR/Cas9-BE3 base editing with the RCAS-TVA system. We recently generated 2 different BE3-TVA mouse strains by crossing Ntv-a and Gtv-a mice with a tetracycline-responsive BE3 mouse model, Tg.tetO-BE3, kindly provided by the Dow Laboratory at Weill Cornell Medicine in New York. In these new strains, BE3 expression is transiently activated by the transduction of a RCAS-Tet-Off vector that carries the tetracycline transactivator (tTA) protein and is subsequently silenced by treating the mice with doxycycline. A continuous expression of the BE3 editor could potentially lead to undesired base pair deletions over time. Together with the RCAS-Tet-Off, mice are injected with RCAS-gRNA constructs for the desired point mutation. Lastly, these vectors can be combined

with either RCAS-PDGFA or RCAS-Sonic Hedgehog (Shh) to model gliomas and medulloblastomas, the most frequent brain tumours in adults and children, respectively. Such a versatile model will now allow us to generate relevant animal models that more closely recapitulate a given patient's tumour. ■

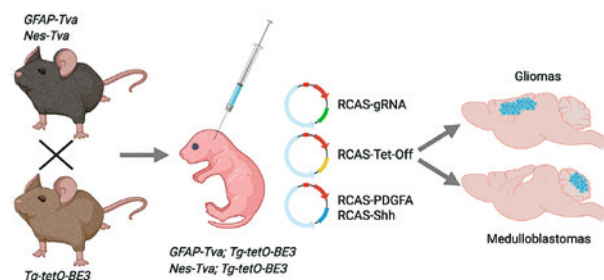


FIGURE BE3-RCAS-TVA system to model brain tumour-associated point mutations.

PUBLICATIONS

- Schmitt MJ, Company C, Dramaretska Y, Barozzi I, Göhrig A, Kertalli S, Großmann M, Naumann H, Sanchez-Bailon MP, Hulsman D, Glass R, Squatrito M, Serresi M, Gargiulo G (2021). Phenotypic mapping of pathologic cross-talk between glioblastoma and innate immune cells by synthetic genetic tracing. *Cancer Discov* 11, 754-777.
- Berg TJ, Marques C, Pantazopoulou V, Johansson E, von Stedingk K, Lindgren D, Jeannot P, Pietras EJ, Bergström T, Swartling FJ, Governa V, Bengzon J, Belting M, Axelson H, Squatrito M, Pietras A (2021). The irradiated brain microenvironment supports glioma stemness and survival via astrocyte-derived transglutaminase 2. *Cancer Res* 81, 2101-2115.
- Marques C, Unterkircher T, Kroon P, Oldrini B, Izzo A, Dramaretska Y, Ferrarese R, Kling E, Schnell O, Nelander S, Wagner EF, Bakiri L, Gargiulo G, Carro MS, Squatrito M (2021). NF1 regulates mesenchymal glioblastoma plasticity and aggressiveness through the AP-1 transcription factor FOSL1. *Elife* 10, e64846.
- Jiménez-Alcázar M, Curiel-García Á, Nogales P, Perales-Patón J, Schuhmacher AJ, Galán-Ganga M, Zhu L, Lowe SW, Al-Shahrour F, Squatrito M (2021). Dihydrogalactitol overcomes multiple temozolomide resistance mechanisms in glioblastoma. *Mol Cancer Ther* 20, 1029-1038.
- Aldaz P, Auzmendi-Iriarte J, Duráñez M, Lasheras-Otero I, Carrasco-García E, Zelaya MV, Bragado L, Ollas-Arjona A, Egaña L, Samprón N, Morilla I, Redondo-Muñoz M, Rico M, Squatrito M, María-Alonso M, Fernández-Irigoyen J, Santamaría E, Larrazóy IM, Wellbrock C, Matheu A, Arozarena I (2021). Identification of a dexamethasone mediated radioprotection mechanism reveals new therapeutic vulnerabilities in glioblastoma. *Cancers (Basel)* 13, 361.

CANCER IMMUNITY JUNIOR GROUP

María Casanova-Acebes
Junior Group Leader

Graduate Students
Federico Lupo (since September),
Enrique Nogueira (since July)



OVERVIEW

Myeloid cells are the major and most diverse component of solid tumours. In our laboratory, we are interested in identifying novel therapeutic targets to block the pathogenicity of tumour-associated myeloid cells, while preserving their homeostatic functions. In particular, we study the biology of macrophages, neutrophils, and their precursors to understand whether their unique plasticity can be reprogrammed to cure cancer.

Our laboratory tackles these challenges by analysing these cells in the tissue in which they reside, i.e., the tumour microenvironment (TME). As immune cells dynamically adapt and establish heterotypic interaction with other cellular components in the TME, we concentrate our efforts on capturing these interactions. By focusing on breast and lung cancer, and through close collaborations between our

“With the advent of scRNAseq technologies, in 2021 we witnessed the remarkable and previously unappreciated complexity of myeloid cells in tumours. Now in the Cancer Immunity Group we will break down that complexity to identify and modulate the immunosuppressive activity of myeloid cells in solid tumours.”

laboratory and the clinic, our goal is to discover novel therapeutic venues for cancer patients.

Technician
Nines Sanguino Acosta

Visiting Students
Ainhoa Muñoz (since November)
(Bachelor's Degree Final Project,

Universidad Autónoma de Madrid,
Spain), Gonzalo Soria (since
September) (Master's Thesis,
Universidad Autónoma de Madrid,
Spain)

RESEARCH HIGHLIGHTS

The Cancer Immunity Group officially started in January 2021 and the team is currently composed of a lab manager, 2 PhD students (one of them supported by the *Fundación Cris Contra el Cancer* and *FPI Severo Ochoa* Programme), and 1 computational Master's student.

We expect that 2022 will be full of scientific discoveries and that we will consolidate our newly established Cancer Immunity laboratory at CNIO. ■

The main research areas of our Group are the following:

- Innate immune cell training in breast cancer metastasis.
- Macrophage-stroma modulation in lung cancer.
- Circadian regulation of tumour immunology.

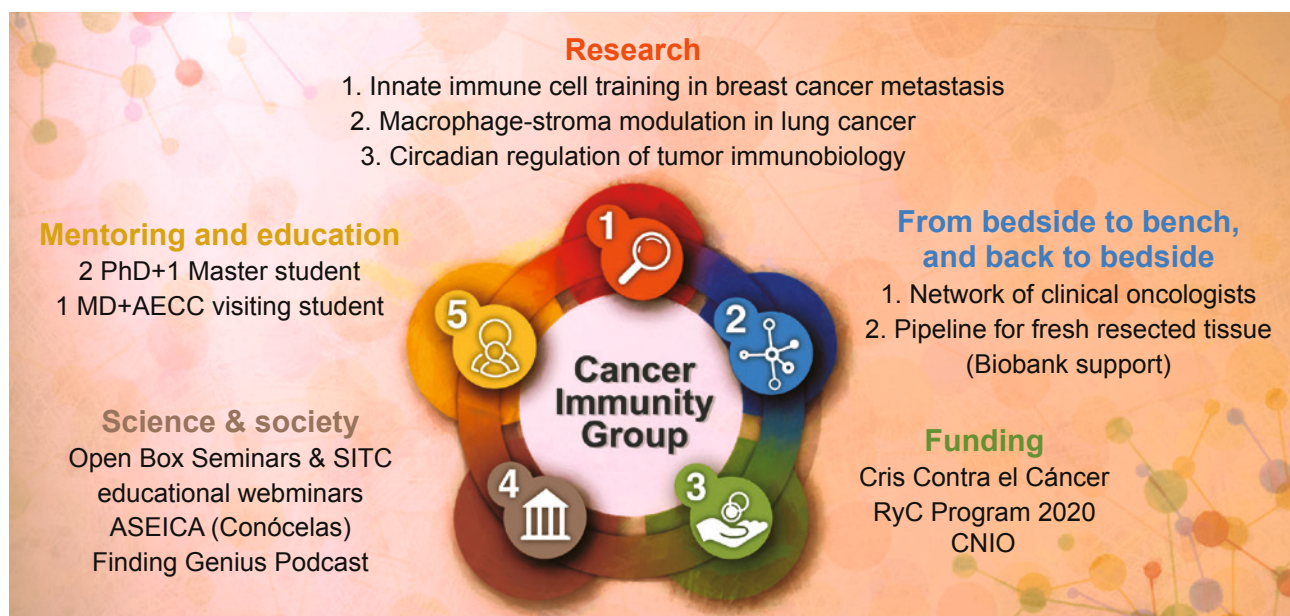


FIGURE Laboratory Strategy of the Cancer Immunity Laboratory.

► PUBLICATIONS

- Hidalgo A, Casanova-Acebes M. Dimensions of neutrophil life and fate. *Seminars in Immunology*. PMID: 34711490.

► PUBLICATIONS AT OTHER INSTITUTIONS

- Casanova-Acebes, M., Dalla, E., Leader, A.M., LeBerichel J, Nikolic J, Morales BM, Brown M, Chang C, Troncso L, Chen ST, Sastre-Perona A, Park M, Tabachnikova A, Dhainaut M, Hamon P, Maier B, Sawai

CM, Agulló-Pascual E, Chober M, Brown BD, Reizis B, Marron T, Kenigsberg E, Moussion C, Benaroch, Aguirre-Ghiso JA, Merad M (2021). Tissue-resident macrophages provide a pro-tumorigenic niche to early NSCLC cells. *Nature* 595, 578-584.

► AWARDS AND RECOGNITION

- María Casanova-Acebes:
- Research contract from the *Ramón y Cajal* Programme, State Research Agency of the Ministry of Science and Innovation, Spain.
- *Cris Talento Post-Doc* Award, *Cris* Cancer Foundation, Spain.

STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA Programme Director



The Programme's research areas and strategic goals

The research performed within the Structural Biology Programme (SBP) focuses on 2 major strategic areas. On the one hand, we use structural and molecular biology to investigate the molecular mechanisms of proteins and macromolecular complexes that contribute to cancer progression. For this, we apply a combination of approaches and methods, but single-particle cryo-electron microscopy (cryoEM) remains one of our core structural technologies. The Programme studies protein kinases, protein complexes involved in the cellular response to DNA damage and genomic instability, proteins participating in DNA replication as well as molecular chaperones. A better understanding of how these macromolecules work and the determination of their atomic structures provides the knowledge needed to understand their roles in cancer and potentially guide new therapeutic opportunities. To achieve these goals, the Groups are supported by specialised Units with expertise in several technologies and methods needed for protein production and characterisation, including cryoEM, NMR, protein crystallography, and biophysical methods such as bio-layer interferometry, SEC-MALS, Surface Plasmon Resonance and others. These platforms are used not only by the Groups within the Programme but also by several others at CNIO. On the other hand, the Programme uses bioinformatics tools, computational cancer genomics and computational oncology to better understand the complexity of cancer, predict therapy responses and develop new therapeutic strategies. As part of these efforts, the Bioinformatics Unit has significant synergies with several groups at CNIO and develops computational tools and methods of interest in cancer research. SBP currently consists of 1 Senior Group, 4 Junior Groups and 5 Units.

Summary of milestones & major achievements in 2021

During 2021, the Structural Biology Programme made substantial contributions in several areas of cancer research. Cryo-EM was used to advance our understanding of the molecular and structural mechanisms of several cellular pathways relevant in cancer. For example, the Programme determined the atomic structures of molecular chaperones involved in mTOR activation and spliceosome assembly, studied mechanisms that transport amino acids across the cell membrane that can be targeted against cancer, and advanced

“Our Programme uses structural biology, computational and genomic tools to improve our understanding of the complexity of cancer and of the proteins involved.”

in the molecular understanding of some of the mechanisms required to repair lesions in the DNA that accumulate and give rise to cancer. On the computational and bioinformatics front, the Programme contributed to the study of genetic interactions in cancer genes as well as the analysis of markers in cancer progression, among several other efforts. We also developed several computational pipelines and tools to help cancer studies such as single-cell RNA-seq analyses.

Several of SBP's Units and Groups made significant contributions to the work of other CNIO groups as part of synergistic collaborations, helping to understand the toxicity of peptides linked to amyotrophic lateral sclerosis, contributing to the study of how melanoma-derived small extracellular vesicles induce metastasis and how centrosome duplication defects cause microcephaly, and helping to characterise trimerbodies as potential therapeutic proteins in cancer. Finally, it is imperative to mention that our expertise in protein structure and purification was put at the service of research against the pandemic. The Structural Biology Programme helped in studies about the prognosis of COVID-19 patients admitted to intensive care units and in the study of mucosal immunotherapy as potential protection against SARS-CoV-2 infection. ■

MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Óscar Llorca
Group Leader

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



Post-Doctoral Fellows
Sofía Cabezero, María
Martínez (since July), Alba Ruiz
(until October)

Graduate Students
Natalia Cuervo, Nayim González

Technicians
Paloma Escudero (since May) (TS)*,
Ana González

**Titulado Superior* (Advanced Degree)

Visiting Scientist
Alberto Palacios (October-
December) (*Instituto de Biología
Molecular y Celular de Plantas*,
Valencia, Spain)

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 chaperones essential for the activation of several macromolecular complexes relevant in cancer such as those formed by the mTOR kinase, and the study of complexes implicated in the repair of DNA double-strand breaks. In addition, and in collaboration with other groups, we are studying the structure and mechanisms of several amino acid transporters.

“We have characterised the structure and the molecular mechanisms of 2 protein complexes (TELO2-TTI1-TTI2 and LAT2/CD98hc) and 1 long non-coding RNA, considered important players in cancer.”

RESEARCH HIGHLIGHTS

Structure of the TELO2-TTI1-TTI2 complex and its role in mTOR activation

As part of a collaboration with Laurence H Pearl and Chrisostomos Prodromou at the Genome Damage and Stability Centre, University of Sussex, UK, we helped to determine the structure of the TELO2-TTI1-TTI2 complex using cryoEM. This complex is essential for the maturation and activation of mTOR, a serine/threonine protein kinase that regulates several essential processes such as cell growth, cell proliferation, cell motility, autophagy, and protein synthesis. The mTOR signalling pathway is often activated in tumours, and the pathway is being studied intensively in the search for anti-cancer therapies. The structure of the TELO2-TTI1-TTI2 complex that we helped to resolve, together with biochemical experiments, revealed some of the mechanisms involved in the activation of mTOR by chaperones.

Long non-coding RNAs in DNA double-strand breaks in hepatocellular carcinoma

Long noncoding RNAs (lncRNA) are now considered essential players in cancer but the mechanisms are poorly understood. As part of a consortium involving several institutions in Europe and the USA, and directed by Puri Fortes at the Centre for Applied Medical Research (CIMA), University of Navarra, in Pamplona (Spain), we contributed to studying the mechanisms of NIHCOLE, a novel lncRNA induced in hepatocellular carcinoma (HCC), whose expression is associated with poor prognosis and survival. In a close partnership between our group at CNIO and the group of Fernando Moreno-Herrero at the CNB-CSIC in Madrid, and with funding from the local Government of Madrid, we used single-molecule imaging methods (AFM and electron microscopy) to characterise the structure of this lncRNA. These images show that NIHCOLE functions as a scaffold promoting the assembly of large multimeric complexes of proteins involved in the repair of DNA double-strand breaks.

Structure of heteromeric transporters of neutral amino acids

Amino acids play a central role in cellular metabolism. The transfer of amino acids across the plasma membrane is performed by proteins that bind and transport these molecules from the extracellular medium into the cell, and vice versa. Heteromeric Amino acid Transporters (HATs) are a family of amino acid transporters that harmonise amino acid concentrations at each side of the plasma membrane, and

they play a significant role in cancer and several inherited diseases. Several loss-of-function mutations in human LAT2/CD98hc are associated with age-related hearing loss and cataracts, and its overexpression in pancreatic cancer cells sustains glutamine-dependent mTOR activation to promote glycolysis and chemoresistance. LAT1/CD98hc is also linked to cancer and autism.

Each member of the HAT family displays a preference for transporting a certain set of amino acids. This specialisation explains the function of each HAT family member in certain physiological processes and diseases. The molecular mechanisms explaining why each family member shows exquisite preference for transporting some amino acids but not others had been mostly unknown. We determined the structure of one such member of the HAT family, LAT2/CD98hc, using cryoEM. This structural information, together with molecular dynamics and mutational and functional studies, enabled us to specify a few residues present in the substrate-binding pocket that contribute to determining substrate preference. ■

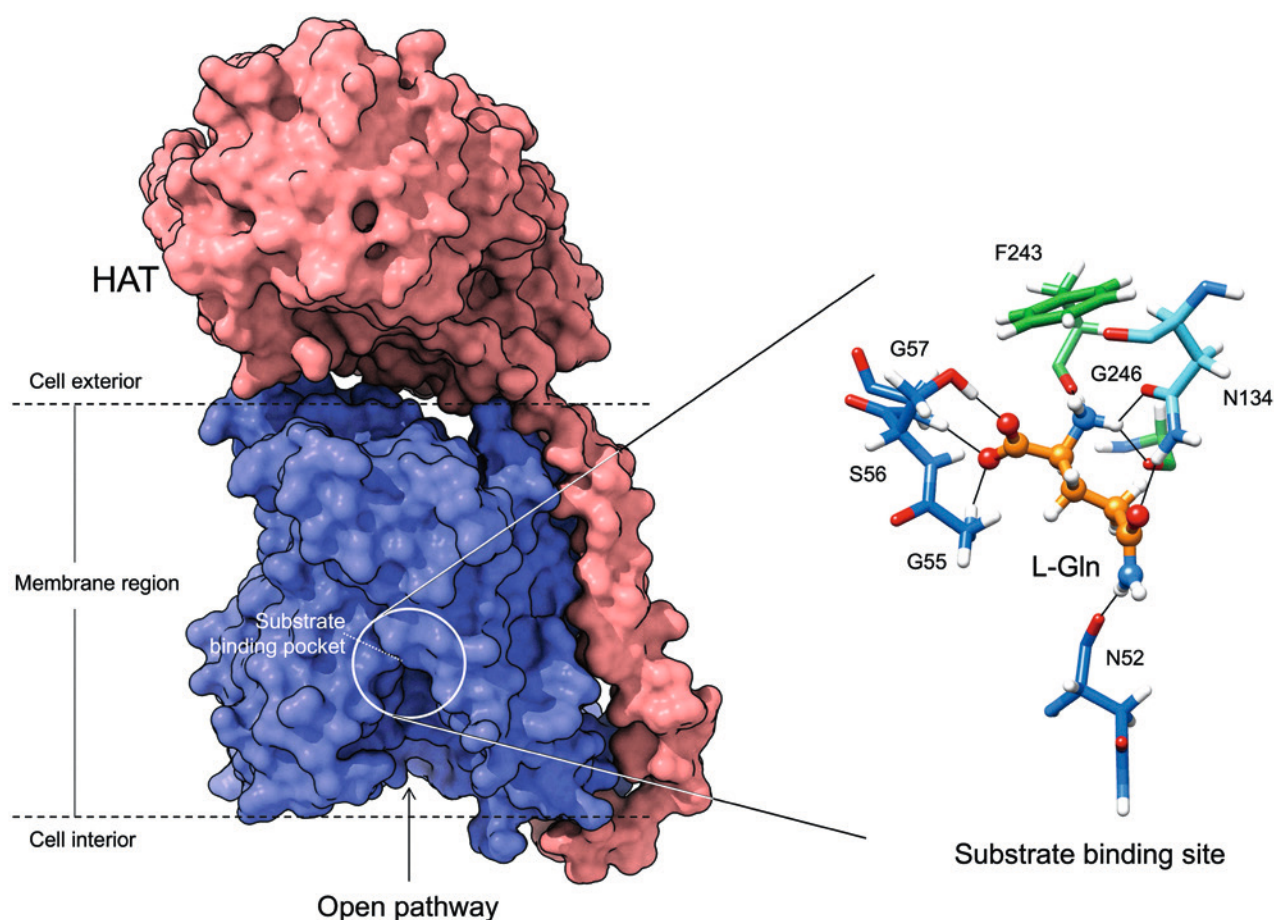


FIGURE 1 Structure of the LAT2/CD98hc HAT amino acid transporter. One view of the cryo-EM structure of LAT2/CD98hc indicating the position

of the substrate binding site, which is open to the cytosol. Molecular dynamics and modelling were used to determine how different amino

acids bind the transporter. Modified from Rodriguez CF *et al.*, *PNAS* 2021.

PUBLICATIONS

- Rivera-Calzada A*, Famelis N, Llorca O, Geibel S* (*co-corresponding authors) (2021). Type VII secretion systems: structure, functions and transport models. *Nat Rev Microbiol.* 19, 567-584.
- Unfried JP, Marín-Baquero M, Rivera-Calzada Á, Razquin N, Martín-Cuevas EM, de Bragança S, Aicart-Ramos C, McCoy C, Prats-Mari L, Arribas-Bosacoma R, Lee L, Caruso S, Zucman-Rossi J, Sangro B, Williams G, Moreno-Herrero F, Llorca O, Lees-Miller SP, Fortes P (2021). Long noncoding RNA NIHCOLE promotes

ligation efficiency of DNA double-strand breaks in hepatocellular carcinoma. *Cancer Res* 81, 4910-4925.

- Rodriguez CF, Escudero-Bravo P, Díaz L, Bartoccioni P, García-Martín C, Gilabert JG, Boskovic J, Guallar V, Errasti-Murgarren E*, Llorca O*, Palacin M* (*co-corresponding authors)(2021). Structural basis for substrate specificity of heteromeric transporters of neutral amino acids. *Proc Natl Acad Sci U S A* 118, e2113573118.
- Pal M, Muñoz-Hernandez H, Bjorklund D, Zhou L, Degliesposti G, Skehel JM, Hesketh EL, Thompson RF, Pearl LH*, Llorca O*, Prodromou C (*co-corresponding authors)

(2021). Structure of the TEL02-TTII-TTII2 complex and its function in TOR recruitment to the R2TP chaperone. *Cell Rep* 36, 109317.

- Del Fresno C, García-Arriaza J, Martínez-Cano S, Heras-Murillo I, Jarit-Cabanillas A, Amores-Iniesta J, Brandi P, Dunphy G, Suay-Corredera C, Pricolo MR, Vicente N, López-Perrote A, Cabezu S, González-Corpas A, Llorca O, Alegre-Cebollada J, Garaigorta U, Gastaminza P, Esteban M, Sancho D (2021). The Bacterial mucosal immunotherapy MV130 protects against SARS-CoV-2 infection and improves COVID-19 vaccines immunogenicity. *Front Immunol* 12, 748103.

AWARDS AND RECOGNITION

- Advisory Board for R&D and Innovation of the Autonomous Government of Navarre, Spain.
- Scientific Advisory Board, Biomedical Research Centre of the Government of Navarre (NAVARRABIOMED), Spain.
- External Scientific Advisory Board, Molecular Biology Institute of Barcelona (IBMB), Spain.

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellows
Julia M. Contreras, Rubén Julio
Martínez-Torres (until December)

Graduate Students
Nicolás Cuesta, Ana Martín, Moustafa
Ahmed Shehata

Students in Practice
Aurane Froux (January-June)



OVERVIEW

Rational and precise targeting of oncogene-driven signalling is a crucial and yet today outstanding challenge in current 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 posttranslational modifications and allosteric inputs, and how they assemble into macromolecular protein complexes to transmit signals inside the cell. We place 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 more potent and specific protein kinase inhibitors, leading

eventually to more effective drugs for the treatment of cancer patients.

We apply an integrated and multidisciplinary approach 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-visualization 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 the design, development, and optimization of protein kinase inhibitors as therapeutic agents in cancer.

(Université de Lorraine, Nancy, France)
Marina Rodríguez (since October)
(Universidad Autónoma de Madrid, Madrid, Spain)

Visiting Scientist
Yanara Astudillo (Universidad Tecnológica Equinoccial - Fundación Carolina, Quito, Ecuador)

RESEARCH HIGHLIGHTS

In 2021, we made significant progress in all our laboratory research projects, which are materializing successfully and, as a result, several papers were submitted for publication and are under review.

1. c-Src codifies a non-receptor tyrosine kinase that is activated by a plethora of signalling receptors that are fundamental in the aetiology of cancer. Despite being the object of intense study over the last 40 years, the precise mechanism by which auto-phosphorylation regulates c-Src intrinsic activity and conformational state independent of external inputs, and how this process is corrupted in cancer, remains elusive. In our work we show that c-terminal Tyr 530 is a *de facto* c-Src auto-phosphorylation site with slow time-resolution kinetics and a strong intermolecular component. By contrast, activation-loop Tyr 419 undergoes very fast kinetics and a cis-to-trans phosphorylation switch that controls c-terminal phosphorylation, substrate specificity and substrate-like properties. In line with these findings, a *Drosophila* mutant at the equivalent residue in the activation loop shows tissue-specific functionality and milder but transforming phenotypes compared with wild-type or constitutive active variants. Furthermore, we provide evidence that the intrinsically disordered N-terminal region of c-Src does not promote direct dimerization in the “apo” or the ATP-complexed states, and that c-Src Tyr 530 auto-phosphorylation is associated with a lowered catalytic status. A crystal structure of the c-Src-Ponatinib complex in a DFG-out state reveals unusual active-like features and provides a clear snapshot of c-terminal Tyr 530 intermolecular phosphorylation between enzyme and substrate acting kinases. Altogether these data indicate that c-Src must adopt an alternative conformation to the inactive-closed state independent of c-terminal Src kinase phosphorylation, and that a sequential and coordinated cis-to-trans phosphorylation switch between the activation and c-terminal segments simultaneously controls c-Src catalytic and non-catalytic functions (Cuesta and Contreras *et al.*, submitted for publication).

2. Gene fusion products are known drivers in human cancers and are current drug targets for personalised therapy. A second research line in the lab was established and directed at dissecting the functional and structural determinants for 2 RET oncogenic fusion products, CCDC6-RET and KIF5B-RET. 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 MS highlighted important roles for catalytic activity and substrate specificity through unexpected allosteric inputs by distant elements to the catalytic site (Hurtado *et al.*, in preparation).

3. A third research line focuses 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 uncovered a cryptic-pocket within the ATP-binding site that is exploited by highly specific second-generation type I RET inhibitors. This information will be crucial to designing and developing highly specific third generation RET inhibitors that are clinically successful and able to overcome refractory RET mutations (Shehata *et al.*, in press).

4. We initiated a new research line focused on the structural and functional characterization of human FAK. We want to explore how auto-phosphorylation drives the functional and conformational landscape of FAK, in a full-length setting, and how phosphorylation interferes with the assembly and interaction with substrates and signalling partners such as RET (both wild-type and oncogenic variants) and c-Src. Using a phospho-proteomic approach we already identified unexpected phospho-sites and revealed a previously unknown switch for FAK catalytic activation by N-terminal elements, which could be therapeutically exploited to design and develop next generation FAK inhibitors.

► PATENT

► Plaza Menacho I (2021). EP21382869.2.

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
Junior Group Leader

Post-Doctoral Fellow
María Dolores Moreno

Graduate Students
Ester Casajús, Samuel Míguez

Technician
Araceli Grande (TS)*

**Titulado Superior (Advanced Degree)*



OVERVIEW

Safeguarding genetic information is essential to all forms of life. Two key cellular processes keep it free from errors: DNA replication and 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 a hallmark of cancer, but it remains unclear how this happens at the molecular level. The devil is in the detail, and we aim to understand what and when something goes wrong with these molecular machines, so that we can act to correct it and prevent it from happening.

These macromolecules are like real-life machines, with intricate mechanisms that allow them to perform their activities. To understand how they work, we use cryo-electron

“Using cryo-EM, we have captured the DNA mismatch repair machinery in multiple functional steps and studied conformational changes that these proteins undergo to recognise the mismatch and license the events that lead to repair.”

microscopy (cryo-EM) and biochemistry in an integrative approach. Beyond fundamental research, this structural information provides the necessary detail for drug development.

Students in Practice

Emma Arean (since September) (*Universidad Politécnica de Madrid, Spain*), Anna Martina Lippert (until June) (*Universidad Internacional Menéndez Pelayo, Madrid, Spain*),

Marta Seijo (*Universidad Complutense de Madrid, Spain*)

Visiting Scientist
Svein Isungset Stove (University of Bergen, Bergen, Norway)

RESEARCH HIGHLIGHTS

Mismatch repair

The DNA mismatch repair machinery (MMR) corrects the errors introduced by DNA polymerases during DNA replication and is critical for genome stability. The MutS protein loads onto newly synthesised DNA and searches for mismatches. Recognition of an error in DNA leads to an ATP-dependent conformational change that transfers MutS into a sliding clamp state. Only this MutS state can activate the MutLATPase, which in turn promotes the removal of the DNA for repair. These protein complexes are incredibly dynamic and flexible. Because of this, critical steps of this process have remained elusive to structural analysis. Using cryo-EM, we captured multiple functional steps and studied the conformational changes that these proteins undergo to recognise the mismatch and license the downstream events that lead to repair. These studies were carried out in collaboration with T. Sixma

(Netherlands Cancer Research Institute) and M. Lamers (Leiden University).

DNA replication & repair – focus on mitochondria

Eukaryotic cells have 2 genomes: nuclear and mitochondrial. 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. By combining *in vitro* reconstitution of DNA replication complexes with cryo-EM imaging, we aim to capture the replication machinery at different functional stages, allowing us to understand in detail its mechanisms and how it is regulated. ■

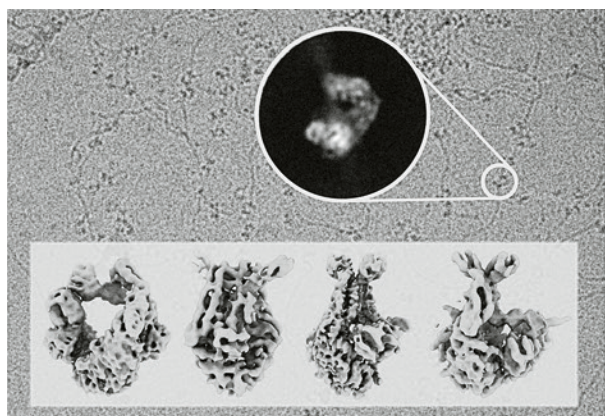


FIGURE 1 Mismatch repair studies. The background of the image shows a cryo-EM micrograph of MutS protein (white circle) on DNA (long strings). Circular insert shows a 2D class average of the protein after image processing. The bottom rectangular insert highlights the multiple structures solved in the

successive steps of the DNA repair process: mutS loading and DNA scanning, mismatch binding, clamp formation, and mutL recruitment and sliding clamp formation. These steps control the licensing of the DNA mismatch repair pathway.

PUBLICATIONS

- Fernandez-Leiro R*, Bhairasing-Kok D, Kunetsky V, Laffey C, Winterwerp HH, Groothuizen F, Fish A, Lebbink JHG, Friedhoff P, Sixma TK*, Lamers MH* (2021). The selection process of licensing a DNA mismatch for repair. *Nat Struct Mol Biol* 28, 373–381. (*) Corresponding authors.
- Gutmann C, Takov K, Burnap SA, Singh B, Ali H, Theofilatos K, Reed E, Hasman M, Nabeebaccus A, Fish M, McPhail MJ, O’Gallagher K, Schmidt LE, Cassel C, Rienks M, Yin X, Auzinger G, Napoli S, Mujib SF, Trovato F, Sanderson B, Merrick B, Niazi U, Saqi M, Dimitrakopoulou K,

Fernández-Leiro R, Braun S, Kronstein-Wiedemann R, Doores KJ, Edgeworth JD, Shah AM, Bornstein SR, Tonn T, Hayday AC, Giacca M, Shankar-Hari M, Mayr M (2021). SARS-CoV-2 RNAemia and proteomic trajectories inform prognostication in COVID-19 patients admitted to intensive care. *Nat Commun* 12, 3406.

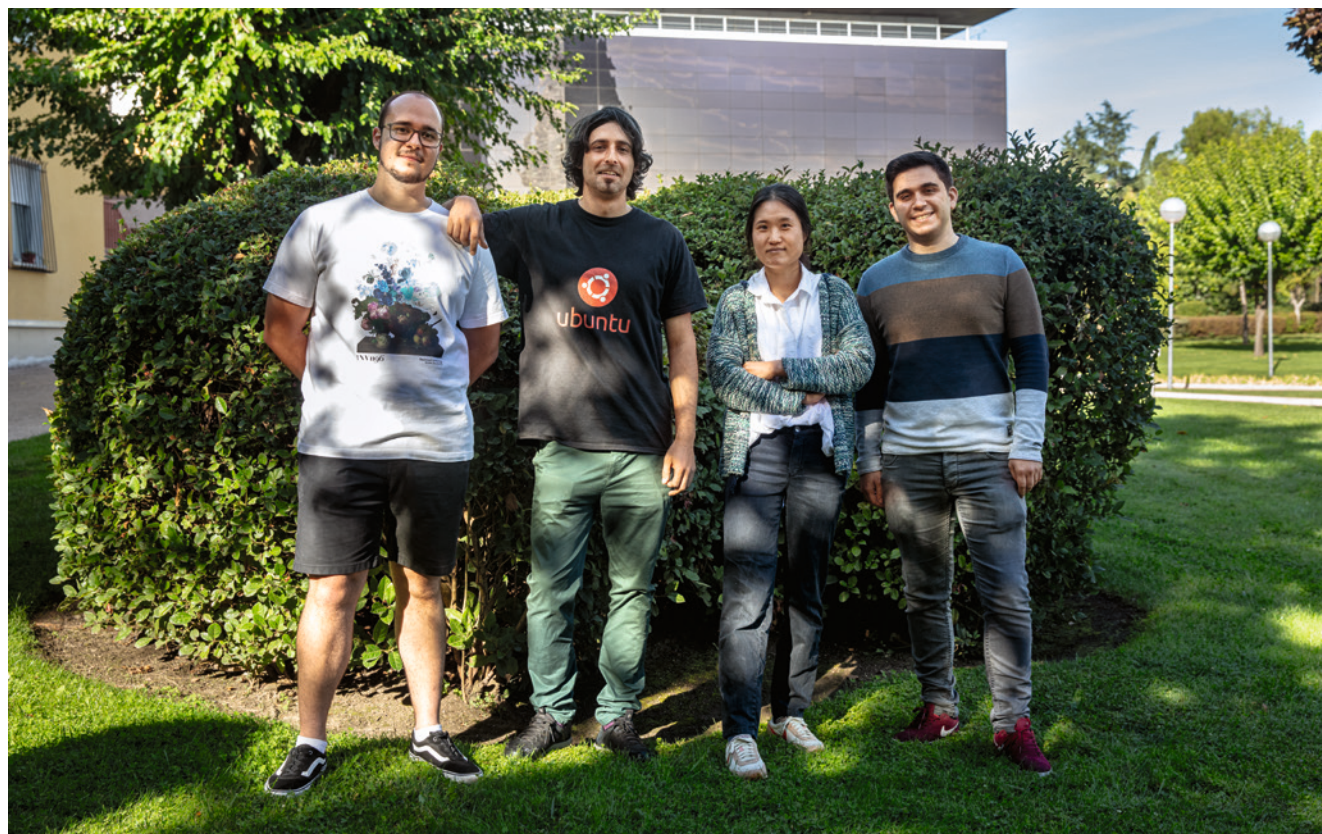
- Lafarga V, Sirozh O, Díaz-López I, Galarreta A, Hisaoka M, Zarzuela E, Boskovic J, Jovanovic B, Fernandez-Leiro R, Muñoz J, Stoecklin G, Ventoso I, Fernandez-Capetillo O (2021). Widespread displacement of DNA- and RNA-binding factors underlies toxicity of arginine-rich cell-penetrating peptides. *EMBO J* 40, e103311.

COMPUTATIONAL CANCER GENOMICS JUNIOR GROUP

Solip Park
Junior Group Leader

Graduate Student
Pelayo González De Lena

Technician
Luis García



OVERVIEW

Cancer is one of the most complex human diseases, involving genetic, environmental, and even unknown factors. Over the past several decades, our knowledge of cancer has rapidly accumulated thanks to different omics technologies, including genomics and proteomics. However, we still lack a complete understanding of the cancer fitness landscape across conditions. For example, how do cancer genes change their working models of tumour progression depending on cancer types or contexts? What kind of trans-interactions exist between 2 genes or many genes beyond single-gene level alterations? How can protein complexes or interactions be perturbed depending on different mutation positions? Based on large-scale genomics and proteomics analyses, we aim to pursue these questions.

“High-order interactions identify mutations that change the dominance and dosage sensitivity of cancer genes. These high-order interactions in the same pathway can be alternative evolutionary paths.”

Visiting Master's Student
Manuel Moradiellos (since
September) (*Universidad Autónoma
de Madrid, Spain*)

Visiting Scientist
Lee Heetak (July-September) (IMBA
- Institute of Molecular
Biotechnology, Vienna, Austria)

RESEARCH HIGHLIGHTS

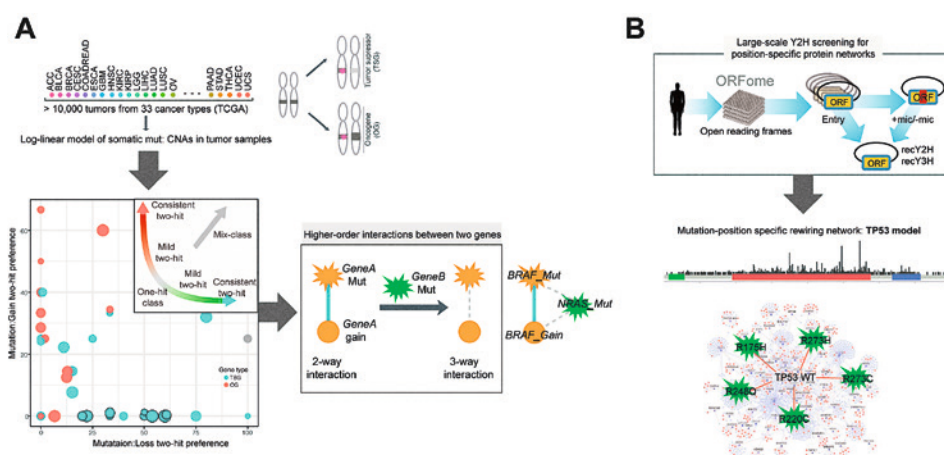


FIGURE Context-specific cancer fitness landscape. **(A)** The optimum model of cancer fitness is diverse across tissues. The third-order interaction refers to the phenomenon of the optimal path for cancer fitness landscape. **(B)** Mutation-specific protein interaction network with the TP53 model.

High-order genetic interactions between two genes

To better understand the dominance and dosage sensitivity of cancer genes, we systematically quantified the interactions between mutations and copy number changes. We found that many cancer genes do not behave like consistent models, but have activity-fitness functions that change across cancer types. To gain a better understanding of this switch, we identified one cause of these changes to be mutations in trans, as higher-order interactions. Most trans interactions were found to be in the same cancer signaling pathways and to share their functions. Our manuscript (in revision) will report the first analysis of high-order interactions in cancer genomics, based on studies conducted in collaboration with F. Supek (*IRB Barcelona, Spain*) and B. Lehner (*CRG, Barcelona, Spain*). Furthermore, we expand this concept to 2 different aspects: (1) time-dependent high-order interaction with germline variants, and (2) condition-specific high-order interactions with cancer-causing factors.

Looking beyond genomics to see cancer using TP53 and KRAS model

Over several years, more than 1,000 somatic drivers have been discovered by analysing huge amounts of genomics data.

However, we need a next-level analysis to obtain a complete view of their working model in cancer. To overcome this missing link, we proposed to map position-specific protein interaction networks by integrating genomics and large-scale Y2H screening. Specifically, we focused on the 2 most important cancer genes, *TP53* and the *RAS* family. In 2021, we created clones for > 10 *TP53* hotspot variants and > 50 *RAS* family variants and conducted large-scale Y2H screening with a complete human library. Our screening results will provide the systematic protein-interaction networks to show how protein interactions can be differentially changed depending on mutations. This will point the way to identifying new precision treatments based on differential protein interaction networks across patients. These studies were carried out in collaboration with Yang's Lab (*CRAG, Barcelona, Spain*). ■

PUBLICATIONS

- Kwon HY, Kumar Das R, Jung GT, Lee HG, Lee SH, Berry SN, Tan JKS, Park S, Yang JS, Park S, Baek K, Park KM, Lee JW, Choi YK, Kim KH, Kim S, Kim KP, Kang NY, Kim K, Chang YT (2021). Lipid-oriented live-cell distinction of B and T lymphocytes. *J Am Chem Soc* 143, 5836-5844.
- Park S, Supek F, Lehner B (2021). Higher order genetic interactions switch cancer genes from two-hit to one-hit drivers. *Nat Commun* 12, 7051.
- Herranz-Montoya I, Park S, Djouder N (2021). A comprehensive analysis of pre-foldins and their implication in cancer. *iScience* 24, 103273.

COMPUTATIONAL ONCOLOGY JUNIOR GROUP

Geoff Macintyre
Junior Group Leader

Staff Scientist
Maria José García



OVERVIEW

In the Computational Oncology Group, we are tackling some of the deadliest cancers by targeting the causes of chromosomal instability. Pancreatic, oesophageal, lung and ovarian cancers have some of the lowest survival rates, but they also share a common trait, which we can exploit – extreme chromosomal instability (CIN). By therapeutically targeting CIN, we aim to improve outcomes in these tumours.

Our main research areas include:

- Using model systems to develop therapeutic strategies to target CIN.
- Predicting therapy response using genomic signatures of CIN in patient biopsies.
- Developing single cell sequencing approaches to detect ongoing CIN.

“We have completed a proof-of-concept study showing that different types of CIN can be studied at high resolution, using single cell DNA sequencing, and induced via genome-editing.”

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

Post-Doctoral Fellow
Bárbara Hernando (since April)

Graduate Student
María Escobar (since February)

Technician
Alice Cádiz (since April) (PEJ, CAM)*

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

Visiting Master's Students
Blas Chaves (*Universidad Complutense de Madrid*, Madrid, Spain), Agustín Sánchez (February-June) (*E.T.S.I. Agronómica, Alimentaria y de Biosistemas*, Madrid, Spain)

RESEARCH HIGHLIGHTS

2021 saw the Computational Oncology Group shed its pandemic shackles and welcome 5 extremely talented new lab members and complete crucial proof-of-concept experiments.

Both Blas Chaves and Agustín Sánchez completed their master thesis projects: Agustín employed machine learning techniques to explore the relationship between DNA methylation and copy number signatures; and Blas quantified patterns of DNA copy number change at single cell resolution induced by knock-out of CDK18. Agustín has since taken a position in the Marcos Malumbres' Lab, and Blas is continuing as a PhD student building a large collection of induced CIN models in collaboration with the CNIO laboratories of Marcos Malumbres, Ana Losada, Felipe Cortés-Ledesma, Óscar Fernández-Capetillo, Juan Méndez, and Miguel Ángel Quintela-Fandino. As part of this project, Blas and Bárbara Hernando developed the first computational tool to come out of the lab, CNpare, which identifies related cell lines based on their genome-wide copy number profile (preprint here: <https://doi.org/10.1101/2021.09.28.462193>).

Following on from her excellent master's thesis identifying mismatch repair deficient ovarian cancers (ongoing project led by Maria José), Maria Escobar started her PhD where she will be using ongoing CIN to predict drug response in organoids and predict risk of progression in premalignant lung lesions. Alice Cádiz joined the lab with a training fellowship and is supporting all lab-based projects and keeping our databases in check.

On the computational side, David, a PhD student from Luis G. Paz-Ares' group, joined the team and will work on mutational signatures in lung cancer. Bárbara joined as a post-doc and has developed bioinformatics pipelines to support analysis

across all projects; she has also carried out crucial analysis for our pan-cancer copy number signature study (see FIGURE 1) correlating signatures with drug response for 1008 drugs across 297 cell lines.

Our early Christmas present — the cellenONE single cell sorter — now allows us to perform single cell DNA sequencing of human tissue samples, so watch this space for exciting new data in 2022! ■

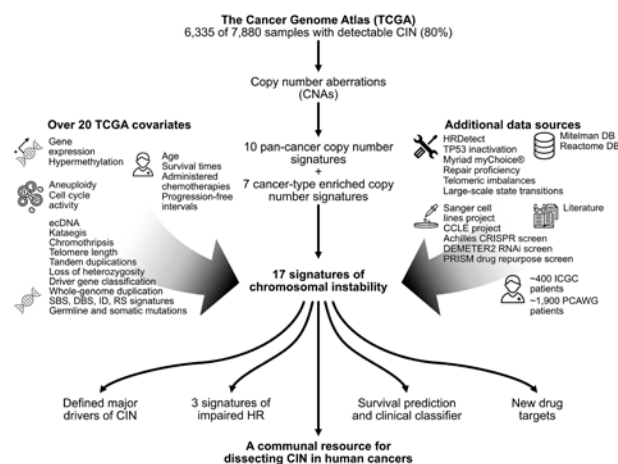


FIGURE 1 A summary of our study where we used DNA profiling of 6,335 tumours to identify copy number signatures of different types of chromosomal instability. We

integrated various data sources to propose signature aetiologies and use signatures to predict treatment response and find new drug targets.

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SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas
Unit Head

Technician
Clara M. Santiveri (TS)*

**Titulado Superior (Advanced Degree)*



OVERVIEW

This Unit focuses on the technical and scientific management of Nuclear Magnetic Resonance (NMR) Spectroscopy and molecular biophysics instrumentation available at the Structural Biology Programme. It provides CNIO researchers with equipment and experimental support for a variety of techniques used in biophysical studies of molecules involved in cancer. This includes the *in vitro* characterisation of the structure and dynamics of proteins by NMR, and of the affinity and kinetics of the interactions of proteins with other biopolymers and small molecules that could represent initial hits in the drug discovery process or research compounds for biophysical and functional studies. Furthermore, we use NMR to characterise the metabolic profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples. In addition, in 2021, we successfully installed a multiple well microplate reader that

“In 2021 we installed a new and versatile multiple well microplate reader that will allow, among other HT applications, quantification, inhibitor discovery, affinity determination and enzyme kinetics on liquid samples, as well as viability and other functional assays on cell samples.”

includes numerous detectors (absorbance, fluorescence intensity, polarisation and time resolved modes, luminescence, alphascreen, etc.) with excellent sensitivity for both 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, isothermal titration and differential scanning calorimeters, a circular dichrograph, dynamic and multi-angle static light scattering devices, two biosensor instruments — surface plasmon resonance (SPR) and biolayer interferometry (BLI) — and a multiple well microplate reader with numerous detectors. Research groups mostly from, but not limited to (i.e., Haematological Malignancies Clinical Research Unit, Monoclonal Antibodies Unit, Molecular Oncology Group and the Experimental Therapeutics Programme – ETP) the Structural Biology Programme used these technologies throughout the year.

The Unit hosts a 700 MHz NMR spectrometer that is equipped with probes and a sample changer to run up to 120 samples automatically. This provides medium throughput for the screening of small molecule protein binders (together with

the Experimental Therapeutics Programme), as well as for metabolite quantification that in 2021 was done in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (ETP), and the Growth Factors, Nutrients and Cancer, and Metabolism and Cell Signalling Groups (Molecular Oncology Programme). In collaboration with the latter group, we also implemented protocols to detect intracellular metabolites derived from the chemotherapeutic drugs 5-F-uracil and 5-F-uridine, using ^{19}F -NMR spectroscopy. For example, FIGURE 1 shows representative spectra that enable characterisation of the metabolic conversion of the drug into different nucleotides and activated sugars, and how it is affected by overexpressing an enzyme involved in purine metabolism and a mutant thereof, as well as the effect of chemical inhibitors acting upstream in the metabolic pathway. 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. ■

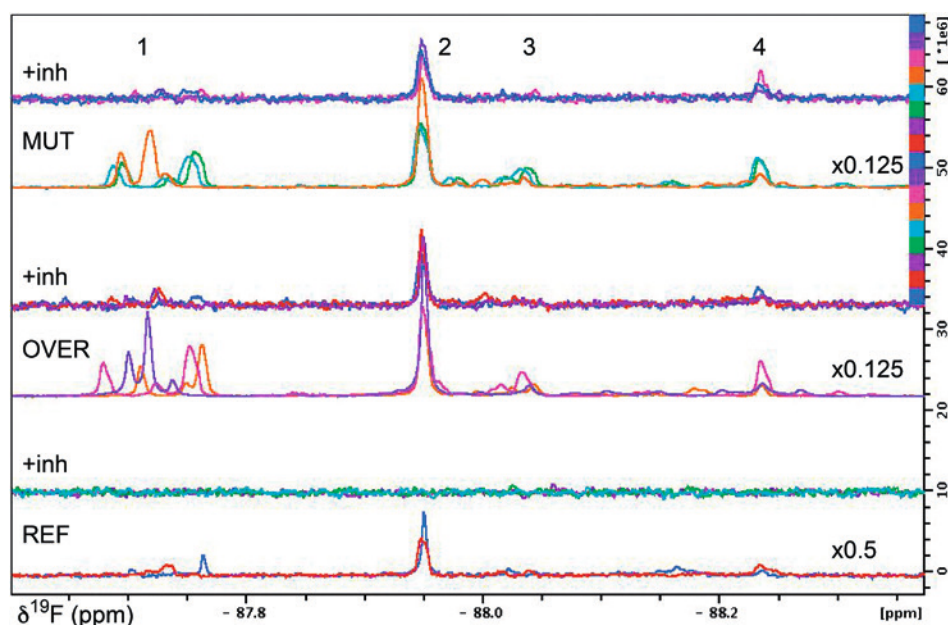


FIGURE 1 Superposition of the ^{19}F -NMR spectra of polar cell extracts following treatment with 100 μM 5-F-uridine for 6 hours. From bottom to top, samples correspond (in triplicate) to control (REF), overexpression of a purine metabolism gene (OVER), and overexpression of an inactive, mutant form of that gene product (MUT). In each of these 3 groups, alternating spectra of control cells (bottom) and of those treated with a chemical inhibitor (+inh, top) of an upstream positive regulator enzyme in the pathway are displayed. The 3 different groups of control spectra (non-treated with inhibitor) are vertically scaled as indicated to facilitate comparison. Tentative assignments of the signals to specific metabolites derived from 5-F-uridine are numbered: 5-F-UDP-hexoses (1), 5-F-UTP (2), 5-F-UDP (3), unknown (4).

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BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Staff Scientist
Michael Tress

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

**Plan de Empleo Joven de la Comunidad de*



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 comprehensive 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

“We have introduced Beyondcell, the first bioinformatics method to define tumour cell subpopulations of differential drug response at single-cell resolution and have proposed cell-specific anticancer treatments.”

analysis and interpretation using computational and statistical methods, (iv) to maintain the scientific computing facilities at the CNIO, and (iv) to provide training in bioinformatics tools and methods.

Madrid (Youth Employment Plan,
Community of Madrid)

Bioinformaticians

Daniel Cerdán (TS) **, Tomas Di
Domenico (TS) **, Coral Fustero (TS)**,

Gonzalo Gómez (TS) **, Osvaldo
Graña (TS) **, Elena Piñeiro (TS) **,
Thomas Anthony Walsh (until April)
(TS) **

***Título Superior* (Advanced Degree)

Students in Practice

Lourdes González (May-December)
and Carlos León (May-December)
(*Master's Programme in
Bioinformatics, Personalised*

Medicine and Health, ISCIII-ENS,
Madrid, Spain)

Visiting Scientist

Paolo Maietta (NIMGenetics, Madrid,
Spain)

RESEARCH HIGHLIGHTS

In 2021, the Bioinformatics Unit published more than 22 peer-reviewed articles as a result of our ongoing research projects and scientific collaborations (see the full list of our activities on our website: <https://bioinformatics.cnio.es/>). We studied cancer cell biology and drug response at single-cell resolution. To this end we developed Beyondcell (FIGURE 1), a computational methodology to identify tumour cell subpopulations under drug exposure, thereby revealing sensitive, innate, and acquired drug resistant cancer cells. Through this method we are able to propose possible treatment strategies to overcome such resistance and to identify drug-response markers. Using Beyondcell, we characterised single-cell variability in drug response in 198 cancer cell lines, finding recurrent patterns of drug heterogeneity and their relationship with the cells' functional status. Our software also allowed us to explore inter- and intra-tumour heterogeneity, linking it to clinical drug response data and to successfully predict responders and non-responders to immunotherapy among melanoma

patients (Fustero-Torre *et al.* 2021). Beyondcell is fully accessible at https://gitlab.com/bu_cnio/beyondcell.

During 2021, our group also assessed the clinical importance of tandem exon duplication-derived substitutions (Martínez-Gómez *et al.* 2021) and implemented Bollito (García-Jimeno *et al.* 2021), a comprehensive bioinformatics pipeline that performs basic and advanced single-cell RNA-seq analysis and TRIFID, a method for classifying the functional importance of splice isoforms (Pozo *et al.* 2021). Additionally, the BU served as an active node of the European network ELIXIR (<https://www.elixir-europe.org/>), leading the ELIXIR Cancer Data Focus Group to provide the framework and expertise for the systematic analysis and interpretation of cancer genomes. With regard to academic and knowledge-transfer activities, we co-organised the Master's degree programme in Biocomputing Applied to Personalised Medicine and Health at the National Institute of Health Carlos III (*Máster en Bioinformática Aplicada a Medicina Personalizada y Salud, ENS-ISCIII*). ■

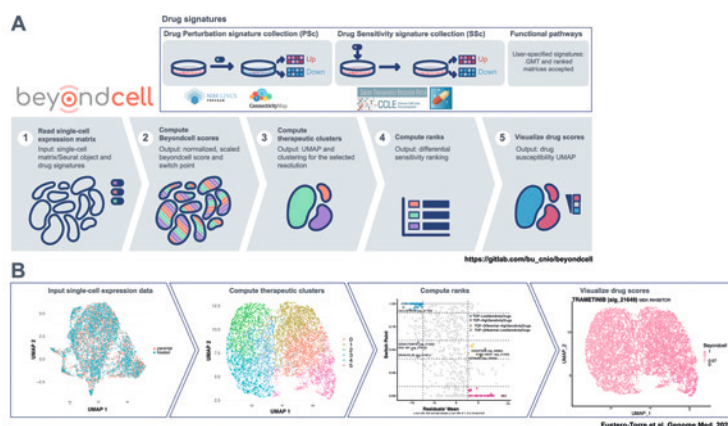


FIGURE 1 (A) Beyondcell workflow. (B) Beyondcell identified the presence of therapeutic clusters (TCs) in BRAF-inhibitor resistant human melanoma cells.

Trametinib had a positive Beyondcell score in all TCs, with a higher sensitivity in TC5, showing it could be proposed to target BRAFi-unresponsive cells.

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FCP, Parker A, Pei B, Pozo F, Riera FC, Ruffier M, Schmitt BM, Stapleton E, Suner MM, Sycheva I, Uszczynska-Ratajczak B, Wolf MY, Xu J, Yang YT, Yates A, Zerbino D, Zhang Y, Choudhary JS, Gerstein M, Guigó R, Hubbard TJP, Kellis M, Paten B, Tress ML, Flicek P (2021). GENCODE 2021. *Nucleic Acids Res* 49, D916-D923.

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tion-derived substitutions. *Nucleic Acids Res* 49, 8232-8246.

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ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Post-Doctoral Fellow
Johanne Le Coq

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

*Titulado Superior (Advanced Degree)

**Plan de Empleo Joven (Youth Employment Plan)



OVERVIEW

The main objective of the Electron Microscopy (EM) Unit is to provide scientific-technical support to researchers to answer their biological questions using different transmission EM techniques. We regularly use negative staining and cryo-EM and help with image processing by performing 2D analysis and 3D reconstruction. We also offer support for choosing adequate EM techniques and performing sample preparation on different types of EM grids. Furthermore, we provide the necessary training for the use of our microscopes and auxiliary equipment. More advanced studies are typically delivered through research collaboration.

“We dedicate our main effort to ensuring efficient access to and use of existing infrastructure in the Unit. We also provide personalised scientific support and training for researchers.”

RESEARCH HIGHLIGHTS

In our studies, we take advantage of the continuous technical advances in cryogenic electron microscopy (cryoEM). Specifically, we use single-particle cryoEM to elucidate the structures of macromolecules at near atomic resolution. At the CNIO we have a 120 kV, Tecnai G2 Spirit microscope equipped with a TVIPS CMOS detector that is used to obtain images of negatively stained samples, to screen vitrified samples, and for small-scale data collection. For medium resolution structural studies, we use a JEM-2200FS cryo-electron microscope equipped with a 200 kV field emission gun and a K3 direct electron detector.

Our scientific activity throughout 2021 involved collaborations with the research groups of the Structural Biology Programme, as well as with groups from other Programmes and with scientists outside the CNIO. For example, together with CNIO's Microenvironment and Metastasis Group, we contributed to the analyses of secreted extracellular vesicles (EVs) that influence the tumour microenvironment and promote distal metastasis. In particular, we imaged melanoma-secreted EVs that have been associated with lymph node, pre-metastatic niche formation in murine models. With the Macromolecular Complexes in DNA Damage Response Group, we pursued our work to structurally characterise several protein complexes e.g., different RUVBL1/2 and DNA repair complexes. Our collaboration also continued with M. Palacín's group (*IRB* Barcelona), with whom we contributed to revealing the molecular mechanisms controlling substrate specificity within the heteromeric amino acid transporter (HAT) family of neutral amino acid transporters. These findings provide the structural bases for mutations in LAT2/CD98hc (HAT member) that alter substrate specificity and that are associated with several pathologies. Finally, in collaboration with the Genome Integrity and Structural Biology Group, we further improved our set-up to use a cryoEM as a tool for drug discovery. ■

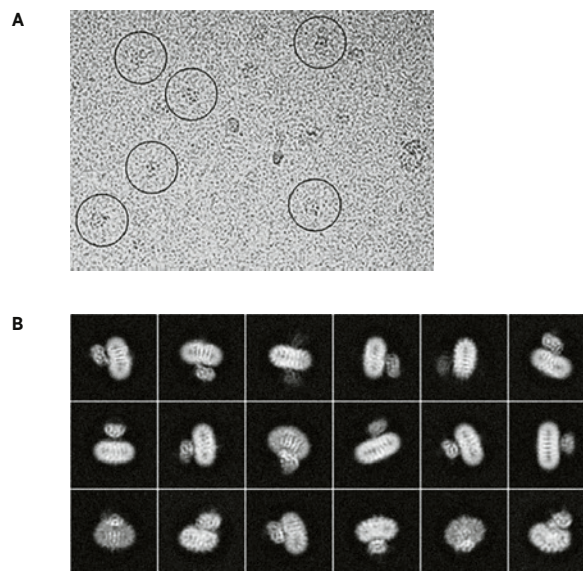


FIGURE CryoEM of the heteromeric amino acid transporter hLAT2/CD98hc embedded in a detergent micelle. **(A)** Representative cryo-electron microscopy field. **(B)** Reference-free 2D class averages.

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CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

Inés Muñoz
Unit Head

Staff Scientist
Jorge L. Martínez

Postdoctoral Fellow
Yudhi Nugraha (since March)

Technicians
Aida Contreras (TS) * (PEJ) **
Diana Martín (until April),



OVERVIEW

The Crystallography and Protein Engineering 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 proteins for different types of biochemical/biophysical/*in vitro*/*in vivo* assays, antibody generation, 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 is regularly used to map the interactions of these small molecules with proteins, speeding up drug discovery projects, not only in industry but also in academic groups.”

Álvaro Otero (until February) (PTA)^{***}
(TS)[†], Lluvia Rebollo (TS)[†] (PEJ)[†],
Pilar Redondo, César Rodríguez
(February-September)

[†] *Titulado Superior* (Advanced Degree)
^{††} *Plan de Empleo Joven* (Youth Employment
Plan)
^{***} *Personal Técnico de Apoyo* (Technical
Support Staff, MCI)

RESEARCH HIGHLIGHTS

The goal of fragment-based screening is to expose protein crystals to libraries of fragments and to solve the crystal structures of the complexes. Our first target was the dimerization domain of TRF1 (Telomeres and Telomerase Group). We identified 3 well-defined fragments bound to the protein that could be further exploited to develop new inhibitors.

Our Unit works closely with the Experimental Therapeutics Programme on several projects: human TRF1 dimerization domain, the kinase domains of human MASTL and human DDR1 for biochemical and structural analyses. Furthermore, to support drug discovery projects, we performed several thermal shift assays (thermofluor) in the presence of compounds developed in the Medicinal Chemistry Section.

We provide the proteins needed by the CNIO Monoclonal Antibodies Unit to generate antibodies, including the CD85 family, CSF3R, CLLU1, RANK, TACI and PILRA, among others. The Unit is also engaged in several internal collaborations with other CNIO groups, providing them with recombinant proteins for biochemical and/or cell-based functional assays.

Throughout 2021, the Unit also sustained its own scientific projects. We continued working on targeting the function of the Mdm2-MdmX E3 complex activity (NIH funded) as well as on the production of a T cell-recruiting bispecific antibody, ATTACK (funded by *Retos Colaboración*). The Unit is also taking part in 2 projects supported by BBVA Foundation grants. The first is a collaborative project with the Biomedical Application of Radioisotopes Unit at *CIEMAT* and the CNIO's

Molecular Imaging Unit to develop new antibody-based positron emission tomography (immunoPET) imaging tools for tumour visualization. The second project, carried out in collaboration with the Immuno-oncology and Immunotherapy Unit at the *Hospital 12 de Octubre*, has resulted in the generation of 2 new synthetic nanobodies capable of targeting the spike protein of the SARS-CoV-2 virus. These nanobodies will soon be tested in mouse models at the Poxvirus and Vaccine Laboratory by M. Esteban's group at the *CNB*. ■



FIGURE Three-dimensional crystal structure of the DDR1 kinase domain (in grey) in complex with the drug ETP-078 (in red).

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García P, Tapia-Galisteo A, Van Bergen En Henegouwen PMP, Sánchez A, Fabregat I, Sanz L, Zapata JM, Álvarez-Vallina L (2021). Case report: an EGFR-targeted 4-1BB-agonistic trimerbody does not induce hepatotoxicity in transgenic mice with liver expression of human EGFR. *Front Immunol* 11, 614363.

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► PATENT

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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 HCGP works in close collaboration with the clinical community.

In 2021, the Human Cancer Genetics Programme was composed of 2 Research Groups: Hereditary Endocrine Cancer and Genetic and Molecular Epidemiology; 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 de Fuenlabrada*.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. In 2021, the Familial Cancer Consultancy at the *Hospital de Fuenlabrada* attended around 397 consultancies, and the HCGP performed 900 genetic diagnoses and carried out 1,106 cytogenetic studies. In addition, the HCGP hosted 3 medical residents from different Spanish hospitals for 3-month training periods. 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 18 national visitors and students were hosted in 2021. In terms of education, 16 national PhD students worked on their research projects, 4 of whom already successfully defended their theses.

One of the main objectives of the Programme is to establish 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 January 2021, several of the HCGP's Group members, including our former HCGP Director Javier Benítez, Ana Osorio and Anna González-Neira, participated in a major international study on the inheritance of breast cancer, published in the prestigious *New England Journal of Medicine*.

Other major achievements of the Programme in 2021 include: COST Action CA20122 - Harmonizing clinical care and research on adrenal tumours in European countries, awarded to Mercedes Robledo; *Ramón Areces* Foundation grant to study the microbiome of high-grade non-muscle invasive bladder cancer, awarded to Núria Malats and the Weizmann Institute in Israel, in collaboration with Francisco X. Real; 1st prize “SETGyC Paper of the Year 2020-2021” in the Gene Therapy category (Molecular Cytogenetics Unit, *Nat. Commun.*); and the 2021 Molecular Cloud “Infinite Possibilities Teams Award” for genome editing (Molecular Cytogenetics Unit).

Upon the retirement of Javier Benítez in 2020, an international search was launched for a new director to lead a new Cancer Genomics Programme. The new programme will incorporate the Groups and Units from the former programme, together with other Groups that use biocomputational tools for the study of cancer, currently under the Structural Biology Programme. This area has the support of Raúl Rabadán, Professor in the Department of Systems Biology and Biomedical Informatics and Director of the Center for Topology of Cancer Evolution and Heterogeneity of Columbia University (New York, USA). In July 2021, Raúl was appointed as Adjunct Professor at the CNIO, an honorary appointment that allows him to maintain an official link with the Centre, reinforcing the presence of the CNIO in the field of computational biology approaches to cancer research.

Maria A. Blasco, Director
Óscar Fernández-Capetillo, Vice-Director

HEREDITARY ENDOCRINE CANCER GROUP

Mercedes Robledo
Group Leader

Staff Scientists
Alberto Cascón, Cristina Rodríguez



Post-Doctoral Fellows

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

Graduate Students

Ángel Fernández (since October), Javier Lanillos, Sara Mellid, María Monteagudo, Juan M. Roldán (until February), María Santos (until November), Carlos Valdivia (since September)

Technicians

Bruna Calsina (TS)¹, Eduardo Gil, Rocío Letón

¹*Titulado Superior* (Advanced Degree)

Students in Practice

Alberto Mora (May-December) (Master's Programme in Bioinformatics, *ISCIII-ENS*, Madrid, Spain), Valeria Piñel (until June) (*Universidad Autónoma de 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 NOP10 as a new metastatic risk marker for paraganglioma, and recommend genetic screening in patients with metastatic non-clear cell renal cancer, as they show high prevalence of pathogenic germline mutations in renal cancer-predisposition genes.”

RESEARCH HIGHLIGHTS

Analysis of telomere maintenance related genes reveals *NOP10* as a new metastatic-risk marker in Pheochromocytoma/Paraganglioma (PPGL)

One of the main problems we face with PPGL is the lack of molecular markers capable of predicting the development of metastases in patients. Telomere-related genes, such as *TERT* and *ATRX*, have been recently described as being associated with disease progression in PPGL. However, the contribution of other genes involving the telomere preservation machinery had not been previously investigated. We analysed the prognostic value of a comprehensive set of genes involved in telomere maintenance in an outstanding series of genetically characterised PPGL samples. In addition to *TERT* and *ATRX*, *NOP10* showed differential expression between metastatic and non-metastatic cases, and alterations in these genes were associated with a shorter time to progression. Analysis of telomere length by Q-FISH in patient samples, and in an *in vitro* model, showed that *NOP10* overexpression is linked to an intermediate-length telomere phenotype without ALT, and *in vitro* results suggested that *NOP10* has a role in telomerase dependent telomere maintenance. Altogether, the results have allowed us to propose *NOP10* as a new risk marker for metastatic disease in patients with PPGL, and to propose the use of *NOP10* immunohistochemistry as a tool for easy implementation in the clinical setting to stratify patients according to their risk at the time of diagnosis (see FIGURE).

DLST is a key regulator of succinylation with tumorigenic potential

Up to 20 genes have been described to be involved in PPGL susceptibility so far, highlighting the remarkable diversity of

biological pathways influencing the development of these tumours. Recently our group identified *DLST*, a gene encoding one of the OGDH complex subunits of the TCA cycle, as a new susceptibility gene for PPGL. Though TCA cycle enzymes are common targets of PPGL predisposing mutations, it seems that the mechanism leading to tumorigenesis in *DLST*-mutated PPGLs is different from what has been previously described. We used a cell model to examine the potential consequences of mutated *DLST* in terms of its cellular location, function and affected molecular pathways. We found a significant decrease in the overall protein succinylation levels in the presence of *DLST* alterations. This post-translational modification (PTM) provides significant chemical and structural changes to proteins, most likely influencing their function. Accordingly, we found that the dysregulation of succinylation seems to have an impact in several essential pathways within cellular metabolism. These results suggest a key role of *DLST* in protein succinylation and support the relevance of this PTM in the development of different types of cancer.

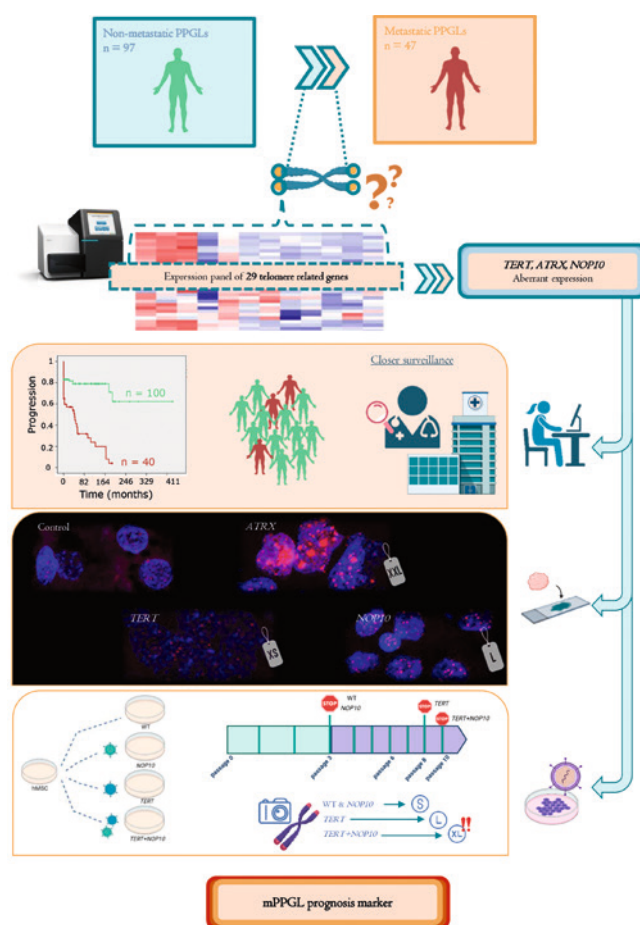
Prevalence of pathogenic germline variants in patients with metastatic renal cancer

Germline mutations are estimated to affect 3-5% of renal cell carcinoma (RCC) patients. However, higher mutational prevalence in non-clear cell histologies and advanced disease has been suggested. To clarify the incidence and spectrum of pathogenic germline mutations in RCC, we recruited 294 unselected metastatic RCC cases plus 21 RCC patients with clinical hereditary features. Germline mutations in RCC-predisposition genes (*FH*, *VHL*) occurred in 1.4% of the unselected patients, with a higher frequency in younger patients

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($P=0.036$) and in non-clear cell histologies ($P=0.0025$). Specifically, 14% of non-type 1 papillary cases carried an *FH* germline mutation with loss-of-heterozygosity and tumour DNA hypermethylation. Mutations in other cancer genes (e.g., *BRCA2*, *CHEK2*) occurred in 5.1% of cases, but had unclear significance for RCC. Our findings confirm high prevalence of pathogenic germline mutations in RCC-predisposition genes in metastatic non-clear cell RCC and highlight that metastatic patients with papillary type 2 or unconventional histologies compatible with *FH* would benefit from genetic screening.



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- ▶ **AWARDS AND RECOGNITION**
- Mercedes Robledo:
 - ▶ Awarded COST Action CA20122 - *Harmonizing clinical care and research on adrenal tumours in European countries* (HARMONISATION), July 2021.
 - ▶ Member of the WG4 Executive Committee focused on hereditary cancer, IMPaCT-Genómica project of the Spanish Institute of Health *Carlos III*, Spain.
 - ▶ Member of the Scientific Advisory Board of the Sant Pau Biomedical Research Institute (IIB Sant Pau) since September 2021, Spain.

GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

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Group Leader

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Jiangchuan He, Francisco J Jurado,



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Titulado Superior (Advanced Degree)

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Sebastián (Sept.-Nov.) (Bachelor's
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Laura Gutiérrez (until May) (*Univ.
Complutense de Madrid*, Spain)

Visiting Scientists

Helena Fidalgo (until May) (*CIBER*,
Madrid, Spain), Isabel A. Martín
(*Univ. CEU San Pablo*, Madrid, Spain),
Ashwag M. Mukhtar (since Dec.) (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.

“Characterising the tumour immune infiltrating repertoire provides insights to understand its immunogenicity and its association with molecular and clinical factors for better patient stratification.”

RESEARCH HIGHLIGHTS

Research Findings

In 2021, GMEG continued to contribute to the pancreatic and bladder cancer epidemiological fields. Regarding **pancreatic cancer** (PC), we aimed to explore the immune repertoire landscape of PC and its association with risk factors and overall survival by extracting the immunoglobulins (IGs) and T cell receptors (TCRs) from the RNA-sequencing of 144 PC from The Cancer Genome Atlas (TCGA) and 180 pancreatic normal tissue from the Genotype-Tissue Expression (GTEx) project. PC presented richer and more diverse IG and TCR infiltration than normal pancreatic tissue. Higher IG infiltration was present in heavy smokers and women, and it was associated with better overall survival (FIGURE 1). In addition, specific IG clonotypes classified samples with better prognosis (FIGURE 2). On the other hand, greater TCR infiltration was present in patients with previous history of diabetes and was associated with lower nonantigen load. We also characterised the risk pattern of diabetes mellitus and BMI associated with PC using causal inference methods. When exploring the association between PC and gallbladder conditions, we concluded that this relationship may be non-causal and partly or largely due to diagnostic attention and/or reverse causation. In the genetic susceptibility field, an integrated GWAS approach allowed us to identify further 57 variants associated with PC. We also participated in international consortia to identify loci increasing the risk of PC among smokers and the association of hepcidin-regulating iron metabolism genes and genetic variants affecting microRNAs with PC. In the clinical field, we were involved in 2 studies on liquid biopsy revealing that KRAS negativity and longer overall survival were associated with hereditary/familial PC and identifying a plasma protein biomarker panel for early detection of

sporadic PC. Regarding **bladder cancer** (BC), in 2021 GMEG participated in several European projects in the clinical domain that have assessed the relationship between patient sex with treatment outcomes after intravesical BCG (bacillus Calmette-Guérin) immunotherapy for T1G3/HG bladder cancer and identified the risk factors for residual disease at re-TUR (repeat transurethral resection) in T1G3 patients and novel genes associated with recurrence and progression in non-muscle-invasive bladder cancer. Furthermore, we have been involved in a multi-omics analysis to identify prognostic molecular subtypes of non-muscle-invasive bladder cancer.

Methodological Contributions

GMEG also continued to explore the analytic strategies and tools to integrate omics and non-omics data into the cancer risk models, and made progress integrating medical image information (radiomics and digital pathology).

Translational Activities

GMEG actively supports several clinical trials on immunotherapy in BC at the methodological level. We continue to sustain the Spanish Familial PC Registry (PanGen-FAM) and the European Registry of PC (PancreOS). We are involved in the PC research platform (PC-CAM) to accelerate the translation of research results into the clinical domain. We lead the Research Work Stream of the Pancreatic Cancer Europe (EPC) multistakeholder platform, and we have moved ahead in increasing awareness about PC. We contributed to the UEG position paper on PC published in 2021. In addition,

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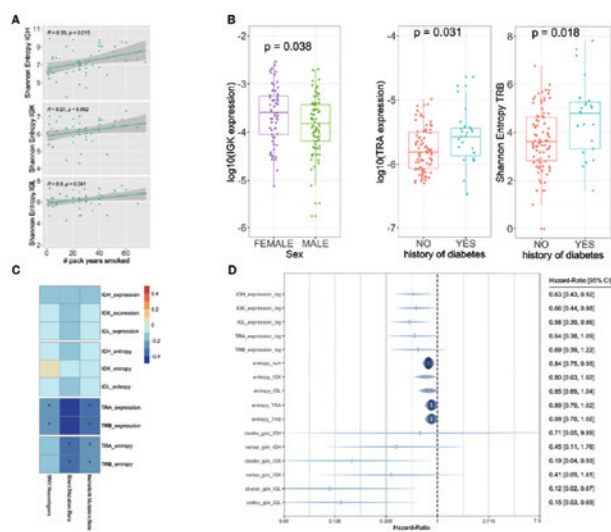


FIGURE 1 Linear regression model showing the association between the IG entropy and smoking (A). Boxplot showing the association between TCR and diabetes and the association between IG expression and sex (B). Pearson correlation showing the association between the TCR and IG

with neoantigen load and mutational rate (C). Forest plot showing the hazard ratios and 95% confidence intervals of IG/TCR measures with overall survival (D). The * shows if the correlation is significant (FDR < 0.05).

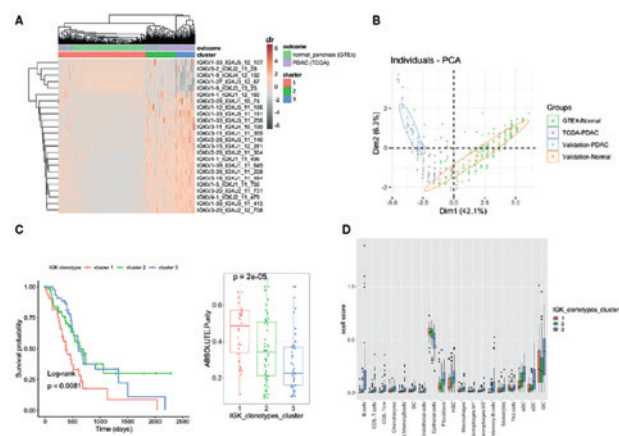


FIGURE 2 Heatmap showing the IGC clonotypes selected to discriminate PDAC (TCGA) vs. Normal Pancreas (GTEx) (A). Bi-plot showing the 2 first principal component analyses using the 24 clonotypes selected and applied to both

discovery and validation datasets (B). Overall survival Kaplan-Meier curves with the 3 clusters obtained and the boxplot with the tumour purity (C). Comparison between the 3 clusters with the cell content deconvoluted using xCell (D).

we joined an initiative of the European Alliance for Personalised Medicine to bring onco-innovation to Europe's healthcare systems by reviewing the potential of biomarker testing in the real world to empower personalised medicine.

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- 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 (2021). Faecal Microbiota Signature for Pancreatic Cancer. *EP21382876.7*.
- AWARDS AND RECOGNITION**
- Núria Malats, together with Francisco X. Real and the Weizmann Institute of Science, received Ramón Areces Foundation Grant to study the microbiome of high-grade non-muscle invasive bladder cancer, *Fundación Ramón Areces*, Spain.

FAMILIAL CANCER CLINICAL UNIT

Miguel Urioste
Clinical Unit Head

Staff Scientist
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OVERVIEW

Mosaic variegated aneuploidy (MVA) is a rare genetic condition that groups together a number of individuals with constitutional mosaic aneuploidies involving different chromosomes, associated with a constellation of clinical features such as developmental delay, microcephaly, and other congenital defects. Cancer predisposition, especially embryonal tumours, is one of the most important clinical signs of MVA. Among several others, spindle assembly checkpoint (SAC) is an important mechanism involved in the correct segregation of chromosomes, thus preventing the appearance of aneuploidies. To date, 3 SAC genes – *BUB1B*, *CEP57*, and *TRIP13* – have been identified as being involved in MVA. Mutations in these genes only account for a proportion of MVA patients. The identification of new genes involved in MVA is not only essential for genetic counselling and for the management of

these patients, but also to unravel the complex relationship between aneuploidy and carcinogenesis.

We also continued our research to try to decipher the genetic bases of familial breast and colorectal cancer, and to apply such knowledge to clinical practice through our Familial Cancer Consultancy in the Fuenlabrada University Hospital. In addition to both identifying new genes involved in the susceptibility to these tumours and quantifying the associated risk, we have also been exploring new therapeutic tools that could be useful in the future.

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Erik Michel Marchena (since April)
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*Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Community of Madrid)

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*Titulado Superior (Advanced Degree)

Student in Practice
Beatriz Benito (January-June)
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CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

Mosaic variegated aneuploidy (MVA). Using WES we found biallelic loss-of-function *MAD1* mutations in a 30-year-old female with multiple cancers, together with a constellation of benign neoplasia derived from the 3 embryonic layers, and developmental defects. The patient showed the highest rate of variegated aneuploidy associated with mutations in any gene involved in SAC (FIGURE 1) and *MAD1* protein absence, a circumstance that is in principle incompatible with embryonic development. In collaboration with other CNIO groups, we are investigating the relationship between aneuploidy and tumorigenesis, trying to identify a second mechanism — a dysfunction in APC/C or in another biological process involved in ensuring chromosome segregation — that could contribute to cell viability or evasion of embryonic lethality.

Deciphering the role of rare variants in Breast Cancer (BC). Earlier in 2021, the 2 largest BC case-control studies published so far confirmed 11 genes associated with BC susceptibility and that therefore should be included in diagnostic testing. All these genes were already considered clinically relevant, except *BARD1*, whose role in BC susceptibility had not been clearly established until now. Based on these results, we included *BARD1* in our diagnostic panel, and we analysed the gene in a large series of 2000 BRCA Spanish families. We found a strikingly high proportion of large deletions in the gene that suggest the existence of regional differences in the spectrum of mutations in the Spanish population.

Identification of new BC susceptibility genes. We previously identified *RECQL5*, a member of the RECQL-helicases family, as a new BC susceptibility candidate. More recently, we

identified 13 additional candidate genes that are currently being analysed by NGS to evaluate their role in the disease in a large set of 3000 Spanish BC families.

DNA glycosylase inhibitors as a new therapeutic approach in hereditary BC patients. We are exploring the possible therapeutic use of OGG1 glycosylase inhibitors in BC patients. We found that the inactivation of BER by the OGG1i TH5487 increases the accumulation of oxidised bases at the telomeres, leading to telomere loss and post-mitotic defects. Moreover, we discovered that TH5487 enhances the activity of the PARP1 inhibitor olaparib in BRCA1 deficient cells.

Clinical and diagnostic activity. During 2021, our consultancy at the Fuenlabrada Hospital was visited by 397 patients and 900 genetic studies were performed in the laboratory.

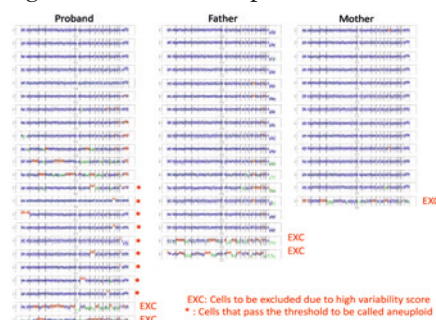


FIGURE 1 Single-cell, low-coverage whole genome sequencing (WES) analysis was performed in blood lymphocytes of the proband and her parents. The analysis demonstrated a high rate of variegated aneuploidy

in the proband's cells. 9 out of 21 cells (42.85%) showed gains of one or two complete chromosomes. Trisomies of chromosomes 21, 12 and 18 were the most frequent. Monosomies were not observed.

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MOLECULAR CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Staff Scientist
Raúl Torres

Graduate Students
Maria Cruz Casado (since May), Pilar Puig

Technicians
M. Carmen Martín, Marta



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 the 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.

“We apply genome engineering approaches to reproduce and eliminate chromosome rearrangements and gene alterations. We also provide 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.

Martínez-Lage (until December) (TS); Francisco José Moya (TS) (PEJ) **

*Titulado Superior (Advanced Degree)

**Plan de Empleo Joven (Youth Employment Plan)

Students in Practice

Alejandro Alonso (since September) (Maastricht University, The Netherlands), Alejandro Nieto (since November) (Universidad Autónoma de Madrid, Spain), Paula M. Ojeda

(February-June) (Universidad de Alcalá de Henares, Spain), Beatriz Olalla (March-August) (Universidad Politécnica de Valencia, Spain)

RESEARCH HIGHLIGHTS

A new protocol to quantify the alterations (copy number changes) in cancer samples. Chromosomal instability (CIN), a type of genomic instability, favours changes in chromosome number and structure and is associated with the progression and initiation of multiple diseases, including cancer. Therefore, CIN identification and analysis represent a helpful tool for cancer diagnosis and treatment. In 2021, we reported an optimised molecular cytogenetic protocol to detect CIN in formalin-fixed, paraffin-embedded mouse and human tissues, using fluorescent *in situ* hybridization to visualise and quantify chromosomal alterations such as amplifications, deletions, and translocations.

Technological and translational activities. Our Unit offers rapid, precise, and affordable technologies to analyse cancer cells at the chromosome level and to functionally interrogate the cancer genome. We provide state-of-the-art molecular cytogenetic and genome editing services. The Unit focuses on making available a complete repertoire of gene editing tools for cellular and genetic manipulation and an array of delivery vehicles, offering a flexible, modular platform for precision genome manipulation. The Unit provides molecular cytogenetics technologies for human and mouse chromosomes analysis, including conventional karyotyping, FISH, SKY and CGH array. In 2021, we carried out over 2,500 assays for experimental and clinically oriented projects.

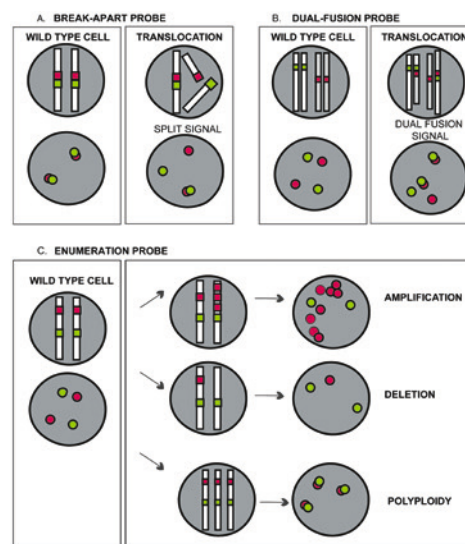


FIGURE Schematic representation of chromosomal abnormalities detected by fluorescence in situ hybridization (FISH). Schematic representation at genomic level of wild-type and rearranged FISH signal patterns detected by: (A) a break-apart probe and (B) a dual-fusion probe. (C) Schematic representation

of amplification, deletion and polyploidy FISH signalling patterns detected by an enumeration FISH probe. Amplification shows multiple red signals, deletion shows loss of a red signal, and polyploidy where the probe signal shows multiple sets of chromosomes, observed by 3 pairs of fusion signals.

PUBLICATIONS

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PATENTS

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- Rodríguez Perales S, Torres Ruiz R, Martínez-Lage M (2018). Gene Editing Based Cancer Treatment. *WO 2020/079243*. *National phase entry* (2021).

AWARDS AND RECOGNITION

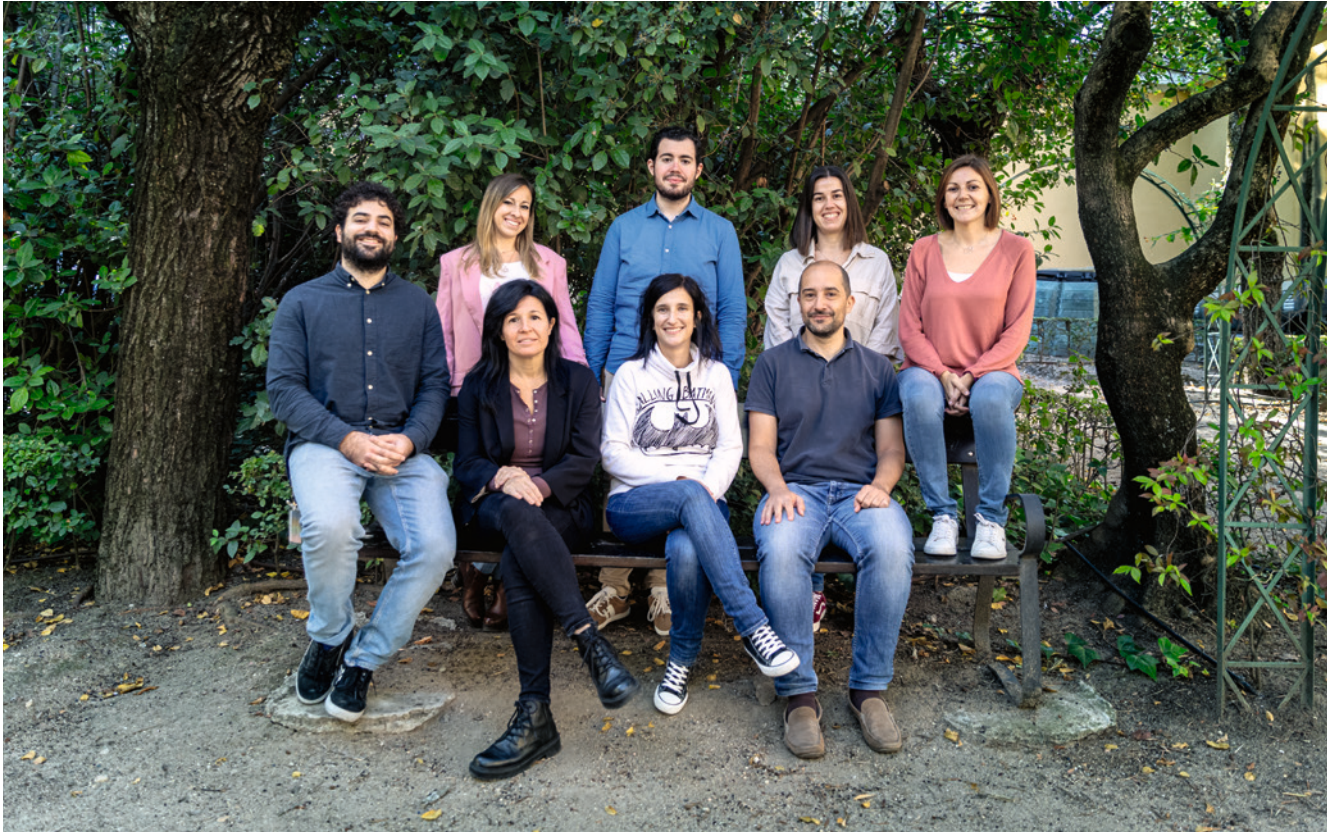
- 1st prize "Paper of the Year 2020-2021" contest in the gene therapy category for the article: *In vivo* CRISPR / Cas9 targeting of fusion oncogenes for selective elimination of cancer cells (*Nature Communications*, 2021), bestowed by The Spanish Society for Gene and Cell Therapy (SETGyC).
- 2021 Molecular Cloud Infinite Possibilities Teams Award, MolecularCloud™ Distinguished Research Awards for Genome Editing 2021.

HUMAN GENOTYPING- CEGEN UNIT

Anna González-Neira
Unit Head

Graduate Students
Hugo Tejera, Alejandro Velasco

Bioinformaticians
Guillermo Pita (TS)*, Jorge Zamora
(May-August) (TS)*



OVERVIEW

In the Unit we implement high-throughput and cost-effective methods to measure from one to millions of genetic variants, mainly single nucleotide variants (SNVs) and copy number variants (CNVs). Epigenetic analyses are also performed. Complementarily, our research focuses on identifying genes associated with cancer risk and response to therapy to understand the underlying molecular mechanisms of cancer susceptibility and drug efficacy/toxicity and to improve individual risk assessment for the identification of high-risk populations.

“Our goal is to identify predictive markers in cancer that allow individual risk assessment, thus integrating personalised medicine into healthcare practice.”

Technicians

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

**Titulado Superior (Advanced Degree)*

RESEARCH HIGHLIGHTS

Breast cancer risk genes – association analysis in more than 113,000 women. This study is the result of the European project BRIDGES (Breast Cancer Risk after Diagnostic Gene Sequencing), in which the Unit participates. The study was based on the analysis of 34 known or suspected breast cancer susceptibility genes in DNA samples from 60,400 women who had developed breast cancer and 53,400 healthy women. The results pinpoint 9 genes for which there is solid evidence of their involvement in the disease: ATM, BRCA1, BRCA2, CHEK2, PALB2, BARD1, RAD51C, RAD51D and TP53. This was already known for some of these genes, but for others, such as RAD51C and D and BARD1, their involvement was not so well established. For all genes, more precise risk estimates can now be calculated, and these estimates are tailored for each tumour subtype. By contrast, the study shows that about 15 of the genes that have been used so far in some tests are not indicative of an increased risk for breast cancer and should therefore not be taken into account in risk estimates, at least at this time (Dorling L *et al.*, 2021).

Characterisation of pharmacogenetic variability in the Spanish population. Pharmacogenetics (PGx) allows for patients to be managed in an individualised manner, leading to the better safety and efficacy of treatments. Nevertheless, genetic differences between populations need to be considered before implementing PGx. We used data from 3006 Spanish individuals to analyse pharmacogenetic variation in 1055 important pharmacogenes for population characterisation. For 21 clinically relevant pharmacogenes, our analysis revealed that 98% of the Spanish individuals harboured at least 1 allele

that leads to a change in the therapy. We also identified 7775 putative pathogenic SNVs and 33 CNVs. This study provided useful information to facilitate PGx implementation in our country by (i) confirming that almost all Spanish individuals could benefit from pharmacogenetics diagnostics; (ii) identifying additional pathogenic pharmacovariants with a possible functional role, data freely available to the public by accessing the Collaborative Spanish Variant Server; and (iii) demonstrating that PGx microarrays can be a cost-effective solution for testing.

POLRMT as a novel susceptibility gene for cardiotoxicity in epirubicin treatment of breast cancer patients. Anthracyclines are among the most used chemotherapeutic agents in breast cancer (BC). However, their use is hampered by anthracycline-induced cardiotoxicity (AIC). To identify novel predictive genes, we conducted a 2-stage genome-wide association study in epirubicin-treated BC patients. The most interesting and replicated finding was rs62134260, located 4kb upstream of POLRMT (OR = 5.76, P = 2.23×10^{-5}). This variant regulates the expression of POLRMT, a gene that encodes a mitochondrial DNA-directed RNA polymerase, responsible for mitochondrial gene expression. Individuals harbouring the risk allele had decreased expression of POLRMT in heart tissue, which may cause impaired capacity to maintain a healthy mitochondrial population in cardiomyocytes under stress conditions, such as is the case with epirubicin treatment. This finding suggests a novel molecular mechanism involved in the development of AIC and may improve our ability to predict which patients are at risk (Velasco-Ruiz A *et al.*, 2021).

PUBLICATIONS

- Breast Cancer Association Consortium; Dorling L *et al.* (incl. González-Neira A, Alonso MR, Álvarez N, Herráez B, Fernández V, Núñez-Torres R, Osorio A, Benítez J) (2021). Breast cancer risk genes - association analysis in more than 113,000 women. *N Engl J Med* 384, 428-439.
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- Park J *et al.* (incl. González-Neira A, Benítez J) (2021). Gene-environment interactions relevant to estrogen and risk of breast cancer: can gene-environment interactions be detected only among candidate SNPs from genome-wide association studies? *Cancers (Basel)* 13, 2370.
- Velasco-Ruiz A *et al.* (incl. Núñez-Torres R, Pita G, Alonso MR, Álvarez N, Herráez B, González-Neira A) (2021). POLRMT as a novel susceptibility gene for cardiotoxicity in epirubicin treatment of breast cancer patients. *Pharmaceutics* 13, 1942.
- Patiño-García A *et al.* (incl. Pita G, González-Neira A) (2021). Whole exome sequencing characterization of individuals presenting extreme phenotypes of high and low risk of developing tobacco-induced lung adenocarcinoma. *Transl Lung Cancer Res* 10, 1327-1337.

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) development of novel agents; 2) study of mechanisms of action of novel compounds and tackling drug resistance; and 3), moving forward in the field of biomarkers, functional taxonomy and precision medicine. Currently, 2 functional objectives summarise the CRP's new operating model: i) generating synergies with ongoing research lines in the basic research programmes; and ii) constituting a bi-directional bridge to facilitate closer interactions 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 de Málaga* and *Hospital de Fuenlabrada*). These agreements foster the interaction between clinicians and scientists and allow scientists from all CNIO Programmes to participate in translational research studies. The ongoing collaborations between CRP Units and Groups from other CNIO Programmes now represent 16 projects and 3 coordinated grants, which accounts for the high translational research activity of the institution. Twelve medical oncology residents from different Spanish hospitals completed their 3-month optional stays at the CNIO during 2021.

Although clinical activity was hampered considerably in 2021 due to COVID-19, the clinical groups managed to produce highly impactful research. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, provided proof-of-concept about the right niches in which NGS profiling is indicated for patients with advanced breast cancer. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, completed a chemo-immunotherapy registration trial in non-small-cell lung cancer, the most common subtype of lung cancer, and its results were recently published in *The Lancet Oncology*. 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 Prostate Cancer Clinical Research Unit (PCCRU), headed by David Olmos, continued working on the development of novel therapies for prostate cancer, building a nomogram that can accurately predict benefit from abiraterone in advanced cases. Additionally, they fine-tuned the technology for disease monitoring with non-invasive techniques in peripheral blood: circulating tumour cells and cell-free DNA. Finally, the Molecular Diagnostics Unit, headed by Luis Lombardía,

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

continued to provide support to hospitals in the diagnosis of different malignancies, performing >1000 diagnoses in 2021. In 2022, we expect major changes in the Programme; 1 Junior Group (PCCRU) is leaving the CNIO and moving to *Instituto i +12* because of the completion of the junior scientist's career development at the CNIO, and the active search for novel candidates to expand our activities is ongoing. ■

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Clinical Research Fellow
Jorge Silva (until August)

Staff Scientists
María José Bueno, Silvana A. Mouron

Post-Doctoral Fellow
Rebeca G. Jimeno



OVERVIEW

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

Our current research areas are:

- Studying the implications of hypoxia for immunotherapies.
- Understanding the individual factors regulating the response to immunotherapy in breast cancer, taking

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

advantage of an advanced, personalised “tumouroid” platform.

- Tackling the mechanisms of resistance against novel therapies in advanced breast cancer.
- Incorporating our findings into concept-driven clinical trials.

Graduate Students

Sara Fernández (until March), José Luis Ruiz

Technicians

Verónica Jiménez, Manuel Muñoz

Students in Practice

Iris Charatsidou (April -October) (University of Ionannina, Greece), Adrián Martínez (July-August) (Summer Trainee, *Universidad Politécnica de Valencia*, Spain), Adrián Sanz (February-June)

(*Universidad de Alcalá de Henares*, Spain)

Visiting Scientists

Ramón Colomer and Ana Garrido (since Nov.) (*Hosp. Univ. de la Princesa*, Madrid, Spain); Elisa Gómez

(since Oct.), Marta Gómez (until Sept.) and Ana López (until June) (*Hosp. Univ. de Fuenlabrada*, Spain); Cristina Merino (since July) and Ana M. Roncero (*Hosp. Univ. 12 de Octubre*, Madrid, Spain); Berta Nassarre (*Apices Soluciones S.L.*, Madrid, Spain)

RESEARCH HIGHLIGHTS

This year, we established a collection of 24 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 own immune cells. 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 expect this to be the core of our research in the coming years.

Next-generation sequencing panels have become widely used tools to try to allocate and individualise treatments in advanced breast cancer. However, considerable controversies exist regarding their usefulness and the specific niches in which they should be used. We issued practical guidelines for the indication of these tests in advanced breast cancer on the basis of our results with 140 patients analysed using NGS and followed-up for clinical outcomes, proving that there are very few clinical niches that justify the use of these panels for now.

Finally, we completed our work regarding predictive factors of sensitivity to paclitaxel in early breast cancer from the perspective of phosphor-proteomics. A CDK4-Filamin A axis

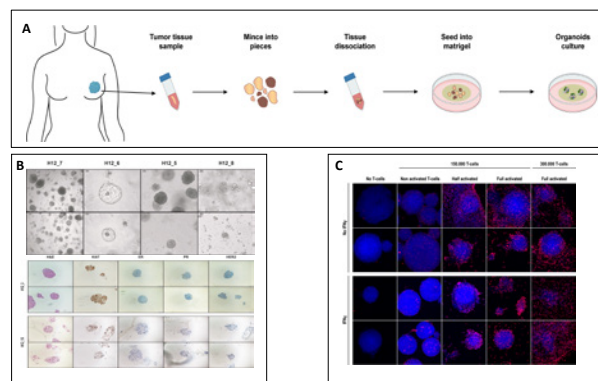


FIGURE (A) General methodology for patient-derived organoid generation. (B) The upper panel illustrates the heterogeneity among organoids – the four presented examples grow in a solid, cystic, discohesive and grape-like pattern, with underlying biological differences in the gene-expression programmes of luminal, basal and stromal cells converging in those morphologies, recapitulating the growth pattern of

the primary tumour. The lower panel shows the differences, between 2 organoids, in classic features such as ER, PR, Ki67, or HER2 levels. (C) Tumoroid culture (organoid plus same patient-derived lymphocytes) showing that different conditions (activation with co-stimulators, plus or minus interferon, or more or less cells) influence the ability to infiltrate and kill the organoid (blue: tumour cells; red: lymphocytes).

that converges in the regulatory machinery of tubulin acetylation is responsible for making cancer cells sensitive to this drug. This pair of markers is highly accurate in predicting sensitivity in the clinical setting. ■

PUBLICATIONS

- Mestre-Farrera A, Bruch-Oms M, Peña R, Rodríguez-Morató J, Alba-Castellón L, Comerma L, Quintela-Fandino M, Duñach M, Baulida J, Pozo ÓJ, García de Herreros A (2021). Glutamine-directed migration of cancer-activated fibroblasts facilitates epithelial tumor invasion. *Cancer Res* 81, 438-51.
- Mouron S, Manso L, Caleiras E, Rodríguez-Peralto JL, Rueda OM, Caldas C, Colomer R, Quintela-Fandino M, Bueno MJ (2021). FGFR amplification or overexpression and hormonal resistance in luminal breast cancer: rationale for a triple blockade of ER, CDK4/6 and FGFR1. *Breast Cancer Res* 23, 21.
- Luna-Dulcey L, Almada da Silva J, Jimenez-Renard V, Caleiras E, Mouron S, Quintela-Fandino M, Cominetti MR (2021). [6]-Gingerol-derived semi-synthetic compound SSi6 inhibits tumor growth and metastatic dissemination in triple-negative breast cancer xenograft models. *Cancers (Basel)* 13, 2855.
- Bueno MJ, Ruiz-Sepulveda JL, Quintela-Fandino M (2021). Mitochondrial inhibition: a treatment strategy in cancer? *Curr Oncol Rep* 23, 49.
- Mouron S, Bueno MJ, Muñoz M, Quintela-Fandino M (2021). Monitoring vascular normalization: new opportunities for mitochondrial inhibitors in breast cancer. *Oncoscience* 8, 1-13.

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. *EP21382061.6*.

AWARDS AND RECOGNITION

- CNIO and Applied Biological Materials Inc. (ABM) license agreement for the commercial sale of HCT1_FASN-knockout cells (developed in our laboratory), for non-human research purposes. December 10, 2021.

PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos (until December)
Junior Clinical Research Unit Head

Staff Scientist
Pedro P. Lopez-Casas (until
November)

Post-Doctoral Fellows
Ylenia Cendón, David Martínez, María
Isabel Pacheco (until June)

Technicians
Mónica Balsells (until May) (TS); Eva



M. Casas, Jeniffer P. Condo (until February), Olga Popova (April–November), Leticia Rivera (until December), Alejandro Sanz (until July) (TS)*

**Titulado Superior* (Advanced Degree)

Visiting Scientists
Daniel Alameda (*Instituto de Investigación Biomédica de Málaga*, Málaga, Spain), Elena Castro (*Instituto de Investigación Biomédica de Málaga*, Málaga, Spain), Carlo

Cattrini (*Università di Genova*, Genoa, Italy), Rebeca Lozano (*Instituto de Investigación Biomédica de Salamanca*, Salamanca, Spain)

OVERVIEW

Prostate cancer is the most common cancer diagnosis in men and, despite its potential to be cured in almost 90% of early stages, its metastatic spread causes about 6,000 deaths every year in Spain alone, whilst in the US over 30,000 men succumb to the disease each year.

During the last few years, our Group has focused precisely on the development of new methods to identify and treat the most aggressive and lethal forms of prostate cancer, in order to accelerate precision medicine for the disease. In particular, over the last 9 years, our group has made significant contributions in:

- i) Establishing and developing several biomarkers based on the concept of liquid biopsy.
- ii) Understanding the implication of gene alterations leading to DNA repair deficiency in this disease.
- iii) Developing new treatments for prostate cancer.

Our work has been widely recognised with several highly cited publications in top journals in our field, including *The New England Journal of Medicine*, *The Lancet Oncology*, *The Journal of Clinical Oncology*, *European Urology*, *Annals of Oncology*, and many others. ■

PUBLICATIONS

- Sweeney C, Bracarda S, Sternberg CN, Chi KN, Olmos D, Sandhu S, Massard C, Matsubara N, Alekseev B, Parnis F, Atduv V, Buchschacher GL Jr, Gafanov R, Corrales L, Borre M, Stroyakovskiy D, Alves GV, Bournakis E, Puente J, Harle-Yge ML, Gallo J, Chen G, Hanover J, Wongchenko MJ, Garcia J, de Bono JS (2021). Ipatasertib plus abiraterone and prednisolone in metastatic castration-resistant prostate cancer (IPATentia150): a multicentre, randomised, double-blind, phase 3 trial. *Lancet* 398, 131–142.
- Lorente D, Llacer C, Lozano R, de Velasco G, Romero-Laorden N, Rodrigo M, Sánchez-Iglesias Á, di Capua C, Castro E, Ferrer C, Sánchez-Hernández A, Olmos D (2021). Prognostic score and benefit from abiraterone in first-line metastatic, castration-resistant prostate cancer. *Eur Urol* 80, 641–649.
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- Smith MR, Thomas S, Gormley M, Chowdhury S, Olmos D, Oudard S, Feng FY, Rajpurohit Y, Urtishak K, Ricci DS, Rooney B, Lopez-Gitlitz A, Yu M, Wyatt AW, Li M, Attard G, Small EJ (2021). Blood biomarker landscape in patients with high-risk nonmetastatic castration-resistant prostate cancer treated with apalutamide and androgen-deprivation therapy as they progress to metastatic disease. *Clin Cancer Res* 27, 4539–4548.
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- Oliva L, Lozano R, Llacer C, Aragón I, Pajares BI, Sáez MI, Herrera-Imbroda B, Montesa A, Hernández D, Villatoro R, Otero A, Correa R, Grau G, Peinado P, Pacheco MI, García-Galisteo E, Rueda A, Machuca FJ, Alba E, Márquez-Aragónés A, Olmos D, Castro E (2021). Risk prediction tools available for germline BRCA1/2 mutations underperform in prostate cancer patients. *Eur Urol Oncol* 4, 315–318.
- Lozano R, Lorente D, Aragón IM, Romero-Laorden N, Nombela P, Mateo J, Reid AHM, Cendón Y, Bianchini D, Llacer C, Sandhu SK, Sharp A, Rescigno P, Garcés T, Pacheco MI, Flohr P, Massard C, López-Casas PP, Castro E, de Bono JS, Olmos D (2021). Value of early circulating tumor cells dynamics to estimate docetaxel benefit in metastatic castration-resistant prostate cancer (mCRPC) patients. *Cancers (Basel)* 13, 2334.
- Sokolova AO, Marshall CH, Lozano R, Gulati R, Ledet EM, De Sarkar N, Grivas P, Higano CS, Montgomery B, Nelson PS, Olmos D, Sokolov V, Schweizer MT, Yezefski TA, Yu EY, Paller CJ, Sartor O, Castro E, Antonarakis ES, Cheng HH (2021). Efficacy of systemic therapies in men with metastatic castration resistant prostate cancer harboring germline ATM versus BRCA2 mutations. *Prostate* 81, 1382–1389.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero



OVERVIEW

The activity of the Molecular Diagnostics Unit (MDU) is primarily aimed at providing an array of reliable and time/cost-efficient molecular diagnostic assays to help our National Health System's clinicians make early diagnosis, detect possible relapses, and/or monitor the response to therapy in patients with different cancers. Therefore, we devote effort to strengthening, updating and expanding the assays that are currently offered by implementing the latest tests available, or by upgrading the most established ones. Likewise, the Unit also provides support to the research needs of CNIO's Clinical Research Units and Research Groups by checking their samples for alterations in the biomarkers included in our catalogue. Furthermore, MDU collaborates with several international and national organisations focused on the standardisation and improvement of molecular diagnostics in cancer. Finally,

“During the last 15 years, MDU has supported over 300 clinicians by providing nearly 7000 specific and sensitive assays, with the aim of improving the diagnosis, prognosis, and response to therapy of more than 3000 cancer patients.”

the MDU is also involved in disseminating knowledge in the field of molecular diagnostics by instructing biomedical students in our techniques and methods.

Students in Practice

Julia Oyón (February-June) (*E.T.S. de Ingeniería Agronómica, Alimentaria y de Biosistemas*, Madrid, Spain), Alicia Hernaiz (February-July) (*Universidad Autónoma de Madrid*, Madrid, Spain)

CORE UNIT HIGHLIGHTS

Extending our portfolio

During 2021, we expanded our offer of assays by adding a new one that will enable the detection of activating mutations in exons 14 and 17 of the *CSF3R* gene encoding the receptor for colony-stimulating factor 3, a cytokine that controls the production, differentiation, and function of granulocytes. Alterations in *CSF3R*, commonly found in patients with chronic neutrophilic leukaemia (CNL) or some atypical chronic myeloid leukaemia (aCML), have been reported as useful prognostic and predictive markers, since patients with altered *CSF3R* showed an aggressive course of CNL and some sensitivity to ruxolitinib, a nonselective JAK inhibitor.

We also increased the detection coverage of a test implemented more than 10 years ago. This test uses qRT-PCR to detect BCL2-IgH fusion gene variants necessary for the diagnosis of follicular lymphoma and some cases of large B-cell lymphomas, as well as to monitor for minimal residual disease after treatment. The former assay detected only 50-60% of the cases with the MBR (major breakpoint region) variant

and 5-10% with the mcr (minor cluster region) variant. However, recent findings have revealed new variants that had not been previously used to evaluate patients with follicular lymphoma. As a result, with the current test, we have improved our capability to notify those patients with follicular lymphoma sharing 3'MBR or ICR variants (10-15% of) and 5'mcr (15-20%) variants (FIGURE 1).

Finally, we also started a pilot study to evaluate the feasibility of implementing a test using Next Generation Sequencing technology that will enable us to analyse the mutational status of the *IGHV* (immunoglobulin heavy chain variable region) gene; this analysis is crucial for the prognosis and response to therapy of patients with chronic lymphocytic leukaemia.

Training

During the first semester of 2021, MDU hosted 2 undergraduate students who carried out their end-of-degree projects. ■

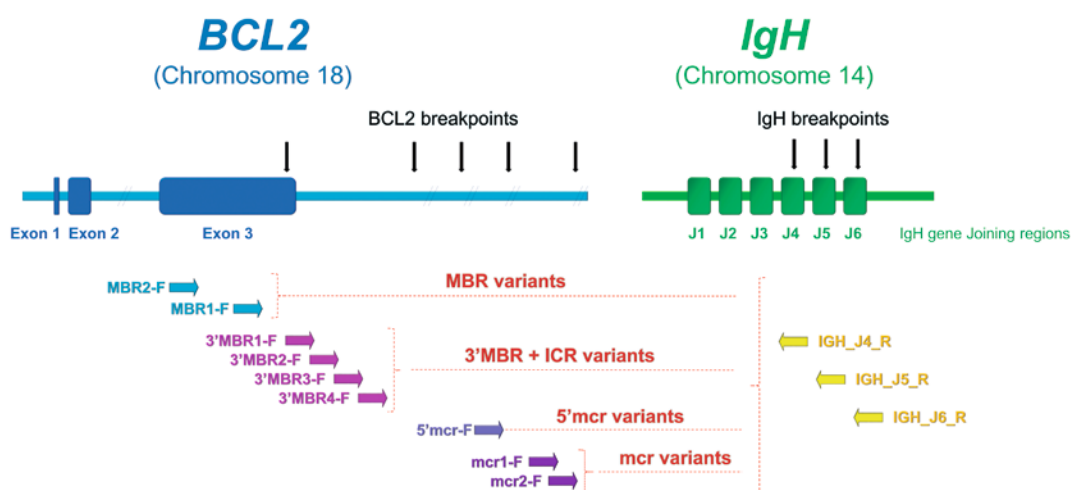


FIGURE 1 Location of primers and probes used in the new assay. Primers and probes were designed to include all known breakpoints in BCL2 and IgH sequences and thus allow us to

detect the different variants of the BCL2-IgH fusion gene. MBR, major breakpoint region; mcr, minor cluster region; ICR, intermediate cluster region.

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head

Staff 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, Alejandra Leivas, Yanira Ruiz-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, Alba Rodríguez, Laura Sánchez

Technicians

Raquel Ancos (since October), Natalia Sofía Buenache, Sara Dorado, Adrián Fernández, Vanesa Garrido, Alicia Giménez, M. Luz Morales, Laura Moreno, Miguel Ángel Navarro, Juan Manuel Rosa, Laura Rufián

Visiting Scientists

María Hernández-Sánchez (*Instituto de Investigación Biomédica de Salamanca*, Salamanca, Spain), Alfonso Navarro (*Instituto de Investigación Hospital Universitario La Paz*, Madrid, Spain)

OVERVIEW

Haematology represents one of the most “hot topics” areas in cancer of the last 5 years, due to society’s growing interest in the immunology that drives one of the biggest discoveries of the 21st century, immunotherapy. In addition, haematology has been gaining traction because of the interest in applying peripheral blood analysis to determine the diagnosis and prognosis of multiple cancers and diseases, and in emerging promising cutting-edge technology such as liquid biopsy, a method currently used to measure minimal residual disease (MRD).

The Haematological Malignancies Clinical Research Unit focuses on 2 research areas: 1) novel immunotherapies against cancer and, more specifically, NK-CAR technology; and 2) the development and improvement of liquid biopsy protocols through next-generation-sequencing.

Moreover, our group investigates the molecular mechanisms of haematological malignancies and then uses the identified molecules and markers (e.g., PIEZO1, HNRNPK) to develop mouse models of the disease that could be exploited therapeutically.

“Our results showed that it was feasible to genetically modify Natural Killer (NK) cells from patients and to safely express CAR-NKG2D, as well as the efficacy of these cells against multiple myeloma.”

RESEARCH HIGHLIGHTS

NKG2D-CAR-transduced natural killer cells efficiently target multiple myeloma

CAR-T-cell therapy is the most common genetically modified cell-based immunotherapy. However, CAR-T therapy usually has high toxicity. In contrast, CAR-NK cells may exert less toxicity. To explore this, we analysed the antitumour activity of activated and expanded NK cells (NKAE) and CD45RA- T cells from multiple myeloma (MM) patients that were engineered to express an NKG2D-based CAR. Although memory T cells were more stably transduced, CAR-NKAE cells exhibited greater *in vitro* cytotoxicity against MM cells, while showing minimal activity against healthy cells. *In vivo*, CAR-NKAE cells mediated highly efficient abrogation of MM

growth, with 25% of the treated mice remaining disease free. Overall, these results demonstrate that it is feasible to modify autologous NKAE cells from MM patients. They also show that it is possible to genetically modify NK cells from patients and to safely express CAR-NKG2D, as well as the efficacy of these cells against multiple myeloma.

Making clinical decisions based on measurable residual disease (MRD) improves the outcome in multiple myeloma

The use of MRD results to make clinical decisions in MM has been underexplored to date. In our study of 400 MM patients,

achievement of MRD negativity at any point was associated with improved progression-free survival (PFS). In addition, patients in whom a treatment change was made showed prolonged PFS, in comparison with patients in which MRD results were not acted upon. In patients with positive MRD during maintenance, a clinical decision (either intensification or change of therapy) resulted in better PFS compared to patients in whom no adjustment was made. Therefore, we find that MRD is useful in guiding clinical decisions during initial therapy and has a positive impact on PFS in MM patients.

Implications of increased mitochondrial content in multiple myeloma

Many studies over the last 20 years have investigated the role of mitochondrial alterations in carcinogenesis. However, the status of the mitochondria in MM and its implication in the

pathogenesis of the disease remain unclear. Our results showed increased mitochondria (DNA content, gene and protein expression, and activity) during the progression of MM. Mechanistically, OXPHOS metabolism was raised by regulation of increased MYC. We tested the efficacy of the mitochondrial inhibitor, tigecycline, to overcome MM proliferation. *In vitro* and *in vivo* therapeutic targeting using the mitochondrial inhibitor tigecycline showed promising efficacy and cytotoxicity in monotherapy and in combination with the MM frontline treatment bortezomib. Thus, we identified new vulnerability in multiple myeloma and provided a novel alternative for MYC inhibition by targeting mitochondrial activity, as an indirect mechanism to avoid MM proliferation. ■

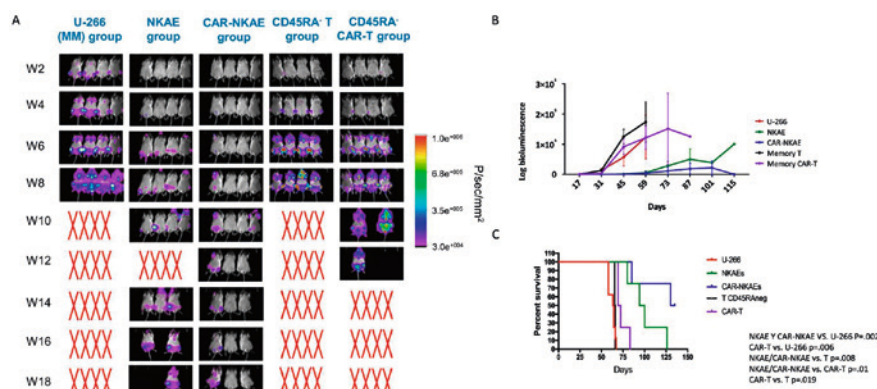


FIGURE 1 NKGD2D-CAR-transduced NKAE cells exhibit potent efficacy *in vivo*. (A) Imaging of tumour burden monitored by bioluminescence at the indicated time points in MM mice, NKAE-cell-treated mice, and CAR-NKAE-cell-treated mice (at day 73 mice 2 and 3 from the NKAE group were accidentally interchanged). (B) Quantification of the bioluminescence signal in MM mice, NKAE-cell-treated mice, CAR-NKAE-cell-treated mice, CD45RA+ T-cell-treated mice, and CD45RA-CAR-T-cell-treated mice at the indicated time points. (C) Kaplan-Meier survival curves.

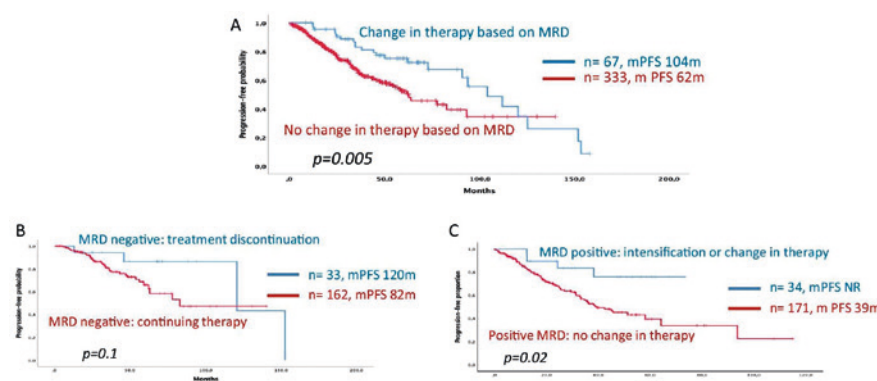


FIGURE 2 Kaplan-Meier curves showing the impact of making clinical decisions based on MRD. (A) PFS from the first MRD data point, comparing patients who underwent a change in therapy based on MRD with those for whom no change in therapy was made. (B) MRD-negative patients: treatment discontinuation (maintenance or transplant) vs. no change in therapy. (C) MRD-positive patients: beginning a new therapy or intensifying therapy vs. no change in therapy.

PUBLICATIONS

Group Original Articles:

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- AWARDS AND RECOGNITION**
- Joaquín Martínez-López, Miguel Gallardo, Teresa Cedena, Lucía Fernández, Ana Jiménez and Santiago Barrio were awarded AES 2021 Health Research Projects (as PIs), *Proyectos de Investigación en Salud*, Spain.
- Ana Jiménez-Ubieto received an AECC grant, the Spanish Association Against Cancer (AECC), Spain.
- María Velasco-Estévez was the recipient of a Marie-Sklodowska Curie Actions Individual Fellowship.

H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

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Ucero



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Graduate Students

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Technicians

Patricia Cozar, Laura García, Beatriz Gil, Patricia Llamas, Alicia Luengo, Patricia Plaza, Arantxa Rosado (TS)*, Rocio Suárez

**Titulado Superior* (Advanced Degree)

OVERVIEW

Lung cancer continues to be the most frequent cause of cancer-related deaths worldwide. Our Unit focuses on the study of lung cancer, from fundamental research proposals to other more clinically oriented, 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 to develop 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 II/III trials in the fields of personalised cancer care and immuno-oncology.

“Our Unit 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) 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, using an EGFR⁺ NSCLC PDX-bearing huPBMC-driven humanized NSG mouse model, we have demonstrated the nontoxic broad antitumour activity of a humanized EGFR-targeted 4-1BB-agonistic trimerbody (4-1BB^{N/C}EGFR) against EGFR⁺ tumours (Compte M *et al.*, *Clin Cancer Res* 2021). Our platform has been expanding in number and histology types (including small cell lung cancer [SCLC] and mesothelioma), as well as cell source (tumours but also SCLC circulating tumour cells), and it includes PDXs and patient-derived organoids. We have also successfully developed a number of huPDX models.

We have evaluated the tumour mutational burden (TMB) and PD-L1 expression obtained with 2 marketed next-generation sequencing panels, TruSight Oncology 500 (TSO500) and Oncomine Tumor Mutation Load (OTML) versus a reference assay (Foundation One, FO) in 96 NSCLC samples. Additionally, we have performed an inter-laboratory reproducibility study and determined adjusted cut-off values. We found that both panels exhibited robust analytical performances for TMB assessment, with stronger concordances in patients with negative PD-L1 expression. TSO500 showed higher inter-laboratory reproducibility. The cut-offs for each assay were lowered to optimal overlap with FO (Ramos-Paradas J, ..., Paz-Ares L, *J Immunother Cancer* 2021).

We have defined novel subgroups of large cell carcinoma (LCC) based on genomic and immune characterisation of 18 early-stage LCC cases and identified a set of biomarkers that

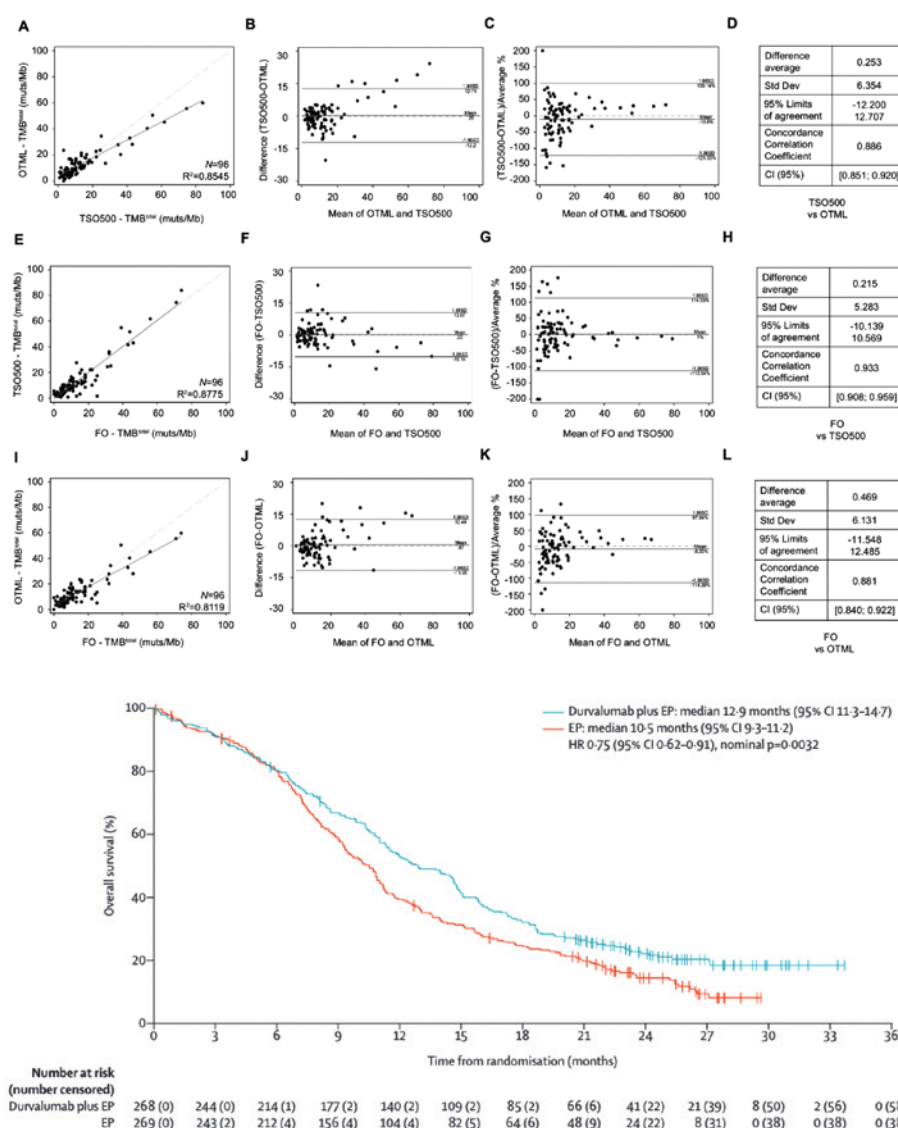


FIGURE 1 Quantification of the degree of agreement between tumour mutational burden (TMB) determination methods. (A–D) Comparison between TSO500 and OTML. (E–H) Comparison between FO and TSO500. (I–L) Comparison between FO and OTML. (A, E, I) Linear regression analyses between methods. (B, F, J) Agreement measurement represented by the difference between the methods against the average of both methods (Bland–Altman plots). (C, G, K) Agreement measurement represented by the difference between the methods divided by the average %, versus the mean of both methods. Transformation of Bland–Altman plots with a correction that allows for easier visualisation. (D, H, L). Degree of agreement shown by the average difference, SD, 95% limits of agreement, concordance correlation coefficient and 95% CI.

FIGURE 2 Updated efficacy outcome of the CASPIAN randomised clinical trial, showing a sustained overall survival (OS) benefit observed at the interim analysis for durvalumab plus platinum-etoposide compared with platinum-etoposide (HR, 0.75 [95% CI 0.62–0.91]; nominal p=0.0032) in treatment-naïve patients with extensive-stage small-cell lung cancer (ES-SCLC).

could potentially predict response to immunotherapy (Ramos-Paradas J, ..., Paz-Ares L, under review in *JCM*). In addition, we have comprehensively characterised the immune and genomic landscape of a cohort composed of 120 resected tumour samples from limited-stage SCLC patients. These studies have led to the categorisation of specific subgroups of SCLC, which will allow us to understand individual immune escape mechanisms and to propose optimal individualised treatment strategies (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 2021, we participated in more than 129 projects in this research area, including 42 new trials. We reported data from the novel combinations ramucirumab plus pembrolizumab (Herbst RS, ..., Paz-Ares L, *J Thorac Oncol*, 2021) and ramucirumab plus osimertinib (Yu HA, Paz-Ares LG *et al.*, *Clin Cancer Res* 2021). These phase I clinical trials demonstrated that both combinations have encouraging safety and antitumour activity in advanced, treatment-naïve NSCLC or T790M-positive EGFR-mutant NSCLC, respectively. We also reported patient outcomes from a global phase II basket study of entrectinib for ROS1 fusion-positive NSCLC and NTRK fusion-positive solid tumours. Findings were consistent with the favourable safety profile of entrectinib and further reinforce the positive benefit-risk profile of this treatment, indicating minimal overall treatment burden.

Changing standard-of-care treatments in clinical practice

The Lung Cancer Clinical Research Unit has led phase III trials whose results have significantly impacted clinical practice in the context of stage IV lung cancer, such as the combination of durvalumab plus etoposide with either cisplatin or carboplatin in extensive small cell lung cancer (ES-SCLC) patients (Goldman JW, ..., Paz-Ares L, *Lancet Oncol* 2021). The updated results of the CASPIAN trial reported that first-line durvalumab plus platinum-etoposide showed sustained

overall survival improvement versus platinum-etoposide, but the addition of tremelimumab to durvalumab plus platinum-etoposide did not significantly improve outcomes versus platinum-etoposide. In addition, we investigated whether adding a limited course (2 cycles) of chemotherapy to first-line nivolumab plus ipilimumab enhances the clinical benefit in patients with advanced NSCLC (Paz-Ares L *et al.*, *Lancet Oncol* 2021). We showed that this combination significantly improved overall survival compared to chemotherapy alone and had a favourable risk-benefit profile. ■

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ROKE I. ORUEZABAL

Director of Innovation (since June)

In 2021 we saw how the Ministry of Science and Innovation launched new calls focused on innovation and development with a very translational perspective, namely the “Strategic Lines” and “Proof of Concept” calls. The CNIO Groups were successful in setting up projects in both scenarios. It is worth noting that 2 Proof of Concept projects were awarded to develop the THankX and MDKinhibitor projects, led respectively by Héctor Peinado and Maria S. Soengas. THankX focuses on the use of THX-B as an antimetastatic therapeutic agent in melanoma in combination with immunotherapy. MDKinhibitor proposes to develop inhibitors and blocking antibodies against the growth factor MDK, a protein secreted in a variety of aggressive tumours, including melanoma. In addition, 2 projects were selected in the Strategic Lines Programme, both with the participation of Marcos Malumbres in collaboration with different public bodies and companies. The CNIO is the coordinating institution of one of them,

entitled mMETonChip, aiming to develop a micrometastasis-on-chip platform for the selection and validation of drugs, a project in collaboration with the Spanish pharmaceutical company PharmaMar. The second project selected in the call, OncoDeePlasm proposes the development of a new ultrasensitive immunodetection platform capable of detecting breast cancer biomarkers in blood. This will be carried out in collaboration with the Institute of Micro- and Nanotechnologies of the CSIC as coordinating institution, the *Hospital Universitario 12 de Octubre* and the company Mecwins.

The CNIO’s Groups participate in many of the most relevant international scientific societies and research networks. A new research network has been established together with Janssen and several European Universities for the study of FGFR in urothelial neoplasms and involving Francisco X. Real’s and Núria Malats’ Groups.

“CNIO’s partnerships with industry exemplify our commitment to knowledge transfer and society.”

International cooperation with the biotech and pharma sectors increased in 2021 with the signature of over €10 million in partnerships with foreign companies, deals managed by the Technology Transfer and Valorisation Office. Besides the strengthening collaborations with pharma companies such as Janssen, Loxo, 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 such as Inflection Biosciences, Refoxy and Totus Medicines, 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, which also generated breakthrough spin-offs as Telomere Therapeutics. Once again, the CNIO broke another barrier thanks to its Biotechnology Programme, signing contracts with industry

for an amount above €1 million, especially in the monoclonal antibodies field.

As a research institution of excellence, the CNIO has developed a strong commitment with 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.

This year, the Biotechnology Programme was able to keep up and running all the operations necessary to guarantee the preservation of the Centre's activities. In fact, the overall activity of our Core Units during 2021 has already returned to the levels achieved in 2019, before the pandemic.

Regarding the projects led by the Core Units, the Mouse Genome Editing Core Unit has been working on the development of mouse models expressing humanized ACE2 to be used for preclinical studies. Having initially received funding through a call for projects focusing on SARS-CoV-2 and Covid19 launched by the *ISCIII* in 2020, the project was expanded to incorporate a collaboration with the CNIO Telomeres and Telomerase Group and Dr Luis Enjuanes' Group at the *CNB-CSIC*, and is now financed through a Synergy Project from the *Comunidad Autónoma de Madrid*. Coordinated by Sagrario Ortega, Head of the Unit, this research uses mouse models to study the effect of short telomeres on the severity of the disease.

On the other hand, our technological capabilities were upgraded during 2021. One example is the acquisition of a high-resolution ultrasound system (Vevo 3100) in the Molecular Imaging Unit, used for imaging studies in animal models, mainly for pancreas, prostate, heart, kidney, and other soft organs. Also, a flow cytometry spectral analyzer (Cytek[®] Aurora) was purchased that will expand the technical capabilities of the Flow Cytometry Unit by making it possible to run cytometry protocols involving more than 40 markers.

This year Javier Muñoz, Head of the Proteomics Unit since 2012, left the CNIO to join the *Instituto de Investigación*

“The activities of the Biotechnology Programme Core Units have again been critical to maintain the outstanding scientific productivity of the CNIO during 2021.”

Sanitaria Biocruces Bizkaia, as group leader. During his time at the CNIO, Javier did a fantastic job, bringing proteomics to the core of the projects undertaken by the CNIO Research Groups, as well as developing his own projects and collaborating with groups outside the CNIO. We thank him for his efforts and excellent work, and wish him the very best for his scientific career from now on.

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. In 2021, and for the first time in the history of the CNIO, the total income derived from this concept exceeded €1 million.

Last but not least, 2021 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 more than 30 publications co-authored by members of the Units, many of them published in top journals. ■

GENOMICS CORE UNIT

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OVERVIEW

The Genomics Unit provides core technological services in the fields of genomics and genetics. Seeking to uncover biological mechanisms, therapeutic targets or prognostic biomarkers, our services cover a broad range of applications. These technologies, with the capacity to interrogate whole genomes and their activities in a single assay, 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. Next-generation sequencing (NGS) is a staple among them. More traditional methodologies like Sanger capillary DNA sequencing are also available. As a side activity, we oversee a genetically engineered mouse genotyping service.

“Our technology portfolio responds to the needs of CNIO’s scientists in the genetics and genomics fields and contributes to the understanding of disease and homeostasis at different levels of biological complexity.”

RESEARCH HIGHLIGHTS

All tumours, even those of the same type and sharing a similar fate, are different and heterogeneous at the genetic level. By employing a distinct set of methodologies with the capacity to analyse a wide number of genetic loci, or even whole genomes in a single assay, genomics reveals basic molecular programmes and helps to dissect biological mechanisms. The Genomics Unit, with its array of molecular services, contributes to the dissection of these biologically complex mechanisms in research projects conducted by multiple CNIO research groups.

For genomic-wide level analysis, we use NGS-based technologies, performed mainly on the Illumina platform. NGS constitutes the final readout for a great variety of different explorations at both the structural and functional levels, including detailed genome or exome tumour characterisations, mutation repertoires, location of bound protein factors, variations in chromatin folding, or on/off functional states. Transcriptional profiles reflect functional choreographies at the genomic level and are useful to decipher tumour compositions, uncover therapeutic targets, or predict disease outcome and guide treatment decisions. Transcriptomes are characterised either from tissue – even from archived FFPE

samples – or from cell culture extracts. Transcriptomes can also be obtained at single cell resolution, through prior separation of individual cells in microdroplet emulsions using the 10xGenomics Chromium platform. A recent implementation at single cell resolution is the multiomic profiling of gene expression and open chromatin regions, opening up new perspectives into the underlying gene regulatory mechanisms that drive cell differentiation and development.

At the single locus level, we provide other services. A traditional DNA capillary sequencing service, based on a 3730xl DNA Analyzer from Applied Biosystems, is being used to find and confirm mutations in candidate genes, or to verify cloned genes or inserts. A relatively simple cell authentication service provides confidence in the identity of the cell lines used for experimentation. The Unit oversees a transgenic mouse genotyping service as well. Its current catalogue includes over 150 genetic modifications, all assayed by custom allele-specific, real-time PCR for a quick and efficient turnaround time. ■

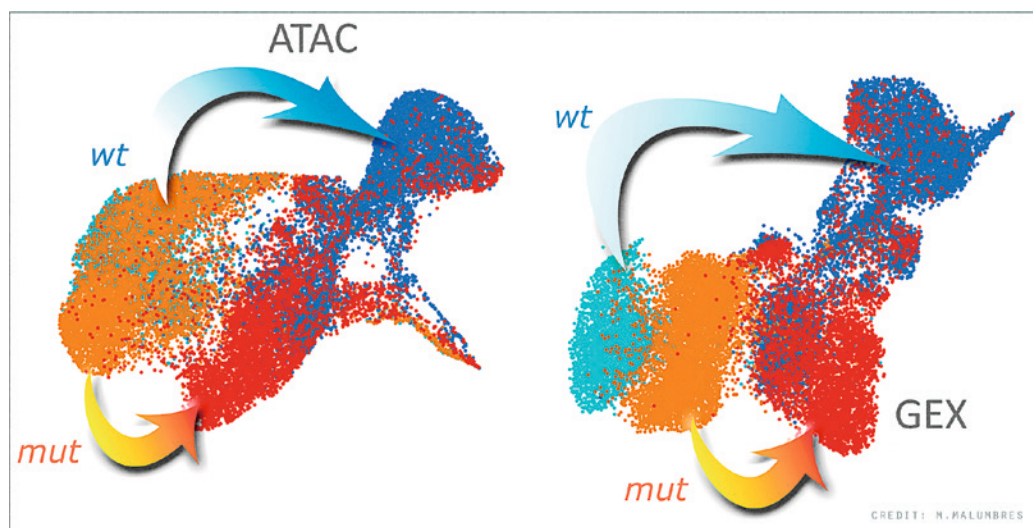


FIGURE Neural differentiation spotted at two time points for both wild type (wt) and *Cdc14a/b*-null (mut) embryonic stem cells. Simultaneous detection of chromatin accessibility patterns (ATAC) and mRNA transcriptomes (GEX) in the same individual cells, shown as dots clustered according to their

similarities, illustrate the coordinated cellular fates of dynamical epigenomic and transcriptomic profiles uncovering gene regulatory programmes. Arrows indicate changes in cell subpopulations' states between days 0 and 5. Data courtesy of Carolina Villarroja, Malumbres Lab, CNIO.

MOUSE GENOME EDITING CORE UNIT

Sagrario Ortega
Core Unit Head

Graduate Students
Aleida Pujol, Daniel Serrano



OVERVIEW

The term “cancer” encompasses a whole spectrum of extremely complex diseases. Genetic and epigenetic modifications in tumour cells lead to the acquisition of a “malignant” phenotype that enables them to escape normal physiological control. We can accurately reproduce many of these modifications in the mouse, creating animal models to study the disease. Tumour cells also interact, at different levels, with other cells in the body such as those of the tumour stroma, immune, cardiovascular or lymphatic systems, which, in turn, modulate tumour growth, invasion and expansion. The study of such complexity requires *in vivo* models that reproduce all the features of cancer in a “whole body” context, including the specific genetic alterations that lead to tumour development in each particular tumour. The precise, targeted and controlled modification of the mouse genome, using the most advanced

“The Mouse Genome Editing 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.”

genome editing tools, sustains the generation of genetic mouse models of cancer that are crucial for understanding the molecular basis of tumour development and the preclinical validation of new and more efficient cancer therapies.

Technicians

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RESEARCH HIGHLIGHTS

COVID19 preclinical mouse models

One limitation in COVID research is the lack of adequate models to study SARS-CoV-2 infection, especially animal models, where the complex interactions established between the virus and its host can be reproduced in a physiological context. During 2021, the Unit focused on generating and characterising mouse models for COVID disease.

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. 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 the study of COVID19, in collaboration with the company Gen-H Genetic Engineering, Heidelberg (Germany).

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. As an alternative, we used a BAC transgene approach to drive expression of human ACE2 under the control of the *Ace2* promoter. In both cases,

the expression of human ACE2 recapitulates the pattern and regulation of endogenous *Ace2* expression.

During 2021, in collaboration with the Coronavirus Laboratory directed by Dr Luis Enjuanes at the *Centro Nacional de Biotecnología (CNB/CSIC)* in Madrid, we tested the susceptibility of our mouse models to SARS-CoV-2 infection. Intranasal inoculation of SARS-CoV-2 in the humanized knockin mice resulted in the accumulation of inflammatory infiltrate (CD45+ cells) surrounding lung alveoli and neighbouring blood vessels (CD31+) close to the cells where the human ACE2 protein is expressed in these mice (FIGURE 1), hence showing that our models are susceptible to SARS-CoV-2 infection. ■

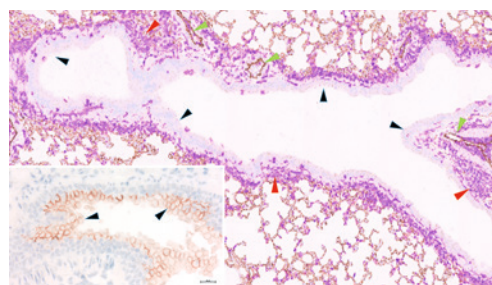


FIGURE 1 Lung inflammation in human ACE2 (hACE2) knockin mice, inoculated with SARS-CoV-2. CD45+ inflammatory cells (magenta; red arrowheads) and CD31+ endothelial cells (brown; green arrowheads) in

the lung of a knockin mouse, 3 days after intranasal virus inoculation. Insert image: hACE2+ expressing cells (black arrowheads in both images) lining the bronchioles.

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MONOCLONAL ANTIBODIES CORE UNIT

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OVERVIEW

Since the discovery of hybridoma technology by Caesar Milstein and Georges Köhler in 1975, monoclonal antibodies (mAbs) have become one of the most relevant methodological advances in biomedicine.

mAbs have provided researchers with the ability to study biological processes reliably and with unprecedented accuracy, improving our knowledge about the processes involved in tumour generation and development. Beyond their applications in the laboratory as research tools, mAbs are also used in the area of diagnostics, and serve as therapeutic agents in the treatment of cancer.

The Monoclonal Antibodies Unit provides CNIO Research Groups with *à la carte* generation of mAbs using hybridoma

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

technology. We are highly specialised in the production of mouse and rat monoclonal antibodies. The Unit also offers mAb characterisation and validation, medium-scale mAb production, as well as a *Mycoplasma* testing service for the cell culture facility.

Student in Practice
Davide Mazzeo (March-June)
(Università degli Studi di Pavia, Pavia,
Italy)

RESEARCH HIGHLIGHTS

During the last 21 years, the Monoclonal Antibodies Unit has generated a large number of mAbs, directed against more than 170 different antigens, mostly targeting molecules for which mAbs are not commercially available. Many of those mAbs have been licensed to external companies, generating royalties that represent an important source of revenue 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

CD229 (LY9). In collaboration with Professor Pablo Engel, from Barcelona University, we produced and characterised a new mAb against the cytoplasmic region of CD229 (Ly9) protein. CD229 is a homophilic receptor that belongs to the SLAM family of cell-surface molecules and acts as a signalling molecule, regulating lymphocyte homeostasis and activation. In our study we investigated the expression of CD229 in normal tissues and B cell malignancies using tissue microarrays. We found CD229 to be restricted to haematopoietic cells, and it is strongly expressed in all cases of myeloma and splenic marginal zone lymphomas. CD229 represents a new biomarker of B cell malignancies, especially in myeloma.

Optimised panel of mAbs for the detection of lymphocyte subpopulations in animal species. Immunohistochemistry (IHC) has proved to be one of the most important ancillary

techniques in the characterisation of neoplastic diseases in humans and, because oncologists demand such diagnostic specificity, it has become equally important in veterinary medicine. The number of immunohistochemical tests offered by veterinary diagnostic laboratories has increased exponentially over the last decade, but the use of this technique has been hampered by the lack of specific mAbs able to work across animal species.

For this reason, in collaboration with the Madrid Zoo, with several departments of veterinary sciences, and with the CNIO Histopathology Unit, we tested more than 100 mAbs in several domestic and wild animal species, generating an extended panel of mAbs able to detect and discriminate different lymphoid subpopulations by IHC. Our study will serve to facilitate further research needed to define the role played by lymphocyte subpopulations in immunological diseases and cancer in animal species. ■

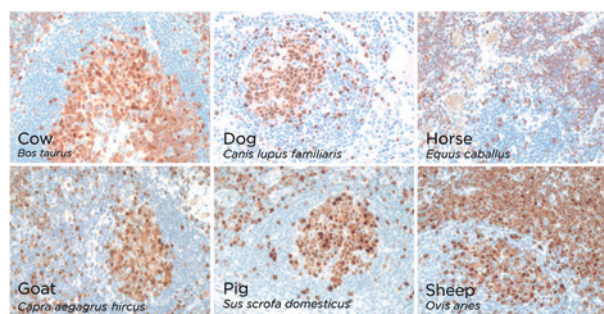


FIGURE Immuno-histochemical staining of TOX mAb in paraffin sections from lymph nodes of domestic animal species.

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lix S, Currás-Freixes M, Rapizzi E, Canu L, Nölting S, Remde H, Fassnacht M, Bechmann N, Eisenhofer G, Mannelli M, Beuschlein F, Quinkler M, Rodríguez-Antona C, Cascón A, Blasco MA, Montero-Conde C, Robledo M (2021). Analysis of telomere maintenance related genes reveals NOP10 as a new metastatic-risk marker in pheochromocytoma/paraganglioma. *Cancers (Basel)* 13, 4758.

PATENT

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MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Tatiana Álvarez, Guillermo
Garaulet (TS)*, Guillermo Medrano,

David Sabador (TS)* (PEJ) **,
Gloria Visdomine

**Titulado Superior* (Advanced Degree)

***Plan de Empleo Joven* (Youth Employment Plan)



OVERVIEW

Molecular Imaging enables the visualisation of cellular function and the follow-up of the molecular processes in living organisms without perturbing them. Molecular imaging offers significant advantages to the scientist over traditional research paradigms. While traditional studies of tumour response to a therapeutic agent involve a large cohort of animals analysed at multiple time points, molecular imaging allows characterisation of tumour development and response to a therapy within the same small set of animals imaged longitudinally at multiple time points. This reduces the number of mice used and increases the statistical power of the study because each animal serves as its own control. Other advantages include the ability to interrogate the whole body, and to visualise the molecular target of interest in 3-dimensional space. Finally, molecular imaging is becoming a key bridging technology for the

**“With the theranostics approach:
we see what we treat, and we treat
what we see.”**

translation of experimental preclinical findings into the clinical environment and, currently, with the theranostic strategy, we can treat the tumours with the same molecule used for PET visualisation by simply changing the isotope for the beta emitter pair.

Visiting Scientist
Marta Oteo Vives (CIEMAT, Madrid,
Spain)

RESEARCH HIGHLIGHTS

The Molecular Imaging Unit continues to provide CNIO researchers with state-of-the-art molecular imaging equipment and human resources in order to guarantee the highest quality studies and to develop and update protocols and imaging techniques to optimise tumour visualisation in both the preclinical and clinical fields. The Unit also assesses and advises researchers on the best-suited imaging modality for their research projects.

In 2021, the BBVA Foundation grant allowed us to work on theranostic applications of radiolabelled antibodies, looking for the best-matched isotope pairs for imaging and therapy and employing the pretargeting approach. We also continued with the rest of our ongoing grant projects. One of our projects, conducted in collaboration with CIEMAT, focuses on developing and labelling nanobodies produced by camelids following the ImmunoPET strategy, where we couple the high specificity and selectivity of the antibodies with the high sensitivity and quantitative capabilities of PET. Another grant project, the Spanish Network for Nanoparticles in Molecular Imaging for developing iron and silver-based nanoparticles for imaging, focuses mostly on optical imaging and multimodality (optical-MRI or PET-MRI) for the detection of primary tumours and distant metastasis.

The results of these research projects, in which the Molecular Imaging Unit is actively involved, will directly benefit CNIO scientists who will be able to use and test these new imaging tools in their own research. One example is a new NIR (near infrared) laser device bought with the RENIM budget.

In 2021, we installed a new ultrasound imaging system, VEVO 3100, to replace one of the old ones, to perform diagnosis and follow-up of tumours, as well as to phenotype different models and organs. The system improves throughput diagnosis by increasing the sensitivity and signal-to-noise ratio of the images. We are also helping to detect pregnancy at early stages, at only 6 weeks, to extract murine embryonic fibroblasts (MEFs) with better accuracy than only with abdominal palpation (FIGURE 1). ■

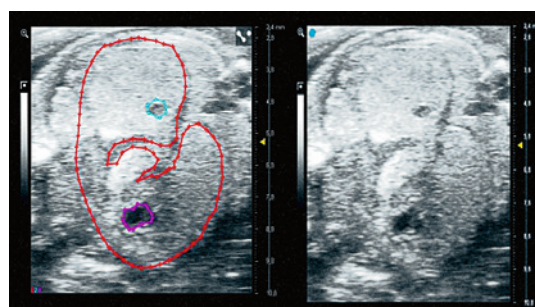


FIGURE 1 Ultrasound imaging of a 13-week embryo where the heart (pink line) and the eye (blue line) are clearly observed.

We could detect pregnancy in the very early stages, at only 6 weeks.

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- de la Calle Arregui C, Plata-Gómez AB, Deleyto-Seldas N, García F, Ortega-Mo-

lina A, Abril-Garrido J, Rodríguez E, Nemazany I, Tribouillard L, de Martino A, Caleiras E, Campos-Olivas R, Mulero F, Laplante M, Muñoz J, Pende M, Sabio G, Sabatini DM, Efeyan A (2021). Limited survival and impaired hepatic fasting metabolism in mice with constitutive Rag GTPase signaling. *Nat Commun* 12, 3660.

- Garlito-Díaz H, Esteban J, Mediero A, Carias-Cáliz RA, Toirac B, Mulero F, Faus-Rodrigo V, Jiménez-Morales A, Calvo E, Aguilera-Correa JJ (2021) A new antifungal-loaded sol-gel can prevent

candida albicans prosthetic joint infection. *Antibiotics (Basel)* 10, 711.

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- Shaikh F, Andersen MB, Sohail MR, Mule-ro F, Awan O, Dupont-Roettger D, Kubassova O, Dehmeshki J, Bisdas S (2021). Current landscape of imaging and the potential role for artificial intelligence in

the management of COVID-19. *Curr Probl Diag Radiol* 50, 430-435.

► AWARDS & RECOGNITION

- Faculty and Mentor of IDEAS2 MIT linQ with Massachusetts Institute of Technology.
- COST Action evaluator, European Cooperation in Science and Technology (COST).
- Guest Associate Editor in Nuclear Medicine, *Frontiers in Medicine*.

FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Julia García Lestón (TS)*, Sara García
García, Marina Quesada Martínez
(until February) (PEJ)**

**Titulado Superior* (Advanced Degree)

***Plan de Empleo Joven* (Youth Employment
Plan)



OVERVIEW

Flow Cytometry is a fast and multiparametric technology, and a very valuable tool in the oncology field. It is an important workhorse for the identification, quantification and isolation of defined subpopulations of cells, based on the expression levels of fluorescent markers and their relationship to each other at the single cell level.

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

We currently have 5 analysers and 3 high-speed cell sorters with different optical configurations to cater to our users' needs. We also have an automated magnetic bead separation system (AutoMACS), 2 automated cell counters (Countess)

“We incorporated a full spectrum cytometer to expand the number of parameters we could study per sample, increasing our knowledge on the role of different immune subsets in cancer progression, and elucidating new biomarkers with potential therapeutic value.”

and a tissue homogenizer (GentleMACS). Analysers are user-operated upon appropriate training, and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6- defined populations simultaneously, as well as perform single cell cloning and index sorting. We can accept human samples to sort under BSL2 regulations.

RESEARCH HIGHLIGHTS

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

- Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- Apoptosis studies (Annexin V, Mitochondrial Membrane Potential, Caspase 3, etc.).
- Multicolour immunophenotyping panels (B and T cell development, Tregs, inflammation, etc.).
- Functional assays (side population detection, Ca^{2+} flux, intracellular pH, etc.).
- Cytometric bead arrays to measure several cytokines from cell extracts and plasma.
- Platelets studies.
- Extracellular vesicles detection (microvesicles and exosomes).
- CTC detection and isolation.
- Single cell sorting for OMICs analysis.

We further optimised our multicolour flow cytometry panels to characterise immune response in various samples from haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Single cell deposition using index sorting into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We also improved the performance characteristics of our instrumentation by creating volttration templates in all our instruments to assess optimal voltage for each detector and expand our training capacities with many more workshops and small practical analysis sessions. This provides our users with more tools to successfully perform their flow cytometry experiments. ■

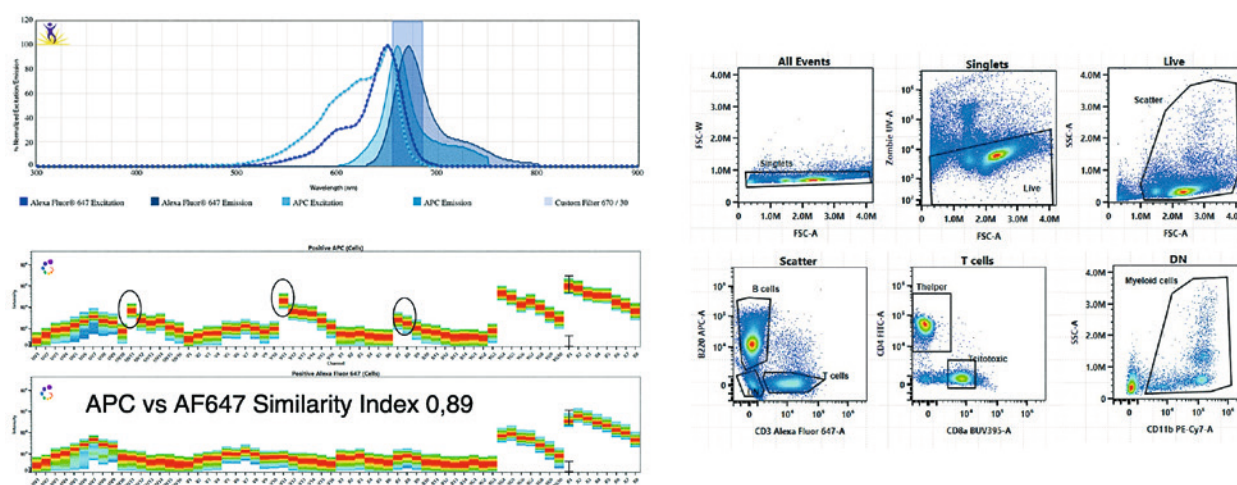


FIGURE Immunophenotyping panel of mouse spleen combining antibodies coupled to AF647 and APC, a

combination not possible with conventional cytometry. Full spectral cytometry opens the door to increase

panel complexity by allowing the use of fluorophore combinations that were not possible before.

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iewicz A, Kalinowska I, Saltari A, Martínez-Gómez JM, Hogan SA, Saragovi HU, Ortega S, García-Martín C, Boskovic J, Levesque MP, Rutkowski P, Hidalgo A, Muñoz J, Megías D, Mehrara BJ, Lyden D, Peinado H (2021). Melanoma-derived small extracellular vesicles induce lymphangiogenesis and metastasis through an NGFR-dependent mechanism. *Nat Cancer* 2, 1387-1405.

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CONFOCAL MICROSCOPY CORE UNIT

Diego Megías (until December)
Core Unit Head

Technicians
Jesús Gómez (PEJ)* (TS) **, Manuel
Pérez (TS) **

*Plan de Empleo Joven (Youth Employment
Plan)

**Titulado Superior (Advanced Degree)



OVERVIEW

Optical microscopy has traditionally been an indispensable tool in cell biology studies. In fact, one of the main challenges in oncology research is the study of specific markers, expression patterns or individual cells in the tumour environment.

The Confocal Microscopy Unit provides the CNIO Research Groups with all the standard methodologies as well as the latest advances in microscopy, offering access to state-of-the-art equipment and software packages related to confocal microscopy, including technical and scientific advice and support to the CNIO scientists. The Unit is also actively involved in developing, testing, and implementing new microscopy technologies, tools and imaging applications that could be of interest to the Research Groups at the CNIO. Training activities are also an essential component of our mission.

“The Confocal Microscopy Unit is fully committed to disseminating advanced microscopy methodologies that are useful for cancer research and society at large; we organised courses, talks and visits, always with the aim of increasing our understanding of cell biology and the disorders of cells that lead to cancer.”

Students in Practice

Ramón Carballas (January-June),
Jorge Padilla (until March), and
Álvaro Román (January-June)
(Universidad Carlos III de Madrid,
Spain)

RESEARCH HIGHLIGHTS

The Confocal Microscopy Unit continues to dedicate significant effort towards developing and implementing High Content Screening (HCS) technology at the CNIO. In 2021, the Unit renewed its equipment in this field, thanks to the funding obtained with a grant awarded through an infrastructures call of the Ministry of Science and Innovation. The Unit already had 1 Opera (Perkin Elmer) HCS system, which enables experiments to be run on fixed and live cells in multiwell plates, and the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence. The acquisition of a new Opera Phoenix HCS microscope with a robotic plate handler will boost screening capacity, making 24/7 operation time possible. This new system will significantly reduce acquisition time and improve sensitivity and excitation flexibility. The platform is equipped with the latest analysis software, getting better results from 3D organoid campaigns and live-cell imaging assays.

In addition to this new system, the Unit is equipped with: 1 super resolution confocal microscope (sp8 STED super-resolution microscope with a white light laser and 3 depletion laser lines); 3 laser scanning confocal systems (Leica SP5) that incorporate UV and multiphoton excitation, as well as a white light laser and hybrid detection; and 2 wide field systems (a THUNDER system with computational clearing algorithms and 8-channel led excitation, and a Leica DMRI6000 system, equipped with microinjection and microfluidics control). All the microscopes are automated and equipped with incubators for live cell imaging.

The Unit implemented the use of high-throughput technologies applied to confocal microscopy using not only the Opera system, but also through a sample navigation application integrated into the SP8 and SP5 confocal systems. This enables high-throughput feeding of the instrument, both in multiwell plates and in tissue sections. These advances allow us to increase the level of information obtained from a sample, as well as carry out the automated screening of cell behaviour under different treatments.

The Unit is involved in promoting and helping its users with novel protocol development for sample preparation, bringing knowledge in tissue clearing as well as in expansion microscopy. Moreover, microfluidics, used for live cell assays in perfusion chambers, has also experienced a great increase in performance and demand. Experiments of intra-vital microscopy are available, and we are now running several projects for studies of metastasis, skin alterations and immune system response. ■

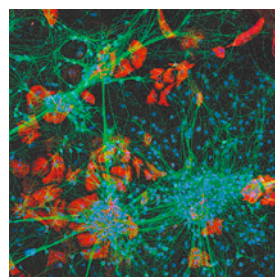


FIGURE Confocal image of cells in culture labelled for neurons (green) and astrocytes (red).

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- González-Martínez J *et al.* (incl. Gómez J, Pérez-Martínez M, Megías D, Boskovic J, Ortega S, Malumbres M) (2021). Deficient adaptation to centrosome duplication defects in neural progenitors causes microcephaly and subcortical heterotopias. *JCI Insight* 6, e146364.

PROTEOMICS CORE UNIT

Javier Muñoz (until August)
Core Unit Head

Graduate Student
Cristina Sayago (until December)

Technicians
Enrique Alonso (until April) (TS) ;
Fernando García (TS) ; Julia Isabel



OVERVIEW

Recent developments in “omics” technologies have revolutionised how biomedical research is conducted. These approaches enable unbiased analyses of biological samples and can be used to generate novel hypotheses. Proteins are the molecular effectors of cells, and mRNA assessment merely represents a proxy to estimate the final levels of the protein product. Moreover, genomics does not provide information about the post-translational modifications of proteins or their interactions. Thus, direct analysis of proteins is paramount to our understanding of how cells work. Proteomics is an emerging and multidisciplinary field that aims to analyse the complex regulation of the proteome and its impact on disease. The CNIO Proteomics Core Unit provides state-of-the-art mass spectrometry-based proteomics to scientists and research groups to better understand, at the proteome level, the molecular basis of cancer.

“In 2021, we developed novel proteomic strategies that could be used to identify potential biomarkers in liquid biopsies.”

Morales (TS) *, Jana Sánchez (TS) *,
Pilar Jiménez De Embún (TS) *,
Eduardo Zarzuela (TS) *

*Titulado Superior (Advanced Degree)

Student in Practice
Gonzalo Pazos (February-June)
(Universidad Autónoma de Madrid,
Madrid, Spain)

RESEARCH HIGHLIGHTS

In collaboration with the Experimental Oncology Group, we used targeted proteomics to accurately identify and quantify different Kras isoforms, which provide valuable information to understand the interplay between these variants. With the DNA Replication Group, we used Affinity Purification Mass Spectrometry (AP-MS) and showed that PrimPol, a primase-polymerase, interacts with factors involved in DNA inter-strand crosslinks. These results have implications for chemotherapy based on DNA crosslinks. In collaboration with the Metabolism and Cell Signalling Group, we used proteomics to analyse expression changes in livers from RagAGTP mice and identified a failed metabolic adaptation to fasting due to a global impairment in the PPAR α transcriptional programme. In addition, in collaboration with the Genomic Instability Group, we used approaches to identify RNA binding proteins and determined that arginine-rich peptides lead to a generalised displacement of factors bound to nucleic acids. These results may provide a plausible mechanism for the pathogenesis of amyotrophic lateral sclerosis. Moreover, we used proteomics, phosphoproteomics, and metabolomics to dissect the series of molecular events that regulate the establishment of naïve pluripotency in embryonic stem cells. These data demonstrated the presence of post-transcriptional regulation, which fine-tune the levels of mitochondrial proteins and enhance their

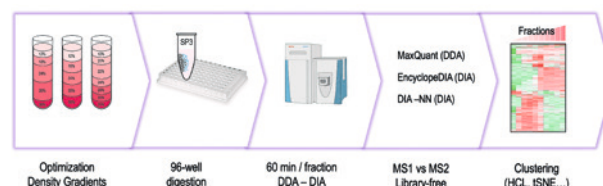


FIGURE 1 Schematic showing the workflow used to reveal the true identity of proteins present in small extracellular vesicles. This figure

depicts some of the steps that have been optimised to improve the confidence of the assignments.

oxphos capacity. Finally, the Unit implemented novel methods aiming to reveal the true identity of proteins present in small extracellular vesicles (sEVs). This is based on high resolution density gradients in conjunction with proteome correlation profiling to deconvolute the origin of proteins (FIGURE 1). Our data revealed that popular markers used to assess the purity of sEVs originate in non-vesicular fractions. This approach could have important applications for identifying potential biomarkers in liquid biopsies. ■

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- ▶ García-Silva S *et al.* (incl. Jiménez-Embún P, Rodríguez-Perales S, Martínez L, Ortega S, Boskovic J, Muñoz J, Megías D, Peinado H) (2021). Melanoma-derived small extracellular vesicles induce lymphangiogenesis and metastasis through an NGFR-dependent mechanism. *Nat Cancer* 2, 1387-1405.
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- ▶ de la Calle Arregui C *et al.* (incl. García F, Caleiras E, Campos-Olivas R, Mulero F, Muñoz J, Efeyan) (2021). Limited survival and impaired hepatic fasting metabolism in mice with constitutive Rag GTPase signaling. *Nat Commun* 12, 3660.
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- ▶ Santos-Coquillat A *et al.* (incl. Peinado H, Muñoz J, Jiménez-Embún P) (2021). Goat milk exosomes as natural nanoparticles for detecting inflammatory processes by optical imaging. *Small* 18, e2105421.
- ▶ Olmeda D *et al.* (incl. Muñoz J, Ortega S, Soengas MS) (2021). Live imaging of neolymphangiogenesis identifies acute antimetastatic roles of dsRNA mimics. *EMBO Mol Med* 13, e12924.
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- ▶ González-Acosta D *et al.* (incl. Muñoz J, Méndez J) (2021). PrimPol-mediated repriming facilitates replication traverse of DNA interstrand crosslinks. *EMBO J* 40, e106355.
- ▶ Lafarga V *et al.* (incl. Boskovic J, Fernandez-Leiro R, Muñoz J, Fernandez-Capetillo O) (2021). Widespread displacement of DNA- and RNA-binding factors underlies toxicity of arginine-rich cell-penetrating peptides. *EMBO J* 40, e103311.
- ▶ Salmón M *et al.* (incl. Caleiras E, Muñoz J, Ortega S, Barbadic) (2021). KRAS4A induces metastatic lung adenocarcinomas in vivo in the absence of the KRAS4B isoform. *Proc Natl Acad Sci USA* 118, e2023112118.
- ▶ Amor López A *et al.* (incl. Jiménez-Embún P, Al-Shahrour F, Muñoz J, Megías D, Peinado H) (2021). Inactivation of EMILIN-1 by proteolysis and secretion in small extracellular vesicles favors melanoma progression and metastasis. *Int J Mol Sci* 22, 7406.
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- ▶ García-Silva S, Jiménez-Embún P, Muñoz J, Peinado, H (2021). Postlymphadenectomy analysis of exosomes from lymphatic exudate/exudative seroma of melanoma patients (2021). *Methods Mol Biol* 2265, 345-359.

HISTOPATHOLOGY CORE UNIT

Vacant
Core Unit Head

Staff Scientist
Eduardo José Caleiras

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

*Plan de Empleo Joven (Youth Employment Plan)



OVERVIEW

Pathology is the branch of science devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs underlying disease. The Histopathology Unit offers support and expertise through a full range of services covering paraffin embedding and tissue sections to histochemical stains; research and diagnostic immunohistochemistry (IHC) testing; antibody validation; *in situ* hybridization techniques (including *in situ* detection of mRNAs by RNAScope); and the generation of tissue microarrays. Furthermore, the Unit offers other value-added services implemented by a team of highly specialised technicians, such as laser-capture microdissection; slide digitalisation; image analysis; and quantification. The Unit collaborates with CNIO researchers in the histopathological characterisation of animal models of disease, providing them with the required pathology expertise. In

“In 2021, despite the Covid-19 pandemic, the Unit was able to return to its usual levels of workload and services, even exceeding expectations in some specific areas.”

addition, the Unit offers its portfolio of services to other institutions, including hospitals, research centres and private companies.

RESEARCH HIGHLIGHTS

In 2021, the Unit was able to return to the standard levels of workload and services recorded before the pandemic and, in some specific areas, such as immunohistochemistry and image digitalisation and analysis, even exceeded expectations. Thus, more than 26,000 paraffin blocks of tissue samples were generated, and *ca.* 21,000 techniques were performed, including histological and IHC techniques (with dual and triple staining being increasingly in demand), *in-situ* chromogenic hybridization, tissue microarrays, slide scanning, etc.

During 2021, we made significant progress in digitalising our material, with approximately 35 % of all the slides generated converted to digital files. In addition, 10% of these were subjected to image analysis and quantification.

We also consolidated the *in situ* hybridization technology for mRNA detection (RNAScope), with 160 cases analysed, some of them with double staining, using the Ventana-Roche automatic platform for IHC stains. This new technique enables efficient detection of specific mRNAs directly on sections from formalin-fixed paraffin-embedded (FFPE) tissues, thus providing a spatial dimension to gene expression analysis. The applications of this new technology are manifold, e.g., as an alternative to IHC whenever it is difficult to find specific antibodies working well on FFPE tissues, or to validate results from other technologies, among others.

The high quality of the techniques run by the Unit continues being endorsed by External Quality Assessment Schemes. In this respect, our histochemical techniques were evaluated by UK NEQAS. Similarly, NordiQC and SEAP have evaluated a subset of our IHC techniques under different modules, including general markers, breast cancer markers and PD-L1; these all obtained good scores.

Training and outreach activities are also a critical component of the Unit's activities. Although some of the usual activities

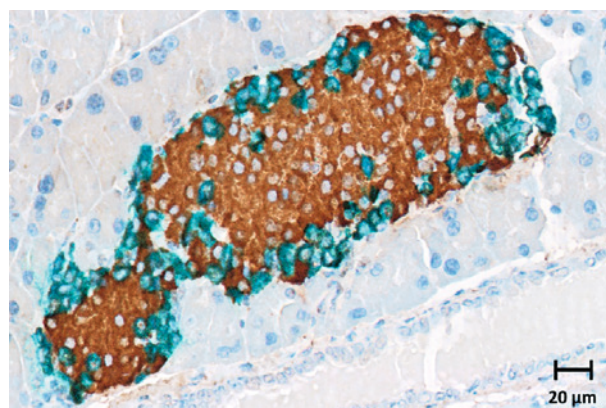


FIGURE Example of dual IHC staining. The image shows a picture of an islet of Langerhans in the pancreas, with double staining for insulin (DAB, brown) and glucagon

(Teal, blue). It can be seen that the insulin staining is homogeneous across the islet, whereas glucagon is localised more in the periphery of the islet.

in this area were compromised due to the pandemic, the Unit was still able to participate in a Master's course on oncology research, in online format, and we hosted a pre-doctoral student for a short training stay on immunohistochemistry techniques during the last quarter of the year. ■

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ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services



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

The CNIO Animal Facility was established to assist researchers in the development and analysis of *in vivo* models. We are currently collaborating with as many as 28 Research Groups, Sections and Units from different Research Programmes.

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 through a comprehensive health surveillance programme. Microbiological and environmental parameters in the animal areas are constantly monitored. All mouse strains

“The Animal Facility provides CNIO researchers with all the support required to work with mouse models, which are essential tools in cancer research, in compliance with the highest standards of animal care and welfare.”

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 dedicated 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 the productivity and ensure the 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 more than 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, Tet transactivator strains and others.

The Animal Facility offers the possibility of running a broad number of experimental procedures in the premises, including the use of gamma irradiation, UV light and volatile carcinogenic agents, as well as surgical procedures, some behavioural studies, a non-invasive blood pressure system, and a laboratory animal monitoring system (Oxylet) that enables measuring a number of physiological parameters for metabolic profiling and phenotyping of mouse models. Last year, the Animal Facility implemented a new climate chamber (HPPlife) that allows keeping mice under controlled environmental conditions of temperature, humidity and light, beyond the standard conditions established at the SPF barrier area. This will allow the study of these environmental factors and their influence on disease development, as well as on the health, behaviour, and welfare of laboratory animals.

Additionally, the monitoring of the mouse models through non-invasive imaging technologies is provided by the Molecular Imaging Unit, which has integrated 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 directed against mouse antigens, as well as for a project of the Experimental Therapeutics Programme aimed at testing the safety of some specific anti-tumour compounds.

All the work carried out by the Animal Facility complies with both national and EU legislation — Spanish Royal Decree RD53/2013 and EU Directive 2010/63/UE — for the protection of animals used for research experimentation and other scientific purposes. Experimental procedures and projects are reviewed and evaluated 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 the corresponding accreditation issued by the competent authority. The Animal Facility offers CNIO's new staff a short course focused on work with laboratory animals, complementary to the online courses that are a requisite to gain access to the facility.

The high standards achieved by the CNIO with regards 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. AAALAC accreditation, considered one of the top international recognitions in this field, was first obtained in October 2016, and renewed in 2019 for a new 3-year period. AAALAC International is a private non-profit organisation that promotes the humane treatment of animals in science through voluntary accreditation and assessment programmes. Recently, the Animal Facility's Head was appointed as an *ad hoc* consultant for the site reviews performed by AAALAC.

In accordance with our commitment to maintain the highest possible standards in relation to animal research issues, 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 research animals can be consulted on the CNIO website.

Until November 2021, the Animal Facility's Head had served as President 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, as well as to promoting refinement, reduction and replacement strategies in research involving animal models. ■

EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director



The following highlights summarise some of the main achievements of the Experimental Therapeutics Programme (ETP) during 2021:

PI3K inhibitors. Several years ago, we developed a series of advanced preclinical PI3K inhibitors. This year, in December 2021, the CNIO and TOTUS Medicines entered into a license agreement to develop a new generation of PI3K inhibitors. Through a unique drug discovery platform that combines different machine learning techniques, TOTUS will use the knowledge generated by the Experimental Therapeutics Programme to develop novel PI3K inhibitors that are more powerful, longer lasting, and with fewer side effects than those developed by other organisations.

CDK8 inhibitors (CDK8-is). We established a collaboration with A. López Martínez at *IdiPaz (Hospital Universitario La Paz, Madrid)* to evaluate the potential activation of NK cells by CDK8-is. The ex-vivo activation of NK cells could improve their immunotherapeutic activity to treat paediatric solid tumours. Next year, we will proceed in this direction.

Mastl inhibitors (MASTL-is). (In collaboration with Marcos Malumbres' Group). In 2021, we further optimised our MASTL-is, paying special attention to their ADMET profiles. We have now identified ETP-715 as a frontrunner inhibitor with improved overall properties (biochemical potency, kinase selectivity, cell activity and ADMET). We are now characterising its pharmacokinetic properties and will continue optimising it in the next stage. Additionally, we have synthesised a collection of around 100 PROTACs-like molecules. During 2021, we optimised the degradation capacity of our initial hits. As a result, we discovered the new MASTL-PROTAC ETP-823, which showed potent and durable MASTL degradation in selected cell lines. For example, in MCF7 cells, ETP-823 showed DC50 ~ 500 nM with maximal (96%) and long-lasting degradation of MASTL (72h in the presence of ETP-823, and 24h after its washout). We will continue their optimisation in 2022.

TRF1. (In collaboration with Maria A. Blasco's Group). In 2021, we focused our activities on our search for direct-TRF1 inhibitors. We had previously set up a "proximity assay" to identify small molecules that disrupt the binding of TRF1 to ds-telomeric DNA. In 2020, we carried out a virtual screening campaign against the DNA-binding domain of TRF1. Subsequent wet screening campaigns led to several hit molecules, which were subjected to triage and validation

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

processes in 2021. The overall results showed that only one compound, ETP-631, qualified as potential hit. The direct interaction of ETP-631 with TRF1 by thermal shift techniques is currently under investigation. We also selected, from our ETP Library, a set of compounds with a preferred functional group present in several literature examples of protein-DNA disruptors. This collection of compounds has been tested in the proximity assay. Interestingly, 3 potential hits have emerged from this screening, which are currently undergoing triage and validation processes.

SETD8 inhibitors. (In collaboration with Oscar Fernández-Capetillo's Group). The main objective of this project is to generate and optimise novel SET8 inhibitors (SETD8-is) as new therapeutic agents. During 2021, we performed biochemical screening campaigns with several ETP-libraries searching for covalent inhibitors. Several compounds were identified as potential hits. We are now characterising their covalent mechanisms of action via detection of the SETD8-ligand adducts by mass spectrometry and their competitiveness against the substrate and/or SAM cofactor. Next, we plan to initiate the SAR exploration of these molecules.

Other projects. ETP has provided support to several CNIO researchers in exploratory projects directed towards the discovery of new therapeutic agents against tyrosyl DNA phosphodiesterase (TDP2) (Felipe Cortés), PrimPol (Juan Méndez), brain metastasis (Manuel Valiente), and malignant peripheral nerve sheath tumours (MPNST) (Héctor Peinado). ETP has also collaborated with Refoxy Pharma (Berlin, Germany) and VIB (Flanders Institute for Biotechnology, Belgium) in drug discovery research projects. ■

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

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** Titulado Superior (Advanced Degree)

*** Plan de Empleo Joven (Youth Employment Plan)

OVERVIEW

The Medicinal Chemistry (MedChem) 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, classical inhibitors, and degraders such as PROTACs, which are then characterised by ETP's Biology Section, in order to evaluate their potency in biological targets *in vitro* and *in vivo* and ultimately to demonstrate their efficacy and mechanisms of action in animal models (*in vivo* proof-of-concept). The Section is also involved in the synthesis of high-quality chemical tools that help to decipher the mechanism of action of an observed phenotype in cellular assays, as well as in the synthesis of reference compounds that assist basic researchers in their investigations.

“In our MASTL project, we generated the first MASTL PROTAC (ETP-823) that potentially degrades MASTL protein via E3 ligase and proteasome recruitment.”

RESEARCH HIGHLIGHTS

Our MedChem activities in 2021 mainly focused on the following projects:

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is led by Maria A. Blasco (Telomeres and Telomerase Group). During 2020, the Biology Section developed an assay to measure the binding of TRF1 to telomeric DNA, and different wet screening campaigns were run. To date we have identified potential “direct” inhibitors of TRF1 that do not interfere with the assay system nor with DNA. We are currently involved in their validation by re-synthesising them to confirm the observed activity with pure compounds, as well as by synthesising some direct analogues to establish a preliminary SAR of the series.

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

This project is being carried out in collaboration with Marcos Malumbres (Cell Division and Cancer Group). We are following 2 different chemical strategies in this project; on the one hand, we are immersed in the fine optimisation of the main chemical series of MASTL inhibitors, in which we have identified a potent, cell active, and selective compound without cardiotoxic alerts (ETP-715). Our objective is to obtain an orally bioavailable compound to carry out efficacy studies in mouse models. On the other hand, we are exploring the development of PROTACs (Proteolysis Targeting Chimeras) molecules to degrade MASTL, not only to inhibit its catalytic activity. Chemically induced degradation of proteins, based on hijacking the natural protein degradation machinery present in cells as a mechanism to degrade proteins of interest (POI) linked to disease, has emerged as an innovative approach in drug discovery (DD) and development. This concept has been put into practice by designing and synthesising hetero-bifunctional small molecules, the so-called PROTACs. Unlike traditional drugs, PROTACs aim to reversibly eliminate the aberrantly functioning protein rather than inhibiting its (catalytic) function. PROTACs follow an event-driven rather than an occupancy-driven pharmacological paradigm and, importantly, act catalytically to degrade super stoichiometric amounts of the target protein. The mechanism of action of PROTACs implies more robust and durable pharmacological effects than just inhibition, providing some advantages, such as the use of lower and/or intermittent dosing regimens, which in turn can lead to improvement of the therapeutic index for PROTACs based drugs, due to reduction or elimination of dose-limiting toxicity adverse events related to off-targets.

These molecules consist of 3 components: a target protein-binding moiety, a degradation machinery-recruiting unit (typically an E3 ubiquitin ligase), and a linker that couples these 2 functionalities. During 2021, we continued our activities in this field by increasing the number of PROTACs synthesised along with the negative probes required to determine their mode of action. As a result of this exploration, we identified 1 PROTAC (ETP-823, FIGURE 1) with a degradation concentration 50 of 0.63 μ M in the MM1.S cell line that will help us to better understand MASTL biology. We are currently involved in the fine optimisation of this PROTAC to improve its potency and confer it with *in vivo* properties.

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

In collaboration with Óscar Fernández Capetillo (Genomic Instability Group), the aim of this project, incorporated into the ETP in 2021, is to generate and optimise SETD8 methyltransferase inhibitors as new therapeutic agents. In 2020, we started our activities by synthesising and acquiring some reference compounds. The initial chemical exploration around some identified hits from Capetillo's Lab rendered several compounds, but in the micromolar range. On the other hand, the screening of irreversible molecules identified hits with low micromolar activity. The hits were synthesised in good purity to validate their mechanisms of action, and in parallel we initiated a chemical strategy based on the synthesis of covalent compounds.

Apart from the drug discovery activities, we give support to several Groups by synthesising reference compounds or purifying compounds to help them with their projects. During 2021, we carried out these activities for the following Groups: Epithelial Carcinogenesis, Microenvironment & Metastasis, Brain Metastasis, Telomeres and Telomerase, Experimental Oncology, Topology and DNA Breaks. ■

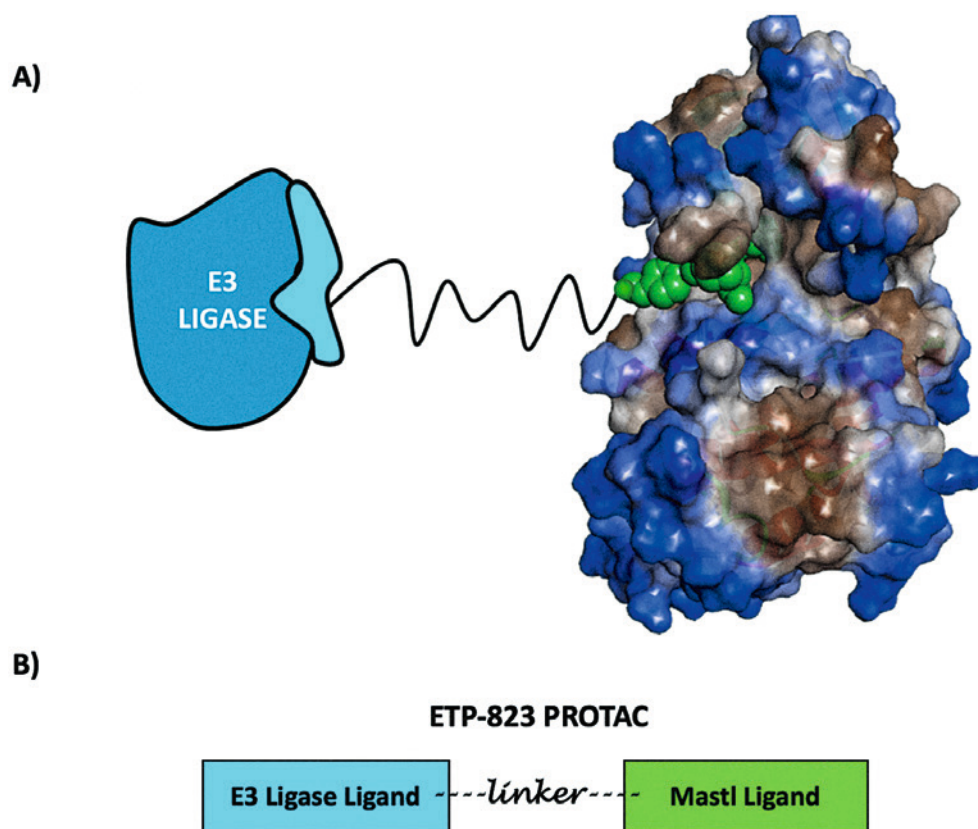


FIGURE 1 (A) Representation of PROTAC ETP-823 binding to the kinase domain of human MASTL protein (PDB 5LOH) through MASTL

Ligand, and to E3 Ligase through the corresponding Ligand. (B) Components of ETP-823 PROTAC.

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***Plan de Empleo Joven* (Youth Employment Plan)

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OVERVIEW

Targeted cancer therapies by means of small molecules or antibodies act on specific molecular targets to block cancer growth and progression. Kinases have become attractive molecular targets for the treatment of numerous cancer types; hence the U.S. Food and Drug Administration (FDA) has approved many small-molecule kinase inhibitors for clinical use, some of them irreversible inhibitors. Similarly, given the importance of epigenetic marks in tumorigenesis, modifiers of DNA or histones have become attractive therapeutic targets; currently, there are 6 epigenetic drugs clinically approved for cancer treatment by the FDA. In collaboration with Óscar Fernández-Capetillo, we recently started an early drug discovery project to develop SETD8 inhibitors, as non-advanced inhibitors have been described so far. By developing a non-radioactive assay, we have been able to perform a screening campaign and identify several molecules as starting points to obtain good SETD8 inhibitors that are both reversible and irreversible.

“We have identified MASTL PROTACs with a nanomolar degradation concentration 50 and a maximum 93% degradation of MASTL via E3 ligase and proteasome recruitment.”

RESEARCH HIGHLIGHTS

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

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. We tested in our biochemical assay using active human full-length MASTL protein, around 250 new compounds, both MASTL-is and MASTL PROTAC-like molecules. For the most potent biochemical inhibitors and PROTACs molecules, we measured MASTL engagement in cells (BRET assay). In the case of PROTACs, we also evaluated their MASTL degradation capacity in cells in order to determine the best linker length and binding to E3 ligase ligand. We identified a nanomolar MASTL degrader (ETP-823) that will be used to study its pharmacological implications (FIGURE 1). In addition, we characterised the ADME-T properties of our most potent MASTL-is to identify the compound with the best pharmacokinetic profile.

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. After virtual screening and wet assay, only 1 compound, ETP-631, qualified as a potential hit; more orthogonal assays to validate the direct binding to hTRF1 are under study. We also performed a new screening of a collection of 1500 molecules selected from our ETP-library that bear a privileged structure to disrupt protein-DNA complexes. After analogue searching, we identified several hits from different chemical series. We are now validating these hits applying orthogonal assays against TRF1 and the TelDNA probe, such as CETSA and the fluorescent displacement assay, respectively, using freshly prepared and/or resynthesized 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. In 2021, we performed a screening campaign with a commercial library of 1500 irreversible molecules, through which several hits were identified and some validated after resynthesis. Moreover, a small internal library of irreversible molecules was tested identifying possible hits with low micromolar activity. The irreversible mechanism of action of all the hits

is being validated by biochemical assays and proteomics in order to gain information to improve activity.

Collaborations with other CNIO Groups

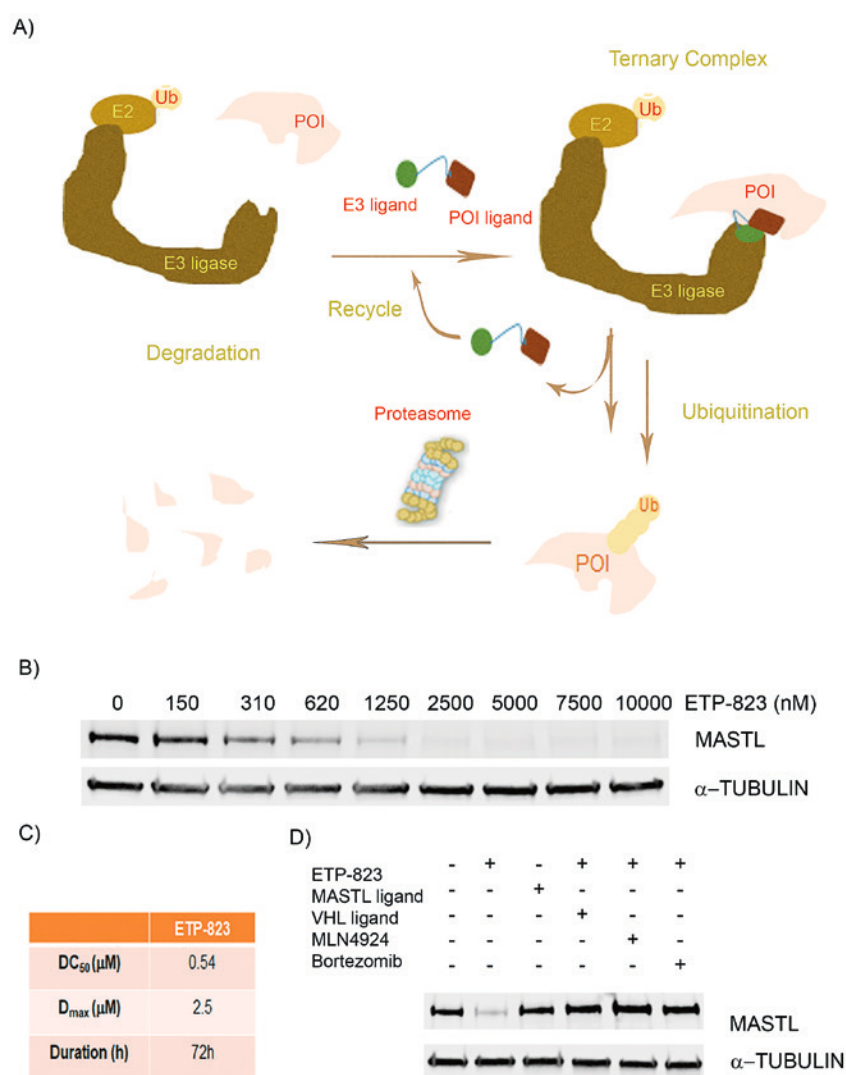
ETP-Biology provided support for *in vivo* studies of selected compounds and drugs, such as pharmacokinetics, distribution and/or antitumour efficacy, performed by the Microenvironment and Metastasis and the Brain Metastasis Groups. Furthermore, in collaboration with the DNA Replication Group, we prepared hits identified in a virtual screening campaign for the validation of PrimPol inhibitors. We also gave technical support by setting up a biochemical assay to be able to perform screening campaigns for the Topology and DNA Breaks Group and the H12O - CNIO Haematological Malignancies Clinical Research Unit. Finally, we collaborated with the Experimental Oncology Group, helping to validate RNAseq data with small molecules.

Collaborations with other institutions

Target X. ETP-Biology performed biomarker evaluation studies against target X in a previous collaboration with VIB (the Flanders Institute for Biotechnology).

Refoxy Pharma collaboration. ETP-Biology gave logistics and data analysis support. ■

FIGURE 1 PROTAC MASTL (ETP-823) downregulates the protein levels of its target. **(A)** Model of PROTAC-induced degradation. **(B)** Dose-dependent downregulation of MASTL levels in MCF-7 after 24h treatment with ETP-823. **(C)** Degradation concentration 50, maximum degradation concentration and duration of MASTL degradation. **(D)** ETP-823's degradation is dependent on the proteasome and the presence of the linkage between both targeting ligands. MCF-7 cells were treated with the indicated compounds for 24 h and analysed by Western blotting. MLN4924: NEDD8-Activating Enzyme inhibitor; Bortezomib: proteasome inhibitor.



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PATENTS

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CNIO - LILLY CELL SIGNALLING AND IMMUNOMETABOLISM SECTION

Susana Velasco
Section Head

Staff Scientists
Ana Cerezo
Sonia Hernández Tiedra
Eva P. Lospitao
Gloria Martínez Del Hoyo
Camino Menéndez



SCOPE OF THE ELI LILLY - CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer immunometabolism. Our Section is funded through a research contract with Eli Lilly and focuses on identifying small molecular weight molecules that regulate the metabolism of malignant cells, with the objective of killing them either directly, by acting synergistically with other antitumour agents, or indirectly by activating the antitumour immune response. Exploring how to better target these mechanisms would lead to better and more efficient therapeutic options.

A combination of *in vitro* and *in vivo* approaches is being utilised to obtain a complete understanding of tumour metabolic reprogramming and the antitumour response. For this purpose, we developed a series of biochemical and cell-

based assays exploiting advanced techniques such as extracellular flux analysis (Seahorse technology), nuclear magnetic resonance, metabolomics and immunophenotyping. Finally, each target goes through an *in vivo* validation process using xenografts, allografts and mouse models developed at the CNIO. This 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 (since November)
Laura Diezma
Roberto Gómez (TS)
Sandra Peregrina (TS)

Natalia Riestra
Patricia Sosa (until December) (TS)
**Titulado Superior (Advanced Degree)*

SCIENTIFIC CONTEXT

Cancer can be defined as the uncontrolled growth and division of cells, leading to tumour formation, invasion, and metastases. Unlike normal cells that require growth factor signals, tumour cells often have mutations that result in constitutively active (“always on”) signaling pathways that drive aberrant cell growth and division. In order to fulfil the high nutrient demand required for their continuous growth, tumour cells have reprogrammed their basal metabolism from an oxidative to a more glycolytic/anabolic one, even in the presence of oxygen. Major molecular mechanisms involved in the process have been identified and characterised. It was found that both oncogenes (Ras, Myc) and tumour suppressor genes (p53, RB, LKB1) impart an altered metabolic phenotype in cancer cells by regulating genes involved in central metabolic pathways.

Cyclins and cyclin-dependent protein kinases (CDKs) are important regulators of the cell cycle. In recent years, several highly effective CDK inhibitor (CDKi) drugs such as abemaciclib and palbociclib have been approved for the treatment of hormone receptor-positive, HER2-negative advanced breast cancer in combination with endocrine therapy, demonstrating substantial improvements in patient progression-free and overall survival. Many tumours eventually develop resistance to these drugs. The precise characterization

of these mechanisms of resistance could lead to the identification of biomarkers predicting the clinical response to CDKis, the identification of other types of tumours also responsive to CDKis, synthetic lethality, and more precise combination therapies.

Our laboratory has collaborated with Eli Lilly Alcobendas (Madrid) and Marcos Malumbres’ Cell Division and Cancer Group at the CNIO to validate molecular targets involved in mechanisms of sensitivity and resistance to CDK4/6 inhibitors identified through a CRISPR/Cas9 library screen. One of our goals was to study how CDKis may affect tumour metabolic reprogramming. FIGURE 1 shows how both abemaciclib and palbociclib specifically target oxidative phosphorylation metabolism while leaving glycolysis unaffected. ■

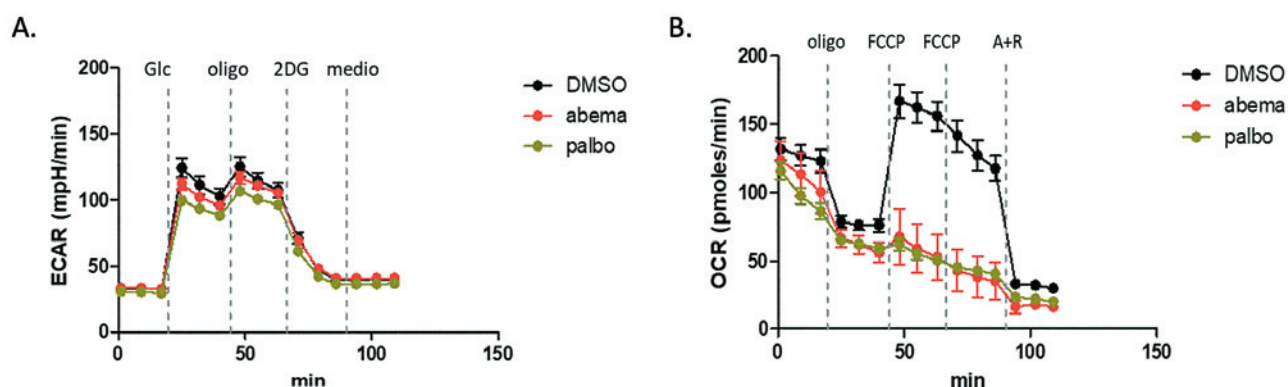


FIGURE 1 Extracellular fluid analysis of breast cancer cell lines treated with CDKi abemaciclib or palbociclib. Both the extracellular acidification rate (ECAR), a measure of the glycolytic status of the cell (A), as well as the oxygen consumption rate (OCR), a mitochondrial test (B), were measured using a Seahorse XPF analyzer. CDKi inhibitors greatly inhibit mitochondrial metabolism

TECHNOLOGY TRANSFER AND VALORISATION OFFICE (TTVO)

Irene Herrera
Director

Technology Transfer Manager
Irene Pino (since September)



At the CNIO, the best science and research efforts join in the desire to make a great impact for cancer patients and the healthcare system. TTVO contributes to this purpose by ensuring the 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 TTVO proactively monitors the progress of the CNIO's scientific activity to identify projects with high transfer potential. In 2021, 12 priority patent applications and 3 divisional applications were filed and another 3 new ideas were incorporated into the TT portfolio and will become priority patent applications in 2022. These cover a wide range of products, including drug inhibitors, new biomarkers, a cell therapy, a therapeutic vector for gene therapy, a composition for treating and preventing cancer and metastasis, a method for diagnosing pancreatic cancer, a method for determining

whether a tumour has deficiencies associated with chromosomal instability, and the identification of a pocket with implications for drug design and personalised therapies for patients with RET-driven cancers. CNIO patents constitute an active portfolio of assets that are carefully prosecuted according to a patent strategy and licensing efforts. In coordination with national and international patent agents, TTVO manages a portfolio of 42 patent families, and provides advice and assistance during the drafting of the patent document, filing, and prosecution process. Two PCT applications for international extension were filed in 2021, and 4 patents with proven commercial interest entered the national phase. Licensed patents make up a remarkable 50% of the CNIO portfolio. Among the licences signed in 2021, in addition to cell lines and antibodies, we highlight a license agreement of the patent family "Inhibitors of PI3 kinase" to the US company Totus Medicines, for a considerable upfront payment and future milestones if achieved, in addition to royalties (terms of the agreement are confidential).

To ensure that scientific ideas and results are transferred to the private sector, a proof-of-concept phase is usually necessary to validate its potential application in the market. The TTVO supports the preparation, coordination and advice of CNIO scientists so that their ideas reach the point of development necessary for potential companies to decide to invest and co-develop. This is the case of calls aimed at technological development projects such as *CaixaImpulse*, among others. Four CNIO projects have been ongoing since 2018: TRF1 inhibitors to treat glioblastoma; FUGE Therapeutics: a gene-editing technology to fight cancers caused by oncogenic fusion genes; cell therapy for effective glucose control in type 1 diabetic patients; and the COVI-PHI test: a novel detection kit for the simple and rapid diagnosis of SARS-CoV-2 infection.

In 2021 CNIO groups actively participated in the new National *AEI* Innovation calls, co-funded by the EU Reconstruction Funds “NextGenerationEU”. Two “Proof of Concept” projects were awarded €150,000 each: “Development of THX-B as a novel anti-metastatic agent” led by Héctor Peinado, and “Rewiring tumour-immune system crosstalk by targeting MIDKINE” led by Maria S. Soengas. In addition, 2 “Strategic Projects” in collaboration with private companies also received grants: “A new patient-derived circulating micrometastases-on-chip platform for drug screen and validation” (*µMETonChip*), coordinated by CNIO (Marcos Malumbres) together with Pharmamar, with a total budget of more than €1.2 million; and “Ultrasensitive optoplasmonic immunoassay platform for early detection of breast cancer based on protein biomarkers at the deep region of the blood proteome” (*OncoDeePlasm*), coordinated by CSIC with the collaboration of CNIO (Marcos Malumbres), Mecwins SA, *HUI2O*, and *FIBH12O*, with a total budget of nearly €1.5 million.

The experience and financial support of the value chain’s actors, from specialised investors to large multinationals in the biopharmaceutical industry and start-up companies, are necessary to develop technologies. The TTVO identifies these partners, negotiates TT agreements, and manages the relationship with licensees, including the payment of royalty fees. In 2021, the TTVO managed 305 agreements (MTAs, CDAs, Research Collaborations, licenses, etc.). Among these industrial partnerships it is worth noting that in 2021, 2 respective addenda were signed to the collaboration contracts between CNIO, UAB, and the spin off Telomere Therapeutics, and between CNIO, CSIC and Refoxy Pharmaceuticals. The first is for a project of gene therapy technology with telomerase in the field of pulmonary and kidney fibrosis co-led by Maria A. Blasco, Head of the Telomere and Telomerase Group, and the second is to develop FOXO target triggers in cancer models and ageing-related diseases, co-led by Joaquín Pastor, Director of the CNIO Experimental Therapeutics Programme. A collaboration study agreement, ESFURN – European Study for FGFR in Urothelial Neoplasms was signed by the CNIO (Francisco X. Real and Núria

Malats) and the following European universities – Erasmus, Birmingham, Erlangen – and Janssen Pharmaceuticals. Other research agreements were signed with Loxo Oncology (Susana Velasco), Boehringer Ingelheim (Mariano Barbacid), and Bristol Myers Squibb (Joaquín Martínez-López).

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, up to €3 million were secured for research activities. Moreover, 6% of the agreements are licences to commercial partners. Patents and unpatented research tools are licensed in order to increase their availability to the scientific community, as well as to create opportunities for our business partners and to provide a financial return on public investment. The net income derived from licences in 2021 increased to €884,466. This income reverts back to CNIO research activities as well as to the inventors themselves. A total of 40 inventors and 10 research groups have contributed towards and benefited from this achievement. Our monoclonal antibody commercial pipeline was also strengthened, and now accounts for more than 110 monoclonal antibodies. CNIO’s monoclonal antibody platform operates not only through licences with leading manufacturers of reagents such as Abcam, EMD Millipore, Becton Dickinson, etc., but also through strategic alliances with companies such as Merck and R-Biopharm and co-development with research institutions (*CNIC*, *HUI2O*, *CRG*, *CICbioGUNE* and *Hosp. Univ. San Carlos*).

Effective transfer of research results to the productive environment requires intensive networking and asset promotion attending international forums and meetings. The Covid-19 pandemic impacted extraordinarily on events promoting technology transfer and partnerships, but with the gradual return to business event activity, our goal in 2022 will be to attend such meetings to achieve collaboration and license agreements. With the aim of fostering the culture of innovation at the end of the year, 6 CNIO researchers were selected to participate in the “Accelerate: Building Business from Science and Technology” course at the *IE* business school in 2022 with the support of *Banco Santander* Foundation.

CNIO’s Technology Transfer Office is actively participating in the recently-created Knowledge Transfer and Innovation Group (KTi SOMMa). The objective is to promote, reinforce and maximise the value of innovation and knowledge transfer generated by the members of the SOMMa alliance, contributing to create a positive social and economic impact at the national and international levels based on scientific excellence. 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-Paño
Director

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

Francisco De Luna (TS)*, Cecilia
Sobrinó (since April), Daniel Alba
(since March) (TS)*

**Titulado Superior* (Advanced Degree)



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 protect donors' rights.

CNIO Biobank is a cross-service platform for CNIO researchers, and in general the wider scientific community, that offers extensive services covering all stages in research project management requiring the use of human samples. CNIO Biobank provides sample handling/processing; collection management; quality, ethical and legal advice; acquisition and design of valuable research collections; and negotiation with different stakeholders to find suitable samples and/or help obtain the ethical approval for any research project (involving human samples).

“Translational research projects should be biobank-centred by using high quality samples and data resulting in better treatments for society that improve health and quality of life.”

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* (ref. nº: B.000848).

Student in Practice
Sheila Artesero (March-June)
(Universidad Rey Juan Carlos,
Madrid, Spain)

RESEARCH HIGHLIGHTS

2021 became a turning point in CNIO Biobank's history. The COVID-19 crisis obliged us not only to adapt and transform our protocols, but also to modify the scope of our activity and support research projects aimed at gaining insights about the virus that may unveil diagnostic markers for detection and/or prognosis as well as the evolution of the infection.

Thanks to *Fundación Ramón Areces* and the coordination with the Brain Metastasis Group at CNIO, we created the *Red Nacional de Metastasis Cerebral (RENACER)*. The objective of *RENACER* is to carry out basic and clinical research that helps to advance the development of therapies for these metastases. Thirteen Spanish hospitals joined the network to create a collection of human samples (12 to date) to further investigate brain metastases, and to improve both their diagnosis and treatment. Through this project, CNIO Biobank collects and stores fully characterised quality samples and clinical information to support excellence in research. As an example of these collections, since November, the Biobank is creating a prospective longitudinal cohort from the work collective of the flight attendants in collaboration with their association (*Asociación Española de Tripulantes de Cabina de Pasajeros*) and thanks to the generous donation of a mobile unit for sample extraction from *Quirón Prevención*. Samples will be taken every two years from the same individuals and in biobank-regime, therefore, accessible to all researchers.

CNIO Biobank acts as national coordinator of the biobanking Hub for the *Plataforma de Biobancos y Biomodelos, Instituto de Salud Carlos III*, a project aimed at integrating and enhancing the role of biobanks as research platforms. CNIO-Biobank's Scientific Director, Eva Ortega-Paño, was also appointed as Scientific Coordinator for the National Node in the European Consortium BBMRI-ERIC.

CNIO Biobank is highly involved in dissemination activities, participating on a regular basis in radio programmes (*RNE*), with a remarkable presence in local and national press, and participating in events such as "*La noche de los investigadores*", "*Día de la mujer y la niña en la ciencia*", "Talent Woman" or "*Mujeres que lideran el futuro*".

CNIO Biobank, in collaboration with the University of Copenhagen (Denmark), Helsinki University (Finland) and the Danish National Biobank, organised a PhD course, "The Future of Biobanking", and a symposium in November entitled "Innovative Approaches in Large-Scale Research Infrastructures". ■

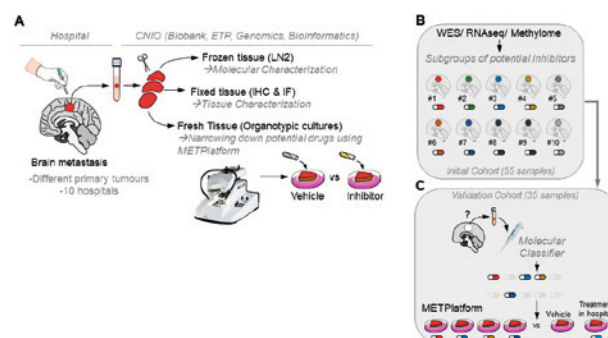


FIGURE RENACER research collection receives samples and clinical information from hospitals across Spain. CNIO Biobank processes frozen tissue for molecular

characterisation; fixed tissue for tissue characterisation; and fresh tissue for organotypic cultures, to analyse potential drugs using METPlatform.

PUBLICATIONS

- Zhu L *et al.* (incl. Blanco-Aparicio C, Martínez S, Sobrino C, Ajenjo N, Artiga MJ, Ortega-Paño E, Rodríguez-Perales S, Muñoz J, Caleiras E, Megías D, Quintela-Fandino M, Pastor J, Valiente M) (2021). A clinically compatible drug-screening platform based on organotypic cultures

identifies vulnerabilities to prevent and treat brain metastasis. *EMBO Mol Med*. PMID: 35174975.

Book Chapter

- Morente MM, Luna-Crespo F, Ajenjo N, Artiga MJ, Ortega-Paño E (2021). National Biobank Networking: The Case of

Spain. p. 153-173. In: *Biobanking of Human Biospecimens*, Hainaut P, Vaught J, Zatloukal K, Pasterk M (eds), Springer International Publishing, 206 pages.

AWARDS AND RECOGNITION

- Eva Ortega-Paño:
• Scientific Coordinator for the National Node

in the European Consortium BBMRI-ERIC, a European research infrastructure for biobanking.

- *Ramón Areces* Foundation Grant to reinforce the implementation, development and coordination of the National Network of Brain Metastasis (*RENACER*), Spain.

Communication

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COMMUNICATIONS

NURIA NORIEGA

SUSANA PÉREZ DE PABLOS

Head of Communications

Science Communication and Social
Media

Rebeca Santamaría (since
September)

Vanessa Pombo (until April)



2021 was undoubtedly a year in which the hottest scientific issue in the news was the Covid-19 pandemic and all SARS-CoV-2-related topics. The Communications Department at the CNIO worked hard to draw attention to the latest developments in cancer research – a disease that continues to be among the leading causes of death worldwide. However, honouring its commitment to public service, the CNIO conducted Covid-19-related research, and the Communications Department publicised the projects carried out in this area, such as the blocking mechanisms of the coronavirus infection and the treatment of associated conditions.

CNIO's scientific news attracted the attention of the main media in Spain and other countries, such as *France Soir* and *Forbes* in Mexico, and also hit the front pages of local and national newspapers, such as *El Mundo* and *Faro de Vigo*. News items included the discovery of the main genes that increase the risk of breast cancer (Javier Benítez, Anna González-Neira and Ana Osorio, *NEJM*); a potential new treatment for kidney fibrosis (Maria A. Blasco, *Nature Aging*); the discovery of a molecular switch that allows the body to adapt to fasting (Alejo Efeyan, *Nature Communications*); a drug already in use in humans that corrects obesity in mice (Nabil Djouder, *Nature Metabolism*); the finding of the cause of neuronal death in a large proportion of familial ALS patients (Óscar Fernández-Capetillo, *The EMBO Journal*); and a mechanism involved in the early stages of melanoma metastasis (Héctor Peinado, *Nature Cancer*).

For the occasion of World Cancer Day in February, the CNIO launched #CNIOStopCancer, an awareness campaign aimed at raising funds for cancer research. This year's campaign had the support of culture and sports celebrities like actor Carlos Hipólito, mountaineer Ederne Pasaban, and World and Enduro Champion Laia Sanz. The campaign received broad media coverage, with impacts on *Canal 24 Horas*, *Telemadrid* and other TV channels.

In September, the CNIO celebrated World Cancer Research Day with an open public event entitled “Vaccines Against Covid-19 and Cancer Control”. The event, supported by “la Caixa” Foundation, featured a keynote lecture by virologist Mariano Esteban, who highlighted the role of research in the fight against SARS-CoV-2 and its impact on the study of other diseases like cancer. Hundreds of users were able to follow the lecture via live streaming, and it appeared in leading media like *Canal 24 Horas* and the *EFE* news agency.

CNIO Arte, a ground-breaking project that explores the common ground where scientists and artists act and interact was again greatly successful in 2021. The project featured two exceptional figures in this 4th edition: computational biologist Sarah Teichmann and artist Daniel Canogar. The spectacular

“In a year marked by the vaccines in the media, CNIO's cancer research made its way to the headlines.”

work created by Canogar (consisting of 12 LED screens, computers, cables and metallic structures) based on Teichmann's research was featured in major media outlets, including *ABC Cultural*, *El País* and *RTVE*. The media impact of this initiative, organised with the support of *Fundación Banco Santander*, is vital to raise funds for cancer research through the sale of the artworks created by the artists who take part in CNIO Arte.

The CNIO is strongly committed to gender equality in science. For International Women's Day, the Centre organised a colloquium discussion with Mary Beard, Professor of Classics at the University of Cambridge and Princess of Asturias Laureate. The event, organised jointly with *IE* University and the British Embassy, was streamed online and covered by *Europa Press* and other media outlets.

In alignment with the EU Directive to replace, reduce and refine the use of animals for scientific purposes, in 2021 the CNIO signed a cooperation agreement with *Fondation Franz Weber* to engage young PhD researchers in research projects of excellence that use alternative methods to animal testing. The agreement was announced by media like the news agency *EFE* at the national level.

These are some of the initiatives in which the CNIO Communications Department participated, adding value to the endeavours and achievements of the Centre's researchers and highlighting the importance of science as a driving force to build a better society. ■

PRESS CLIPPINGS



1 La Razón, January 17, 2021

2 El Diario.es, January 21, 2021

3 La Tarde, Canal 24h, February 1, 2021

4 Faro de Vigo, February 23, 2021

5 Forbes, March 12, 2021

6 El Correo Gallego, April 7, 2021

7 La Voz de Galicia, April 12, 2021

8 ABC, April 16, 2021

9 El País, May 13, 2021



10 La Razón, July 4, 2021
11 News Medical Life Sciences, July 8, 2021

12 El Mundo, July 16, 2021
13 El Español, August 23, 2021
14 France Soir, August 30, 2021

15 El Faro, Cadena Ser, September 6, 2021
16 La tarde, Canal 24h, September 24, 2021

17 El Periódico, September 26, 2021
18 National Geographic TV, October 4, 2021



19 Telemadrid, October 14, 2021

20 El Mundo, October 17, 2021

21 La Rioja, October 19, 2021

22 El Mundo, November 10, 2021

23 Forbes México, November 18, 2021

24 El Faro de Vigo, November 22, 2021

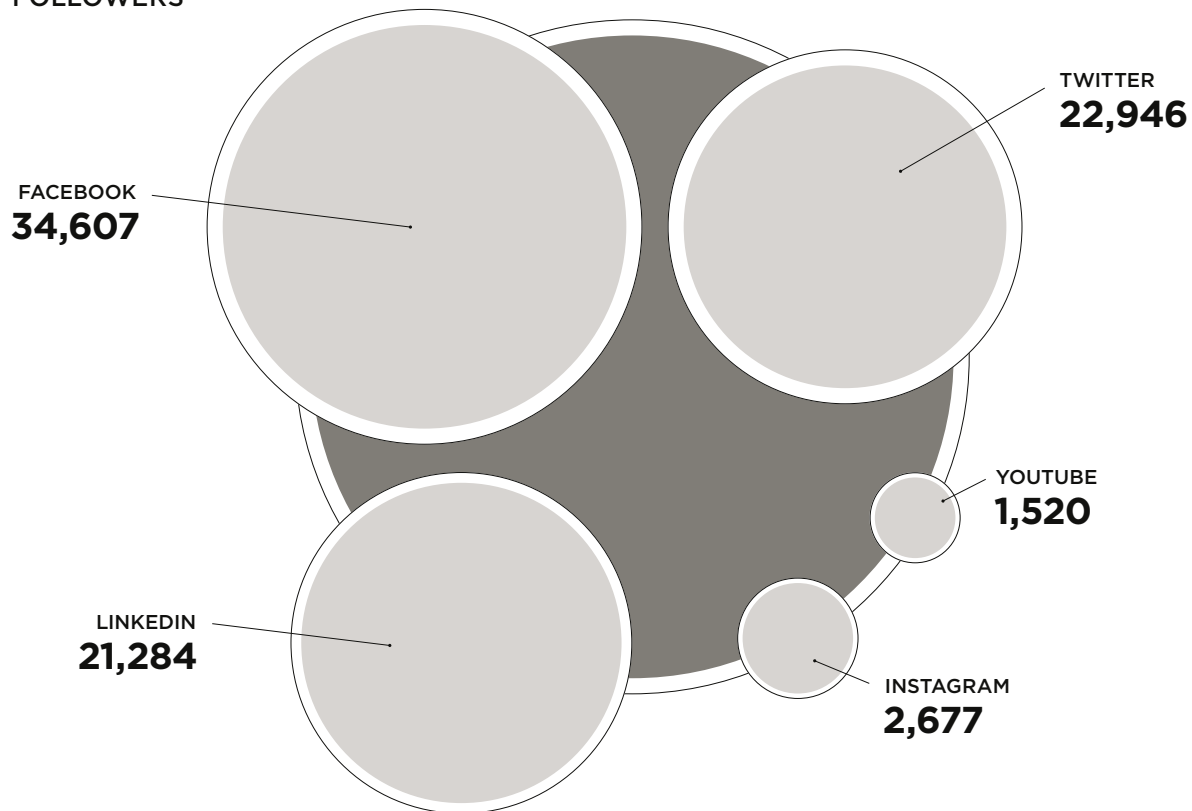
25 El Mundo, November 26, 2021

26 La Sexta Noticias, November 26, 2021

27 Sinc, December 29, 2021

28 Órbita Laika, La 2, December 29, 2021

2021 SOCIAL NETWORK DATA FOLLOWERS



LINKEDIN

IMPRESSIONS	817,202
INTERACTIONS	33,217
LINK CLICKS	21,269
INTERACTION RATE	3.83%

YOUTUBE

UPLOADED VIDEOS TOTAL	156
UPLOADED VIDEOS 2021	30
VIEWS IN 2021	42,141
WATCH TIME (HOURS)	4,75
AVERAGE VIEW DURATION	6:42

TWITTER CNIO

IMPRESSIONS	2,510,800
RETWEETS	3,279
LIKES	8,517
MENTIONS	4,832
VISITS TO THE PROFILE	189,923

FACEBOOK

IMPRESSIONS	2,975,460
REACH	1,428,261
LINK CLICKS	32,298
INTERACTIONS	45,448

SOCIAL EVENTS



In 2021, Spain's Minister for Health, Carolina Darias, held a technical meeting with the European Commissioner for Health and Food Safety, Stella Kyriakides, during a visit to the Spanish National Cancer Research Centre (CNIO). The meeting was also attended by Raquel Yotti, Director of the Spanish Institute of Health *Carlos III* (ISCIII); María A. Blasco, CNIO Director; and María Jesús Lamas, Director of the Spanish Agency of Medicines and Medical Devices (AEMPS). Those appearing in the picture are from left to right: María Jesús Lamas, Stella Kyriakides, Carolina Darias, Raquel Yotti, María Blasco and Cristóbal Belda (Director of ISCIII). July 9, 2021.



On International Women's Day, the CNIO, IE University and the British Embassy organised the colloquium discussion "Women in Power," featuring three great leaders in their fields: Mary Beard, Professor of Classics at the University of Cambridge and Princess of Asturias Laureate; María Blasco, molecular biologist and Director of the CNIO; and Susana Torres, Professor of Humanities at IE University; along with journalist Fiona Govan as moderator. During the colloquium, which took place via Zoom, these 3 leaders highlighted that, despite the progress made to incorporate women into different social spheres, there is still a long way to go to achieve real equality and outlined the challenges that remain. March 9, 2021.



With the support of "la Caixa" Foundation, we celebrated World Cancer Research Day with the symposium "Vaccines against COVID-19 and cancer control," in which CNIO director Maria A. Blasco and immunologists Mariano Esteban and Margarita del Val participated. "The development of new vaccines against infectious diseases and cancer should be obligatory in industrialised countries," urged Mariano Esteban, the

immunologist who is currently developing one of the CSIC's vaccines against COVID-19. After his keynote lecture, there was a roundtable moderated by journalist Cristina Villanueva in which the following speakers took part: Maria A. Blasco, Mariano Esteban, Margarita del Val, Rosario Perona (Deputy Director for Research Assessment and Promotion at *ISCIII*) and Sergio Recio (CNIO Friend). September 24, 2021.



Although the use of animals is necessary for the study of certain pathological processes, the development of techniques that are alternatives to animal experimentation will make it possible to reduce the use of experimental animals and replace them with these techniques. That was made clear at the "III Workshop on Philosophy and Science: The use of animals in science. Ethical and epistemic problems

and alternatives" that we organised with the support of *Banco Sabadell* Foundation. This meeting took place at the CNIO and was attended by more than 300 people who registered online. Scientists Manuel Valiente, Lluís Montoliu, Raúl Rabadán and Maria A. Blasco debated with philosophers Fabiola Leyton, Marta Tafalla and Guillermo Repetto, and journalist Lucía Arana. November 23, 2021.



CNIO participated, for the 11th year in a row, in the European Researchers' Night, which is funded by the EU Framework Programme for Research & Innovation, Horizon 2020 - Marie Skłodowska-Curie actions. In Madrid, it is promoted by the Department of Education and Research and coordinated

by *Fundación madri+d*. In this special year, the meeting had to be held online, but the distance did not diminish the enthusiasm of the researchers nor that of our 300 attendees, who had a lot of fun conducting a scientific experiment in their homes. September 24, 2021.

Institutional Image & Outreach to Society

INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY

AMPARO GARRIDO Coordinator

Science Outreach Officer
Paula Calderón (since July)



“This past year saw us attracting the keen interest of visitors to ARCO, Spain’s best known venue for Contemporary Art, highlighting the work of 4 creators who took part in CNIO’s previous art events. The display stand received special praise for its vibrancy and originality.”

In 2021, the CNIO celebrated the fourth edition of CNIO Arte carried out, as in previous years, with the collaboration of *Fundación Banco Santander*. Its commissioner was once again Amparo Garrido, coordinator of the CNIO Office for Institutional Image and Outreach to Society. This year featured visual artist Daniel Canogar, whose work has had a great impact across the continents, and computational biologist Sarah Teichmann, from the Wellcome Sanger Institute (Cambridge, UK), co-founder of the international Human Cell Atlas project. Following their encounter, the artist produced the work “Fulguraciones”, which was on display at CNIO from June 14th to September 11th. All proceeds from artwork sales go directly to the CNIO Friends philanthropic platform, which funds cancer research at the CNIO.

For the first time in Spain, we had the honour of being the first research centre to be awarded an exhibition space of our own in the ARCO International Contemporary Art Fair. The CNIO

Art initiative had a stand at the fair to exhibit the artworks of artists Daniel Canogar, Eva Lootz, Chema Madoz and Carmen Calvo, our participants in CNIO Arte throughout the years. The Fair was held between the 7th and the 11th of July. Our stand was a great success and was very well received by the public. There were many visitors, ordinary citizens as well as representatives from well-known corporations, foundations and institutions. These encounters had a positive impact on disseminating knowledge about the CNIO, opening the door to prospective donations and collaborations.

CNIO Arte was presented in ARCO on July 9th, in the ARCO Presentations Room. A panel was held with Maria A. Blasco, CNIO Director; Hugh Elliott, Ambassador of the United Kingdom to Spain; Borja Baselga, President of the *Banco Santander* Foundation; Daniel Canogar, featured artist from CNIO Arte 2021; and with CNIO Arte Commissioner Amparo Garrido as the moderator. The media showed great interest in the event, as was the case in the previous years. The following media outlets were present: *EL PAÍS*, *ABC Cultural*, *ABC*, *La Razón*, *20 Minutos*, *Diario Vasco*, *EL Correo*, *La Verdad*, *El Diario Montañés*, *Ideal*, *Hoy*, *Sur*, *Norte de Castilla*, *El Comercio*, *Las Provincias*, *La Voz de Cádiz*, *Diario Siglo XXI*, *RTVE*, *RNE 1*, *EFE*, *COLPISA*, *Crónica de Cantabria*, *Infosalus.com*, Biotech-Spain, among others.

Owing to the great popularity achieved with CNIO Arte, Eva Lootz was chosen to participate and show her work, along with several other Spanish artists, in the exhibition “Synopsis: Science and Art in Spain, from Ramón y Cajal to the 21st century” in Sweden’s Nobel Prize Museum, held between 24 November - 05 December 2021.

Another initiative that our Office worked on in 2021 is a joint project between the CNIO, Columbia University in New York, theoretical physicist and computational biologist Raúl Rabadán — who collaborates with CNIO — and Daniel Canogar, the CNIO Arte 2021 featured artist. The project foresees the creation of new artwork by Daniel Canogar inspired by Raúl Rabadán’s work, which will then be exhibited first at Columbia University. This would help boost CNIO’s exposure, as well as speak for the importance of scientific research. Initiatives such as this one advance the two fundamental missions of our Office: to consolidate the “CNIO stop cancer” brand and to promote outreach activities, both cultural and scientific.

In our efforts to bring art and science closer together, we have also signed a cooperation agreement with the *Amigos del Reina Sofía* Foundation. Its aim is to promote joint activities and to amplify the dissemination of the Centre’s information. There



Daniel Canogar, visual artist; Eva Lootz, visual artist; Maria A. Blasco, CNIO Director; and Amparo Garrido, CNIO Arte curator.



Upper picture: Guided tour of the CNIO Arte stand at ARCO 2021; collectors looking at our stand.

Lower picture: Presentation of CNIO Arte at ARCO 2021. Roundtable moderated by project curator Amparo Garrido, with participants Hugh Elliot, British Ambassador to Spain; Daniel Canogar, visual artist; María A. Blasco, CNIO Director; and Borja Baselga, Director of *Fundación Banco Santander*.

are also benefits for CNIO staff, namely a permanent 50 % discount on the *Amigos del Reina Sofía* membership fee.

In order to reinforce the CNIO Arte initiative, this Office held the II Art and Science Symposium in 2021 as well. The purpose of the Symposium is to enrich and deepen the connections between art and science, whereby leading experts invite us to reflect on this matter. In accordance with the dialogue between Daniel Canogar and Sarah Teichmann in CNIO Arte, the theme for this second edition of the Symposium is “Cartography in the Digital Age”. We were pleased to welcome Carlos Jiménez, Professor Emeritus of Aesthetics, historian, architect, author, art critic and Director of the Symposium; Alfonso Valencia, ICREA Research Professor Director of the Life Sciences Department at Barcelona SuperComputing Center; Elena Castro, philosopher and researcher; and Nerea Calvillo, architect, lecturer and researcher, along with artist Daniel Canogar. Together, they reflected on society’s current huge appetite for data and information, the challenges posed by the availability of so much information and the importance of representing this visually. This event, as well as the CNIO Arte Presentation, are available on our YouTube channel. The Office was also in charge of recording via Zoom and post-producing the campaign videos for the “CNIO stop cancer DONA 2021” series, featuring mountaineer Edurne Pasaban, actor Carlos Hipólito and motorcycle champion Laia Sanz, in collaboration with the Development & Philanthropy Office. Our Office also directed and supervised all the photographs used in CNIO’s Annual Report, coordinated its graphic design, as well as the printing of the Report, together with the Scientific Publications Office.

In 2021 we also continued to update the content on 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 various events, supporting them and collaborating with other entities with the aim to boost our visibility, such as the European Researchers’ Night and World Cancer Research Day, among others.

Finally, towards the end of 2021, our Office Coordinator, Amparo Garrido, travelled with Susana Solano — the CNIO Arte 2022 featured artist — to Mozambique, to visit the hospital where scientist Pedro Alonso works. This trip was required by the artist to find inspiration, connect with the subject, and create the work that will be exhibited at CNIO Arte 2022. Amparo documented this trip visually, with photographs, video and interviews. These materials will be used to promote and support CNIO Arte 2022.

Our Office is responsible for the design and printing of this Annual Report and other institutional publications, the creation and adaptation of outreach materials, brochures and merchandising that reflects the new brand identity, as well as launching new outreach initiatives in the fields of art, culture and science. 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



The Office of Philanthropy and Development continues to grow and design innovative new opportunities to create partnerships with both Spanish and international organisations. In addition, our ‘CNIO Friends’ crowdfunding programme offers individuals the opportunity to directly support cancer research in one of the best cancer centres worldwide.

We have continued to take a strategic and proactive approach to securing funds for cancer research this year, cultivating new prospects, and working with companies, foundations and associations to develop new collaborative partnerships. Since the development of the office in September 2019, we are building a new “major gifts” programme for the CNIO (€100k+) and we have been energised by the enthusiastic response from organisations to develop partnerships with us. As every year, in 2021 we worked in conjunction with our colleagues in the Institutional Image & Outreach to Society and Communications teams to develop an annual campaign to grow the brand of the CNIO and encourage the public at large to support the critical work of cancer research.

The CNIO Friends Programme has raised more than €2.8 million in total donations since 2014. 100% of these donations are used to recruit excellent scientists from around the world to conduct a 2-year postdoctoral research project. These contributions have thus far enabled the CNIO to hire 26 new researchers since 2016 with a biannual contract. In 2021, CNIO Friends raised €670k, which will be used to engage more scientific talent in 2022. The legacy programme continues to gain attention, raising a cumulative total of €1.33 million since 2015. In 2021, CNIO received charitable bequests of €80k, with €528k pending to be executed.

The Philanthropy Office works to identify and cultivate new donor relationships, and steward (i.e. recognise and thank) our existing supporters. This can be rewarding work, and although major gifts can take up to 2 years to close, the sense of achievement when a significant and impactful agreement is signed is worth the effort.

As we suspected, fundraising proved challenging in 2021. Fundraising totals always tend to reflect the situation a year behind reality, and so the effect the second year of the pandemic made a difference to our donations this year. However, we remain optimistic about the future. For organisations and individuals who feel passionate about working together to stop cancer, the CNIO provides a wonderful partnership opportunity. We look forward to developing new partnerships and alliances in 2022 to continue to enable the brilliant work of the CNIO. ■

“Now, more than ever, research is critical. Philanthropy gives every individual the opportunity to be part of the solution. Together, we can stop cancer.”

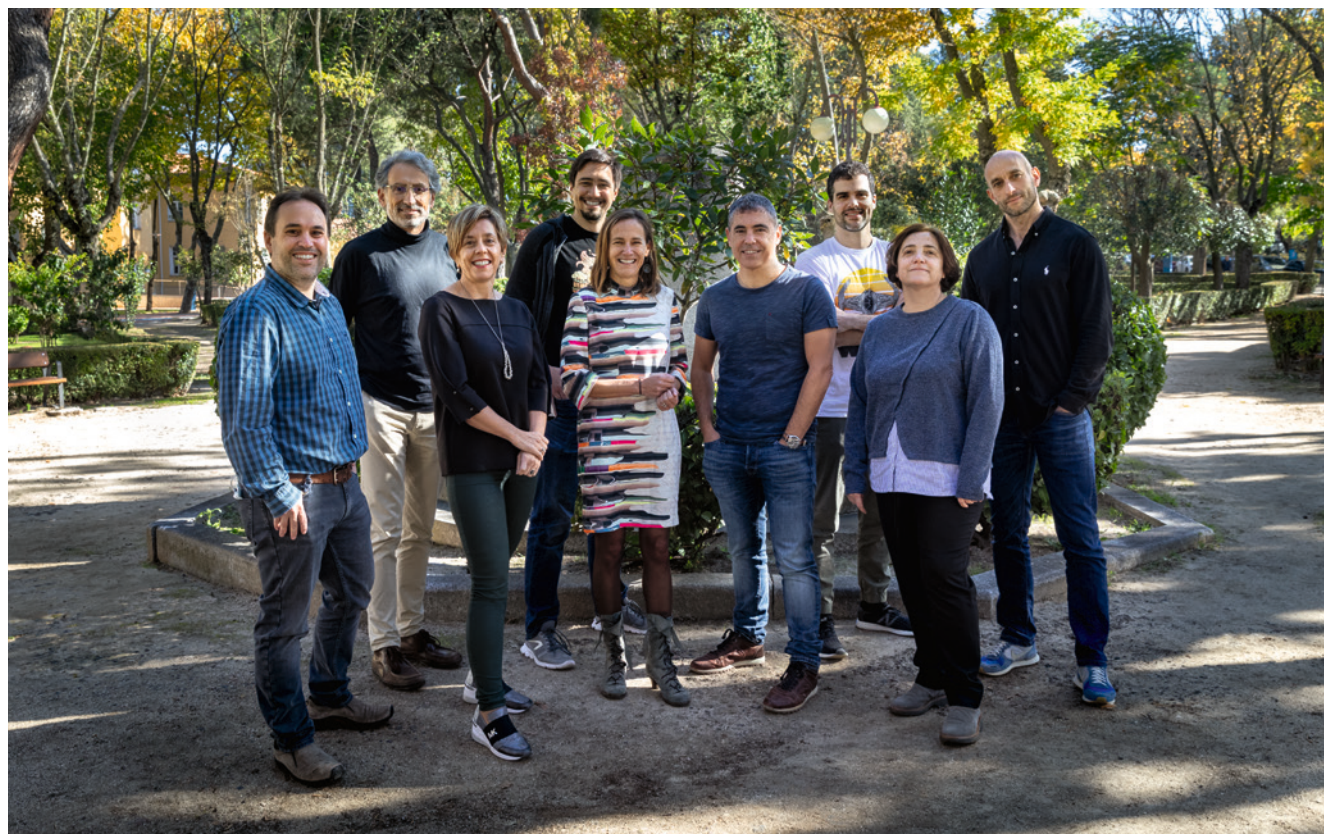
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DEAN'S OFFICE

María S. Soengas
Dean for Academic Affairs

PARTICIPANTS
Personnel in training:
Nicolás Cuesta
Fernando Pozo



One of the elements that contribute to the international projection of the CNIO is the commitment of our established investigators to foster new generations of scientists. In fact, over 60% of the work force at our institution comprises young researchers, including undergraduate students, predoctoral and postdoctoral fellows, medical residents and a broad spectrum of visiting scientists. As such, 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. We are also most grateful to the *Fundación Jesús Serra* for their continuous support to career development programmes at the CNIO.

At the Dean's Office, we pay particular attention to ensuring 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 careers.

We have also kept our commitment to outreach activities, one of the most exciting being the *Marie Skłodowska Curie European Researchers' Night*. In 2021 we participated for the 8th time in an event organised in various countries throughout Europe and in multiple centres in Spain. Our contribution at the CNIO was "*Meet a Scientist, Become a Scientist*". Due to safety precautions related to the Covid-19 pandemic, we organised

Staff/Faculty:
Nabil Djouder
Rafael Fernández-Leiro
Eva González-Suárez
Óscar Llorca
Ana Losada

Jorge L Martínez
Héctor Peinado

this event in a virtual format, but this format did not preclude a very active and engaging event. We mailed a “research kit” with informative material to over 350 registered guests so they could perform an experiment in their own homes. It was truly gratifying to see whole families following and talking live with our scientists.

Another of our key events in 2021 was our Annual CNIO Lab Day. We held an ePoster session with over 80 communications, which were evaluated by an *ad hoc* committee to select 15 “elevator pitch” presentations from each of the CNIO scientific programmes. These talks highlighted the broad spectrum of topics that bring our Centre to the forefront of research in the cancer field. We also hosted external speakers, this year focusing on different aspects of communication. We thus learned from **Ignacio Crespo** (editor at *La Razón*) about different styles of conveying scientific findings through social media; **Javier Carmona** (editor at *Nature Medicine*) gave us tips about how to effectively submit manuscripts for publication in highly competitive journals; and **David Goodsell** (Investigator at the Center for Computational Structural Biology, Scripps Research Institute USA) spoke about data presentation through graphical modelling.

We also enjoyed the “*Director’s List Awards*”, an event that recognises outstanding contributions 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 2021 the “*Antonia Nieto Award*” went to **Celia de la Calle** for impressive work published in *Nature Communications* on new discoveries regarding the impact of fasting and cancer. Additional awards in the PhD category went to **Javier Ramos-Paradas** (*J Immunother Cancer*), **Ana Teijeiro** (*Nat Metab*), **Lucía Zhu** (*EMBO Mol Med*), **Antonio Galarreta** (*EMBO J*) and **Daniel González-Acosta** (*EMBO J*) for their contributions to the understanding of different aspects of tumour initiation, progression and response to therapy.

“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.”

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

The awardee was **Vanessa Lafarga** for exciting new findings in underlying mechanisms linked to the development of amyotrophic lateral sclerosis (*EMBO J*).

3. Outstanding Contribution to Outreach and Awareness

The recipient was **José Luis Fernández** on behalf of the whole team in charge of maintaining Computer Services at the CNIO, for the extra dedication to ensure efficient online connections, and for volunteering in the various activities related to dissemination of science to society carried out at our Centre, such as European Researchers’ Night and Lab Day. The award was presented by the *Fundación Bandera Rosa*, which actively supports women with breast cancer and collaborates with our Centre via the CNIO Friends platform.

Lab Day proceeded with additional Awards from the Dean’s Office: 4 awards for Best Oral Presentations and 3 for Best Posters. In addition, we had the Awards for Scientific Videos (**María Escobar Rey**) and for T-Shirt Design (**María J. Jiménez Santos**), both portraying the mission of the CNIO to get research closer to the bedside.

In summary, at the CNIO we are as proud as ever of the achievements of our young investigators. We thank all those public and private contributors who help fuel their efforts, and we will strive in our commitment to being useful to other investigators and to the society at large. ■

CNIO WOMEN IN SCIENCE OFFICE

Isabel López De Silanes
Coordinator

Seminars and Events Coordinators:
Maria Blasco
M^a Jesús Alcamí
Isabel López de Silanes



Work-Life Balance Coordinator:
Raquel García-Medina

MEMBERS
Maria A. Blasco
Maria Jesús Alcamí
Celia de la Calle
Lola Martínez

Diego Megías
Francisca Mulero
Eva Ortega
Fernando Peláez
Sandra Rodríguez-Perales

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 WISE Office is composed of CNIO volunteers from across all the areas present in the Centre, including the Director.

Despite the SARS-Cov2 pandemic still ongoing in 2021, the WISE Office continued to work actively to make the CNIO a better place to work and to reconcile work and private life. We continued our activity as part of the Monitoring Committee of the Equality Plan (CSPI) and, as a result, the “remote working” pilot programme was finally approved as well as the catalogue of permits, licences and other licence reconciliation measures. The “Workplace Harassment” Protocol was also prepared and submitted to the CNIO Direction for final approval. The WISE Office was also involved in preparing the Human Resources Excellence in Research Award (HRS4R) from the European Commission.

We also continued organising the WISE seminar series, in which we invite numerous top female and male leaders from different areas to give a talk. Because of the pandemic, all the talks were held online via the Zoom platform. The following talks were given during the year:

- Sara Giménez, Member of the Congress and of the European Commission against Racism and Intolerance. Title: “*Avanzar en igualdad de trato y oportunidades, aproximación al pueblo gitano*”. 18/01/2021.
- Gemma Robles, journalist and Deputy Director of *EL PERIÓDICO*. Title: “*Prensa, ciencia y la deuda con Matilda*”. 09/02/2021.
- Mary Beard, Professor of Classics at the University of Cambridge. Title: “*Mujer y poder*”. 08/03/2021.
- Soraya Saenz de Santamaría, former Vice President of Spain. Title: “*Talento femenino y gestión de crisis*”. 16/03/2021.
- Cani Fernández, President of the National Authority for Markets and Competition (CNMC). Title: “*La presencia de la mujer en la abogacía*”. 12/04/2021.
- Zulema Altamirano, Director of the Women and Science Unit of the Ministry of Science and Innovation. Title: “*Trabajando en igualdad: empoderar, crear, transformar*”. 18/05/2021.
- Marta Sanz, writer. Title: “*La escritura: un viaje por el cuerpo humano*”. 17/05/2021.

Here at the WISE Office, we share what the ethologist Jane Goodall said: “What you do makes a difference, and you have to decide what kind of difference you want to make.”

- Rigoberta Bandini, actress and singer. Title: “*Dialogo con Rigoberta Bandini*”. 08/07/2021.
- Maria Neira, Director of the Department of Environment, Climate Change and Health at the WHO. Title: “*Salud pública en femenino*”. 14/09/2021.
- Maria Pagés, choreographer. Title: “*Bailar necesita algo de talento y mucho trabajo*”. 03/11/2021.
- Blanca Li, Director of *Teatros del Canal*. Title: “*Estar en la acción*”. 10/11/2021.

On February 11, the International Day of Women and Girls in Science, we released a video featuring 6 of our scientists who listed the reasons why women should become scientists using the hashtag #HazteCientífica. This initiative was selected among the finalists for the STEM Talent Award for “Best Company” in 2021. We were also involved in other educational initiatives to promote scientific careers among students (Universities, SRUK Cambridge, etc.).

For the occasion of International Working Women’s Day, we organised a joint event with the British Embassy and IE University with the distinguished historian Mary Beard as the featured speaker. In addition, on that day, we broadcasted the documentary “Picture a Scientist” to all CNIO staff. ■

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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-"la Caixa" Foundation 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 progress report seminars and the CNIO guided visits.

“All our efforts are dedicated 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 scientific publications, including the CNIO Annual Report, booklets of the Scientific Advisory Board (SAB) meeting and those of other annual 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 non-specialised audiences. ■

COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies and is used to finance not only the outstanding R&D activities of the Centre, but also strategic actions in innovation together with industry partners, as well as all other relevant activities related to dissemination and scientific outreach, which aim to promote public awareness. In 2021, researchers at the CNIO were involved in 159 projects that received extramural funding.

CNIO is actively participating in 65 collaborative projects in total: 16 are international collaborative projects (4 of which are coordinated by the CNIO), and 49 are collaborative projects conducted at the national level (14 of them coordinated by the CNIO). The international collaborative projects are funded by institutions such as the European Commission through the Horizon 2020 Framework Programme, the US National Institutes of Health (NIH), the US Department of Defense (DoD), the International Human Frontier Science Program Organization, the Paradifference Foundation, Worldwide Cancer Research, and the Lustgarten Foundation-Stand-up to Cancer Initiative. At the national level, collaborative projects have received important public grants of the Strategic Research Action, managed by the Institute of Health Carlos III (*ISCIII*)

and the State Research Agency, Spanish Ministry of Science and Innovation (*AEI/MCIN*), and the R&D Activities Programmes of the Community of Madrid, most of them co-funded by European Structural and Investment Funds (European Regional Development Fund and European Social Fund). Private funders and charities have also recognised the excellence of our scientific projects, such as the Scientific Foundation of the Spanish Association Against Cancer (*Fundación Científica AECC*), the *Ramón Areces* Foundation, *La Marató de TV3* 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 2021, 17 of these projects received international funds, while 77 of them received national funding (mainly from the *AEI/MCIN*, the *ISCIII* and private foundations). The international individual projects are funded by the European Commission (8 European Research Council ERC grants and 5 Marie Skłodowska-Curie Actions), Worldwide Cancer Research, the Cancer Research Institute, the US DoD, the Prostate Cancer Foundation, the American Thyroid Association, and the Mark Foundation for Cancer Research.

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

EUROPEAN COMMISSION



EU FRAMEWORK PROGRAMME: HORIZON 2020 (2014-2020)

SOCIETAL CHALLENGE 1: HEALTH, DEMOGRAPHIC CHANGE AND WELLBEING

PRINCIPAL INVESTIGATOR

PROJECT TITLE

Benítez, Javier

BRIDGES: Breast cancer risk after diagnostic gene sequencing (Ref.: 634935)

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)

ERANET ERA PERMED [SPANISH GROUPS ARE COFUNDED BY INSTITUTO DE SALUD CARLOS III (ISCIII) AND AECC SCIENTIFIC FOUNDATION (FC AECC)]

PRINCIPAL INVESTIGATOR

PROJECT TITLE

Barbacid, Mariano

ERA PerMed: Personalized multimodal therapies for the treatment of lung cancer (ISCIII Ref.: AC20/00114; FC AECC Ref.: PERME20707BARB)

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)

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)

**INTERNATIONAL HUMAN
FRONTIER SCIENCE
PROGRAM ORGANIZATION
(HFSP)**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Llorca, Óscar (Coordinator)	Photochemical trap and high-resolution imaging of transient chromatin complexes from living cells (Ref.: RGP0031/2017)

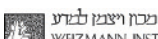
**LUSTGARTEN FOUNDATION
& STAND-UP2CANCER**


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**


FUNDACIÓN
RAMÓN ARECES

*** stop cancer



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.	MIT-BC Study: Tumor 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)
Efeyan, Alejo	ERC Starting Grant NutrientSensingVivo: The Physiology of Nutrient Sensing by mTOR (Ref.: 638891)
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: Tumor compartment-specific effects of P70S6K in early triple negative breast cancer: regulation of antitumor immune response and therapeutic implications (Ref.: 893597)
Soengas, María S. Frago, Susana	METMEL: Long range-acting drivers of premetastatic niches in melanoma (Ref.: 753442)
Real, Francisco X. Zagorac, Sladana	PDASwITch: Super-enhancer modules controlling plasticity and response to therapy in pancreatic cancer (Ref.: 895943)

MELANOMA RESEARCH ALLIANCE (MRA)



PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	Blocking melanoma brain metastasis by targeting the environment (REF.: 498103)

PROSTATE CANCER FOUNDATION

PRINCIPAL INVESTIGATOR

Castro, Elena;
Olmos, David

PROJECT TITLE

Prospective study of lethal prostate cancer clinical and genomic evolution in DNA repair deficient tumours

WORLDWIDE CANCER RESEARCH (WCR; FORMERLY AICR)

PRINCIPAL INVESTIGATOR

Malumbres, Marcos

PROJECT TITLE

Exploring the use of CDK4/6 inhibitors in combination with classical chemotherapy (Ref.: 20-0155)

CANCER RESEARCH INSTITUTE

PRINCIPAL INVESTIGATOR

Valiente, Manuel

PROJECT TITLE

Brain-specific strategies to improve responses to immunotherapy (Ref.: 54545)

AMERICAN THYROID ASSOCIATION (ATA), COFUNDED BY BITE ME CANCER (BMC)

PRINCIPAL INVESTIGATOR

Montero, Cristina

PROJECT TITLE

Discovering novel molecular nodes involved in MTC development and evolution (Ref.: GRANT2020-0000000150)

**MARK FOUNDATION FOR CANCER RESEARCH**

PRINCIPAL INVESTIGATOR

Valiente, Manuel

PROJECT TITLE

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)**



**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 SALUD¹**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	<i>Plataforma de Bioinformática. Instituto Nacional de Bioinformática</i> (Group Ref.: PT17/0009/0011)
Artiga, M ^a Jesús; Ortega, Eva	<i>Plataforma de Biobancos</i> (Group Ref.: PT17/0015/0004)
González-Neira, Anna	<i>Plataforma de proteómica, genotipado y líneas celulares. Plataforma de recursos biomoleculares, PRB³</i> (Group Ref.: PT17/0019/0020)
Muñoz, Javier	<i>Plataforma de proteómica, genotipado y líneas celulares. Plataforma de recursos biomoleculares, PRB³</i> (Group Ref.: PT17/0019/0005)
Ortega, Eva	<i>Plataforma de Biobancos y Biomodelos</i> (Group, Ref.: PT20/0070)

IMPACT PROJECTS: PRECISION MEDICINE INFRASTRUCTURE²

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

RESEARCH PROJECTS IN HEALTH / PROYECTOS DE INVESTIGACIÓN EN SALUD³

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Artiga, M ^a Jesús (Coordinator)	OPTIMARK project: Optimization of tissue samples for the development and validation of disease biomarkers (Ref.: PI16/00946)
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)
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)
Olmeda, David (Coordinator)	Visualization and molecular characterization of new biomarkers of resistance to immunotherapy (Ref.: PI18/01057)
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 tumors. Predictive markers of sensitivity to treatment (Ref.: PI20/01169)

1, 2, 3 This Programme is cofunded by the European Regional Development Fund (ERDF) and European Social Fund (ESF)



**STATE RESEARCH AGENCY.
MINISTRY OF SCIENCE AND
INNOVATION / AGENCIA
ESTATAL DE INVESTIGACIÓN.
MINISTERIO DE CIENCIA E
INNOVACIÓN**



NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION

STRATEGIC LINES PROJECTS (PUBLIC-PRIVATE COLLABORATIVE PROJECTS)⁴

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos (Coordinador)	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)

EXCELLENCE NETWORKS / REDES DE EXCELENCIA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos (Coordinador)	Research Network iDIFFER: Balancing proliferation and differentiation: mechanisms and relevance in human disease (Ref.: RED2018-102723-T)

CHALLENGES-COLLABORATION PROJECTS / PROYECTOS RETOS-COLABORACIÓN⁵

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	New approaches for treatment of lung cancer. (Ref.: RTC-2017-6576-1)
Martínez-Torrecuadrada, Jorge L.; Muñoz, Inés	ATTACK: Cancer immunotherapy with bispecific antibodies that engage T-lymphocytes (Ref.: RTC-2017-5944-1)
Real, Francisco X.	IMMOPDL2: Preclinical development of antibodies against the immunomodulator PD-L2 for the treatment of diseases caused by cellular damage. Validation of the strategy in residual tumors and fibrosis (Ref.: RTC-2017-6123-1)

4 Funded by MCIN/AEI/10.13039/501100011033 and the European Union "NextGenerationEU"/PRTR"



5 This Programme is cofunded by the European Regional Development Fund (ERDF)



**COMMUNITY OF MADRID /
COMUNIDAD AUTÓNOMA DE
MADRID**

Comunidad de Madrid
R&D ACTIVITIES PROGRAMME IN BIOMEDICINE⁶:

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 TECHNOLOGIES⁷:

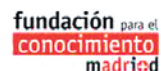
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Llorca, Óscar	<i>Programa Tec4BioCM: Tecnologías Aplicadas al Estudio de Nanomáquinas Biológicas</i> (Ref.: P2018/NMT4443)

SYNERGY PROJECTS:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Llorca, Óscar	<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-Clinico 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)

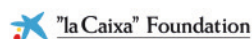


**MADRI+D FOUNDATION
/ FUNDACIÓN PARA EL
CONOCIMIENTO MADRI+D**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Dean's Office for Academic Affairs Soengas, María S.	European Researchers' Night 2021, organized by Madri+d Foundation and funded by EU-H2020 Programme. Marie Skłodowska-Curie actions GA 101036010

**SCIENTIFIC FOUNDATION OF
THE SPANISH ASSOCIATION
AGAINST CANCER /
FUNDACIÓN CIENTÍFICA DE
LA ASOCIACIÓN ESPAÑOLA
CONTRA EL CÁNCER (AECC)**


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)
Real, Francisco X. (Coordinator); Malats, Núria	Invasive bladder cancer: towards precision medicine (Ref.: GCB14142293REAL)
Valiente, Manuel	Study of the molecular mechanisms involved in primary (glioblastoma) and secondary (metastasis) brain tumors to identify novel therapeutic targets and anti-cancer agents, biomarkers to select treatments and novel non-invasive methods for molecular diagnosis (Ref.: GCTRA16015SEOA)

**"LA CAIXA" BANKING
FOUNDATION / FUNDACIÓN
BANCARIA "LA CAIXA"**

HEALTH RESEARCH PROJECTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo (Coordinator)	NUTRITHELIUM: Decoding the paracrine control of metabolic fitness by endothelial nutrient signaling (Ref.: HR21-00046)
Llorca, Óscar	ASC4Neuro: Amino acid transporter structure to target glutamate transmission in neuro diseases (Ref.: HR20-00081)
Llorca, Óscar	lncRNAs-RS-CRC: Understanding lncRNAs in replicative stress and colorectal cancer: from cancer biology to single-molecules (Ref.: HR21-00176)
Peinado, Héctor	OncoExoPeptides: Defining The Role of Exosome-Secreted Micropeptides in Pancreatic Cancer (Ref.: HR18-00256)
Real, Francisco X.	RBM10across: RBM10, a novel splicing regulator and tumor suppressor: from mechanisms to therapies (Ref.: HR21-01208)
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)

**ASOCIACIÓN ESPAÑOLA DE
INVESTIGACIÓN SOBRE EL
CÁNCER (ASEICA)**

PRINCIPAL INVESTIGATOR

Peinado, Hector

PROJECT TITLE
Análisis de la secreción de neoantígenos en exosomas tumorales de pacientes con cáncer microcítico de pulmón metastásico obtenidos de sangre periférica
**BBVA FOUNDATION /
FUNDACIÓN BBVA**

COVID-19 SCIENTIFIC RESEARCH TEAMS
PRINCIPAL INVESTIGATOR

Muñoz, Inés

PROJECT TITLE

Synthetic immunology to engineer pan-coronavirus immunity (Ref.: Biomed-COVID-19_0155)

**LA MARATÓ DE TV3
FOUNDATION / FUNDACIÓ
LA MARATÓ DE TV3**

PRINCIPAL INVESTIGATOR

González-Suárez, Eva

PROJECT TITLE

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)

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)
Guerra-González, Carmen	The stroma as a therapeutic target of pancreatic cancer (Ref.: PI19/00514)
Olmos, David	Identification of lethal subtypes of prostate cancer by integration of transcriptomic, genomic and clinical data (Ref.: PI19/01380)
Ortega, Ana	Targeting dysregulated nutrient-sensing pathway in follicular lymphoma (Ref.: PI18/00816)
Quintela, Miguel Ángel	Longitudinal, single-cell analysis of immunomodulator/antiangiogenic therapies in advanced breast cancer: a refined tool for precision medicine (Ref.: PI19/00454)
Robledo, Mercedes	Progression related mechanisms in endocrine and neuroendocrine tumours (Ref.: PI17/01796)
Rodríguez, Sandra	Study of the role of epigenetic modifications in the development of Ewing sarcoma: High-throughput screening of epigenetic genes using CRISPR libraries in human (11; 22) + t cells (Ref.: PI17/02303)
Rodríguez, Sandra	Use of CRISPR/Cas13 system for a programmable diagnosis and inhibition of fusion oncogenes (Ref.: PI20/01837)

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)

SARS-COV-2 AND COVID-19 RELATED ILLNESS RESEARCH PROJECTS¹⁰

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Sagrario	<i>Modelos pre-clínicos en ratón para el estudio de Covid19 y ensayo de estrategias terapéuticas</i> (Ref.: COV20/00532)

8, 9, 10 This Programme is cofunded by the European Regional Development Fund (ERDF)



**STATE RESEARCH AGENCY.
MINISTRY OF SCIENCE AND
INNOVATION /AGENCIA
ESTATAL DE INVESTIGACIÓN.
MINISTERIO DE CIENCIA E
INNOVACIÓN**



NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION

CENTRES OF EXCELLENCE “SEVERO OCHOA” AND UNITS “RAMIRO DE MAEZTU” SUB-PROGRAMME/ SUBPROGRAMA DE APOYO A CENTROS DE EXCELENCIA “SEVERO OCHOA” Y UNIDADES “RAMIRO DE MAEZTU”

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Center of Excellence “Severo Ochoa” (Ref.: CEX2019-000891-S)

R&D EXCELLENCE PROJECTS / PROYECTOS DE I+D EXCELENCIA¹¹

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Leiro, Rafael	Macromolecular complexes in the mitochondrial DNA replication and repair pathways: structural and molecular mechanisms by cryo-EM (Ref.: BFU2017-87316-P)
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-INVESTIGACIÓN¹²

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	CANTHERHET: Computational targeting of cancer heterogeneity: in silico drug prescription for tumor clonal populations (Ref.: RTI2018-097596-B-I00)
Barbacid, Mariano	RAFTING: c-RAF, a key mediator of K-RAS driven cancers: Therapeutic approaches (Ref.: RTI2018-094664-B-I00)
Blasco, Maria	TELOHEALTH: Telomeres and Disease (Ref.: SAF2017-82623-R)
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)
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)
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)

CHALLENGES-RESEARCH PROJECTS / PROYECTOS RETOS-INVESTIGACIÓN¹²

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	NewCDKtarget: Validation of a New Subfamily of Cyclin-dependent Kinases as Cancer Targets (Ref.: RTI2018-095582-B-I00)
Méndez, Juan	REP&TOL: Mechanisms of DNA replication and damage tolerance (Ref.: PID2019-106707RB-I00)
Muñoz, Javier	EPI-MASS: Epigenetic modifiers in pluripotency: a proteomic analysis of non-histone protein methylation (Ref.: SAF2016-74962-R)
Park, Solip	CancerFitness: Systematic analysis of the cancer fitness landscape for cancer genes across cancer types (Ref.: PID2019-109571RA-I00)
Peinado, Héctor	EXO-NGFR: Analyzing the relevance exosome-derived NGFR during pre-metastatic niche formation (Ref.: SAF2017-82924-R)
Peinado, Héctor	OUTANERVE: Role of NGFR regulating the immunoevasive phenotype of melanoma metastasis initiating cells (Ref.: PID2020-118558RB-I00)
Piedrafita, Gabriel	uroCellDynasts: Cell competition dynamics and clonal evolution during urothelial regeneration and carcinogenesis (Ref.: PID2020-116163GA-I00)
Plaza, Iván	ESFORET: Structure-function studies of oncogenic RET kinase fusions in human cancers: from mechanism of action to targeted therapy (Ref.: BFU2017-86710-R)
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)
Rodríguez, Cristina	RCC-MARKER: Improving the clinical management of advanced renal cell carcinoma through genomic technologies (Ref.: RTI2018-095039-B-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)
Squattrito, 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)

EXPLORA PROJECTS / PROYECTOS EXPLORA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	Improving naive pluripotency and the generation of insulin-producing cells with a single microRNA (Ref.: SAF2017-92729-EXP)
Rodríguez, Sandra	Delivery of functional CRISPR component by pseudotyped virus-like particles (Ref.: BIO2017-91272-EXP)

RESEARCH EUROPE / EUROPA INVESTIGACIÓN

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	LiquidEVs: Use of liquid biopsy of extracellular vesicles and cfDNA in plasma and lymphatic fluid as a novel diagnostic and prognostic test in melanoma patients (Ref.: EIN2019-103379)

12 This Programme is cofunded by the European Regional Development Fund (ERDF)



SCIENTIFIC INFRASTRUCTURES / INFRAESTRUCTURAS CIENTÍFICO-TECNOLÓGICAS¹³

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Megías, Diego	Implementation of automated microscopy platform for high-performance screening (High Content Screening) (Ref.: EQC2021-007743-P)
Mulero, Francisca	High Resolution Magnetic resonance 3T based in superconducting magnet without helium as cryogenizer (Ref.: EQC2021-006797-P)

PROOF OF CONCEPT¹⁴

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Hector	THankX: Development of THX-B as a novel anti-metastatic agent (Ref.: PDC2021-121102-I00)
Soengas, María S.	MDK-INHIBITORS: rewiring tumor-immune system crosstalk by targeting MIDKINE (Ref.: PDC2021-121831-I00)

SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)

**GRANTS FOR RESEARCH PROJECTS IN CHILDHOOD CANCER**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	Targeting telomeres in neuroblastoma (Ref.: CICPF18004BLAS)

GRANTS FOR EMERGING GROUPS (AECC LAB):

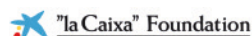
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	Nutrient signaling in the pathogenesis and treatment of B cell Lymphoma (Ref.: LABAE16001EFEY)
Peinado, Héctor	Defining the mutational landscape in plasma and lymphatic fluid-derived exosomes in melanoma patients (Ref.: LABAE19027PEIN)
Rodríguez, Sandra	Programmable detection and inhibition of fusion oncogenes using CRISPR/Cas13 (Ref.: LABAE20049RODR)
Squatrino, Massimo	Novel therapeutic approaches for therapy-resistant malignant brain tumors (Ref.: LABAE16015SQUA)
Valiente, Manuel	New treatments for brain metastasis based on the study of their biology (Ref.: LABAE19002VALI)

GRANTS FOR RESEARCH PROJECTS IN CANCER:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
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)
Olmos, David	Genomic epidemiology and clinical implications of DNA-repair genes and other oncogenic drivers in metastatic hormone-sensitive prostate cancer (Ref.: PROYE19054OLMO)

"IDEAS SEMILLA" GRANTS (SEED FUNDING):

PRINCIPAL INVESTIGATOR	PROJECT TITLE
González-Suárez, Eva	RANK, instead of RANKL, as a new therapeutic target in Triple Negative Breast Cancer (Ref.: IDEAS19093GONZ)
Rodríguez, Cristina	Bypassing Nonsense Mediated mRNA Decay to enhance immunotherapy response in cancer (Ref.: IDEAS20138RODR)

"LA CAIXA" BANKING FOUNDATION / FUNDACIÓN BANCARIA "LA CAIXA"**HEALTH RESEARCH PROGRAMME**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Targeting Telomeres in Cancer (Ref.: HR18-00023)
Soengas, María S.	Immunomodulatory drivers in melanoma progression and therapy response (Ref.: HR20-00465)

CAIXAIMPULSE PROGRAMME

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	TRF1 inhibitors as a first-in-class therapy for glioblastoma and lung cancer (Ref.: CI18-00016)
Rodríguez, Sandra	Gene therapy for human cancers driven by fusion genes (Ref.: CI18-00017)
Malumbres, Marcos	miRNA-based strategy to expand cell therapy potential for treating diabetes (Ref.: CI19-00001)

CRIS FOUNDATION AGAINST CANCER / FUNDACIÓN CRIS CONTRA EL CÁNCER**EXCELLENCE GRANTS**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Casanova, María	Network of myeloid vulnerabilities at metastatic site (Ref.: PR_TPD_2020-09)
Olmos, David	Addressing the biological and clinical role of RB1 loss and DNA repair defects in lethal prostate cancer (Re.: EXCELLENCE 19-26)

**SPANISH ASSOCIATION OF
PANCREATOLOGY /
ASOCIACIÓN ESPAÑOLA DE
PANCREATOLOGÍA**

CARMEN DELGADO/MIGUEL PÉREZ-MATEO GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Guerra, Carmen	<i>Nueva estrategia terapéutica: estroma e inmunoterapia</i>
Malats, Núria	<i>Marcadores microbianos para el diagnóstico del adenocarcinoma ductal de páncreas</i>

**FERO FOUNDATION /
FUNDACIÓN FERO**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	Exploiting signaling dependencies and metabolic vulnerabilities of B cell lymphoma

**BBVA FOUNDATION /
FUNDACIÓN BBVA**

LEONARDO GRANTS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo	<i>Nuevos modelos animales de translocaciones a la carta para el desarrollo y estudio linfomas de células B (Ref.: PR[19]_BIO_MET_0029)</i>
Peinado, Hector	<i>Análisis de la mutación BRAF en exosomas circulantes de pacientes de melanoma (Ref.: IN[21]_BBM_TRA_0050)</i>

SCIENTIFIC RESEARCH TEAMS

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Mulero, Francisca	TherAbnostic: Radioimmunotheragnostics for metastatic lung cancer with pretargeted clickable Ab fragments (Ref.: PR[19]_BIO_IMG_0096)

**RAMÓN ARECES
FOUNDATION / FUNDACIÓN
RAMÓN ARECES**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Eva	<i>Red Nacional de Metástasis Cerebral: Implantación, Desarrollo y Coordinación (Ref.: CIVP20S10662)</i>
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

**GRUPO ESPAÑOL
MULTIDISCIPLINAR DE
MELANOMA**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Ana	Estudio de la implicación de ruta de señalización de mTORC1 en la patología del Linfoma Folicular y autoinmunidad

**SPANISH ASSOCIATION OF
GASTROENTEROLOGY /
ASOCIACIÓN ESPAÑOLA DE
GASTROENTEROLOGÍA**

**TAMARITE — AESPANC — ACANPAN
GRANT:**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	<i>Radiomics en cáncer de páncreas para una medicina estratificada y de precisión: un estudio piloto.</i>

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. During 2021, the CNIO signed several new agreements with Spanish Universities and other institutions, namely with the *Universidad Complutense de Madrid, Universidad Autónoma de Madrid, Universitat Rovira i Virgili, Universidad de Castilla-*

La Mancha, Universidad de Comillas, Universidad de Lleida, IES Opea, IES Moratalaz, IES Jaime Ferrán Clúa, IES Villaverde, IES Benjamín Rua, IES Jose Luis Sampedro, IES Juan de Mairena, Centro Educativo Aloya, Centro Valle del Miro, Instituto Técnico de Enseñanzas Profesionales, Colegio Alemán, and Colegio Hastings.

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2017	2018	2019	2020	2021
Training of PhD students	112	109	100	109	135
Post-doctoral training	44	50	49	52	62
Training for MDs	21	12	20	7	10
Laboratory training for MSc/BSc students	99	128	150	85	106
Laboratory training for technicians	20	13	15	5	9

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 2021, 1 student from Spain participated in this programme.
- Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO individual Research Groups or Units. This year, 106 students participated in these programmes, of whom 2 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, 14 students obtained their PhD degrees in 2021 and 39 others joined the CNIO in the same year. Over 15% of the 137 students working at the CNIO in 2021 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 2021, 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 2021 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	119
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science and Innovation / <i>Ministerio de Ciencia e Innovación</i> (Predoctoral Fellowships)	55
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)	12
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (I+D Projects)	6
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (Fellowships)	4
Institute of Health Carlos III (<i>ISCIII</i>) / <i>Instituto de Salud Carlos III (ISCIII)</i>	1
PFIZER	1
<i>Grupo Español de Tumores Neuroendocrinos y Endocrinos (GETNE)</i>	1
<i>Fundación Ramón Areces</i> / CNIO	1
CRIS Foundation / <i>Fundación CRIS</i>	3
<i>Financiación empresa privada</i>	1
Community of Madrid / <i>Comunidad de Madrid</i>	11
Comillas University / <i>Univesidad de Comillas</i>	1
CNIO	5
"la Caixa" Banking Foundation / <i>Fundación Bancaria "la Caixa"</i> (I+D Projects)	3
"la Caixa" Banking Foundation / <i>Fundación Bancaria "la Caixa"</i> (Predoctoral Fellowships)	11
<i>Centro de Investigación Biomédica en Red CIBERISCIII</i>	1
INTERNATIONAL ORGANISATIONS	16
AXA	2
China Scholarship Council (CSC)	2
European Research Council	7
GENCODE	2
ESMO	1
Marie Skłodowska-Curie actions of the European Commission	2
MIT International Science and Technology Initiatives (MISTI)	1
TOTAL	135

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 2021, 61 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 sixth call of the "CNIO Friends" Postdoctoral Contract Programme, launched in 2021, resulted in the recruitment of 7 scientists for a 2-year period each.

FUNDING SOURCES OF POST-DOCTORAL CONTRACTS	NO.
SPANISH ORGANISATIONS	50
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science and Innovation / <i>Ministerio de Ciencia e Innovación</i> (Postdoctoral Fellowships)	4
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)	6
Spanish Association Against Cancer (<i>AECC</i>) / <i>Fundación Científica de la AECC</i> (Fellowships)	6
"la Caixa" Banking Foundation/ <i>Fundación Bancaria "la Caixa"</i> (Postdoctoral Junior Leader - INCOMING)	1
"la Caixa" Banking Foundation/ <i>Fundación Bancaria "la Caixa"</i> (I+D Projects)	4
<i>ISCIII</i>	1
Cris Cancer Foundation (<i>CRIS</i>) / <i>Fundación Cris Contra el Cáncer (CRIS)</i>	2
Community of Madrid / <i>Comunidad de Madrid</i>	4
CNIO	20
<i>Banco Santander</i> Foundation / <i>Fundación Banco Santander</i>	1
<i>BBVA</i> Foundation / <i>Fundación BBVA</i>	1
INTERNATIONAL ORGANISATIONS	12
AIRC	1
EMBO	1
ESMO	1
European Commission	1
European Research Council	3
Marie Skłodowska-Curie actions of the European Commission	4
Worldwide Cancer Research UK	1
TOTAL	62

POSTGRADUATE PROGRAMMES

In addition, the CNIO — in collaboration with academic institutions across Spain — provides access to a variety of postgraduate programmes that cover the areas of Cellular &

Molecular Biology, Molecular Biomedicine, Biotechnology, Biocomputing, Clinical & Applied Cancer Research, and Therapeutic Targets.

Official Postgraduate Programmes in Molecular Biosciences

The majority of the international postgraduate trainings offered at the CNIO are developed in collaboration with the Faculty of Medicine and Faculty of Sciences at the Autonomous University of Madrid (UAM). These trainings fall under 4 official Postgraduate Programmes, namely, the Doctorate in Molecular Biosciences, Master's in Biomolecules & Cell Dynamics, Master's in Molecular Biomedicine, and Master's in Biotechnology. CNIO also collaborates with the UAM as a partner institution of UAM's Doctoral School (EDUAM), and is a member of the Management Committee.



Master's Degree in Biocomputing Applied to Personalised Medicine and Health

The Master's in *Bioinformática Aplicada a la Medicina Personalizada y la Salud* is organised together with the National School of Health of the National Institute of Health Carlos III (*Escuela Nacional de Sanidad del Instituto de Salud Carlos III, ENS-ISCIII*).



Official Master's Degree in Clinical and Applied Cancer Research

The CNIO and the CEU-San Pablo University in Madrid (USP-CEU) co-organise a Postgraduate Training Programme in Clinical and Applied Cancer Research: the *Máster Universitario en Investigación Clínica y Aplicada en Oncología*.



Official Master's Degree in Therapeutic Targets of Cell Signalling: Research and Development

The CNIO collaborates with the Biochemistry and Molecular Biology Department at the University of Alcalá de Henares (UAH) for the *Máster Oficial en Dianas Terapéuticas en Señalización Celular: Investigación y Desarrollo*.



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 9 students who participated in this programme in 2021, 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 2021, 10 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 2021, the *Ramón y Cajal* Programme supported 7 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. Sixteen 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 (2 contracts) of the Institute of Health *Carlos III*; and the Spanish Association Against Cancer (*AECC*, 10 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 2021, Raúl Rabadán, from Columbia University in New York (USA), was beneficiary of the *Jesús Serra* Foundation's Visiting Researchers Programme.



"SCIENCE BY WOMEN" PROGRAMME

Thanks to the "Science by Women" Programme, launched by the Spanish "Fundación Mujeres por África", Ashwag Mohammed Mukhtar, from Al-Neelain University, Sudan, was awarded a grant to join the CNIO's Genetic and Molecular Epidemiology Group for a 6-month stay as visiting scientist.



SCIENTIFIC EVENTS

MEETINGS & CONFERENCES, 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

18-19 JANUARY 2021

12-13 APRIL 2021

1-2 MAY 2021

24-25 MAY 2021

5-6 JULY 2021

6-7 SEPTEMBER 2021

18-19 OCTOBER 2021

29-30 NOVEMBER 2021

SPEAKERS:

- **Lola Martínez**, Head of the Flow Cytometry Unit. CNIO
- **Sara García García**, Flow Cytometry Unit Technician. CNIO
- **Julia García-Lestón**, Flow Cytometry Unit Technician. CNIO
- **Andrea Valle**, European Applications Specialist DeNovo Software

MULTICOLOR FLOW CYTOMETRY COURSE

15-16 MARCH 2021

20-21 SEPTEMBER 2021

SPEAKERS:

- **Andrea Valle**, European Application Specialist. DeNovo Software
- **Jose Manuel Ligos**, Technical Application Specialist. Cyttek Biosciences
- **Lola Martinez**, Head of the Flow Cytometry Unit. CNIO
- **Julia García Lestón & Sara García**, Flow Cytometry Unit Staff. CNIO

ADVANCED CELL CYCLE DATA ANALYSIS WITH FCS EXPRESS 7

13 MAY 2021

ORGANISERS:

- **Lola Martinez**, Head of the Flow Cytometry Unit. CNIO
- **Andrea Valle**, European Applications Specialist DeNovo Software

III CNIO-BANCO SABADELL FOUNDATION WORKSHOP ON PHILOSOPHY, SCIENCE AND MEDICINE: THE USE OF ANIMALS IN SCIENCE. ETHICAL AND EPISTEMIC PROBLEMS AND ALTERNATIVES.

23 NOVEMBER 2021

WITH THE SUPPORT OF:**Sabadell Foundation****ORGANISERS:**

- **Maria A. Blasco (CNIO)**
- **Antonio Diéguez (UMA)**
- **Arantza Etxeberria (UPV/EHU)**

SPEAKERS:

- **Fabiola Leyton**, University of Barcelona
- **Manuel Valiente**, Spanish National Cancer Research Center
- **Lucía Arana**, *El caballo de Nietzsche, elDiario.es*
- **Marta Tafalla**, Autonomous University of Barcelona
- **Lluís Montoliu**, National Centre for Biotechnology (CNB-CSIC)
- **Guillermo Repetto**, Pablo de Olavide University
- **Raúl Rabadán**, Columbia University



In total, the CNIO hosted 13 online distinguished speakers in 2021.



DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
29/01/2021	Christopher Vakoc	Cold Spring Harbor Laboratory, New York, US	Aberrant transcription in pancreatic cancer
FEBRUARY			
05/02/2021	Elena Conti	Max Planck Institute of Biochemistry, Martinsried Germany	The RNA exosome complex: the Dr Jekyll and Mr Hyde of RNA degradation
26/02/2021	Raúl Rabadán	Columbia University, New York, US	A Topological Data Analysis perspective on coronavirus evolution
MARCH			
05/03/2021	Lluís Fajas	Center for Integrative Genomics, CIG, Lausanne, Switzerland	THE CDK4 REVOLUTION: The fight against hunger
12/03/2021	Titia Sixma	The Netherlands Cancer Institute, Amsterdam, The Netherlands	DUB gymnastics: allosteric regulation of DUBs in DNA regulation
MAY			
07/05/2021	Agata Smogorzewska	The Rockefeller University, New York, US	Tumorigenesis in the setting of DNA interstrand crosslink repair deficiency
21/05/2021	Sara Buhrlage	Dana-Farber Cancer Institute, Boston, US	Integrated platform for DUB inhibitor discovery
JUNE			
04/06/2021	Pascale Cossart	Pasteur Institute, Paris, France	Infection biology in the era of microbiomes: the Listeria paradigm
11/06/2021	Asifa Akhtar	Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany	X chromosome: A paradigm to study epigenetic regulation
18/06/2021	Nuria Sebastián	Pompeu Fabra University, Barcelona, Spain	Languages before language: Infants' early steps to learn language(s)
OCTOBER			
08/10/2021	Markus Grompe	Oregon Health & Science University, Portland, US	The role of polyploid hepatocytes in injury, aging and oncogenesis
05/11/2021	Clara Montagut	Hospital del Mar, Barcelona, Spain	Clinical utility of Liquid biopsy in colorectal cancer
DECEMBER			
17/12/2021	Serena Nik-Zainal	Hutchison/MRC Research Centre - University of Cambridge, UK	Recent insights into somatic mutagenesis in human cancers and stem cell systems

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 having the following purpose: 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 16 *ad-hoc* seminars were organised by CNIO researchers in 2021.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
12/01/2021	Fernando Calvo	Institute of Biomedicine and Biotechnology of Cantabria (IBBTEC), Spain	Harnessing cancer-associated fibroblasts to deter tumour progression
15/01/2021	Lluís Cordón Barris	Keck School of Medicine, USC, Los Angeles, USA	Drawing a microcephalic brain picture from different perspectives
FEBRUARY			
4/02/2021	Elva Martín Batista	University of La Laguna, San Cristóbal de La Laguna. S/C de Tenerife, Spain	The anticonvulsant and neuroprotective roles of SGK1.1
22/02/2021	Miguel F. Sanmamed	Clínica Universidad de Navarra, Pamplona, Spain	New tools for cancer immunotherapy development
25/02/2021	Juan Pablo Unfried	Center for Applied Medical Research (CIMA), University of Navarra, Spain	Long non-coding RNA NIHCOLE promotes DNA damage repair in liver cancer cells
MARCH			
11/03/2021	Javier Tamayo	Institute of Micro and Nanotechnology (IMN-CNM), CSIC, Madrid, Spain	Optonomechanical technologies for early cancer detection and new avenues in cancer research
25/03/2021	Carles Ubeda	FISABIO, Valencia, Spain	Role of the microbiome in infectious and autoimmune diseases
APRIL			
13/04/2021	Branavan Manoranjan	University of Calgary, Calgary, Canada	Bi-directional vulnerability of malignant brain tumors to Wnt signaling
MAY			
12/05/2021	Antonin Morillon	Institut Curie, Paris, France	Long non coding RNA, fate and function
JUNE			
14/06/2021	Javier Redondo Muñoz	Center for Biological Research (CIB), Madrid, Spain	The interplay between epigenetics and cytoskeleton in mechanics and migration of leukemia cells
SEPTEMBER			
16/09/2021	Anders Nordell	Collective Minds Radiology	Collaborative Research and a central, cloud based, multi-modal data repository with analytics and data curation

DATE	SPEAKER	ORGANISATION	TITLE
OCTOBER			
04/10/2021	Roser Vento-Tormo	Wellcome Sanger Institute, U.K.	Mapping tissues <i>in vivo</i> and <i>in vitro</i>
27/10/2021	Israel S. Fernández	St Jude Children's Research Hospital, Memphis, TN, USA.	Structural basis of DNA Targeting by a transposon encoded CRISPR-Cas SYSTEM
NOVEMBER			
16/11/2021	Hugo Muñoz Hernández	Biozentrum, University of Basel, Switzerland	Transient states of substrate transfer in polyketide synthases visualised by Cryo-EM
22/11/2021	Gregorio Rubio Navarro and Antonio Falcó Montesinos	Politécnica de Valencia University and CEU Cardenal Herrera University, Madrid, Spain	Systems Biology based Digital-Twins: A constructive approach for recommendation systems in precision medicine
24/11/2021	Maria Mittelbrunn Herrero	Center for Molecular Biology "Severo Ochoa", Madrid, Spain	How can aged-related changes in T cell metabolism drive systemic multi-tissue ageing?

WOMEN IN SCIENCE SEMINARS

In 2021, 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
18/01/2021	Sara Giménez	Member of the Congress and of the European Commission against Racism and Intolerance	Advancing equal treatment and opportunity, approaching the Roma people
09/02/2021	Gemma Robles	Deputy Director of <i>El Periódico</i>	Press, science and the debt with Matilda
09/03/2021	Mary Beard, Maria Blasco, and Susana Torres	University of Cambridge; CNIO; IE University	Women and Power: Round table
11/03/2021	Online film	Women in Science Office	"Picture a Scientist"
16/03/2021	Soraya Sáenz de Santamaría	Former Vicepresident of the Government of Spain	Female talent and crisis management
12/04/2021	Cani Fernández	President of the National Commission of Markets and Competition (CNMC)	The Presence of Women in the Legal Profession
18/05/2021	Zulema Altamirano Argudo	Spanish Ministry of Science and Innovation	Working in equality: empower, believe, transform
24/06/2021	Marta Sanz	Writer and Philologist	Writing: a journey through the human body
08/07/2021	Rigoberta Bandini	Artist	Dialogue with Rigoberta Bandini
14/09/2021	Maria Purificación Neira	Environment, Climate Change and Health, (ECH) UHC/HEP World Health Org., Geneva, Switzerland	Public Health in feminine
03/11/2021	María Pagés	choreographer of flamenco and Spanish dance	Dancing needs some talent and a lot of work
10/11/2021	Blanca Li	Director of the <i>Teatros del Canal</i>	Colloquium between Blanca Li, Director of the <i>Teatros del Canal</i> and Maria Blasco, CNIO Director

SCIENCE DISSEMINATION EVENTS

“VACCINES AGAINST COVID-19 AND CANCER CONTROL”
 24 SEPTEMBER 2021
**ORGANISER:**
Centro Nacional de Investigaciones Oncológicas (CNIO)
WITH THE SUPPORT OF:
“la Caixa” Foundation
KEYNOTE LECTURE:

- **Mariano Esteban**, Poxvirus and Vaccines Group
Director- CNB-CSIC
- “Visión de un virólogo: cómo las vacunas contra la pandemia COVID-19 nos ayudan al control del cáncer”

ROUND TABLE

- **Maria A. Blasco**, Spanish National Cancer Research Center
- **Mariano Esteban**, Poxvirus and vaccines Group - CNB-CSIC
- **Rosario Perona**, The Spanish National Research Council (CSIC)
- **Sergio Recio**, CNIO Friend
- **Margarita del Val**, Center of Molecular Biology “Severo Ochoa”

CHAIR

- **Cristina Villanueva**, Journalist

OPEN DOORS DAY: EVERYONE UNRAVELLING CANCER
 4 NOVEMBER 2021


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 (XXI *Semana de la Ciencia y de la Innovación*, 1-14 November 2021).

In November 2021, the CNIO participated in the Science and Innovation Week under the motto “*Todos y todas desarmando al cáncer*” (“Everyone Unravelling Cancer”). The event was held online with over 200 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.

RESEARCHERS' NIGHT

24 SEPTEMBER 2021

The Centre opened its doors to the public on September 24 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 live 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 or doubts. Altogether, more than 300 people signed up for the day, a new success in terms of participation.

To be able to carry out the experiment, in which they learned how to extract DNA from a tomato, the registered participants received a "scientist's kit" with all the necessary materials at home.

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.

The European Researchers' Night is an initiative of the European Union, held simultaneously in 350 European cities to disseminate the importance of scientific knowledge. In Madrid, it is promoted by the Regional Ministry of Science, Universities and Innovation and coordinated by the Madrid Knowledge Foundation. The Project is funded by the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie actions.



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
- **Rosa Menéndez López**
President of the Spanish National Research Council (CSIC)
Presidenta del Consejo Superior de Investigaciones Científicas (CSIC)
- **José Alarcón Hernández**
Director of the Department of Public Policies of the Cabinet of the Presidency of the Government
Director del Departamento de Políticas Públicas del Gabinete de la Presidencia del Gobierno
- **Margarita Blázquez Herranz**
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III
Subdirectora 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**
Representative: Rafael Pardo Avellaneda, Director
- **Fundación Bancaria Caixa d'Estalvis i Pensions de Barcelona, "la Caixa"**
Representative: Antonio Vila Bertrán, General Director
Alternate Representative: Àngel Font Vidal, Corporate Director of Research and Health
- **Grupo PRISA**
Representative: Ignacio Polanco Moreno (until October), Honorary Chairman

→ Secretary

- **Margarita Blázquez Herranz**
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III
Subdirectora 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 €252,708 in 2021 (€281,950 in 2020). This amount was received as base salary and position salary supplements: €226,896 (€222,447 in 2020); variable remuneration: €23,770 accrued during 2019 (€55,054 accrued in 2020); 0.9% increase: €2,042. Like every year, there is a provisional amount of €113,942 for the variable accrued in 2020 and 2021.
- 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
Head of the Chromatin Dynamics Team, Nuclear Dynamics Research 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

 - **Sara Courtneidge, PhD, DSc (hc)**
Professor, Departments of Cell, Developmental and Cancer Biology and Biomedical Engineering, School of Medicine
Oregon Health & Science University
Portland, 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
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

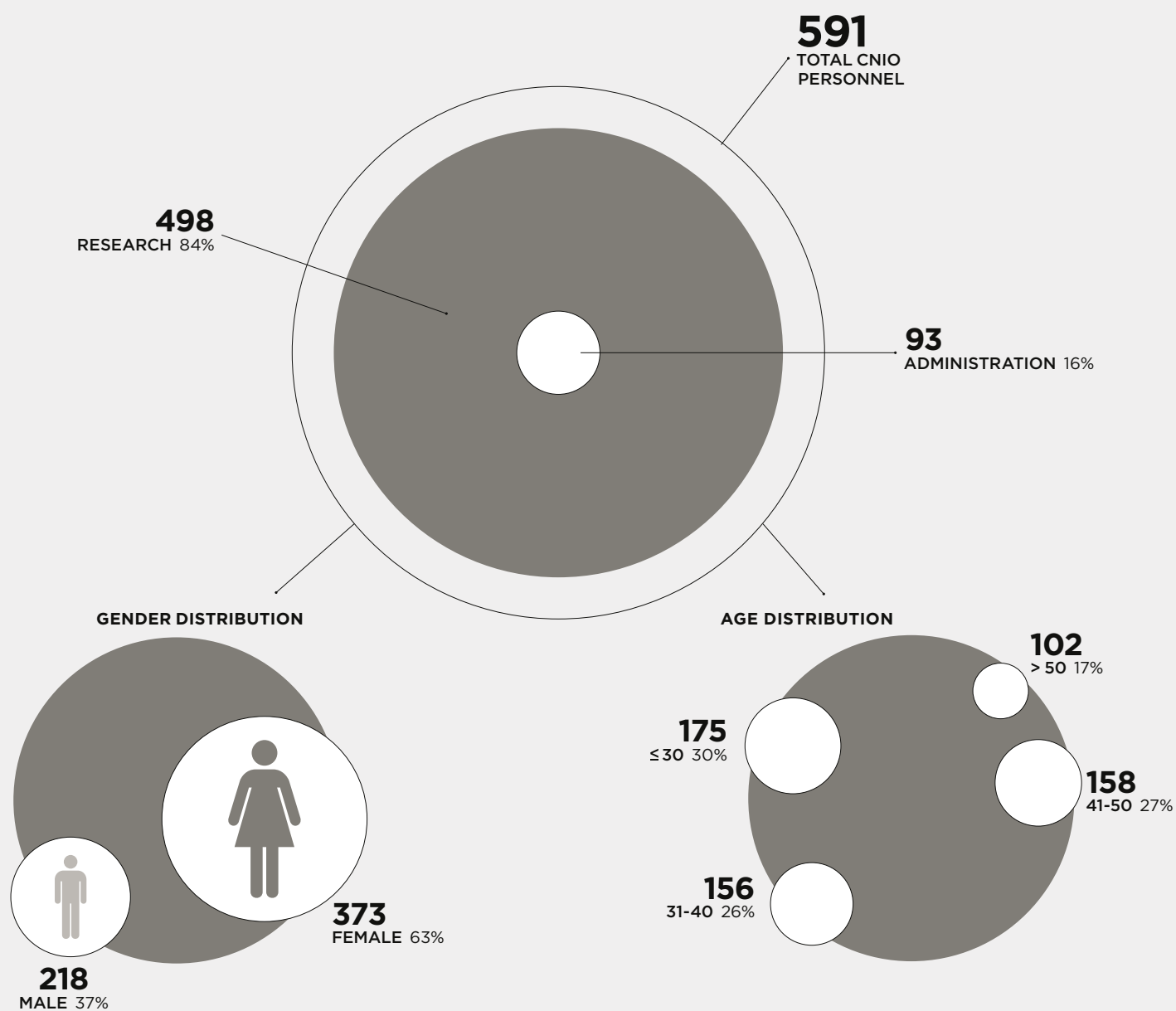
-
- **Scott W. Lowe, PhD**
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Chair, Geoffrey Beene Cancer Research Center
Investigator, Howard Hughes Medical Institute
Memorial Sloan-Kettering Cancer Center
New York, USA
 - **Ángela Nieto, PhD**
Full Professor and Head of Developmental Neurobiology
Neuroscience Institute *CSIC-UMH*
Alicante, Spain
 - **Andre Nussenzweig, PhD**
Chief, Laboratory of Genome Integrity
NIH Distinguished Investigator, Head of the Molecular
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Bethesda, USA
 - **Daniela Rhodes, PhD, FRS**
Emeritus Research Leader, LMB Division of Structural
Studies
MRC Laboratory of Molecular Biology
Cambridge, United Kingdom
 - **Josep Tabernero, MD PhD**
Director, Vall d'Hebron Institute of Oncology (VHIO)
Head, Medical Oncology Department of Vall d'Hebron
University Hospital
Director of Clinical Research at VHO
Barcelona, Spain

MANAGEMENT



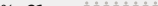
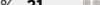

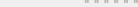
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CNIO PERSONNEL 2021



GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

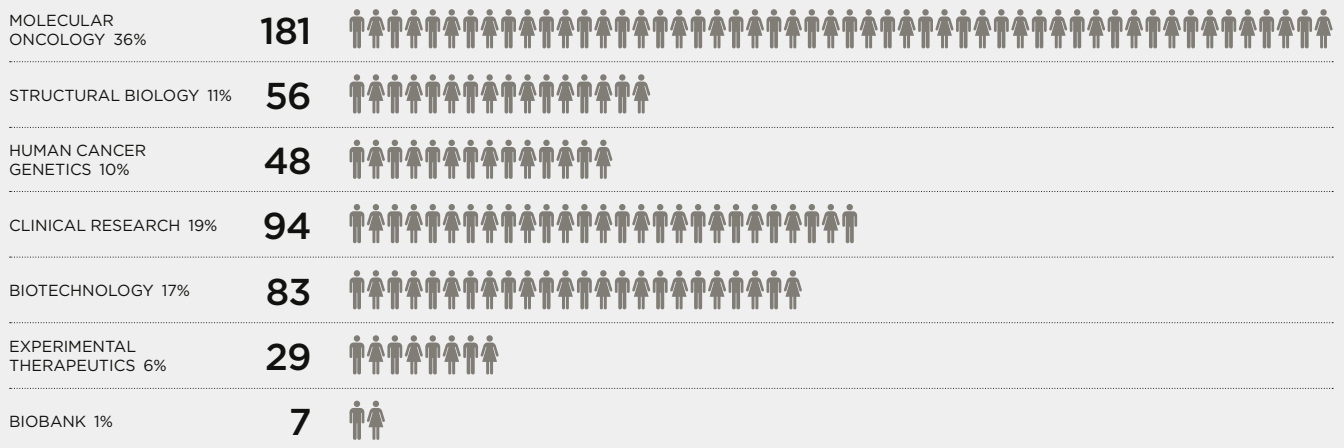
GROUP LEADERS, HEADS OF UNIT/SECTION	FEMALE	52%	23	
	MALE	48%	21	
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	FEMALE	65%	11	
	MALE	35%	6	
MANAGEMENT: DIRECTORS, HEADS OF AREA	FEMALE	29%	4	
	MALE	71%	10	

SCIENTIFIC PERSONNEL 2021

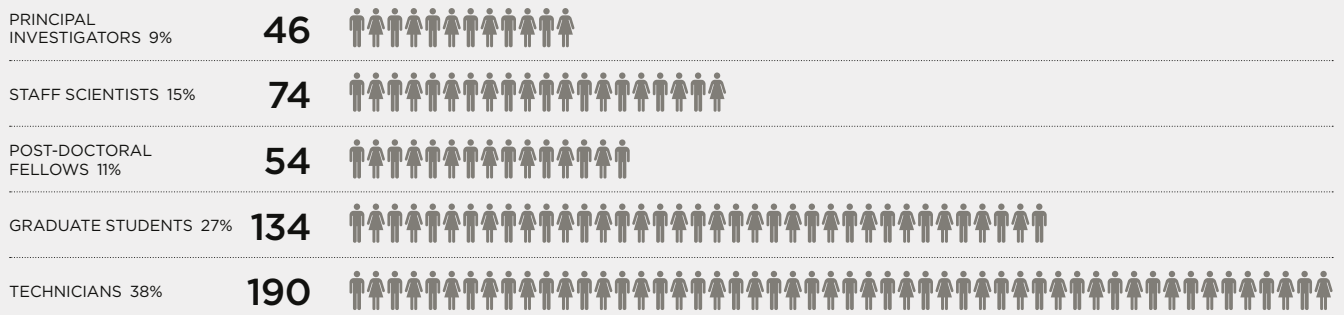
TOTAL SCIENTIFIC
PERSONNEL

498

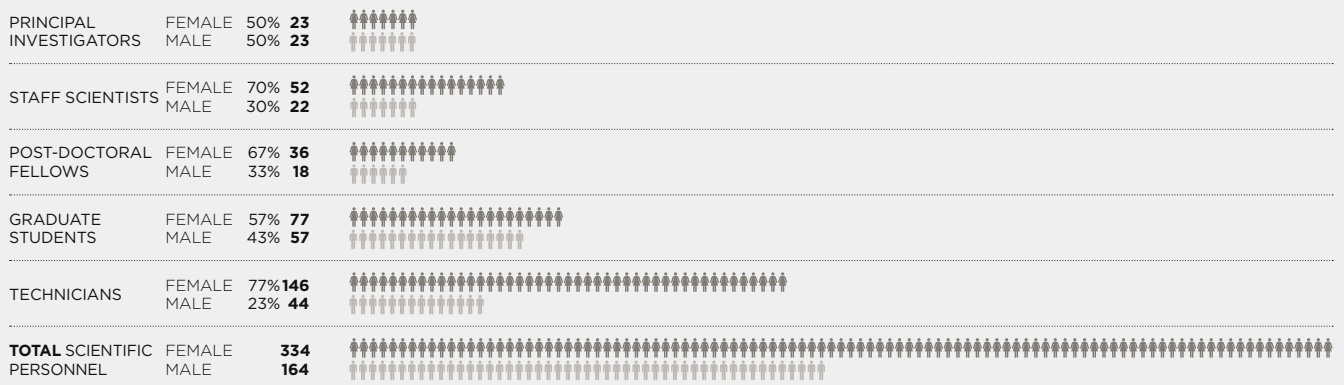
DISTRIBUTION BY PROGRAMMES



DISTRIBUTION BY PROFESSIONAL CATEGORY



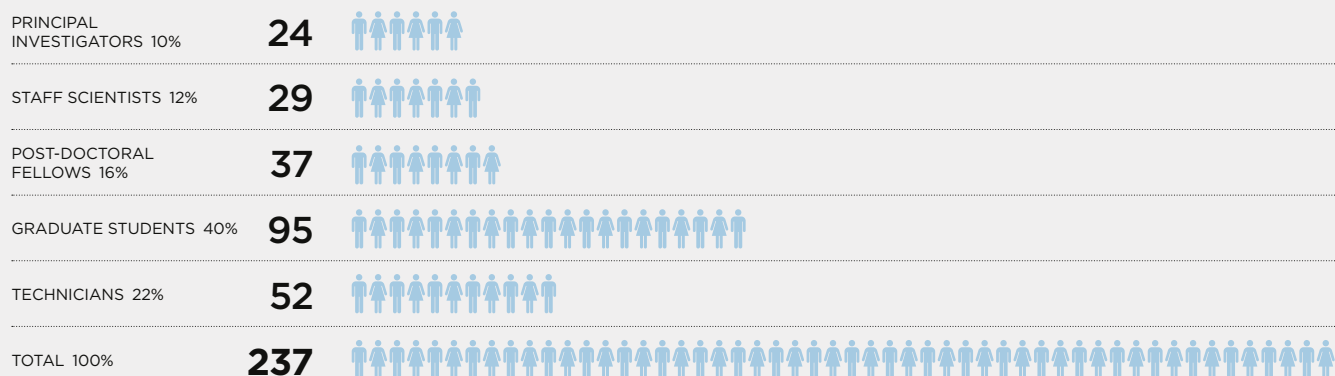
GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY



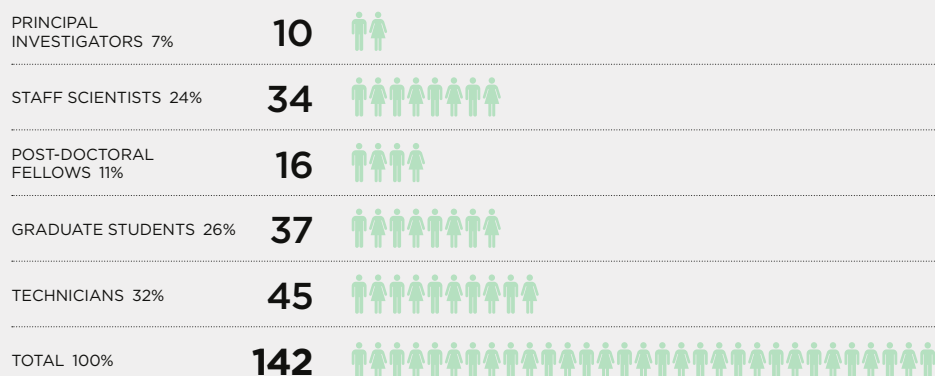
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC
PERSONNEL

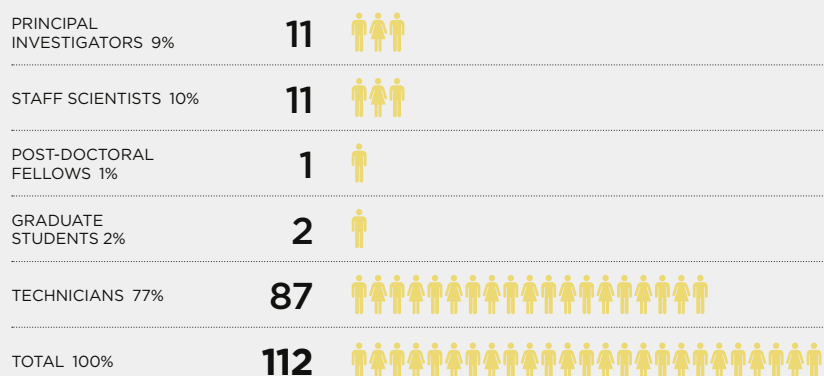
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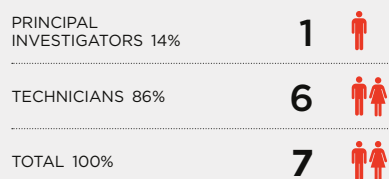
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH



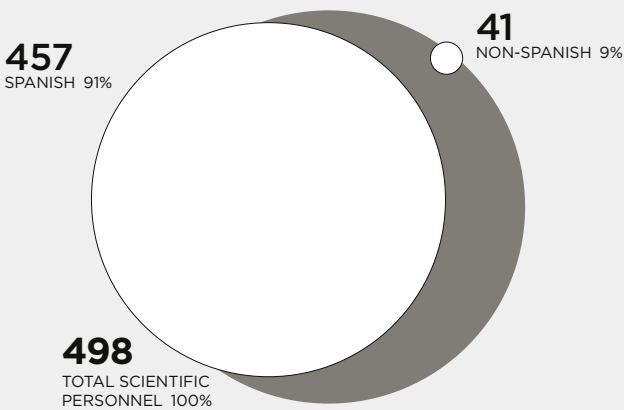
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION



DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BIOBANK



SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

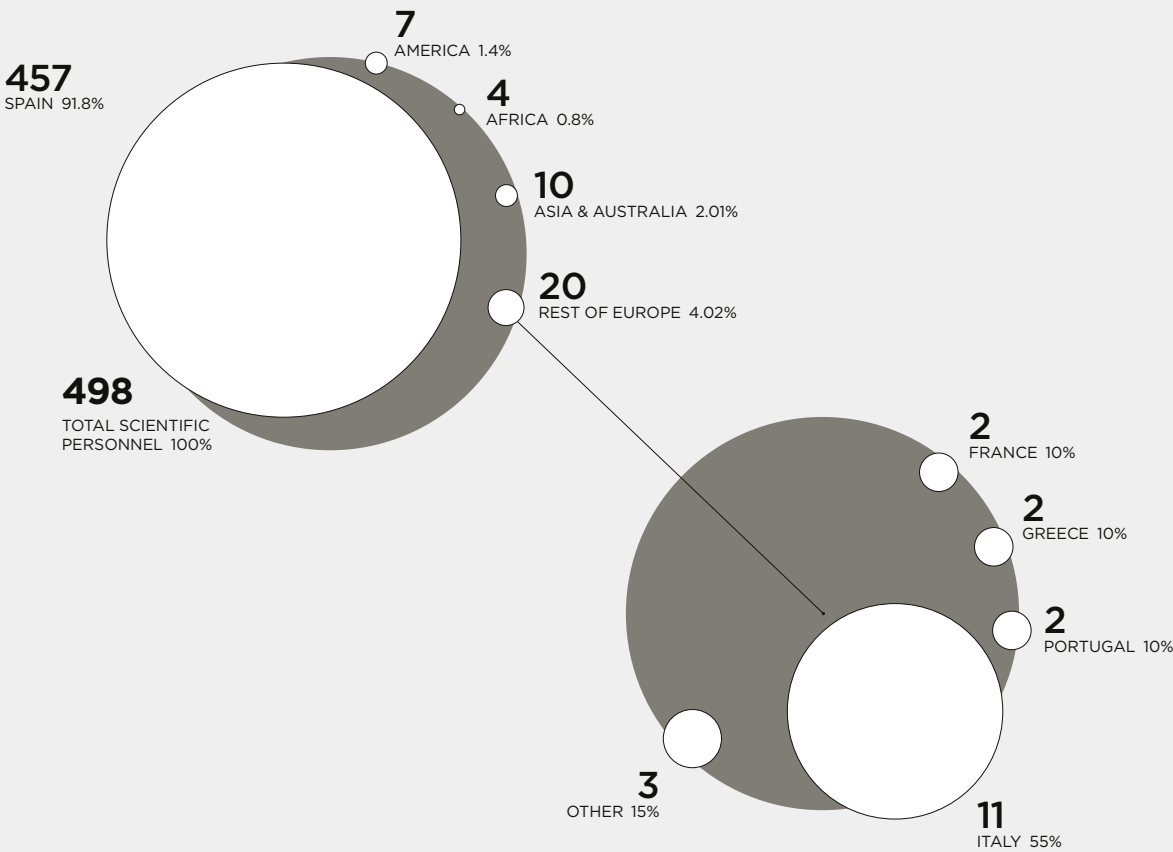


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS 11%	5	
STAFF SCIENTISTS 6%	4	
POST-DOCTORAL FELLOWS 19%	10	
GRADUATE STUDENTS 12%	16	
TECHNICIANS 3%	6	

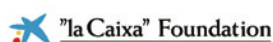
Total foreign scientific personnel 41
Percent values represent percentages of foreign employees of the total CNIO personnel within each category

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN



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 2021. They play an inherent role in our present and future successes.”



The *Fundación “la Caixa”* helps finance our most prominent international conferences, the CNIO-“la Caixa” Foundation 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 2021, 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 2 research groups at the CNIO: the Cancer Immunity Group, led by María Casanova; and the Prostate Cancer Clinical Research Unit (CRU), headed by David Olmos. 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 6 researchers will receive training on managerial and entrepreneurial skills in 2022, in collaboration with the *IE Business School*. *Banco Santander* Foundation also supports our successful outreach project, CNIO Arte.



The *Fundación Seve Ballesteros* is a private not-for-profit institution focused on securing, financing, and promoting research projects centred on brain tumours. *Fundación Seve Ballesteros* has been supporting the *Seve Ballesteros* Foundation-CNIO Brain Tumour Group, headed by Massimo Squatrito, since 2012. This Group focuses on identifying markers for brain tumours as its principal activity.



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 recipient of the *Jesús Serra* Foundation's Visiting Researchers' Award in 2021 was Raúl Rabadán, Professor in the Department of Systems Biology and Biomedical Informatics and Director of the Center for Topology of Cancer Evolution and Heterogeneity of Columbia University (New York, USA).



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 2021, our research activities and seminars were also supported, among others, by *Fundación Investigación Biomédica Hospital Universitario 12 de Octubre*, *Fundación Española de Hematología y Hemoterapia*, *Fundación Banco Sabadell*, *the French Embassy*, and *the British Embassy*.

CNIO Friends Philanthropic Donations

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Art & Science Join Forces Through ‘CNIO Arte’	252
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Donors to the CNIO 2021	255

The CNIO Friends Programme enjoyed another successful year, in which we were able to open a competitive application process for 7 new post-doctoral researchers. We received a generous €100k donation from *Fundación Domingo Martínez* in honour of their 50th Anniversary in order to fully-finance one of the contracts, and received €20k from *Fondation Franz Weber* towards the funding of a contract focusing on alternative methods to animal models.

In all, the CNIO Friends programme raised in excess of €669k in 2021. Fundraising around the world has been affected by the COVID-19 pandemic, with the financial effects felt more keenly in 2021 than 2020, especially in relation to corporate and foundation donors.

We launched our multichannel branding and fundraising World Cancer Day campaign in February, encouraging people to donate to the CNIO to help us stop cancer. We were joined this year by mountaineer Edurne Pasaban, actor Carlos Hipólito, and motorcycle champion Laia Sanz, who participated in videos to spread awareness and encourage people to become friends of the CNIO.

The annual CNIO Arte initiative, now in its 4th year, showcased a collaboration between visual artist Daniel Canogar, author of exceptional artworks exhibited all around the world, and the computational biologist Sarah Teichmann, from the Wellcome Trust Sanger Institute (Cambridge, UK) and cofounder of the international project “Human Cell Atlas”. The CNIO Arte project is based on the fundamental principle that science and art are indispensable for understanding and interpreting the world and that each can inspire the other. Any profits from the sale of artworks from this initiative go directly to the CNIO Friends programme.

In 2021, we strengthened our partnership with *Fundación Solidaridad Carrefour* and were delighted to receive another donation of €39k to purchase valuable Real-Time PCR equipment. For International Women’s Day, we hosted 30 teenage daughters of Carrefour workers for an online lab experiment to extract the DNA from a tomato. This event was an opportunity to further engage one of our donors, and also to promote scientific vocations among girls to positively change the gender stereotypes that exist around science and research.

Brother Iberia made a second generous donation of €21k to support cancer research, and we were delighted to once again receive €25k from the Simonia Trust. We developed a new alliance with SantaLucia this year in which we received a donation from their company and also were one of the winning finalists in a public vote securing an additional donation from

“CNIO Friends continues to inspire people to join us in our mission to stop cancer.”

SantaLucia to support our cancer research. We were also delighted to receive donations this year from associations such as *Rosae*, *Bandera Rosa*, and *Las Supernenas*, companies such as Petroplast, and some very generous individual donations and other individuals to support the CNIO Friends Programme.

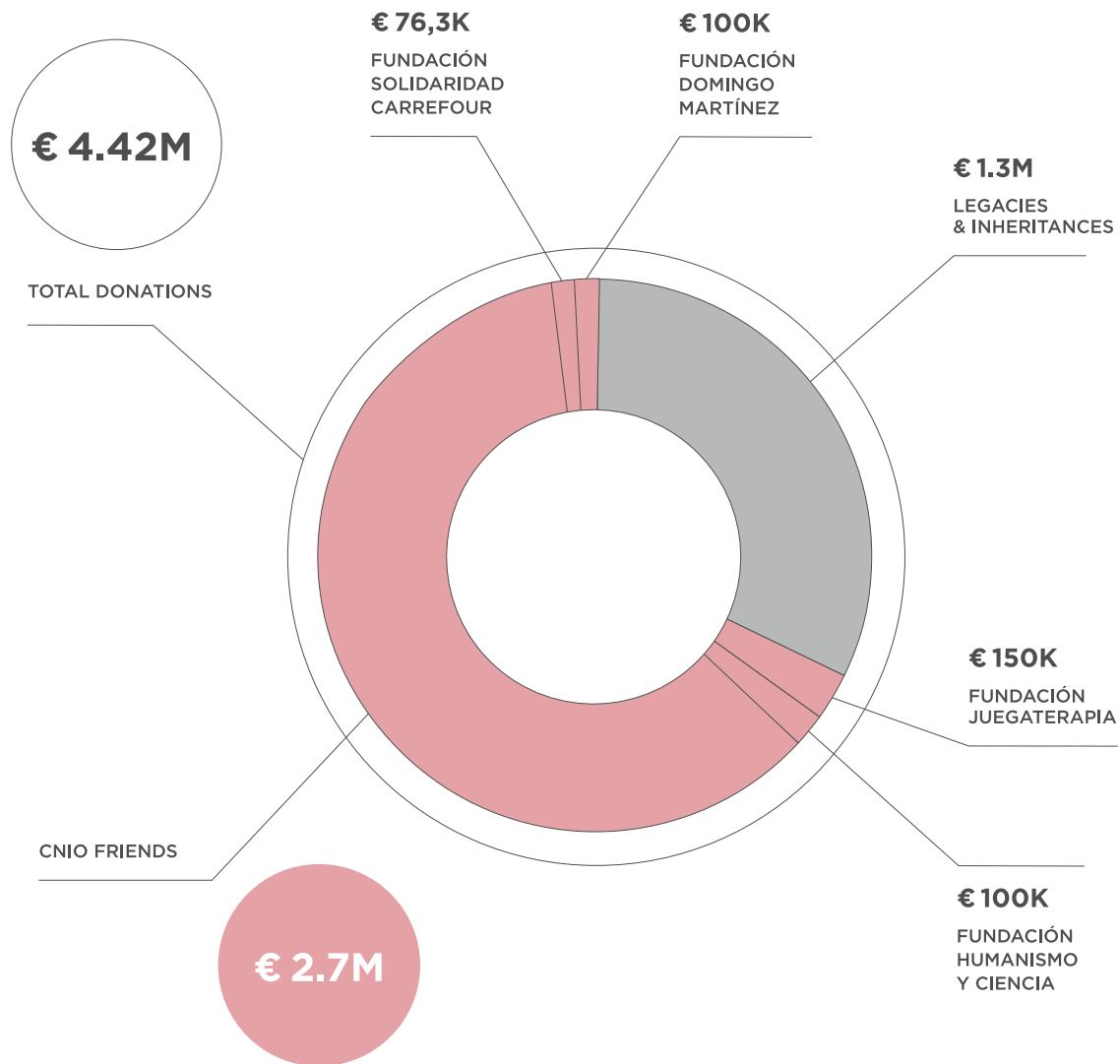
Our CNIO Friends Meeting Day was held by Zoom to thank our donors for their support and give them an update on the impact of their donations. After a Centre overview from Maria Blasco, our Friends had the pleasure of hearing from Rubén Julio Martínez, Moustafa Shehata (via his group leader Iván Plaza), Sofía Cabezudo, Eunjeong Kim, Magdalena Leal, María José Andreu, and Sergio Muñoz about their philanthropy-funded research projects.

Edurne Pasaban, Spanish Mountaineer and world record holder for the first woman to climb all 14 eight-thousanders, joined Maria Blasco at the end of the session to discuss her journey, why she joined CNIO Friends herself to support the campaign, and the importance of resilience and a robust mindset in overcoming obstacles.

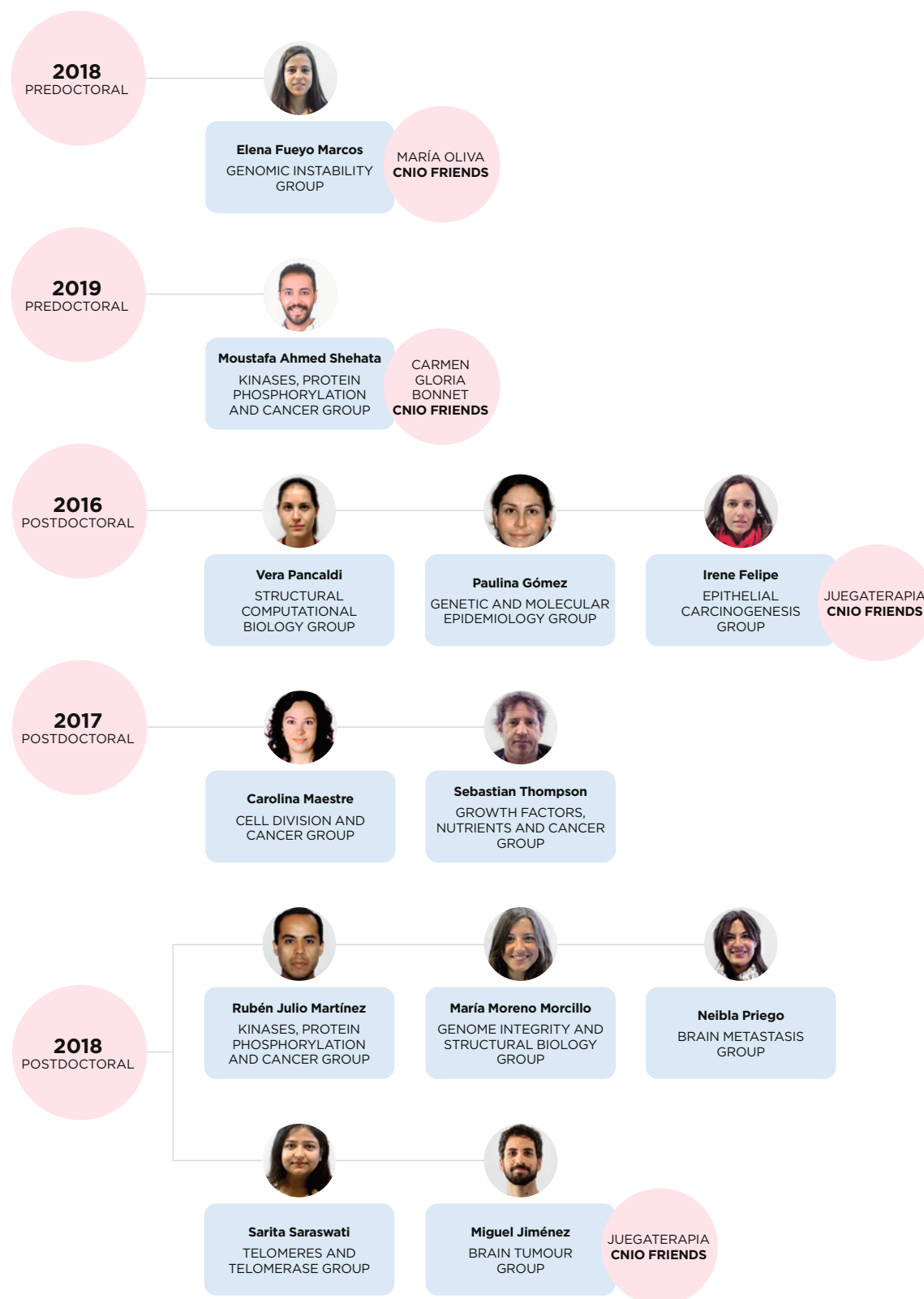
Another pillar of philanthropy that continues to grow for the CNIO is our legacy programme. This year marks a cumulative total of around € 1.33m in legacies over 7 years, with €80k received in 2021 and €528k pending to be executed.

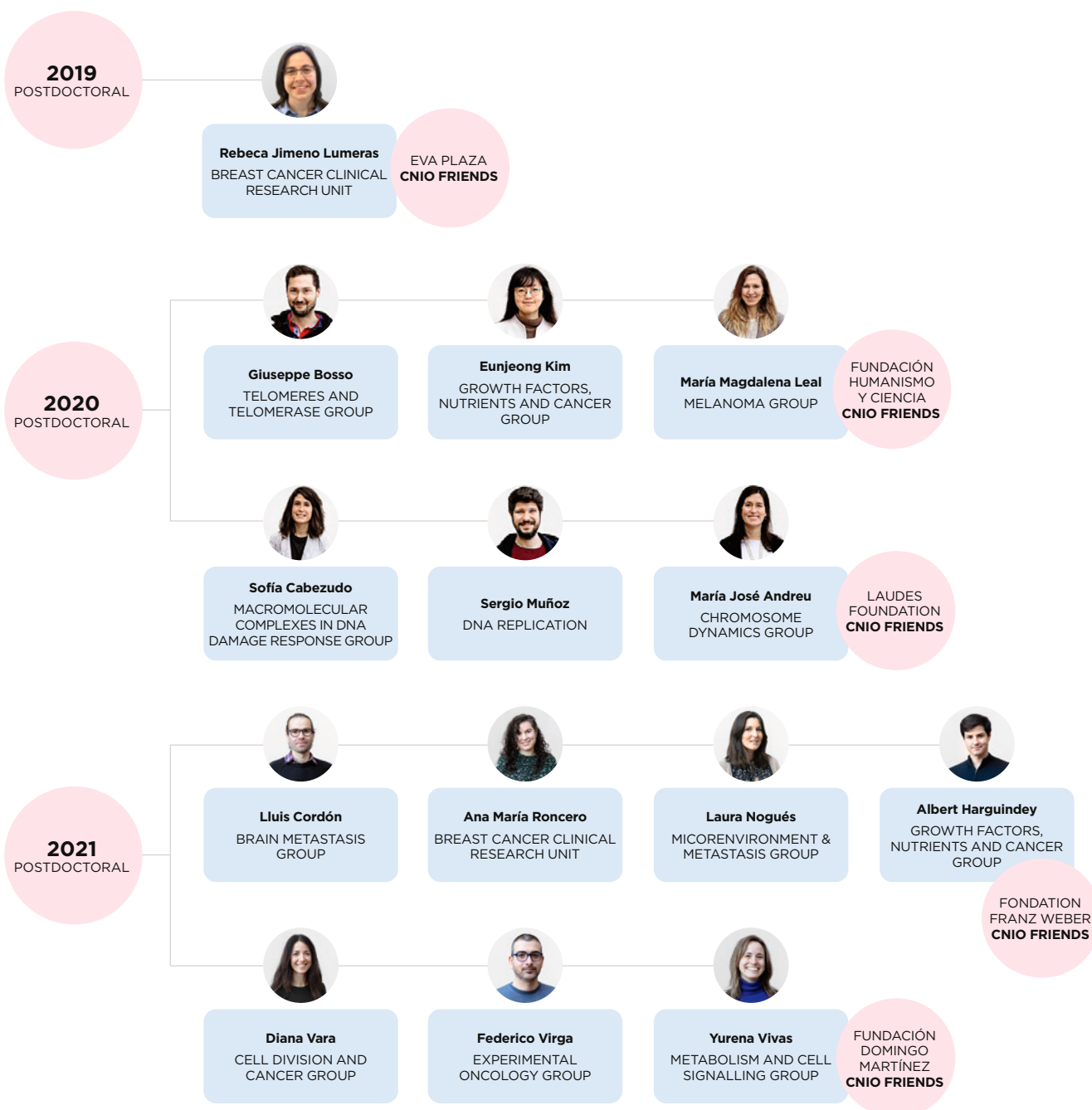
The pandemic has meant that fundraising teams have had to think of new and innovative ways to support their causes. This year has undoubtedly been challenging without the usual opportunities to create new alliances and collaborations via events and networking opportunities. However, people have continued to give generously and our CNIO Friends and donors have continued to demonstrate support and enthusiasm for our work, despite the difficult economic and social situation that COVID-19 has rendered for citizens and organisations alike. We look forward to the opportunity to more in-person in 2022 and will continue to develop and grow our philanthropic programme. Most importantly, we extend a heartfelt thank you to our donors for their commitment to cancer research. Together, we can stop cancer. Philanthropy enables everyone to be part of the solution.

DONATIONS TO THE CNIO



'CNIO FRIENDS' INTERNATIONAL PREDOCTORAL/ POSTDOCTORAL CONTRACTS





'CNIO FRIENDS' DAY

Our CNIO Friends Meeting Day was held by zoom to thank our donors for their support and give them an update on the impact of their donations. After a Centre overview from Maria Blasco, our Friends had the pleasure of hearing from Rubén Julio Martínez, Moustafa Shehata (via his group leader Iván Plaza), Sofía Cabezudo, Eunjeong Kim, Magdalena Leal, María José Andreu, and Sergio Muñoz about their philanthropy-funded research projects.

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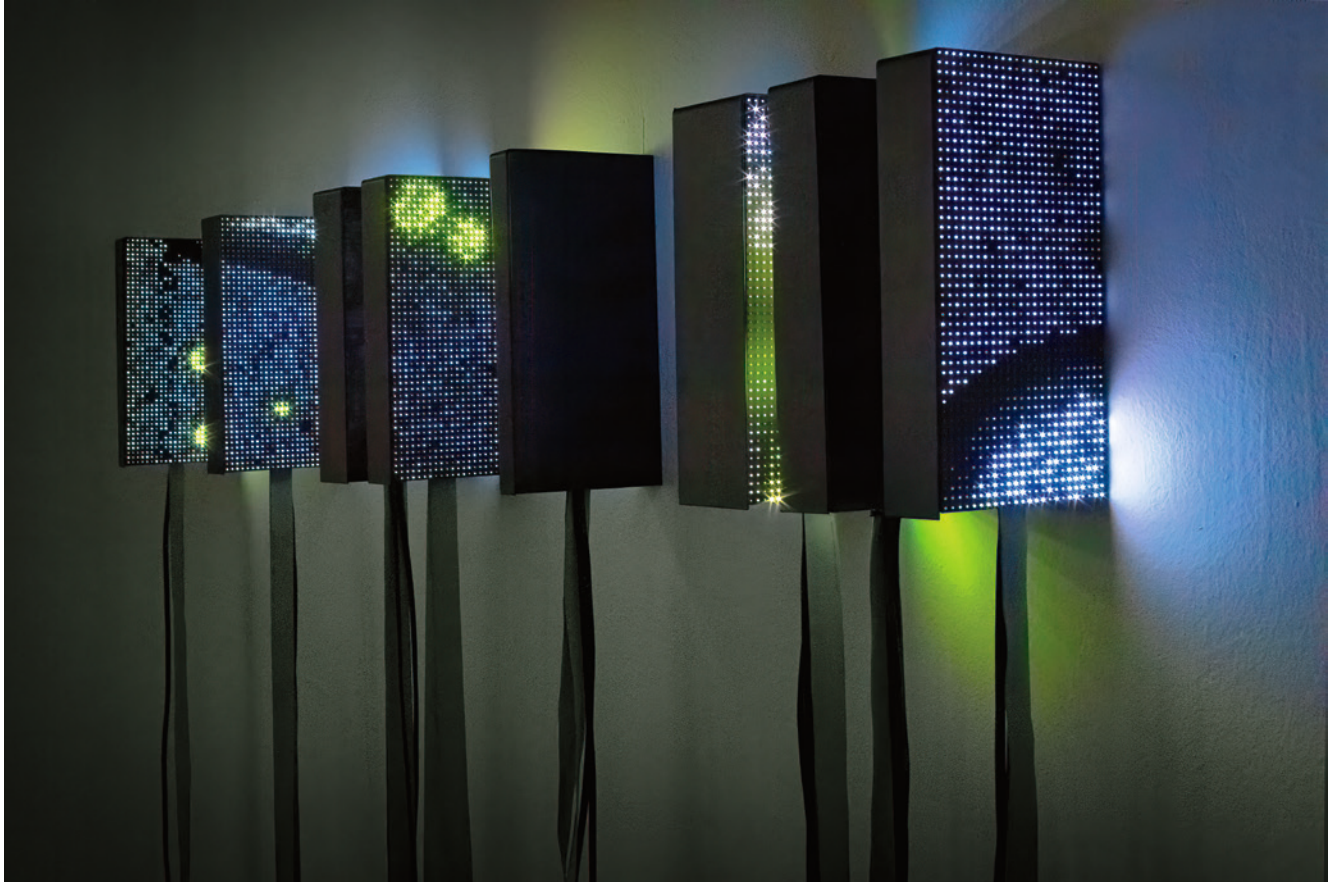
WORLD CANCER DAY CAMPAIGN



We launched our multichannel branding and fundraising campaign on World Cancer Day to build awareness of our #CNIO StopCancer initiatives and invite new donations for cancer research, with special thanks to the collaboration of our partners Exterior Plus and JCDecaux.

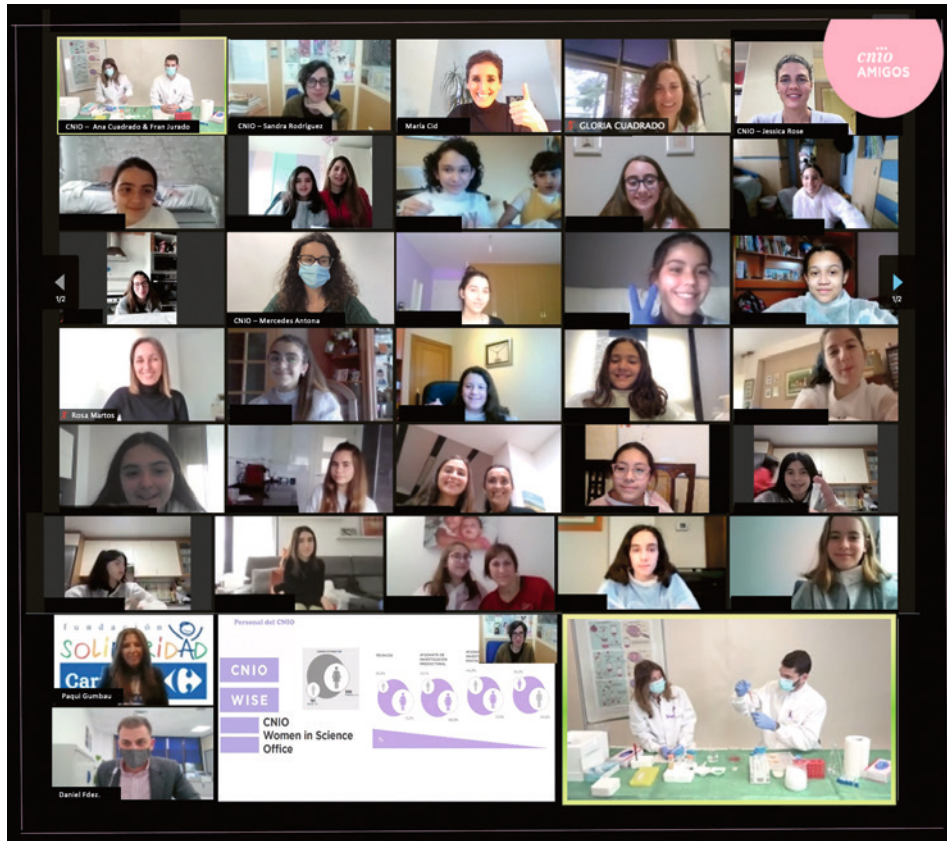


ART & SCIENCE JOIN FORCES THROUGH ‘CNIO ARTE’



The CNIO Arte initiative, now in its 4th year, showcased a collaboration between visual artist Daniel Canogar, author of exceptional artworks exhibited all around the world, and the computational biologist Sarah Teichmann, from the Wellcome Trust Sanger Institute (Cambridge, UK) and cofounder of the international project “Human Cell Atlas”. The CNIO Arte project is based on the fundamental principle that science and art are indispensable for understanding and interpreting the world and that each can inspire the other. Any profits from the sale of artworks from this initiative go directly to the CNIO Friends programme.

GENDER EQUALITY AND FUNDACIÓN SOLIDARIDAD CARREFOUR



For International Women's Day, we hosted 30 teenage daughters of Carrefour workers for an online lab experiment. This event was an opportunity to further engage one of our donors and also to promote scientific vocations among girls to positively change the gender stereotypes that exist around science and research.

ROSAE'S 20TH ANNIVERSARY

To celebrate the 20th anniversary of their breast cancer association, we visited Valdepeñas to collect *Rosae's* annual donation to the CNIO.



BANDERA ROSA PATIENT ASSOCIATION

Despite having their finances affected by the pandemic, *Bandera Rosa* still made their annual donation. We were invited to their new office to collect it in person.



DONORS TO THE CNIO 2021

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Almendralejo, Badajoz

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· ***Asociación Recreativo-Cultural de apoyo a personas afectadas de cáncer “Esperanza”***
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· ***Marcha contra el cáncer Puebla del Maestre***
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· ***Plataforma de Enfermos de Cáncer y Solidaridad.***
Carolina Riesco
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Algeciras, Cádiz | · Asociación para la Atención Integral de Afectados de Cáncer
Llerena, Badajoz |
| · Asociación de Mujeres Afectadas de Cáncer de Mama “Rosae”
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Madrid, Madrid |
| · Fundación Solidaridad Carrefour
Madrid, Madrid | |

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.

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