

**ANNUAL
REPORT 2020**

All the portraits in this year's Annual Report were captured via Zoom. Multiple optical devices were used: mobile phones, tablets, computers... But each portrait was captured and chosen with the same sensitivity by the same "artist's eyes".

ANNUAL REPORT 2020

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**“This year’s
Covid-19 pandemic
has increased the
importance of
scientific research;
it is thanks to science
that the world is
slowly returning
to normality.”**

MARIA A. BLASCO
Director

FOREWORD

Maria A. Blasco Director

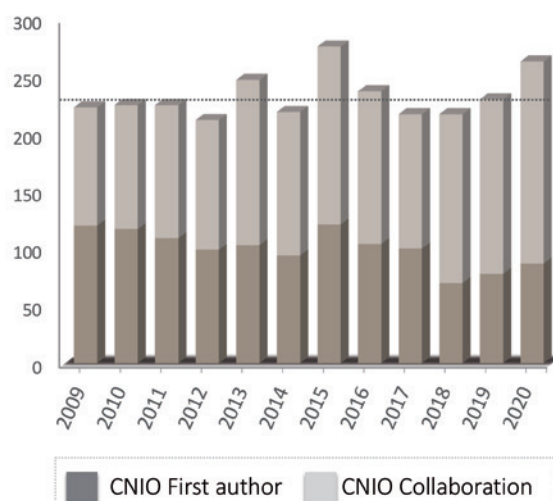
This year started with the arrival of the COVID-19 pandemic in early 2020 which, although it has changed our lives in so many ways, has not stopped, but instead increased the importance of scientific research. Indeed, it is thanks to science that the world is slowly returning to normality.

Despite the initial limitations imposed in the workplaces by this health crisis, I am proud to convey that the CNIO has been able to overcome those difficulties and to achieve our goal of scientific leadership and international impact. In 2020, CNIO researchers authored a total of 263 papers, 36 of which were published in journals with impact factor between 10 and 15, and 36 publications in journals with impact factor greater than 15. This year was very productive in terms of number of high-quality publications, which increased by 26% with respect to 2019. 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 are a testimony of our scientific efforts in basic and translational cancer research.

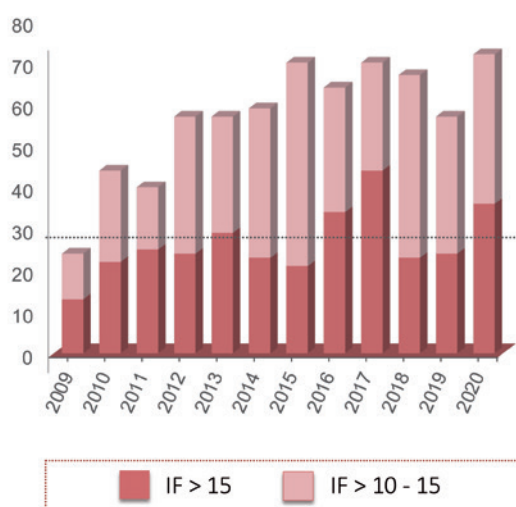
In 2020 the CNIO was awarded for the third time the Spanish Government accreditation as Centre of Excellence within the *Severo Ochoa / María de Maeztu* call from the Ministry of Science and Innovation. The CNIO is thus one of the only 6 research centres in Spain that have been granted this distinction without interruption since the launch of this flagship programme of Spanish science in 2011.

Also, 2020 was particularly successful for CNIO researchers in getting access to top-level funding opportunities in international calls, with 3 Group Leaders being awarded prestigious ERC Grants during 2020: Maria Blasco, Telomeres and Telomerase Group Leader (ERC Advanced Grant, project SHELTERIN); Marisol Soengas, Melanoma Group Leader (ERC Advanced Grant, project METALERT-STOP); and Manuel Valiente, Brain Metastasis Junior Group Leader (ERC Consolidator Grant, project ALTER-BRAIN).

TOTAL NUMBER OF PUBLICATIONS



TOP PUBLICATIONS



Aiming to contribute to the fight against the COVID-19 pandemic by using our best resource, which is excellent science, this year several Groups and Units at the Centre became involved in projects focusing on different aspects of the disease, such as the association of telomere length with the severity of the pathologies related to COVID-19; the identification of genetic variants correlated with disease severity; the generation of humanised mouse models susceptible to SARS-CoV-2 infection for preclinical studies; the development of a new and rapid diagnostic method using phi29 polymerase amplification; and the evaluation of the modulation of the viral receptor ACE2 with drugs already approved for medical use, to name a few. It is clear that understanding the molecular mechanisms of life can be useful not only to solve cancer 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 2020 we carried out a selection process that allowed us to incorporate a new Research Group into the Molecular Oncology Programme, which will join the Centre in January 2021: the Immunity of Cancer Group, led by Maria Casanova, coming from the 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 immunoncology area. We wish this new recruit the best of luck in developing her scientific projects and professional duties at the CNIO, which will ultimately increase our research portfolio and scientific achievements. On the other hand, Javier Benítez, Director of the Human Cancer Genetics Programme, retired after many years of excellent work and dedication to the CNIO. We want to wholeheartedly thank him for his contribution in making the CNIO a Centre of excellence of international reputation and wish him the best for this new step in his life.

In line with our efforts to provide value to society and to foster the visibility of the science of excellence carried out in our country, the CNIO further committed to the SOMMa Alliance of Centres and Units of Excellence *Severo Ocha/María de Maeztu*, through my appointment as new SOMMa President in late 2020 for the period 2021-2022, and by assuming the coordination of the activities of the alliance from the CNIO. At CNIO we are strongly committed to defending the values of research centres of excellence in Spain, and to underscoring the need to provide the proper ground for science and the prosperity of our society.

Another indicator of our societal and scientific impact is the breadth and relevance of our innovation activities. In 2020, our efforts in promoting collaborations with industry resulted in €2.4 million secured through research agreements (an amount 34% higher than in 2019). As much as 60% 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.

A key achievement in this area this year was the launch of a new spin-off company, Telomere Therapeutics, which is focused on the development of gene therapy based on telomerase, using AAV9 virus as vector, for the treatment of pulmonary fibrosis and other fibrosis conditions, which currently have no effective treatments. This new company is based on the results generated by the Telomeres and Telomerase Group of Maria Blasco at the CNIO, in collaboration with the Group of Fátima Bosch, from the *Universidad Autónoma de Barcelona*. The company has received seed capital from the firm InVivo Capital Partners. It is also worth mentioning the research agreement established with Bionam Biotech and *CRIS contra el cáncer* for a project on pancreatic cancer of the Experimental Oncology Group, led by Mariano Barbacid.

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. In 2020 an ERC Proof of Concept Grant was awarded to Oskar Fernández-Capetillo, Head of the Genomic Instability Group, to work on the development of SETD8 protein inhibitors with potential for the treatment of neuroblastomas and medulloblastomas, paediatric tumours that currently have poor prognosis. In addition, a project from the Topology and DNA Breaks Group, led by Felipe Cortés, was awarded a *CaixaImpulse* CONSOLIDATE COVID-19 grant to develop a novel detection kit for the simple and rapid diagnosis of SARS-CoV-2 infection. The CNIO holds a total of 4 prestigious *CaixaImpulse* projects that aim to accelerate the transfer of technology from basic research centres to industry.

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. In 2020 ETP started a collaboration with the company Foxy Pharma and the *Consejo Superior de Investigaciones Científicas* (CSIC), in order to develop compounds triggering a proprietary target relevant in cancer models and ageing-related diseases.

Our training programmes are also a key feature of the Centre, as an essential part of our mission. To provide training to the new generations 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 2020, Sonia Laín, from the *Karolinska Institutet* in Stockholm (Sweden), was beneficiary of the *Jesús Serra* Foundation's Visiting Researchers Programme. In addition, thanks to the "Science by Women" programme of the *Mujeres por Africa* Foundation, Mai Tolba, from the Faculty of Pharmacy of the Ain Shams University, in Abbasiya, Egypt, was awarded

a grant as Visiting Scientist to join the Breast Cancer Unit at the CNIO for a 6-month stay.

In a year marked by COVID-19 in the media, CNIO news hit the headlines, keeping the good pace of previous years and marking over 3,400 appearances in press (printed and online) and over 250 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 third edition of CNIO Arte, were also covered by general interest media, which we do not usually reach.

But raising awareness about cancer research must go further. In spite of the situation generated by the pandemic, we did not want to miss the celebration of World Cancer Research Day. Thus, we organised an online session entitled “A New Era of Cancer Research: Towards the Engagement of the Entire Society” on September 24, with the participation of renowned scientist Francis Mojica as keynote speaker, and a discussion panel on the challenges of cancer research and the need for society to get involved in order to face those challenges. With initiatives like this, we keep working towards our goal of bringing science closer to society.

The Institutional Image and Outreach Office is leading several projects that aim to open new avenues to gain society's trust and attention, as well as to emphasise the value of science. Thus, in 2020, the third 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 Spanish paleoanthropologist Juan Luis Arsuaga and the visual artist Carmen Calvo. The funds raised from this initiative totalled €100,000, which contributed directly to our CNIO Friends philanthropic initiative. To increase the impact of this initiative, in 2020 we organised the first 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 an open and enriching discussion on a selected topic. The symposium was held jointly with the presentation of the 3rd edition of the CNIO Arte project, under the title “The relations between art and archaeology”, chaired by Prof. Carlos Jiménez (Emeritus Professor of Aesthetics, historian and art critic). Also in 2020, the second event in the series “CNIO Workshop on Philosophy, Science and Medicine, Socio-environmental factors of health and disease” was held at CNIO, with the support of the *Banc Sabadell* Foundation. Discussion topics illustrated the social impact of current molecular biology: longevity, human enhancement and transhumanism, personalised medicine, and gene editing.

The recently created CNIO Office of Development and Philanthropy kept working on identifying and providing new philanthropic funding sources that contribute to hiring new research talent for the CNIO. All donations to the CNIO go

directly to the CNIO Friends initiative, to cover 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 2020, CNIO Friends received €813,000 in philanthropic donations and €258,000 from legacies and bequests. The funding derived from the CNIO Friends initiative, implemented in 2015, has thus far enabled the hiring of 17 postdoctoral fellows and 2 PhD students, with a record 6 new postdoctoral positions hired through philanthropy this year. We would like to thank all of our generous donors for their support of the CNIO. Every single donation directly supports cancer research and will contribute to society for generations to come.

Our commitment to gender equality was consolidated in 2020 through the activities spearheaded by our CNO 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. Despite the pandemic and thanks to the Zoom platform, our WISE office was able to keep bringing outstanding female speakers to the CNIO who inspire and encourage us to explore new perspectives and visions on the role of women in society. In our efforts to educate the future generations without gender bias, the WISE Office organised the science workshop CNIOptics# with the title “The power of the light” in which the most talented students from the Technovation platform participated.

With great pleasure and pride, we provide our society once again with a story of our achievements in 2020, 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.

Finally, I would also like to wholeheartedly thank visual artist Amparo Garrido for kindly contributing the fabulous artwork that illustrates the cover of our Annual Report.



Presentation of CNIO Arte at ARCOmadrid 2020.



OSCAR FERNÁNDEZ-CAPETILLO
Vice-Director

“Despite the difficulties, in 2020 we adapted and kept doing what we do best. These are times when society needs us more than ever, and CNIO scientists stood up to the challenge.”

Year after year, writing this summary makes me revisit the works of our scientists, and my conclusion is always the same: wow. We now have a better understanding of how resistance to cancer therapies occurs and have provided new ideas as to how this can be overcome. We discovered new mutations that predispose to breast cancer, and conditions such as type 3 diabetes that are associated to pancreatic cancer. We have a better atomic understanding of molecular machineries that regulate cancer cell growth, and improved gene-editing technologies to develop targeted cancer therapies. Our efforts to further collaborate with hospitals are also on the rise, exemplified by the creation of a national brain metastasis network that should facilitate clinical

and basic research. Besides cancer, CNIO scientists have made relevant advances in areas such as developing a new protocol that greatly facilitates the generation of reprogrammed stem cells, identifying new regulators of cardiac development, discovering how certain viral infections trigger diabetes, and demonstrating the efficacy of telomerase-based therapies for pulmonary fibrosis. This output in COVID-19 times is impressive. Thanks to all of you. In 2020 the world faced one of the biggest pandemics in recent times and many families lost loved ones. My last thought inevitably goes to all of them. I very much hope that, before 2021 ends, we will all be able to enjoy life and the CNIO experience in its full. Stay safe, stay strong.

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
Seve-Ballesteros Foundation-CNIO Brain Tumour 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 Junior Group

Geoffrey John Macintyre
Computational Oncology Junior Group

Ramón Campos-Olivas
Spectroscopy and Nuclear Magnetic Resonance Unit

Fátima Al-Shahrour
Bioinformatics Unit

Jasminka Boskovic
Electron Microscopy Unit

Inés Muñoz
Crystallography and Protein Engineering Unit

TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Javier Benítez Programme Director (until July)
Gema Moreno Secretary

Javier Benítez (until July)
Human Genetics Group

Mercedes Robledo
Hereditary Endocrine Cancer Group

Núria Malats
Genetic and Molecular Epidemiology Group

Miguel Urioste
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
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

BIOTECHNOLOGY PROGRAMME

CAROLINA POLA DIRECTOR OF INNOVATION (until November)

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
Confocal Microscopy Core Unit

Javier Muñoz
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 expertise relevant in oncology, including DNA and chromosome stability (Maria A. Blasco, Óscar Fernández-Capetillo, Massimo Squatrito, Felipe Cortés Ledesma, and Ana Losada), oncogenes and cell cycle kinases (Mariano Barbacid), DNA replication (Juan Méndez), mitosis (Marcos Malumbres), melanoma (María S. Soengas), molecular pathophysiology of epithelial tumours (Francisco X. Real), metabolism and cell signalling (Nabil Djouder and Alejo Efeyan), and metastasis (Manuel Valiente, Eva González Suárez and Héctor Peinado). Our Programme will also soon incorporate a group working in cancer immunotherapy, which will certainly be of help as many of our existing groups have projects related to cancer immunity.

It goes without saying that 2020 has not been the easiest of times in any respect, including for scientific research. Yet, despite the limitations, scientists at the MOP continued to make significant contributions. Thanks to their work, we now have a better understanding of the mechanisms of resistance to cancer therapies and better ideas as to how to optimise treatments to overcome this resistance. We also made interesting discoveries related to how chromosome topology and structure influence cancer onset and overall adult health, and revealed key insights that could help to optimise the efficacy of cancer immunotherapies in tumours such as melanoma or breast cancer. While most of our scientific projects are strictly related to oncology, scientists at the MOP have also made very significant findings related to other age-

“2020 has shown that MOP scientists are still productive in very challenging days. I sincerely wish that 2021 brings us all back the opportunity to enjoy the experience of being a scientist in its full.”

related diseases, such as the discovery of a new mechanism linking viral infections to diabetes or advances in gene therapy for pulmonary fibrosis. Significantly, this last approach has led to the establishment of a new spin-off company that will try to advance the clinical development of telomerase-based gene therapy. Congratulations to Maria Blasco and her team for this important milestone. Finally, I want to note that while virology is not our area of expertise, scientists at the MOP have also tried to be of help and use their knowhow in the battle against covid-19. This exemplifies a common value among scientists; we are here to help.

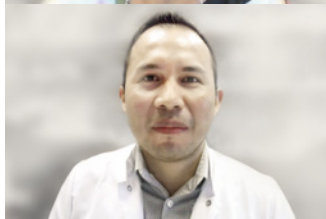
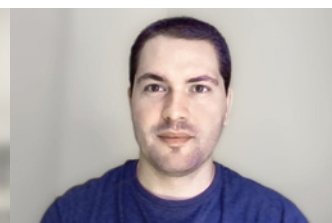
My last sentence from the 2019 annual report was: “Let 2020 be our year”. Well, I was certainly not thinking of this. Yet, in some respects, it was our year. It was the year that we had to reinvent ourselves and overcome many limitations to show that we can still be productive even in the most challenging times. I am always proud to be the Director of the MOP, but even more so this year. Thank you all for making it possible.

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 (since December),
Buyun Ma (since November), Sergio
Piñeiro, Sarita Saraswati



Graduate Students

José Carlos González, Paula Infantes, Óscar Laguía (since November), Jessica Louzame, Amparo Sánchez (since October), Raúl Sánchez

Technicians

Ana Guío (TS)*, Rosa M. Serrano
**Titulado Superior* (Advanced Degree)

Visiting Students

Alejandro Bernardo

(January-February) (Bachelor's Student, *University of Oviedo*, Spain), Sarah M. Gutiérrez (until June) (BS Thesis, *Complutense University of Madrid*, Spain), Aristeidis Michail (until March) (Erasmus Fellowship, *National and Kapodistrian University*

of Athens, Athens, Greece), M. José Romero (July-August) (Summer Traineeship, *Universitat Autònoma de Barcelona*, Spain), Irene Sánchez (since September) (Bachelor's Student, *University of Huddersfield*, UK)

OVERVIEW

We study the mechanisms by which tumour cells are immortal and normal cells are mortal. Immortality is one of the most universal characteristics of cancer cells. The enzyme telomerase is present in more than 95% of all types of human cancers and absent in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes, essential for chromosome protection and genomic stability. Progressive shortening of telomeres associated with organism ageing leads to ageing. When telomeres are altered, adult stem cells have a maimed regenerative capacity.

Our research focuses on:

- Generating mouse models to validate telomeres and telomerase as therapeutic targets for cancer and age-related diseases.
- Interplay between telomeres and DNA repair pathways.
- Role and regulation of non-coding telomeric RNAs or TERRA.
- Testing telomerase gene therapy in “telomere syndromes” and age-related diseases.
- Role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

“We have shown that the targeting of telomere maintenance mediated through the microRNA miR-490 could be therapeutically important in the treatment of glioblastoma.”

RESEARCH HIGHLIGHTS

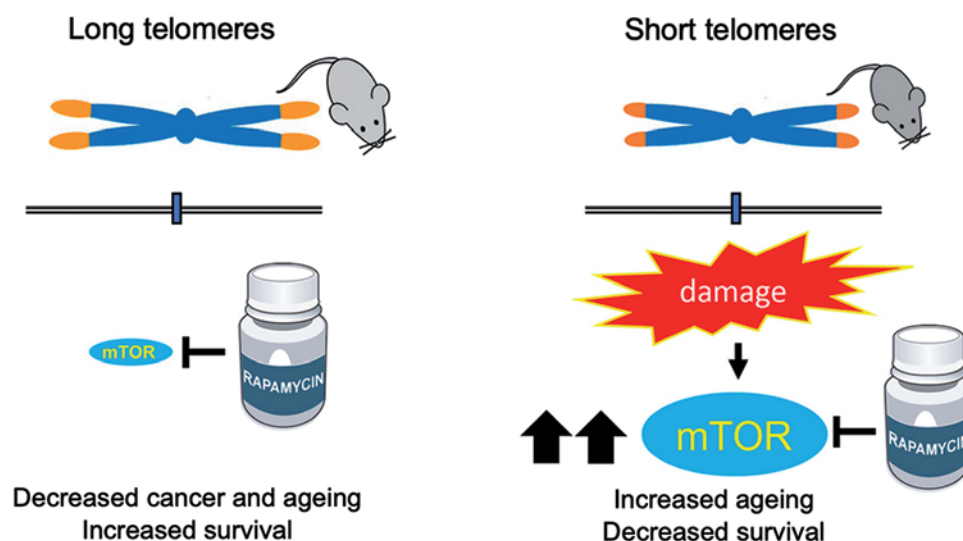
Short telomeres mice need active mTOR pathway for survival

The mechanistic target of rapamycin (mTOR) pathway is a central regulator of cell growth and metabolism. A variety of signals, including growth factors and nutrients, regulate mTOR activity. Inhibition of this nutrient-sensing pathway is considered a therapeutic target to delay ageing and age-related pathologies. mTOR exists in two distinct complexes, mTORC1 and mTORC2, each with different substrates and activities. Of the two, mTORC1 is the only one sensitive to acute rapamycin treatment.

Genetic or pharmacological inhibition of mTORC1 with rapamycin, or with rapamycin-derived compounds, delays ageing and increases the lifespan of mice. There is evidence suggesting that lifespan extension by dietary restriction may partly arise from mTORC1 inhibition. Rapamycin significantly decreases cancer incidence in wild-type mice and it also has immunosuppressant properties.

Since mTOR inhibitors could represent potential treatments for human patients afflicted with telomere syndromes, we addressed whether rapamycin treatment could ameliorate the premature ageing phenotypes and the decreased longevity of telomerase-deficient mice with short telomeres. We found that while chronic rapamycin treatment in mice with telomeres of normal length inhibits mTOR activity and leads to a decrease of cancer and ageing and to increased survival (FIGURE 1), in telomerase-deficient mice with short telomeres it leads to the upregulation of the mTOR pathway and, quite unexpectedly, to the decreased longevity of these mice, a stark contrast to the lifespan extension observed in similarly treated wild-type mice (FIGURE 1). Altogether, our findings demonstrate that hyperactivation of the mTOR pathway as the consequence of short telomeres constitutes a compensatory survival mechanism. In agreement with this, inhibition of this pathway has deleterious effects in telomerase-deficient mice.

FIGURE 1 Opposite outcomes of rapamycin treatment depending on telomere length.



PUBLICATIONS

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Telomerase treatment prevents lung fibrosis associated with physiological ageing

Idiopathic pulmonary fibrosis (IPF) is a potentially lethal disease associated with certain mutations or advanced age, currently lacking a cure. We had shown that specific induction of telomere dysfunction alveolar type II (ATII) cells sufficed to induce progressive and lethal pulmonary fibrosis in mice, demonstrating that dysfunctional telomeres ATII cells are at the origin of IPF. We had also demonstrated that the presence of short telomeres in lung cells triggered IPF in telomerase-deficient mice upon treatment with a low dose of the lung-damaging agent bleomycin. We had also shown that treatment with a telomerase gene therapy that activated telomerase in the lungs stopped lung fibrosis progression in these mice. Evidence from human patients and mouse models with short telomeres indicates that short/dysfunctional telomeres are at the origin of IPF.

It remained unknown whether physiological ageing leads to short telomeres in the lung, and whether this increases the risk of IPF with ageing. We have now found that physiological ageing in wild-type mice leads to telomere shortening and a reduced proliferative potential of ATII cells and club cells, increased cellular senescence and DNA damage, increased fibroblast activation and collagen deposits, and impaired lung biophysics, suggestive of a fibrosis-like pathology. Treatment of ageing wild-type (FIGURE 2) and telomerase-deficient mice with telomerase gene therapy prevented the onset of lung profibrotic pathologies. Short telomeres associated with physiological ageing are at the origin of IPF; a potential treatment for IPF based on telomerase activation would be of interest both for patients with telomerase mutations and sporadic cases of IPF associated with physiological ageing. ■

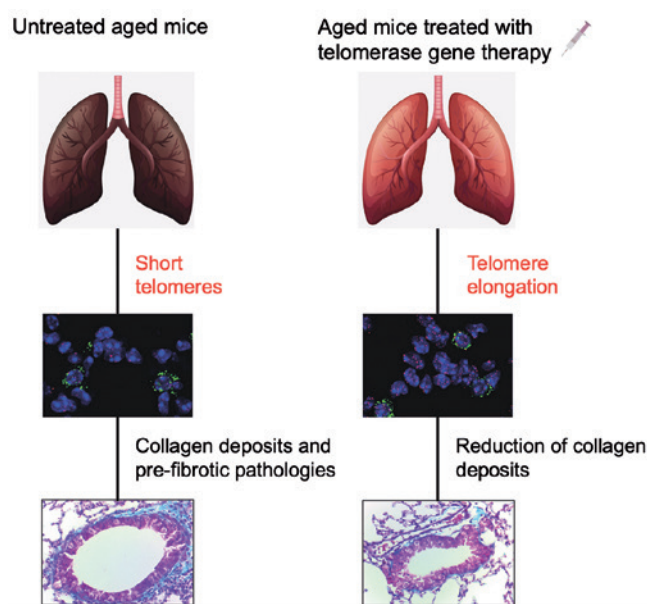


FIGURE 2 Development of ageing-associated initial stages of pulmonary fibrosis in untreated mice (left) is prevented by telomerase gene therapy treatment (right).

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AWARDS AND RECOGNITION

- ▶ Chair of SOMMa ("Severo Ochoa" Centres and "María de Maeztu" Units of Excellence Alliance), Spain.
- ▶ ERC-Advanced Grant SHELTERINS, European Research Council.
- ▶ Spin-off company *Telomere Therapeutics*, a joint venture arising from the laboratories of Maria A. Blasco at CNIO and of Fátima Bosch (Universitat Autònoma de Barcelona) to develop telomerase-based

gene therapies for the treatment of pulmonary and renal fibrosis, Spain.

- ▶ Member of the Scientific Advisory Board of the Institute of Genetics and Molecular and Cellular Biology-IGBMC, Illkirch, Strasbourg, France.
- ▶ Member of the Scientific Advisory Board of the Marseille Cancer Research Centre – CRCM, Marseille, France.
- ▶ Editorial Board Member, *International Journal of Cancer*.

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Marina Malumbres (until May) (Master
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Musteanu (*Universidad Complutense
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OVERVIEW

KRAS oncogenes have been identified in one-fifth of all human cancers. In 2020, selective inhibitors against one of the *KRAS* oncogenic isoforms, *KRAS*^{G12C}, have been developed. Yet all the other isoforms remain undruggable. Moreover, selective inhibitors of *KRAS* signalling pathways have failed in the clinic due to unacceptable toxicities. Previous work in our laboratory, allowed us to identify RAF1 as the only effector within the MAPK pathway whose elimination induced significant tumour regressions without causing major toxicities. In 2020, we have identified CDK4 as a second potential therapeutic target. Combined RAF1 ablation and expression of a CDK4 kinase dead isoform completely prevented tumour progression of *KRAS*/*TRP53* driven lung adenocarcinomas and led to complete regression of a quarter of these tumours without increasing overall toxicity. We trust that the identification of additional targets with potential therapeutic activity may eventually lead to the complete elimination of these aggressive tumours.

“Combined RAF1 ablation and expression of a CDK4 kinase dead isoform completely prevented tumour progression of *KRAS*/*TRP53* driven lung adenocarcinomas and led to complete regression of a quarter of these tumours.”

RESEARCH HIGHLIGHTS

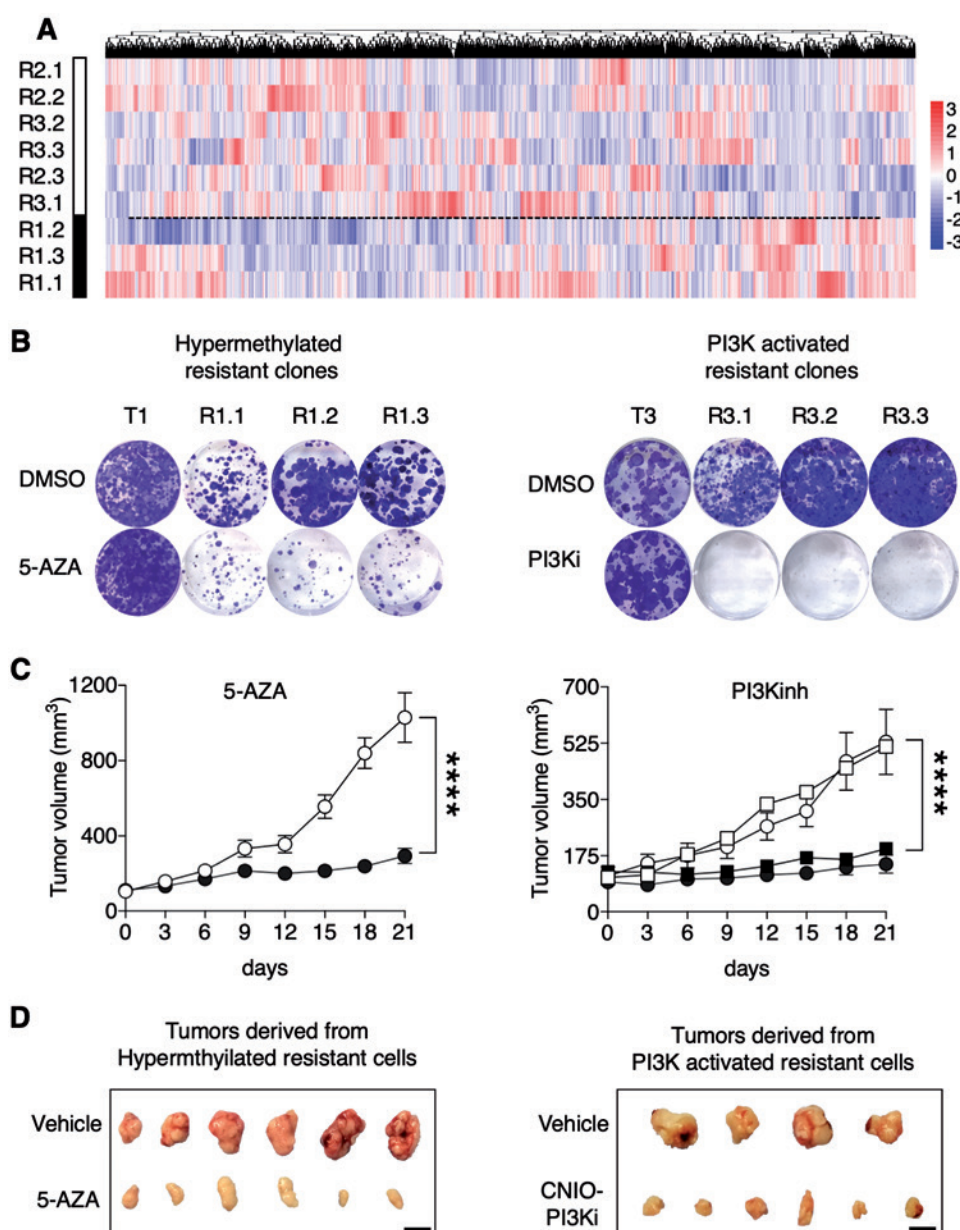
CDK4 and RAF1 are essential for progression of *Kras/Trp53* driven lung adenocarcinomas

Most KRAS mutant lung adenocarcinomas remain intractable for targeted therapies. During 2020, 2 companies, Amgen and Mirati, developed selective inhibitors against one of the KRAS oncogenic isoforms, KRAS^{G12C}, based on the unique properties of the cysteine residue to form covalent bonds. Yet, all other KRAS isoforms remain undruggable. Likewise, no selective inhibitor against KRAS downstream effectors has been approved by the FDA due to their unacceptable toxicities. Genetic interrogation of members of the MAPK pathway along with the interphase CDKs identified CDK4 and RAF1 as the only targets capable of inducing therapeutic responses without causing significant toxicities. We have interrogated the therapeutic consequences of expressing a kinase dead isoform of CDK4 in combination with RAF1 ablation in advanced *Kras/Trp53* driven lung adenocarcinomas. This combination induced complete regression in 25% of the tumours. Moreover, it completely prevented tumour progression. To pharmacologically validate our genetic studies, we compared the therapeutic effect of expressing CDK4^{KD} with that of 2 independent CDK4/6 inhibitors, palbociclib and abemaciclib, in the context of RAF1 ablation. Unfortunately, both inhibitors failed to phenocopy the cooperative effect observed upon genetic inactivation of CDK4. Likewise, we also attempted to pharmacologically validate the therapeutic effect of RAF1 ablation by inhibiting its kinase activity with 4 independent RAF kinase inhibitors, including MLN2480 (Millennium), GW5074 (GSK), PLX8394 (Plexicon), and LSN3074753 (Eli Lilly). Only the latter displayed a sub-micromolar IC₅₀ against cell lines derived from 2 independent KRAS mutant lung PDX models. Treatment of tumour-bearing *Kras^{+/FSFG12V};Trp53^{F/F}* mice for 4 weeks with a combination of abemaciclib and LSN3074753 reduced, but did not prevent tumour growth and only induced partial regressions in 10% of the tumours. No complete regressions were observed. Therefore, pharmacological validation of our genetic results will require the generation of more potent and selective inhibitors.

Characterisation of CDK4/RAF1 resistant tumour cells

In spite of the significant therapeutic response observed upon CDK4 and RAF1 inactivation, most tumours (66%) only underwent partial responses (PRs), indicating the presence of resistant cells. To interrogate those mechanisms implicated in the lack of response to CDK4/RAF1 inactivation, we selected cells able to proliferate in the absence of wild type CDK4 and RAF1. Comparison of the transcriptional profiles of these resistant clones with that of their parental cell line correlated with the presence of 2 independent resistant mechanisms. They included a “hypermethylated” phenotype leading to a significant decrease in the expression of a series of tumour suppressor genes and a transcriptional profile suggestive of a PI3K activated phenotype. To pharmacologically validate this bioinformatic analysis, we exposed the CDK4/RAF1 resistant clones with the hypermethylated phenotype to the demethylation agent 5-azacytidine (5-AZA). These resistant clones, but not their parental cells, were exquisitely sensitive to this drug. Likewise, exposure of resistant clones displaying a transcriptional profile consistent with a PI3K activated phenotype were effectively inhibited by PI3K inhibitors. Interestingly, *Kras/Trp53* mutant lung tumour cells were not sensitive to either 5-AZA or PI3K inhibitors unless CDK4 and RAF1 were previously inactivated, thus demonstrating that these pharmacological vulnerabilities to methylation or PI3K inhibitors represent *bona fide* resistance mechanisms. Nevertheless, the high toxicities displayed by 5-AZA and PI3K inhibitors in the clinic underscores the need for better compounds to combat the resistance to CDK4/RAF1 inhibition. Hopefully, the design of more potent and selective inhibitors against these targets should allow the translation of these results to the clinic in a not-too-distant future. ■

FIGURE 1 Pharmacological validation of the hypermethylated and PI3K activated resistance mechanisms. (A) Heatmap representing colour-coded expression levels of differentially expressed genes among the indicated CDK4/RAF1 resistant clones. (B) Colony formation assay of parental cell lines and CDK4/RAF1 resistant clones treated with DMSO (upper panel) or with 2 μ M of 5-azacytidine (5-AZA) (left panel) or with 1 μ M of PI3K inhibitor (right panel) for 9 days. (C) Tumour growth of CDK4/RAF1 resistant clones treated with vehicle (open circles) or 5-AZA (left panel) or PI3K inhibitor (right panel) (solid circles). Student's T test. **** p < 0,0001. (D) Representative tumours derived from a hypermethylated resistant clone treated with vehicle or with 5-AZA (left panel) and tumours derived from a PI3K activated resistant clone treated with vehicle or with the PI3K inhibitor (right panel). Scale bar: 1 cm.



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AWARDS AND RECOGNITION

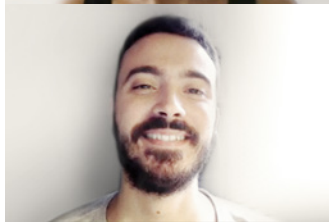
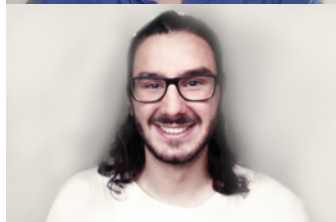
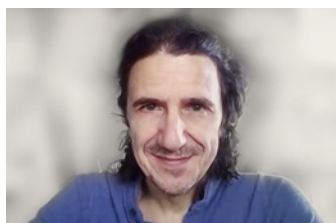
- *Reservista de Honor*, Spanish Ministry of Defense, Madrid, Spain.

- *Echegaray Medal*, Spanish Academy of Sciences, Madrid, Spain.
- *Paget Memorial Lecture*, London, UK.
- *Gordon Peters Lecture*, ESMO MAP Oncology Congress, Amsterdam, The Netherlands.
- *Closing Lecture*, 17th International ASE/ICA Congress, Santiago de Compostela, Spain.
- *Keynote Lecture*, III *CIBERONC* Young Researchers Meeting, Madrid, Spain.

CELL DIVISION AND CANCER GROUP

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Villarroya



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(until June) (*Universidad Carlos III de Madrid*, Spain)

Visiting Scientist

Senn Wakahashi (Kobe University, Kobe, Japan)

OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated in mammalian cells. Our scientific interests are:

- to understand the basic control mechanisms that regulate the cell division cycle.
- to characterise the physiological and therapeutic consequences of cell cycle deregulation.
- understanding self-renewal and pluripotency in stem cell biology and tumour development.
- 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.

“Our group has proposed new therapeutic uses of CDK4/6 inhibitors in metastatic cancer, as well as new strategies to improve the function of pluripotent cells in regenerative medicine.”

RESEARCH HIGHLIGHTS

Cell cycle inhibition in cancer in cancer therapy

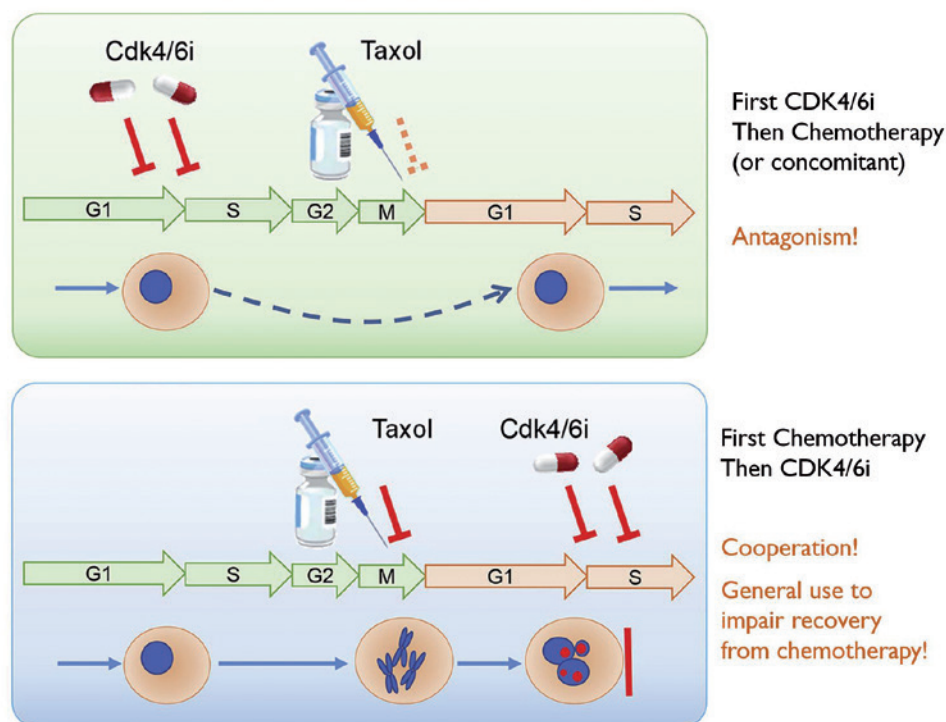
Inhibition of the cell cycle kinases CDK4 and CDK6 is currently part of the standard-of-care for the treatment of hormone receptor-positive, metastatic breast cancer. Inhibiting CDK4/6 activity is also considered an attractive therapeutic intervention for multiple other malignancies. However, it is generally assumed that these inhibitors should not be used in combination with classical chemotherapy, given that CDK4/6 inhibition arrests cells in G1, thereby protecting tumour cells from the cytotoxic effect of classical chemotherapy acting either in S-phase or mitosis in proliferating cells. Unfortunately, classical chemotherapy (DNA damaging agents, topoisomerase inhibitors, taxanes etc.) remains the treatment of choice for most patients with advanced disease. Using pancreatic adenocarcinoma (PDAC) as a model, we recently generated data suggesting that, both *in vitro* and *in vivo*, applying CDK4/6 inhibitors right after taxanes strongly cooperates to prevent tumour cell proliferation (FIGURE 1). We also demonstrated that the mechanism behind these observations is different from the classical model in which CDK4/6 are required for S-phase entry. We described that CDK4/6 activity is required for homologous recombination and DNA repair, and the recovery from the chromosomal damage imposed by taxanes or DNA damaging agents. This mechanism immediately suggests that CDK4/6 inhibitors could be efficiently used after a variety of classical chemotherapies, including nucleotide analogues, topoisomerase poisons and other DNA damaging agents, microtubule poisons, targeted anti-mitotic therapies, etc., as well as radiation. These results may have a major impact on the application of cell cycle inhibitors in the clinic in a variety of tumour types, and we are currently evaluating possible scenarios to move this strategy into clinical trials in breast cancer patients.

Improving the use of pluripotent cells in regenerative medicine

How pluripotent cells control their self-renewal and differentiation potential is becoming a major research topic in our laboratory. Our recent work suggests that a microRNA expressed in early development, miR-203, is able to induce naive pluripotency in both murine and human induced pluripotent cells (iPSC) and embryonic stem cells (ESC), thereby enhancing the potential of these cells *in vitro* and *in vivo*. Mechanistically, this effect is mediated through the repression of *de novo* DNA methyltransferases Dnmt3a and Dnmt3b and, global, but transient, genome demethylation. Application of miR-203 to iPSCs or ESCs mediates the resetting of the epigenetic memory and improves the developmental potential of these cells in multiple assays, including generating or live mice, complementation of tetraploid embryos, or interspecies assays in which human pluripotent cells are aggregated into mouse embryos. Exposure to miR-203 enhances the differentiation of mesenchymal cells from pluripotent cells *in vitro*, and improves the recovery from heart injuries in a model of cardiac infarctions in mice. These findings may have important potential implications in regenerative medicine that we plan to study in the upcoming years.

Finally, we are using a variety of genetically-modified mice and iPS/ES cells with specific mutations in cell cycle regulators to understand the basic mechanisms of control of cell cycle progression and self-renewal in pluripotent cells. Our preliminary data suggest interesting connections between cell cycle kinases and phosphatases, the developmental potential of neural progenitors, and the generation of developmental syndromes with defects in the nervous system, including primary microcephaly, a developmental defect resulting in smaller brain at birth. The molecular connections between centrosome dynamics, cell cycle regulation, and cell fate in neural progenitors are currently under analysis in these models. ■

FIGURE 1 A new strategy for the use of CDK4/6 inhibitors (CDK4/6i) in cancer therapy. It is well established that CDK4/6i impair entry into the cell cycle, thus antagonising the cytotoxic effect of classical chemotherapy (upper panel). However, our recent data suggest the sequential use of CDK4/6i “after” chemotherapy (bottom), thereby preventing DNA repair and recovery from the cytotoxic effect of many drugs that are the standard-of-care in a wide variety of metastatic cancers.



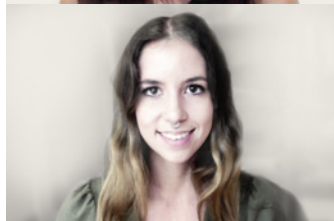
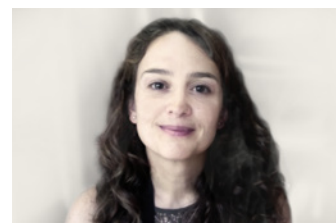
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GENOMIC INSTABILITY GROUP

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**Titulado Superior (Advanced Degree)*

Visiting Student

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OVERVIEW

In the Genomic Instability Group, our main goal is to understand the molecular mechanisms underlying cancer and other age-related diseases, and then use this knowledge for the development of new therapies. To this end, we combine molecular and cellular biology approaches with chemical and genetic screens that allow us to identify cancer cell vulnerabilities and new druggable targets. In parallel, we develop mouse models of disease, which we can later use as platforms for testing new treatments. With all these tools, in recent years, we have made exciting discoveries in several areas, from basic to translational research. We have contributed to the understanding of fundamental aspects of DNA replication, unveiled new mechanisms of resistance to cancer therapies, and developed anticancer drugs that are now in clinical development. Ultimately, our objective is to translate our findings into better treatments for human disease. During 2020 we made significant advances in several areas related to cancer ontogeny and therapy. For instance, we revealed a tumour suppressor role for the RNA-binding protein EWSR1 and made significant advances in the development of chemical inhibitors of the histone methyltransferase SETD8.

RESEARCH HIGHLIGHTS

EWSR1 loss drives nucleolar stress and cancer development

Metastatic Ewing Sarcoma (ES) is a paediatric bone tumour driven by translocations that frequently involve the RNA-binding protein EWSR1 (e.g., EWSR1-FLI1). We previously demonstrated that ES shows remarkable susceptibility to a treatment with ATR inhibitors that were developed by our Group and the CNIO Experimental Therapeutics Programme. While most of the focus in ES research has been placed on understanding the role of EWSR1-harbouring translocations, it is becoming clear that loss of EWSR1 could also potentially contribute to carcinogenesis. To address this possibility, we developed constitutive and conditional knockout mouse models of EWSR1. Constitutive EWSR1 nullzygosity leads to anaemia and is embryonic lethal, indicating a particular impact on the immune system. Consistently, ubiquitous deletion of EWSR1 in

adult animals leads to a fully penetrant early onset of thymomas to which mice succumb within the first 6 months of life (FIGURE 1). These results are consistent with recent large-scale cancer genomic studies that have identified recurrent EWSR1 gene deletions in human thymomas. EWSR1 deficient MEF or thymoma cell lines presented an accumulation of nucleolar stress, which increased their sensitivity to RNA Polymerase I inhibitors. Altogether, this work identified EWSR1 as a tumour suppressor and revealed vulnerabilities in EWSR1-deficient cells that could be exploited for potential treatments.

Developing new SETD8 chemical inhibitors

Drugs targeting the epigenetic machinery are a promising avenue for cancer therapy, particularly in the context of paediatric tumours where the mutational load is low and they are frequently

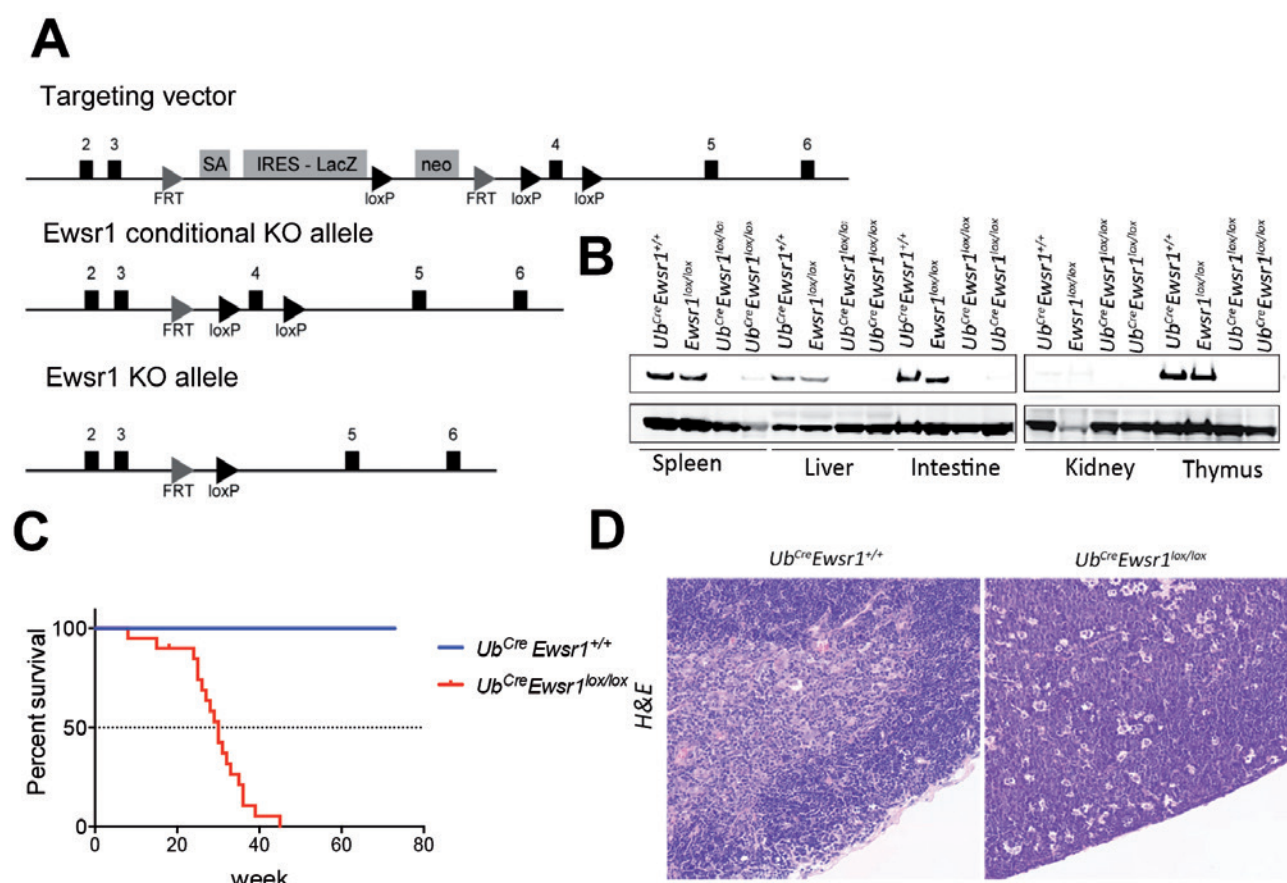
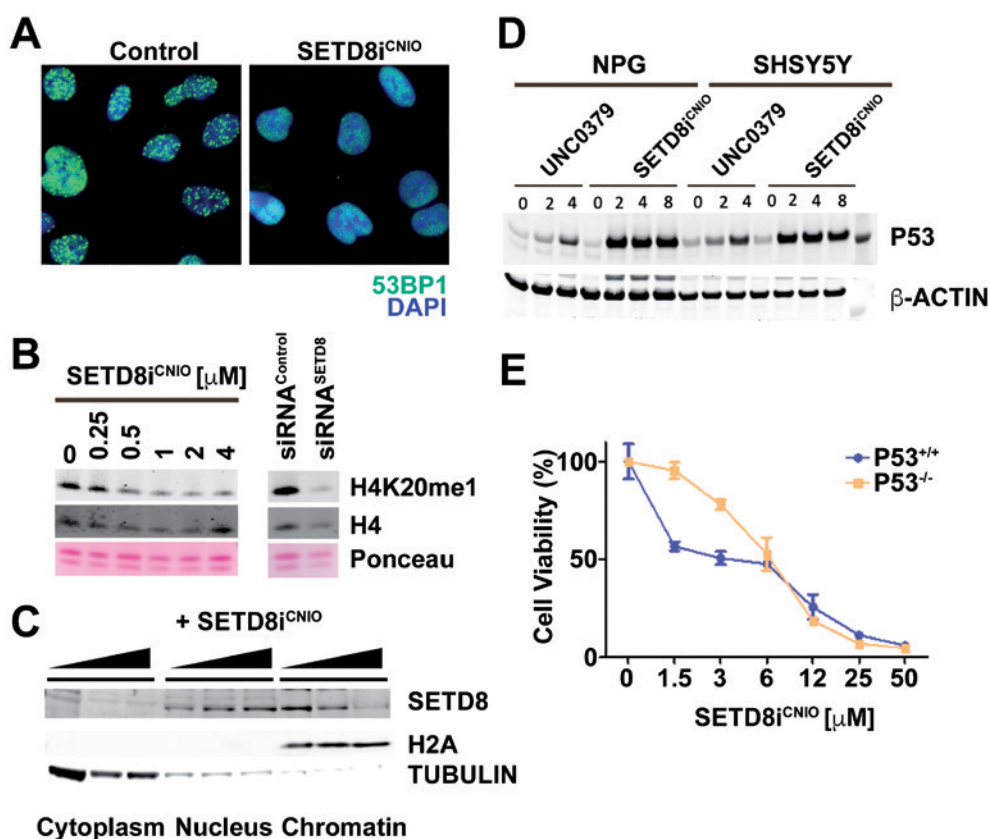


FIGURE 1 EWSR1 deletion in adulthood leads to thymoma formation in mice. (A) Scheme of the *Ewsr1* conditional knockout targeting strategy and alleles generated. (B) Depletion of EWSR1 expression (upper band) in several tissues of the *Ewsr1* conditional

knockout mice after treatment with tamoxifen for 1 week to activate a ubiquitously expressed Cre-ER recombinase. The expression of β -ACTIN (lower band) is shown as a loading control. (C) Survival curves for *Ub^{Cre}Ewsr1^{+/+}* and *Ub^{Cre}Ewsr1^{lox/lox}* mice

after treatment with tamoxifen since week 6. (D) Representative examples of the haematoxylin/eosin staining of thymuses obtained from tamoxifen-treated 4 month old *Ub^{Cre}Ewsr1^{+/+}* and *Ub^{Cre}Ewsr1^{lox/lox}* mice.

FIGURE 2 Properties of CNIO SETD8 inhibitors. (A) SETD8^{iCNIO} inhibits the formation of irradiation induced 53BP1 foci in U2OS cells (10Gy, 1hr). (B) Dose-dependent reduction of H4K20me1 in U2OS cells treated with SETD8^{iCNIO}. The effect of SETD8 depletion by siRNA is shown as control. (C) SETD8^{iCNIO} leads to a dose-dependent displacement of SETD8 from chromatin. (D) Comparison of the effect of SETD8^{iCNIO} and the commercial SETD8 inhibitor UNC0379 in increasing P53 levels in 2 neuroblastoma cell lines. Note the bigger effect of the CNIO compound at the same doses. (E) Comparison of the toxicity of SETD8^{iCNIO} in wild type and P53-deficient HCT116 cells. Similar effects were observed with all SETD8 inhibitors tested.



associated with stem-cell differentiation anomalies. In this context, recent evidence has indicated that targeting the histone methyltransferase SETD8 might have antitumour effects in certain paediatric tumours of poor prognosis, such as neuroblastoma or medulloblastoma. However, the few currently available SETD8 inhibitors show poor potency and pharmacological properties, and none has reached clinical trials. Our group, through a previous collaboration with the laboratory of Dr Modesto Orozco at the *IRB* (Barcelona), identified a new class of chemical SETD8 inhibitors, with the main compound (SETD8^{iCNIO}) significantly reducing histone 4 monomethylation (H4K20me1) levels in cellular assays. The compounds also trigger the known cellular effects associated to SETD8 inhibition, such as increasing the expression of P53

or inhibiting the function of DNA repair factors like 53BP1 (FIGURE 2). Hence, we currently have a new class of SETD8 inhibitors with cellular activity in the mid-nanomolar range. Regarding their mechanism of action, our current data already reveal that, contrary to current proposals, and despite the increase in p53 levels observed when cells are exposed to SETD8 inhibitors, the cell death induced by these compounds is unrelated to P53 and instead related to other aspects of cellular metabolism such as nucleolar integrity. As we did in the past for ATR inhibitors, we are currently exploring other essential aspects to optimise the clinical application of these new agents as anticancer drugs, such as to define the type of tumours that are more likely to respond to the therapy (biomarkers), and the mechanisms of resistance that might emerge upon treatment. ■

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AWARDS AND RECOGNITION

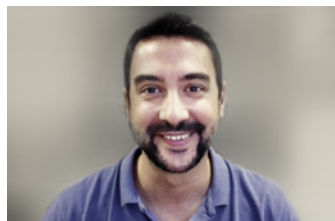
- ERC Proof of Concept (PoC) Grant to develop SETD8 inhibitors, European Research Council.
- Section Editor, *Molecular Oncology*.

TOPOLOGY AND DNA BREAKS GROUP

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OVERVIEW

DNA topoisomerases have a dual relationship with the genome. They are essential to solve the 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 proven a causal link between spontaneous DNA breaks induced by topoisomerase II and tumorigenesis in mouse models of lymphoid and prostate cancer.”

RESEARCH HIGHLIGHTS

In 2020, our work mostly focused on understanding DNA topoisomerase II (TOP2) function and how double-strand breaks (DSBs) derived from its aberrant action can compromise genome integrity and drive tumorigenesis.

Machine learning to predict topoisomerase II function genome wide

We have performed an unbiased analysis of available chromatin and DNA sequence features in order to establish which of them determine TOP2 binding genome wide. We achieved highly accurate predictions, with accessible chromatin and architectural factors being the most informative features. Strikingly, we found that TOP2 is sufficiently explained by only 3 features: DNase I hypersensitivity, CTCF and cohesin binding, for which genome-wide data are widely available. Based on this, we developed a predictive model for TOP2 genome-wide binding that can be used across cell lines and species, and generate virtual probability tracks that accurately mirror experimental ChIP-seq data. These results deepen our knowledge on how the accessibility and 3D chromatin organisation of TOP2 function and constitute a proof of principle regarding the *in silico* prediction of sequence-independent chromatin-binding factors. The methodology may now be used to predict TOP2 function in multiple cell-types, organisms and conditions, boosting our understanding of TOP2 biology and its implications in the origin of oncogenic translocations and other types of chromosomal rearrangements as potential cancer drivers.

Topoisomerase II-induced DNA breaks and lymphoid cancer linked to the human cancer-prone genetic syndrome Ataxia Telangiectasia

The ATM kinase is a master regulator of the DNA damage response to DSBs and a well-established tumour suppressor whose loss is the cause of the neurodegenerative and cancer-prone syndrome Ataxia-Telangiectasia (A-T). A-T patients and

Atm^{-/-} mouse models are particularly predisposed to develop lymphoid cancers derived from deficient repair of RAG-induced DSBs during V(D)J recombination. We have unexpectedly found that specifically disturbing the repair of TOP2-induced DSBs by genetically removing the highly specialised repair enzyme TDP2 strongly increases the incidence of thymic tumours in *Atm*^{-/-} mice, but without changing their molecular characteristics or underlying genomic rearrangements, including a significant association with *Tcr* loci. Furthermore, we found that TOP2 strongly colocalises with RAG, both on a genome-wide scale and specifically at sites undergoing V(D)J recombination, in a manner that is consistent with its involvement in solving topological problems associated to 3D genome organisation, and that results in an increased chromosomal fragility of these regions. Thus, our findings demonstrate a strong causal relationship between spontaneous TOP2-induced DSBs and cancer development, confirming these lesions as major drivers of ATM-deficient lymphoid malignancies, and potentially other conditions and cancer types.

Topoisomerase II-induced DNA breaks and prostate cancer

TOP2 has been previously linked to the regulation of hormone-induced transcription, and in particular, to the activation of androgen-responsive genes. The mechanism by which this occurs, and whether the induction and repair of DSBs is involved, remain, however, poorly understood. In collaboration with the group of Hiroyuki Sasanuma and Shunichi Takeda (University of Kyoto) we have addressed the involvement of TOP2 and TDP2 in the response to androgen. We found that physiological concentrations of androgens induce TOP2-mediated DSBs that are repaired by TDP2 in human prostate cancer cells and prostate epithelium in mouse models. Furthermore, we found that TDP2-deficient mice spontaneously develop higher levels of prostate hyperplasia when compared to wild-type animals. These results suggest that endogenous TOP2-mediated DSBs resulting from androgen signalling can drive prostate hyperplasia and influence the development of prostate cancer. ■

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FIGURE 1 Comparison between experimentally determined (ChIPseq) and machine learning-predicted TOP2 binding genome wide. (A) Representative USSC genome browser views of experimental and predicted TOP2 binding in a representative genomic region in the indicated mouse tissues or cell types. (B) Representative USSC genome browser views of experimental (two independent ChIP-seq experiments; R1 and R2) and predicted TOP2 binding in the human MCF7 breast cancer cell line. Annotated genes in each region are indicated.

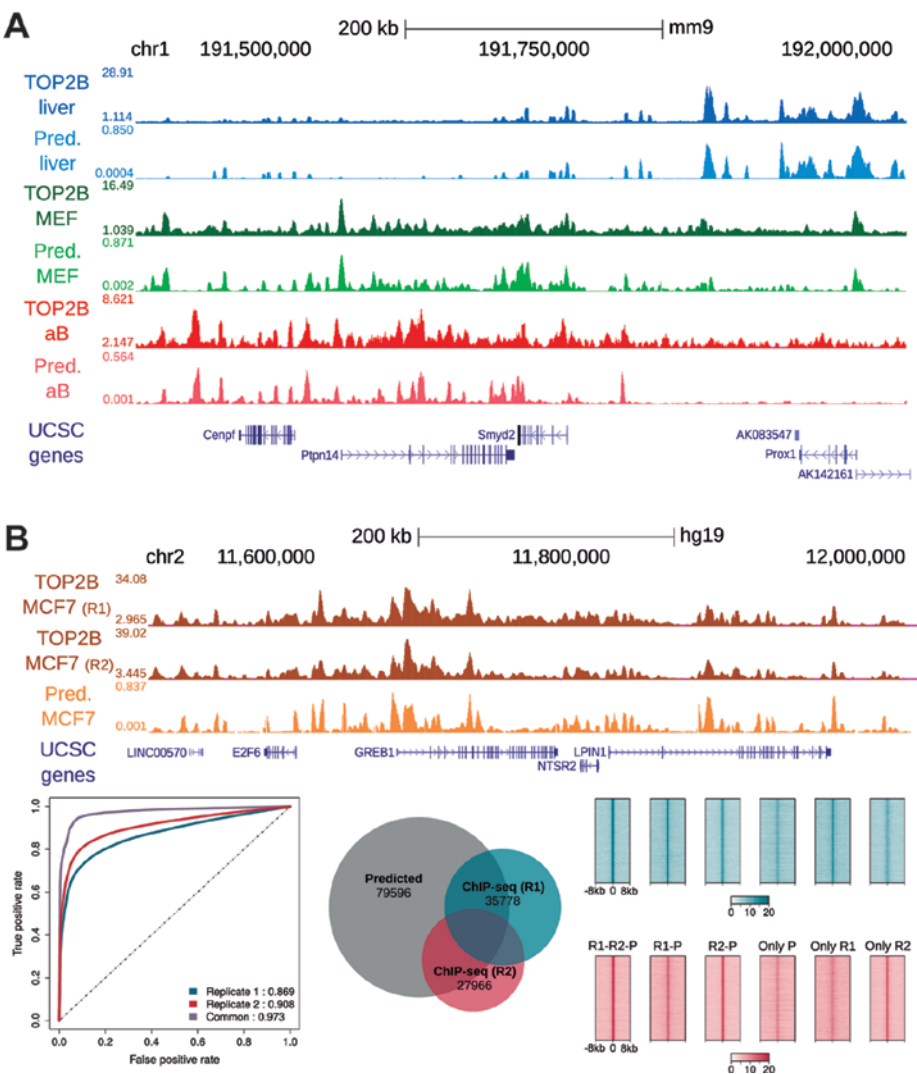
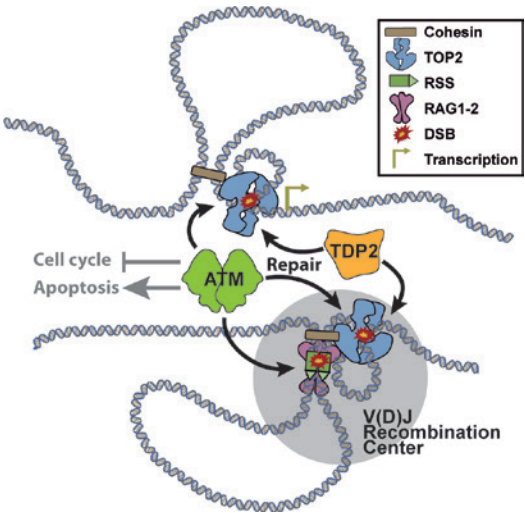


FIGURE 2 Model to explain aberrant TOP2 activity as a driver of ATM-deficient thymic tumours. TOP2 activity accidentally results in DSBs throughout the genome (top). Additionally, TOP2-DSBs arise associated to V(D)J genome reorganisations (bottom), concurring with RAG-mediated DSBs. TDP2 and ATM limit the oncogenic potential of these lesions.

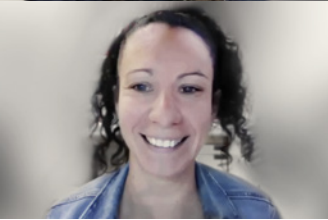
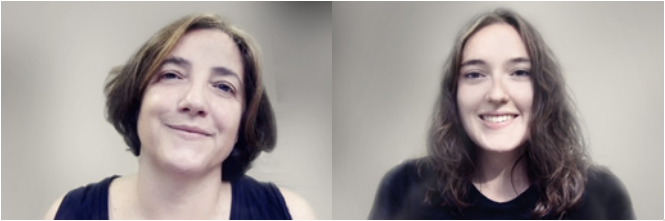


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OVERVIEW

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 have found that cohesin STAG2 is essential during embryonic development, while its loss in adult mice reduces fitness without increasing tumour incidence.”

RESEARCH HIGHLIGHTS

Cohesin STAG2 is essential for mouse embryonic development

Cohesin consists of 4 core subunits, SMC1, SMC3, RAD21 and STAG/SA. There are two versions of the STAG subunit in vertebrate somatic cells, STAG1 and STAG2, giving rise to two distinct complexes. These are present in all tissues and cell types, but their functional specificity is not well established. *STAG2* is commonly mutated in cancer, and germline mutations in both *STAG1* and *STAG2* have been recently identified in cohesinopathy patients. To identify specific functions of STAG2 at the cellular and organismal levels and to better understand the pathological consequences of its loss, we generated and characterised a *Stag2* conditional knock out mouse strain in collaboration with the groups of F. X. Real (CNIO) and M. Manzanera (CNIC, CBMSO). We found that embryos lacking cohesin-STAG2 die by mid-gestation, showing global developmental delay and a selective defect in the developing heart, most prominently in structures derived from secondary heart field progenitors. Both decreased proliferation and altered transcription of tissue-specific genes likely contribute to these defects. In contrast to the embryonic lethality, STAG2 is largely dispensable in adults, and its tissue-wide inactivation does not lead to tumours but reduces fitness and affects both haematopoiesis and intestinal homeostasis. We also analysed the consequences of *Stag2* deletion in mouse embryo fibroblasts (MEFs). *Stag2*-null MEFs show mild centromeric cohesion defects and proliferate more slowly than wild type MEFs, but they are viable. Likewise, we had previously reported that *Stag1*-null MEFs display telomere cohesion defects that impair chromosome segregation, but they are also viable. Thus, cells growing in culture can survive with a single cohesin complex carrying either STAG1 or STAG2, while the two complexes are required to fulfil embryonic development (FIGURE 1).

Different dynamics of the two cohesin variants underlie their differential contribution to 3D genome organisation

Cohesin and the proteins that regulate its association to chromatin (NIPBL, PDS5A/B, WAPL, ESCO1/2, CTCF) are key for shaping genome architecture. Our previous studies in human epithelial cells and mouse embryonic stem cells knocked down for STAG1 or STAG2 identified differential contributions of the two complexes. Cohesin-STAG1, together with the architectural protein CTCF, plays a more important role in the demarcation of topological associated domains (TADs) while cohesin-STAG2 promotes more local chromatin contacts that are relevant for tissue-specific transcription independently of CTCF. Analysis of the distribution of cohesin and its regulators in MEFs confirmed that the two variants occupy CTCF-bound positions while STAG2 can be additionally found at non-CTCF sites (FIGURE 2). PDS5A and PDS5B are located at the former positions while NIPBL is enriched preferentially at the latter. Unlike previous observations in *Stag1* deficient MEFs, in which the distribution of cohesin changed to include new non-CTCF positions, the number of cohesin binding sites detected in *Stag2* deficient MEFs is restricted to those overlapping with CTCF. This situation, which occurs in several tumours with loss of function mutations in *STAG2*, alters gene expression. Salt extraction of chromatin fractions and fluorescence recovery after photobleaching (FRAP) experiments in MEFs show that cohesin-STAG2 binding to chromatin is more dynamic than binding of cohesin-STAG1. In addition, we have observed a preferential association of STAG2 and the cohesin releasing factor WAPL, while others have reported a stronger interaction between STAG1 and CTCF. We are currently exploring the molecular determinants of these preferences and how they contribute to shape chromatin architecture. ■

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FIGURE 1 Scheme that summarises our findings in the characterisation of *Stag1* KO and *Stag2* KO cells (MEF, mouse embryo fibroblasts, top) and embryos (bottom). Both STAG1 and STAG2 cohesins are required for embryonic development while either one is sufficient for cell viability.

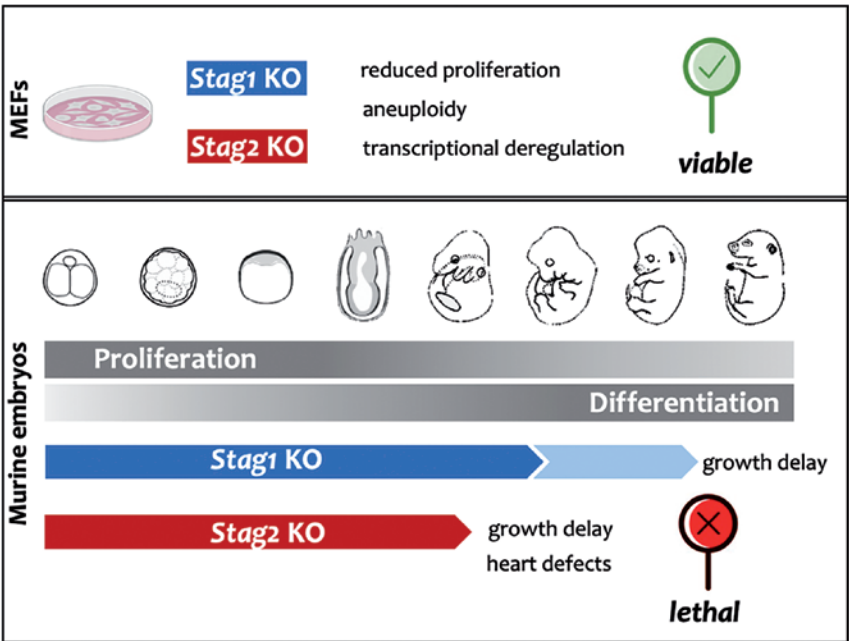
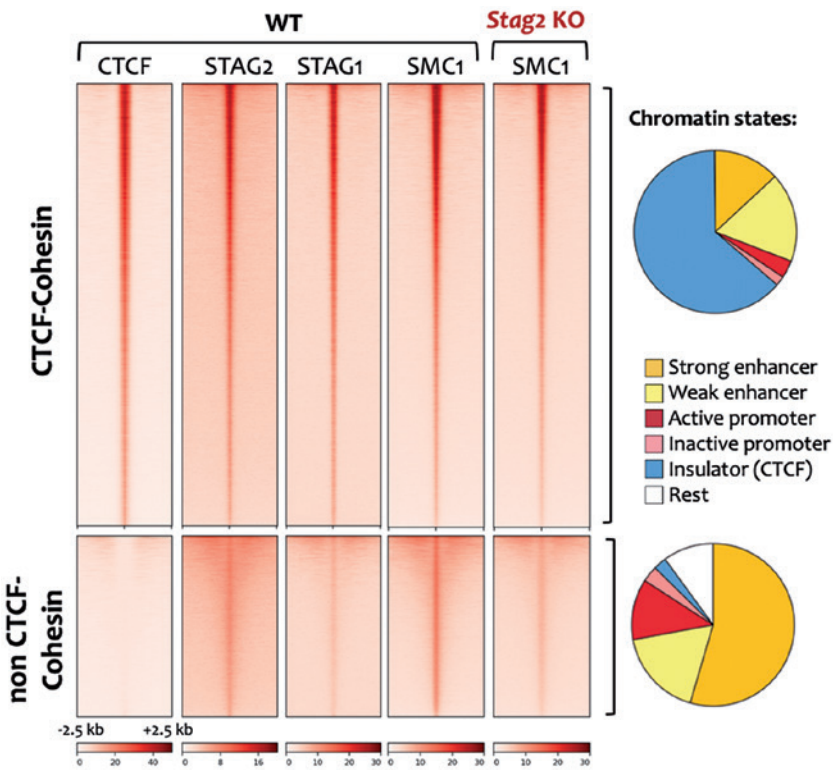


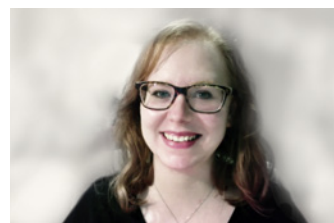
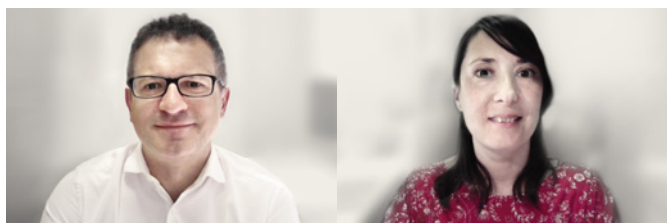
FIGURE 2 Heatmaps showing the distribution of CTCF and cohesin subunits in wild type (WT) MEFs. Two types of positions are found, with or without CTCF. STAG2 is the preferred variant in non-CTCF cohesin positions, which are located at enhancers and promoters (pie charts on the right). Cohesin occupancy in these positions decreases in *Stag2* KO MEFs (compare heatmaps for SMC1 in WT and *Stag2* KO MEFs), suggesting that they cannot be occupied by cohesin STAG1, even in the absence of STAG2.



DNA REPLICATION GROUP

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

OVERVIEW

The process of DNA replication is responsible for many of the genomic alterations underlying the activation of oncogenes or the inactivation of tumour suppressor genes. While some of these alterations are inherent to life, e.g. the introduction of mutations due to DNA polymerase errors, others are caused by environmental agents – UV light, ionising radiation, toxic chemicals in tobacco smoke, and other pollutants – that induce chemical modifications in the DNA and complicate its replication. The capacity to generate difficult-to-replicate DNA modifications, e.g. covalent links between the two strands of the double helix, is the basis for the cytotoxic effect of cisplatin and other drugs used in cancer therapy. Our laboratory studies how the “replisome” machinery is capable of operating through these lesions, a step that normally leads to the activation of specific DNA repair pathways. In 2020 we focused on the study of the PrimPol enzyme that mediates the replicative tolerance of DNA crosslinks generated by common chemotherapy agents.

“We have identified that PrimPol facilitates the tolerance and repair of DNA inter-strand crosslinks, making it a suitable molecular target to enhance the efficacy of chemotherapy.”

RESEARCH HIGHLIGHTS

The slowdown of DNA synthesis caused by DNA lesions, hard-to-replicate special structures or collisions with transcription proteins is referred to as “replicative stress” (RS). In previous years we reported that human PrimPol protein mediates the bypass of UV-generated DNA lesions by synthesising primers that allow re-initiation of DNA synthesis from a downstream point, leaving behind short non-replicated gaps. In 2020 we found that PrimPol-mediated repriming is involved in the tolerance and repair of inter-strand crosslinks (ICLs), one of the most cytotoxic DNA lesions. We also participated in collaborative studies that underscore the importance of repriming in cancer cells deficient in BRCA proteins, and in the response to DNA lesions induced by benzo[*a*]pyrene, an ubiquitous environmental carcinogen.

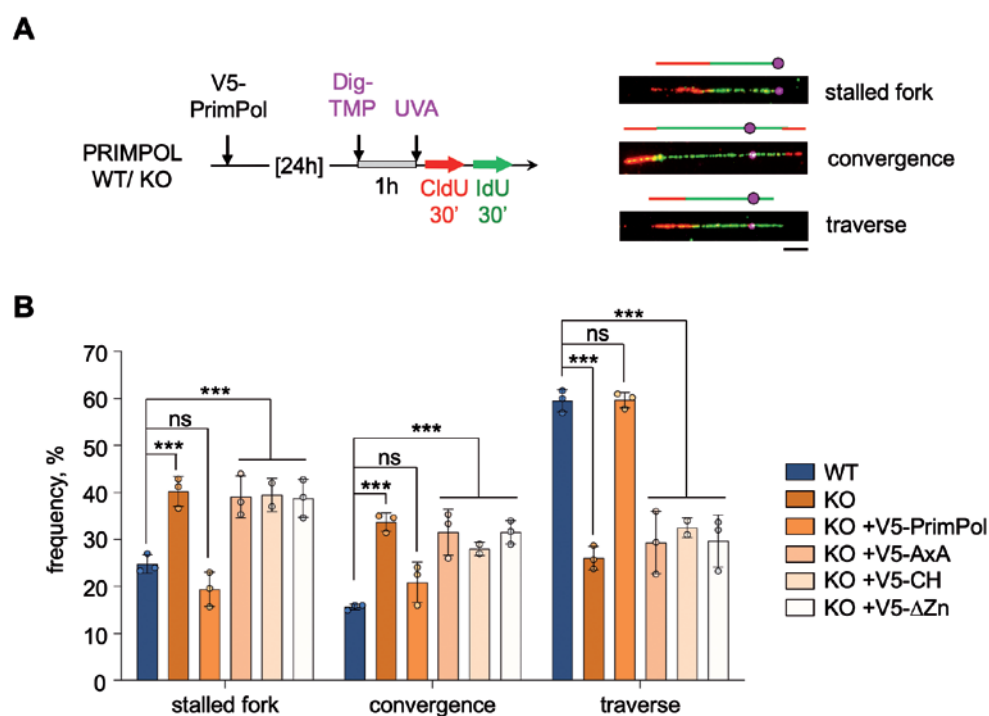
Replicative tolerance mediated by PrimPol

PrimPol is the only enzyme with primase activity identified in mammalian cells besides the Pol α /primase that initiates DNA synthesis at replication origins. It facilitates replication through UV-induced CPD and 6,4pp DNA adducts. During the last year we found that PrimPol is required to elicit the repair of DNA ICLs caused by endogenous aldehydes, chemotherapy agents (e.g. cisplatin), and chemicals used to treat certain skin conditions (e.g. trimethylpsoralen). ICL recognition and repair

requires DNA replication and a combination of homologous recombination, translesion synthesis and nucleotide excision repair. Mutations in ICL repair genes cause Fanconi Anaemia, a rare but severe disease associated to congenital abnormalities, bone marrow failure, and predisposition to leukaemia and solid tumours.

Our recent research indicates that PrimPol interacts with proteins that recognise ICLs such as the BTR complex (Bloom’s-Top3A-RMI1-RMI2) and the FANCM translocase complex (FANCM-MHF1-MHF2-FAAP24), and plays an important role in the progression of the replisome through ICLs, also called “ICL traverse”. Using an assay to monitor DNA replication in stretched DNA fibres in the presence of ICLs, we have found that the catalytic activity of PrimPol is required for efficient ICL traverse (FIGURE 1). Genetic ablation of PRIMPOL in human cells and mice leads to hypersensitivity to ICL-inducing agents, as indicated by the higher incidence of chromosomal lesions (FIGURE 2), and delays ICL repair. Of note, the FA pathway can be activated without PrimPol by an alternative way that requires the convergence of two replication forks at each ICL lesion. The role of PrimPol in ICL traverse reveals a new molecular element in the complex pathways leading to ICL repair (González-Acosta *et al.*, 2020).

FIGURE 1 PrimPol mediates ICL traverse. (A) Left, experimental design. Right, individual DNA fibres with different patterns of DNA synthesis around an ICL lesion. (B) Percentage of each pattern in the experimental conditions tested. AxA, PrimPol catalytic mutant. CH and Δ Zn, primase-null, polymerase-proficient PrimPol mutants. Statistical analysis: two-way ANOVA and Bonferroni post-test. ***, $p < 0.001$. Adapted from González-Acosta *et al.* (2020).



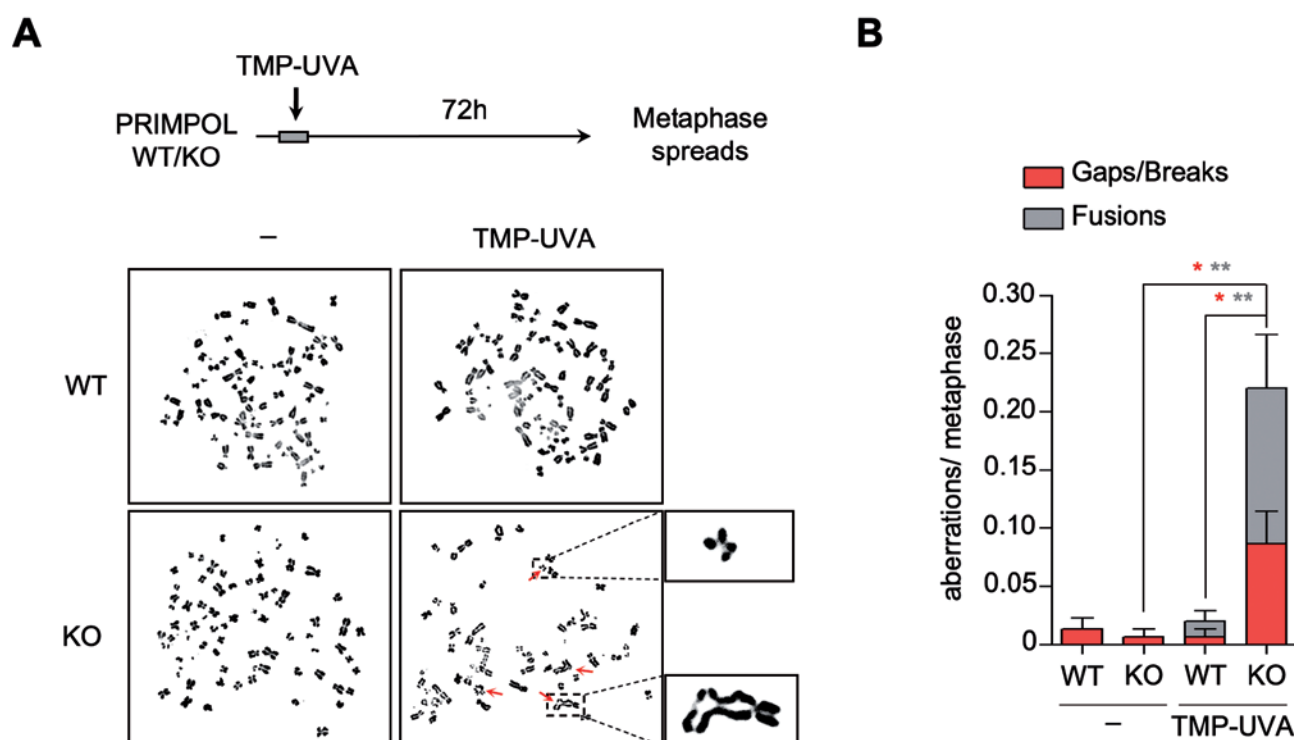


FIGURE 2 PrimPol loss sensitises cells to ICL-inducing agents. (A) Experimental design and metaphase spreads from control or TMP-UVA-treated WT and PrimPol KO cells.

Examples of chromosome breaks and fusions are highlighted. (B) Quantification of chromosome alterations per metaphase (average and SEM) in each condition. Statistical

analysis: one-way ANOVA and Bonferroni post-test. *, p<0.05; **, p<0.01. Adapted from González-Acosta *et al.* (2020).

In collaboration with A. Vindigni (Washington University, St Louis, USA), we learnt that PrimPol mediates an adaptive response to cisplatin in BRCA-deficient cells that have lost the ability to stabilise stalled forks in situations of RS (Quinet *et al.*, 2020). In addition, we participated in a study led by E. Petermann (University of Birmingham, UK), showing that PrimPol acts on bulky DNA adducts caused by benzo[α]pyrene-diol-epoxide (BPDE). In this case, Rad51 protein is recruited to the ssDNA gaps generated by repriming and mediates the post-replicative repair of BPDE-induced adducts by homologous recombination.

Because PrimPol counteracts the cytotoxic effect of DNA crosslinks, we hypothesise that it could be targeted to increase the efficacy of chemotherapy based on crosslinking

agents. A screening for PrimPol small molecule inhibitors is underway, supported by the CNIO Experimental Therapeutics Programme.

DNA replication in Pds5-deficient cells

We are also interested in the molecular connections between DNA replication and the cohesin complex involved in sister chromatid cohesion, chromatin organisation, transcriptional regulation, and DNA repair. In collaboration with Ana Losada's Group (CNIO), we learnt that the cohesin-associated factors PDS5A and PDS5B are required for proper replication fork progression and BRCA2-mediated protection of stalled forks (Morales *et al.*, 2020). ■

PUBLICATIONS

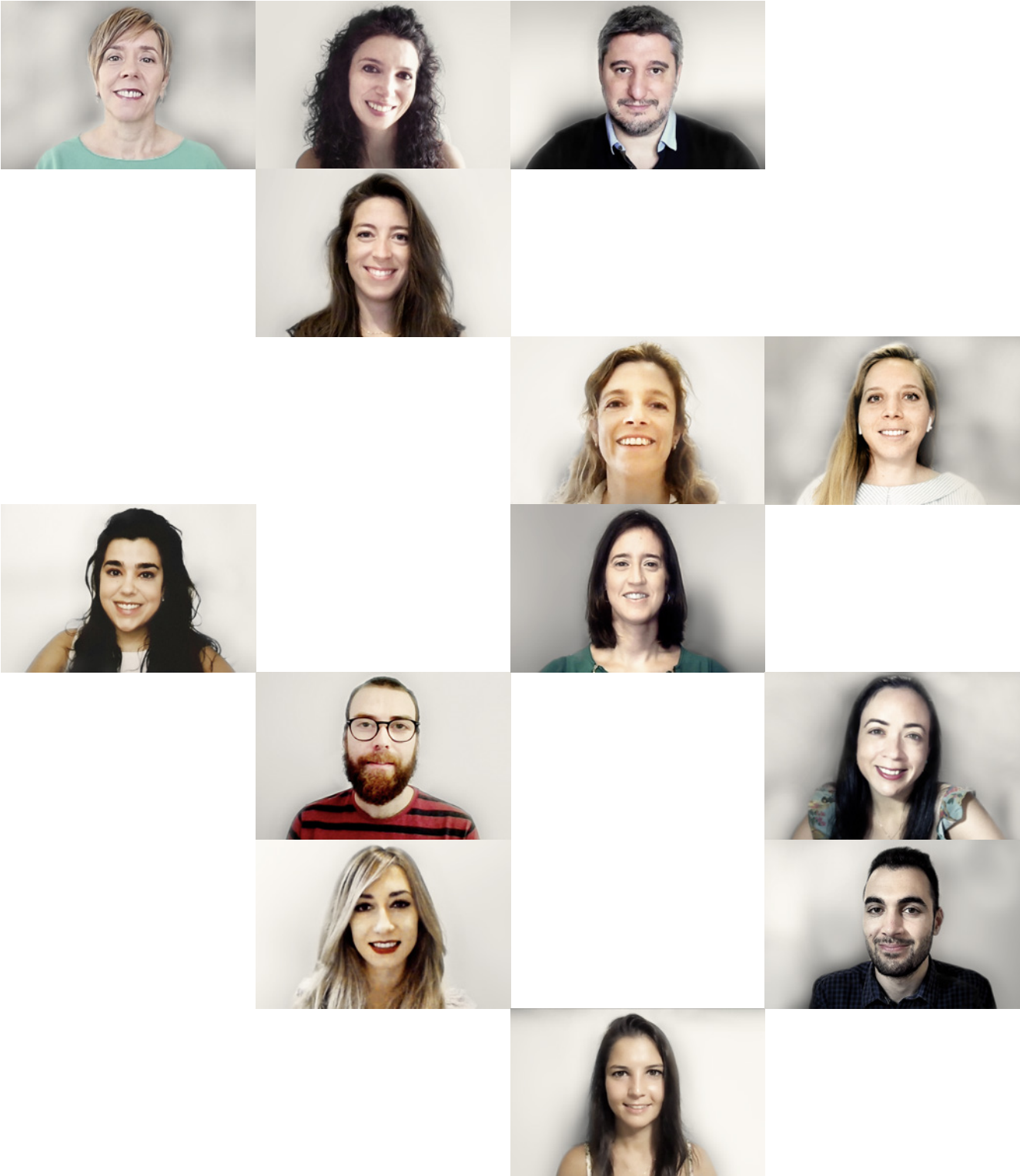
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MELANOMA GROUP

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OVERVIEW

Melanomas are the only tumours where lesions barely over one millimetre in depth can be at risk for metastasis. An increasing number of (epi)genetic alterations and mechanisms of immune evasion have been identified in this disease. 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 identify new progression biomarkers and anticancer agents. We are particularly interested in defining lineage-specific vulnerabilities that distinguish melanomas from other tumours with lower metastatic potential (publications in *Nature*, *Cancer Cell*, *Nature Cell Biology*, *Nature Communications*, among others). Our laboratory has also generated first-in-class lymphoreporter mice for non-invasive imaging of pre-metastatic niches in melanoma (*Nature*) and has identified actionable immune suppressive mechanisms with implications for patient treatment (*Nature Medicine*). 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

CNIO Melanoma Group: objectives and model systems

Melanomas are aggressive solid tumours and a paradigm of how basic and clinical research have significantly improved patient prognosis. Nevertheless, despite great success with targeted and immune-based therapies, sustained clinical responses are still limited. Moreover, the field lacks molecular markers of diagnosis, and the knowledge about how melanomas progress is largely incomplete. One of the main objectives of our Group is to define modulators of this aggressive behaviour. In particular, we are interested in identifying mechanisms that drive (pre) metastatic niche formation *in vivo*, specifically those acting in a systemic manner already from early stages of melanoma development, creating “permissive” microenvironment(s) for tumour progression.

Our Group’s main aims are to:

- define when and how melanomas act “at a distance” (on stromal and immune compartments) before tumour cell dissemination.
- determine how melanoma cells evade the immune system, and whether distinct mechanisms may be activated at different anatomical sites.
- 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). All these proteins had potent autocrine effects on the tumour cells where they were expressed. However, we were also interested in 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, revealed the

growth factor MIDKINE (MDK) as a new driver of melanoma metastasis. We have now performed loss- and gain-of-function studies of downstream effectors of MDK *in vitro* and *in vivo* (mouse xenograft models), combined with expression studies in large patient cohorts. These studies have revealed yet a new function of MDK in immune suppression. Specifically, we identified a MDK-associated gene set that was able to separate melanoma patients with a differing transcriptomic profile, involving in particular a variety of immunomodulators (Cerezo-Wallis *et al.*, *Nature Medicine* 2020). Curiously, although MDK promoted an inflammatory secretome (driven in part by NF- κ B), the ultimate outcome was 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 (FIGURE 1, left part). This “Jekyll and Hyde syndrome” described above, whereby the immune system can shift from an anti-tumoural to a pro-tumoural phenotype depending on MDK expression, was recently published in *Nature Medicine* (Cerezo-Wallis *et al.*, 2020) and featured on the cover of the journal.

Gene signatures that define response to immune checkpoint blockade in melanoma patients

Having found that MDK promoted immune suppression, our next approach was to block its function genetically or pharmacologically. Using various murine systems, we found that MDK inhibition favoured the response to vaccination treatments, and importantly, promoted an interferon (IFN)-driven secretome that enhanced the effect of immune checkpoint blockers (ICB) (summarised in FIGURE 1, right part). This IFN-signalling resulting from MDK blockade was enriched in 6 independent clinical cohorts of melanoma patients treated with ICB (see examples in FIGURE 2). Therefore, these results provided proof of principle for MDK inhibition as a strategy to prime immunologically unresponsive tumours into “hot” lesions with an improved response to ICB. The novelty and physiological relevance of these data received considerable attention in the media (TV, press, radio) and were echoed in independent News & Views in *Nature Reviews Cancer*, *Cancer Discovery* and in *Pigment Cell and Melanoma Research*. ■

FIGURE 1 Immune suppressive roles of Midkine in melanoma progression and metastasis. Summary of MDK-dependent polarisation of macrophages towards tolerogenic phenotypes that ultimately lead to T cell dysfunction (from Cerezo-Wallis *et al.*, *Nature Medicine* 2020).

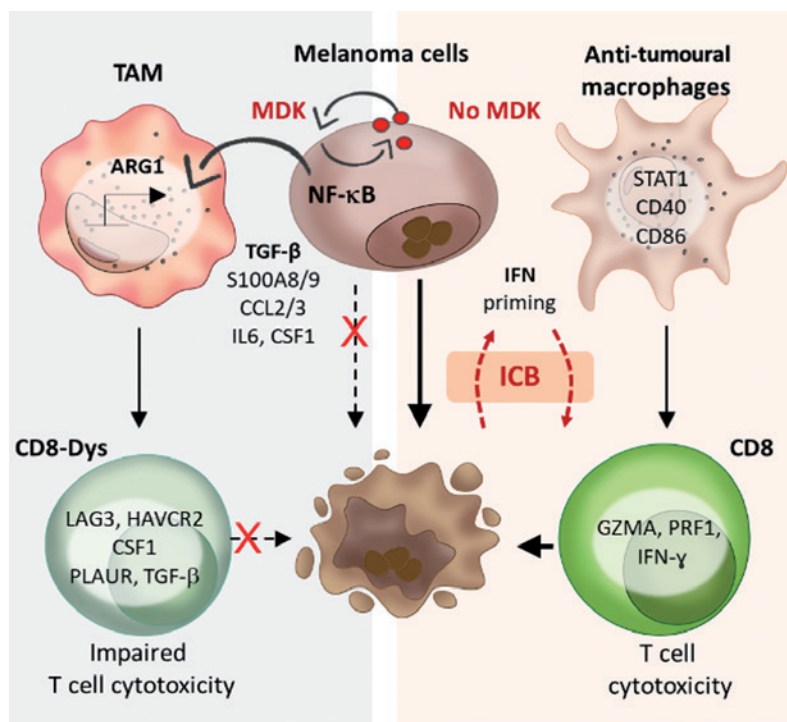
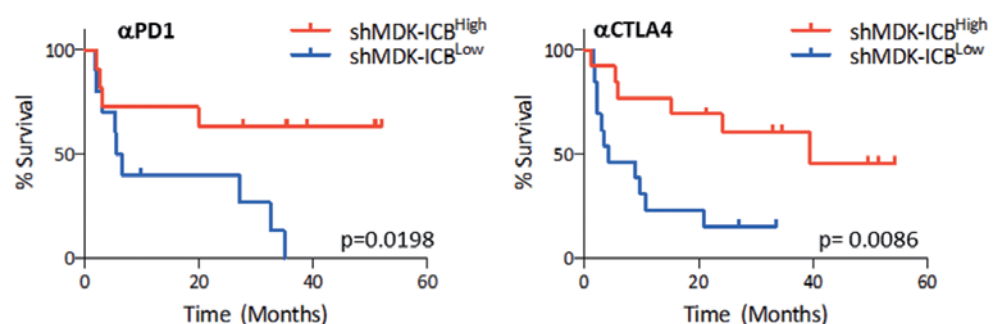


FIGURE 2 Therapeutic implications of dual inhibition of MDK and immune checkpoint blockade. Differential survival of melanoma patients treated with anti-PD1 or anti-CTLA4 antibody depending on whether or not their expression profile is enriched in a gene signature identified by dual blockade of MDK and ICB in mouse xenograft models (here labelled as shMDK-ICB^{High} and ^{Low}, respectively).



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AWARDS AND RECOGNITION

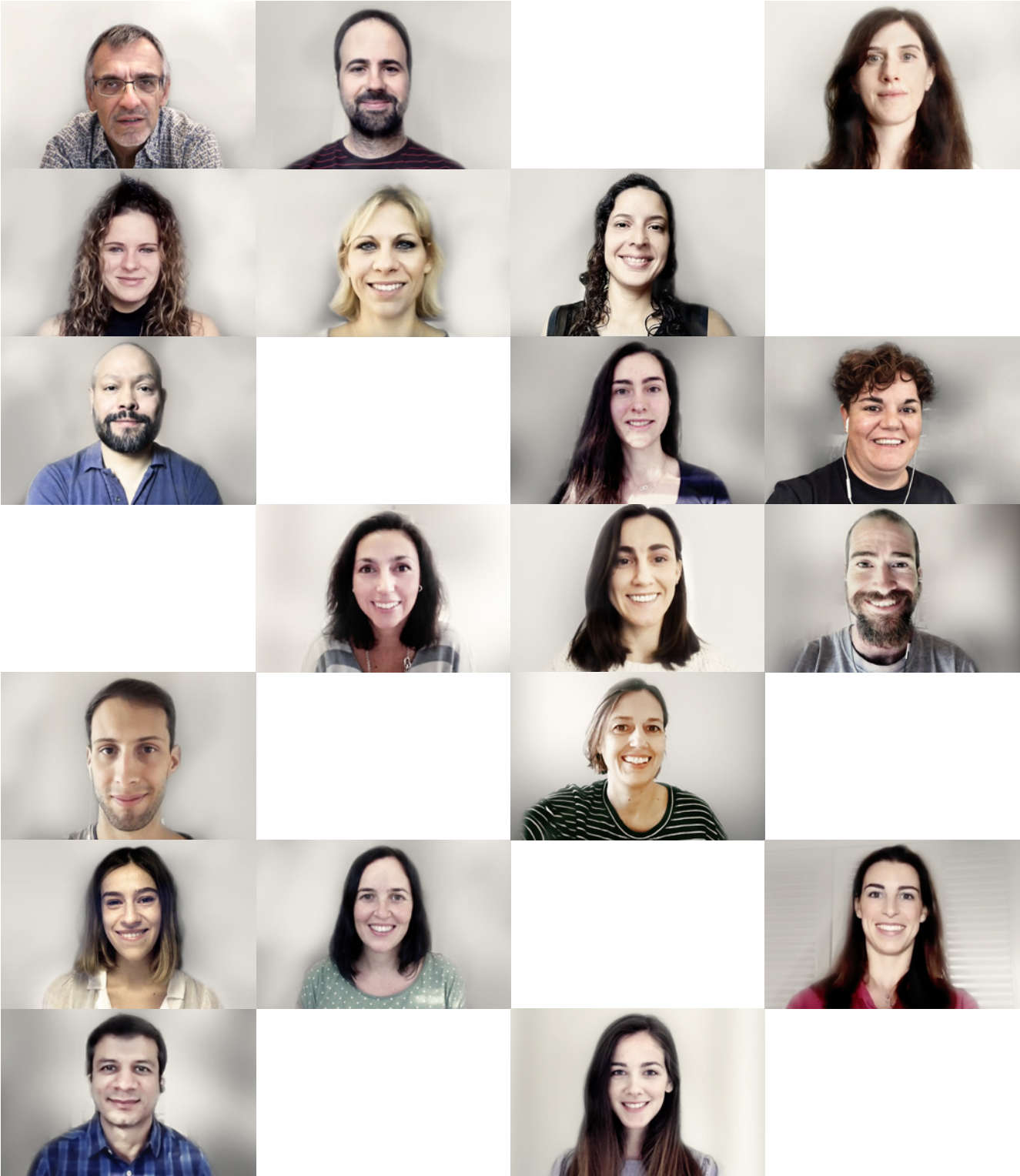
- Vicepresident/President Elect of ASE/CA, the Spanish Association for Cancer Research.
- ERC-Advanced Grant METALERT-STOP, European Research Council.

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Pérez, María Ramal (since Oct.)

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(University of Porto, Portugal), Mariona Cubells (Feb.-Sep.) (Universidad Complutense de Madrid, Spain)

Visiting Scientists

Beatriz V. Blas (Jan.- March) (Hospital

Universitario de Guadalajara, Spain), Luis C. Fernández (Universidad Europea de Madrid, Spain), Mark Kalisz (until July) (CIBER, Madrid, Spain), Fernando Burdío (Aug.-Dec.) (Hospital del Mar-Parc de Salut Mar, Barcelona, Spain)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial bladder carcinoma (UBC) with a disease-oriented approach. We use patient samples, cultured cells, and genetically modified mice, giving a similar weight to the 3 model systems. Observations made at either of these levels are then extended through additional work. To translate the findings, we bring this knowledge to a “population” level – leveraging on information and samples from large patient cohorts – in close collaboration with Núria Malats’ Group (CNIO).

In PDAC, a main hypothesis is that cell differentiation is a potent tumour suppressor mechanism acting early in carcinogenesis.

We use the excellent genetic mouse models available because these processes cannot be readily studied in humans. In mice, PDAC can originate in pancreatic progenitors and in adult acinar and ductal cells. Understanding the contribution of early molecular events is crucial to design better strategies for prevention and early tumour detection.

In UBC, we focus on identifying new genes, using them for improved tumour taxonomy, characterising the mechanisms of action, and applying this knowledge for improved prediction of outcome and therapy.

RESEARCH HIGHLIGHTS

Pancreatic cancer molecular pathophysiology

The genetic/genomic changes associated with PDAC have been extensively described by the genome consortia, and there is increasing interest in defining the molecular changes that precede tumour development. Our lab 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 regulators involved - including GATA6, GATA4, NR5A2, HNF1A, and NFIC. Dysregulation of these genes is associated with a scenario of pre-inflammation or inflammation, dependent on a functional interaction with the microbiome: antibiotic administration to deplete gut bacteria enhances the activity of the acinar programme and rescues the inflammatory predisposition of *Nr5a2* heterozygous mice, with lesser effects on wild type mice. The relevance of these findings to PDAC development are being analysed. These studies provide the basis for the pharmacological and genetic manipulation of acinar differentiation as a tumour preventative strategy.

GATA6 is a master regulator of the “classical” PDAC transcriptional programme and its loss is associated with poor patient outcome. In mice, GATA6 loss promotes metastasis and immune evasion (with P. Martinelli). GATA4 loss also favours PDAC development/progression in mice. However, these proteins play opposite roles in inflammation and they contribute differently to tumour initiation. In collaboration with an international consortium, we have shown that tumours that lose both GATA6 and GATA4 have the worst outcome and we are assessing the hypothesis that *GATA6* amplifications are associated with long-term survivorship, possibly by locking cells in a differentiated state. We are focusing on deciphering their overlapping and unique transcriptional programmes using a combination of mouse models and genomic approaches (i.e., RNA-Seq and ChIP-Seq).

New conditional knockout mouse models of *Hnf1a*, developed with J. Ferrer (CRG, Barcelona) and Sagrario Ortega (CNIO), show that HNF1A can act as a tumour suppressor in PDAC initiation. Using a dual recombinase system, we are assessing the role of HNF1A and its partner NR5A2 in tumour maintenance.

The activity of these transcription factors is intertwined, and our overarching goal is to establish the rules and hierarchies governing the control of acinar differentiation and their contribution to preneoplasia and cancer.

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

We focus on understanding 2 new UBC tumour suppressor genes that we identified through exome sequencing: *STAG2* and *RBM10*. *STAG2* codes for a cohesin subunit and *RBM10* codes for a splicing regulator. We have generated conditional mouse models for these 2 genes and are exploring their role in development and urothelial biology and 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. In normal urothelial cells, the genomic effects of *STAG2* loss are dependent on the differentiation state

of cells, in agreement with the strong association of *STAG2* inactivation with a luminal/urothelial phenotype.

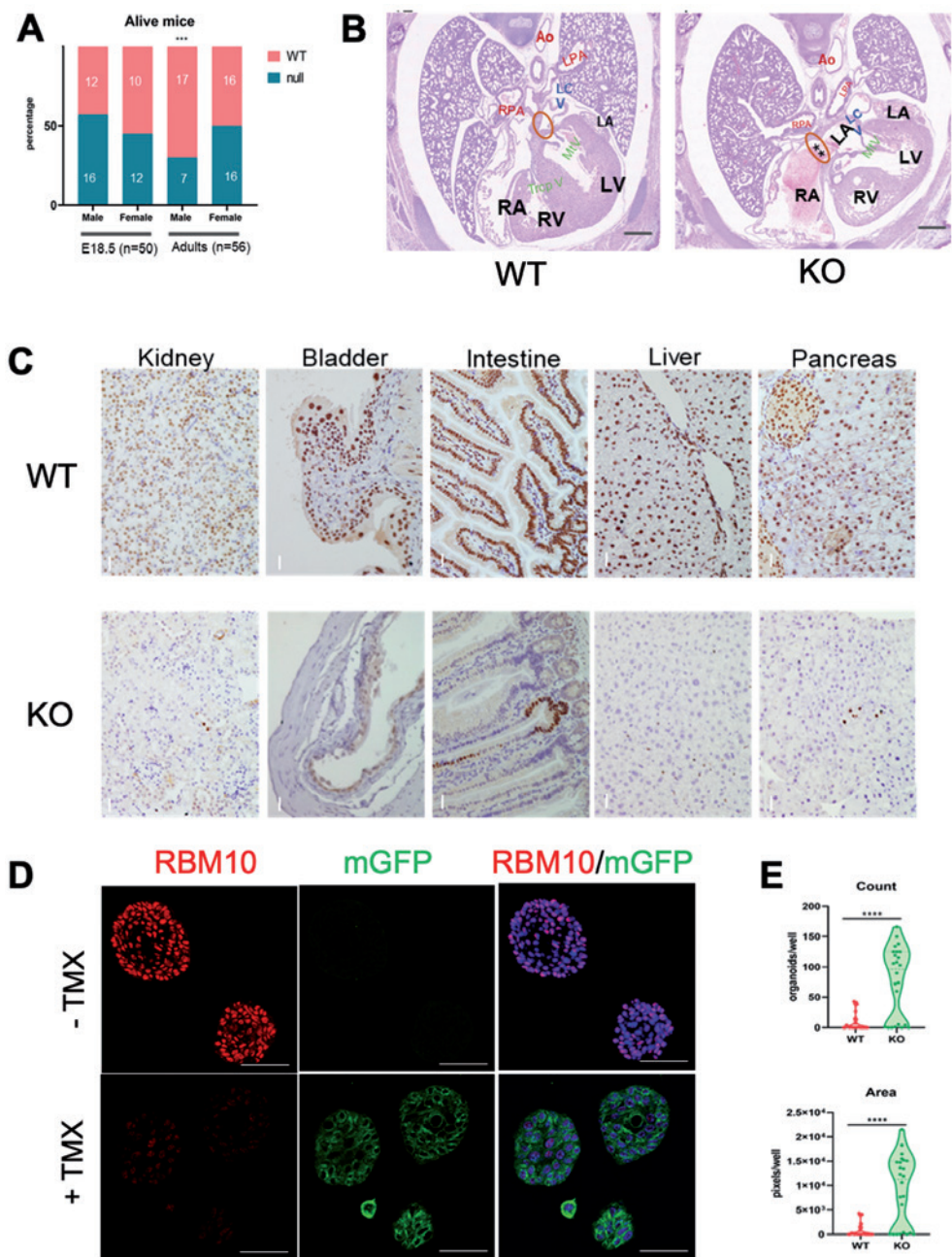
Regarding *RBM10*, somatic mutations occur in a variety of human tumours, and germline mutations cause TARP syndrome, characterised by developmental heart and craniofacial abnormalities. Constitutive inactivation of *Rbm10* in mice partially recapitulates these features and leads to partial embryonic male lethality and multiple heart defects, indicating functional conservation across species (with S. Martin Puig, CNIC). In contrast, *Rbm10* inactivation during adulthood is well tolerated (FIGURE 1). *Rbm10* knockout urothelial organoids display partial growth factor independence, pointing to a role of RBM10 in the regulation of EGFR pathway activity.

Our translational studies focus on the prediction of response to cisplatin-based chemotherapy and to immune checkpoint blockade (ICB). In collaboration with Núria Malats and Spanish uro-oncologists, we are assessing the value of immune signatures to stratify patients to receive neoadjuvant therapy (cisplatin-based chemotherapy vs. ICB) in a randomised clinical trial. ■

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FIGURE 1 Conditional *Rbm10* knockout mice as a model for the study of TARP syndrome and UBC. (A) *Rbm10^{lox/y};CAG-Cre* mice show a partially penetrant embryonic-lethal phenotype in males. Percentage of *Rbm10* WT and null male and female mice that were alive at E18.5 and at 30 weeks of age (***p-value < 0.001). (B) Transversal heart sections of *Rbm10* WT and null embryos. Atrial defects are shown with ** inside red circles (scale bar, 500µm). RA (Right Atrium), LA (Left Atrium). (C) RBM10 expression in tissues from 11 week-old control (WT) and *Rbm10^{lox/y};Ubi-CreERT2;Rosa26^{mT/mG}* mice (KO) mice after 3 weeks of tamoxifen (TMX) treatment showing extensive recombination (scale bar, 50µm). (D) Immunofluorescence staining of urothelial organoids 12 days after TMX treatment, highlighting the loss of RBM10 expression. (E) Raw count and area of *Rbm10* WT and KO organoids cultured in medium without EGF (****p< 0.0001, Student's T-test).



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► AWARDS AND RECOGNITION

► Member, Discovery Research Committee, Cancer Research UK, London, UK.
► Full Associate Editor, *Gut*.

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NUTRIENTS AND
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Master's Students

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OVERVIEW

Our laboratory focuses on understanding mechanisms of diseases associated to the digestive system, including liver, intestine and pancreas. Our work aims to integrate mouse models mimicking human disease with state-of-the-art genomics, proteomics, bioinformatics, metabolic pathways and gut microbiome analyses, and therapeutic technologies combined with human data, to: find out what goes wrong in diseased and cancerous tissues; understand how organs can regenerate; and, if regeneration goes awry, to determine how it contributes to cancer.

We put a special emphasis on studying the mechanobiology of tissue development in a health and disease context, from the physical and mechanical perspective at the molecular, cellular, and tissue levels, with the eventual goal to understand how an injured liver progresses to a cancerous tissue, in order to find new therapeutic targets. Additionally, the application of mathematical models to quantitatively study and analyse mechanical forces and cellular plasticity is an important focus in collaboration with other research groups. Finally, the use of nanotechnology combined with *in vivo* disease models generated in our laboratory might provide additional opportunities to complement our work and impact the field of medicine in diagnosis and treatment.

“We put much effort into understanding the mechanisms of diseases by generating and using genetically engineered mouse models that recapitulate the pathological features of human syndromes in order to guide the design of novel medicines.”

RESEARCH HIGHLIGHTS

Our interest is mainly driven by the discovery of two components initially identified in our laboratory to be downstream targets of the growth factor and nutrient signalling cascades: the **URI (Unconventional prefoldin RPB5 Interactor)** and **MCRS1 (Microspherule protein 1)** proteins. URI and MCRS1 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 with non-oncogene addiction functions and dependence, 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 the URI prefoldin-like complex has some co-chaperone activities, both complexes seem to be critical for chromatin dynamics and remodelling, and are most likely involved in cellular plasticity and tissue regeneration.

Using **genetically engineered mouse models (GEMMs)** 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. We put emphasis on studying diseases associated to the liver, intestine, and pancreas, as these organs are physiologically interconnected and influenced through their exocrine and/or endocrine functions (FIGURE).

Research in the last decade has focused mainly on understanding the functions and roles of newly discovered mutated genes in the development of cancer and associated diseases. With this focus, less attention has been paid to environmental factors leading to the expression of virulent eukaryotic proteins or tissue damage that also present a permanent challenge for an organism. How environmental factors can alter the host's eukaryotic epithelial cells to cause various pathologies, potentially progressing to cancer, remains largely unknown. Identifying the likely causal links between environmental stresses and diseases progressing to cancer will help to elucidate mechanisms of disease and develop new therapeutic strategies.

Recently, we made the key discovery that URI marks the slow-cycling, label-retaining (LR) cells in the intestinal crypt, which are essential for organ regeneration following ionising radiation. Reduced URI levels render LR cells

highly proliferative by activating the β -catenin/c-MYC axis. Consequently, LR cells become radiosensitive, thereby increasing gastrointestinal syndrome severity. We conclude that: (1) URI protects LR cells to promote intestinal tissue regeneration in response to high-dose irradiation; and (2) LR cells represent the facultative stem cell pool essential for organ regeneration following ionising radiation. This work was published in *Science* (Chaves *et al.*, 2019).

We intend in the near future to continue deconstructing the mechanisms of pathologies associated to the digestive system in response to environmental stressors. We will focus on understanding the mechanisms of liver diseases to find out what goes wrong in diseased and cancerous tissues, and to understand how the organ can regenerate; we will also investigate how regeneration in chronic injury can impact hepatocellular carcinoma (HCC) development, the most common and one of the most lethal and aggressive human liver cancers. A complete understanding of the mechanisms and temporal and spatial kinetics of signalling pathways and cell types involved, as well as the chronological evolution of the regenerative response during disease progression, will help us to understand what controls liver regeneration in chronic injury and HCC.

This work will be facilitated by our long-standing research interest in liver diseases and tissue regeneration, and impacted by the generation of sophisticated mouse models, recapitulating clinical features of the disease. Moreover, new approaches for the quantitative assessment, mathematical modelling, and bioinformatics analysis of single cell RNA sequencing will be specifically developed. Special effort will also be made to elucidate mechanisms of liver diseases from a patho-physiological standpoint which implicates organ crosstalk via metabolic pathways, gut microbiome, and cross-immune reactions. Recently, we have also developed an interest in mechanobiology and nanotechnology-based theranostics combined with the latest imaging technologies. This will allow us to associate conceptual advances arising in our laboratory, together with the GEMMs we generate, with these new technologies to guide the design of new therapeutic approaches to prevent and treat liver diseases and cancer. The final goal will be to promote healthy liver regeneration and to identify and functionally validate targets with potential preventive and therapeutic values. Doing so will enable us to treat frequent human lethal disorders with increased worldwide incidence and unmet medical needs. ■

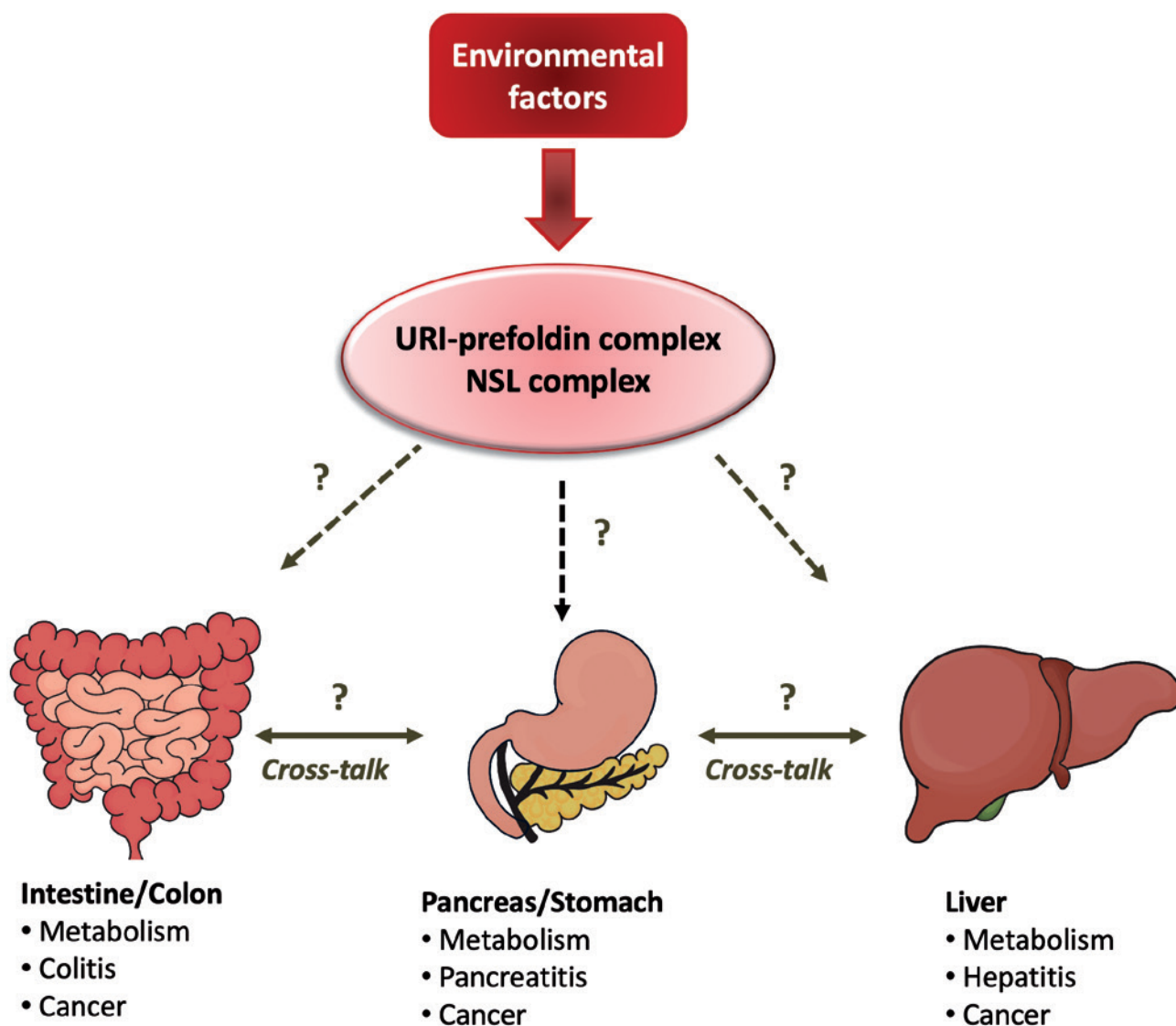


FIGURE Representation of our future research direction. By focusing on URI and MCRS1 mouse models generated in our laboratory, we

mainly focus on deconstructing the mechanisms of pathologies associated to the digestive system.

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- **AWARDS AND RECOGNITION**
- Member of the evaluation committee to assess the research quality of the Institute for Advanced Biosciences in Grenoble, France (IAB <https://iab.univ-grenoble-alpes.fr/>).
- Member of the High Council for the Evaluation of Research and Higher Education (HCERES), France.
- Member of the European Association for the Study of Diabetes (EASD).
- Editorial Board Member, *Translational Oncology*.

TRANSFORMATION AND METASTASIS GROUP

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Group Leader

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Alejandro Collado, Jaime Redondo

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 work correctly in the 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.

One of our research lines aims to characterise the role of the TNF family member RANK in mammary gland development and breast cancer and to elucidate its therapeutic potential.

“Clinical and preclinical findings support that activation of RANK signalling in breast cancer cells induces immunosuppression and that its blockage leads to a T cell dependent anti-tumour response.”

RESEARCH HIGHLIGHTS

Therapeutic impact of targeting RANK or RANKL in breast cancer and the tumour-immune crosstalk

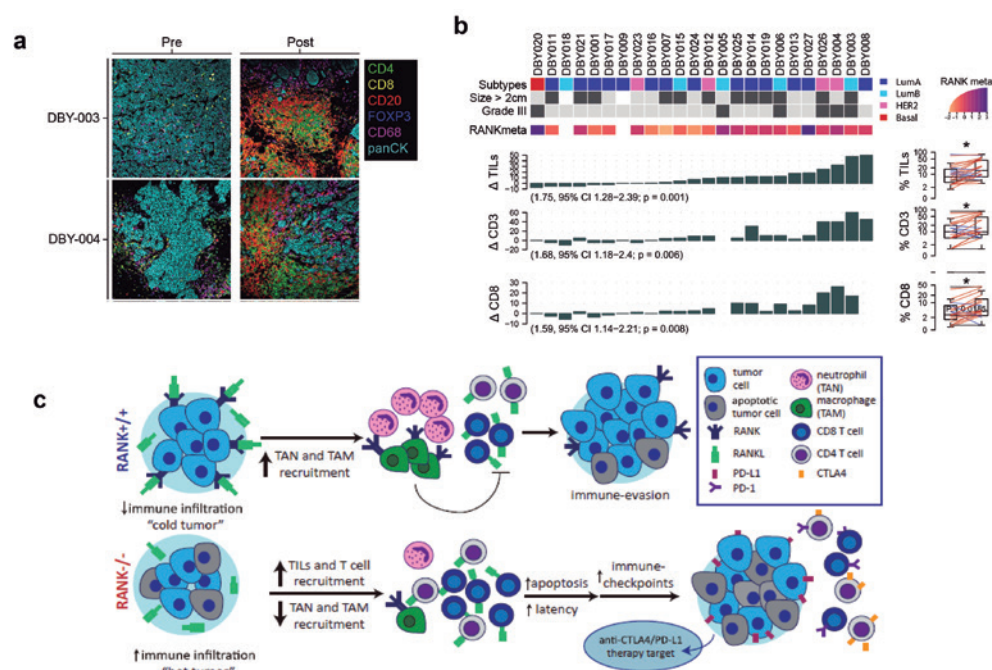
Most breast cancers exhibit low immune infiltration and are unresponsive to immunotherapy. We hypothesised that inhibition of the RANK signalling pathway may enhance anti-tumour immune response. Using preclinical mouse models, we found that loss of RANK signalling in tumour cells increases infiltration by leukocytes, lymphocytes, and CD8⁺ T cells, and reduces macrophage and neutrophil infiltration. CD8⁺ T cells mediate the attenuated tumour phenotype observed upon RANK loss, whereas neutrophils, supported by RANK-expressing tumour cells, induce immunosuppression. Moreover, RANKL inhibition increases the anti-tumour effect of immunotherapies in mouse mammary tumours through a tumour cell mediated effect. Comparably, pre-operative single-agent denosumab in premenopausal early-stage breast cancer patients from the Phase-II D-BEYOND clinical trial (NCT01864798) was well tolerated, inhibited RANK pathway, and increased tumour infiltrating lymphocytes and CD8⁺ T cells. Higher RANK signalling activation in tumours and serum RANKL levels at baseline predict the immune-modulatory effects driven by denosumab.

Altogether, our preclinical and clinical findings reveal that tumour cells exploit the RANK pathway as a mechanism to evade immune surveillance and support the use of RANK pathway inhibitors to prime luminal breast cancer for immunotherapy (Gomez-Aleza *et al.*, *Nat Commun*, 2020).

RANK signalling increases after anti-HER2 therapy contributing to the emergence of resistance in HER2-positive breast cancer

Around 15-20% of primary breast cancers are characterised by HER2 protein overexpression and/or *HER2* gene amplification. Despite the successful development of anti-HER2 drugs, intrinsic or acquired resistance represents a major hurdle. RANK and RANKL proteins are more frequently detected in HER2-positive tumours that have acquired resistance to anti-HER2 therapies than in treatment-naïve ones. *RANK* (but not *RANKL*) gene expression increased after dual anti-HER2 neoadjuvant therapy in the cohort from the SOLTI-1114 PAMELA trial. Results in HER2-positive breast cancer cell lines recapitulate the clinical observations, with increased RANK expression after short-term treatment with anti-HER2 therapies and enhanced NF- κ B activation in lapatinib resistant HER2+ breast cancer cells. Moreover, we found that overactivation of the RANK signalling pathway enhances ERK and NF- κ B signalling and increases lapatinib resistance in different HER2-positive breast cancer cell lines. Our results indicate that ErbB signalling is required for RANK/RANKL-driven activation of ERK in several HER2-positive cell lines. In contrast, lapatinib is not able to counteract the NF- κ B activation elicited after RANKL treatment in RANK-overexpressing cells. Finally, we show that enhanced RANK pathway activation alters HER2 phosphorylation status and RANK binding to HER2 in breast cancer cells. Altogether, our

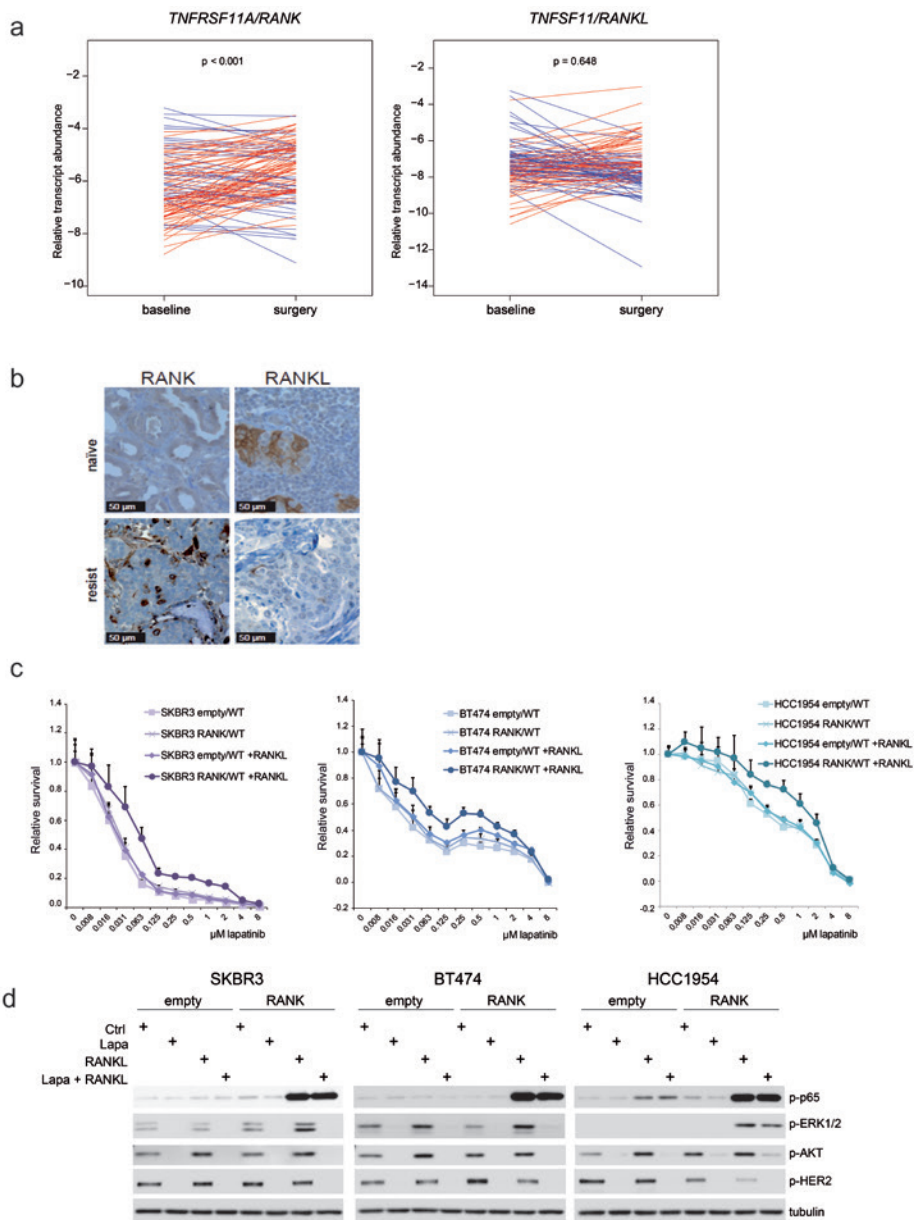
FIGURE 1 The immunomodulatory role of anti-RANKL in breast cancer. (a) Representative micrographs of multiplex IHC of pre- and post-treatment tumour sections. (b) Bar-plots showing the change from baseline (Δ ; post- minus pre-treatment values of TILs CD3+ and CD8+ T cells). For each measured parameter, the corresponding boxplot is displayed on the right-hand side. (c) RANK expression in breast cancer cells favours recruitment of TAMs and TANs, immunosuppressive population which interferes with lymphocyte T cell recruitment and/or activity. Denosumab (anti-RANKL) or RANK signalling inhibition results in increased TILs, lymphocytes and CD8+ T cell infiltration, transforming immune “cold” tumours into “hot” ones and attenuating tumour growth.



data support a physical and functional link between RANK and HER2 signalling in breast cancer and demonstrate that increased RANK signalling may contribute to the development

of lapatinib resistance through NF- κ B activation (Sanz-Moreno *et al.*, *Breast Cancer Research*, 2020). ■

FIGURE 2 (a) *RANK* but not *RANKL* expression increased after dual anti-HER2 therapy in patient samples ($n = 151$) from the PAMELA trial. Ladder plots showing gene expression before (baseline) and after (surgery) dual anti-HER2 treatment. (b) Representative images of RANK and RANKL in treatment-naïve and anti-HER2-resistant HER2 breast cancer tumour samples. (c-d) Overactivation of RANK signalling in HER2-positive cell lines increased NF- κ B activation and lapatinib resistance. Relative survival in lapatinib of HER2+ breast cancer cells overexpressing RANK (c) and downstream pathways (d).



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MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Staff Scientist
Susana García

Post-Doctoral Fellows
Marta Hergueta, Laura Nogués

Graduate Students
Elena Castellano, Teresa González,
Alberto Hernández, Lucía Robado



OVERVIEW

Our Group aims to understand the crosstalk between tumour cells and their microenvironment during metastatic progression. Microenvironmental cues are important at all steps of the metastatic process, for which the recruitment of a variety of stromal cells is crucial. Secreted factors play an essential role in this mechanism including soluble factors and extracellular vesicles. These mechanisms of cell-cell communication have become as a *novel language* of cancer that we aim to decode. We are interested in: 1) understanding how tumour cells crosstalk with stromal cells involved in lymph node and distal metastasis in melanoma, lymphoma, prostate cancer and malignant peripheral nerve sheath tumours; 2) the influence of obesity in melanoma and breast cancer metastasis; and 3) the use of secreted extracellular vesicles (EVs) as surrogate markers of tumour progression. Our studies

“We are interested in understanding how tumour cells corrupt the tumour microenvironment along metastatic progression and the main mechanisms involved, with the aim to develop novel anti-metastatic therapies.”

are focused on deciphering novel biomarkers of metastatic progression and the molecular mechanisms involved, with the aim to define novel therapeutic targets to block metastatic spread.

Technicians

Sara Sánchez-Redondo, Vanesa Santos

Students in Practice

Eduardo Garvín (July-September)

(*Universidad Francisco de Vitoria*, Madrid, Spain), Ane Rubio (since September) (*Universidad Complutense de Madrid*, Madrid, Spain)

Visiting Scientists

Alberto Carretero (*Hospital Universitario 12 de Octubre*, Madrid, Spain), María Isabel González (until March) (*Hospital Gregorio Marañón*, Madrid, Spain)

RESEARCH HIGHLIGHTS

Novel approaches in liquid biopsies

We are developing state-of-the-art technologies to implement EV-based liquid biopsies in the diagnosis and prognosis of patients with melanoma. We have found that the detection of BRAFV600E mutation in circulating EVs from the lymphatic exudate obtained post-lymphadenectomy can be used to identify melanoma patients at risk of relapse (FIGURE).

Novel mechanisms driving in local and distal metastasis

We are investigating the mechanisms involved in melanoma and prostate cancer metastasis. We found that nerve growth factor receptor (NGFR) is overexpressed in metastatic melanoma cells, secreted in EVs, and that it is shuttled to lymphatic endothelial cells inducing lymphangiogenesis and metastasis. We are also studying the use of NGFR inhibitors as a new strategy to block melanoma metastasis. Finally, we are defining the role of secreted EVs in prostate cancer lymph node metastasis.

Impact of high fat diet in metastasis

We are currently analysing how obesity influences metastasis through systemic and local changes in melanoma and breast cancer. We are interested in defining how obesity impacts breast cancer lung metastasis by reinforcing pro-coagulant activities. We are testing novel approaches to reduce tumour-platelet interactions and develop anti-metastatic therapies.

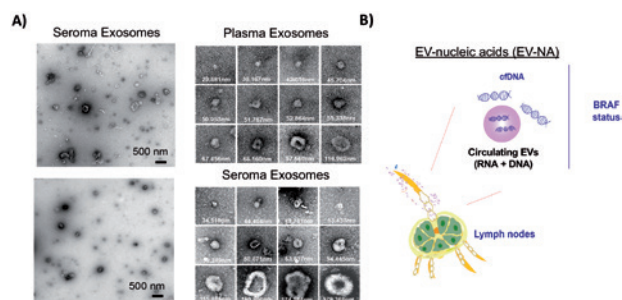


FIGURE Use of EVs in liquid biopsies. (A) Electron microscopy images of exosomes derived from plasma and exudative seroma from

melanoma subjects. (B) Scheme of the analysis performed for the detection of BRAF mutations in EV nucleic acids (EV-NA) in EVs.

We are also analysing how adipose tissue reinforces melanoma metastasis by promoting tumour cell homing and metastatic behaviour.

Tumour-stroma interactions in metastasis

We are studying how alterations in the lymph node microenvironment influence lymphoma progression. We are analysing the role of NGFR in lymph node stromal cells and its influence in follicular lymphoma. We are also exploring novel therapeutic strategies against malignant peripheral nerve sheath tumours (MPNSTs). We are currently testing a combination therapy with MEK inhibitors and anti-angiogenic antibodies as a novel treatment for MPNSTs. ■

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BRAIN METASTASIS JUNIOR GROUP

Manuel Valiente
Junior Group Leader

Post-doctoral Fellows
Mariam Al-Masmudi, Neibla Priego



OVERVIEW

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is poor given that local therapies (i.e., surgery and radiation) have limited benefits 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.

“The many branches of our research programme have evolved into a solid strategy that is producing results that could be translated into real benefits for patients with brain metastases.”

Graduate Students

Laura Adriana Álvaro, Ana de Pablos Aragonese, Pedro García, Carolina Hernández (since July), Pablo Sanz (until September), Lucía Zhu

Visiting Graduate Student

Lauritz Miarka (Boehringer Ingelheim Fonds MD Fellowship) (*Christian-Albrechts-University Kiel*, Kiel, Germany)

Technicians

María Isabel García (since February), María Perea (since September), Diana Patricia Retana

RESEARCH HIGHLIGHTS

A “white book” for brain metastasis research

Together with 19 laboratories worldwide, we have built a public resource for organotropic cancer cell lines that are metastatic to the brain (The Brain Metastasis Cell Lines Panel: <https://apps.cnio.es/app/BrainMetastasis/CellLines>). This is the most valuable research tool available to interrogate brain metastasis. In addition, we jointly describe the main strategies to study brain metastasis, their current problems, and the open key questions.

Modelling the aggressive growth of brain metastasis

During 2020, the Group participated in an international effort to interrogate the growth of aggressive tumours with mathematical models that have helped to obtain key principles that govern cancer cell colonisation in experimental models and in patients. ■

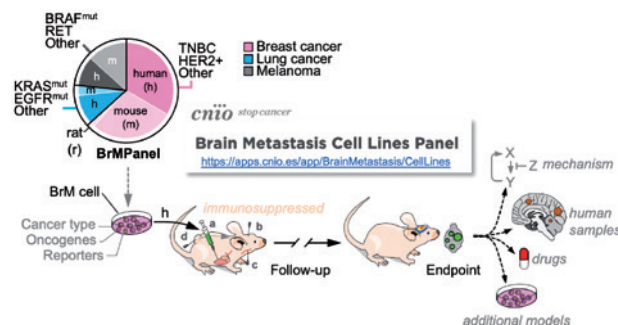


FIGURE The Brain Metastasis Cell Lines Panel is the first effort to collect existing information on existing brain tropic cancer cell lines, their

behaviour *in vivo*, and their potential use for research on metastasis. A publicly available webpage describes this open resource.

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(*) Corresponding authors.

AWARDS AND RECOGNITION

- Manuel Valiente:
- ERC Consolidator Grant, European Research Council (ERC).
- La Marató TV3 Grant, *Fundació La Marató TV3*, Spain.
- ESMO Faculty Member, CNS tumours faculty group (2020-2024), European Society for Medical Oncology (ESMO).
- Appointed Next Chair of the EANO Scientific Committee, European Association of Neuro-Oncology (EANO; expected mandate 2022-2024).
- Lauritz Miarka was recipient of a Boehringer Ingelheim Fonds MD Fellowship.

METABOLISM AND CELL SIGNALLING JUNIOR GROUP

Alejo Efeyan
Junior Group Leader

Staff Scientists
Bárbara Martínez, Ana Ortega

Post-Doctoral Fellow
Melania Zauri



OVERVIEW

In the Metabolism and Cell Signalling Lab we study the links between nutrients, cancer, and ageing. All 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, 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. In the lab, we combine mouse genetics and cell biological tools to gain insight into the genetic and environmental corruptions of nutrient signalling cascades, aiming to conceive therapeutic interventions in the context of cancer, obesity and the process of ageing.

“Our studies support the targeting of nutrient signalling as a novel, efficacious and safe approach against the aberrant metabolism of cancer cells and to combat the process of ageing.”

Graduate Students

Celia de la Calle, Lucía de Prado
(since September), Nerea Deleyto,
Ana Belén Plata

Technicians

Cristina Lebrero (TS)*, Ana Sagrera
(TS)*, Alba Sanz

**Título Superior* (Advanced Degree)

Student in Practice

Lucía de Prado (January-June)
(*Universidad Autónoma de Madrid*,
Madrid, Spain)

Visiting Scientist

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

RESEARCH HIGHLIGHTS

To understand the negative impact of chronic nutrient overload in systemic metabolism, and because the liver has a key role in metabolic homeostasis, we generated mice that have chronically high nutrient signalling only in hepatocytes (by liver-specific expression of an active RagA allele: RagA^{GTP}). RagA^{GTP} livers exhibited high phosphorylation of mTOR targets (S6K1 and 4EBP1; FIGURE A) and, importantly, the sole activation of RagA in the liver, without altering nutrient intake, impaired glucose homeostasis, as revealed by loss of glucose tolerance (FIGURE B). This result highlights the relevance of a chronic nutrient surplus – liver Rag GTPase signalling axis in metabolic complications of the obesity state.

Studying the connections of nutrients and cancer, we previously found that activating mutations in the gene called RagC (key player, together with RagA, in the signal transduction of cellular nutrient levels) result in a subtype of B cell lymphoma, follicular lymphoma. Thus, a lot of interest to develop pharmacological inhibitors of this nutrient signalling pathway has recently spurred, but these drugs are still to be developed. Thus, to determine both the efficacy and safety of inhibition of nutrient signalling against follicular lymphomas, we undertook a genetic approach: we now generated mice expressing a hypomorphic allele of RagC, and asked whether 1) decreased nutrient signalling could suppress the development of follicular lymphoma; and 2) unanticipated side effects could preclude the use of such inhibitors. Hypomorphic RagC mutant mice (RagC^{Q119L}) showed a significant extension of survival when follicular lymphomas were induced (FIGURE C), and an exhaustive analysis of potential side effects revealed that B cells were selectively affected (FIGURE D), importantly, without detectable undesirable trade-offs in other organs (not shown). These results support both the efficacy and safety of nutrient signalling inhibitors in the treatment of B cell neoplasms. ■

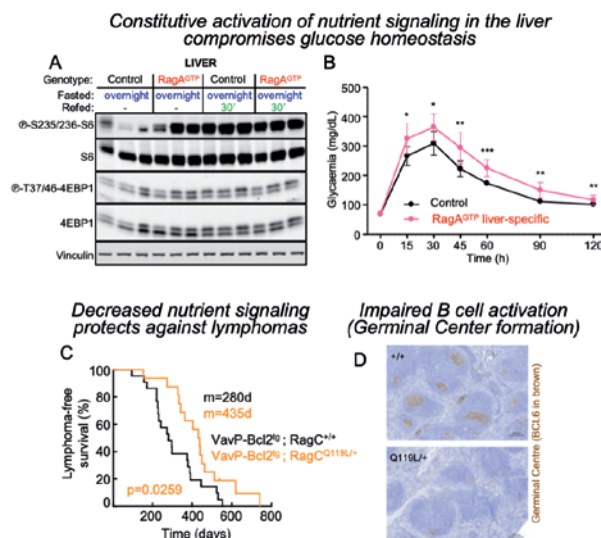


FIGURE (A) Increased nutrient signalling - mTOR activity in RagA^{GTP} liver samples. (B) Glucose intolerance in liver-specific Rag^{GTP} mice. (C) Endogenous expression of RagC^{Q119L}

protects against lymphomas induced by Bcl2. (D) Activation of B cells (Germinal Centre stain in brown) is impaired in RagA^{Q119L/+} mice.

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AWARDS AND RECOGNITION

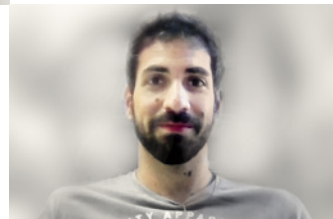
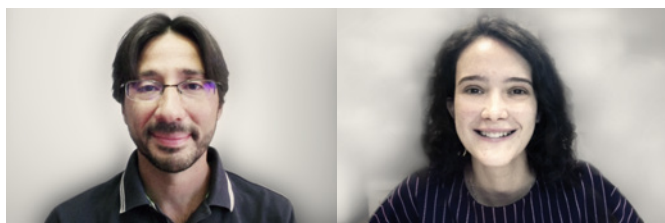
- ▶ Alejo Efeyan: EMBO Young Investigator Award, European Molecular Biology Organization.
- ▶ Ana Ortega was awarded a *Ramon y Cajal* Research Contract (MCI), Spain.
- ▶ Lucía De Prado received an *FPI* PhD Student Fellowship (MCI), Spain.

SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

Staff Scientist
Bárbara Oldrini

Post-Doctoral Fellow
Miguel Jiménez



OVERVIEW

Malignant brain tumours represent about 3% of all cancers, and annually about 100,000 new cases are diagnosed worldwide. In Spain, there are about 4,000 new cases a year. Gliomas are a large collection of brain tumours of which Glioblastoma Multiforme (GBM) is the most frequent and aggressive primary central nervous system (CNS) tumour in adults. Regardless of the recent advances in treatment modalities, GBM patients usually respond weakly to all therapeutic approaches, and prognosis remains dismal (approximately 15 months).

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

“The central focus of our Group is to uncover the genetic alterations present in GBM patients that are responsible for the aggressiveness of this tumour type, with particular interest in the identification of the signalling pathways that lead to poor treatment response.”

Graduate Students

María del Mar Gardeazabal, Verónica Matía

Technicians

Alicia Marie Gaëlle Leblond (since February), Paula Kroon (until March) (TS)*

*Titulado Superior (Advanced Degree)

Visiting Scientist

Ernesto Mejías (since September) (Ludwig Maximilian University of Munich, Munich, Germany)

RESEARCH HIGHLIGHTS

The molecular basis underlying Glioblastoma (GBM) heterogeneity and plasticity are not fully understood. GBM is a very heterogeneous disease for which multiple transcriptional subtypes have been described. Among these subtypes, the Mesenchymal (MES) GBMs tend to have the worst prognosis. The most frequent genetic alterations — Neurofibromatosis type 1 gene (*NF1*) copy number loss or mutation — and important regulators of the MES subtype, such as *STAT3*, *CEBPB* and *TAZ*, have been identified. Nevertheless, the mechanisms regulating MES GBMs are still not fully understood. Even though each subtype is associated with specific genetic alterations, there is also considerable plasticity among them: different subtypes co-exist in the same tumours, and shifts in subtypes can occur over time. This plasticity may be explained by the acquisition of new genetic

and epigenetic abnormalities, by stem-like reprogramming or by clonal variation. Using transcriptomic data of patient-derived brain tumour stem cell lines (BTSCs), classified according to GBM-intrinsic signatures, we identified the AP-1 transcription factor *FOSL1* as a key regulator of the mesenchymal subtype. We provided a mechanistic basis for the role of *NF1*, a negative regulator of the RAS/MAPK pathway, in GBM mesenchymal transformation through the modulation of *FOSL1* expression. Depletion of *FOSL1* in *NF1*-mutant human BTSCs and *Kras*-mutant mouse neural stem cells results in loss of the mesenchymal gene signature, reduction in stem cell properties and *in vivo* tumorigenic potential. Our data demonstrated that *FOSL1* controls GBM plasticity and aggressiveness in response to *NF1* alterations. ■

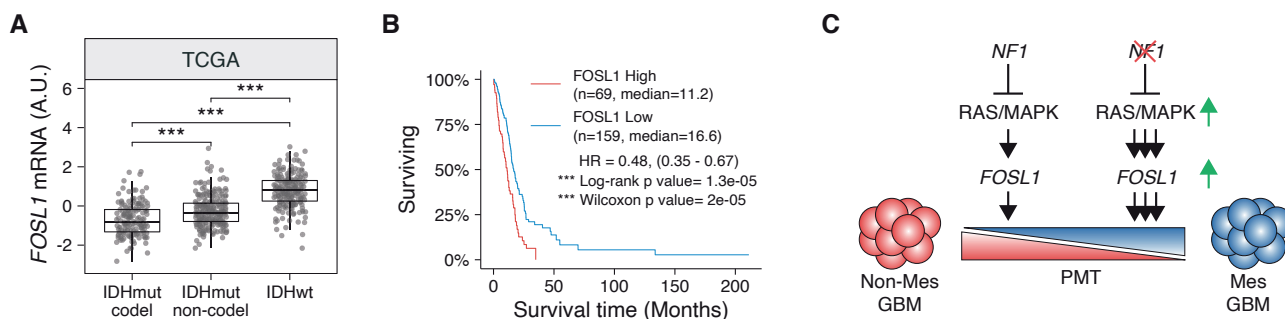


FIGURE 1 NF1 regulates mesenchymal glioblastoma plasticity and aggressiveness through the AP-1 transcription factor FOSL1. (A-B) *FOSL1* is upregulated in IDH-

wt gliomas and is associated with worst prognosis. (C) NF1-MAPK-FOSL1 signalling modulates MES GBM plasticity.

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STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA Programme Director



Description of the Programme's areas of research and strategic goals

The Structural Biology Programme (SBP) has two strategic goals. 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. The Programme studies protein kinases as well as protein complexes involved in the complicated cellular response to DNA damage and genomic instability. 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. 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. SBP is currently composed of 1 Senior Group, 5 Junior Groups and 4 Units.

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

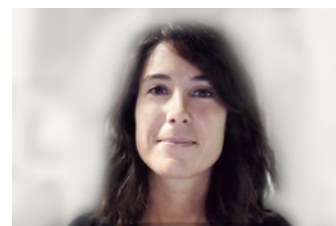
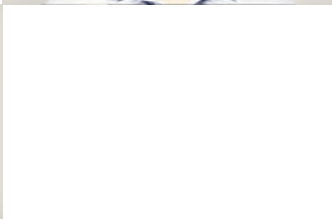
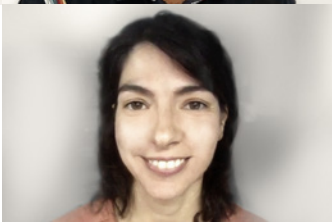
Summary of milestones & major achievements during 2020

2020 has been a difficult year for the Programme due to the Covid-19 pandemic, and this struggle was especially challenging for 2 new junior groups that had just started to assemble their team at CNIO. Despite the difficulties, everyone in the Programme has made a substantial effort to keep our research moving; we made some important contributions to the mechanistic understanding of the mismatch repair machinery in response to DNA replication errors, to the role of RUVBL1 and RUVBL2 ATPases in the regulation of essential cellular processes, and to the understanding of the activation of cytosolic hybrid histidine kinases and the Focal Adhesion Kinase (FAK). In addition, work by the groups in the Programme has contributed to the analysis of tissue-specific alternative splicing, to the characterisation of tumour-immune heterogeneity in advanced ovarian cancer, and to creating new tools for drug repositioning and prioritisation. ■

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 Cabezudo, Alba Ruiz

Graduate Students
Natalia Cuervo, Carlos Fernández,
Nayim González (since December)

Technician
Ana González

Student in Practice
Razvan Borza (until June)
(*Universidad Autónoma de Madrid*,
Madrid, Spain)

OVERVIEW

Our current work dedicates special attention to study RUVBL1 and RUVBL2, 2 highly conserved AAA+ ATPases that are essential for several cellular processes relevant in cancer, including Fanconi anaemia, chromatin remodelling, nonsense-mediated mRNA decay (NMD), and the assembly and activation of large macromolecular complexes such as the those formed by mTOR and ATR kinases. Interestingly, RUVBL1-RUVBL2 inhibitors show anti-oncogenic potential, and cancer cells with high mTOR activity are dependent on the functions of RUVBL1-RUVBL2 for survival. How RUVBL1 and RUVBL2 perform their functions is only partially understood. Our work provides novel structural and mechanistic understanding of how these ATPases work, which will be useful for exploring new ways to target these proteins. For this, we combine biochemistry, and molecular and cell biology with cryo-electron microscopy methods that allow us to visualise individual macromolecular complexes and to resolve their structure at high resolution.

“We have improved the structural understanding of how RUVBL1 and RUVBL2 are regulated, information needed to explore new ways to target these ATPases as a therapeutic opportunity against cancer.”

RESEARCH HIGHLIGHTS

RuvB-like 1 (RUVBL1) and RuvB-like 2 (RUVBL2) are 2 highly conserved AAA+ ATPases, which have been found to be essential in a wide range of unrelated cellular processes, including transcriptional regulation, chromatin remodelling, DNA repair, Fanconi anaemia, nonsense-mediated mRNA decay (NMD), and the assembly and activation of complexes formed by the kinases of the phosphatidylinositol 3-kinase-related kinase (PIKK) family, such as mTOR and ATR. RUVBL1 and RUVBL2 are involved in cancer through their contribution to these cellular processes. They are essential for tumour cell growth, and they are overexpressed in many cancer types such as hepatocellular carcinoma, colon, breast or lung cancer. Interestingly, recent years have seen the development of several inhibitors of RUVBL1 and RUVBL2 ATPase activity for their use against cancer cells.

RUVBL1 and RUVBL2 work as a heterohexameric complex, but how this complex is capable of performing such a diversity of functions remains poorly understood. During 2020 we used cryo-electron microscopy (cryo-EM) studies combined with other techniques to characterise 3 cellular processes in which these ATPases are essential.

On the one hand, we characterised in detail how a domain of the RNA polymerase II-associated protein 3 (RPAP3) protein can specifically recognise the ATPase domain of RUVBL2. RPAP3 interacts not only with RUVBL1-RUVBL2, but also with HSP90 and other proteins that are needed to assemble and activate a growing list of complexes, including RNA polymerase II and complexes of the PIKK family of kinases such as mTOR, ATR, and SMG1. In addition, in collaboration with Jens Luders at the *IRB* (Barcelona), we found that RUVBL1-RUVBL2 participates in the assembly of the human γ -tubulin ring complex, a macromolecular assemblage that regulates microtubule formation. This finding was used to describe, for the first time, a procedure for the reconstitution of the human γ -tubulin ring complex that will be useful for functional and structural studies.

Furthermore, we started to characterise what is the function of RUVBL1 and RUVBL2 in nonsense-mediated mRNA decay (NMD), in collaboration with the Electron Microscopy Unit. NMD is a surveillance pathway that regulates gene expression by targeting some RNAs for degradation, especially aberrant mRNAs containing premature termination codons. RUVBL1-RUVBL2 ATPase activity is essential for the initiation of NMD but why and how this happens is still unknown. In 2020 we showed that DHX34, an RNA helicase that participates in the initiation of an NMD response, interacts with RUVBL1-RUVBL2, inducing conformational changes that regulate their ATPase activity. These findings suggest that factors required

for NMD are coupled to RUVBL1 and RUVBL2 to regulate their ATPase activity. Further work will be needed to understand in full how RUVBL1 and RUVBL2 regulate NMD. ■

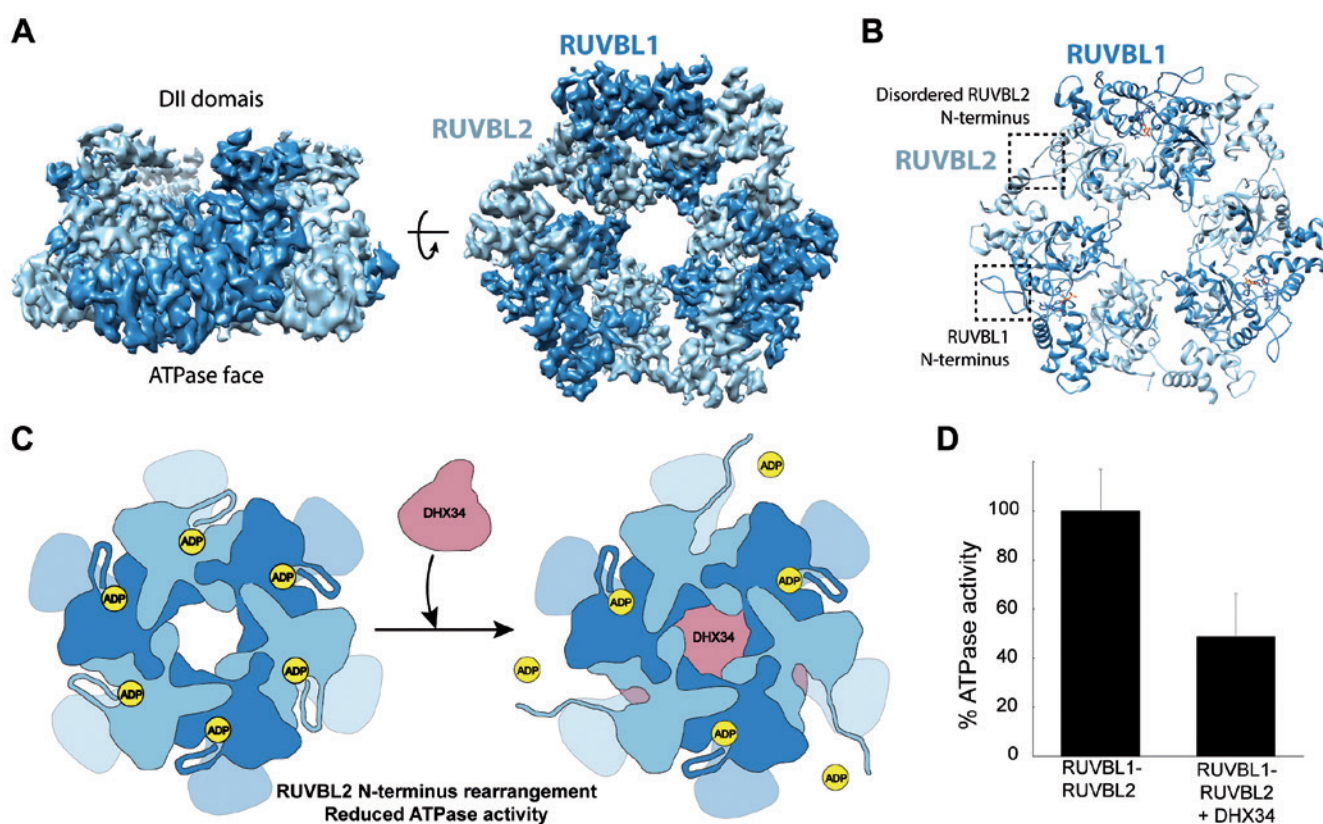


FIGURE RUVBL1 and RUVBL2 participate in nonsense-mediated mRNA decay (NMD). RUVBL1 and RUVBL2 form heterohexameric complexes that regulate NMD initiation by unknown mechanisms. We used

cryo-EM to visualise how DHX34, a helicase required for NMD, interacts with RUVBL1-RUVBL2 and regulates ATP hydrolysis. (A) Two views of the cryo-EM map of the RUVBL1-RUVBL2 complex after its interaction with

DHX34. (B) Atomic model of RUVBL1-RUVBL2 after interaction with DHX34. (C) Model of how DHX34 regulates the ATPase activity of RUVBL1-RUVBL2. (D) DHX34 inhibits RUVBL1-RUVBL2 ATPase activity.

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KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellows
Irene Ayuso (since February), Pablo Soriano (until February), Rubén Julio Martínez



OVERVIEW

Rational and precise targeting of oncogene-driven signalling is a crucial but still pending 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 quantification of posttranslational modifications, X-ray crystallography, and *in vivo* validation using *Drosophila* models. Furthermore, we use structure-guided drug discovery and MD simulation approaches to exploit structural and functional vulnerabilities for the design, development, and optimisation of optimal protein kinase inhibitors.

Graduate Students

Nicolás Cuesta, Ana Martín (since February), Moustafa Ahmed Shehata

Visiting Master's Student

Carlos Vega (February-November)
(*Universidad Complutense de Madrid*, Madrid, Spain)

Student in Practice

Marta Iglesia (February-July)
(*Universidad Autónoma de Madrid*, Madrid, Spain)

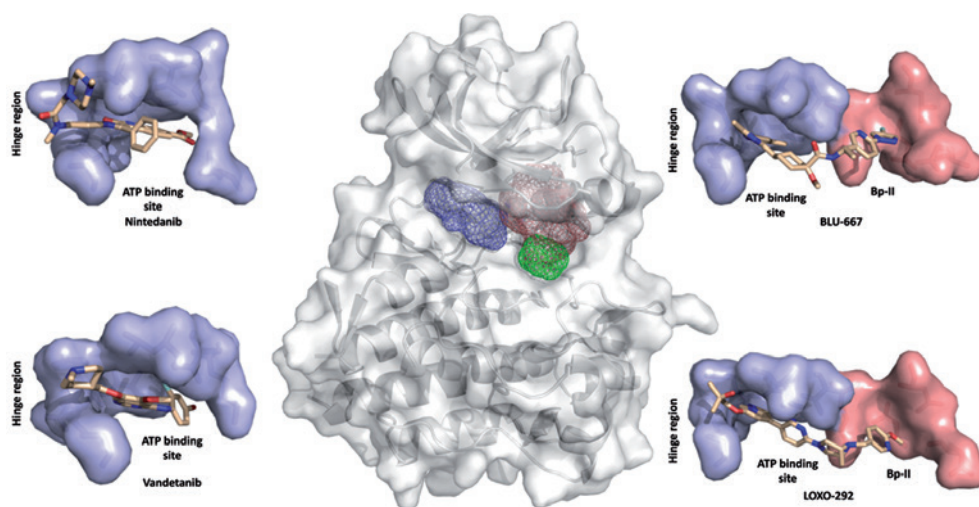
RESEARCH HIGHLIGHTS

In 2020, we made significant progress on the research lines initially established in the laboratory:

1. We elucidated important structural and molecular details about the precise mechanism of the catalytic activation and auto-regulation of the c-Src oncogene. By applying a systemic phospho-proteomic approach, we identified new c-Src autophosphorylation sites and revealed that “canonical” activating and repressive tyrosine residues actually play other important roles and functions not previously envisioned.
2. Another research line was directed at dissecting the function of CCDC6-RET, a RET oncogenic fusion and driver in NSCLC. We successfully purified this challenging protein in different isoforms and length-variants and, by applying an integrated approach, demonstrated that the full-length construct behaves as an active dimer in solution. Auto-phosphorylation assays demonstrated fast kinetics compared to RET wild-type constructs. Further phospho-site mapping by MS and dissection of the activation mechanism highlighted important roles for catalytic activity and substrate specificity through unexpected elements.

3. A third research line focused on the exploitation of structural and functional vulnerabilities in RET for the rational design and development of highly specific inhibitors. Our current paradigm is based on 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 their 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, screening with both virtual and chemical libraries, together with biophysical and biochemical tools for functional validation. Following this multidisciplinary approach, we identified an allosteric interface in RET with a good druggability score that can potentially be targeted with allosteric inhibitors. Furthermore, we uncovered a new sub-pocket within the ATP-binding site that is exploited by highly specific second-generation type I RET inhibitors (FIGURE). This information will be crucial for the design and development of highly specific, clinically successful third generation RET inhibitors able to overcome refractory RET mutations. ■

FIGURE Structure-based drug-discovery for new druggable pockets in RET. Cartoon representation of RET kinase domain with mapped druggable pockets within the active site depicted in mesh representation. The pockets are ranked from highest to lowest based on the colour code: red, green, and blue. We identified a new pocket within the ATP-binding site that is exploited by highly potent and specific type I RET inhibitors (Shehata *et al.*, submitted).



GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno

Graduate Students
Ester Casajús, Samuel Míguez



OVERVIEW

Safeguarding genetic information is essential to all forms of life. Two key cellular processes keep it free from errors: DNA replication and repair. Importantly, when they 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 on it to correct it as well as to 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

“Combined with other approaches already established at the CNIO, we use cryo-Electron Microscopy to study diverse macromolecular complexes involved in cancer to an unprecedented level of detail.”

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

Technician
Araceli Grande (TS)
**Titulado Superior* (Advanced Degree)

Students in Practice
Alba De Haro (until July)
(*Universidad Autónoma de Madrid*, Madrid, Spain), Alberto López Francos (February-July)
(*Universidad Autónoma de Madrid*,

Madrid, Spain), Anna Martina Lippert (since April) (*Universidad Internacional Menéndez Pelayo*, Madrid, Spain)

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

RESEARCH HIGHLIGHTS

Mismatch repair

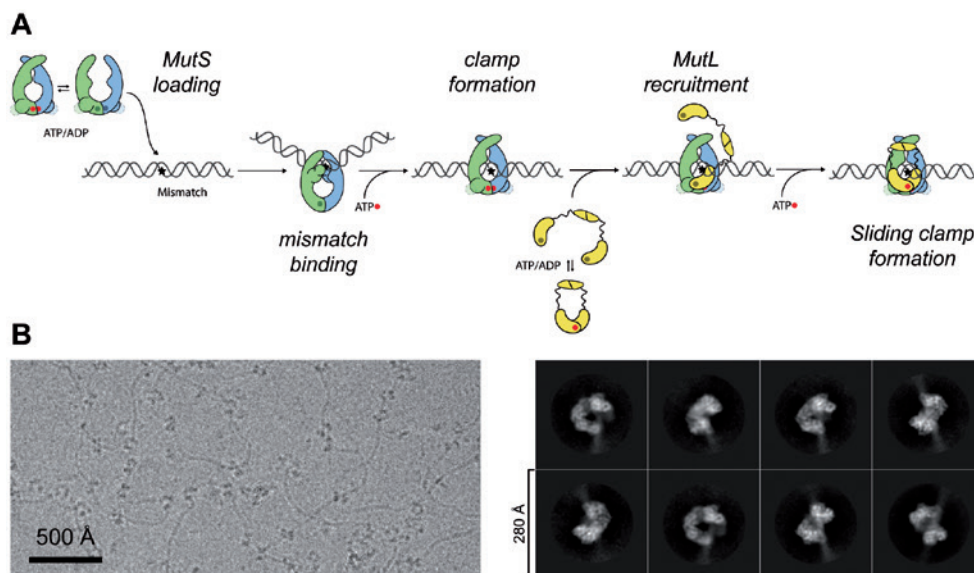
The DNA mismatch repair machinery (MMR) corrects the errors introduced by DNA polymerases during DNA replication and is critical for genome stability. The MutS protein loads onto newly synthesised DNA and searches for mismatches. The recognition of an error in the DNA leads to an ATP-dependent conformational change that transfers MutS into a sliding clamp state. Only this MutS state can activate the MutL ATPase, 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-Electron Microscopy (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 Titia Sixma (Netherlands Cancer Research Institute) and Meindert Lamers (Leiden University).

DNA replication and 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 and, in particular, 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 Mismatch repair studies. (A) Scheme representing the initial stages of the DNA mismatch repair pathway: mutS loading and DNA scanning, mismatch binding, clamp formation, mutL recruitment, and sliding clamp formation. These steps control the licensing of DNA repair. (B) Cryo-EM micrograph of MutS protein on DNA (long strings) (left) and 2D class averages of the protein after image processing (right). These images are used for high-resolution structural analysis.



PUBLICATIONS

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COMPUTATIONAL CANCER GENOMICS JUNIOR GROUP

Solip Park
Junior Group Leader

Graduate Student
Pelayo González de Lena (since November)

Technician
Luis García (since July)



OVERVIEW

According to the conventional point of view of the 1980s, cancer drivers were classified into tumour suppressor genes (TSGs) and oncogenes (OGs) based on their functional roles in cancer. Mutations in TSGs were considered recessive while mutations in OGs were expected to be dominant gain-of-function mutations. However, importantly, the success of large-scale cancer genomics has led to debate around the dogma in cancer genetics that all cancer genes should behave according to the same model, even in different contexts (e.g., cancer types).

By analysing large-scale cancer genomics data, many exceptions have been observed, including haploinsufficiency in TSGs or amplification-linked mutation in OGs and even in dual-functional genes. It is clear that activity levels of genomic alterations in cancer genes are disparate across cancer types, and

“By analysing large-scale cancer genomics data, we aim to further pursue novel questions about cancer-type and context-specific tumour progression to understand tumour heterogeneity.”

optimal models for tumour progression may also vary depending on contexts or cancer types. The pioneering cancer genomics studies referenced above have triggered many interesting questions about how cancer genes change their models of tumour progression depending on cancer types or contexts.

Visiting Student

Sulki Song (January- March) (Seoul
National University Cancer Research
Institute, Seoul, South Korea)

RESEARCH HIGHLIGHTS

Cancer fitness landscape: from within a gene (cis) to between genes (trans)

The classic 2-hit model postulates that both alleles of a tumour suppressor gene must be inactivated by a combination of 2 different alterations for tumour progression. However, some cancer genes increase tumour fitness after only a single hit and, in some cases, a second hit may actually be detrimental. To comprehensively understand the cancer fitness landscape, we analysed > 10,000 tumours and classified cancer genes as 2 hits, 1 hit, or having optimal activity levels, which is a dangerous approximation because the activity-fitness functions of individual cancer genes are often diverse depending on the context. Specifically, mutations in other cancer genes frequently switch individual drivers from requiring 2 hits to 1 hit being sufficient to promote tumour progression. These results will provide the correct genetic model for a cancer gene, depending on their contexts, and emphasise a frequent redundancy between a second hit occurring in the same gene or in a second gene in a pathway during tumour progression. These studies were conducted in collaboration with Fran Supek (*IRB*, Barcelona) and Ben Lehner (*CRG*, Barcelona).

Inherited variants of Mendelian disease-associated genes in cancer genomics

Hereditary diseases are caused by pernicious mutations in certain genes or chromosomes. Usually, the abnormalities appear in newborns or during infancy, but sometimes they also occur in adults, such as is the case with Huntington's disease. In cases of late onset, it is reported that not only does it cause a single disease, but it also changes the concomitant pathways or affects cancer development if the stress from toxicity is sustained. The occurrence of cancer is apparent when there is an accumulation of additional variations. Using large-scale cancer genomics data, we identified the contribution of Mendelian disease-associated genes to cancer risk across more than 30 cancer types. These results will enable cancer prevention through genetic testing aimed at reflecting individual disease susceptibility to various diseases. These studies were carried out in collaboration with Young-il Goh (Seoul National University, South Korea). ■

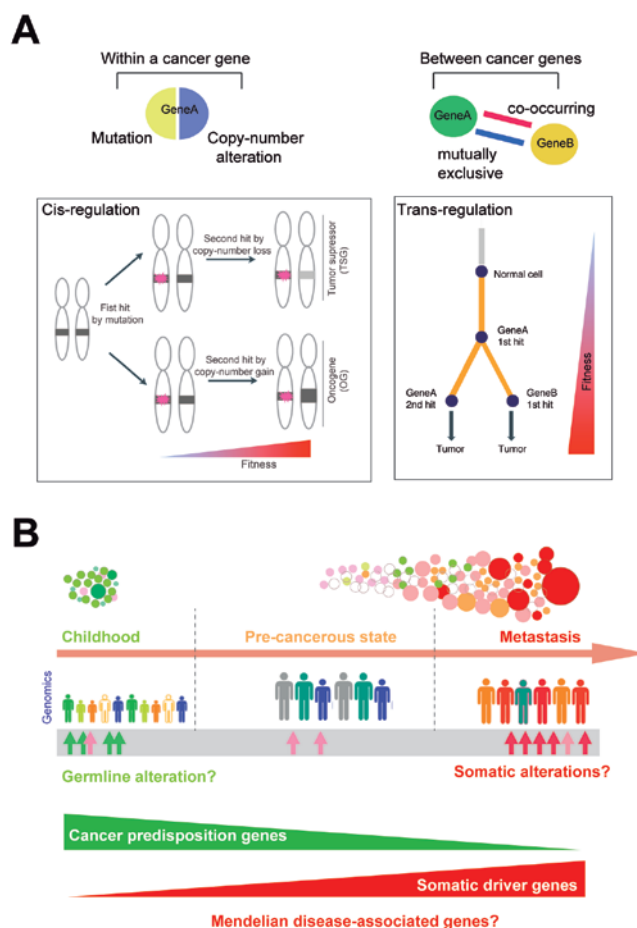


FIGURE A systematic understanding of context-dependent cancer fitness landscape by analysing large-scale cancer genomics. **(A)** A cancer gene will change its optimal model of tumour progression depending on the context from cis-regulation to trans-regulation. **(B)** Novel insight into the increment of cancer risk by Mendelian disease-associated genes.

COMPUTATIONAL ONCOLOGY JUNIOR GROUP

Geoffrey John Macintyre
Junior Group Leader

Staff Scientist
María José García (since August)

Visiting Master's Student
Blas Chaves (since November)
(*Universidad Complutense de Madrid*,
Madrid, Spain)



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 that 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.

“In our first year of operation we have established computational and laboratory infrastructure that will allow us to observe chromosomal instability at the earliest stages of tumour evolution.”

- Developing single cell/nucleus sequencing approaches to detect ongoing CIN.

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.

RESEARCH HIGHLIGHTS

2020 started well for the Computational Oncology Group with the publication of work the group was involved in as part of the pan-cancer analysis of whole-genomes project. However, the onset of the Covid-19 pandemic meant the first year of operations for the Computational Oncology Group did not go as planned. It did, however, make some of the small wins a lot more important!

We now have a brand-new laboratory setup designed specifically to interrogate tumour DNA copy number using a low-cost, low-pass whole-genome sequencing strategy. We have established our computational lab and have worked closely with the Bioinformatics Unit to upgrade CNIO's scientific computing infrastructure to handle the imminent influx of sequencing data.

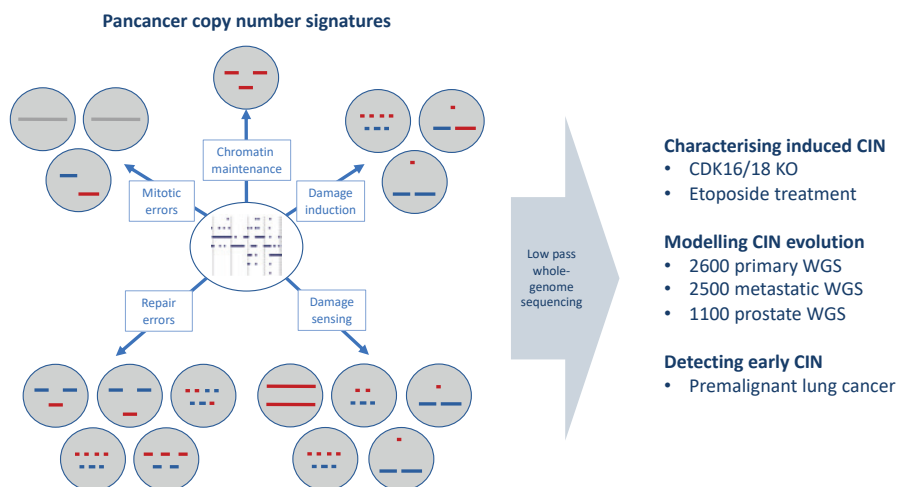
The Group is also slowly growing size – María José García joined from Javier Benítez's Group upon his retirement and has been the driving force behind getting the Computational Oncology Group's laboratory operations up and running. She brings with her a wealth of experience preparing clinical

samples for DNA sequencing and her own funded project on DNA mismatch repair in ovarian cancer. Blas Chaves has joined for his Master's project at *Universidad Complutense de Madrid (UCM)* and has demonstrated his aptitude in the lab and in front of the computer. He will be characterising different types of chromosomal instability in model systems using copy number signatures.

We have developed collaborations with Marcos Malumbres' laboratory where we will determine the types of CIN caused by knockout of CDKs, and with Felipe Cortés' laboratory looking at CIN induced by etoposide treatment. We have started our project with Sam Janes at University College London (UCL) detecting CIN in premalignant lung cancers. Finally, we have made significant progress characterising pan-cancer patterns of CIN and how they relate to drug response – look out for a publication in 2021!

It looks an exciting year moving ahead with 3 new members and a chance to finally put our new laboratory infrastructure into action to tackle some of the deadliest cancers. ■

FIGURE A schematic of the 17 pancancer copy number signatures we have identified using >10,000 tumour samples across 32 tumour types and how we will combine these with low-pass whole genome sequencing to interrogate different types of CIN at different stages of tumour evolution.



► PUBLICATIONS AT OTHER INSTITUTIONS

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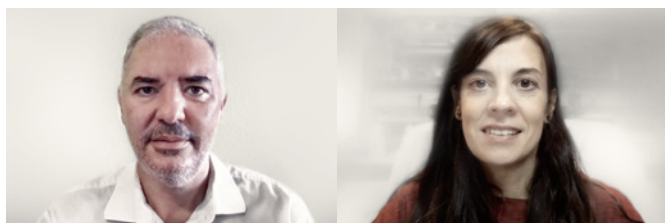
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SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

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 characterisation 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 serve as 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

“In 2020 we initiated work with a QTOF mass spectrometer that will allow the quality control of purified proteins from their intact mass spectrum, as well as the targeted characterisation or profiling of metabolites in liquid samples of cancer model systems.”

human samples. In addition, in 2020, we adopted a mass spectrometer for the characterisation of intact proteins and for metabolite studies using HPLC-MS methods.

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, and two biosensor instruments: surface plasmon resonance (SPR), and biolayer interferometry (BLI). Research Groups mostly from, but not limited to, the Structural Biology Programme used these technologies throughout 2020 (i.e., the Haematological Malignancies Clinical Research Unit, the Monoclonal Antibodies Unit, and the Experimental Therapeutics Programme – ETP).

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 ETP), as well as for metabolite quantification that, in 2020, 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). During 2020, we incorporated a QTOF mass spectrometer that will complement our battery of techniques for the quality control of purified proteins with the information contained in their intact mass spectra. For example, we examined several reference proteins (see FIGURE), verifying that the instrument can determine the mass with high precision and accuracy employing nanogram amounts. In addition, HPLC-MS measurements of biofluids were also initiated. 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. ■

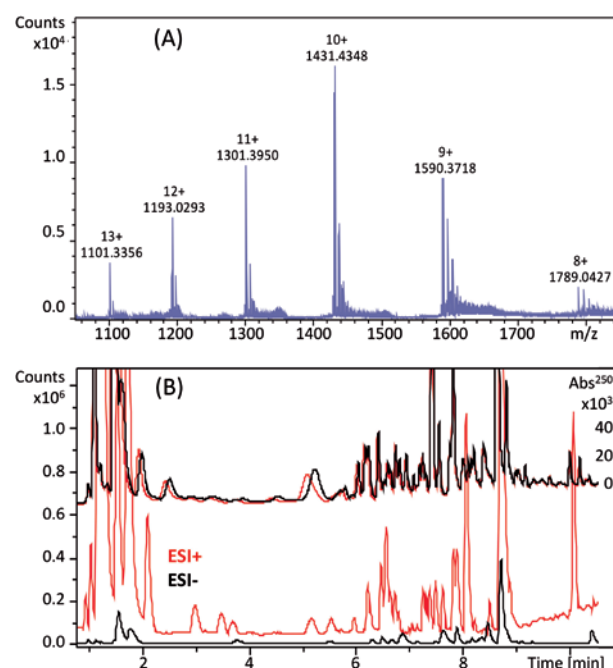


FIGURE (A) Mass spectrum of perfused lysozyme (20 µg/mL at 3 µL/min) with indication of the identified cationic forms (+13 to +8), which allows determination of a mass of 14304 Da (expected 14313 Da). (B) HPLC-MS chromatograms of human urine injected in a reverse-phase

column as followed by base peak ion counts (lower traces, left scale) and UV absorbance (upper traces, right scale). Two consecutive runs of the same sample measured in positive (red) and negative (black) Electro Spray Ionization mode are shown.

PUBLICATIONS

- Izquierdo-García JL, Comella-Del-Barrio P, Campos-Olivas R, Villar-Hernández R, Prat-Aymerich C, De Souza-Galvão ML, Jiménez-Fuentes MA, Ruiz-Manzano J, Stojanovic Z, González A, Serra-Vidal M, García-García E, Muriel-Moreno B, Millet JP, Molina-Pinargote I, Casas X, Santiago J, Sabriá F, Martos C, Herzmann C, Ruiz-Caballo J, Domínguez J (2020). Discovery and validation of an NMR-based metabolomic profile in urine as TB biomarker. *Sci Rep* 10, 22317.
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BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Staff Scientist
Michael Tress

Graduate Students
Santiago García (since March) (PEJ, CAM) , María José Jiménez, Laura Martínez, Fernando Pozo, Kevin Troulé

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



OVERVIEW

Bioinformatics is a key discipline for understanding the cancer genome and for the future of cancer therapeutics. Bioinformatics-based approaches have the ability to transform the vast amount of biological data into 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

“DREIMT provides the largest drug-immune expression signature associations database available, allowing the users to generate hypotheses and explore druggable targets throughout the immune system.”

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

Bioinformaticians

Daniel Cerdán (TS)^{*,} Tomas Di Domenico (TS)^{*,} Coral Fustero (TS)^{*,} Gonzalo Gómez (TS)^{*,} Osvaldo Graña (TS)^{*,} Elena Piñero (TS)^{*,} Thomas Anthony Walsh (TS)^{*,}

^{*,}Titulado Superior (Advanced Degree)

Students in Practice

Ruth Álvarez (since July) and Agustín J García (since July) (*Master's Programme in Bioinformatics, ISCIII-ENS, Madrid, Spain*), Luis García (until May) (*Universidad Autónoma*

de Madrid, Spain), Jorge Lemos (February-June) (*Universidad Autónoma de Madrid, Spain*)

Visiting Scientists

Enrique Carrillo (until April) (*IMDEA Food Institute, Spain*), Jose María

Fernández (*BSC-CNS, Spain*), Paolo Maietta (*NIMGenetics, Spain*), Javier Perales (until June) (*Heidelberg University, Germany*)

RESEARCH HIGHLIGHTS

In 2020, the CNIO Bioinformatics Unit published more than 20 peer-reviewed articles (see full list on our web site <https://bioinformatics.cnio.es/>) as a result of our ongoing research projects and scientific collaborations. We studied the effect of drugs on the biology and activity of the immune system and its interaction with cancer cells. To this end we developed DREIMT (Troulé K *et al.*, 2020), a new hypothesis-generation web tool that performs drug prioritisation analysis for immunomodulation. DREIMT provides immunomodulatory drugs targeting up to 70 immune cell subtypes through a curated database that integrates 4,960 drug profiles and ~2.6K immune gene expression signatures. DREIMT is the largest database for drug-immune expression signature associations currently available. DREIMT also provides tools to suggest potential immunomodulatory drugs targeting user-supplied gene expression signatures. DREIMT is fully accessible to the scientific community at <http://www.dreimt.org>. Additionally, we applied our method PanDrugs (<https://www.pandrugs.org/>) in the context of Pan-Cancer analysis of whole genomes (The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium, 2020).

During 2020, the Unit also analysed alternative splicing variants in vertebrates and found that they played a significant role in the evolution of brain and heart tissues (Rodriguez JM *et al.*, 2020). Furthermore, our results in the analysis of SINE Alu genomic elements confirmed that such elements contribute to the expansion of the human proteome despite little evidence of their biological relevance (Martinez-Gomez L *et al.*, 2020). With regard to academic and knowledge transfer activities, we co-organised the Master's degree in *Bioinformática Aplicada a Medicina Personalizada y Salud (ISCIII-ENS)* (visit our web page for a full list of activities). ■

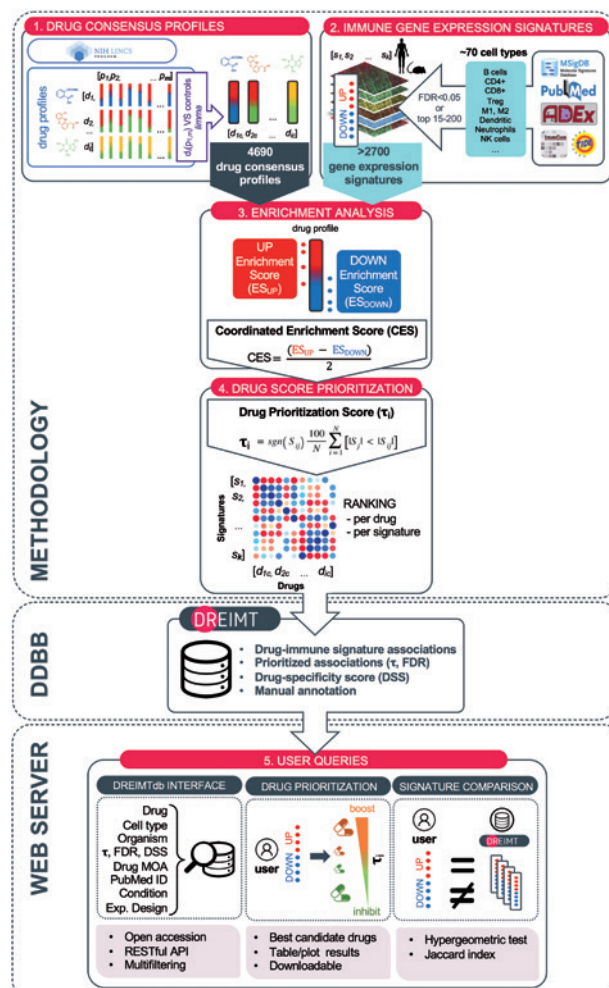


FIGURE DREIMT consists of a methodology, a database and a web tool for hypothesis generation and prioritisation of drugs capable of modulating immune cell activity

from transcriptomics signatures. DREIMT output includes drug-signature association scores, FDRs and downloadable plots and results tables.

▶ SELECTED PUBLICATIONS*

- ▶ The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium (2020). Pan-cancer analysis of whole genomes. *Nature* 578, 82-93.
- ▶ Dimitrov-Markov S, Perales-Patón J, Bockorny B, Dopazo A, Muñoz M, Baños N, Bonilla V, Menendez C, Duran Y, Huang

L, Perea S, Muthuswamy SK, Al-Shahrour F, Lopez-Casas PP, Hidalgo M (2020). Discovery of new targets to control metastasis in pancreatic cancer by single cell transcriptomics analysis of circulating tumor cells. *Mol Cancer Ther* 19, 1751-1760.

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- ▶ Rodríguez JM, Pozo F, Di Domenico T, Vazquez J, Tress ML (2020). An analysis of tissue-specific alternative splicing at the protein level. *PLoS Comput Biol* 16, e1008287.

- ▶ Martinez-Gomez L, Abascal F, Jungreis I, Pozo F, Kellis M, Mudge JM, Tress ML (2020). Few SINEs of life: Alu elements have little evidence for biological relevance despite elevated translation. *NAR Genom Bioinform* 2, lqz023.

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

ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Post-Doctoral Fellow
Johanne Le Coq (since December)



OVERVIEW

The main goal of the Electron Microscopy (EM) Unit is to provide scientific-technical support to CNIO researchers to solve their biological questions using various transmission EM techniques. We routinely use negative staining and cryo-EM, and we also help in image processing by performing 2D analysis and 3D reconstruction. We also offer support for selecting adequate EM techniques and performing sample preparation. Moreover, we provide the necessary training on the use of our microscopes and auxiliary equipment. More advanced studies are typically delivered through research collaboration.

“We devote our main effort to allowing efficient access to, and use of, existing infrastructure in the Unit. We provide scientific support and training to researchers tailored to their research needs.”

Technicians

Carmen García (TS)* (PEJ)** , Pilar Redondo (until December)

*Titulado Superior (Advanced Degree)

**Plan de Empleo Joven (Youth Employment Plan)

Student in Practice

Sofía Sonsoles Doyagüez (February-June) (*Universidad Politécnica de Madrid*, Madrid, Spain)

RESEARCH HIGHLIGHTS

Technical advances in the last decade have positioned cryogenic electron microscopy (cryo-EM) as one of the most powerful and effective technologies available to investigate the structures of macromolecules at near-atomic resolution. Among several cryo-EM structural determination methods, single-particle analysis is the most popular for structural biologists, as it has relatively well-established methods for sample preparation, data collection, image processing, and structural determination. At the CNIO we have in place a 120 kV, Tecnai G2 Spirit microscope equipped with a TVIPS CMOS detector that is used to obtain images of negatively stained samples and to screen vitrified samples. For medium resolution structural studies, the Unit is equipped with a JEM-2200FS cryo-EM and a K3 direct electron detector camera. Our scientific activity throughout 2020 involved collaborations with all the Research Groups from the Structural Biology Programme, several Groups from other Programmes, as well as with scientists outside the CNIO. For instance, in collaboration with the Cell Division and Cancer Group, we monitored centriole structure and organisation as a consequence of lack of Cep135, a protein involved in centrosomal and spindle dynamics; in collaboration with the Microenvironment and Metastasis Group, we studied the morphological changes of extracellular vesicles, mainly exosomes isolated from prostate cancer cells; in collaboration with the Macromolecular Complexes in DNA Damage Response Group, we continued our collaboration on the structural characterisation of several protein complexes, e.g., RUVBL1/2 complexes and DNA repair complexes; together with Manuel Palacín's group (*IRB*, Barcelona), we also continued the collaboration on high-resolution structural characterisation of amino acid transporter complexes; and, finally, in collaboration with Genome Integrity and Structural Biology Group, we have been setting-up a pipeline to use a cryo-EM as a tool for drug discovery. ■

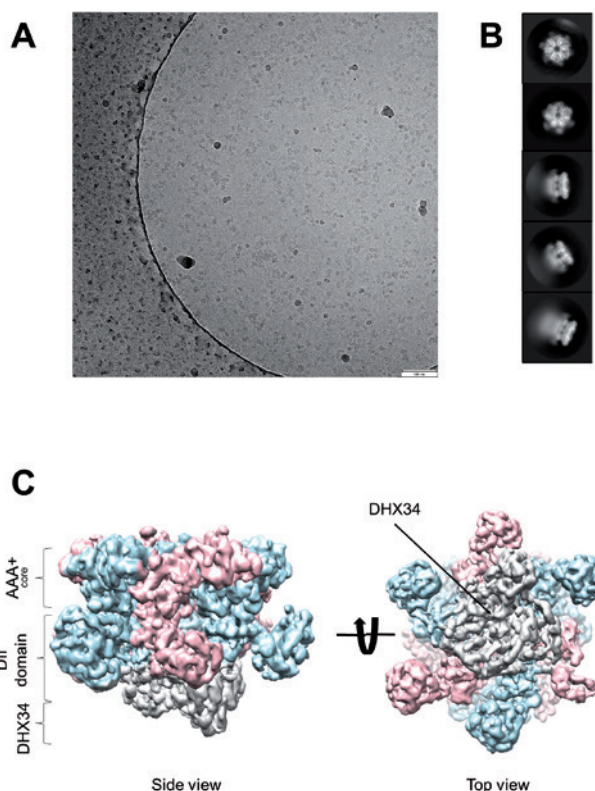


FIGURE Architecture of RUVBL1-2-DHX34 complex revealed by cryo-EM. (A) Representative cryo-EM field. (B)

Reference-free 2D class averages. (C) Views of cryo-EM structure of the complex.

► PUBLICATIONS

► Acebrón I, Righetto RD, Schoenherr C, Buhr S, Redondo P, Culley J, Rodríguez CF, Daday C, Biyani N, Llorca O, Byron A, Chami M, Gräter F, Boskovic J, Margaret C Frame, Stahlberg H, Lietha D (2020).

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RUVBL1-RUVBL2 AAA-ATPases by the nonsense-mediated mRNA decay factor DHX34, as evidenced by Cryo-EM. *Elife* 9, e63042.

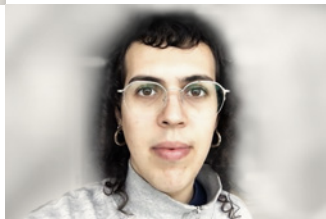
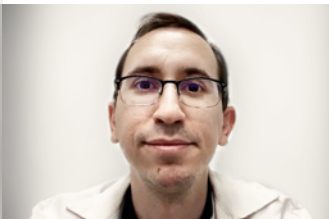
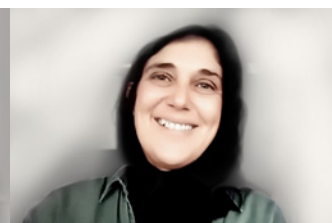
► González-Martínez J, Cwetsch AW, Martínez-Alonso D, López-Sainz LR, Almagro J, Megías D, Boskovic J, Gilabert-Juan

J, Graña-Castro O, Pierani A, Behrens A, Ortega S, Malumbres M (2020). Lack of adaptation to centriolar defects leads to p53-independent microcephaly in the absence of Cep135. *BioRxiv*, doi: <https://doi.org/10.1101/2020.05.07.082032>.

CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

Inés Muñoz
Unit Head

Staff Scientist
Jorge Luis Martínez



OVERVIEW

The Crystallography and Protein Engineering Unit (XTPEUnit) is a core facility created to provide on-demand services at different levels to fulfil the needs of our users. By offering services ranging from protein cloning to solving the 3D structures of proteins, we help our users to further comprehend how target proteins work. With this purpose in mind, we produce proteins for different types of biochemical/biophysical/*in vitro/in vivo* assays and for monoclonal antibody production, also offering macromolecular structural determination at high-resolution (atomic) by X-ray crystallography, and at low-resolution in solution by small-angle X-ray scattering (SAXS). Protein co-crystallisation, in the presence of inhibitors or small fragments, is routinely done in our laboratory in combination with studies on protein thermal stability (thermofluor assay), to accelerate the guided drug discovery process.

“By fragment screening on crystals, we visualise direct interactions between small molecules and proteins, speeding up the identification of new targetable sites in drug discovery projects.”

Postdoctoral Fellows

Raquel S. Correia (until March)
Johanne Le Coq (until December),
Virginia Sandonis (since October)

Technicians

Aida Contreras (TS) * (PEJ) **, Diana
Martín (since February), Álvaro
Otero (TS) *, Eduardo Rebollo (TS) *

(PEJ) **, Pilar Redondo (since
December)

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

***Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

Our Unit works closely with the Experimental Therapeutics Programme on several projects. To fulfil the need of recombinant proteins, we produced, throughout the year, full-length and kinase domain human MASTL, full-length mouse TRF1 and human TRF1 dimerization domain, for biochemical, *in vitro*, thermo-stability and structural analyses. Furthermore, to support drug discovery projects, we performed several thermal shift assays (thermofluor) in the presence of compounds developed and tested at the Medicinal Chemistry Section and the Biology Section, respectively.

We also continued our close collaboration with the CNIO Monoclonal Antibodies Unit on the production of proteins to generate highly specific antibodies against several cancer-associated proteins such as HASPIN, RANK, CD85C, CD85G and CD85J, and other protein tools such as Cas9. Additionally, we ran a number of internal collaborations with other CNIO Groups and Units, providing them with recombinant proteins for biochemical and/or cell-based functional assays; this was the case, for example, with the Telomeres and Telomerase Group, the Experimental Oncology Group, the Genomic Instability Group, the Cell Division and Cancer Group, the Melanoma Group, the H12O-CNIO Lung Cancer Clinical Research Unit, the Macromolecular Complexes in DNA Damage Response Group, the H12O-CNIO Haematological Malignancies Clinical Research Unit, and the Transformation and Metastasis Group.

The Unit maintained collaborations with various external groups: the Environmental Biology Department, *CIB-CSIC*, Spain; the Pharmacology and Therapeutics Department, Roswell Park Cancer Institute, USA; the Department of Biomedicine, University of Bergen, Norway; the Department of Crystallography and Structural Biology, *Instituto*

Química-Física Rocasolano, CSIC, Spain; the Department of Immunology, Genetics and Pathology, Uppsala University, Sweden; the Cancer Immunotherapy Unit; *12 de Octubre* University Hospital, Spain; and the Division of Pulmonary and Critical Care Medicine, Fibrosis Research Center, and Center for Immunology and Inflammatory Diseases, Harvard Medical School, USA.

Throughout 2020, the Unit also proceeded with its own scientific projects. We continued working on targeting the function of the Mdm2-MdmX E3 complex activity in the context of an NIH-funded collaborative project with the Department of Pharmacology and Therapeutics at Roswell Park Cancer Institute. In addition, we are recombinantly producing a T cell-recruiting bispecific antibody (named *ATTACK*) for structural and functional purposes, in collaboration with the company LeadArtis, the Department of Microbiology (Immunology) of the *Complutense* University of Madrid, and the Cancer Immunotherapy Unit of the *12 de Octubre* University Hospital; a project funded by the *Retos Colaboración* programme of the Spanish Ministry of Science, Innovation and Universities. The Unit is also taking part in 2 collaborative projects with the Biomedical Application of Radioisotopes Unit of *CIEMAT*, the Bioactive Nanostructured Materials Group of the *Complutense* University of Madrid, and the CNIO's Molecular Imaging Unit to develop new antibody-based positron emission tomography (immunoPET) imaging tools for tumour visualisation and pretargeted clickable antibody fragments for theranostic applications; both projects are supported by *BBVA* Foundation grants. Finally, in 2020 we were awarded a *BBVA* Foundation grant, jointly with the Cancer Immunotherapy Unit of the *12 de Octubre* Hospital's Research Institute (i+12), to design a new immunotherapy method to fight Covid-19. ■

PUBLICATIONS

- Esteban-Burgos L, Wang H, Nieto P, Zheng J, Blanco-Aparicio C, Varela C, Gómez-López G, Fernández-García F, Sanclemente M, Guerra C, Drosten M, Galán J, Caleiras E, Martínez-Torrecuadrada J, Fajás L, Peng SB, Santamaría D, Musteanu M, Barbacid M (2020). Tumor regression and

resistance mechanisms upon CDK4 and RAF1 inactivation in KRAS/P53 mutant lung adenocarcinomas. *Proc Natl Acad Sci USA* 117, 24415-24426.

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Rho-mediated homeostatic control of lymphatic endothelial cell junction integrity. *Elife* 9, e57732.

- Martínez-González S, García AB, Albarrán MI, Cebriá A, Amezquita-Alves A, García-Campos FJ, Martínez-Gago J, Martínez-Torrecuadrada J, Muñoz I, Blanco-Aparicio C, Pastor J (2020). Pyrido[2,3-b][1,5]benzoxazepin-5(6H)-one

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PATENT

- Paz-Ares L, Martínez JL, Roncador G, Ojeda L, Ferrer I (2020). Interleukin 11 receptor alpha subunit (IL11RA) neutralizing antibodies and uses thereof. *EP20382631.8*

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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 2020, the Human Cancer Genetics Programme was composed of 3 Research Groups: Hereditary Endocrine Cancer, Genetic and Molecular Epidemiology and Human Genetics; 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 2020, the Familial Cancer Consultancy carried out around 75 consultancies, and the HCGP performed 622 genetic diagnoses and 605 cytogenetic studies. In addition, the HCGP's Groups hosted 10 residents from different Spanish hospitals for a 3-month training. The HCGP also offers short-stay opportunities of 2-6 weeks for professionals from different international research centres; a total of 15 national visitors and students were hosted. In terms of education, 14 national PhD students worked on their research projects, 3 of whom already successfully defended their theses.

The Programme participates in many international and national Consortia. In 2020, the HCGP collaborated with 18 international consortia and led or participated in 17 national and 5 international European projects. Milestones and major achievements of the HCGP in 2020 include: "Ideas Semilla" project from the *AECC* (Spanish Association Against Cancer), granted to Cristina Rodríguez; ATA (The American Thyroid Association) research grant for young scientists, awarded to Cristina Montero Conde; and H2020 project PANCAIM, Pancreatic cancer AI for genomics and personalised Medicine, awarded to Núria Malats. These projects were awarded for their highly innovative projections.

We would like to take this opportunity to thank our former Programme Director, Javier Benítez, who has served as Director of the Human Cancer Genetics Programme since 2005. We are thankful to him for his important contributions as well as for his inestimable dedication and commitment to the Programme. Thank you Javier for having been a part of our CNIO community and we wish you the best on your retirement!

A new Director for the Human Cancer Genetics Programme will be appointed soon. We are confident that, under a new leadership, the Programme will continue to grow and further develop translational research with the overarching goal of improving the diagnostics, prevention and treatment of cancer.

Maria A. Blasco, *Director*

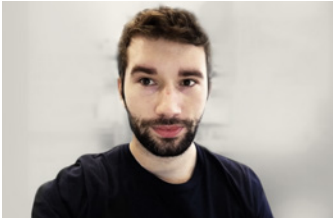
Óscar Fernández-Capetillo, *Vice Director*

HUMAN GENETICS GROUP

Javier Benítez (until July)
Group Leader

Staff Scientists
Maria José García (until July), Ana
Osorio (until July)

Post-Doctoral Fellow
Oriol Calvete (until November)



Graduate Students

Juan Miguel Baquero (until December), Paloma Martín (until December)

Technicians

Alicia Barroso (until July), Victoria Fernández (until August) (TS)*

**Título Superior* (Advanced Degree)

Students in Practice

María Escobar (February-July) (*Universidad Autónoma de Madrid*, Spain), Rocio Mirabet (February-June) (*Universidad Politécnica de Madrid*, Spain), Anne B. Parra

(January-June) (*Universidad Complutense de Madrid*, Spain), Eneida Turiján (until April) (*Universidad Autónoma de San Luis Potosí*, Mexico)

OVERVIEW

Our work focusses on deciphering the genetic bases of hereditary and sporadic breast and ovarian cancer and other rare tumours. During 2020, we contributed to the definition of the main *bona-fide* breast cancer (BC) susceptibility genes through our substantial participation in an ambitious international collaboration (BRIDGES, a European project). In addition, we obtained funding to develop two new projects focussed on breast and ovarian cancer genetics. We have also been exploring the currently unknown genetic basis of testicular cancer. More recently, we started a study to elucidate the common genetic origin of different autoimmune endocrinopathies, such as chronic gastritis atrophy, thyroiditis, diabetes, or arthritis, and we identified several genes that open a new scenario for diagnosis and treatment. Finally, we found interesting results suggesting a synthetic lethal interaction between *BRCA1* and *OGG1* and a synergistic effect between *OGG1* and *PARP1* inhibitors.

“In 2020, we contributed to determining the most relevant genes in BC susceptibility, we found several candidate genes explaining the possible common origin of autoimmune endocrinopathies, and we identified a synergistic effect between *OGG1* inhibitors and *PARP1* inhibitors.”

RESEARCH HIGHLIGHTS

Hereditary breast and ovarian cancer*Deciphering the role of rare variants in BC*

The first publication reporting results obtained by the European project BRIDGES, to which we made a major contribution, was released this year. Thirty-four confirmed or putative BC susceptibility genes were sequenced in 60,466 cases and 53,461 controls. Nine genes were confirmed as *bona-fide* BC susceptibility genes, and the risk conferred by their mutations to develop the disease was refined. These results, published in the *New England Journal of Medicine* (Dorling *et al.*, 2020), are highly relevant to improving the genetic diagnosis, counselling and follow-up of BC patients and their families. In addition, we participated in the largest collaborative international study evaluating the risk conferred by mutations in the *RAD51C* and *RAD51D* genes to develop breast and ovarian cancer (Yang *et al.*, 2020).

Identification of 14 new candidate BC susceptibility genes

As a complementary approach to the large collaborative projects, we are conducting a study using next-generation sequencing (NGS) technologies in a few, very well selected families, to identify new BC susceptibility genes. This approach led to the identification of *RECQL5*, a member of the RECQL-helicases family, as a new BC susceptibility candidate (Tavera-Tapia *et al.*, 2019). Besides *RECQL5*, we identified 13 additional candidate genes that are extremely interesting because of their function, the potential deleteriousness of the variants, and their rarity in the general population. We developed an NGS panel containing this set of genes and will evaluate their role in BC by sequencing a large set of 3000 Spanish BC families.

*Modifier genes in *BRCA1/2* genes*

We continue to play an active role in the CIMBA consortium (Consortium of Investigators of Modifiers of *BRCA1* and

BRCA2), which made important contributions in 2020 i) showing the modification of ovarian cancer risk by the PRS (Polygenic Risk Score) in *BRCA1* and *BRCA2* mutation carriers (Barnes *et al.*, 2020), ii) identifying the spectrum of mutations in male BC patients harbouring mutations in the *BRCA* genes (Silvestri *et al.*, 2020), and iii) associating mutations in different domains of *BRCA1* and *BRCA2* with prostate cancer risk (Patel *et al.*, 2020). We also started collaborating in the ambitious CONFLUENCER project, funded by the National Cancer Institute, the aim of which is to perform new Genome-Wide Association Studies (GWAS).

DNA glycosylase inhibitors as a new therapeutic approach in hereditary BC patients

In 2014, we reported that SNPs located in genes encoding for DNA glycosylases involved in the Base Excision Repair (BER) pathway could act as breast and/or ovarian risk modifiers in *BRCA1/2* mutations carriers (Osorio *et al.*, 2014). Since then, we have been exploring the mechanisms of action of these SNPs (Benítez-Buelga *et al.*, 2016; and 2017; Baquero *et al.*, 2019), as well as of the OGG1 glycosylase inhibitor TH5487 and its possible therapeutic use in BC patients. We found that the inactivation of BER by TH5487 increases the accumulation of oxidised bases at the telomeres, leading to telomere loss and post-mitotic defects (Baquero *et al.*, 2020 under review) (FIGURE). Moreover, we discovered that TH5487 enhances the activity of the PARP1 inhibitor olaparib in *BRCA1* deficient cells. These preliminary results might represent the proof-of-concept for new alternative or complementary therapies for hereditary breast and ovarian cancer patients.

Ovarian cancer

Endometrioid (EOC) and clear cell (CCOC) ovarian carcinomas are considered refractory to chemotherapy and present bad outcomes once disseminated. About 15% show defects in

mismatch repair (MMRd) and microsatellite instability (MSI). MMRd is a predictor of immunotherapy response, and the global burden of instability has been associated with survival in other tumour types. We compiled more than 200 EOCs and CCOCs and assessed the global load of microsatellite instability and tumour mutation burden using an “*ad hoc*” NGS ovarian cancer panel (OvaSeq-MSI). OvaSeq-MSI includes a large set of MS sequences to improve MSI determination and provides information about mutations in ovarian carcinogenesis genes, ovarian cancer susceptibility genes, or therapeutic targets. Preliminary results show a good correlation between global instability and mutation burden, and an association between the extent of both indicators and improved disease evolution in patients (lack of relapse). Altogether, we expect to find markers that will enable more rational therapeutic decision-making for EOC and CCOC patients.

Familial cancer exome project

Autoimmune polyneuroendocrine syndrome (APS)

In 2015, we published research identifying the *ATP4A* gene as being responsible for families with achlorhydria-mediated gastric neuroendocrine tumours (gNET) (Calvete *et al.*, 2015 and 2016). In 2017, we extended this study to a new gNET family that presented with hypothyroidism and arthritis explained by two mutations in the *ATP4A* and *PTHRI* genes (Calvete *et al.*, 2017). In addition, we explored the pathogenic mechanism underlying tumour progression mediated by mutations affecting achlorhydria (Benítez *et al.*, 2020). We have further investigated the apparent relation of gastric autoimmune disease (gastric neuroendocrine tumour or chronic atrophic gastritis) co-occurring with other autoimmune diseases (hypothyroidism, arthritis, DM1, vitiligo). Using an NGS custom panel including 12 new candidate genes, we found

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several mutations putatively affecting the cell's internal acid-base balance function, not only in parietal cells from the stomach but also in other tissues (thyroid, epithelium, pancreas, skin), explaining this autoimmune polyendocrine syndrome (Calvete *et al.*, under review).

Testicular Germ Cell Tumour (TGCT)

During the past year, we explored TGCT in familial, bilateral, and sporadic patients, by evaluating their clinical history and by identifying moderate susceptibility genes and pathways involved in the disease. Familial and bilateral patients develop TGCT significantly earlier, and their relatives have a lower percentage of other tumour types compared to sporadic patients, suggesting a more relevant genetic background in their origin. A gene ontology analysis was performed with the variants obtained by WES, and the glycosylation pathway, a post-translational modification that consists of a carbohydrate attaching to different functional groups, was found to be over-represented in all cohorts of patients and thus might be involved in the development of the disease. In addition, the glycosylation genes with variants uncovered by WES were significantly located in the same cytoband as the susceptibility loci previously described by GWAS studies. ■

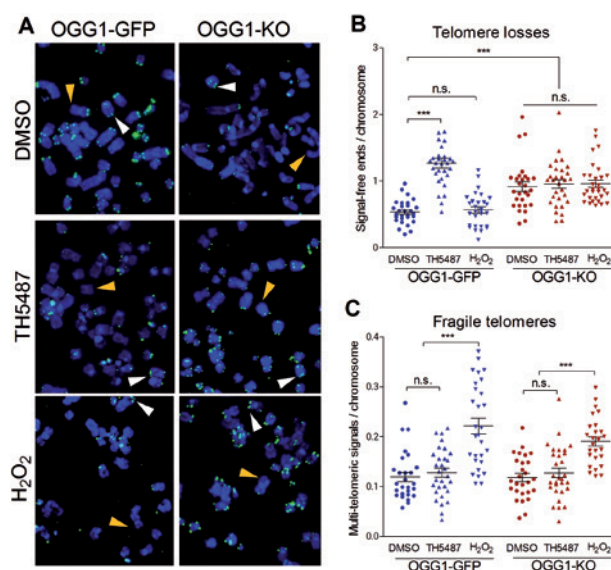


FIGURE The inactivation of OGG1 with TH5487 inhibitor causes telomere losses. (A) Telo-FISH images of metaphase chromosomes from U2OS OGG1-GFP or OGG1-KO cells for non-treated (DMSO), TH5487, and oxidative treatment. Chromosomes were stained with DAPI (blue) and telomeres were stained with PNA telomeric probe (green). An example of a telomere loss (orange arrowhead) and a fragile telomere

(white arrowhead) is indicated in each image. (B) Quantification of telomeric signal-free ends for the conditions shown in (A). (C) Quantification of fragile telomeres. Comparative analysis for the frequency of multi-telomeric signals for the conditions shown in (A). Each dot represents a metaphase. More than 30 metaphases per condition from two independent experiments.

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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 a miR-483-5p/ALCAM axis as a new player in pheochromocytoma at the metastatic niche and showed that mTOR pathway mutations correlate with poor prognosis in chromophobe renal cell carcinoma.”

RESEARCH HIGHLIGHTS

mTOR pathway alterations in chromophobe renal cell carcinoma associated with poor outcome

Chromophobe renal cell carcinoma (chRCC) is a histologically and molecularly distinct class of rare renal tumour. Knowledge on drug targets is limited and treatments do not follow a molecular rationale. In the largest series of chRCC (n=92) so far, we performed an in-depth characterisation of the mTOR pathway through targeted NGS and immunohistochemistry (Roldán-Romero *et al.*, 2020). Furthermore, we investigated mutations in the electron transport chain Complex I genes by developing a bioinformatics approach able to identify mitochondrial variants using NGS off-target data (Lanillos *et al.*, 2020). Mutations in key components of the mTOR pathway (MTOR, TSC1, TSC2) occurred in 17% of primary tumours and were associated with the immunohistochemistry staining of phospho-S6 and PTEN, and with chRCC eosinophilic variant, supporting their biological relevance. Patients with mTOR pathway mutations had worse outcomes (overall survival: HR=5.5 and P=0.027; confirmed in TCGA with HR=10.3, P=0.006), and mutations in TP53 and telomere maintenance genes were enriched in metastatic cases. Overall, we showed that mTOR pathway mutations correlate with poor prognosis in chRCC, suggesting that mTOR inhibitors might be a good therapeutic option for patients with these alterations.

Overexpression of miR-483-5p is confined to metastases and linked to high circulating levels in pheochromocytoma/paraganglioma patients

Pheochromocytoma/paraganglioma (PPGLs) are rare neuroendocrine tumours. Approximately 15% of PPGL patients present with metastasis at diagnosis or over a long period after resection of the primary tumour, and there is a lack of prognostic molecular tumour markers that may improve the risk stratification. A comprehensive characterisation of miRNA profiles in primary tumours, metastases and liquid biopsies of PPGL patients, allowed us to identify high expression levels of miR-483-5p in metastatic tissues versus matched primary tumours (P=6.5 x 10⁻⁴), and in the serum of metastatic patients compared to non-metastatic cases (P=2.0 x 10⁻³). Moreover, circulating miR-483-5p discriminated metastatic patients with high accuracy (AUC=0.81, 95%CI=0.651-0.972, P=4.0 x 10⁻³). Integrative analyses of transcriptome data suggest that miR-483-5p plays a role in angiogenesis, wound healing and extracellular matrix organisation, while it regulates ALCAM expression in metastatic PPGL (FIGURE). ALCAM/CD166 is member of a subfamily of immunoglobulin receptors that promotes T-cell activation and proliferation via its interaction with CD6 and is involved also in the processes of cell adhesion and migration. It has been reported that ALCAM expression

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decreases with the progression of numerous tumours, which entails a poor prognosis. In fact, ALCAM-ALCAM interaction is dynamically regulated to turn tumour cells from a benign to a malignant phenotype by disrupting cell-cell adhesion and enabling tumour cell invasion and metastasis. In this regard, we observed a lower expression of ALCAM in metastases than in primary tumours (as opposed to miR-483-5p) in the published PPGL series, as well as in the whole TCGA PANCAN cohort extracted from the UCSC Xena browser (n=396 metastatic tissues and n=9712 primary tumours; $P=5.8 \times 10^{-102}$). Importantly, previous reports demonstrated that miR-483-5p directly binds ALCAM and regulates its expression in lung and in liver cancer. Hence, the functional interaction miR-483-5p/ALCAM may shed light on the understanding of PPGL metastatic niche and warrants further investigation. The main conclusion of the study is that overexpression of miR-483-5p is linked to metastatic colonisation, and pinpoints circulating levels as a promising biomarker of metastatic PPGL. ■

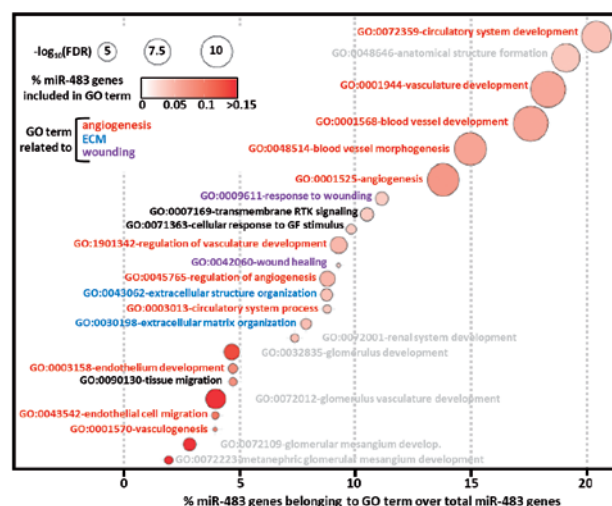


FIGURE 5 Functional enrichment and integrative analysis reveal a link between miR-483-5p and angiogenesis, wounding and extracellular matrix differential traits, and uncovers miR-483-5p/ALCAM as new player in PPGL metastatic niche. Diameter of bubbles indicates the % of genes correlated with miR-483-

5p levels (miR-483 genes) included in each specific GO term. Diameter is proportional to the $-\log_{10}(\text{FDR})$; X axis shows the % miR-483 genes present in each GO term over the total of miR-483 genes. GO terms: in red, genes related to angiogenesis; in blue, extracellular matrix (ECM); and in purple, wounding.

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AWARDS AND RECOGNITION

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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, its 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.

“The integrative effort of the epidemiology, statistics, bioinformatics, and molecular biology fields has allowed us to gain additional insight into the inherited basis of pancreatic cancer.”

RESEARCH HIGHLIGHTS

Research Findings

In 2020, and despite the challenges the COVID-19 pandemic posed, GMEG made important contributions to the epidemiological fields of pancreatic and bladder cancers. Regarding pancreatic cancer (PC), we completed the PanGenEU GWAS and complemented it with genome spatial autocorrelation analysis and Hi-C map interactions, enabling us to identify low frequency variants not detected by GWAS (FIGURE 1). This multi-step strategy, combined with an in-depth *in-silico* functional analysis, allowed us to gain additional insight into the inherited basis of PC. We also further characterised the association between smoking and PC risk using 2,009 cases and 1,532 controls recruited in the PanGenEU study. This study revealed differences in PC risk by tobacco type and other habit characteristics, as well as non-linear risk associations, emphasising potential differences in the underlying mechanisms leading to PC. Importantly, by using the PanGenEU study resources, we also analysed the association between type 2 diabetes mellitus (T2DM) subtypes (new-onset-NODM or long-standing-LSDM) and PC risk, we explored the direction of causation through Mendelian Randomisation analysis, and we assessed the mediation role of BMI (FIGURE 2). The findings of this study did not support a causal effect of LSDM on PC but suggest that PC is the cause of NODM. The interplay between obesity and T2DM is complex and warrants further examination. In addition, by participating in national and international collaborative efforts, we pursued the identification of urine and serum biomarkers to diagnose PC at earlier stages, and the characterisation of the germline and the somatic alteration landscapes of PC.

Regarding bladder cancer (BC), GMEG participated in a European-based effort to subclassify non-muscle-invasive bladder cancer (NMIBC) through a large integrative multi-omics analysis including gene expression, chromosomal instability, and spatial proteomics. The integrated classifier had independent prognostic value beyond established prognostic clinicopathological parameters. Under the umbrella of the AECC muscle invasive bladder cancer (MIBC) study, we reported that patients with BASQ-like tumours have a higher likelihood of experiencing a pathological complete response to neo-adjuvant chemotherapy. In addition, through an international collaboration, we observed that *FGFR3* mutations play a functional role distinct from *FGFR3* over-expression, pointing to the possibility that patients with *FGFR3* mutations may be more likely to benefit from anti-*FGFR3* therapy than patients with over-expression only.

Methodological Contributions

GMEG continued to explore the analytic strategies and tools to integrate omics and non-omics data into the cancer risk models, and started considering the integration of medical image information (radiomics and digital pathology) through an H2020-funded project (PanCaim).

Translational Activities

GMEG actively provides support in several clinical trials on immunotherapy and vitamin D in BC at the methodological level. We continue to sustain the Spanish Familial PC Registry

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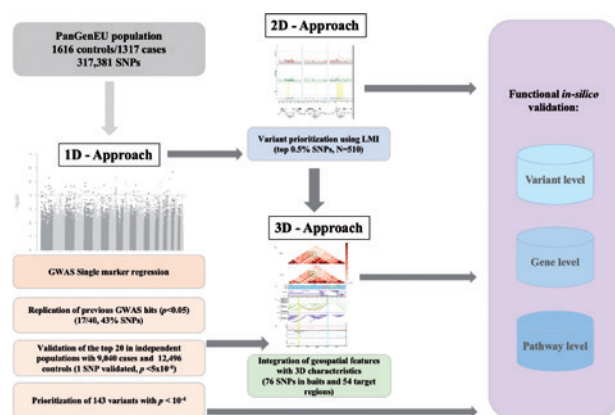


FIGURE 1 Pancreatic Cancer GWAS Study Flowchart: overview of the complementary approaches

adopted in this study to identify new pancreatic cancer susceptibility regions.

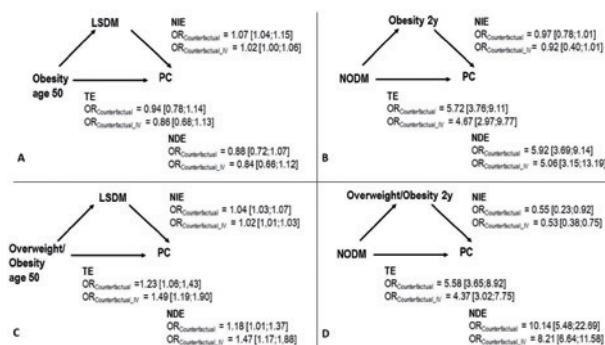


FIGURE 2 DACs showing results from causal mediation analyses within 2,018 cases and 1,540 controls evaluating mediator effects of obesity or T2DM on PC risk associations by T2DM subtypes. The natural indirect (NIE), direct (NDE) and total effect (TE) of the associations are

shown. **A** and **B** for LSDM or obesity (mediators) in association analyses between obesity or NODM and PC risk, respectively. **C** and **D** for LSDM or overweight/obesity (mediators) in association analyses between overweight/obesity or NODM and PC risk, respectively.

(PanGen-FAM) and the European Registry of PC (PancreOS). We launched a 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 (PCE) multi-stakeholder platform and we moved forward in increasing awareness of PC among health policy makers and in translating the urgent need to invest on PC research by joining efforts with EC IPAAC Joint Action and with EAPM. ■

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 - **AWARDS AND RECOGNITION**
 - Member of the working group of experts to evaluate the carcinogenicity of opium consumption, IARC Monographs 126.

FAMILIAL CANCER CLINICAL UNIT

Miguel Urioste
Clinical Unit Head

Staff Scientist
Ana Osorio (since August)

Technicians
Alicia Barroso (since August),
Victoria Fernández (since



OVERVIEW

The SARS-CoV2 pandemic created an unprecedented situation affecting all human activities and it forced alliances between healthcare workers, academics, scientists, and administrative and government entities around the world, to accelerate our knowledge about the disease and the search for efficient treatments and immunisation. COVID-19 exhibits great clinical and, possibly, populational heterogeneity, in which our genes probably play an important role.

In April 2020, our Unit, together with the Genotyping Unit, set out to identify prognostic markers that could help to stratify the population and allow us to identify *a priori* those subjects who will have a more severe course of Covid-19, in order to focus preventive resources on them and prioritise future immunisation. Since the beginning of the pandemic, various

national and international projects and initiatives have been launched with similar objectives. The *COVID-19 Host Genetics Initiative* (<https://www.covid19hg.org/>) is an international consortium that integrates more than 150 participants. Together with other groups from Spain, we are participating in exploring the role of host genetic factors in the severity of the disease. At the national level, the *ScourGe* consortium was created, made up of more than 70 clinical, research and biobank groups. We are currently analysing the genome of more than 10,000 individuals with different clinical forms of Covid-19.

The goal is to ultimately gather genomic data from many thousands of individuals from different populations to try to identify clinically relevant markers in a disease like Covid-19, whose genetic bases will be difficult to unravel.

September) (TS)*, Verónica García (TS)*, Maika González-Neira, Fátima Mercadillo

**Titulado Superior* (Advanced Degree)

CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

Since the summer of 2020, the Unit has expanded with 3 new people joining from the Human Genetics Group: Ana Osorio, Alicia Barroso and Victoria Fernández, all 3 involved in the research and diagnosis of hereditary forms of breast cancer. This is the most important change since the Unit's creation and represents a huge reinforcement for our diagnostic and research activity.

Clinical and diagnostic activity during 2020 was also disrupted by the Covid pandemic. For several months the Consultancy in the Fuenlabrada University Hospital had to remain closed. Even so, throughout the year we saw a total of 365 patients (32.1% decrease over 2019). Also, because of the pandemic, the number of genetic studies carried out decreased from over 572 performed in 2019 to 344 during 2020 (39.8% decrease).

Laura Pena left the Unit in November 2019. However, she defended her doctoral thesis in January of 2020. In her work — “Clinical and genetic characterisation of 145 Spanish patients diagnosed with PTEN hamartoma tumour syndrome” —, she characterised the disease in a wide series of Spanish patients, at both genetic and clinical levels, reviewing the patients' features, comparing them with other studied populations, and assessing the usefulness of the diagnostic criteria. The second objective of the work was to look for other genetic factors that could be involved in the phenotype of patients with PHTS who do not harbour *PTEN* mutations. The results of this work were used to formulate several recommendations: for the diagnosis, the selection of the most useful clinical features to drive genetic testing; and for the follow-up, obesity check-ups and anticipation of cancer screenings. Overall, this

work contributes to accelerate and improve the diagnosis and management of PHTS patients.

During 2020 we continued our work on early-onset colorectal cancer (EOCRC). Our goal is to build partnerships with patients, clinicians, and researchers. The increase in EORC incidence, its global dimension, and the many aspects distinguishing it from colorectal cancer that develops at older ages, make it necessary to bring attention to this problem and understand the causes of this striking increase. In June we launched the 2nd International Symposium on EOCRC in collaboration with Fight Colorectal Cancer (Fight CRC), a leading patient-empowerment and advocacy organisation in the United States. This collaboration was established based on the priorities that emerged from the 1st EOCRC Working Group held in Denver, CO (USA) in February 2019, to align research priorities in exploring the causation and aetiology of sporadic EOCRC and to support their ongoing work in convening a workgroup of now over 100 active participants. A result of these efforts has been the implementation of the Spanish EOCRC Group and the European Study of EOCRC Group (see in publications list Perea *et al.*, 2020). We believe that these initiatives will help to better develop the fight against EOCRC. ■

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MOLECULAR CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Staff Scientist
Raúl Torres

Graduate Student
Pilar Puig



OVERVIEW

Recurrent chromosomal rearrangements — changes in the structure of native chromosomes — are very common and well-known hallmarks of cancer. A better understanding of these cancer-causing mechanisms will lead to novel therapeutic regimens to fight cancer. The research activity of the Molecular Cytogenetics Unit focuses on increasing the knowledge about the role of chromosomal rearrangements in cancer development and progression and the discovery of 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

“We apply genome engineering approaches to reproduce and eliminate chromosome rearrangements and gene alterations. We provide access to the latest cytogenetic and CRISPR technologies.”

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

Technicians

M. Carmen Martín, Marta Martínez-Lage (TS)*, Francisco José Moya (TS)* (PEJ)**

*Titulado Superior (Advanced Degree)

**Plan de Empleo Joven (Youth Employment Plan)

Visiting Scientist

Doron Tolomeo (until March)
(Università degli Studi di Bari Aldo Moro, Bari, Italy)

RESEARCH HIGHLIGHTS

***In vivo* CRISPR/Cas9 targeting of fusion oncogenes for selective elimination of cancer cells**

Fusion oncogenes (FOs) are common alterations found in around 20% of cancer types and are powerful drivers of tumour development. Because their expression is exclusive to cancer cells and their elimination induces apoptosis in FO-driven cancer cells, FOs are attractive therapeutic targets. However, specifically targeting the resulting chimeric products is challenging. Based on CRISPR/Cas9 technology, we devised a gene-editing strategy targeting 2 introns of the genes involved in the rearrangement, allowing for robust disruption of the FO specifically in cancer cells. As a proof-of-concept of its potential, we demonstrated the efficacy of intron-based targeting of FOs in reducing tumour burden/mortality in *in vivo* Ewing sarcoma and chronic myeloid leukaemia models. The FO targeting approach might open new horizons for the selective elimination of cancer cells.

Technological and translational activities

We provide state-of-the-art Molecular Cytogenetic and Genome Editing services. The Unit makes available a complete suite of tools for cellular and genetic manipulation to research groups; these tools can be used interchangeably with an array of delivery vehicles, offering a flexible, modular platform for precision genome manipulation. The Unit offers molecular cytogenetics technology analysis of human and mouse chromosomes, including conventional karyotyping, FISH, SKY and CGH array.

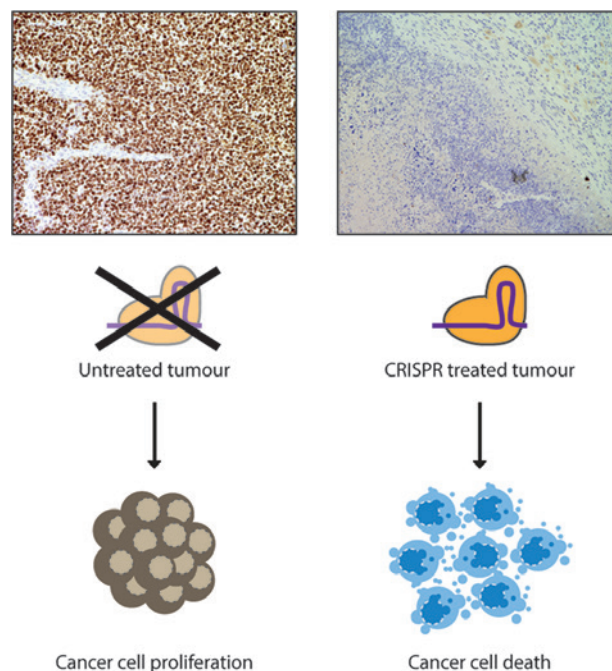


FIGURE Representative images of non-edited (left) and CRISPR edited tumours for the elimination of fusion genes (right). Cells are stained with the Ki67 proliferation

marker. Brown staining indicates a high proliferation rate, while the absence of staining (cells in blue) indicates no proliferation.

Our Unit offers rapid, precise, and affordable technologies to analyse cancer cells at the chromosome level and to functionally interrogate the cancer genome. In 2020, we carried out over 2,700 assays for experimental and clinically oriented projects. ■

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HUMAN GENOTYPING- CEGEN UNIT

Anna González-Neira
Unit Head

Graduate Students
Hugo Tejera, Alejandro Velasco



OVERVIEW

In the Unit we implement high-throughput methods for detection of genetic variation (single nucleotide variants, indels, structural variants) and methylation analysis using DNA microarray and next-generation DNA sequencing technologies. Complementarily, research focused on identifying predictive biomarkers for precision medicine is undertaken.

“Our aim is to identify predictive biomarkers in cancer patients in order to implement precision medicine in clinical practice.”

Technicians

Charo Alonso, Núria Álvarez,
Belén Herráez, Rocío Nuñez (TS),
Guillermo Pita (TS)*

**Título Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

Novel predictive genetic markers for adverse drug reactions in breast cancer (BC) patients. Persistent chemotherapy-induced alopecia (pCIA) and capecitabine-induced hand-foot syndrome (CiHFS) are 2 common adverse drug reactions in cancer treatment. pCIA occurs in its most severe form in up to 10% of BC patients treated with docetaxel-based therapies, having a profound psychological impact on them. CiHFS is a dermatological toxicity affecting around 30% of patients, and the main cause of dose reductions and chemotherapy delays. By GWAS, we identified a regulatory variant associated with pCIA appearance in patients; this finding was validated in the replication cohort (ORcombined 4.05; 95% IQR, 2.46-6.67; $P=3.946 \times 10^{-8}$). This variant affects ABCB1 mRNA expression, being the risk allele associated with decreased expression. The ABCB1 gene encodes P-glycoprotein, an efflux pump responsible for the elimination of docetaxel, and lower expression could cause decreased drug elimination and thus its intracellular accumulation. Carriers of the risk allele would experience high drug exposure in the hair follicle and alopecia may become permanent, owing to the destruction of hair follicle stem cells. In addition, we discovered and replicated a cluster of 4 variants associated with decreased levels of CDH4 mRNA and the protein it encodes, R-cadherin, which localises in the granular layer of the epidermis. This resulted in reduced expression of involucrin, a protein of the cornified envelope, an essential structure for skin barrier function.

Identifying variants of pharmacogenomic interest using CSVS, a crowdsourcing database of the Spanish population genetic variability. Genetic differences between human populations are becoming increasingly recognised as important factors accounting for interindividual variations in drug responsiveness. Using data from the CSVS repository, we addressed how population-specific differences in genes involved in drug absorption, distribution, metabolism, excretion and toxicity (ADMET) could affect the rates and risks of drug inefficacy and/or adverse drug reactions in the Spanish population. We studied the Spanish genetic variability in a total of 421 pharmacogenes and, interestingly, a non-negligible percentage of private variation was observed in genes encoding proteins involved in drug metabolism, transport, and response.

Detection of mutations in liquid biopsies from paediatric CNS tumours. Paediatric CNS tumours are the most fatal cancer diseases in childhood. Due to their localisation and infiltrative nature, some tumour resections or biopsies are not feasible. We conducted the first study to compare different sources of liquid biopsies in paediatric cancers, an unmet need for clinical practice. We found serum to be more promising than plasma for BRAF V600E by dPCR detection in liquid biopsy of CNS paediatric cancers. ■

PUBLICATIONS

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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) developing novel agents; 2) studying 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: a) generating synergies with ongoing research lines in the basic research programmes; and b) 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 enable scientists from all CNIO Programmes to participate in translational research studies. The number of ongoing collaborations between units of the CRP and research groups of other CNIO Programmes have increased to 26 projects and 3 coordinated grants, which translates into the high translational research activity of the institution. Eleven residents in medical oncology from different Spanish hospitals completed their 3-month optional visiting stays at CNIO during 2020.

Although clinical activity was hampered considerably this year due to COVID-19, the clinical groups managed to produce highly impactful research. The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, validated for the first time in a clinical trial the preclinical observation of synergy between mitochondrial inhibitors and antiangiogenics. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, brought novel approaches for the treatment of small-cell lung cancer to the clinic: chemo-immunotherapy with durvalumab in first-line treatment, and lurbinectedin for pre-treated patients, 2 unmet clinical needs. These findings were published in 2 separate papers in *The Lancet*, and supported FDA and EMA approval, respectively. The Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez-López, made relevant contributions towards the understanding of COVID-19 in haematological malignancies. The Prostate Cancer Clinical Research Unit (PCCRU), headed by David Olmos, significantly contributed to the development of the first 2 targeted therapies for metastatic prostate cancer: olaparib in patients with HRRm prostate cancer, recently

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

approved by FDA and EMA, and ipatasertib in PTEN null prostate cancer, both findings were published in top journals such as the *New England Journal of Medicine* and *The Lancet*. Additionally, they described that poor outcomes within a significant proportion of *BRCA2* mutant prostate cancers are linked to the co-deletion of *BRCA2* and *RBI* genes; this codeletion identifies a subgroup of particularly aggressive prostate cancers at diagnosis that may benefit from novel treatment approaches. The PCCRU was awarded one of the two *CRIS* Cancer Grants for Junior Researchers endowed with 1.25 million euro. Finally, the Molecular Diagnostics Unit, headed by Luis Lombardía, continued to provide support to hospitals in the diagnosis of different malignancies, performing >1000 diagnoses this year. With the large number of ongoing translational research collaborations, the arrival of novel immune-oncology drugs, and the search for novel groups for the CRP, we face an exciting year 2021 for patient-oriented oncology research at CNIO. ■

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Clinical Research Fellow
Jorge Silva (since July)

Staff Scientists
María José Bueno, Silvana Andrea Mouron

Post-Doctoral Fellow
Rebeca Gema 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. Specifically, our research areas cover the:

- Study of the mechanisms of resistance against CDK inhibitors.
- Characterisation of aberrant signalling axes in triple-negative breast cancer.
- Role of FGFR1 in cancer progression and therapeutic resistance in hormone-positive cancer.
- Study of the implications of hypoxia in response to immunotherapy.

“We have provided clinical proof-of-concept of the synergy between antiangiogenics and mitochondrial inhibitors in breast cancer, which we previously described in animal models.”

Graduate Students
Sara Fernández, José Luis Ruiz

Technicians
Verónica Jiménez, Manuel Muñoz

**Titulado Superior (Advanced Degree)*

Student in Practice
Katarina Mick (January-May)
(University of Zagreb, Croatia)

Visiting Scientists
Ramón Colomer (Hospital

Universitario de la Princesa, Madrid, Spain), Liany Luna (Universidade Federal de São Carlos, Brazil), Ana López (Hospital Universitario de Fuenlabrada, Spain), Lucas Moreno (Hospital Vall d'Hebron, Barcelona,

Spain), Berta Nassarre (Apices Soluciones S.L, Madrid, Spain), Ana Roncero (Hospital Universitario 12 de Octubre, Madrid, Spain)

RESEARCH HIGHLIGHTS

The following highlights some of the achievements of the Breast Cancer Clinical Research Unit during 2020:

- We demonstrated that PD-L1 inhibitors in combination with antiangiogenics in advanced breast cancer restrict their activity to those cases in which antiangiogenics normalise tumour hypoxia and vasculature.
- We confirmed our preclinical findings regarding acquired resistance against antiangiogenics: in those cases where antiangiogenics normalise tumour hypoxia and vasculature, a targetable mitochondrial switch takes place. Consequently, mito-inhibitors were found to synergise with antiangiogenics. We confirmed the findings in a clinical trial in early breast cancer.
- In parallel, in the preclinical setting, we are solving the mechanism of escape against antiangiogenics that increases vascular abnormality and tumour hypoxia. Preliminary data suggested that hypoxic areas are excluded from the antitumor immune response. Diverse therapeutic avenues are being explored to induce immune re-infiltration in hypoxic areas.
- We characterised FGFR1 as a driver of acquired resistance to combined treatment with hormonal and CDK inhibitors. We found that in FGFR1-amplified or overexpressed tumour models, the triple combination of FGFR1, CDK4/6 and ER blockade is the only one able to completely suppress RB phosphorylation.
- Completion of the first phosphoproteomic screening in a randomised clinical trial in early breast cancer treated with paclitaxel monotherapy revealed that elevated P70S6K and CDK4 are highly reliable biomarkers of sensitivity to this drug. ■

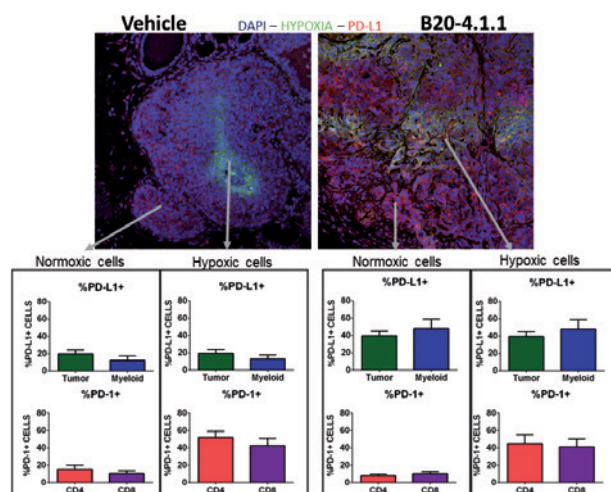


FIGURE Tumours treated with antiangiogenic (B20-4.1.1) become hypoxic (green), and express high PD-L1 (red). The immune infiltrate is

excluded from hypoxic areas (upper charts); a detailed analysis shows that PD-L1 is mostly expressed by tumour cells in hypoxic areas (lower charts).

PUBLICATIONS

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in a patient with cancer? *EClinicalMedicine* 25, 100487.

AWARDS AND RECOGNITION

- ▶ Scientific Advisor, the Kærtor Foundation Cancer Innova Program, Spain.

PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Staff Scientist
Pedro P. Lopez-Casas

Clinical Research Fellows
Rebeca Lozano, Carlo Cattrini

Post-Doctoral Fellows
Isabel Aragón, David Martínez, María
Isabel Pacheco



OVERVIEW

Prostate cancer (PrCa) 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 8 years, our group has made significant contributions in:

→ Establishing and developing several biomarkers based on the concept of liquid biopsy.

→ Understanding the implication of gene alterations leading to DNA repair deficiency in this disease.
→ 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.

Graduate Students
Ylenia Cendón, Lorena
Magraner (until April)

Technicians
Mónica Balsells (TS)*, Eva M.

Casas (since March), Jeniffer P.
Condo (since February), Teresa
Garcés, Carles Moreno (until May),
Leticia Rivera, Alejandro Sanz (TS)*

**Titulado Superior* (Advanced Degree)

Visiting Scientists
Daniel Alameda (*Instituto de
Investigación Biomédica de Málaga,
Spain*), Elena Castro (*Instituto de
Investigación Biomédica de Málaga,
Spain*), Francesca M. Vitrone

(until September) (*Instituto de
Investigación Biomédica de Málaga,
Spain*)

RESEARCH HIGHLIGHTS

During 2020, our Group was recognised with the 1st *CRIS* Excellence in Research Award. This award will help, among other aspects, to continue our work on understanding gene alterations that could be synergistic with DNA repair defects promoting oncogenesis and prostate cancer progression, and therefore to be exploited as potential new targets. For example, at the 2020 American Society of Clinical Oncology meeting, we presented the results of the PROREPAIR-A study, in which we reported that *BRCA2* defects are frequently associated to *RB1* loss and/or *MYC* amplification, and that their combination is associated to poor outcomes. We also advanced in our understanding of *ATM* defects in prostate cancer. As part of the thesis project of our student Ylenia Cendón, we established that *ATM* may contribute or not to cancer oncogenesis and progression depending on the genetic background (i.e., it may be synergistic in an *RB1* suppression or *MYC* overexpression context, and the opposite in a *PTEN* loss context). Our aim is to publish these results during 2021.

In addition, the group actively participated in several clinical trials, and this included our participation in the steering committees of large phase II trials. We particularly contributed to the approval of olaparib as the first targeted treatment for precision medicine in metastatic prostate cancer. Like everyone around the world, we were also affected by the Covid-19 pandemic. Our research efforts, especially in the clinic, had to be slowed down as doctors in our teams and

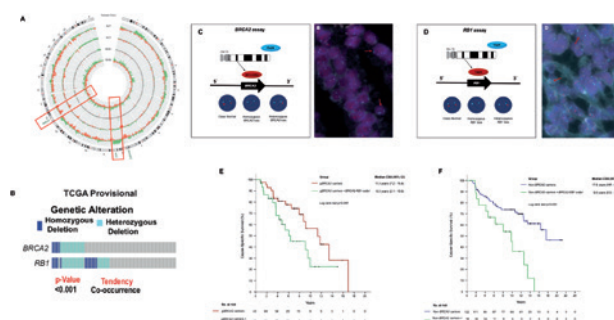


FIGURE PROREPAIR-A study: (A) aCGH plot comparing PrCa tumours from patients with and without germline *BRCA2* mutations; (B) *BRCA2* and *RB1* loss co-occur also in sporadic PrCa shown by TCGA data; (C) and (D) FISH assays illustrating *BRCA2* and *RB1* co-deletion; (E) gBRCA2 PrCa patients; and (F) sporadic PrCa patients. In both, Kaplan-Meier curves showed that patients with somatic co-deletion of *BRCA2* and *RB1* had worse survival outcomes.

other associated researchers had to focus on delivering patient care. Still, we also tried to contribute through international collaborations to understanding the role of *TMPRSS2*, a key AR-regulated gene, which could be involved in SARS-CoV-2's entry into the cell. ■

PUBLICATIONS

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- Rescigno P *et al.* (incl. Olmos D, Castro E) (2020). Early post-treatment prostate-specific antigen at 4 weeks and abiraterone and enzalutamide treatment for advanced prostate cancer: an international collaborative analysis. *Eur Urol Oncol* 3, 176-182.

AWARDS AND RECOGNITION

- David Olmos:
- Member of the Board of Directors, European Organization for Research and Treatment of Cancer (EORTC).
- Impact Award (Partnering PI). US Department of Defense, Congressionally Directed Medical Research Programs.
- CRIS Excellence in Research Award, Spain.
- Elena Castro: Faculty Board Member, ESMO Preceptorships in PrCa.
- Rebeca Lozano: awarded the "Merit Award", ASCO Annual Meeting 2020.
- Carlo Cattrini: awarded the "Merit Award", ASCO Genitourinary Cancers Symposium 2020.
- Nuria Romero: awarded the "Merit Award", ASCO Annual Meeting 2020.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero



OVERVIEW

The Molecular Diagnostics Unit (MDU) is entrusted to provide our National Health System's hospitals with a wide range of sensitive, specific, reliable and updated assays. By using golden standard molecular techniques, we routinely identify alterations in the sequence or expression of key genes that are involved in cancer and that could in turn be used in the diagnosis and/or prognosis of patients, the detection of minimal residual disease in patients showing clinical remission, or for monitoring response to therapy. Our Unit also provides support to CNIO's Clinical Research Units and Research Groups by developing and implementing novel solutions for their research needs. Involved in the global efforts to standardise and improve molecular diagnostics testing in cancer, we work in partnership with international and national consortia dedicated to these objectives. Finally, our Unit

“Despite the fact that the COVID-19 pandemic notably disrupted the testing status quo in the area of oncology diagnosis, MDU not only continued to supply our NHS hospitals with assays but also established new ones.”

remains fully committed to promoting laboratory training and mentoring for students, technicians and medical residents.

CORE UNIT HIGHLIGHTS

Broadening our genetic testing catalogue

During 2020, we implemented 3 new molecular diagnostic tests based on bi-directional Sanger sequencing. These tests will allow us to provide more comprehensive molecular diagnostics of some haematological malignancies like myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML), for which we were already offering a sizable panel of clinically applicable markers (FIGURE).

The first 2 assays aim to detect mutations in the *ASXL1* (additional sex combs-like 1) gene that plays a role in both embryonic development and chromatin remodelling, and in the *SF3B1* (splicing factor 3b subunit 1) gene, which encodes a catalytic core component of the RNA splicing machinery and is involved in transcription and mRNA processing. Somatic mutations in *ASXL1* and *SF3B1* genes have been observed, among many other cancers, mostly in MDS, CMML and AML. Mutually exclusive, *ASXL1* mutations are prognostic of high-risk MDSs, acute transformation in CMML, and shorter overall survival of patients with AML, while *SF3B1* structural alterations are predictive of a longer overall survival of MDS patients but shorter overall survival in *de novo* AML and chronic lymphocytic leukaemia (CLL) patients.

The third test developed seeks to detect mutations in the *SETBP1* (SET-binding protein 1) oncogene, encoding a binding partner for the multi-function SET oncoprotein involved in apoptosis, transcription and nucleosome assembly. *SETBP1*

overexpression is associated with a worse overall survival of elderly AML patients. Furthermore, somatic gain of function mutations of *SETBP1* are associated with myeloid leukemic transformation and convey poor prognosis of MDS and CMML patients.

Tutoring

In 2020 we hosted, in the framework of our training policy, one medical resident.

Diagnostic marker	Targeted exon(s)	MPN	MDS	CMML	AML
ASXL1	13 & 14	✓	✓	✓	✓
SETBP1	12		✓	✓	
SF3B1	14, 15 & 16	✓	✓	✓	✓
CALR	9	✓			
CEBPA	Whole gene		✓		✓
WT1	7 & 9				✓
TP53	5-8		✓	✓	✓
FLT3	13-15			✓	✓
IDH1	4			✓	✓
IDH2	4	✓	✓	✓	✓
JAK2	12 & 14	✓	✓	✓	✓
KIT	9, 11, 13 & 17				✓

Diagnostic marker	Targeted exon(s)	MPN	MDS	CMML	AML
KRAS	2 & 3			✓	
MPL	10	✓		✓	
TET2	3, 6, 7, 9, 10 & 11	✓	✓	✓	✓
NPM1	12		✓	✓	✓
NRAS	2 & 3		✓	✓	✓
RUNX1	4, 5, 6 & 8		✓	✓	✓
SRSF2	Whole gene		X	X	X
CBL	7 & 8	X	X	X	
U2AF1	Whole gene		X	X	X
DNMT3A	15-23	X	X	X	X
EZH2	15		X	X	X
PHF6	Whole gene		X		X

FIGURE The addition of 3 new markers (in red) to those already provided (in green) gets us closer to the whole panel of known markers used for the diagnosis/prognosis of

myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS), chronic myelomonocytic (CMML) and acute myeloid (AML) leukaemias.

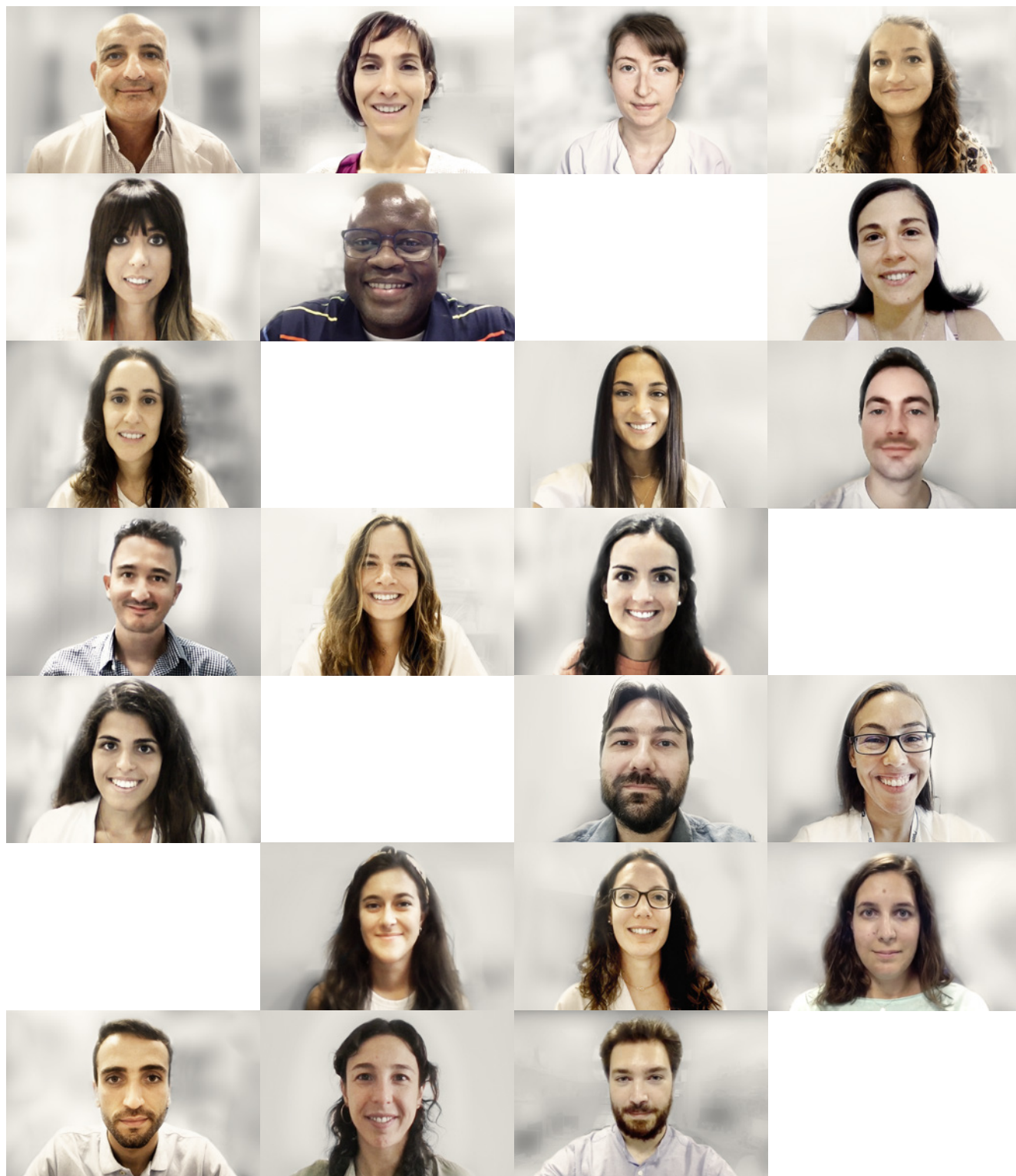
H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

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
(since October), 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 Ruíz-Heredia, Antonio Valeri, María Velasco-Estévez (since February) (PEJ, CAM)*

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

Graduate Students
Pedro Aguilar, Noemí Álvarez, Eva Castellano, Laura Córdoba (since February), Isabel Cuenca, Jessica Encinas, Roberto García (since October), Elena Maroto, Michael Ochieng (since August), Alejandra Ortíz, Alba Rodríguez, Laura Sánchez

Technicians
Natalia Sofía Buenache (since August), Adrián Fernández, Vanesa Garrido, Alicia Giménez, M. Luz Morales, Laura Moreno, Miguel Ángel Navarro, Laura Rufián

Master's Students
María Balaguer (until May) (*Universidad de Barcelona*, Spain),

María Cruz Casado (until April) (*Universidad Carlos III de Madrid*, Spain)

Visiting Scientists
María Hernández (since March) (*IBSAL*, Salamanca, Spain), Alfonso Navarro (since February) (*IDIPAZ*, Madrid, Spain)

OVERVIEW

Recently, the field of haematology has been gaining traction in cancer research, not only for the study of critical disease affecting human health, but also for its implications in solid cancers and the applicability of haematological tools to other areas.

In terms of cancer implications, immune cells play a remarkable role in metastasis, inflammation, and immune surveillance. In fact, this concept triggered the development of the most promising cancer therapy of the 21st century, immunotherapy.

Moreover, the haematology research area has been developing cutting-edge applications such as liquid biopsy, an easy peripheral blood/plasma analysis that can anticipate the appearance of disease, or the emergence of tumour clones in relapsed patients.

The following main lines of research define our laboratory:

- Liquid biopsy, minimal residual disease, and next-generation sequencing.
- Immunotherapy: NK/T-CAR, BITES and immune checkpoints in haematological and paediatric cancers.
- Role of hnRNP K, master regulator of tumourigenesis.
- Viral infection and cancer.

“We improved our in-house deep-sequencing analysis for measurable residual disease (MRD), revealing its value in decision-making for clinical trials and as a prognosis marker.”

RESEARCH HIGHLIGHTS

Next-generation sequencing of measurable residual disease in multiple myeloma by immunoglobulin repertoire analysis

Measurable residual disease (MRD) is a poor study variable in routine practice. The most common measure of MRD was developed through multiparametric flow cytometry, of which our laboratory has conducted previous studies in multiple myeloma (MM) and other haematological malignancies. However, preliminary next-generation sequencing data from MRD studies show an increase in sensitivity, specificity, and applicability. In 2020, we measured MRD by next-generation sequencing of immunoglobulin genes with a sensitivity of 10^{-6} . Here we present our single-institution experience assessing MRD in 234 MM patients, both newly diagnosed (159) and relapsed (75). We describe the impact of depth, duration, and direction of response on prognosis. Those patients achieving MRD negativity at 10^{-6} , as well as 10^{-5} , had good prognosis and higher progression-free survival (PFS). In the MM diagnosis cohort, 40% of the patients achieved MRD negativity at 10^{-6} and 59% at 10^{-5} . Median PFS in this cohort was superior in those achieving MRD at 10^{-5} vs $<10^{-5}$ (PFS: 87 months vs 32 months; $P < .001$). In the MM relapsed cohort, 36% achieved MRD negativity at 10^{-6} and 47% at 10^{-5} . Median PFS was superior for the cohort achieving MRD at 10^{-5} vs $<10^{-6}$ (PFS: 42 months vs 17 months; $P < .01$). Serial MRD monitoring identified 3

categories of MM patients at diagnosis: (A) patients with ≥ 3 MRD 10^{-6} negative samples, (B) patients with detectable but continuously declining clonal numbers, and (C) patients with stable or increasing clonal number (≥ 1 log). PFS was superior in groups A and B vs C ($P < .001$).

This work validates the importance of MRD evaluation as part of clinical care, both as an important prognostic marker at diagnosis and at relapse in multiple myeloma disease. Our data support its use as an endpoint in future clinical trials as well as for clinical decision-making. (Work published in *Blood Advances*).

Impact of prolonged maintenance of the immunomodulatory drug lenalidomide in multiple myeloma

Lenalidomide is an immunomodulatory drug approved for maintenance treatment in newly diagnosed multiple myeloma, and it has been shown to improve progression-free survival (PFS) and, in several studies, overall survival. Nevertheless, the impact of prolonged treatment with lenalidomide on the kinetics of minimal residual disease (MRD) and its prognostic impact have not been studied in depth. To obtain better knowledge in this regard, we retrospectively analysed 139 patients who received lenalidomide maintenance in real-world

PUBLICATIONS

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- Medina A *et al.* (incl. Cedena MT, Martínez-López J) (2020). Comparison of next-generation sequencing (NGS) and next-generation flow (NGF) for minimal residual disease (MRD) assessment in multiple myeloma. *Blood Cancer J* 10, 108.
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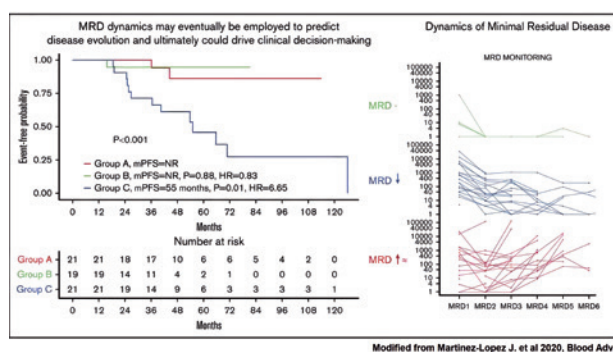
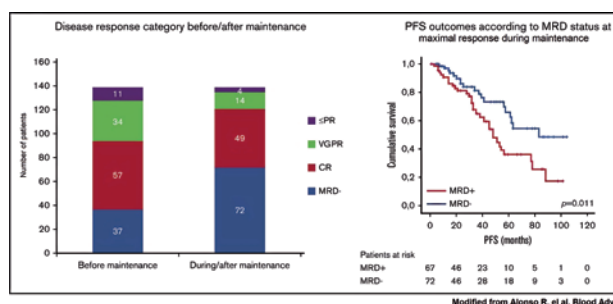


FIGURE 1 MRD deep-sequencing in house evaluation provides prognostic value and accurate monitoring of multiple myeloma disease: Left: PFS curves from (A) patients with ≥ 3 MRD 10^{-6} negative samples, (B) patients with detectable but continuously

declining clonal numbers, and (C) patients with stable or increasing clonal number (≥ 1 log). PFS was superior in groups A and B vs C ($P < .001$). Right: Dynamic of MRD values during multiple myeloma progression.



clinical practice and whose MRD levels were observed during the treatment period by multiparametric flow cytometry or next-generation sequencing with a sensitivity of at least 10^{-4} . Lenalidomide maintenance correlated with an increased depth of the disease response, with 38.1% of patients achieving maximal response during lenalidomide treatment. Moreover, 34.3% of patients who were MRD positive after induction treatment achieved MRD-negative status during maintenance and ultimately had improved PFS. Sequential MRD assessments identified patients with progressively decreasing MRD levels who also had better PFS outcomes, compared with patients not showing a decreasing pattern of MRD.

These results support the role of maintenance therapy, not only to sustain, but also to increase the depth of disease response with a PFS benefit. In addition, MRD monitoring during maintenance identifies patients with better prognosis and may help in their clinical management. (Work published in *Blood Advances*).

FIGURE 2 Lenalidomide maintenance treatment provides better response and fewer relapse events. Left: Disease response category before

and after lenalidomide maintenance treatment. Right: PFS curves according to MRD positive or negative during maintenance.

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- Joaquín Martínez-López:
- CRIS Contra el Cáncer research project (2020/0063, PI), CRIS Cancer Foundation, Spain.
- Astellas Pharma S.A. Sponsorships (2020/0028, PI), Astellas Pharma S.A., Spain.
- Miguel Gallardo: Miguel Servet Fellowship (CP19/00140, PI), Miguel Servet Programme of the Spanish National Institute of Health Carlos III (ISCIII), Spain.
- María Linares: Ideas Semilla AECC 2020 research project (IDEAS20014LINA, PI), the Spanish Association Against Cancer (AECC), Spain.
- Rosa M. Ayala: PETHEMA study research project (2020/0070, PI), Programme for the Study of Haematological Malignancies (PETHEMA), Spain.
- Lucía Fernández, EMBO short-term Fellowship, INSERM laboratory, Paris, France.

H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

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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 ones, 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 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 contributed remarkably to the development of predictive biomarkers that have impacted the currently available selection of targeted therapies (e.g., EGFR mutation, NTRK rearrangements) and novel immunotherapeutics (e.g., tumour mutational burden in the clinic). We have led controlled clinical trials with novel agents as well as combinations of targeted therapies (e.g., ramucirumab plus pembrolizumab) or checkpoint inhibitors (e.g., chemotherapy plus nivolumab plus ipilimumab) in lung cancer that have impacted clinical practice worldwide.”

RESEARCH HIGHLIGHTS

Biomarker discovery and implementation

We currently own an extensive patient-derived xenograft (PDX) platform that has led to the deciphering of the role of the tyrosine kinase receptors, FGFR1 and FGFR4, and the adhesion molecule N-cadherin in non-small cell lung cancer (NSCLC), and to develop new biomarkers with a predictive role for anti-FGFR therapy in NSCLC (Quintanal-Villalonga A *et al.*, *EBioMedicine* 2020). In this study, only co-expression of FGFR1 and/or FGFR4 with N-cadherin in different lung cancer patient cohorts inferred a poorer outcome. Treatment of high FGFR1- and/or FGFR4-expressing lung cancer cell lines and PDXs with selective FGFR inhibitors showed high efficacy, but only in models with high FGFR1/4 and N-cadherin expression. We therefore provide *in vitro* and *in vivo* evidence showing that expression of the adhesion molecule N-cadherin is key for the oncogenic role of FGFR1/4 in NSCLC. In addition, our data

show that the complementary determination of N-cadherin and FGFR1/4 expression may further optimise patient selection for anti-FGFR therapy efficacy. Moreover, our PDX platform has also contributed to discovering Notch as a novel therapeutic target in lung adenocarcinoma osimertinib-treated patients after disease progression (Bousquet Mur E *et al.*, *JCI* 2020), as well as to test novel combination therapies, including a novel neutralising anti-HER3 antibody, to overcome resistance to EGFR targeted therapies (Romanello D. *et al.*, *Cancers (Basel)* 2020).

In 2020, we performed a harmonisation study to determine the tumour mutational burden (TMB) in a clinically well-annotated cohort of 96 resected patients with NSCLC. We evaluated the TMB assessment concordance of 2 novel next generation sequencing (NGS) panels, TSO500 and Oncomine TML (OTML), compared to a reference assay

(Foundation One - FO). Both panels showed strong agreement with FO, with Concordance Correlation Coefficients (CCC) of 0.933 (TSO500; 95%CI=0.908;0.959) and 0.881 (OTML; 95%CI=0.840;0.922). Additionally, we adjusted the cut offs for each of the in-house panels (ESMO 2020).

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; in 2020, we participated in more than 45 projects in this research area, including 10 new trials. We reported data from novel combinations of first-line ramucirumab plus pembrolizumab (Herbst RS, ..., Paz-Ares L, *JTO* 2020), and second-line ramucirumab plus osimertinib (Yu HA, Paz-Ares L, *et al.*, *Clin Cancer Res* 2020). Recently, we tested the safety and efficacy of bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of the transforming growth factor β (TGF- β) receptor II (a TGF- β “trap”) fused to a human immunoglobulin G1 antibody blocking programmed death-ligand 1 (PD-L1) in patients with advanced NSCLC (Paz-Ares L, *JTO* 2020). In addition, we published a phase II trial of lurbinectedin, a novel transcription inhibitor, in small cell lung cancer with encouraging activity on the second-third line setting (Trigo J, ..., Paz-Ares L, *Lancet* 2020). At present, combination studies with irinotecan and atezolizumab are ongoing. Bintrafusp and lurbinectedin are now being tested in a phase III trial led by Luis Paz-Ares.

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 pembrolizumab plus chemotherapy in NSCLC patients (Paz-Ares L. *et al.*, *JTO* 2020). In the protocol-specified final analysis of KEYNOTE-407, this combination continued to exhibit a clinically meaningful improvement in overall survival (OS), progression-free survival (PFS), second PFS (PFS-2), overall response rate (ORR), and duration of response (DOR), compared with placebo plus carboplatin-paclitaxel/nab-paclitaxel in patients with previously untreated metastatic squamous NSCLC. In addition, the exploratory results of the PACIFIC trial of outcomes by tumour cell (TC) programmed death-ligand 1 (PD-L1) expression showed that PFS benefit with durvalumab was observed across all subgroups, and OS benefit across all but TC <1%, for which limitations and wide HR CI preclude robust conclusions (Paz-Ares L *et al.*, *Ann Oncol* 2020). Finally, the 9LA trial showed the superiority (including prolonged survival) of a short course of chemotherapy plus ipilimumab and nivolumab, compared to chemotherapy alone in advanced NSCLC.

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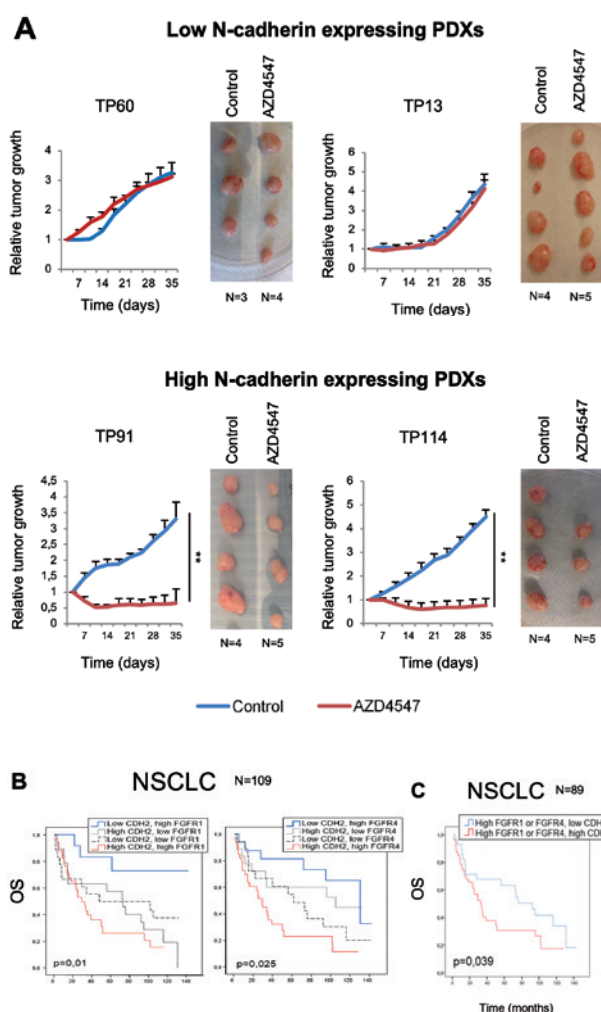


FIGURE 1 (A) AZD4547 treatment of low (upper graphs) and high (lower graphs) N-cadherin-expressing patient-derived xenografts (PDXs), demonstrating that treatment of high-FGFR1 and/or FGFR4-expressing PDXs with selective FGFR inhibitors showed high efficacy, but only in models with high N-cadherin expression. (B) Kaplan-Meier curves of overall survival (OS) showing that patients whose tumours exhibited

high expression of FGFR1 or 4 and N-cadherin genes had the poorest prognosis, while the group with high FGFR1 or 4 and low N-cadherin expression had the best prognosis. (C) OS curve of patients showing that those with high FGFR1/4 and low N-cadherin expression had increased overall survival (Hazard Ratio of 1.89 [1.023-4.9], $p = 0.039$) compared to patients with elevated expression of both genes.

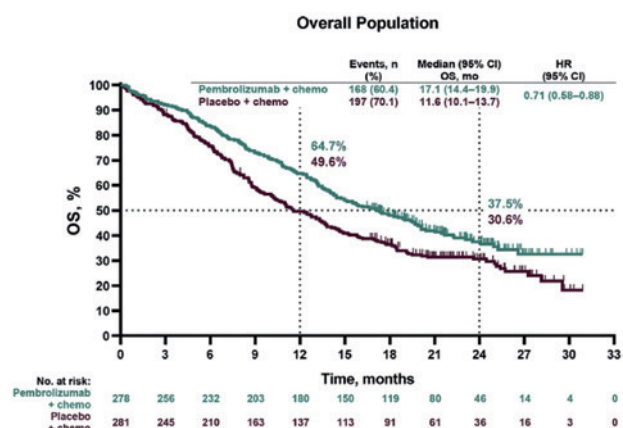


FIGURE 2 Updated efficacy outcome of the KEYNOTE-407 randomised clinical trial, showing an improvement in overall survival (OS) with pembrolizumab plus chemotherapy, compared to

chemotherapy alone (HR, 0.71 [95% CI: 0.58-0.88]) in treatment-naïve patients with metastatic squamous non-small cell lung cancer (NSCLC).

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- President of the Spanish Association for Cancer Research ASEICA (2021-2022).

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CAROLINA POLA

Director of Innovation (until November)

In 2020, the world took one of the hardest hits in history, but modern medicine has proven to be an invaluable tool to face the economic and health hurdles brought upon us by the COVID-19 pandemic. Whereas remaining challenges exist, innovation took the central stage everywhere and created new processes and tools that have allowed the world to move forward despite the strict social confinement and travel bans across the globe. Innovation is a priority, and at CNIO we share this value as one of the ways to bring solutions to society.

In 2020, the broad knowledge of biological processes and late-stage technologies developed by investigators at CNIO resulted in 2 projects being awarded grants through the *ISCI* extraordinary call for proposals to develop solutions for COVID-19. One of the projects aims to develop a humanised mouse model to test new drugs and vaccines for the disease. The second project focuses on a rapid and sensitive novel diagnostic

test to allow point-of-care diagnostics for COVID-19 – this innovative undertaking was also supported by a *CaixaImpulse* Consolidate grant to ensure its further development. Innovation support and actions for the research teams were put into place to secure collaborations with the private sector to speed up the adoption of project outputs by the market.

The efforts to support investigators in their innovation activities and facilitate the transfer of technology to the market resulted in an economic return of €728,000 for CNIO from royalties, the highest in the last 5 years. The technologies licensed have an impact on the research ecosystem and the drug development industry.

In 2020 we witnessed a milestone for CNIO with the launch of the Telomere Therapeutics spin-off to develop a telomerase gene therapy for the treatment of lung and kidney fibrosis.

“2020 marked the year for science and innovation worldwide – we aligned our efforts to respond to the needs of a global health problem while continuing to bring value and solutions for cancer and other diseases.”

The company will develop an innovative, advanced therapy that can provide a curative solution for a clinically unmet need worldwide. The operation entailed an investment of €1.5 million for the development of the technology in collaboration with the *Universitat Autònoma de Barcelona*, cofounder of the spin-off.

In 2020 we also achieved another milestone by establishing a substantial funding agreement with *CRIS Cancer* to develop new mouse models of pancreatic cancer and innovative targets that are expected to have a high impact for this incurable disease. Altogether, the CNIO was able to secure up to €2.4 million in research contracts with the private sector.

Despite the barriers to establishing contact with international industry, the CNIO participated in 2 calls from international pharmaceutical companies and was asked to be part of Pfizer's

selective network to join the Centre for Therapeutic Innovation call. We will continue to work with private collaborators that can help speed up the development of new therapies and technologies in biomedicine.

As a result of the scientific discoveries and continuous scouting at CNIO in the recent years, in 2020 we filed 1 priority patent application and entered the international phases with another patent. This will surely result in future developments and agreements with industry and venture capital to launch new companies that will develop products to help society and contribute to the productive biomedical sector as a whole. As a driver of knowledge creation, the CNIO will continue to explore new ways to rapidly transfer this knowledge to society and the market, contributing to the growth of our innovation ecosystem and the technology surge in the upcoming years.

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 mainly focus on providing support and collaborating with the CNIO Research Groups, they also work with groups from other public research institutions as well as with private companies.

It is impossible to avoid mentioning the impact of the COVID19 pandemic on the research activity of our laboratories in 2020. However, despite the hurdles and difficulties imposed by the sanitary crisis, particularly during the first months of the pandemic, the Biotechnology Programme was able to keep up and running all the essential operations necessary to guarantee the maintenance and preservation of the critical activities of the Centre. In particular, the Animal Facility made an extraordinary effort to preserve the integrity of the mouse colonies during the worst days of the pandemic and the general confinement. Likewise, the rest of the Core Units (Histopathology, Molecular Imaging, Genomics, Flow Cytometry, etc.) maintained a set of minimal services that helped to avoid wasting the efforts made by CNIO researchers in costly and lengthy projects. New ways of providing assistance to customers using telematic tools were promptly put into place by the Units (particularly Flow Cytometry, Confocal Microscopy, Histopathology), which allowed us to maintain the required support to users, while minimising physical interactions.

Moreover, the Mouse Genome Editing Core Unit is working on a project to develop a mouse model expressing humanised ACE2, the protein that is used by the coronavirus to enter into the host cells, which could be used for preclinical studies of the disease. The project has received funding following the successful application to a call for research projects focusing on SARS-CoV-2 and Covid-19 launched by the Institute of Health Carlos III (*ISCiii*).

On the other hand, the technological capabilities of the Units were upgraded during 2020, with the acquisition of a Luminex IVIS Optical system for small animal models at the Molecular Imaging Unit, and the purchase of a STED platform for super-

“By swiftly adapting their procedures and workflows to the new conditions imposed by the pandemic, the Core Units of the Biotechnology Programme have been able to continue meeting the needs and expectations of CNIO researchers.”

resolution microscopy. This was co-funded with support obtained from a successful grant application for scientific infrastructures from the Ministry of Science and Innovation (*MCI*), which was deployed during the year.

As usual, the Core Units were active in attracting funding from external sources through activities related to innovation, 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 to represent a significant funding source for the CNIO. In 2020, despite the impact of the pandemic on the global economy, the total income derived from royalties was maintained at essentially the same level as in 2019, close to €1 million.

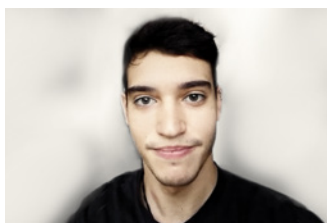
Last but not least, 2020 was again a very productive year scientifically for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in the nearly 30 publications co-authored by members of the Units, many of them in top journals. ■

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Laura Conde,
José Luis Espadas (PEJ)*, Guadalupe
Luengo, Jorge Monsech, Ángeles
Rubio

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



OVERVIEW

The aim of the Genomics Unit is to provide technological services in the fields of genomics and genetics. Technologies with the capacity to interrogate genomes and their activities in a single assay are available. Methodologies able to dissect active molecular hierarchies and pathways (transcriptomic RNA profiling) or structural variations (mutation landscapes, chromatin structure) for the study of cancer or other biological processes. Our services cover a broad range of applications. Next-generation sequencing (NGS) is a staple among them. It is being used for chromatin structure studies, chromosomal protein location analysis, and for transcriptome determinations - even at the single-cell level. More traditional methodologies like Sanger capillary DNA sequencing are also provided. As a side activity, the Unit supports a genetically engineered mouse genotyping service.

“The Genomics Unit offers services in the genetics and genomics fields, which contribute to the understanding of disease and homeostasis at different levels of molecular complexity.”

RESEARCH HIGHLIGHTS

The Genomics Unit, with its portfolio of services that survey different aspects of biological complexity, contributes to the research projects of CNIO Groups. The activities most in-demand are related to applications based on deep-sequencing technologies (NGS). NGS permits a variety of different explorations, such as whole genome and whole exome tumour characterisation, transcriptomic analysis, and the determination of genome structure and of chromatin functional states through the location of interacting factors or epigenomic modifications.

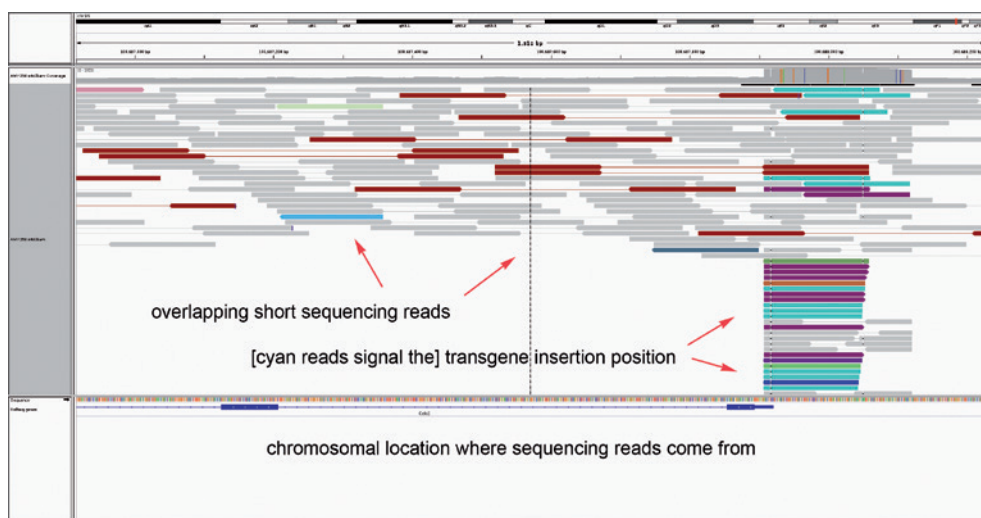
Some of our contributions in 2020 led to the publications referenced below, with some of the Unit's members as authors:

To better characterise colorectal cancer, Costales-Carrera *et al.* examined gene expression profiles in organoids generated from endoscopic biopsies. Rectal tumour organoids differ in gene expression with respect to colon tumour organoids. Interestingly, calcitriol (vitamin D) upregulates stemness genes in rectum and colon organoids, which is consistent with homeostatic action on the large intestine crypt stem cell

compartment. Moreover, differences in biosynthetic processes between rectal tumour and colon tumour organoids suggest that the malignant transformation of stem cells differs even between neighbouring intestinal locations.

In the second reference, a pilot biomarker and efficacy study in advanced HER2-negative breast cancer patients, Quintela-Fandiño *et al.* discuss the potential priming role of a prior antiangiogenic therapy on subsequent immunotherapy. Vascular normalisation was seen after the antiangiogenic bevacizumab treatment in non-progressors' tumours, which also showed gene expression profiles with both increased T-effector and T-memory features and decreased Treg signatures. In summary, the antiangiogenic activity was found to immuno-prime at least a fraction of patients who will more likely benefit from subsequent immunotherapy. ■

FIGURE Among its multiple applications, next-generation sequencing can be used to ascertain the position of the chromosomal insertion of transgenes. This figure highlights fragments whose sequencing reads map both in an endogenous locus and in the transgene (not shown).



► PUBLICATIONS

- Costales-Carrera A, Fernández-Barral A, Bustamante-Madrid P, Domínguez O, Guerra-Pastrián L, Cantero R, Del Peso L,

Burgos A, Barbáchano A, Muñoz A (2020). Comparative study of organoids from patient-derived normal and tumor colon and rectal tissue. *Cancers (Basel)* 12, 2302.

► Quintela-Fandiño M, Holgado E, Man-

so L, Morales S, Bermejo B, Colomer R, Apala JV, Blanco R, Muñoz M, Caleiras E, Iranzo V, Martínez M, Domínguez O, Hornedo J, González-Cortijo L, Cortes J, Gasol Cudos A, Malon D, López-Alonso

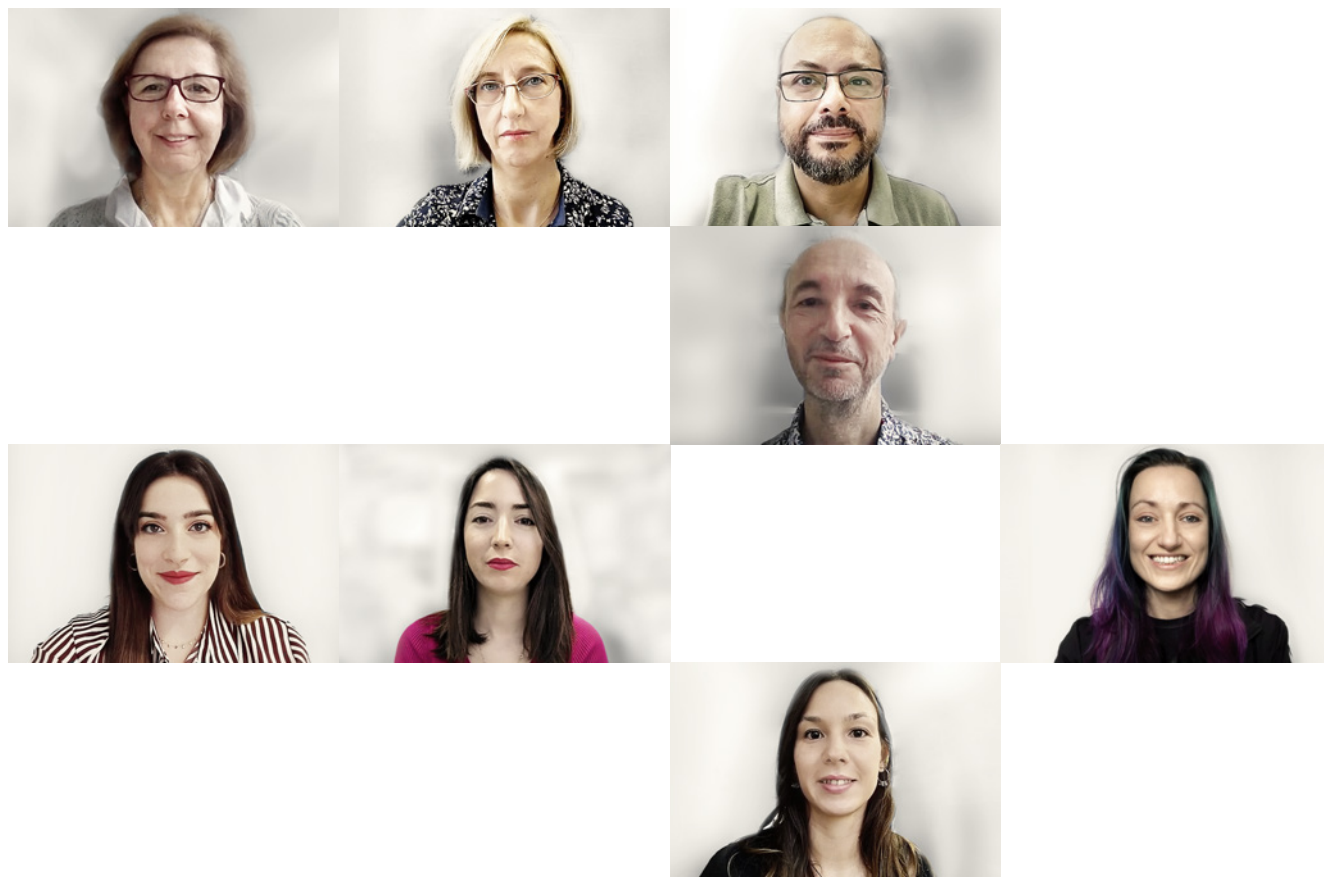
A, Moreno-Ortiz MC, Mouron S, Mañes S (2020). Immuno-priming durvalumab with bevacizumab in HER2-negative advanced breast cancer: a pilot clinical trial. *Breast Cancer Res* 22, 124.

MOUSE GENOME EDITING CORE UNIT

Sagrario Ortega
Core Unit Head

Graduate Student
Aleida Pujol

Technicians
Estefanía Ayala, Marina Cabrerizo
(PEJ-L)*, Carmen Gómez,



OVERVIEW

Genetically modified mouse models are an essential part of any discipline of biomedical research, including cancer. Our Unit has created about 500 mouse models to support cancer research, using state-of-the-art mouse genetics and gene editing technologies.

The term “cancer” encompasses a whole spectrum of extremely complex diseases in which tumour cells interact, at different levels, with various physiological components, such as the immune system, blood vessels or stromal tissue, 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 type. The precise,

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

The Mouse Genome Editing Unit has more than 20 years of experience in the design, generation, and validation of genetically modified mouse models. In addition, it has created a collection of currently over 1,000 cryopreserved mouse strains from which the entire scientific community may benefit in many different research disciplines.

Jaime Muñoz (TS)**, Patricia Prieto (TS)**
 Pierfrancesco Vargiu (TS)**

*Plan de Empleo Joven-Licenciado (Youth
 Employment Plan-Graduate)

**Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

COVID19 preclinical mouse models

The emergence in 2019 of the new coronavirus strain SARSCoV2/SARS-2 and the expansion of the pandemic called COVID-19, prompted the global scientific community to dedicate an unprecedented effort to developing strategies aimed at halting SARS-2 spread and protecting against COVID disease. However, one of the main limitations in COVID research is the lack of adequate models to study SARSCoV2 infection, especially animal models, where the complex interactions established between the virus and its host are reproduced in a physiological context.

The laboratory mouse is the most widely used animal model in biomedicine, but it is not a permissive species for SARSCoV2 infection. Structural differences between the human Angiotensin Converting Enzyme-2 (ACE2) protein, the virus cellular receptor, and its murine orthologous are the cause of the different response in human and mouse. Supported by a dedicated grant from the Spanish Institute of Health *Carlos III*, and with the collaboration of Gen-H Genetic Engineering, Heidelberg, during 2020, the Unit created “humanised” mouse models optimised for the study of COVID-19 that, once characterised, will be available for the scientific community. These models were created using the latest gene editing technologies, and present unique advantages over the models available so far for COVID-19.

We created 2 different types of mouse models. In the first one, using a knockin approach, the human ACE2 protein is expressed under the transcriptional control of the endogenous mouse *Ace2* promoter, interrupting simultaneously the *Ace2* coding sequence resulting in the knockout of the mouse *Ace2*

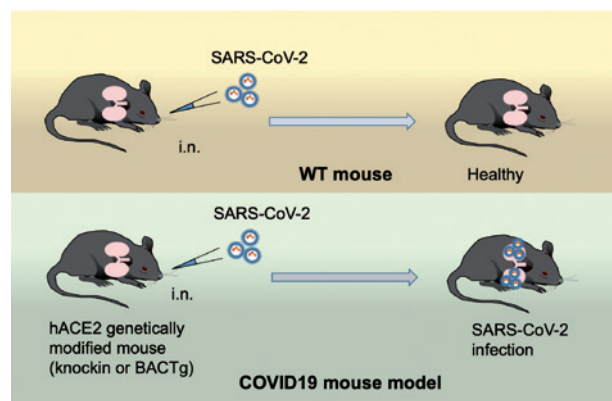


FIGURE Humanised mice as animal models of COVID-19. Expression of the human form of the ACE2 protein in mice makes them susceptible to SARS-CoV2 infection by intranasal (i.n.) inoculation of the virus. We

have used two different strategies to express human ACE2 with the same transcriptional control of the endogenous *Ace2* gene, knockin and BAC-transgenesis.

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. Both models are currently being characterised for SARS-CoV2 infection response. ■

PUBLICATIONS

- ▶ Lynch CJ, Bernad R, Martínez-Val A, Shahbazi MN, Nóbrega-Pereira S, Calvo I, Blanco-Aparicio C, Tarantino C, Garreta E, Richart-Ginés L, Alcazar N, Graña-Castro O, Gómez-López G, Aksoy I, Muñoz-Martín M, Martínez S, Ortega S, Prieto S, Simboeck E, Camasses A, Stephan-Otto Attolini C, Fernandez AF, Sierra MI, Fraga MF, Pastor J, Fisher D, Montserrat N,

Savatier P, Muñoz J, Zernicka-Goetz M, Serrano M (2020). Global hyperactivation of enhancers stabilizes human and mouse naive pluripotency through inhibition of CDK8/19 Mediator kinases. *Nat Cell Biol* 22,1223-1238.

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- ▶ Kalisz M, Bernardo E, Beucher A, Maestro MA, Del Pozo N, Millán I, Haeberle L, Schlenz M, Safi SA, Knoefel WT, Grau V, de Vas M, Shpargel KB, Vaquero E, Magnuson T, Ortega S, Esposito I, Real FX, Ferrer J (2020). HNF1A recruits KDM6A to activate differentiated acinar cell programs that suppress pancreatic cancer. *EMBO J* 39, e102808.

- ▶ Salazar-Roa M, Trakala M, Álvarez-Fernández M, Valdés-Mora F, Zhong C, Muñoz J,

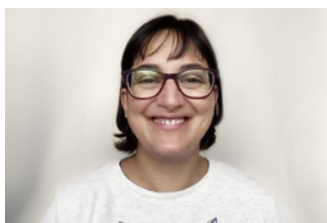
Yu Y, Peters TJ, Graña-Castro O, Serrano R, Zapatero-Solana E, Abad M, Bueno MJ, de Cedón MG, Fernández-Piqueras J, Serrano M, Blasco MA, Wang DZ, Clark SJ, Izpisua-Belmonte JC, Ortega S, Malumbres M (2020). Transient exposure to miR-203 enhances the differentiation capacity of established pluripotent stem cells. *EMBO J* 39, e104324.

MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

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

*Titulado Superior (Advanced Degree)



OVERVIEW

One of the greatest advances of the last decades in the field of biotechnology has been the development of hybridoma technology to generate monoclonal antibodies (mAbs). mAbs have revolutionised the field of research and medicine. Antibodies are essential tools in a broad array of laboratory techniques and procedures, and they are used to address basic research questions, leading to a better understanding of life processes. Their outstanding specificity makes them exquisite tools that enable researchers to explore and dissect biological processes in detail; they are particularly helpful in the investigation of new approaches for the diagnosis, prevention, and treatment of cancer.

The Monoclonal Antibodies Unit provides CNIO Research Groups with an *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 give added value to 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.

RESEARCH HIGHLIGHTS

During the last 20 years, the Monoclonal Antibodies Unit has generated a large number of mAbs directed against more than 160 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

hXCR1 and hCLEC9a mAbs: a specific tool for the detection of conventional type 1 DCs

Dendritic cells (DCs) are key orchestrators of immune responses. A specific DC subset, conventional type 1 DCs (cDC1s), has been recently associated with human cancer patient survival and, in preclinical models, is critical for the spontaneous rejection of immunogenic cancers and for the success of T cell-based immunotherapies.

These recent findings suggest an important role for cDC1 in anticancer immunity in humans. New developments in the understanding of sDC1s biology have identified CLEC9A (DNGR-1) and XCR1 as specific markers for the cDC1 subtype. We therefore developed and validated 2 monoclonal antibodies against human XCR1 and CLEC9a that can detect the expression of these proteins in human paraffin tissue.

These novel antibodies will allow this specific subtype of dendritic cells to be identified and studied, clarifying the role of cDC1s in human cancer.

EuroMabNet, a European consortium of experts in monoclonal antibody technology

In 2008, in collaboration with Oxford University, we founded EuroMabNet (www.euromabnet.com), a non-profit organisation that currently spans 12 European countries. EuroMabNet's primary goal is to provide an arena for people working in the field of monoclonal antibody production and technologies to exchange knowledge and updated methodologies, as well as to create common strategies to

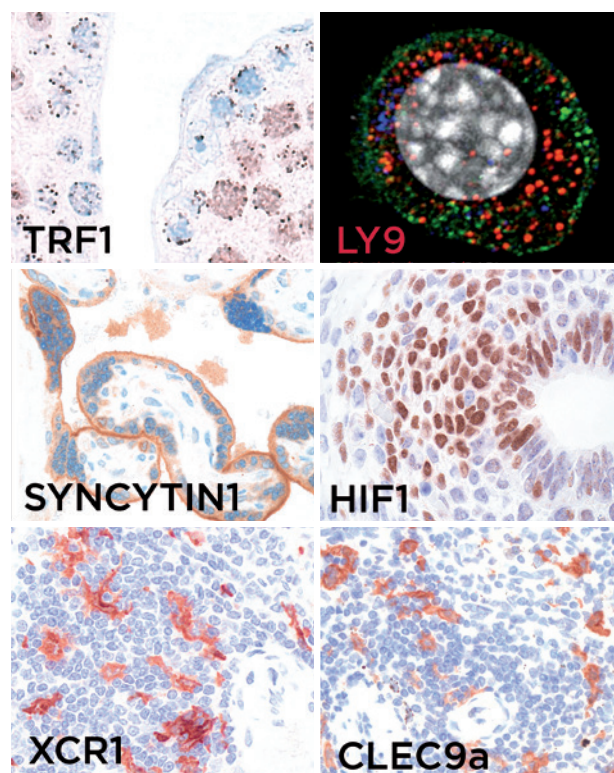


FIGURE Immunohistochemistry staining in human and mouse paraffin tissues using some of the antibodies generated in the last few years by the Monoclonal Antibodies Unit.

improve and standardise the production of properly validated antibodies. Such a platform will provide the scientific and medical communities with antibodies that are “fit for use” and can be used with confidence in terms of both their specificity and technical performance. ■

PUBLICATIONS

- ▶ Voskuil JLA, Bandrowski A, Begley CG, Bradbury ARM, Chalmers AD, Gomes AV, Hardcastle T, Lund-Johansen F, Plückthun A, Roncador G, Solache A, Taussig MJ, Trimmer JS, Williams C, Goodman (2020). The Antibody Society's antibody validation webinar series. *MAbs* 12, 1794421.
- ▶ Maestre L, García-García JF, Jiménez S, Reyes-García AI, García-González Á, Montes-Moreno S, Arribas AJ, González-García P, Caleiras E, Banham AH, Piris MÁ,

Roncador G (2020). High-mobility group box (TOX) antibody a useful tool for the identification of B and T cell subpopulations. *PLoS One* 15, e0229743.

PATENT

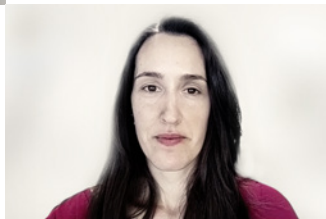
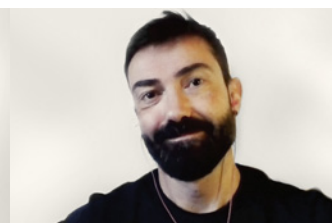
- ▶ Paz-Ares L, Martínez JL, Roncador G, Ojeda L, Ferrer I (2020). Interleukin 11 receptor alpha subunit (IL11RA) neutralizing antibodies and uses thereof. *EP20382631.8*.

MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians

Tatiana Álvarez, Guillermo Garaulet (TS)*, Silvia Leal (until July), Sergio Mateos (until August) (TS)* (PEJ)*, Guillermo Medrano, David Sabador



OVERVIEW

A new strategy involving molecular imaging is Theranostics, which integrates diagnostics and therapy for patient management. In the oncology field, this technique combines tumour diagnostic imaging and therapeutics, trying to improve existing techniques. The possibility of detecting the disease, guiding therapy and conducting follow-up is opening a new chapter in precision medicine.

In molecular imaging medicine, theranostics refers to the use of molecules labelled with either diagnostic radionuclides (e.g. gamma-ray or positron emitters) or with therapeutic radionuclides (beta-particle emitters) for diagnosis and treatment of a particular disease. Thus, molecular imaging and disease diagnosis can effectively lead to personalised treatment using the same study molecules.

“Theranostics gives you the possibility of using exactly the same molecular targeting compound for both diagnostic and therapeutic purposes. ‘If you can see it, you can treat it’.”

(since September) (TS)* (PEJ)**,
Gloria Visdomine

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

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

RESEARCH HIGHLIGHTS

In 2020, the Molecular Imaging Unit received a new grant from the BBVA Foundation to start working on theranostics applications of radiolabelled antibodies. We also continued with the rest of our ongoing projects. One of our projects, granted in collaboration with *CIEMAT*, focuses on developing and labelling nanobodies produced by camelids using the ImmunoPET strategy (FIGURE). This strategy combines the high specificity and selectivity of the antibodies with the high sensitivity and quantitative capabilities of PET. We also continued our participation in the *RENIM* network programme. Our project, supported by a grant from the *Comunidad de Madrid (RENIM-CM)*, focuses mostly on developing nanoparticles to perform optical imaging and multimodality imaging (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.

In 2020, we also installed a new optical imaging system, IVIS Lumina III, to replace the old one, 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.

The Molecular Imaging Unit continues to provide CNIO researchers with state-of-the-art molecular imaging equipment

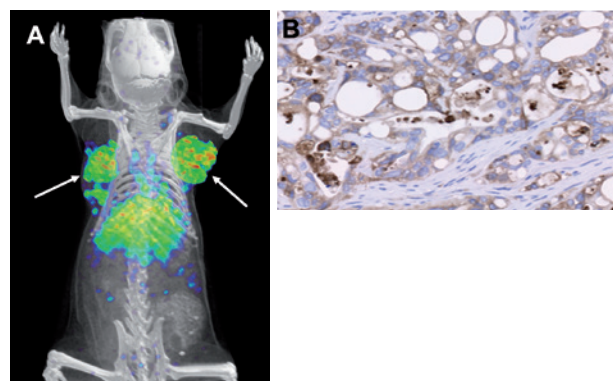


FIGURE (A) Representative PET/CT images, with ^{89}Zr -DFO-LEM2/15 at 24 h, of a mouse with subcutaneous

CAPAN-2 cells. White arrows indicate the tumour. (B) IHC MT1-MMP was detected using the LEM2/15 antibody.

and human resources in order to guarantee the highest quality studies and to develop and update protocols and imaging techniques that serve 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. ■

PUBLICATIONS

- ▶ Quintela-Fandino M, Morales S, Cortés-Salgado A, Manso L, Apala JV, Muñoz M, Gasol Cudos A, Salla Fortuny J, Gion M, Lopez-Alonso A, Cortés J, Guerra J, Malón D, Caleiras E, Mulero F, Mouron S (2020). Randomized phase 0/I trial of the mitochondrial inhibitor ME-344 or placebo added to bevacizumab in early HER2-negative breast cancer. *Clin Cancer Res* 26,35-45.
- ▶ Aguilera-Correa JJ, García-Casas A, Mediero A, Romera D, Mulero F, Cuevas-López I, Jiménez-Morales A, Esteban J (2020). A new antibiotic-loaded sol-gel can prevent bacterial prosthetic joint infection: from *in vitro* studies to an *in vivo* model. *Front Microbiol* 10, 2935.
- ▶ Conesa-Buendía FM, Mediero A, Fujikawa R, Esbrit P, Mulero F, Mahillo-Fernández I, Mues AO (2020). Beneficial effects of manually assisted chiropractic adjusting instrument in a rabbit model of osteoarthritis. *Sci Rep* 10, 13237.
- ▶ Shaikh F, Andersen MB, Sohail MR, Mulero F, Awan O, Dupont-Roettger D, Kubassova O, Dehmeshki J, Bisdas S (2020). Current landscape of imaging and the potential role for artificial intelligence in the man-

agement of COVID-19. *Curr Probl Diagn Radiol*. PMID: 32703538.

Book Chapter

- ▶ Shaikh F, Franc B, Mulero F (2020). Radiomics as Applied in Precision Medicine. In: Clinical Nuclear Medicine, Ahmadzadehfard H, Biersack HJ, Freeman L, Zuckier L (eds). Springer, Cham. DOI: https://doi.org/10.1007/978-3-030-39457-8_3. Print ISBN: 978-3-030-39455-4. Online ISBN: 978-3-030-39457-8.

AWARDS AND RECOGNITION

- ▶ Faculty of MIT *linQ* with Massachusetts Institute of Technology and Advisory Board Member of *Catalyst Europe*, a programme that belongs to the EIT Health Campus pillar.
- ▶ Member of Spanish PET Group, Spanish Society of Nuclear Medicine and Molecular Imaging (*SEMNUM*).
- ▶ Project evaluator of *Junta de Andalucía* and *Agencia de Qualitat Universitaria de les Illes Balears*, Spain.
- ▶ Editorial Board Member, *PLOS ONE* Journal.

FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Julia García-Lestón (TS)*, Sara
García García, Marina Quesada
Martínez (since September) (PEJ)**

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



OVERVIEW

Flow Cytometry is a fast and multiparametric technology, and an extremely valuable tool in the oncology field. It is an important workhorse able to identify, quantify and isolate defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other at the single cell level.

Our aim is to provide 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 4 analysers and 3 high-speed cell sorters with different optical configurations to cater to users' needs. We also have an automated magnetic bead separation system

(AutoMACS), 2 automated cell counters (Countess) 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.

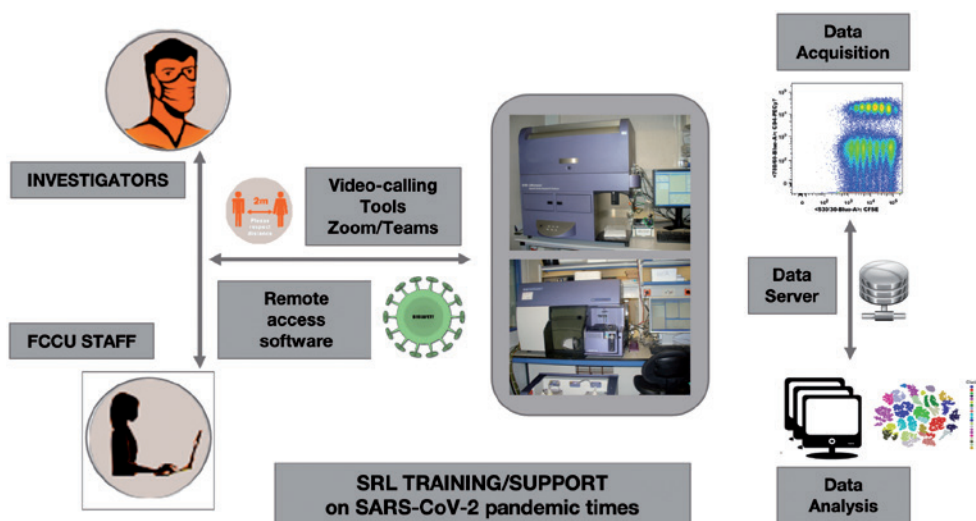
RESEARCH HIGHLIGHTS

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 of the applications developed and validated by our Unit include:

- Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- Apoptosis studies (Annexin V, Mitochondrial Membrane 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 characterisation of our instrument performance by creating volttration templates in all our instruments to assess optimal voltage for each detector, and expanded 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 At the Flow Cytometry Unit, we put in place a successful workflow for remote training and support in all our analysers and some of our cell sorters so we could continue to work throughout the Covid-19 pandemic, while complying with new social distancing rules.



PUBLICATIONS

- ▶ Cerezo-Wallis D, Contreras-Alcalde M, Troulé K, Catena X, Mucientes C, Calvo TG, Cañón E, Tejedo C, Pennacchi PC, Hogan S, Kölblinger P, Tejero H, Chen AX, Ibarz N, Graña-Castro O, Martínez L, Muñoz J, Ortiz-Romero P, Rodríguez-Peralto JL, Gómez-López G, Al-Shahrour F, Rabadán R, Levesque MP, Olmeda D, Soengas MS (2020). Midkine rewires the melanoma

microenvironment toward a tolerogenic and immune-resistant state. *Nat Med* 26, 1865-1877.

- ▶ Palma F, Affinito A, Nuzzo S, Roscigno G, Scognamiglio I, Infenito F, Martinez L, Franzese M, Zanfardino M, Soricelli A, Fiorelli A, Condorelli G, Quintavalle C (2020). miR-34c-3p targets CDK1 a synthetic lethality partner of KRAS in non-small cell lung cancer. *Cancer Gene Ther*. PMID: 32948832.

- ▶ Daniels K, Conway A, Gardner R, Martinez L, Price KM, Schneider S, Sheridan S, Srivastava J, Thornton S (2020). Remote training of SRL users and staff in a global pandemic. *Cytometry A*. PMID: 33175460.
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AWARDS AND RECOGNITION

- ▶ Member of the International Society for Advancement of Cytometry (ISAC) Shared Resource Laboratories (SRL) Task Force.
- ▶ Treasurer of the European Association "Core Technologies for Life Sciences (CTLIS)".

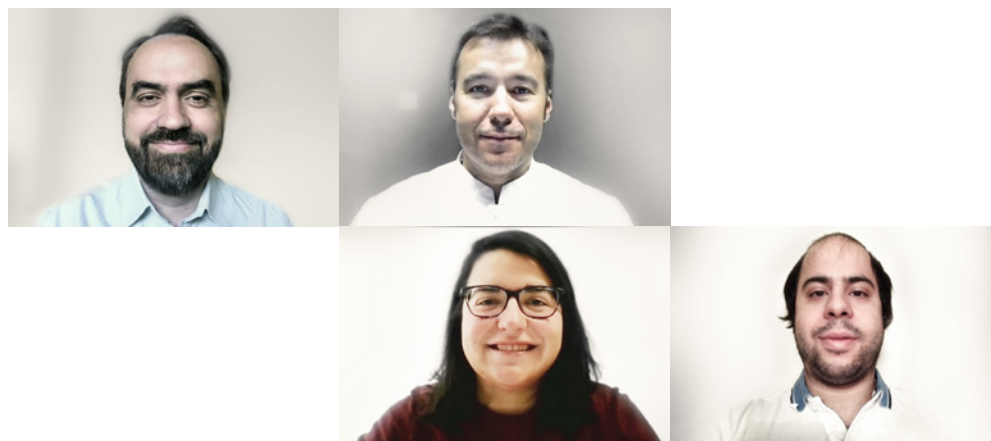
CONFOCAL MICROSCOPY CORE UNIT

Diego Megías
Core Unit Head

Technicians
Jesús Gómez (PEJ)* (TS)**, Gadea
Mata (until September) (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 CNIO's 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 CNIO scientists. The Unit is actively involved in developing, testing, and implementing new microscopy technologies, tools and imaging applications that could be of interest to CNIO Research Groups. 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 have 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.”

RESEARCH HIGHLIGHTS

In 2020, the Unit significantly renewed its equipment. First, we installed a new sp8 STED super-resolution microscope with a white light laser and 3 depletion laser lines. This will make it possible to surpass the conventional optical resolution limit to explore and quantify with more detail biological sub-cellular structures such as DNA damage, telomeres, etc. Additionally, we equipped the Unit with a new live cell imaging, wide-field platform (Thunder) to allow higher throughput, but, more importantly, to increase acquisition speed and increase the possibilities in multiple colour experiments.

In addition to these 2 new systems, the Confocal Microscopy Unit is equipped with 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 Deltavision 4D deconvolution station 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 high throughput technologies applied to confocal microscopy using 2 different systems:

- An Opera (Perkin Elmer) High Content Screening (HCS) system, which allows running HCS experiments on fixed and live cells in multi-well plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence.
- A sample navigation application integrated into the SP8 and SP5 confocal systems, enabling high throughput feeding of the instrument, not only in multi-well plates but also in tissue sections.

These advances enable us to increase the level of information obtained from a sample as well as to carry out the automated screening of cell behaviour under different treatments.

The Confocal Microscopy Unit continues to dedicate significant efforts towards developing and implementing HCS technology at the CNIO.

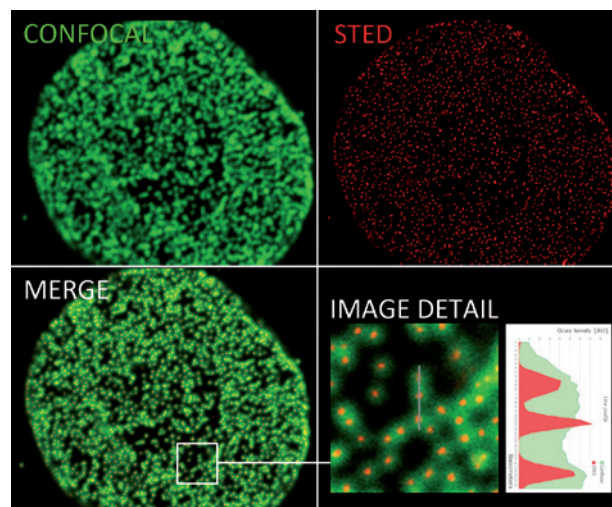


FIGURE Example of a super resolution image. Nuclear pore protein acquired in conventional confocal mode (green) compared with the super resolution STED image (red).

The Unit promotes and helps with the protocol development for novel 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. ■

► PUBLICATIONS

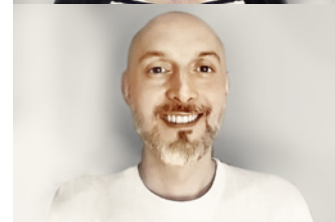
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PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Post-Doctoral Fellow
Francisco J. Sánchez (until June)

Graduate Students
Cristina Sayago



OVERVIEW

Current advances in high-throughput techniques represent a paradigm-shift and have revolutionised biomedical research. Omics technologies provide an unbiased view of a biological system and can be used to test and generate novel hypotheses. Proteins are the molecular effectors of cells and transcriptomics merely represents a proxy to estimate the final protein product. Moreover, genomic data do not provide information about the post-translational modifications of proteins or their interactions. Thus, direct interrogation of proteins is of paramount importance. Proteomics is a discipline that aims to understand the complex regulation of the proteome and its impact on disease. However, global analysis of proteins is challenging owing to their high complexity and high dynamic range. To tackle these analytical challenges, proteomics uses a combination of sample preparation, mass spectrometry

“Direct analysis of proteins through mass spectrometry-based proteomics is essential to fully understand the underlying mechanisms of cancer.”

(MS) and bioinformatics. The CNIO Proteomics Core Unit provides MS-based proteomics to research groups in order to better understand, at the proteome level, the molecular basis of cancer.

Technicians

Enrique Alonso (TS)*, Fernando García (TS)*, Julia Isabel Morales (since December) (TS)*, Jana Sánchez (TS)*, Álvaro Soriano (until January) (PEJ, CAM)*, Pilar Ximénez

De Embún (TS)*, Eduardo Zarzuela (TS)*

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

RESEARCH HIGHLIGHTS

In 2020, the Proteomics Unit participated in multiple projects that demanded advanced proteomics. In collaboration with the Melanoma Group, we investigated the role of MIDKINE in the tumour micro-environment. We analysed the downstream effects (loss-of-function and gain-of-function) of this secreted factor with respect to inflammatory processes. Together with the former Tumour Suppression Group, we showed that inhibition of CDK8, a negative regulator of the Mediator complex, activates super-enhancers of key identity genes and stabilises the so-called naïve pluripotent state. Using phosphoproteomics, we analysed the early effects of a CDK8i to identify downstream effectors that could play a role in this process. Moreover, we collaborated in other projects related to, among others, the identification of biomarkers for lymphatic-promoted disorders (with the Microenvironment and Metastasis Group) and the impact of BCL7A mutations in the interactome of the SWI/SNF complex in diffuse large B-cell lymphoma (DLBCL). Finally, the Unit worked on implementing new technologies to the range of available services. Recent advances in machine learning have made it possible to predict fragmentation spectra from peptide sequences. This has pushed forward the implementation of Data-Independent Acquisition (DIA)-based methods. Unlike Data Dependent Acquisition (DDA), DIA sequentially fragments the entire m/z range using wide isolation windows (8-20 m/z). The deconvolution of high multi-plexing spectra is achieved by software like EncyclopeDIA. Empirically-corrected libraries can be easily generated from a representative pool of samples that is analysed by Gas Phase Fractionation (GPF) using lower isolation windows. This strategy enables 6000-

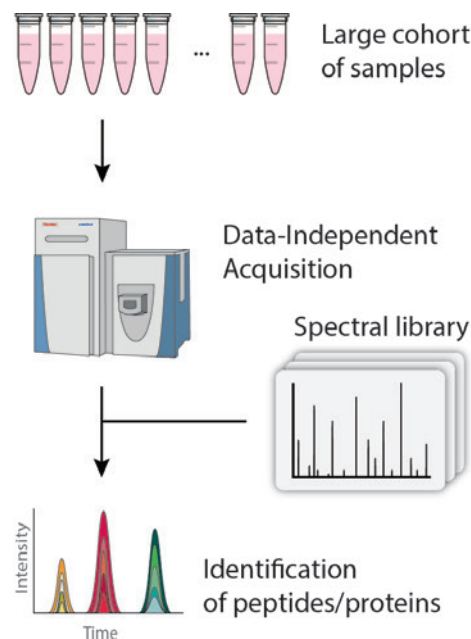


FIGURE Data-Independent Acquisition enables fast and cost-effective profiling of proteomes with a significant coverage depth. This

will facilitate studies that require comparisons for a large number of conditions and biological replicates.

8000 proteins from cell lysates to be identified in 90 minutes of instrument time, representing a promising approach for quantitative proteomic studies. ■

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HISTOPATHOLOGY CORE UNIT

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Core Unit Head

Staff Scientist
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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 set of services ranging from paraffin embedding and tissue sections to histochemical stains, research and diagnostic immunohistochemistry (IHC) testing, antibody validation, *in situ* hybridisation techniques (including *in situ* detection of mRNAs by RNAScope), as well as the generation of tissue microarrays. Furthermore, the Unit offers other value-added services assisted 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. Also, the Unit offers its portfolio of services to other institutions, including hospitals, research centres and private companies.

“Despite the difficult situation generated by the Covid-19 pandemic, the Unit was able to adapt its workflows and procedures to meet the needs and demands of our customers.”

RESEARCH HIGHLIGHTS

Despite all the difficulties and restrictions imposed by the Covid-19 pandemic, the Unit has maintained the portfolio of services demanded by its users in accordance with the needs of their projects. Particularly during the initial months of the pandemic, the Unit established a system to ensure that the most basic activities were covered, to avoid losing valuable research materials and to guarantee the provision of essential services to the CNIO community. Thus, more than 25,000 paraffin blocks of tissue samples were generated, and *ca.* 22,000 techniques were performed, including histological and IHC techniques, *in-situ* chromogenic hybridisation, tissue microarrays, slide scanning, etc. These numbers are very close to those recorded in 2019, before the pandemic.

During 2020, the Unit placed a particular focus on optimising and consolidating in its portfolio the *in situ* hybridisation technology branded as RNAScope, using the Ventana-Roche automatic platform for IHC stains. This new technique allows us to efficiently detect 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 that work well on FFPE tissues, or to validate results from other technologies, among others. This technology has already been used in numerous projects and has proved its value for CNIO researchers.

The high quality of the techniques run by the Unit continues being endorsed by External Quality Assessment Schemes. Thus, our histochemical techniques were evaluated by UK

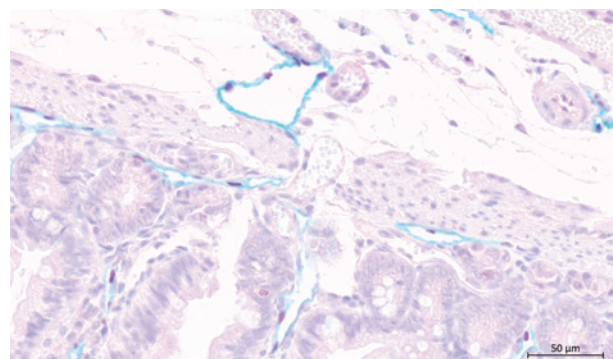


FIGURE Detection of lymphatic vessel endothelium using as markers for dual staining PROX1 (Purple), localising the nuclei, and LYVE1 (Teal, in blue) at the cytoplasm. Blood vessel endothelium (visible at the

right edge of the main lymphatic vessel in the figure, with erythrocytes inside) is negative for this dual staining (in collaboration with the CNIO Melanoma Group).

NEQAS. On the other hand, NordiQC and SEAP 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 most of the usual activities in this area were compromised due to the pandemic, the Unit was still able to participate in a Master's course on oncological research, providing a practice session on the technologies offered by the Unit. We hope that these activities will resume in full next year. ■

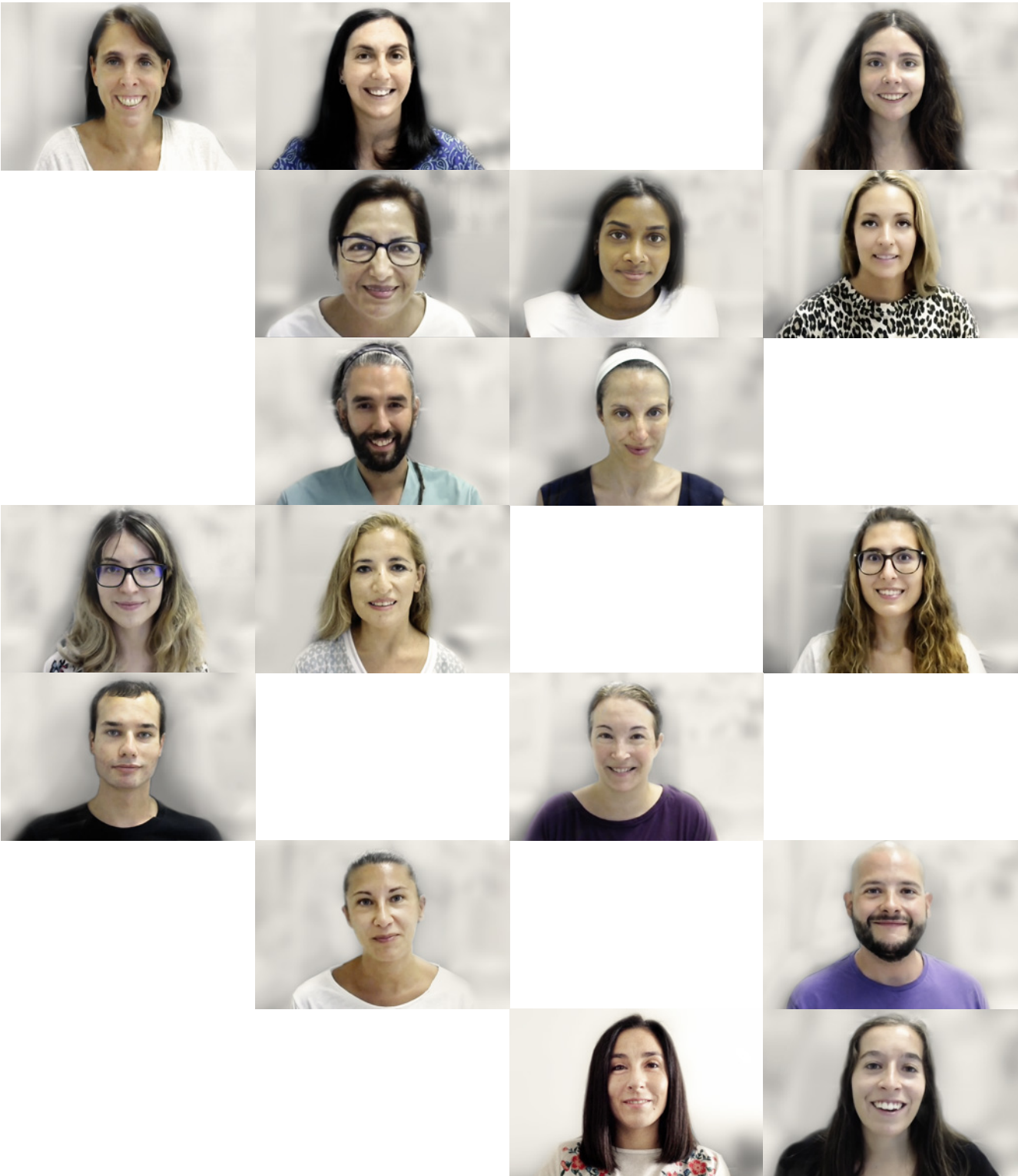
PUBLICATIONS

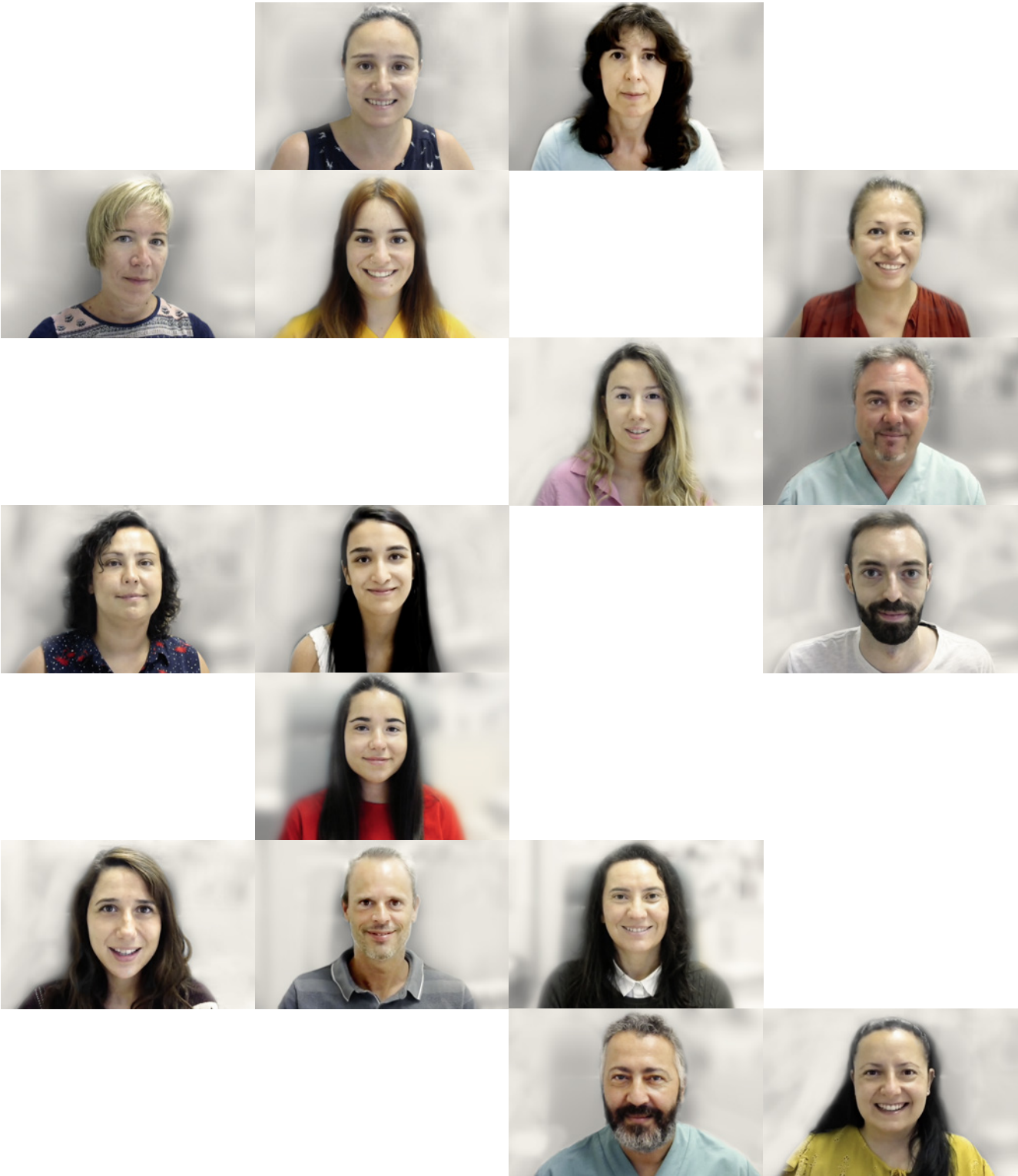
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ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Vivotecnia Management & Services





The CNIO has a state-of-the-art Animal Facility, managed by Vivotecnía 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 high standards achieved by the CNIO with regard to the use and care of animals for experimentation have been recognised by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. AAALAC accreditation, considered one of the top international recognitions in this field, was first obtained in October 2016, and was 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.

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 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

“Mouse models are essential tools in cancer research. The Animal Facility offers to CNIO researchers all the capabilities needed in this area, in compliance with the highest standards of animal care and welfare.”

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. In 2020 the Animal Facility implemented a new climate chamber (HPPlife) that allows mice to be kept under controlled environmental conditions of temperature, humidity and light, beyond the standard ones established at the SPF barrier area. This will allow the study of these environmental factors and their influence on the development of disease, 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 for the generation of monoclonal antibodies directed against mouse antigens, as well as for a project of the Experimental Therapeutics Programme that aims to test 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 must 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 the work with laboratory animals, which is complementary to the online courses that are a requisite to gain access to the facility.

In line with our commitment to maintaining 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.

2020 was especially hard for us due to the Covid-19 pandemic crisis. In March, we had to implement, in a very short time, contingency plans to ensure the health of our staff, the welfare of the animals, and the continuation of the procedures in process. Thanks to the collaboration and professionalism of all the staff, including caretakers, technicians, supervisor and veterinarian, and as a result of the new and effective safety measures put in place, the Animal Facility was able to continue providing the support demanded by CNIO users with the same excellence level as usual, without any outbreaks across the staff.

During 2020, the Animal Facility's Head continued serving 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 2020:

CDK8 inhibitors (CDK8-is). We carried out preliminary non-regulatory toxicity studies in rats with our lead compound ETP-18. It showed preclinical toxicity results, which could allow its inclusion in further regulatory development stages.

Mastl Inhibitors (MASTL-is). (In collaboration with Marcos Malumbres's Group). In 2020, we evaluated the selectivity (+ 460 kinases) of 3 previously explored chemical series. One of them showed an acceptable profile and was selected for further SAR exploration. As a result, and to the best of our knowledge, we have discovered the most potent MASTL-is to date. For example, we identified ETP-184 as a potent inhibitor of MASTL at the biochemical ($IC_{50} < 30$ nM full length h-MASTL) and cellular levels (BRET technology $EC_{50} < 100$ nM). ETP-184 also showed an acceptable kinase selectivity profile (KinomeScan) and metabolic stability (microsomes). We will continue the hit to lead exploration of this series in 2021. Additionally, we have been working on the discovery of PROTACs to target the degradation of MASTL in cells. Such compounds would allow the inhibition of both kinase and non-kinase roles of MASTL. We synthesised a collection of around 80 PROTACs-like molecules. Two compounds were identified as moderate degraders. We will continue their characterisation and optimisation in 2021.

TRF1. (In collaboration with Maria A. Blasco's Group). In 2020 we carried out RNAseq experiments with ETP-946 to shed light on its (molecular) mode of action. The comparison of up- and down-regulated genes with similar RNAseq profiles of known compounds led us to postulate the most probable mechanism of action (MoA) by which ETP-946 modulates TRF1. Furthermore, we achieved the experimental validation of this MoA for ETP-946 and its series. We also demonstrated that known drugs, acting through this mechanism, were able to modulate TRF1, and other proteins of the shelterin complex, as ETP-946. These results will be reported accordingly. Meanwhile, in 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 led to several hit molecules, which are currently undergoing triage and validation processes.

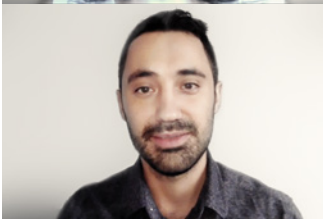
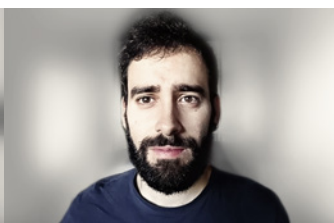
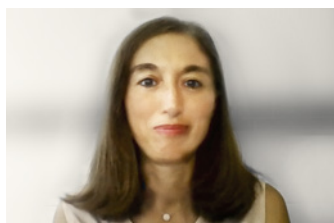
"ETP has evolved along the years into a 'Swiss army knife profile'. Our adaptability is essential to tackle the diversity of Drug Discovery and Chemical Biology projects at CNIO."

SETD8 inhibitors. This project was recently internalised and incorporated into the ETP pipeline, and will be carried out in collaboration with the CNIO Genomic Instability Group. Our main objective is to generate and optimise novel SET8 inhibitors (SETD8-is) as new therapeutic agents. We have set up a biochemical assay that has been validated with previously reported SETD8 inhibitors. We plan to perform screening campaigns with several ETP-libraries to identify new chemotypes that inhibit SETD8 for the development and design of novel SETD8-is with intellectual property. ■

MEDICINAL CHEMISTRY SECTION

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Section Head

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Technicians

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Bruno Di Geronimo (TS)* (PEJ)**,
Francisco Javier García (TS)* (PEJ)**

Student in Practice

Luis De Castilla (January-June)
(*Universidad Rey Juan Carlos*,
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* *Titulado Superior* (Advanced Degree)

** *Plan de Empleo Joven -Licenciado* (Youth
Employment Plan-Graduate)

OVERVIEW

The Medicinal Chemistry Section is part of the multidisciplinary Experimental Therapeutics Programme (ETP) that is focused on early drug discovery activities. Our aim is to generate advanced chemical compounds that could be further developed into drugs to treat cancer. The Section deals with the design, synthesis, and optimisation of compounds that are characterised in the Biology Section of ETP to evaluate their potency in biological targets, *in vitro* and *in vivo* drug-like properties and, finally, to demonstrate their efficacy and mechanism of action in animal models (*in vivo* proof-of-concept). As a complementary strategy to the classic inhibitors, we also contemplate the degradation of particular targets using different chemical approaches such as the use of proteolysis targeting chimeras (PROTACs). Additionally, we have entered the chemical biology field in order to discover and identify novel drugs and targets from phenotypic screenings. In this regard, we contribute by synthesising high quality chemical tools needed for interrogating the observed phenotype.

“In our MASTL project we generated high quality inhibitors that are to be used as chemical tools for target validation studies and to serve as the basis for the development of advanced lead compounds.”

RESEARCH HIGHLIGHTS

In 2020, ETP was involved in several drug discovery projects (FIGURE). A summary of some of them is provided below.

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

This CNIO project yielded advanced optimised molecules with a “first-in-class” profile. Preliminary toxicity studies in rats were carried out using the leading product of our chemical series. The results showed a preclinical toxicity profile compatible with its potential development as a drug.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is led by Maria A. Blasco (CNIO Telomeres and Telomerase Group). During 2020, new biotinylated chemical probes that do not require “click chemistry” were used to identify the molecular targets of ETP-946. Unfortunately, no conclusive results were obtained. By contrast, studies performed in the ETP Biology Section and focused on the cellular level of the altered genes, after treatment with ETP-946, allowed us to identify the potential mechanism of action of ETP-946 (and its series, Series 2) by which the levels of TRF1 in telomeres are modulated. Currently, resynthesis of several compounds is required to validate this new hypothesis.

Additionally, during this year, the Biology Section developed an assay to measure the binding of TRF1 to telomeric DNA, and a wet screening campaign was run with the aid of virtual screening techniques. We identified potential “direct” inhibitors of TRF1 and we are currently involved in their validation by resynthesizing them to confirm the observed activity. Once we have confirmed the hits, we will start SAR activities around them.

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

This project is undertaken in collaboration with Marcos Malumbres (CNIO Cell Division and Cancer Group). In 2020, we characterised the selectivity (+ 460 kinases) of 3 previously explored chemical series. One of them showed quite acceptable selectivity, while the other 2 proved to be excessively promiscuous. The selective series was chosen for further development, and we significantly advanced its SAR exploration by synthesising more than 100 compounds. As a result, we obtained nanomolar MASTL inhibitors biochemically and in cells, and with good metabolic stability

in liver microsomes. Fine optimisation of this series is currently ongoing. Additionally, we are exploring the development of PROTAC molecules to degrade MASTL and not only to inhibit its catalytic activity. We synthesised a collection of more than 80 PROTAC-like molecules using CRBN and VHL as E3 ligands. Two compounds were identified as moderate degraders in the MDAMB231 cell line. This exploration is still ongoing to identify more potent degraders. Other strategies are also being explored, for example, the use of hydrophobic tagging (HyT) in our molecules to induce degradation of MASTL.

Discoidin domain receptor (DDR) 1/2 inhibitors

This project was carried out in the context of a doctoral thesis by an FPI “*Severo Ochoa*” Fellowship student. In this thesis project a chemical series of DDR1/2 inhibitors was generated that led to the identification of potent compounds at a biochemical and cellular level, and with acceptable selectivity. The results will be protected by a patent application.

HistoneH4-lysine20 N-methyltransferase (SETD8) inhibitors

In collaboration with Óscar Fernández Capetillo (CNIO Genomic Instability Group), the aim of this project, recently internalised in the ETP, 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 has started and will help us to understand their binding mode and to design *de novo* SETD8-is, including intellectual property in their structures.

Finally, we also gave support to the Experimental Oncology Group by the synthesis of reference compounds needed for their projects. ■

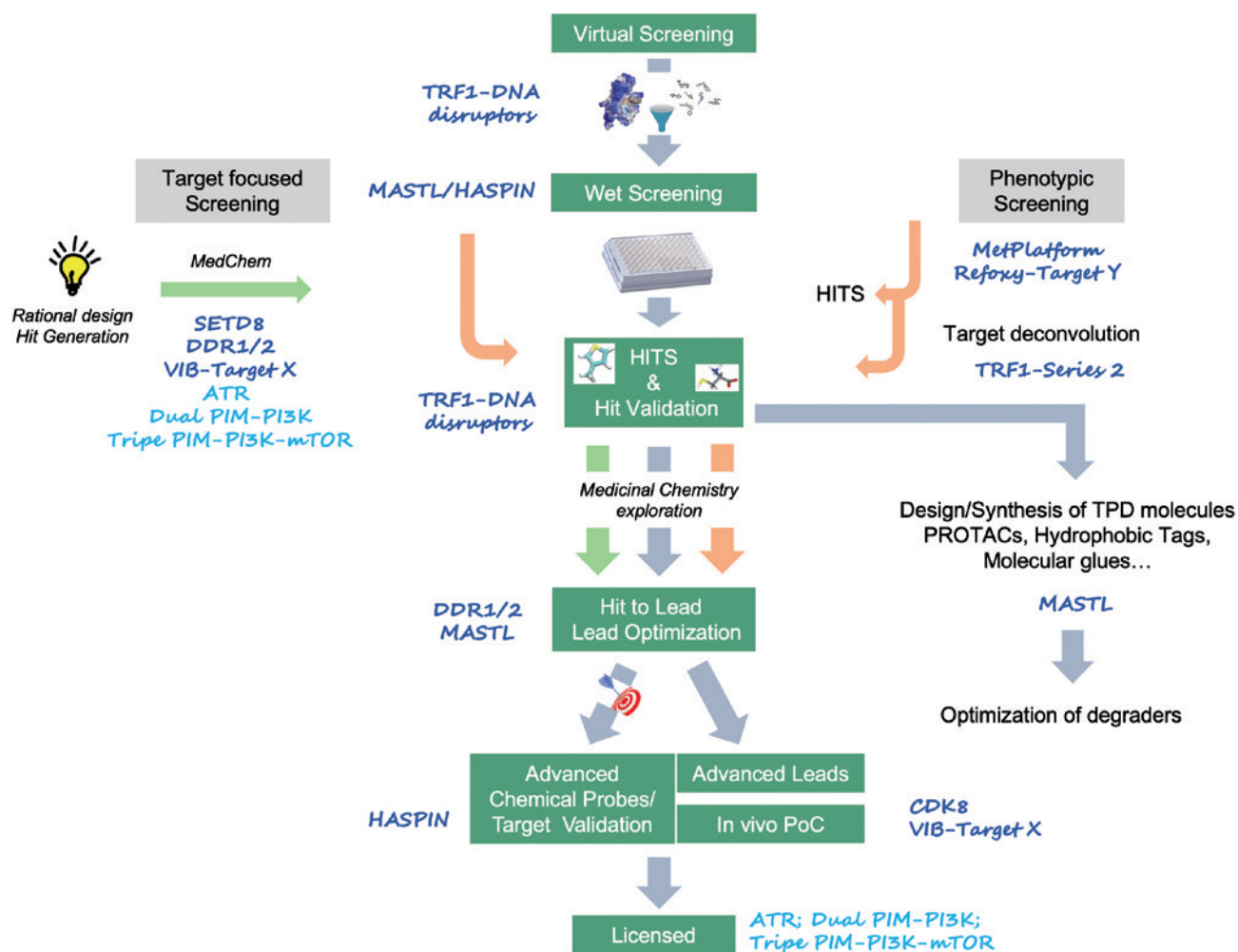


FIGURE Drug Discovery phases for the different projects investigated in ETP. The project is indicated in blue colour, close to its status (dark blue, current projects with activities in ETP; light blue past projects).

PUBLICATIONS

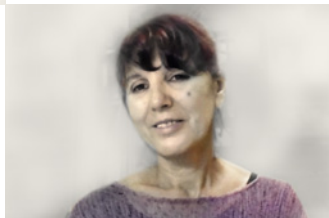
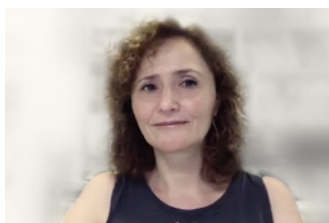
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BIOLOGY SECTION

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

Unfortunately, María was killed in a road accident. May she rest in peace.

Students in Practice
 Marina Obdulia Moreno (March-July) (*Universidad Carlos III*, Madrid, Spain), Antonio Segovia

(February-June) (*Universidad Autónoma de Madrid*, Spain), Juan María Carralero (February-June) (*Universidad Politécnica de Madrid*, Spain), Paula Losada (January-June) (*Universidad Complutense de Madrid*, Spain)

Visiting scientist
 Sonia Lain (until October) (*Karolinska Institutet*, Stockholm, Sweden)

OVERVIEW

Target Engagement assays measure compound binding to a selected target protein inside living cells and enable making correlations with biochemical activity. BRET or bioluminescence resonance energy transfer can be used to study cellular target engagement. NanoBRET assays are based on the fact that NanoLuc luciferase fusion proteins expressed in cells can transfer energy to a proximal fluorophore called tracer. Compound affinity to a given protein can be measured by competitive displacement of the tracer reversibly bound to the corresponding NanoLuc luciferase fusion protein in cells. In the case of PROTACs (Proteolysis Targeting Chimeras), target engagement assays are essential to evaluate the affinity of PROTACs to both the target protein and the E3 ligase, as binding to both is required for the formation of the ternary complex, which triggers the degradation of the target protein. We apply BRET assays routinely in our projects to help in the optimisation of our molecules.

“We successfully established a cellular target engagement assay, based on BRET technology, in our MASTL project; this assay enables us to measure the affinity of compounds for their proteins of interest in cells, as well as to establish correlations with their biochemical activities.”

RESEARCH HIGHLIGHTS

During 2020, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

Preliminary toxicity studies in rats with our lead compound showed a preclinical toxicity compatible with its potential drug development. Next we want to validate its immunotherapeutic potential in *in vitro* models that evaluate the activation of NK cells.

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 150 new compounds, both MASTL-i and MASTL PROTAC-like molecules. We measured MASTL engagement in cells (BRET assay) for the most potent biochemical inhibitors and PROTACs molecules. In the case of PROTACs, we also evaluated their cell affinity against the E3 ligases selected in their design. In addition, we started to characterise the ADME-T properties of our more potent MASTL-is, identifying soluble, permeable, and metabolically stable molecules for further characterisation in *in vivo* pharmacokinetic studies.

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. We continued our efforts to decipher the molecular target/mechanism of action (MoA) of our series 2 of TRF1 inhibitors, represented by hit ETP-946. The results of RNAseq experiments with ETP-946 retrieved a potential MoA of ETP-946. After initial validation experiments with orthogonal assays, we are now carrying out an in-depth characterisation that will be reported accordingly. We are also currently working to identify disruptors of TRF1 binding to ds telomeric DNA. In this direction, wet screening has been performed with the hits identified after virtual screening activities. Now, we are validating these hits applying orthogonal assays against TRF1 and the TelDNA probe with freshly prepared and/or resynthesized samples. We anticipate new screening campaigns using a selected library of ETP compounds that bear a privileged structure to disrupt protein-DNA complexes. Finally, it is worth mentioning that we implemented modifications in our initial TRF1 dimerization assay to avoid interferences with the detection system, which will allow the reduction of the false positive rate in future screening campaigns.

SET domain containing lysine methyltransferase 8 (SETD8)

This project was recently internalised and incorporated into the ETP pipeline, and will be carried out in collaboration with the CNIO Genomic Instability Group. Our main objective is to generate and optimise novel SET8 inhibitors as new therapeutic agents. We set up a biochemical assay that has been validated with previously reported SETD8 inhibitors. Next, we will perform screening campaigns with several ETP-libraries in order to identify new chemotypes that inhibit SETD8, for the development and design of novel SETD8-i with intellectual property.

Collaborations with other CNIO Groups

ETP-Biology provided ongoing support in screening activities performed by the Brain Metastasis Group. We also supported *in vivo* studies of selected compounds and drugs, such as pharmacokinetics, distribution and/or antitumour efficacy, performed by the Microenvironment and Metastasis and the Metabolism and Cell Signalling Groups. Furthermore, we provided analogues of the hits identified in a screening campaign for the validation of PrimPol inhibitors, in collaboration with the DNA Replication Group. Finally, we collaborated with the Experimental Oncology Group, validating the hits obtained after a screen with the ETP-antitumour library to identify novel treatments of mutant KRas NSCLC mouse cell lines that regrow after knocking down mutant KRas.

Collaborations with other institutions

Target X. ETP-Biology performed pharmacokinetic studies of more advanced inhibitors developed against target X in a previous collaboration with *Vlaams Instituut voor Biotechnologie VIB* (the Flanders Institute for Biotechnology). ■

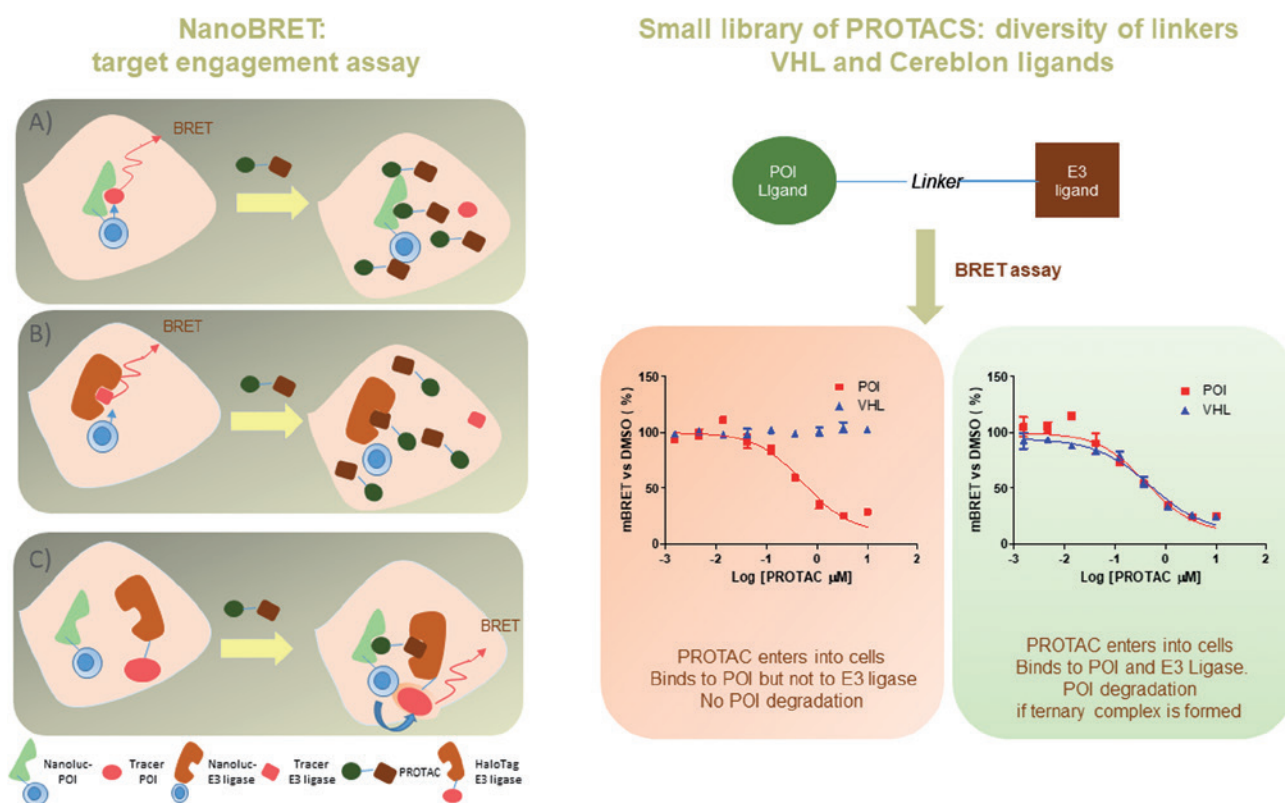


FIGURE Target engagement assays based on BRET technology (Promega). Nanoluc POI or E3 ligase excite a tracer that emits BRET; competition with the tracer by PROTACs will decrease BRET

signal. Ternary complex formation is measured by BRET emission brought about by the proximity of Nanoluc POI and HaloTag E3 ligase mediated by the PROTAC molecule. (A) BRET binding to POI. (B) BRET binding to E3 ligase.

(C) BRET mediated by formation of the ternary complex. These 3 assays can be used to characterise a library of PROTACS to identify those that bind to POI and E3 ligase and select them for ternary complex formation.

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CNIO - LILLY CELL SIGNALLING AND IMMUNOMETABOLISM SECTION

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**Titulado Superior (Advanced Degree)*



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 the identification of small molecular weight molecules that regulate the metabolism of malignant cells, with the objective of killing them either directly, acting synergistically with other antitumour agents, or 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 has been used to obtain a complete understanding of tumour metabolic reprogramming and the antitumour response. For this purpose, we have developed a series of biochemical and cell-based

assays exploiting advanced techniques such as extracellular flux analysis (Seahorse technology), NMR, 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, as well as the immunohistochemical characterisation of tumours for different metabolic, immune and tumour markers. The final step is the validation in human samples from healthy donors or patients using PBMCs or tumour tissue arrays.

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”) signalling 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, known as the Warburg effect. Both oncogenes (Ras, Myc) and tumour suppressor genes (p53, RB, LKB1) impart an altered metabolic phenotype in cancer cells through the regulation of genes involved in central metabolic pathways such as glycolysis, fatty acid metabolism, oxidative phosphorylation, nucleotide synthesis and the one carbon pool (reviewed by Gilmour & Velasco, *SLAS Discov* 2017). All these alterations lead tumours to rely heavily on specific metabolic pathways to obtain their energy, while using other pathways to grow in order to give them a growth advantage. This situation may leave tumour cells in a frail position under certain treatments, while normal cells may be able to compensate, adapt and survive. Our laboratory is searching for this metabolic weakness in order to stop tumour growth.

Furthermore, the high requirements of nutrients and other soluble factors as well as the release of metabolites with immunosuppressive properties, together with the hypoxic conditions found in tumours, create a “non-friendly” microenvironment for an anti-tumour immune surveillance,

while facilitating the growth of other tumour-promoting cells such as stroma and myeloid cells (FIGURE 1A). Thus, the mechanistic understanding of cancer metabolism has led to renewed interest in developing therapeutics that target key enzymes involved in these processes. One such enzyme is Indoleamine 2,3-dioxygenase 1 (IDO1), which catalyses the initial and rate-limiting step of kynurenine synthesis from tryptophan. Tumour cells selectively upregulate IDO1 as an immune evasion mechanism through the synthesis of kynurenine, either through intrinsic expression of IDO1, or in response to IFN- γ . Our laboratory has further characterised the participation of IDO1 as an immune checkpoint by analysing two different aspects of its biology (Cerezo *et al.*, AACR Annual Meeting 2019): i) the metabolomic analysis of tryptophan metabolism using an NMR-based readout approach. This assay allowed us to detect the contribution of tryptophan catabolism to purine synthesis, suggesting further roles of tryptophan catabolism in tumours through its participation in the one carbon pool pathway (FIGURE 1B); and ii) analysing the IDO1 pattern of expression in the tumour microenvironment. It was observed that IDO1 is mostly expressed in a wide range of highly inflamed tumours, the so called “hot tumours”, together with other immune checkpoint targets such as CD73 and PDL-1 (FIGURE 1C). Furthermore, the differential expression of these immune checkpoints in separate spatial compartments of the tumour adds a new level of complexity in the dynamics of the tumour/stroma/immune cell interactions. These findings justify the use of anti-immune checkpoint combination therapy and aid to a more refined patient stratification. ■

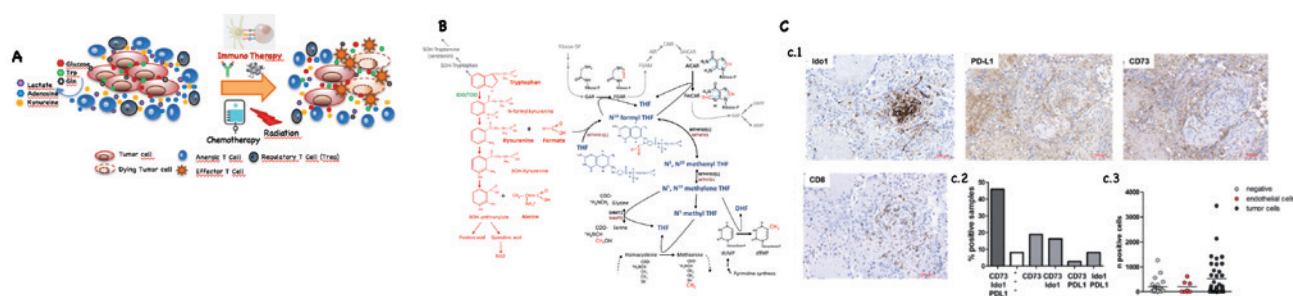


FIGURE 1 Targeting cancer metabolic immune suppression. **(A)** Tumour cells produce a battery of immunosuppressive metabolites such as lactic acid, kynurenine or adenosine that result in an anergic T cell phenotype, while consuming key metabolites such as glucose or tryptophan necessary for a proper Teff (CD8⁺) activity. As a result, T cells are metabolically incapable of mounting an antitumour immune response. Metabolic regulation, together with immunotherapy and other classical therapies (radiation, chemotherapy), would diminish the production of immune suppressive metabolites

and increase the levels of metabolites such as glucose, or the tryptophan necessary for a proper anti-tumour T cell response. **(B)** Tryptophan/one carbon pool metabolism connection. Serine (in black) and tryptophan (in red) metabolism can feed into the one-carbon metabolic pathway to support nucleotide biosynthesis. ¹³C-labeled tryptophan and serine were used to assess the relative contribution of each amino acid to the one carbon pool by NMR. **(C) c.1**-Immunohistochemical analysis of the expression of the immune suppressors IDO, CD73 and PDL1, versus CD8, a marker for Teff, in a panel

of 37 lung tumours. **c.2** and **c.3**- High expression of these immune checkpoints correlates with more inflamed tumours, “hot tumours”. They tend to be co-expressed in different areas of the same tumour, showing a complementary pattern of expression. There are also differences in tissue distribution, while in some tumours they are homogeneously expressed in all the tissue compartments, in others they are preferentially expressed either in the tumour or in stromal or endothelial cells.

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

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Technology Transfer Manager
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**Plan de Empleo Joven (Youth Employment Plan)*



“Our office is strongly committed to translating new discoveries in cancer research into outputs for the benefit of patients and the health care system. To this end, we identify, protect, and develop projects with commercial potential, always with the mindset of co-developing them with private and public entities to increase the value of potential products.”

At the CNIO, the best science and research efforts join in the desire to make a great impact for cancer patients and the health care system. The Technology Transfer and Valorisation Office (TTVO) contributes to this purpose by ensuring appropriate protection of intellectual property and by channelling the technologies that arise from our research to companies and entrepreneurs to develop them further and thereby impact society.

The TTVO proactively monitors the progress of the CNIO’s scientific activity to identify projects with high transfer potential. In 2020, 8 new ideas were incorporated into the technology transfer portfolio, of which 1 turned into a priority patent application, and 7 will become priority patent applications in 2021. These cover a wide range of products, including a monoclonal therapeutic antibody, drug inhibitors, new biomarkers, a cell therapy, a therapeutic vector for gene therapy and a SARS-CoV-2 diagnostic kit.

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 34 patent families, and provides advice and assistance during the drafting of the patent document, the filing, and the prosecution process. One PCT (Patent Cooperation Treaty) application for international extension was filed in 2020, and 1 patent with proven commercial interest entered the national phase. Licensed patents make up a remarkable 41% of the CNIO portfolio. Among those signed in 2020, was a license agreement with the newly created spin-off company Telomere Therapeutics, in which both the CNIO and the *Universitat Autònoma de Barcelona (UAB)* are partners, encouraging technology transfer through academic spin-offs. This new company, which received investment from InVivo Capital Partners, will develop gene therapy technology with telomerase to treat pulmonary and kidney fibrosis. The CNIO and UAB licensed 2 patent applications in co-ownership, and 1 more patent will be submitted in 2021. These patents protect the results obtained by the laboratory of Maria A. Blasco (CNIO Telomeres and Telomerase Group) and by the research group of Fátima Bosch (Animal Genetic Engineering Laboratory, UAB).

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 and coordination of this phase, and advises 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. Besides the 3 CNIO projects ongoing since 2018, a new project was awarded a *CaixaImpulse* CONSOLIDATE COVID-19 grant in 2020 and thereby benefits from funding and mentoring by experts of the national bio-ecosystem. This project, co-led by Felipe Cortés Ledesma (CNIO Topology and DNA Breaks Group) and Luis Blanco Dávila (*CBMSO-CSIC*), aims to develop a novel detection kit for the simple and rapid diagnosis of SARS-CoV-2 infection. It is also worth mentioning that an ERC Proof of Concept Grant was awarded to Óscar Fernández-Capetillo (CNIO Genomic Instability Group) in 2020 to develop his TARGETSET research project. This project aims to advance the development of novel SETD8 inhibitors — linked to the development of some childhood tumours associated with a poor prognosis — and to study the preclinical potential of a compound that could help treat these tumours. In addition, 3 projects selected in pharmaceutical industry calls — *Farmaindustria*, Merck KGaA Research Grants, and Pfizer Call for Proposals — are being evaluated, and 1 project is currently under evaluation by a venture capital firm.

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 technology transfer agreements, and manages the relationship with licensees, including the payment of royalty fees. In 2020, the TTVO managed 261 agreements (MTAs, CDAs, research collaborations, licenses, etc.). Among these industrial partnerships is worth noting a research contract between the CNIO, *CRIS contra el cáncer*, and Bionam Biotech for a pancreatic cancer project led by Mariano Barbacid (CNIO Experimental Oncology Group). Other partnerships include the collaboration of the CNIO Experimental Therapeutics Programme, led by Joaquín Pastor, with Foxy Pharma and *CSIC*, to develop FOXO target triggers in cancer models and ageing-related diseases.

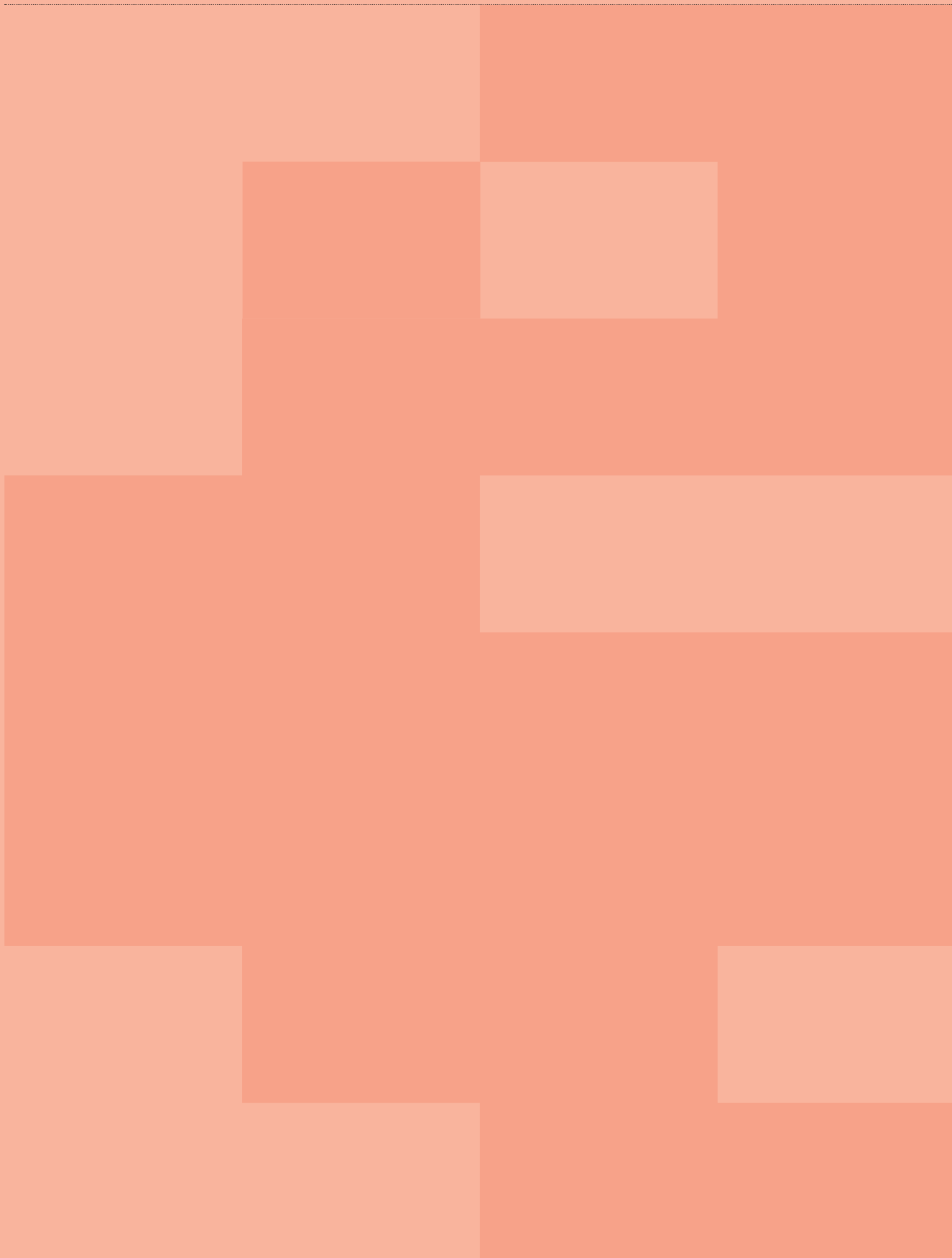
Most of these agreements (60%) were established with international entities, which is an indicator of the internationalisation of the CNIO's research activity. Through collaborations with industry, up to €2.4 million were secured for research activities. Moreover, 4% of the agreements are licences to commercial partners. Patents and unpatented research tools are licensed 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 2020 increased to €728,000. This income reverts 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 100 monoclonal antibodies.

In February 2020, TTVO jointly with the *Instituto de Salud Carlos III* participated in *Transfiere*, the European Meeting on Science, Technology, and Innovation. Then, the Covid-19 pandemic had an extraordinary impact on events promoting technology transfer and partnerships, many of which, such as BIO2020 (USA) or BioSpain, were scheduled in 2021 in a virtual format. In these circumstances, we decided to seize the opportunity offered by the increase in virtual meetings and organised a training seminar on the protection of IP in bioinformatics, AI, and big data, for nearly 50 CNIO researchers, in collaboration with the intellectual property firm Mewburn Ellis. 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 *Instituto de Empresa* business school that will be developed in 2021 with the support of *Banco Santander* Foundation.

All the above-mentioned achievements stand testament to the excellence and hard work of CNIO scientists and to the 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)*

**Titulado Superior* (Advanced Degree)



OVERVIEW

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

“CNIO Biobank is heading towards modern, dynamic biobanking, facilitating the use of quality human samples for biomedical research, while protecting donors' rights and contributing to CNIO's research excellence.”

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).

RESEARCH HIGHLIGHTS

In 2020, CNIO Biobank incorporated 6,500 cases to enlarge several existing collections (3,353 lymphoid cases, 507 gynaecologic cases, 955 digestive cases, 1220 cases in the basic collection, 545 non neoplastic cases) and acquired 2 new collections: COVID-19 collection (272 cases) and PDX collection (219 cases). Tissue samples were shared from 730 cases to support 12 research projects.

The CNIO Biobank's Virtual Catalogue includes 7,563 images; the tissue micro-array (TMA) catalogue holds 288 images, 156 corresponding to histological H&E stains and the rest IHQ images.

The knowledge generation impact related to activity in 2020 resulted in 7 (Q1) publications acknowledging CNIO's Biobank contribution, with a mean impact factor (IF) of 9.044.

CNIO Biobank also managed 3 project-derived collections, offering its services of custody and storage that provide traceability and GDPR compliance, and collaborated with the Familial Cancer Unit in requesting 19 new cases for genetic studies.

CNIO Biobank supported 12 CNIO project submissions for ethical evaluation by the *Instituto de Salud Carlos III (ISCIII)* Research Ethics Committee. This year, we also opened a new information inquiry option (<https://apps.cnio.es/app/Biobanco/Asesoria/Consulta>) accessible to researchers or even the wider community, located on the Biobank-CNIO website, to address any questions regarding biobanking.

CNIO Biobank is participating in the *Plataforma de Apoyo a la Investigación en biobancos* project, corresponding to the *Acción Estratégica en Salud 2017-2020*, promoted by the *ISCIII*. CNIO Biobank is also leading the Optimark (PI16/00946) research project focused on identifying quality markers for tissue samples sensitive to pre-analytical variables.

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*, to name a few.

CNIO Biobank in collaboration with Lund University (Sweden), University of Copenhagen (Denmark) and the Danish National Biobank organised a PhD course, "The Future of Biobanking", and a symposium entitled "Scaling Omics Approaches to Population Size" held in Copenhagen and Lund in November 2020.

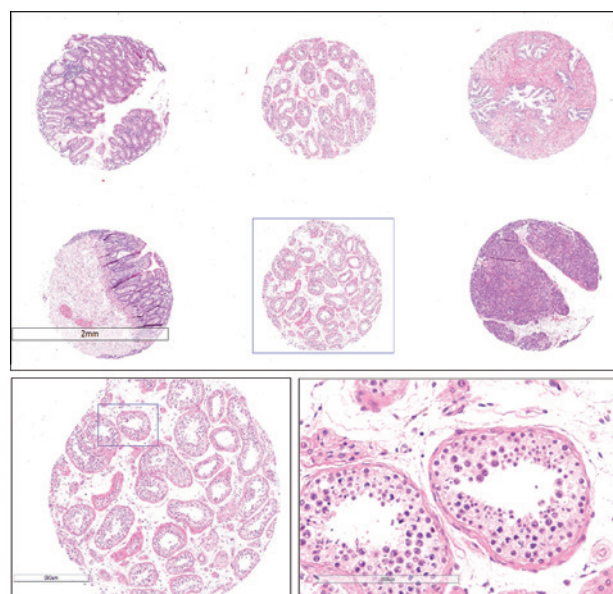


FIGURE H&E staining images of a TMA built at CNIO Biobank containing non-tumour tissue from different anatomic locations. Different magnifications are shown: 2x (upper panel), 5x (lower left), 20x (lower right). Blue squares indicate the area amplified in the next panel.

Communication

Communications

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COMMUNICATIONS

NURIA NORIEGA

SUSANA PÉREZ DE PABLOS (since July)

Head of Communications

Science Communication
and Social Media
Vanessa Pombo



**“In a year marked by
Covid-19 in the media,
CNIO’s cancer research hit
the headlines.”**



In a year marked by the coronavirus pandemic in the media, CNIO's Communications Department continuously reached out to patients, family members and society at large, sharing news of the Centre's research activity and hitting the headlines with its stories.

The trend in media coverage of CNIO's activities and papers was optimal: over 3,400 appearances in online and print newspapers and over 250 mentions on TV and radio.

In addition to specialist media — *Diario Médico*, *Gaceta Médica*, SINC News Agency —, the CNIO also attracted great interest from the top Spanish newspapers — *El País*, *El Mundo*, *ABC*, *El Español*, *La Razón* —, radio stations — *RNE*, *Cadena Ser*, *Onda Cero*, *Radio 5*, *COPE*, *Capital Radio* — and TV channels — *TVE*, *La 2*, *La Sexta*, *Antena 3*, *Telecinco*, *Canal 24 Horas* —, as well as online newspapers — *Público*, *elDiario.es*, *El Imparcial* —, general interest and popular magazines and radio stations — *Muy Interesante*, *Cadena 100*, *Esquire*, *Hola*, *Jot Down*, *Cadena Dial*, *Elle* — as well as websites from the USA, the UK, France, Mexico, Cuba and Uruguay, among others.

The new developments that received broadest coverage were the following: the combination of 2 drugs for the effective treatment of malignant mesothelioma in mice (Francisco X. Real, *Cancer Research*); the finding that type 3c diabetes can be an early sign of pancreatic cancer (Núria Malats, *Gut*); the development of a simple, fast technology for more effective stem cell therapies in regenerative medicine (Óscar Fernández-Capetillo, *The EMBO Journal*); the new path to future gene therapy for age-related pulmonary fibrosis (Maria A. Blasco, *Journal of Cell Biology*); cell reprogramming with CRISPR gene editing to eliminate tumour cells without affecting normal cells (Sandra Rodríguez-Perales, *Nature Communications*); and the description of the molecular mechanism underlying melanoma and how it “tricks” the immune system into playing a role in its development (Marisol Soengas, *Nature Medicine*). Furthermore, Covid-19-related research at CNIO was covered by leading media channels, such as *TVE* (*Comando Actualidad*), *El Mundo*, *La Sexta* (*Al rojo vivo*, *El objetivo de Ana Pastor*) and the Beijing-based news channel CGTN in Spain.

Moreover, the #CNIOStopCancer campaign, carried out by CNIO Friends in February to honour World Cancer Day, was advertised on *Telemadrid*, *Onda Cero* (*Julia en la Onda*), *COPE* (*Herrera en COPE*), *RNE* (*24 Horas*, *Las mañanas de RNE con*

Íñigo Alfonso), *El Mundo*, *ABC* and *La Razón*. It also reached other media where the CNIO is not mentioned very often, including *Hola*, *Elle*, *Pronto* and *20 Minutos*.

Other projects also contributed to bringing CNIO's activity to the general interest media in 2020. CNIO Arte, for instance, brought together paleoanthropologist Juan Luis Arsuaga and artist Carmen Calvo. Now in its third edition, this project, organised with the help of *Fundación Banco Santander*, explores the common ground of scientific research and artistic creation. The works created by Calvo based on Arsuaga's findings and ideas were shown in *La 2* (*Atención, obras!*), *Canal 24 Horas* (*La hora cultural*, *La noche en 24 horas*), *TVE* (*Los desayunos de TVE*), National Geographic, *RNE* (*Por tres razones*) and *Radio 3* (*Hoy empieza todo*).

As we have done for the past few years, in 2020 we wanted to celebrate World Cancer Research Day on September 24 as well. Our fourth annual conference, organised with the support of “la Caixa” Foundation and held online only in 2020, was entitled “A New Era of Cancer Research: Towards the Engagement of the Entire Society”. It featured Francis Mojica as keynote speaker. Dr Mojica is a professor at the University of Alicante, known for his discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), which later developed into the first widespread genome editing tool. His presentation, “Solving Conundrums, Fighting Ailments, Having Fun with CRISPR”, was followed by a panel discussion on the challenges of cancer research and the need for society to get involved to face those challenges. The panel featured Maria A. Blasco, CNIO Director and Head of the CNIO Telomeres and Telomerase Group; Luis Gonzaga Paz-Ares, Head of the H12O-CNIO Lung Cancer Clinical Research Unit and Head of the Medical Oncology Service at *12 de Octubre* University Hospital; Sandra Rodríguez-Perales, Head of the CNIO Molecular Cytogenetics Unit; and María Luisa Villafranca, breast cancer patient and President of the *ROSAE* Breast Cancer Association, a CNIO Friend.

Finally, in 2020 CNIO sealed its partnership with L'Oréal Group Spain to launch the #ResearchIsLife campaign with La Roche-Posay on World Cancer Day in February. For 2 weeks, CNIO Friends was present in pharmacies all over the country. In addition, there was advertising in social media and emailing to clients, consumers, and the media, in an effort to engage new supporters for cancer research at the CNIO.

PRESS CLIPPINGS

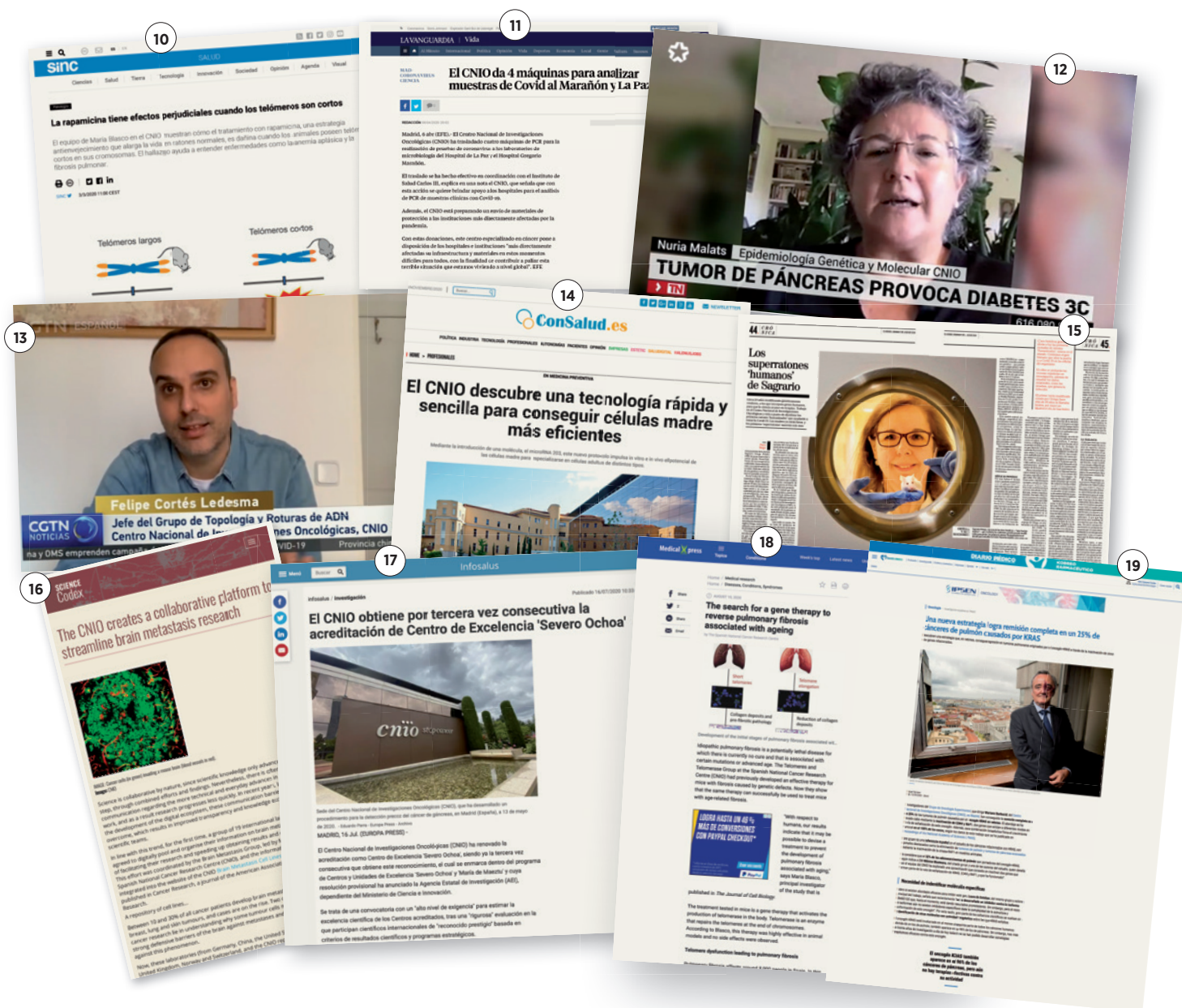


1 *Diario de León*, January 14, 2020
2 *Telediario 2*, TVE, January 15, 2020

3 *El País Semanal*, January 26, 2020
4 *Gaceta Médica*, January 27, 2020

5 *La aventura del saber*, La 2, February 4, 2020
6 *Madrid Directo*, Telemadrid, February 4, 2020

7 *El País*, February 22, 2020
8 *La hora cultural*, Canal 24 Horas, February 27, 2020
9 *ABC*, February 27, 2020

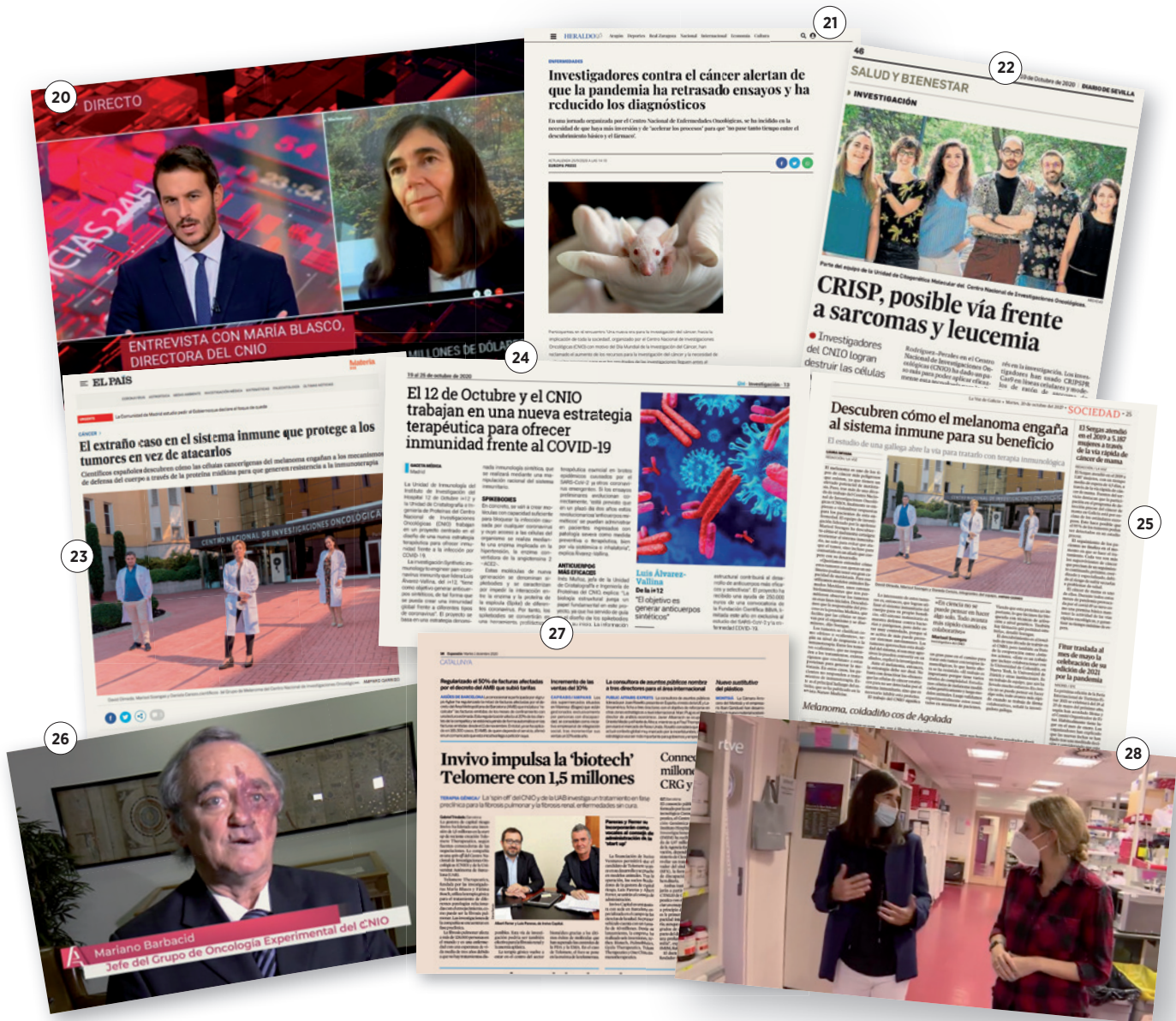


- 10 Agencia SINC, March 3, 2020
 11 La Vanguardia, April 6, 2020
 12 Telenoticias 1, Telemadrid, May 13, 2020

- 13 CGTN Noticias, CGTN Español, May 20, 2020
 14 Con Salud, July 2, 2020

- 15 Crónica El Mundo, July 5, 2020
 16 Science Codex, July 8, 2020
 17 Infosalus, July 16, 2020

- 18 MedicalXpress, August 10, 2020
 19 Diario Médico, September 12, 2020



20 La tarde en 24 horas, Canal 24 Horas, September 24, 2020

21 Heraldo de Aragón, September 25, 2020

22 Diario de Sevilla, October 10, 2020

23 El País, October 19, 2020

24 Gaceta Médica, October 19, 2020

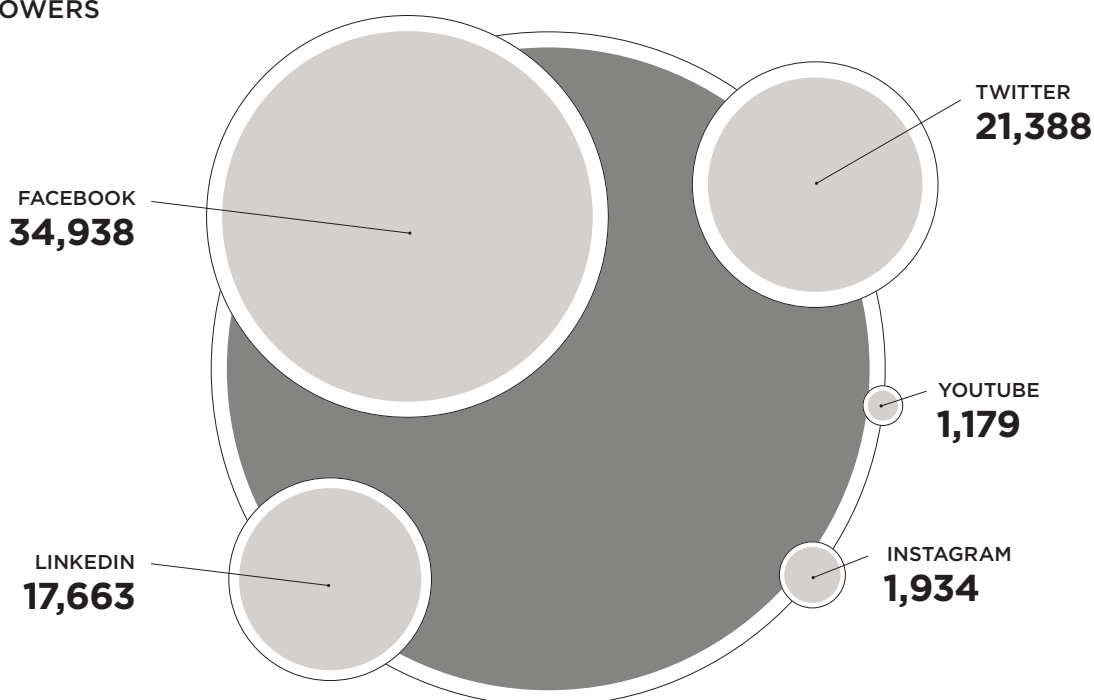
25 La Voz de Galicia, October 20, 2020

26 Audiencia Abierta, TVE, November 14, 2020

27 Expansión, December 1, 2020

28 Comando Actualidad, TVE, December 9, 2020

2020 SOCIAL NETWORK DATA FOLLOWERS



LINKEDIN

IMPRESSIONS	882,131
INTERACTIONS	39,232
LINK CLICKS	22,581
INTERACTION RATE (AVERAGE)	3.87%

YOUTUBE

UPLOADED VIDEOS (TOTAL)	117
UPLOADED IN 2020	14
VIEWS IN 2020	44,531
WATCH TIME (IN HOURS)	5,315
AVERAGE VIEW DURATION (IN MINUTES)	7:09

TWITTER CNIO

LIKES	11,993
RETWEETS	4,180
IMPRESSIONS	3,106,900
MENTIONS	5,809
VISITS TO THE PROFILE	45,333

FACEBOOK

IMPRESSIONS	2,130,887
REACH	1,911,143
LINK CLICKS	33,514
INTERACTION (REACTIONS, COMMENTS, SHARES)	46,821

The engagement of our followers on CNIO's social media channels is a good thermometer of our most impactful research news for society. In 2020, some of the highlighted topics – among many others – were science news, such as a new strategy to prevent the most aggressive tumours from generating resistance to chemotherapy (Marcos Malumbres, *Cancer Cell*, February); the confirmation that diabetes type 3c is an early manifestation of pancreatic cancer (Núria Malats, *Gut*, May); or the discovery of how melanoma deceives the immune system, increasing resistance to immunotherapy (Marisol

Soengas, *Nature Medicine*, October). Other relevant news included items such as Maria A. Blasco and Marisol Soengas each being awarded an ERC Advanced Grant to tackle the immortality of cancer cells and metastasis; Óscar Fernández-Capetillo receiving an ERC Proof of Concept Grant to study a new treatment for childhood cancer; the launch of the spin-off *Telomere Therapeutics* by Maria A. Blasco (CNIO) and Fàtima Bosch (UAB); the recruitment of Geoff Macintyre and María Casanova-Acebes... and, of course, the renewal of our accreditation as “*Severo Ochoa*” Centre of Excellence.

SOCIAL EVENTS



The Minister of Science and Innovation, Pedro Duque, and the Director of the National Institute of Health *Carlos III* (ISCIII), Raquel Yotti, visited the CNIO facilities to get first-hand information on several projects being carried out to study Covid-19. Maria A. Blasco outlined the progress of these projects to the Minister and, in addition, informed him about the CNIO's working policies during the lockdown. *May 4, 2020.*



José Luis Rodríguez Zapatero, former President of the Spanish Government, participated in the seminar series of the CNIO Office for Women in Science (WISE). Under the title "Science and Politics" of his online talk, he called for the creation of a high-level commission to propose projects to be funded by European recovery funds because of the effects of the Covid-19 pandemic. To the vision of science to improve life expectancy and health, the former president wanted to add the role that science plays as an element of coexistence based on principles and values. He thus stressed that the 21st century must be decisive in favour of gender equality. *September 15, 2020.*



With the support of "la Caixa" Foundation, we celebrated World Cancer Research Day with an online event entitled "A new era of cancer research: towards the engagement of the entire society". Francis Mojica, Professor at the University of Alicante, gave the keynote speech "Solving enigmas, combatting disease, delighting in CRISPR". After his talk, a roundtable discussion was held with the participation of Maria A. Blasco, Luis Paz-Ares, Sandra Rodríguez-Perales, and María Luisa Villafraña (Rosae Association, CNIO Friends). *September 24, 2020.*



Do we really understand cancer in terms of the molecular processes underlying it? Do we know how a patient's socio-environmental factors affect this understanding? Four scientists (Maria A. Blasco, Luis Paz-Ares, Miquel Porta, and Fernando Valladares) and five philosophers (Arantza Etxeberria, Antonio Diéguez, Marta Bertolaso, Anna Estany, and Elena Casetta) participated in a debate on this question in the online "Workshop on Philosophy, Science and Medicine: socio-environmental factors of health and disease" organised by the CNIO with the support of the *Banco Sabadell* Foundation. November 24, 2020.



CNIO participated, for the eighth 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 and nor that of the 280 attendees, who enjoyed doing a scientific experiment in their homes. November 27, 2020

International Affairs



INTERNATIONAL AFFAIRS

CAROLINA POLA Director of International Affairs (until November)



The current pandemic will be remembered as the event that put global travel on hold. Whereas this has undoubtedly created new barriers for international activities, the limitations have been overcome by the birth of new opportunities and innovative means of collaborations. The International Affairs (IAs) department has leveraged and joined the efforts of partners around the world to maximise the opportunities for CNIO to continue its activities in the international space. During 2020, we participated remotely in more than 10 events organised by both European bodies and national coordinators of cancer and science missions. Some of them include roundtables and meetings organised by the UK Embassy on Innovation and EC policy groups in Europe Unite against Cancer. This strategic participation in discussions regarding the future of the Cancer Mission and a deep understanding of the upcoming topics and calls is at the core of IAs aim to maximise CNIO's participation in the future Horizon Europe.

In 2020, we actively participated in the ERC Proof of Concept (PoC) call, successfully obtaining 1 grant to develop novel inhibitors for cancer. We also contributed to the submission of 5 Future and Emerging Technologies (FET) open proposals in collaboration with multiple partners in international consortia. These efforts help us prepare the ground for future collaborations, which we hope will bear fruit in upcoming calls.

Our participation in and commitment to the *Severo Ochoa* and *Maria de Maetzu* Alliance (SOMMa) continued and, starting in 2021, the CNIO will hold the Presidency of the Alliance to keep bringing the mission and values of SOMMa to the different

“Maintaining our international impact and reach remains a priority, despite the hurdles and challenges faced in 2020.”

stakeholders in the science and innovation ecosystem. Now more than ever, science is playing a central part in the life of society and, as an Alliance, we will continue working to improve the path for science and technology as a driver of sustainable and innovative growth for Spain and the world.

2020 also marked the second call for collaborative projects in our tripartite alliance with the *Ramon Areces* Foundation and the Weizmann Institute of Science. The large numbers of proposals, which grew by 20% compared to the last call, is a clear indicator of the enthusiasm of researchers for such international initiatives. In 2021 we will launch our third joint symposium and continue nurturing a fruitful global collaboration.

As a reference for science in Spain, the CNIO will strive to maintain its position abroad and harness its capabilities to catapult into being new ways of collaboration, both with public actors in the framework of Horizon Europe, and with private entities to enable public-private collaborations.

Institutional Image & Outreach to Society



INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY

AMPARO GARRIDO Coordinator

Science Outreach Officer
Bárbara González (until December)
(TS)*

**Titulado Superior (Advanced Degree)*



“It’s a rewarding experience for sure, curating works of art for the CNIO. You get to explore deep inside that common ground where Scientist and Artist act and interact, as you acquire greater first-person familiarity with their disciplines. That insight is what seeps through my senses as it reaches inward toward the deepest recesses of the heart.”



In 2020, CNIO held the third annual CNIO Arte, curated for the second year in a row by visual artist Amparo Garrido, who also serves as Coordinator of Institutional Image and Outreach to Society at the Centre. This initiative was once again supported by the *Banco Santander* Foundation.

The 2020 event featured artist Carmen Calvo, who was awarded Spain's National Prize for Visual Arts in 2013 and, in 2014, was elected Academic Member of the San Carlos Royal Academy of Fine Arts in Valencia. Her outstanding work can be seen today in many of the world's leading museums and collections. Calvo was paired off with Juan Luis Arsuaga, professor of Palaeontology at the Complutense University of Madrid, who also serves as

Scientific Director of the Museum for Human Evolution and has been named laureate of the Prince of Asturias Award for Technical and Scientific Research, as part of the Atapuerca research team. Following this encounter, the artist created 4 works that were exhibited to the public at CNIO on February 20th and then presented at the Madrid International Contemporary Art Fair, *ARCO*, hosted at the IFEMA grounds. All sales proceeds were allocated in full to funding cancer research projects via the "CNIO Friends" philanthropic initiative.

In 2020, the first Art and Science Symposium was held to promote CNIO Arte, with the aim of expanding and deepening dialogue between the arts and science. It also brought together renowned



CNIO Arte 2020 exhibition. In this image, from left to right: visual artist Carmen Calvo and palaeontologist Juan Luis Arsuaga. Photo: copyright of Antonio Tabernero.



experts to inspire collective reflection on the world in which we live. Carrying forward the dialogue established between Carmen Calvo and Juan Luis Arsuaga, the theme of this first Symposium focused on the connections between art and archaeology. The Symposium was led by Carlos Jiménez, Professor Emeritus of Aesthetics, historian, architect, writer, and art critic. Jiménez was joined by Fernando Castro Flórez and Aurora Fernández Polanco, and together these 3 art world experts, accompanied by artist duo Bleda & Rosa, shared their views on the Symposium's theme in an event that was open to the public.

CNIO Arte received positive media coverage, as was the case in previous years, such as in 2018 when pioneering molecular biologist Margarita Salas was brought together with visual artist Eva Lootz, or the 2019 tandem formed by Chema Madoz, laureate of the Spanish National Photography Award for the year 2000, and Juan Ignacio Cirac, Director of the Max Planck Institute of Quantum Optics.

In 2020, the Institutional Image and Outreach to Society Office directed and produced a series of campaign videos to support our outreach actions for World Cancer Research Day, featuring artists and actors Luz Casal, Christina Rosenvinge, María Hervás, and Enrique Arce. Another accomplishment was our livestream of the European Researchers' Night, including a set of videos produced to support this otherwise live event. Despite taking place via Zoom, the evening ended up being a great success.

Because of the COVID-19 pandemic, a new task for this Office was to find post-production solutions to multiple CNIO events that inevitably had to be hosted via Zoom. This ensured that the resulting videos, which were subsequently uploaded to our website, were of good quality and attractive to potential viewers. Furthermore, we also spent a lot of time in 2020 updating the content of our organisation's website (www.cnio.es), including newly produced images and infographics as well as further information about the Centre and its activities. Our office worked together closely with other CNIO departments to help familiarise them with our new corporate identity handbook.

We were also responsible for designing and printing this Annual Report, as well as other outreach institutional publications.

This area involves the creation and adaptation of research dissemination materials, the production of flyers and merchandise in accordance with our current corporate identity, and launching new outreach initiatives around science, art, and culture. Other outreach events in which we collaborated and continued to support include Science Week and CNIO Open Doors Day. All of the aforementioned work builds on one of CNIO's key strategic pillars, namely to widen the Centre's reach and impact on society and, in doing so, to encourage philanthropic financial contributions to the institution.

Lastly, our Office also participated in other cultural and outreach initiatives that aim to increase CNIO's global visibility, such as the *Diverciencia* fair with its 14th year of International Online Science Day, where CNIO was present with a virtual stand featuring the Centre and its activities; and the science outreach project CNIO & The City. Due to the exceptional circumstances caused by the COVID-19 pandemic, such events took place online. CNIO & The City in 2020 could count on the strong involvement of CNIO researchers sharing their knowledge with more than 300 students and 20 teachers from 14 centres.

Carmen Calvo and Juan Luis Arsuaga at the excavations in the Valley of the Neanderthals archaeological site (Pinilla del Valle, Madrid) / Photograph by Amparo Garrido.

1st Art and Science Symposium, CNIO Arte 2020. In this image, from left to right: Carlos Jiménez, Amparo Garrido, María Bleda, Aurora Fernández Polanco, José María Rosa, and Fernando Castro Flórez. Photograph by Antonio Tabernero.

Development & Philanthropy



DEVELOPMENT & PHILANTHROPY

JESSICA J. ROSE Director

Development & Philanthropy Officer
Mercedes Antona



“Philanthropy gives individuals the power to make a difference. 100% of our donations fund the salaries of outstanding researchers – which means that every single donation directly helps us stop cancer.”



Philanthropic fundraising could still be considered in its infancy in Spain – especially compared to the industry in other parts of the world. The opportunity to develop a new and innovative fundraising function within a globally established centre of excellence such as the CNIO is rare, and we have been excited to grow this exciting new function for the Centre.

The CNIO has established itself as a world-class cancer research institute and benefits from recognition by the scientific community and the public as a leader in improving health outcomes. The institute's reputation has significant potential to attract philanthropic gifts as already demonstrated by the “Friends of the CNIO” crowdfunding programme. This reputation, combined with the opportunity to develop a structured new major gifts programme, uniquely positions the institute to be a world leader, not just in cancer research but in fundraising.

The CNIO has successfully attracted gifts to the CNIO Friends Programme (*Amigos del CNIO*) for a number of years now. The addition of a dedicated philanthropy office for the Centre has enabled us to take a strategic and proactive approach to securing funds for cancer research. This approach involves cultivating new prospects, and working with companies, foundations and associations to develop new collaborative partnerships in an effort to secure major gifts (€100k+) for the CNIO. In addition, we work with our colleagues in the Institutional Image & Outreach to Society team and the Communications team 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 was a success from the start, and has been growing each year to reach over €2.2 million in total donations thus far. 100% of these donations have been channelled into funding to recruit excellent scientists, opening new lines of research across the CNIO. These contributions have thus far enabled the CNIO to close 20 new 2-year research contracts. In 2020 alone, CNIO Friends raised €813k, which will be used to engage more scientific talent in 2021. In addition, the Office is growing the legacy programme, which has thus far received a cumulative total of €1.2 million since 2015.

In 2020, CNIO received charitable bequests of €258k, with €527k pending to be executed.

The Philanthropy Office was established to optimise the efforts to identify and cultivate new donor relationships, in addition to continuing to recognise and thank our existing supporters. In support of this goal, we were delighted to welcome 4 new CNIO Ambassadors from the entertainment industry, who joined the CNIO Stop Cancer campaign and kindly participated in our video campaign to invite new Friends to support our cancer research.

2020 has been an interesting year for philanthropic organisations and fundraising in general. For the CNIO, the year provided a robust funding total, however the potential effects of COVID-19 on society and on the economy may well be felt more keenly in 2021. Despite the challenges, the Philanthropy Office is optimistic about the future. The CNIO provides a wonderful partnership opportunity for organisations and individuals who feel passionate about working together to stop cancer. We look forward to working together with industry and society to enable the CNIO to continue to be one of the best cancer research institutions in the world.

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DEAN'S OFFICE

María S. Soengas
Dean for Academic Affairs

PARTICIPANTS
Personnel in training:
Daniela Cerezo, Marta Contreras, Ana Cuadrado, Nicolás Cuesta, Adrián del Rincón, David Olmeda, Cristina Tejedo, Fernando Pozo



One of the key missions of the CNIO is to ensure the most productive scientific environment for our personnel in training. This is a main commitment of our faculty, since over 60% of the work force at our institution are undergraduate students, predoctoral and postdoctoral fellows, medical residents and a broad spectrum of visiting scientists. We also have a variety of exchange and visitor programmes. In this context, we are most grateful to the *Fundación Jesús Serra*, for their continuous support to strengthen career development programmes at the CNIO.

As for other research centres, and as is the case for the population at large, the COVID-19 pandemic has represented

a challenge for the CNIO. We had to set safety measures, reorganise our teams and prioritise efforts but, still, our commitment to our investigators remained undeterred. After a short break, our weekly Progress Report seminars by students and postdocs proceeded in a virtual format, allocating time and resources for formal feedback sessions afterwards. Distinguished speaker seminars were also reinstated, so CNIO personnel could attend in a remote manner. We recognised, however, that this SARS-CoV2 situation took a personal toll. Therefore, we organised a Meet-the-Expert online session with a professional coach precisely on the topic of “Recognising and overcoming stress at the workplace”.

Staff/Faculty:

Nabil Djouder, Rafael Fernández-Leiro, Eva González-Suárez, Óscar Llorca, Ana Losada, Lola Martínez, Jorge L. Martínez, Francisca Mulero, Héctor Peinado, Manuel Valiente

We also kept our outreach activities active, one of the most exciting being the *Marie Skłodowska Curie European Researchers' Night*. This was our seventh participation in an event happening in various countries in Europe and in multiple centres in Spain. Our contribution at the CNIO was “*Meet a Scientist, Become a Scientist*”, this time in a virtual format. The event was a tour-de-force from an organisational perspective, but truly rewarding. In brief, we set up a “research kit” with informative material that was mailed to over 250 registered guests so they could perform an experiment in their own homes, following and talking live with our scientists. It was a wonderful experience that we will certainly repeat in the future.

Another highlight of the year was our Annual CNIO Lab Day, in 2020 celebrating its 10th anniversary. This also took place online, but was quite special. An online ePoster session was set up, whereby a record high of over 85 communications were discussed among CNIO members so that they could showcase their projects and get feedback. Some of the “best-to-come” in each of the scientific programmes of the Centre were selected for video presentations and also for oral discussion. Importantly, we wanted to celebrate this Lab Day with CNIO alumni. We were fortunate (and proud) of seeing how Marta Shahbazi, co-founder of the CNIO PhD Students Association, and Donatello Castellana, leader of CNIO-Postdocs, have progressed to very productive careers at the University of Cambridge (UK) and at the CIC bioGUNE, respectively. Similarly exciting was to hear from Cristina Mayor (trained in Oscar Fernández-Capetillo's Group), on the quite successful projects she is developing in her new laboratory at the Institute for Research in Biomedicine (IRB, Barcelona). We were also inspired by Damià Tormo, trained in the Melanoma Group in our Centre, seeing how he has set up multiple and highly successful start-up companies and a hedge fund, as well as the not-for-profit Columbus Foundation (USA) in support of children with rare diseases. Not less impressive was to learn from Ignacio Dolado, once at the laboratory of ex-CNIO investigator Ángel Nebreda, and now leading Global Medical Oncology programmes at Roche (Switzerland).

A main highlight of Lab Day was the announcement of the recipients of our “*Director's List Awards*”. These are recognitions for outstanding contributions made by our personnel in 3 categories: (1) predoctoral fellows with publications of the highest scientific impact; (2) excellence in research by postdoctoral and staff investigators; and (3) altruistic volunteering to further the mission of the Centre related to training, scientific divulgation, and outreach.

1. Awards to 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 2020 the “*Antonia Nieto Award*” went to Daniela Cerezo for an impressive work in *Nature Medicine* on new discoveries on the modulation of the immune system in cancer. Additional awards in the PhD category went to Marta Martínez Lage (*Nat Commun*), Isabel Ferrara-Romeo (*Nat Commun*), Laura de Esteban Burgos (*Proc Natl Acad Sci USA*), and María Santos (*Genet Med*).

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

The awardee was Bárbara Oldrini for exciting new findings in acquired resistance to chemotherapy in gliomas (*Nature Communications*).

3. Outstanding Contribution to Outreach and Awareness

The recipient was Ana Cuadrado for her continued contribution as a volunteer in the various activities related to dissemination of science to society carried out by the CNIO, such as European Researchers' Night, among others. The award was presented by Javier López Sedó, from the *Fundación Humanismo y Ciencia*, which in 2020 signed a collaboration agreement with CNIO Friends.

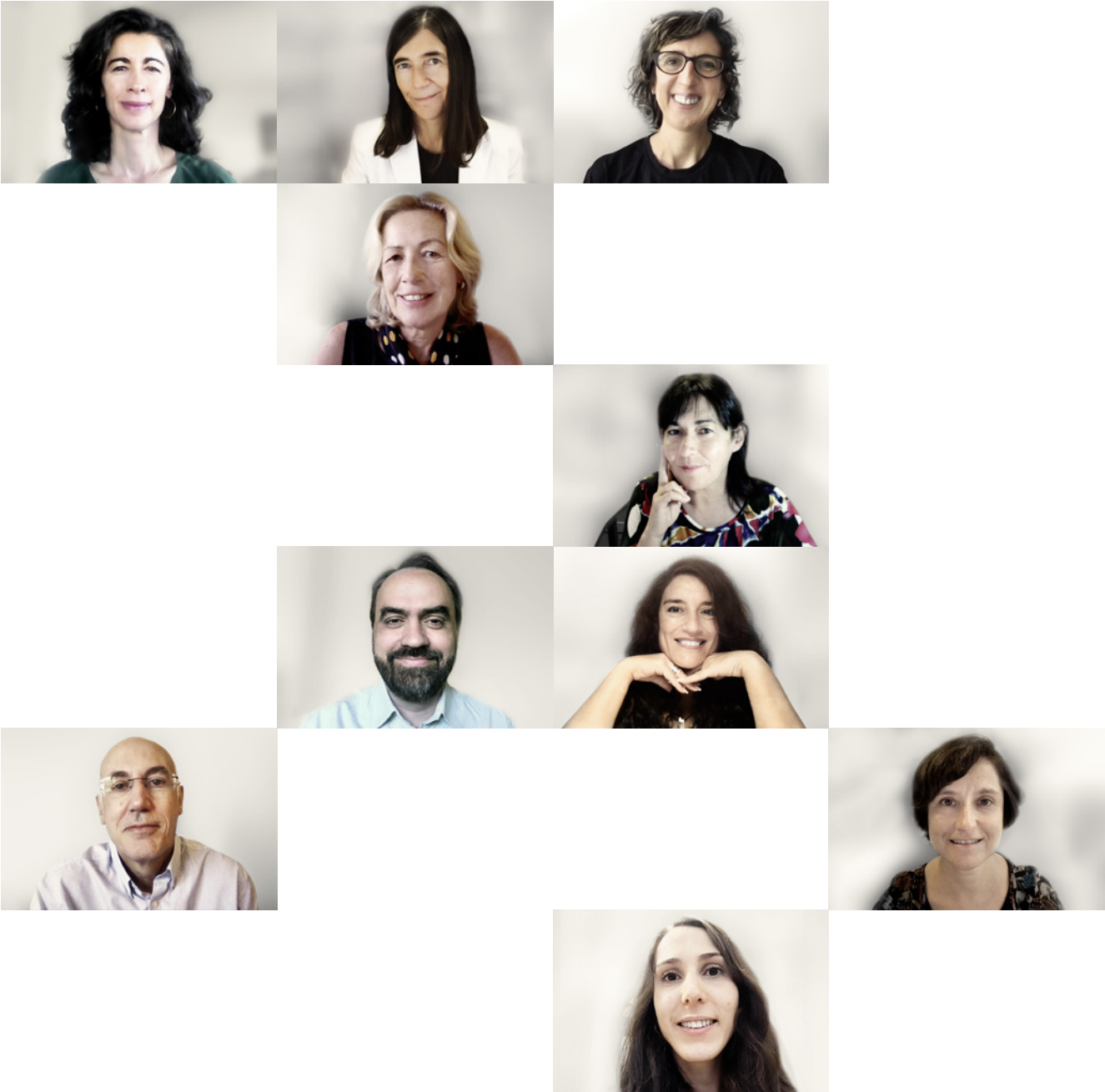
The Lab Day proceeded with additional Awards from the Dean's Office for the Best Oral presentations and the Best Posters. The closure included yet an additional Award for T-Shirt Design, this year to Alberto Cascón for an emotive “Imagine a world without research”. It was beautiful allegory that illustrates the impact of science on human health (whether in SARS-CoV2 infections or cancer).

In summary, the COVID-19 pandemic certainly impacted our Groups personally and scientifically, but the commitment at CNIO is to continue being at the forefront of research in cancer, and to strive in our commitment to promote our young investigators and help them succeed.

CNIO WOMEN
IN SCIENCE OFFICE

Isabel López De Silanes
Coordinator

MEMBERS
Seminars and Events Coordinator:
Isabel López de Silanes
Work-Life Balance Coordinator:
Raquel García-Medina



Maria A. Blasco, María Jesús Alcamí,
Celia de la Calle, Lola Martínez,
Diego Megías, Francisca Mulero, Eva
Ortega, Fernando Peláez, Sandra
Rodríguez-Perales

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

Despite the SARS-Cov2 pandemic of 2020, the WISE Office continued to work actively to make the CNIO a better place to work and to reconcile work and private life. Thanks to the “remote working” pilot programme implemented in February 2020, as part of the actions of the Equality Plan approved in 2019 (prepared by the WISE Office, among others), the CNIO adapted quickly to the restrictions imposed by the Government regarding physical presence at the workplace. In addition, using telematics tools and, in collaboration with the CNIO Direction and Management and the Works Council, the WISE Office worked on the preparation of a catalogue of permits and licences for CNIO workers to be added to the Equality Plan. It was also involved in the preparation of the Human Resources Excellence in Research Award (HRS4R) from the European Commission.

In 2020, we continued organising the WISE seminar series, in which we invite numerous top female and male leaders from different areas to speak. All the talks from March onwards were held online via the Zoom platform. The talks given during 2020 were the following:

- Laura Freixas, writer. Title: “*Cuando hablo de mí, hablo de vosotras... y de vosotros*”. 28/01/2020.
- Amelia Valcárcel, philosopher. Title: “*El don de la duda*”. 25/02/2020.
- Inés Cobo, curator of the contemporary art collections of the Friends of the Prado Museum Foundation. Title: “*El Museo del Prado y las artistas*”. 15/06/2020.
- Jose Luis Rodríguez Zapatero, former President of Spain. Title: “*Ciencia y Política*”. 15/09/2020.
- Ana de Miguel, philosopher. Title: “*Filosofía y feminismo: si la filosofía lo mueve todo... ¿por qué encerró a las mujeres y cada filósofo puso su propio cerrojo?*”. 13/10/2020.
- Marieta Jimenez, European President of Merck Healthcare. Title: “*El potencial de la mujer como fuente de crecimiento y líder del proceso de transformación social*”. 10/11/2020.
- Pepa Bueno, journalist and broadcaster. Title: “*Periodismo y feminismo*”. 12/11/2020.

Here at the WISE Office, we share what the twice Nobel prize winner laureate Marie Curie said: ‘Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained.’

In addition, before the pandemic started, we organised the science workshop CNIOptics# entitled “The power of the light”. The most talented students from the Technovation platform participated in the event, which took place at the CNIO on February 25th. We were also involved in other educational initiatives, through the *11defebrero.org* platform, to promote scientific careers among students from the *Comunidad de Madrid* and other locations in Spain (e.g., *IES Cristo Rey de Valladolid*). This initiative was carried out in collaboration with the “CNIO and The City” science outreach project, which was funded by *FECYT*, with the aim of creating closer links between society and the educational system. As part of this project, WISE members mentored high school students in carrying out what is called “My first project”. The mentoring was done on an online basis because of the pandemic.

Facts & Figures

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SCIENTIFIC MANAGEMENT

ISABEL BARTHELEMY Scientific Management Director

Raquel Ares (until March), Sonia Cerdá, Almudena del Codo, M. Dolores Liébanes, Victoria López, Mercedes Moro, Leyre Vergés, Helena Zamora



“All our efforts are dedicated towards providing our scientists with the best possible framework and to taking care of all formalities so that they, in turn, can focus on making a difference through top-notch science.”



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 (SAB) 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 those for scientific outreach.

The Library administers the electronic subscriptions of over 300 scientific journals at the CNIO and manages journal article requests for journals to which the CNIO is not subscribed to. The Library also provides information regarding reference management software, manages the open-access repository *REPISALUD* and organises the CNIO guided visits.

The Scientific Publications Office is responsible for the preparation of institutional scientific publications, including the CNIO Annual Report, booklets of the Scientific Advisory Board meeting and those of other symposia, as well as scientific dissemination books and leaflets. The Office also provides support for the scientific editing of 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 it 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 all other relevant activities related to dissemination and scientific outreach, which are aimed to promote public awareness. In 2020, researchers at the CNIO were involved in 142 projects that received extramural funding.

CNIO is actively participating in 56 collaborative projects in total: 14 are international collaborative projects (3 of which are coordinated by the CNIO), and 42 are collaborative projects conducted at the national level (16 of them coordinated by the CNIO). The international collaborative projects are funded by institutions such as the European Commission through the 7th Framework Programme and Horizon 2020, the US National Institutes of Health (NIH), the US Department of Defense (DoD), the International Human Frontier Science Program Organization, the Melanoma Research Alliance, the Paradifference Foundation, the Worldwide Cancer Research, Lustgarten Foundation - Stand-up 2 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/MCI*), 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 2020, 19 of these projects received international funds, while 67 of them received national funding (mainly from the *AEI/MCI*, 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), the Worldwide Cancer Research, the Cancer Research Institute, the Prostate Cancer Foundation, the US DoD, the Prostate Cancer Foundation, the European Foundation for the Study of Diabetes, the American Thyroid Association, and the Melanoma Research Alliance.

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

EUROPEAN COMMISSION



HORIZON 2020 (2014-2020)

SOCIETAL CHALLENGE 1: HEALTH, DEMOGRAPHIC CHANGE AND WELLBEING

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier (until July 2020); González-Neira, Anna	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)

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 2: Integrated human genome annotation: generation of a reference gene set (Ref.: U41HG007234)

**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



מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, Maria S. (Coordinator)	Heterogeneity in melanoma metastasis and resistance to immune checkpoint blockade

INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

EUROPEAN COMMISSION



7TH FRAMEWORK PROGRAMME (2007-2013)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	ERC Consolidator Grant RSHEALTH: Investigating the causes and consequences of replication stress in mammalian health (Ref.: 617840)

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)
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
Soengas, María S. Frago, Susana	METMEL: Long range-acting drivers of premetastatic niches in melanoma (Ref.: 753442)
Real, Francisco X. Zagorac, Slađana	PDASwTch: Super-enhancer modules controlling plasticity and response to therapy in pancreatic cancer (Ref.: 895943)
Efeyan, Alejo Fernández-Capetillo, Óscar Zauri, Melania	METLINK: Identification of links between cancer cell growth and metabolism genes (Ref.: 794177)

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

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)

US CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS (CDMRP)/US DEPARTMENT OF DEFENSE

PRINCIPAL INVESTIGATOR

Peinado, Héctor

PROJECT TITLE

Role of exosomes and Endoglin in Neurofibromatosis Progression (Ref.: W81XWH-16-1-0131)

CANCER RESEARCH INSTITUTE

PRINCIPAL INVESTIGATOR

Valiente, Manuel

PROJECT TITLE

Brain-specific strategies to improve responses to immunotherapy (Ref.: 54545)

BEUG FOUNDATION FOR METASTASIS RESEARCH

PRINCIPAL INVESTIGATOR

Valiente, Manuel

PROJECT TITLE

Altered brain vessels as a novel target in brain metastasis

EUROPEAN FOUNDATION FOR THE STUDY OF DIABETES/ JUVENILE DIABETES RESEARCH FOUNDATION/LILLY

PRINCIPAL INVESTIGATOR

Djouder, Nabil

PROJECT TITLE

Elucidating mechanisms of epigenetic changes in type 1 diabetes and treatment with DNA demethylating drugs

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)

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)
Benítez, Javier (until July 2020); 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)

RESEARCH PROJECTS IN HEALTH / PROYECTOS DE INVESTIGACIÓN EN SALUD²

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, Núria (Coordinator)	Building and validation of risk prediction models for pancreas cancer. The application of a multi-omics approach (Ref.: PI15/01573)
Malats, Núria (Coordinator)	Study of the environmental and genetic risk profiles and clinical behaviour of the basal-like phenotype of pancreatic cancer. Comparison with bladder, breast and endometrial cancers (Ref.: PI18/01347)
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)

1, 2 This Programme is cofunded by the European Regional Development Fund (ERDF)



TECHNOLOGICAL DEVELOPMENT PROJECTS / PROYECTOS DE DESARROLLO TECNOLÓGICO³

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria (Coordinator)	Translational Studies for the Development of Telomerase Gene Therapies as Treatments for Myocardial Infarction and Pulmonary Fibrosis (Ref.: DTS17/00152)

SARS-COV-2 AND COVID-19 RELATED ILLNESS RESEARCH PROJECTS⁴

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cortés-Ledesma, Felipe (Coordinator)	SARS-COV-2 diagnosis by phi29 polymerase amplification (Ref.: COV20/01036)

**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 (2013-2016 AND 2017-2020)

EXCELLENCE NETWORKS / REDES DE EXCELENCIA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar (Coordinator)	Research Network UBIRed: Ubiquitin like proteins in signalling, proliferation and cancer (Ref.: SAF2017-90900-REDT)
Malumbres, Marcos (Coordinator)	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)

3, 4, 5 This Programme is cofunded by the European Regional Development Fund (ERDF)



**COMMUNITY OF MADRID /
COMUNIDAD AUTÓNOMA DE
MADRID⁶**

Comunidad de Madrid
R&D ACTIVITIES PROGRAMME IN BIOMEDICINE:

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, Maria	<i>Programa RyPSE-CM: RNA y proteínas de unión a RNA. Implicaciones en salud y enfermedad</i> (Ref.: B2017/BMD-3770)
Djouder, Nabil	<i>Programa TomoXliver-CM: Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar</i> (Ref.: B2017/BMD3817)
Malumbres Marcos (Coordinator); Barbacid, Mariano	<i>Programa iLUNG-CM: Terapias personalizadas y nanotecnología en cáncer de pulmón</i> (Ref.: B2017/BMD-3884)
Mulero, Francisca	<i>Programa RENIM-CM: Red Madrileña de Nanomedicina en Imagen Molecular</i> (Ref.: B2017/BMD-3867)
Quintela, Miguel Ángel	<i>Programa IMMUNOTHERCAN-CM: Inmunidad tumoral e inmunoterapia del cáncer</i> (Ref.: B2017/BMD-3733)
Robledo, Mercedes	<i>Programa TIRONET2-CM: Fisiopatología Tiroidea. Mecanismos implicados en cáncer, autoinmunidad y acción de las hormonas tiroideas</i> (Ref.: B2017/BMD-3724)
Soengas, María S.	<i>Programa NANODENDMEDII-CM: Nanosistemas dendríticos como agentes y vectores terapéuticos en distintas aplicaciones biomédicas (II)</i> (Ref.: B2017/BMD-3703)

R&D ACTIVITIES PROGRAMME IN TECHNOLOGIES:

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/BI04747)

⁶ These Programmes are co-funded by the European Regional Development Fund (ERDF) and the 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 2020, organized by Madri+d Foundation and founded by EU-H2020 Programme. Marie Skłodowska-Curie actions GA 953820

**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)
Soengas, María S. (Coordinator); Gómez, Gonzalo Peinado, Héctor	Distinct routes of metastatic dissemination in different melanoma subtypes. Implications in the validation of new tumor biomarkers and therapeutic targets (Ref.: GCB15152978SOEN)
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
Llorca, Óscar	Amino acid transporter structure to target glutamate transmission in neuro diseases (Ref.: HR20-00081)
Peinado, Héctor	Defining The Role of Exosome-Secreted Micropeptides in Pancreatic Cancer (Ref.: HR18-00256)
Soengas, María S.	Exploiting post-transcriptional regulation to uncover novel vulnerabilities of metastatic cells (Ref.: HR17-00232)

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 HEALTH⁷**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier (until July 2020); Urioste, Miguel	Massive sequencing contributes to decipher the genetic bases of families with rare tumors (Ref.: PI16/00440)
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)

TECHNOLOGICAL DEVELOPMENT PROJECTS / PROYECTOS DE DESARROLLO TECNOLÓGICO⁸

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 PROJECTS⁹

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)

**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)

7, 8, 9 This Programme is cofunded by the European Regional Development Fund (ERDF)



R&D EXCELLENCE PROJECTS / PROYECTOS DE I+D EXCELENCIA¹⁰

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)
Llorca, Óscar	Structural and molecular mechanisms regulating the PIKK family of kinases, including DNA- PKcs, SMG1 and mTOR (Ref.: SAF2017-82632-P)

CHALLENGES-RESEARCH PROJECTS / PROYECTOS RETOS-INVESTIGACIÓN¹¹

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	CANTHERHET: Computational targeting of cancer heterogeneity: in silico drug prescription for 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)
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)
Losada, Ana	COHESIN2: Molecular mechanisms of variant cohesin function (Ref.: BFU2016-79841-R)
Losada, Ana	COHESIN3D: Cohesin functions in development, differentiation and disease (Ref.: PID2019-106499RB-I00)
Macintyre, Geoffrey J.	TTCIN: Therapeutic targeting of chromosomal instability in cancer (Ref.: PID2019-111356RA-I00)
Malumbres, Marcos	NewCDKtarget: Validation of a New Subfamily of Cyclin-dependent Kinases as Cancer Targets (Ref.: RTI2018-095582-B-I00)
Méndez, Juan	REPLICON2: Control of eukaryotic DNA replication (Ref.: BFU2016-80402-R)
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)
Ortega, Sagrario	ESSENCE: Extrinsic control of the skin stem cell niche in homeostasis and cancer (Ref.: BFU2015-71376-R)

10, 11 This Programme is cofunded by the European Regional Development Fund (ERDF)



CHALLENGES-RESEARCH PROJECTS / PROYECTOS RETOS-INVESTIGACIÓN¹²

PRINCIPAL INVESTIGATOR	PROJECT TITLE
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)
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)
Real, Francisco X.	TF-PDAC Transcription factors in pancreatic cancer: from biology to therapy (Ref.: RTI2018-101071-B-I00)
Rodríguez, Cristina	PREDICT: Identification of genetic markers and physiopathologic factors predictive of the peripheral neuropathy of paclitaxel and of other oncologic drugs: massive sequencing of candidate genes (Ref.: SAF2015-64850-R)
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)
Squatrito, Massimo	GLIO-TRK: TRKing down oncogenic genetic rearrangements in gliomas (Ref.: RTI2018-102035-B-I00)
Valiente, Manuel	Stat3 ReACTIVE: Biology of Stat3+ reactive astrocytes in brain metastasis (Ref.: SAF2017-89643-R)

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)

SCIENTIFIC INFRASTRUCTURES / INFRAESTRUCTURAS CIENTÍFICO-TECNOLÓGICAS¹³

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Megías, Diego	Grant for acquiring scientific equipment: high resolution microscope based on Stimulated Emission Depletion (STED) (Ref.: EQC2019-006679-P)

12, 13 This Programme is cofunded by the European Regional Development Fund (ERDF)



**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.: LABAE1600IEFEY)
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)
Squatrito, 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
Losada, Ana	Identification of a gene signature associated with aggressive Ewing Sarcoma for diagnostic and therapeutic purposes (Ref.: PROYE20046LOSA)
Fernández-Capetillo, Óscar	Targeting the histone methyltransferase SETD8 in cancer: from biomarker identification to drug development and mechanisms of resistance (Ref.: PROYE2010IFERN)
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, Maria 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; Salazar, María (until July 2020)	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
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>Desarrollo de estrategias terapéuticas dirigidas contra el estroma del cáncer de páncreas</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

**FUNDACIÓN PROYECTO
NEUROFIBROMATOSIS**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	<i>Combinación de terapias antiangiogénicas e inhibidores de MEK en neurofibromatosis</i>

**BBVA FOUNDATION /
FUNDACIÓN BBVA**

Fundación BBVA

LEONARDO GRANTS

PRINCIPAL INVESTIGATOR

PROJECT TITLE

Olmeda, David

*Nuevas estrategias para el tratamiento preventivo de la enfermedad metastásica en Melanoma (Ref.: IN18_BBM_TRA_0293)***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**FUNDACIÓN
RAMÓN ARECES

PRINCIPAL INVESTIGATOR

PROJECT TITLE

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

**“L'ORÉAL-UNESCO FOR
WOMEN IN SCIENCE”
PROGRAMME**

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**

Asociación Española de Gastroenterología

TAMARITE — AESPANC — ACANPAN GRANT

PRINCIPAL INVESTIGATOR

PROJECT TITLE

Molina, Esther

Radiomics en cáncer de páncreas para una medicina estratificada y de precisión: un estudio piloto

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 2020, the CNIO signed several new agreements with Spanish Universities and other institutions, namely with the *Universidad de Salamanca, Universidad de Valencia, Universidad de Alicante, Universidad de Alcalá, Universidad Rey Juan Carlos, Universidad Carlos III de Madrid, Universidad*

Internacional Menéndez Pelayo, Universidad Politécnica de Madrid, Universidad Politécnica de Valencia y Universidad del País Vasco, IES Siglo XXI, IES Lope de Vega, IES Antonio Machado, IES San Juan de la Cruz y el Instituto Técnico de Estudios Profesionales, Centro Educativo CEU-Instituto Superior de Estudios Profesionales, and Sociedad Cooperativa Madrileña de Enseñanza Alhucema.

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2016	2017	2018	2019	2020
Training of PhD students	110	112	109	100	109
Post-doctoral training	51	44	50	49	52
Training for MDs	17	21	12	20	7
Laboratory training for MSc/BSc students	95	99	128	150	85
Laboratory training for technicians	26	20	13	15	5

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 2020, 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's individual Research Groups or Units. This year, 85 students participated in these programmes, of whom 4 ended up joining the CNIO as pre-doctoral students.

TRAINING OF PHD STUDENTS

The training of PhD students in cutting-edge cancer research is of key importance to the CNIO. The Centre offers many opportunities for bright and dynamic university graduates, of all nationalities, to pursue an ambitious PhD project. Attesting to this, 14 students obtained their PhD degrees in 2020 and 21 others joined the CNIO in the same year. Over 15% of the 109 students working at the CNIO in 2020 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 2020, 2 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 2020 was as follows: 68 % of students worked in the Molecular Oncology Programme, 13% in the Structural Biology Programme, 11% in the Human Cancer Genetics Programme, 0% in the Experimental Therapeutics Programme, 2% in the Biotechnology Programme, and 6% in the Clinical Research Programme.



FUNDING OF PHD TRAINING	NO.
SPANISH ORGANISATIONS	93
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)	7
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (I+D Projects)	3
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (Fellowship)	1
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i>	2
Cris Foundation / <i>Fundación Cris</i>	1
Community of Madrid / <i>Comunidad de Madrid</i>	4
CNIO	3
<i>Fundación Española de Hematología y Hemoterapia (FEHH)</i>	1
“la Caixa” Banking Foundation/ <i>Fundación Bancaria “la Caixa”</i> (I+D Projects)	1
“la Caixa” Banking Foundation/ <i>Fundación Bancaria “la Caixa”</i> (Predoctoral Fellowships)	14
Comillas University / <i>Universidad de Comillas</i>	1
INTERNATIONAL ORGANISATIONS	16
EMBO	1
China Scholarship Council (CSC)	1
<i>Consejo Nacional de Ciencia y Tecnología</i> (Mexico)	2
European Society for Clinical Nutrition and Metabolism (ESPEN)	1
European Research Council	3
GENCODE	2
ESMO	1
Columbia University / <i>Universidad de Columbia</i>	1
Weizmann Institute of Science (WIS)	1
Marie Skłodowska-Curie actions of the European Commission	3
TOTAL	109

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 2020, 52 postdoctoral fellows worked at the CNIO. Notably, about one third of these fellows were from outside of Spain, many coming from very prestigious international institutions.

In 2020, the *Fundación Banco Santander* funded a highly competitive fellowship programme aimed at supporting outstanding young scientists who have been trained in the UK or in the USA, and who wish to start or continue their postdoctoral training at the CNIO. This last call resulted in contracting 1 postdoctoral scientist.

Thanks to the donations received through the "CNIO Friends" platform, the fifth call of the "CNIO Friends" Postdoctoral Contract Programme, launched in 2020, resulted in the recruitment of 6 scientists for a 2-year period each.

FUNDING SOURCES OF POST-DOCTORAL CONTRACTS	NO.
SPANISH ORGANISATIONS	43
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science and Innovation / <i>Ministerio de Ciencia e Innovación</i> (Postdoctoral Fellowships)	6
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)	5
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (Fellowships)	5
Community of Madrid / <i>Comunidad de Madrid</i>	7
CNIO	15
Banco Santander Foundation / <i>Fundación Banco Santander</i>	1
"la Caixa" Banking Foundation/ <i>Fundación Bancaria "la Caixa"</i> (Postdoctoral Fellowships)	2
Cris Cancer Foundation (CRIS) / <i>Fundación Cris Contra el Cáncer (CRIS)</i>	2
INTERNATIONAL ORGANISATIONS	9
AIRC	1
European Commission Framework Programme / H2020	3
European Research Council	1
Lilly Foundation / <i>Fundación Lilly</i>	1
Marie Skłodowska-Curie actions of the European Commission	2
Worldwide Cancer Research UK	1
TOTAL	52

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 5 students who participated in this programme in 2020, 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 2020, 7 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 2020, the *Ramón y Cajal* Programme supported 6 scientists. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (currently the State Research Agency of the Spanish Ministry of Science and Innovation) aims to encourage Spanish or foreign scientists working abroad to return to or relocate to Spain. Successful candidates are selected on the basis of their potential capacity to lead independent projects and groups, or to contribute

successfully to the ongoing research in the existing groups. Fourteen other scientists are funded by similar programmes, including the *Juan de la Cierva* programme (Spanish Ministry of Science and Innovation, 6 contracts); the *Miguel Servet* programme (2 contracts) of the Institute of Health *Carlos III*; and the Spanish Association Against Cancer (*AECC*, 6 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 2020, Scott Lowe, from the Memorial Sloan Kettering Cancer Centre in New York (USA), and Sonia Laín, from the *Karolinska Institutet* in Stockholm (Sweden), were beneficiaries of the *Jesús Serra* Foundation's Visiting Researchers Programme.



"SCIENCE BY WOMEN" PROGRAMME

Thanks to the "Science by Women" Programme, launched by the Spanish *Fundación Mujeres por África*, the CNIO selected Mai Tolba, from Ain Shams University (Egypt), to carry out a 6-month stay at the CNIO during 2020.



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

28-30 JANUARY 2020

6-9 JULY 2020

15-16 OCTOBER 2020

16-17 NOVEMBER 2020

ORGANISER:

Flow Cytometry Unit, CNIO, Madrid, Spain

SPEAKERS:

- **Lola Martinez**, Head of the Flow Cytometry Unit. CNIO
- **Sara García García**, Flow Cytometry Unit Technician. CNIO
- **Andrea Valle**, European Applications Specialist DeNovo Software

II CNIO-BANCO SABADELL FOUNDATION WORKSHOP ON PHILOSOPHY, SCIENCE AND MEDICINE: SOCIO-ENVIRONMENTAL FACTORS OF HEALTH AND DISEASE
19 NOVEMBER 2020

WITH THE SUPPORT OF:

Banc Sabadell Foundation

ORGANISERS:

- **Maria A. Blasco (CNIO)**
- **Antonio Diéguez (UMA)**
- **Arantza Etxeberria (UPV/EHU)**

SPEAKERS:

- **Marta Bertolaso**, University Campus Bio-Medico of Rome, Italy
- **Luis Paz-Ares**, H12O-CNIO Lung Cancer Clinical Research Unit, Madrid, Spain
- **Anna Estany**, Autonomous University of Barcelona, Spain
- **Miquel Porta**, Hospital del Mar Institute of Medical Research (IMIM), and Autonomous University of Barcelona, Spain
- **Elena Casetta**, University of Turin - Italy
- **Fernando Valladares**, National Museum of Natural Sciences, CSIC, Madrid, Spain



CNIO DISTINGUISHED SEMINARS

The purpose of the Distinguished Seminars Series is to invite outstanding and internationally renowned scientists to give a seminar and to meet with researchers at the CNIO. Distinguished Seminars are recurrent events that are open to the public and are held throughout the year, usually on Fridays at noon in the CNIO Auditorium. Each Distinguished Seminar series includes world-leading scientists who address topics that are of general interest to the CNIO faculty. All the seminars from October onwards proceeded in a virtual format due to the current pandemic.

In total, the CNIO hosted 14 online and onsite distinguished speakers in 2020.



DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
17/01/2020	Núria Lopez Bigas	IRB Barcelona, Spain	The mutational footprints of cancer therapies
31/01/2020	Cedric Blanpain	Université Libre de Bruxelles, Brussels, Belgium	Mechanisms regulating tumor transitional states
FEBRUARY			
07/02/2020	Victoria Sanz-Moreno	Barts Cancer Institute - Queen Mary University of London, UK	Cytoskeletal dynamics: the Achilles heel of cancer
21/02/2020	Anna Veiga	Reproductive Medicine Service at Dexeus Women's Health; Bellvitge Biomedical Research Institute (IDIBELL); Universitat Pompeu Fabra (UPF); Barcelona, Spain	Barcelona Stem Cell Bank: iPS generation as disease model
MARCH			
06/03/2020	Werner Kühlbrandt	Max-Planck-Institute of Biophysics, Frankfurt am Main, Germany	High-resolution cryoEM of membrane protein complexes
OCTOBER			
16/10/2020	Fatima Gebauer	Centre for Genomic Regulation, Barcelona, Spain	RNA binding proteins in cancer progression and suppression: goggling through UNR/CSDE1
23/10/2020	Antonio Pérez-Martínez	Autonomous University of Madrid, University Hospital La Paz, Spain	A road to overcome hurdles in paediatric cancer: from natural killers to artificial executors
30/10/2020	Raúl Pérez-Jiménez	CIC nanoGUNE, San Sebastián, Spain	Ancestral enzymes: evolution towards biotechnology
NOVEMBER			
06/11/2020	Fatima Mechta-Grigoriou	Institut Curie, Paris, France	Role of fibroblast heterogeneity in metastatic spread and immunotherapy resistance in cancer
13/11/2020	Gaëlle Legube	Chromatin and DNA repair group CBI (Centre De Biologie Integrative) CNRS-University of Toulouse, France	Chromosome and chromatin dynamics in DNA double strand break repair
20/11/2020/	Samra Turajlic	The Francis Crick Institute, London, UK	Understanding cancer evolution through studies in renal cancer and melanoma
27/11/2020	Nicola Aceto	Swiss National Science Foundation Assistant Professor of Oncology Investigator - European Research Council (ERC), Basel, Switzerland	Biology and vulnerabilities of circulating tumour cells
DECEMBER			
11/12/2020	Caroline Dive	CRUK Manchester Institute Cancer Biomarker Centre, UK	Liquid biopsies to support better outcomes for lung cancer patients
18/12/2020	Luis Enjuanes	The National Centre for Biotechnology (CNB), Madrid, Spain	Human coronaviruses: pathogenesis and protection

AD-HOC SEMINARS

In addition to the CNIO Distinguished Seminar Series, the CNIO also hosts numerous *ad-hoc* seminars throughout the year. *Ad hoc* seminars are organised for the purpose of: academic interactions, academic elevation and enrichment, as well as academic *vis-a-vis* social networking. In addition, they facilitate socialising with colleagues from other institutions. A total of 21 *ad-hoc* seminars were organised by CNIO researchers in 2020.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
09/01/2020	Miguel Foronda	Associate Editor, <i>Nature Cancer</i> , London, UK	The ins and outs of publishing in <i>Nature Cancer</i>
20/01/2020	Manuel Collado Rodríguez	Health Research Institute of Santiago de Compostela (IDIS), SERGAS, Spain	Cellular senescence in development, regeneration, and cancer
27/01/2020	David Fernández-Antorán	Sanger Institute, Cambridge, UK	Game of clones: a story of winners and losers
FEBRUARY			
10/02/2020	Paulina Prorok	Technical University of Darmstadt, Germany	Role of G-rich sequences in regulation of replication origin activity
11/02/2020	Israel Salguero Corbacho	Gurdon Institute Cambridge, UK	DNA double strand break repair: there is life beyond gH2AX
13/02/2020	Oscar Yanes	<i>Rovira i Virgili</i> University, Reus, Spain	Metabolomics as a unique biochemical approach to understand cell metabolism
13/02/2020	Alessandro Scandurra	Bio-Techne Brands, UK	The simple Western technology: a complete protein analysis solution (gel free)
25/02/2020	Christopher Pin	University of Western Ontario, Canada	The contribution of cell stress to initiation and progression of pancreatic cancer
MARCH			
10/03/2020	Gerard Cantero	Centre for Genomic Regulation (CRG), Barcelona, Spain	Targeting mucin secretion to control chemoresistance in colorectal cancer
JULY			
27/07/2020	Luca Lignitto	Laura and Isaac Perlmutter Cancer Center, NYU Langone Medical Center, US	Deciphering the heme code in physiology and disease
SEPTEMBER			
01/09/2020	Johnny Kim	Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany	Spatio-temporal control of cellular plasticity for regeneration and against cancer risk
02/09/2020	Tereza Andreou	Stem Cell and Neurotherapies Laboratory, University of Manchester Manchester, UK	From mitochondria, to brain tumours... and back
03/09/2020	Eduardo Balsa	IIBm (CSIC-UAM). Madrid, Spain	Molecular and metabolic mechanisms underlying mitochondrial dysfunction
18/09/2020	Fevzi Demircioglu	Famagusta State Hospital, Cyprus	Cancer associated fibroblasts FAK regulates malignant cell metabolism

22/09/2020	Arnau Sebé-Padrós	Centre for Genomic Regulation (CRG), Barcelona, Spain	Cell type phylogenetics: mapping and comparing early metazoan cell type regulatory programmes
OCTOBER			
20/10/2020	Christopher Casley and Camille Terfve	Partner Patent Attorney Life Sciences and Associate Patent Attorney Life Sciences. Mewburn Ellis, UK	Protecting intellectual property in bioinformatics, artificial intelligence and big data: challenges and opportunities
23/10/2020	Antonio Pérez-Martínez	Autonomous University of Madrid, University Hospital La Paz, Spain	A road to overcome hurdles in paediatric cancer: from natural killers to artificial executors
26/10/2020	Laia Richart – Gines	<i>Institut Curie</i> , Paris, France	Dosage matters: xist acts as a gatekeeper of epithelial cell homeostasis
NOVEMBER			
05/11/2020	Thomas Miller	The Francis Crick Institute, London, UK	How to licence an origin of replication: Mechanism of MCM double-hexamer formation revealed by cryo-EM
27/11/2020	Stefano Gambera	<i>ISCIII & UCM</i> , Madrid, Spain	Modelling sarcomagenesis: from tumour initiation to progression to treatment
DECEMBER			
16/12/2020	Faiyaz Notta	Ontario Institute for Cancer Research, Canada	Pancreatic cancer - origins and subtypes

WOMEN IN SCIENCE SEMINARS

In 2020, 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		TITLE
28/01/2020	Laura Freixas	Writer	<i>Cuando hablo de mí, hablo de vosotras... y de vosotros</i>
25/02/2020	Amelia Valcárcel	Professor of Moral and Political Philosophy at UNED	<i>El don de la duda</i>
15/06/2020	Inés Cobo Calleja	Museo del Prado Friends Foundation	<i>El Museo del Prado y las artistas</i>
15/09/2020	José Luis Rodríguez Zapatero	Former President of the Government of Spain	<i>Ciencia y Política</i>
13/10/2020	Ana de Miguel	Philosopher (<i>Profesora Titular de Filosofía Moral y Política de la URJC. Responsable del Programa de Doctorado de Estudios Interdisciplinarios de Género y del Observatorio de Género de la URJC</i>)	<i>Filosofía y feminismo: si la filosofía lo mueve todo... ¿por qué encerró a las mujeres y cada filósofo puso su propio cerrojo?</i>
10/11/2020	Marieta Jiménez Urgal	European President of Merck Healthcare	<i>El potencial de la mujer como fuente de crecimiento y líder del proceso de transformación social</i>
12/11/2020	Pepa Bueno	Journalist and broadcaster	<i>Periodismo y feminismo</i>

SCIENCE DISSEMINATION EVENTS

WORLD CANCER RESEARCH DAY

“UNA NUEVA ERA PARA LA INVESTIGACIÓN DEL CÁNCER: HACIA LA IMPLICACIÓN DE TODA LA SOCIEDAD”
24 SEPTEMBER 2020

ORGANISER:

Centro Nacional de Investigaciones Oncológicas (CNIO)

WITH THE SUPPORT OF:

”la Caixa” Foundation

SPEAKERS:

- **Francis Mojica**, Associate Professor of the Department of Physiology Genetics and Microbiology at the University of Alicante
- **Maria A. Blasco**, CNIO Director and Head of the Telomeres and Telomerase Group
- **Luis Paz-Ares**, Head of the H12O-CNIO Lung Cancer Clinical Research Unit
- **Sandra Rodríguez-Perales**, Head of the Molecular Cytogenetics Unit
- **María Luisa Villafranca**, breast cancer patient and president of the Asociación Rosae, members of CNIO Friends
- **Moderadora: Cristina Villanueva**, Journalist

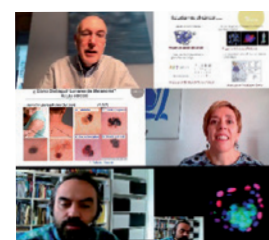


OPEN DOORS DAY: EVERYONE UNRAVELLING CANCER

12 NOVEMBER 2020

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 (XX *Semana de la Ciencia y de la Innovación*, 2-15 November 2020).

In November 2020, the CNIO participated in the Science and Innovation Week under the motto “*Todos y todas desarmando al cancer*” (“Everyone Unravelling Cancer”). The event was held online with over 70 attendees, who took the opportunity to learn more about a top research institution like the CNIO.



Drs. Fernando Peláez, Marisol Soengas and Diego Megías.

RESEARCHERS' NIGHT

27 NOVEMBER 2020

The Centre opened its doors to the public on November 27 to show its commitment to society and to promote scientific culture.

Between 5pm and 11pm, 4 groups of people of all ages shared a live science session with young researchers who volunteered for this initiative. The researchers guided participants through a science experiment that they could perform in their own homes with household products, and answered any questions participants had about why they decided to devote themselves to research and what their day-to-day work consists of. In total, 98 connections were made with 280 people, a record number.

In order to carry out the experiment (in which DNA extraction from a tomato was demonstrated), the participants received a “science kit” in their homes with all the necessary materials.

The aim of the activity is to awaken scientific curiosity among youngsters, to erase the stereotypes associated with people involved in research, and to show what CNIO's cancer research involves.

European Researchers' Night is a European Union initiative aimed at raising awareness and interest in science among the public at large, held simultaneously in 350 European cities. In Madrid, it is promoted by the Science, Universities and Innovation Department of the Regional Government of Madrid, and coordinated by the *Fundación para el Conocimiento madri+d*. The project is funded by the European Union under the Horizon 2020 Research and Innovation Programme – Marie Skłodowska-Curie Actions.



GUIDED VISITS

Throughout the year, the CNIO provides tailor-made opportunities to visit its installations and to learn about the essentials of cancer research. During 2020 (before the COVID-19 pandemic reached Spain), more than 269 people participated in such guided visits; most of them were ESO and *Bachillerato* student groups, but also professionals in the health sector.

ADMINISTRATION

BOARD OF TRUSTEES

→ Honorary President

- **Pedro Francisco Duque Duque**
Minister of Science and Innovation
Ministro de Ciencia e Innovación

→ President

- **Rafael Rodrigo Montero**
Secretary General for Research of the Spanish Ministry of Science and Innovation
Secretario General de Investigación del Ministerio de Ciencia e Innovación

→ Vice-President

- **Raquel Yotti Álvarez**
Director of the National Institute of Health Carlos III
Directora 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)
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Subdirectora General de Redes y Centros de Investigación Cooperativa del Instituto de Salud Carlos III
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Directora Gerente del Instituto Aragonés de Ciencias de la Salud

- **Antonio Fernández-Campa García Bernardo**
Beatriz Allegue Requeijo (until end November 2020)
Director of the Galician Health Knowledge Agency - *ACIS*
Director/a de la Agencia Gallega del Conocimiento en Salud- ACIS

- Representative of the Science, Technology and Innovation Advisory Council appointed by the plenary session of this Council (appointment pending)
Un representante del Consejo Asesor de Ciencia, Tecnología e Innovación designado por el Pleno de este Consejo (pendiente de nombramiento)

→ Elected Members

- **BBVA Foundation**
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, 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 €281,950 in 2020 (€359,124 in 2019). This amount was received as base salary and position salary supplements: €222,447.12 (€218,004 in 2019); 2% increase approved in the General State Budget for 2018: €4,448.88. To these amounts, the variable remuneration for directors of €55,054 accrued during 2018 and 2019 (€135,954 accrued in 2019), must be added. Like every year, there is a provisional amount of €56,986 for the variable accrued in 2020.
- Members of the CNIO Board of Trustees are not remunerated.

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MRC Laboratory of Molecular Biology
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* Plan de Empleo Joven (Youth Employment Plan)

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(since February)Becerra, Carolina (since
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Luongo, Victoria

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Echeverria, Pedro
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Ortega, CarlosVerde, Begoña (since
February)

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Carbonel, David

Izquierdo, David

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Moreno, José
Moreno, José Antonio
Poza, Javier
Rivas, JoséSánchez, Alejandro
Serrano, Alberto
Varynytskyi, Ihor
Vázquez, Manuel
Yague del Ejido, Alberto

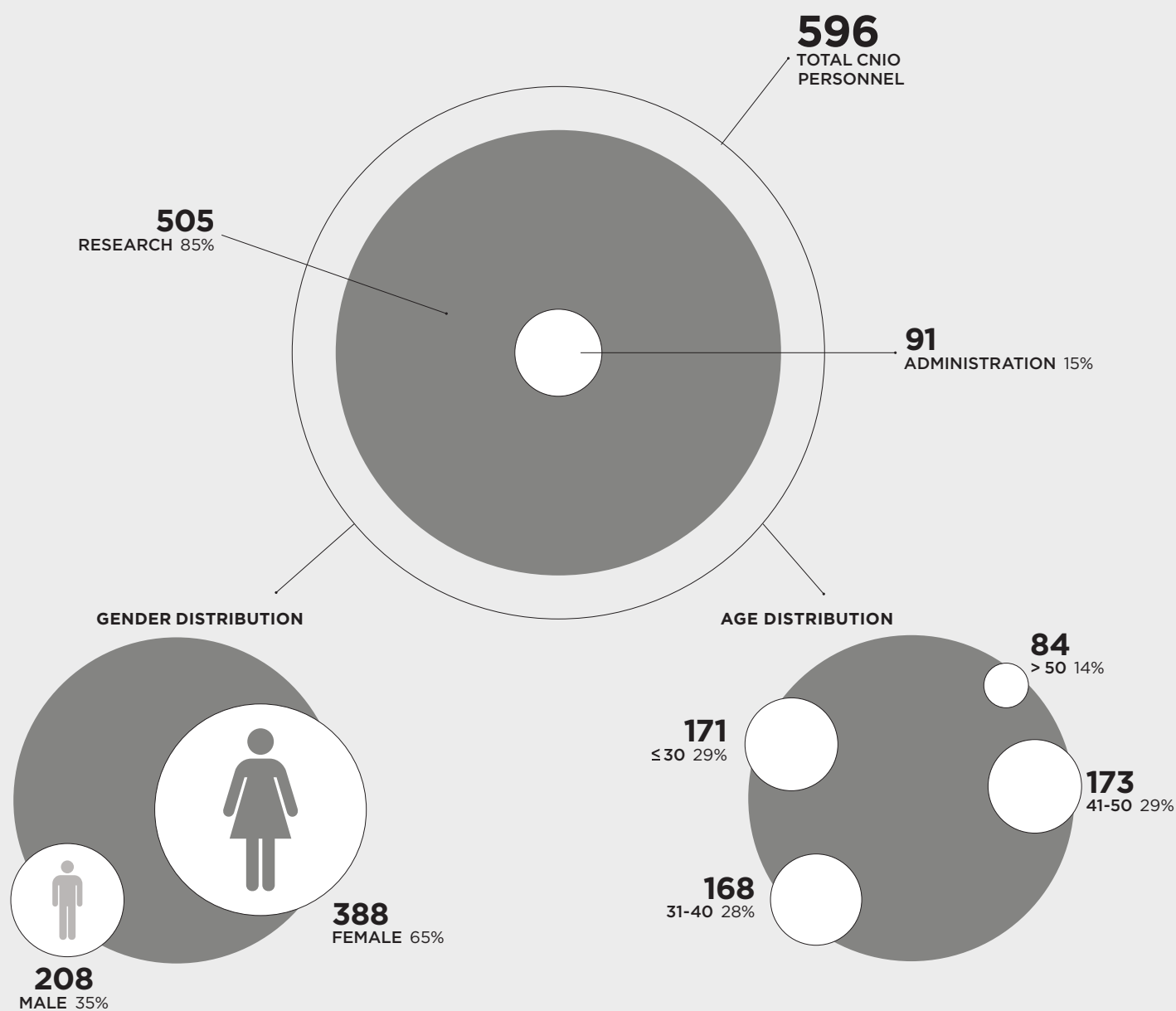
PREVENTION & BIOSECURITY

**Cespón,
Constantino** HeadBertol, Narciso
Giménez, Andrés
Gómez, VíctorMartínez, Jose L.
Oset, Francisco J.
Tendero, María

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**Fernández, José
Luis** Headde Miguel, Marcos
Barjola, Eva
García, Jose Manuel
Hernández, JulioMartín, Félix
Martínez, Rubén
Reviriego, PabloEXTRAMURAL CLINICAL
RESEARCH**López, Antonio** Director

CNIO PERSONNEL 2020



GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

Category	Gender	Percentage	Count	Visual Representation
GROUP LEADERS, HEADS OF UNIT/SECTION	FEMALE	46%	21	21 female icons
	MALE	54%	25	25 male icons
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	FEMALE	67%	10	10 female icons
	MALE	33%	5	5 male icons
MANAGEMENT: DIRECTORS, HEADS OF AREA	FEMALE	29%	4	4 female icons
	MALE	71%	10	10 male icons

SCIENTIFIC PERSONNEL 2020

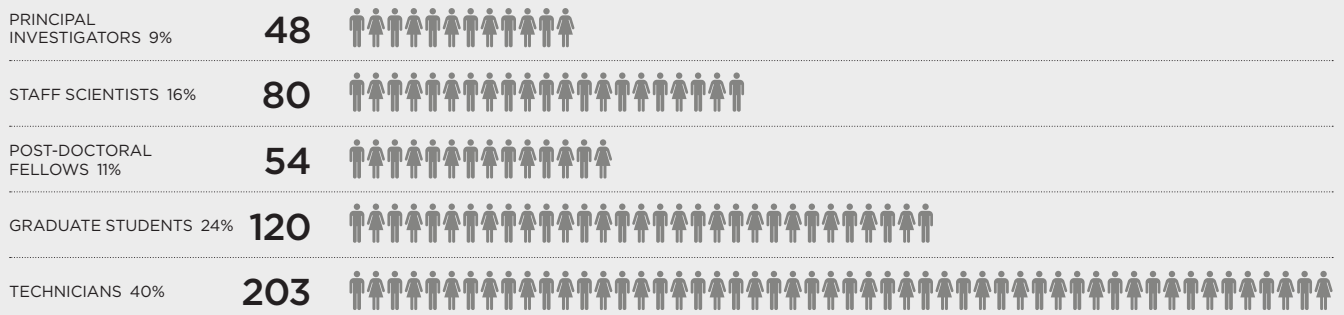
TOTAL SCIENTIFIC
PERSONNEL

505

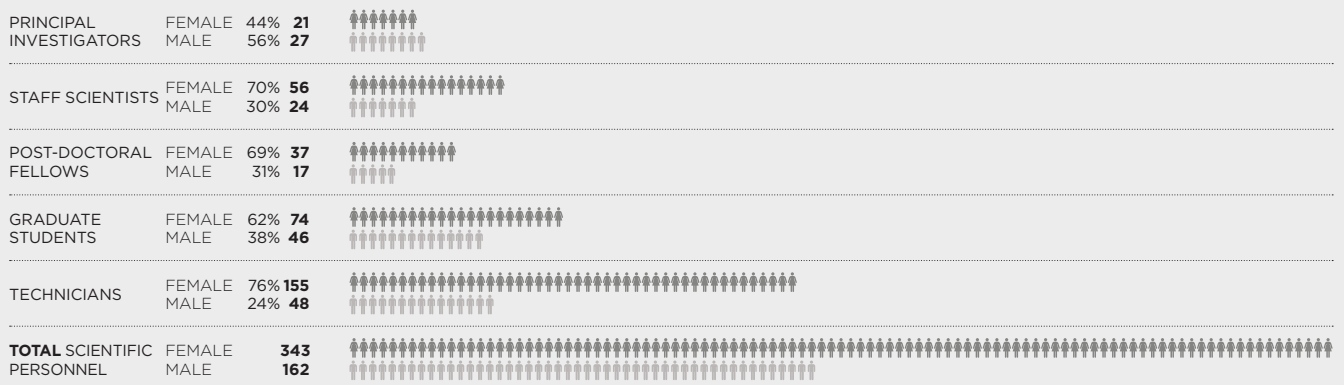
DISTRIBUTION BY PROGRAMMES



DISTRIBUTION BY PROFESSIONAL CATEGORY



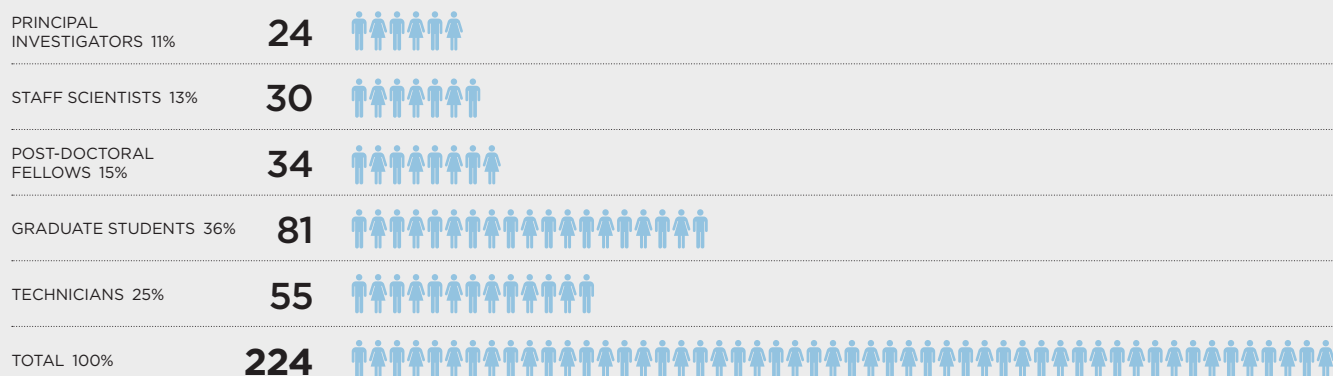
GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY



DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC
PERSONNEL

505



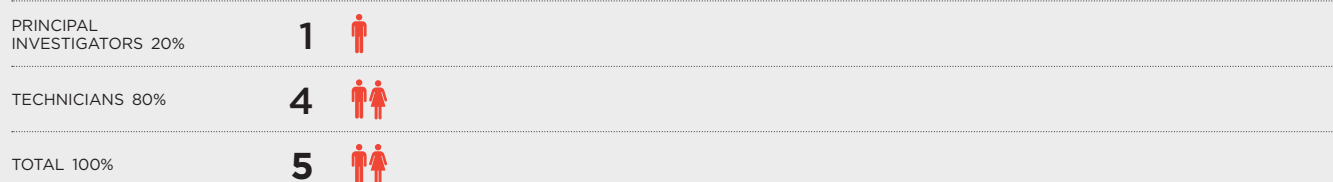
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH



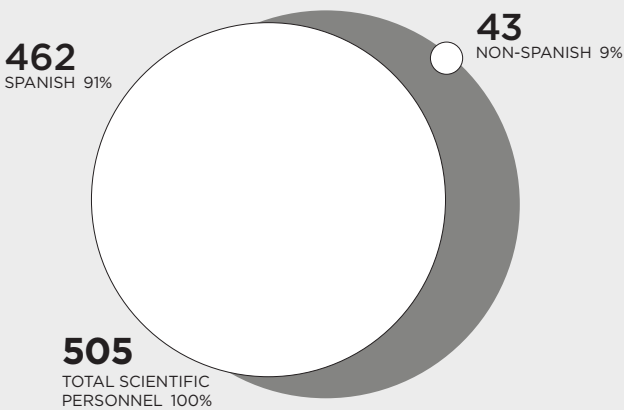
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION



DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BIOBANK



SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

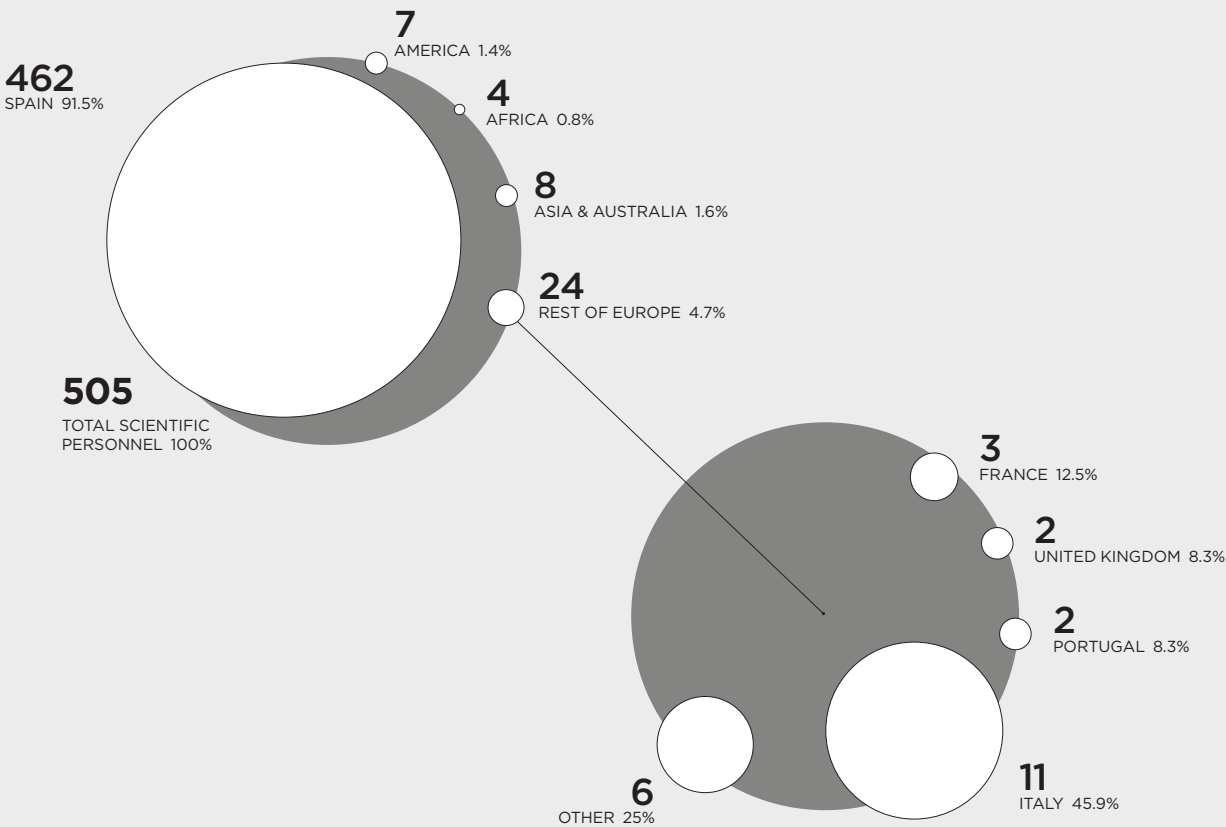


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS 13%	6	
STAFF SCIENTISTS 6%	5	
POST-DOCTORAL FELLOWS 24%	13	
GRADUATE STUDENTS 8%	9	
TECHNICIANS 5%	10	

Total foreign scientific personnel 43
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 2020. They play an inherent role in our present and future successes.”



“la Caixa” Foundation

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. The CNIO has been participating in a doctoral fellowship programme of the “la Caixa” Foundation, named INPhINIT, since 2017; the aim of this programme is to attract outstanding international students to carry out doctorates at top Spanish research centres.



Fundación de Investigación para vencer el cáncer

www.criscancer.org

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 4 research groups at the CNIO: the Experimental Oncology Group, led by Mariano Barbacid; the Prostate Cancer Clinical Research Unit (CRU), headed by David Olmos; the Breast Cancer CRU, headed by Miguel Quintela; and the H12O-CNIO Haematological Malignancies CRU, led by Joaquín Martínez-López. These Groups focus on the translation of advances in cancer research into novel therapeutics and improvements in patient care.



The *Fundación Marcelino Botín* and the *Banco Santander* are committed to supporting scientific research and knowledge transfer from academia to the market through science programmes; this transfer is regarded as one of the main driving forces for Spain’s economic

and social development. These 2 well-recognised organisations collaborate with the CNIO in this regard by supporting the research group led by Maria A. Blasco.

FUNDACION

Banco Santander

The *Fundación Banco Santander* funds the *Banco Santander* Foundation – CNIO Fellowships for Young Researchers Trained in the

UK/USA. These fellowships have the aim to support highly talented and motivated young scientists who have been trained in the UK or in the USA, and who wish to pursue their postdoctoral training at the CNIO. One young scientist, Jonathan Barroso-González from the University of Pittsburgh in Pennsylvania, was the recipient of a Santander Foundation-CNIO Fellowship in 2020. Additionally, thanks to the support of the *Fundación Banco Santander*, a group of 6 researchers will receive training on managerial and entrepreneurial skills in 2021, in collaboration with the IE Business School. *Banco Santander* Foundation also supports our CNIO Arte project.



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.



The *Fundación Jesús Serra-Catalana Occidente* continues to fund the Visiting Researchers' Programme that was established to

support prestigious international professors for short stays at the CNIO. The recipients of the *Jesús Serra* Foundation's Visiting Researchers' Award in 2020 were Scott W. Lowe, Chair of the Cancer Biology and Genetics Program and the Geoffrey Beene Cancer Research Center at Memorial Sloan Kettering Cancer Center in New York (USA), and Sonia Laín, Group Leader at the *Karolinska Institutet* in Stockholm (Sweden).



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 2020, 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

Donations to the CNIO	255
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Donors to the CNIO 2020	263

CNIO Friends, the landmark philanthropic programme for the CNIO, celebrated our most successful year to date, raising over €813k in philanthropic donations in 2020. The programme grows from strength to strength each year, with more than €2.2m raised in 6 years. Donations to the CNIO enable the Centre to recruit outstanding talent from around the world, and in 2020 we opened a competitive application process for 6 new post-doctoral researchers to explore new ways of diagnosing and treating cancer.

We were particularly delighted to raise 3 generous €100k donations to create 3 new fully-funded post-doctoral contracts; many thanks to *Fundación Humanismo & Ciencia*, *Fundación Domingo Martínez* and the *C&A Together Program* for their commitment to cancer research. We were also pleased to receive a donation from *Fundación Solidaridad Carrefour* to purchase valuable nucleic acid extraction equipment.

For the occasion of World Cancer Day on the 4th of February, we carried out a multi-channel fundraising campaign under the slogan #CNIOStopCancer, in which notable personalities from the artistic world, such as Luz Casal, Christina Rosenvinge, María Hervás and Enrique Arce participated. This campaign highlighted the importance of research as the only way to achieve new and more efficient ways of curing cancer and encouraged people to be part of the solution by joining CNIO Friends. The campaign was carried out thanks to the support of several companies that provided us with their resources and services: Exterior Plus, Act One Producciones, Lanaja Factory, JCDecaux and L’Oreal.

We welcomed Brother Iberia as a donor to CNIO Friends, and continue to receive valuable annual support from Petroplast and Fressia Group. Once again, the CNIO and La Roche-Posay (L’Oréal Group) carried out the *#InvestigaciónEsVida* campaign to recognise World Cancer Day. In addition, the national trade union Fetico organised the “Stop Cancer” event, which was attended by more than 1,200 attendees belonging to some of the biggest companies in Spain.

In 2020, our donors joined us in a slightly different CNIO Friends Meeting Day. Over 100 Friends and their companions joined us for a moving online conference in which Scientific Director Maria Blasco gave an overview of the latest news and major developments at CNIO. Our Friends then had the pleasure of hearing from Rebeca Jimeno, María Moreno, Rubén Martínez, and Paula Martínez on behalf of Sarita Sariswati on the progress of their research projects, funded by CNIO

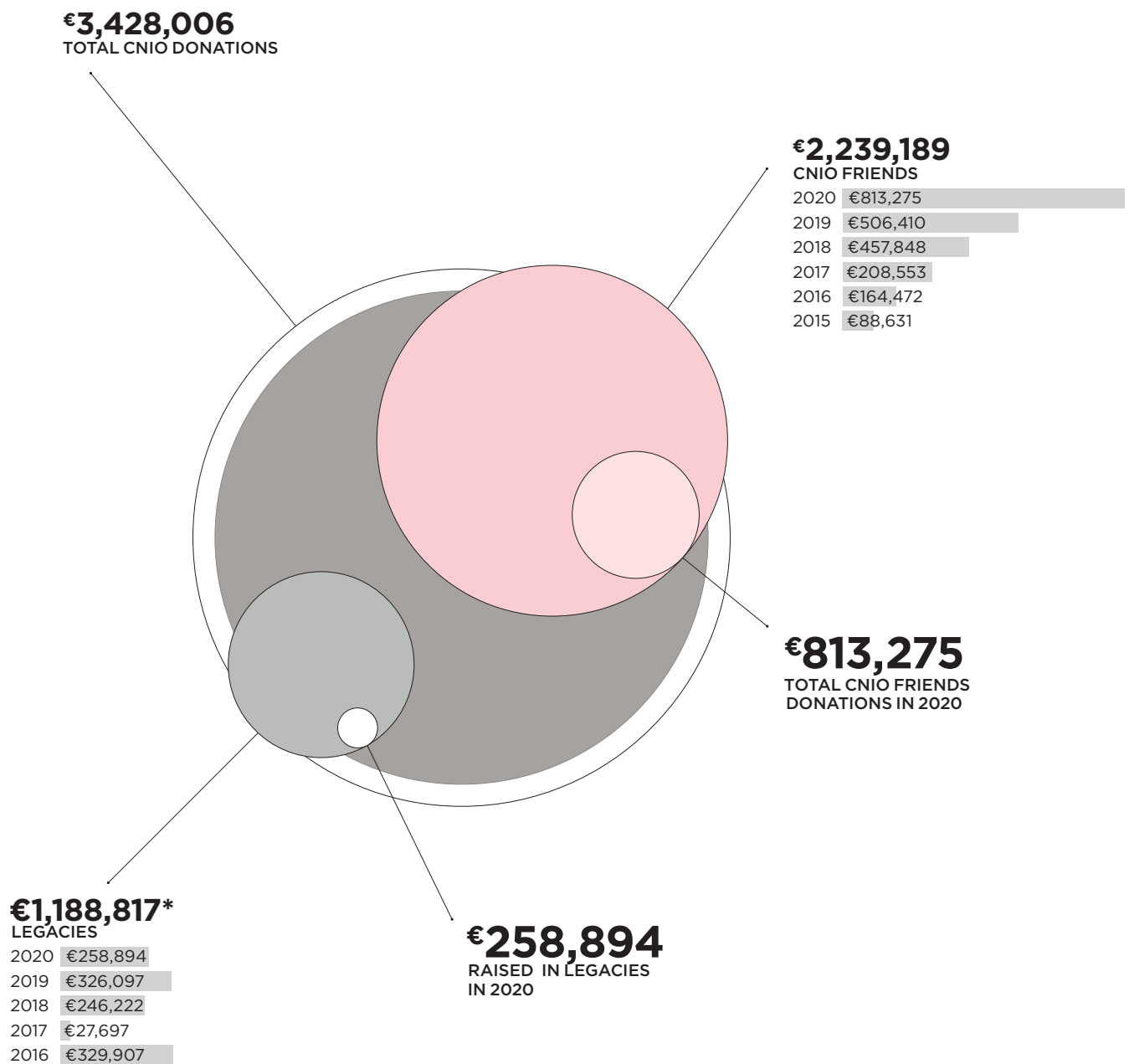
“CNIO Friends marks its most successful year yet, enabling 6 outstanding post-doctoral researchers to join the CNIO.”

Friends. We were then joined by special guest, singer Luz Casal, who spoke with Maria Blasco about solidarity and the initiative she carried out over the lockdown period and then thrilled the audience with a snippet of song.

Another pillar of philanthropy that continues to grow for the CNIO is our legacy programme. This year marks a cumulative total of around €1.2m raised in legacies over 5 years, with €258k raised in 2020 and €527k pending to be executed.

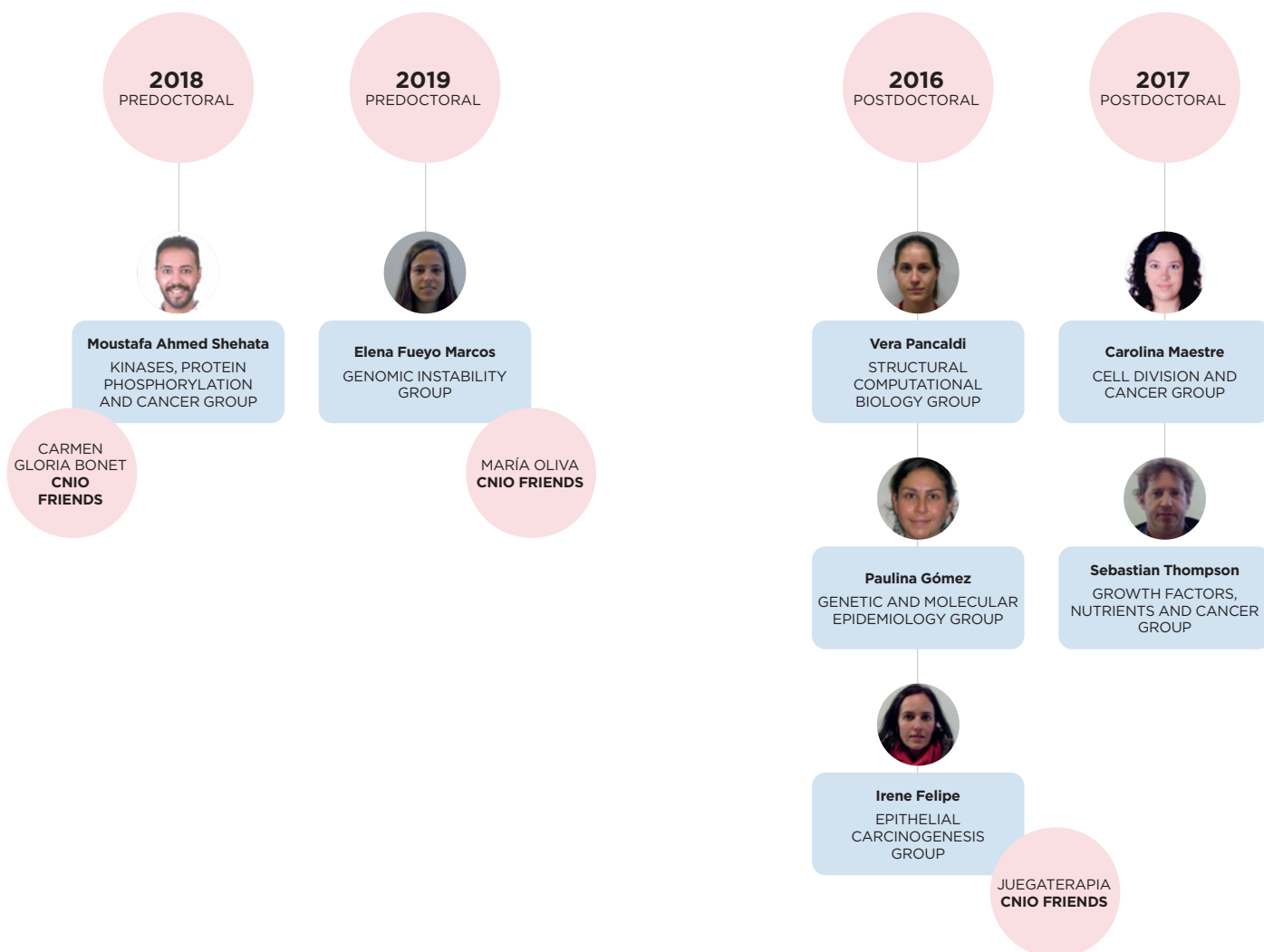
2020 has no doubt been a challenging year for fundraising activities, without any of the usual opportunities to get together, network, meet new donors or host events. However, people have continued to give generously and our CNIO Friends and donors have demonstrated more support and enthusiasm for our work than ever. We would like to take this opportunity to thank our donors once again for their commitment to cancer research. Now, more than ever, it is critical to encourage and support scientific endeavours. Together, we can stop cancer.

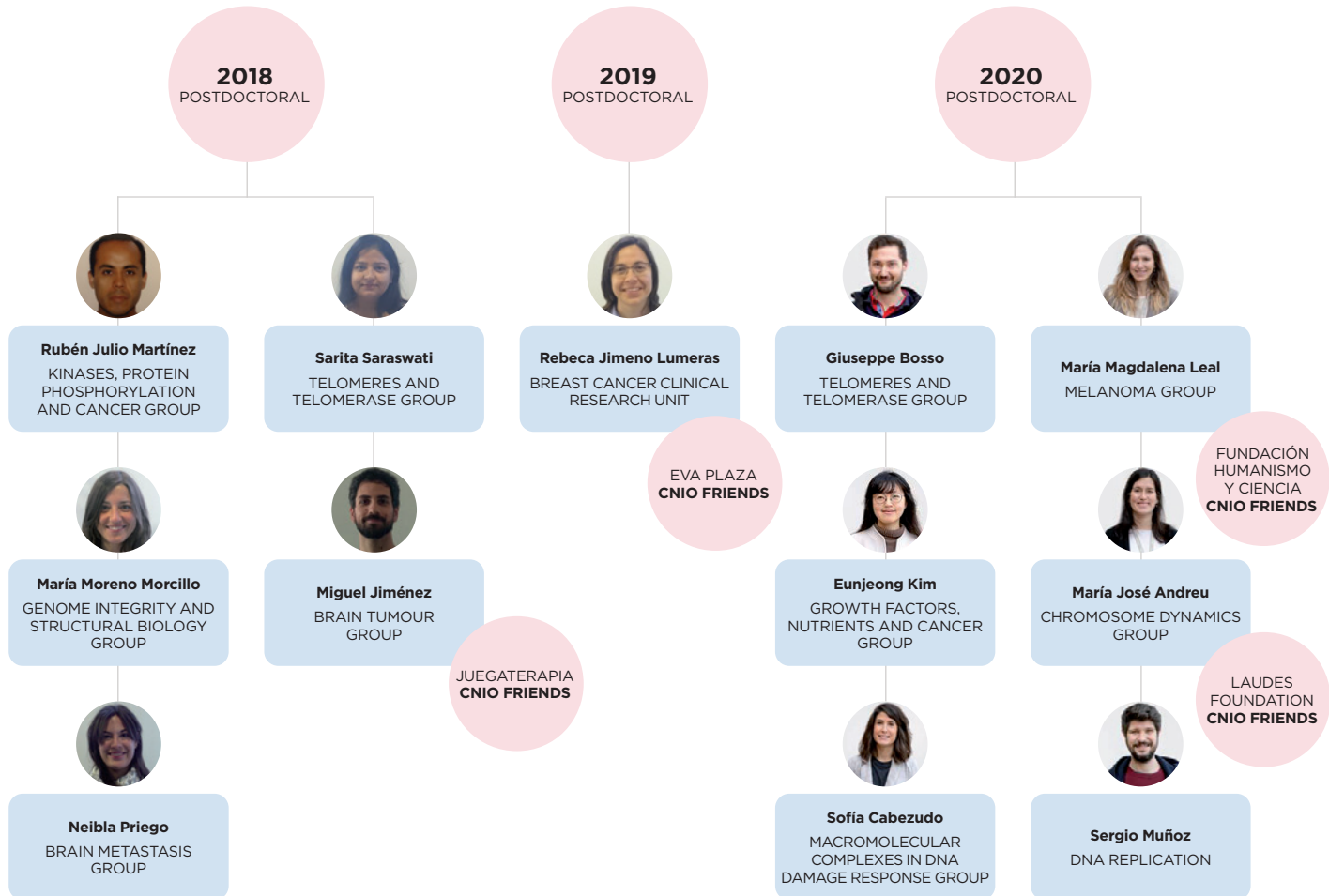
DONATIONS TO THE CNIO



* €527,912 pending to be executed.

'CNIO FRIENDS' INTERNATIONAL RESEARCH CONTRACTS





“FUNDACIÓN HUMANISMO Y CIENCIA- CNIO FRIENDS” CONTRACT

Fundación Humanismo y Ciencia generously funded a 2-year contract to carry out a project for the development of preclinical models for the study of tumours and the design of new therapeutic tools. As a result, the new *Fundación Humanismo y Ciencia* Research Fellow has joined the group of Marisol Soengas to begin an exciting and innovative project on melanoma and immunology in cancer.



“FUNDACIÓN DOMINGO MARTÍNEZ- CNIO FRIENDS” CONTRACT

In celebration of its 50th anniversary, *Fundación Domingo Martínez* signed an agreement to fund a 2-year CNIO Friends postdoctoral contract, which will join the call for 2021. This project will conduct research in cancer via the tumour microenvironment.



CNIO FRIENDS DAY



In a virtual meeting held on July 2, the CNIO Friends, who are made up of all of the philanthropic donors to the CNIO, had the opportunity to learn about the cancer research projects that are being carried out at the Centre thanks to their generous donations. All philanthropic donations to the CNIO go directly to the CNIO Friends Fellowship Programme which enables the CNIO to hire new research talent. The event was a moving opportunity for our donors, also joined by special guest and CNIO Ambassador Luz Casal, to meet the researchers and hear first-hand about their innovative projects.

BROUGHT TOGETHER BY CNIO ARTE



“Nosotros somos los pájaros que se quedan”, Carmen Calvo, 2019. CNIO Arte is an initiative that establishes contact between renowned international scientists and artists to explore common territories between scientific research and artistic creation. In 2020 we welcomed two outstanding figures from the fields of art and science: visual artist Carmen Calvo and paleoanthropologist Juan Luis Arsuaga. The benefits of the sale of the artworks are generously donated to CNIO Friends.

FUNDACIÓN SOLIDARIDAD CARREFOUR VISITS CNIO



On the occasion of International Women's Day in March, the CNIO hosted one of our CNIO Friends, *Fundación Solidaridad Carrefour*, by inviting daughters of their workers for a visit to our labs. This event was an opportunity to work with one of our donors to promote scientific vocations among girls and adolescents, as well as positively changing the cultural and gender stereotypes that exist around science and research.

CNIO-LA ROCHE-POSAY #INVESTIGACIÓNESVIDA CAMPAIGN



LA ROCHE-POSAY
LABORATOIRE DERMATOLOGIQUE

SÚMATE
A LA LUCHA CONTRA EL CÁNCER
#InvestigaciónEsVida
4 DE FEBRERO - DÍA MUNDIAL DEL CÁNCER

cnio stop cancer

EN
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PIEL

La Roche-Posay supported cancer research for the second consecutive year as part of their *#InvestigaciónEsVida* (*#ResearchIsLife*) campaign by making a donation to CNIO Friends. In addition, the collaboration involved the distribution of CNIO leaflets in pharmacies throughout Spain.

DONORS TO THE CNIO 2020

BENEFACTOR FRIENDS

· Alberto Heras Hermida Majadahonda, Madrid	· Guillermo Alonso Borrego Galdakao, Vizcaya
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· Anunciación de los Milagros García Calvo Madrid, Madrid	· Luis David Sanz Navarro Madrid, Madrid
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· **Sergio Recio España**
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· **Vicente Belenguer Tarín**
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· **Asociación Cultural “Certamen Nacional de Nanas”**
Rojales, Alicante

· **Asociación Oncológica Tierra de Barros**
Almendralejo, Badajoz

· **Asociación Recreativo-Cultural de apoyo a personas afectadas de cáncer “Esperanza”**
Burela, Lugo

· **Colectivo de Mujeres Afectadas de Cáncer “Las Supernenas”**
Tomelloso, Ciudad Real

· **Plataforma de Enfermos de Cáncer y Solidaridad**
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|---|---|
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| · Asociación de Mujeres Afectadas de Cáncer de Mama “Rosae”
Valdepeñas, Ciudad Real | · Colectivo de Afectados “El Árbol de la Vida”
Las Pedroñeras, Cuenca |
| · Asociación para la Atención Integral de Afectados de Cáncer
Llerena, Badajoz | |

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- | | |
|--|--|
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Madrid, Madrid |
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Madrid, Madrid |
| · Fetico Federación
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Madrid, Madrid |
| · Fundación Humanismo y Ciencia
Madrid, Madrid | · Petroplast
Logroño, La Rioja |

Last but not least, we would also like to extend our 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.

CREATIVE TEAM

In order to pour the Annual Report into a more creative concept, the CNIO works closely with selected professionals in the artistic and creative sectors who ensure delivery of an end product that is attractive in more ways than one. We extend our

thanks to the creative team, the visual artist Amparo Garrido, and the graphic design studio underbau whose invaluable work created the images and design that illustrate this Annual Report.

AMPARO GARRIDO PHOTOGRAPHY



A Madrid-based visual artist working with photography and video, Amparo Garrido has been represented in individual and group shows both in Spain and abroad since 1998. Her work has been honoured in several prestigious competitions. She obtained the first place in the 2001 edition of the ABC Photography Prize, and second place in the 2007 *Purificación García* Prize. Other honourable mentions include the *Pilar Citoler* and *Ciudad de Palma* prizes. Her work can be found in major collections, including the *Museo Nacional Centro de Arte Reina Sofía* in Madrid, the photographic holdings of the Madrid regional authority, the Coca-Cola Foundation, the *Es Baluard* Museum of Modern and Contemporary Art in Palma de Mallorca, and the Galician Centre of Contemporary Art (CGAC) in Santiago de Compostela, among many others.

Amparo's most recent work, feature film "The Silence that Remains", was selected to be part of the Documentary Feature Film section of the Málaga Spanish Film Festival in its 22nd edition, Málaga 2019, as well as for competition in the 37TFF Torino Film Festival – Best International Documentary section – Torino, Italy 2019. Since then, her feature film has garnered selections at major film festivals including DOXA Documentary Film Festival, Canada, *SanFic*, Chile, *eDOC Festival Internacional de Cine Documental*, Ecuador, Kathmandu International Mountain Film Festival (KIMFF), Nepal, *Suncine* Environmental Film Festival/Human Ecology Award, Spain, among others that have not yet unveiled their film selection.

UNDERBAU DESIGN



Underbau is a design studio that emerged in 2008 from professional designers with 20 years of experience in the field of corporate design, publishing and advertising. From the very beginning, the studio has sought to maintain its primary focus on art and culture, working together with Spanish and international bodies such as the *Orquesta y Coro Nacionales de España*, *Museo Picasso Málaga*, *Fundación de Amigos de Museo del Prado*, *Instituto Cervantes* and *Museo*

Thyssen-Bornemisza. Underbau's total-design approach puts the emphasis on coherency. To achieve that, the studio assumes full responsibility for the entire creative process, from the initial concept to the final product.

Special Thanks to the visual artist Amparo Garrido,
for giving up the rights of the photography for the cover.
This piece belongs to the series: *Tiergarten. A German
Romantic Garden.*

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