

**ANNUAL
REPORT 2019**

ANNUAL REPORT 2019

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“Our commitment to gender equality was consolidated in 2019. The CNIO together with the Works Council elaborated a very ambitious CNIO Equality Plan. This is an exciting time for the Centre and a step forward on the road to gender equality.”

MARIA A. BLASCO
Director

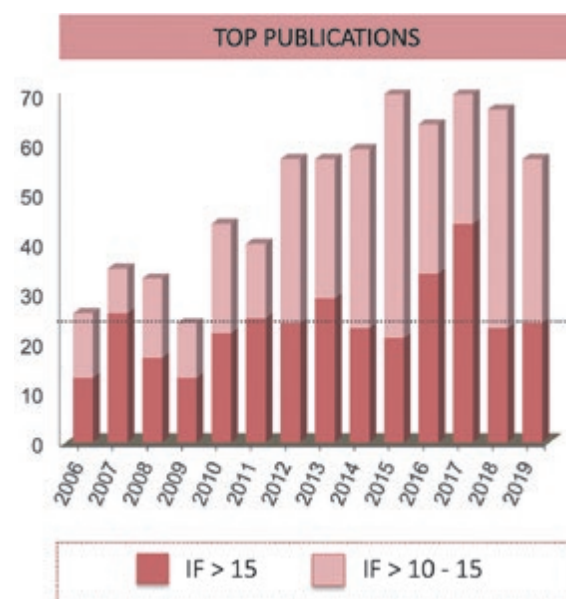
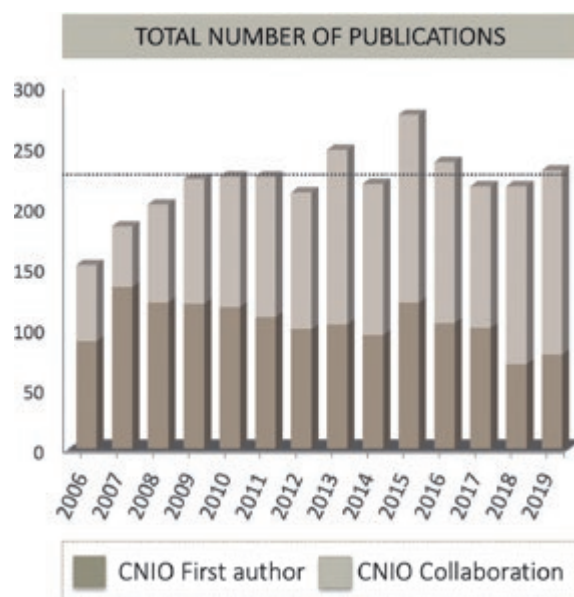
FOREWORD

Maria A. Blasco Director

This year, once again, I am proud to convey how the CNIO has moved forward in so many directions, pushed by the extraordinary work of our people. In our goal to achieve scientific leadership and international impact, we succeeded in 2019 to place the CNIO on the map as one of the top cancer research centres in Europe and worldwide. In 2019, CNIO researchers authored a total of 230 papers, 33 of which were published in journals with impact factor between 10 and 15, and 24 publications in journals with impact factor greater than 15. This year, 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. These indicators are a testimony of our scientific impact on basic and translational cancer research.

Science is constantly evolving, and so do we as a Centre, by incorporating new groups working on new research lines that best complement those already existing. Thus, in 2019 we reinforced our basic research arm by recruiting 2 new Research Groups to the Molecular Oncology Programme: the Transformation and Metastasis Group, led by Eva González Suárez, coming from IDIBELL, Barcelona; and the Topology and DNA Breaks Group, led by Felipe Cortés, formerly at CABIMER-CSIC, Sevilla. Both researchers hold prestigious ERC Consolidator Awards. These 2 Groups will contribute to reinforcing the already prominent role of the CNIO in 2 areas considered as critical in cancer research, namely genome integrity and metastasis.

The Structural Biology Programme also incorporated 2 Junior Research Groups: the Computational Cancer Genomics Group, led by Solip Park, formerly at the Centre for Genomic Regulation, Barcelona; and, towards the end of the year, the Computational Oncology Group, led by Geoffrey John Macintyre, coming from the Cancer Research UK Cambridge Institute, UK. These 2 Junior Groups will focus their efforts on the field of systems biology and computational genomics applied to the study of cancer. During the recruitment process we counted on the unparalleled support of Raúl



Rabadán, Professor at Columbia University, and one of the top international experts in computational genomics. We believe this will constitute one of the key areas for the CNIO in the years to come, and we expect to develop this new expertise at the CNIO, fostering fruitful collaborations with researchers working in other fields within our institution and elsewhere.

Finally, in 2019 Eva Ortega-Paíno was recruited as new Director of the CNIO Biobank, after the retirement in 2018 of the former Director, Manuel Morente. Eva was formerly Scientific Coordinator of Biobank Sweden for the Escania region, bringing to the CNIO a long trajectory and experience in the management of biobanks at the international level.

We wish all these new recruits the best of luck in the development of their scientific projects and professional duties at the CNIO, which will ultimately increase our portfolio of achievements.

As a national centre and a flagship for cancer research in Spain, we strive to achieve the largest societal impact, and to do so we believe in the benefit of creating new bridges with scientists and institutions throughout the world. In 2019, we witnessed how our successful and exemplary partnership with the Weizmann Institute of Science (WIS) and the *Ramón Areces* Foundation bore fruit. We launched our first collaborative project to advance immunotherapy research in melanoma, and we held our second joint symposium on cancer research, hosted by an outstanding group of scientists of the WIS in Rehovot, Israel. This experience is a testimony to how science can also be a driver of diplomacy, nurturing long-term collaborations.

In line with our efforts to provide value to society and increase excellent science visibility, the CNIO further committed to the SOMMa Alliance of Centres and Units of Excellence, by our Director Maria Blasco holding the SOMMa vice-presidency and leading the Outreach workpackage. We committed ourselves to defend the values of all research centres in Spain and to underscore the need to provide the proper ground for science and society prosperity. In 2019, SOMMa's second 100xciencia meeting was held in San Sebastián, creating an exciting environment for engagement with citizens through a science fair and science talks open to the public, oriented towards showing our discoveries and their real-world applications. We hope that those initiatives will have a real impact, so that the research activity carried out in our country can permeate beyond the scientific community to the whole of society.

Another indicator of our societal and scientific impact is the breadth of our innovation activities, in which we also strive to excel every year. In 2019, our efforts in promoting collaborations with industry resulted in 1.7 million euros secured through research agreements, and 2 patent applications in partnership with international biotechnology companies. Our close partnership with Lilly was extended for another 3 years, during

which we expect other collaborations to flourish as a result of this ongoing relationship. Our transference activities for CNIO assets resulted in royalties for CNIO totalling 674,900 euros, the highest amount in the last 3 years, which is in part reinvested in our own research and translational activities. This creates a virtuous cycle that contributes to the generation of new discoveries with an impact on the business ecosystem.

Furthermore, we continue promoting the participation of CNIO investigators in national and international calls from private foundations and public funding agencies supporting projects with a strong innovation component. In 2019 a project focused on the development of a novel cell therapy for diabetes was awarded a *Caixaimpulse*; and this same project was competitively chosen to be part of the programme MIT Global IDEA². This international initiative supports the creation of a broad network and close mentoring with experts from the Boston ecosystem. The CNIO also obtained its first FET-OPEN for the use of novel probes and heat-based approaches for the diagnosis and treatment of brain cancers. Our Experimental Therapeutics Programme continues to co-develop drug candidates from novel targets validated by CNIO investigators, contributing to the generation of new agents to fight cancer and ageing-related diseases, which also represents assets for the CNIO. Our drug discovery pipeline gained additional visibility through our participation in the Milner Therapeutics Symposium, an international forum for research centres of excellence and multinational drug companies, as well as in multiple bio-business forums held in Spain and Europe. Our substantial activity in innovation at the international level reflects the leadership and influence of the CNIO and our researchers worldwide.

Our training programmes are among the key features of the Centre, and an essential part of our mission. Creating new generations of highly prepared researchers is a priority for the CNIO, and we warmly thank our sponsors as they contribute to support all our training initiatives, as well as our collaborations with investigators abroad. During 2019, 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 Researcher Programme. In addition, thanks to the 'Science by Women' programme of the *Mujeres por Africa* Foundation, Hayet Rafa, from the University of Science and Technology Houari Boumediene in Algiers, joined the CNIO's Melanoma Group for a 6-month stay as a visiting scientist.

Societal progress and prosperity is the main reason for our existence, and this is why we are strengthening our initiatives to create the appropriate channels for dissemination and communication with citizens, including youngsters who could evolve into the next generation of scientists. To achieve this goal, our strategy for media relations and communications

plays a pivotal role. In the last two years, our media coverage increased by almost 75%, featuring stories that have a strong impact worldwide, including front pages in widespread national and regional newspapers. But we also want to create science and research awareness among our citizens through actions such as the event ‘New horizons in cancer research: from bench to bedside’, held at CaixaForum Madrid with the support of “la Caixa” to celebrate World Cancer Research Day. In addition, to provide visibility to the obstacles often found by professional women scientists, we ran the social media campaign *#YoRompoTechoCristal* [IBreakTheGlassCeiling], which was spread on social media and picked up by a large number of media outlets. These are only some examples of the activities we set up to bring science closer to the public, and we will continue working on this in the future.

The still young Institutional Image and Outreach Office, created in 2018, is leading several projects that aim to open up new avenues to gain society’s trust and attention as well as emphasise the value of science. Our new website, a portal between the scientific community and society, was awarded an honourable mention in the XXXII *Premios Prismas* of scientific dissemination. In April 2019 CNIO and “la Caixa” celebrated in Madrid’s CaixaForum a roundtable entitled ‘Science rescues Victor Frankenstein. Mary Shelley, lover of Science’, with the discussion moderated by author and screenwriter Fernando Marías, CNIO Director Maria A. Blasco, the writer Lorenzo Luengo, and the novelist, actress and theatre producer Cristina Higuera. In 2019 our CNIO Arte project co-funded by *Banco Santander* Foundation, which explores the common territories between scientific research and artistic creation, brought together the Spanish scientist Ignacio Cirac, Director of the Max Planck Institute, Germany, and the photographer Chema Madoz. The funds obtained from this initiative totalled more than 100,000 euros, which contributes directly to our CNIO Friends philanthropic initiative. In 2019 the first event in the series ‘CNIO Workshop on Philosophy & Biomedical Sciences: Debates on Conceptual and Social Issues’ 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.

In September 2019, CNIO established a new Development and Philanthropy Office aimed at identifying and providing new funding sources that may contribute to the sustainability of the Centre, as well as involving all of society in the mission and activities of the CNIO. The newly established office manages the CNIO Friends initiative and seeks to build new collaborative partnerships with corporate partners, individuals and philanthropic foundations. In 2019, CNIO received 515,000 euros through the ‘CNIO Friends’ platform and 284,000 euros from legacies and bequests. The funding derived from the CNIO Friends initiative since its implementation in 2015 has

allowed us to incorporate 11 postdoctoral fellows and 2 PhD students, and 4 new postdoctoral positions are expected in the near future. We enthusiastically thank all of our CNIO Friends for their support and commitment to cancer research.

Our commitment to gender equality was consolidated in 2019 through the many activities spearheaded by our CNIO Women and Science Office (WISE). This is an effort from CNIO volunteers who have contributed to impactful changes that are facilitating both cultural changes and institutional improvements. As part of these efforts, our WISE office brought outstanding female speakers who inspire and encourage us to explore new perspectives and visions on the role of women in society. The WISE Office also organised at the CNIO, in collaboration with the British Embassy, the 1st event on Equality for the SOMMa Centres and Units of Excellence, on October 29. Several Directors and Managers of the 53 SOMMa centres and units participated in the event, which aimed to share best practices in relation to gender equality, in order to promote in a coordinated and institutional manner the cultural change required to integrate gender equality in any strategic decisions related to science in our country. Finally, the WISE Office contributed, together with the Works Council and the CNIO Direction, to elaborating the CNIO Equality Plan, which was approved by the Delegated Commission of our Board of Trustees in October 2019. This Equality Plan sets up specific objectives expected to be reached in this matter in the short and medium term, as well as the strategies and actions to facilitate their achievement.

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 (‘Tiergarten’ exhibited at *Museo del Romanticismo*, Madrid 2012).

With great pleasure and pride we provide our society once more with a story of our achievements in 2019 and new expectations and visions for the future.



CNIO Arte 2019. Artwork by photographer Chema Madoz.



OSCAR FERNÁNDEZ-CAPETILLO
Vice-Director

“Discoveries made by CNIO scientists are constantly expanding the frontiers of our knowledge, and 2019 was no exception.”

During 2019, our scientists made great contributions towards a better understanding of how cancer originates and its potential treatments. We have revealed new strategies that could help to treat pancreatic tumours and contributed to clinical efforts in testing the efficacy of immunotherapy in lung cancer. We have proven that mutations affecting how cells sense the presence of nutrients can drive carcinogenesis and provided some initial ideas as to how to treat these tumours. We now also know that the synthesis of certain lipids is particularly essential during the initial steps of breast carcinogenesis and developed new biomarkers based on the lymph that could be taken to clinical practice. We have been able to establish primary uroepithelial organoids, which was a long-sought aim in the bladder cancer research and revealed how mutations in the cohesin subunit SA2 might drive carcinogenesis by regulating gene expression. We

have also identified strategies that are particularly toxic for cells with more chromosomes than normal, something very frequent in tumour cells. Besides from cancer, contributions from CNIO scientists often have implications in other areas that are of high relevance for human health. For instance, we have revealed new clues about why different species age at different rates and obtained an atomic-view understanding of some key pathogens, such as those that drive tuberculosis. We also have discovered strategies that increase the survival of mice exposed to ionising radiation, by protecting stem cells. Writing this annual overview is always a gratifying moment of reflection for me, as it reminds me of the reasons that made me join CNIO in the first place. I am in great company. Thanks to you all for your hard work and for making it possible. We should all be proud of where we are.

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 (since August)
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 (since August)
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 (since September)
Computational Cancer Genomics Junior Group

Geoffrey John Macintyre (since December)
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
Gema Moreno Secretary

Javier Benítez
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 and Genome Editing
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

CAROLINA POLA DIRECTOR OF INNOVATION

BIOTECHNOLOGY PROGRAMME

Fernando Peláez Programme Director
Celia María Ramos Secretary

Orlando Domínguez
Genomics Core Unit

Sagrario Ortega
Mouse Genome Editing Core Unit

Giovanna Roncador
Monoclonal Antibodies Core Unit

Francisca Mulero
Molecular Imaging Core Unit

Lola Martínez
Flow Cytometry Core Unit

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 (since June)
Anabel Sanz Head (until April)

BIOBANK

Eva Ortega-Paíno Director (since September)
Miguel Quintela-Fandino Acting Director (until August)

Basic Research

Molecular Oncology	
Programme	18
Telomeres and Telomerase Group	20
Experimental Oncology Group	24
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MOLECULAR ONCOLOGY PROGRAMME

ÓSCAR FERNÁNDEZ-CAPETILLO Programme Director



The Molecular Oncology Programme (MOP) is the largest research programme at the CNIO, hosting 11 Senior and 4 Junior Groups. Scientists at the MOP focus on trying to obtain a mechanistic understanding of how cells in our body work, as well as to identify the molecular alterations at the cellular level that drive carcinogenesis. In addition, they try to generate new ideas and strategies to combat the disease. To do so, MOP Research Groups use a wide range of technologies including molecular and cellular biology, mouse models, genomics and patient material. The Programme encompasses expertise related to some of the most active areas of research in molecular oncology, including DNA and chromosome stability (Maria A. Blasco, Óscar Fernández-Capetillo, Massimo Squatrito 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 of the bladder and pancreas (Francisco X. Real); growth factors, metabolism and cell signalling (Nabil Djouder and Alejo Efeyan); and metastasis (Manuel Valiente and Héctor Peinado). During 2019, 2 additional Senior Groups joined the MOP: the Transformation and Metastasis Group, led by Eva González Suárez; and the Topology and DNA Breaks Group, led by Felipe Cortés Ledesma – both supported by an ERC Consolidator grant. My warm welcome to both, and best wishes for a bright future at CNIO.

In terms of scientific publications, this year once again, the Molecular Oncology Programme has continued to be on the frontline of oncology research. The top-level quality of the

“Many of our scientists are making an effort to translate their discoveries into therapies. We are not there yet, but I am firmly convinced that we are advancing on solid grounds. Let 2020 be our year.”

research conducted by each of these Groups is exemplified through 11 papers published in *Nature* journals (*Nature*, *Nature Cell Biology*, *Nature Communications*, *Nature Genetics*, *Nature Medicine*, *Nature Metabolism*), 3 papers in *Cell* Journals (*Cancer Cell*, *Molecular Cell*) and 1 paper in *Science*; additional excellent contributions were made to *Annals of Oncology*, *EMBO Journal*, *EMBO Molecular Medicine*, *European Urology*, *Gut*, *iScience*, *JNCI*, *The Journal of Clinical Investigation*, *The Journal of Clinical Oncology*, and *The Journal of Experimental Medicine*.

Scientists at the MOP are at the forefront of their respective fields of research and, besides from their publications, they contribute to, participate in and/or lead multiple scientific events aimed at both specialists and the general public.

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, Sergio



Piñeiro, Sarita Saraswati, Kurt Whittemore (until Aug.)

Graduate Students
Leire Bejarano (until July), Iole Ferrara (until Oct.), José Carlos González, Paula Infantes (since

Sept.), Jessica Louzame, Juan José Montero, Miguel A. Muñoz (until June), Raúl Sánchez

Technicians
Aksinya Derevyanko (until June),

Ana Guío (since Feb.) (TS)*, Rosa M. Serrano

Visiting Students
Tommaso Vicanolo (until Sept.) (*Università di Roma Sapienza*),

Omkar Vinchure (April-June) (Indian Institute of Technology Delhi), Nora Giello (March-Aug.) (*Università degli Studi Napoli Federico II*)

**Titulado Superior* (Advanced Degree)

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.
- The interplay between telomeres and DNA repair pathways.
- The role and regulation of non-coding telomeric RNAs or TERRA.
- Testing telomerase gene therapy in ‘telomere syndromes’ and age-related diseases.
- The role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

“Our studies showed that combination therapy with TRF1 inhibitors might become an effective strategy to inhibit cancer growth and combat drug resistance in glioblastoma and other tumours.”

RESEARCH HIGHLIGHTS

New effective drug combinations for glioblastoma in mice

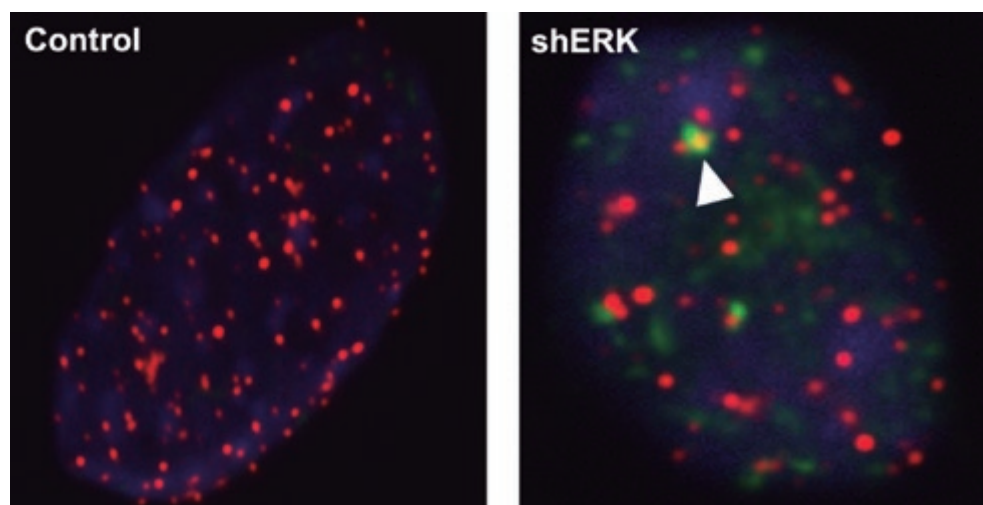
Since the maintenance of telomeres, the eukaryotic chromosome ends, is essential to sustain cancer cell growth, they are considered a universal anti-cancer target. Telomeres are protected by the shelterin complex; targeting the shelterin complex may be an effective anti-cancer strategy. Glioblastoma is the most aggressive brain tumour, with very poor prognosis. Patients with glioblastoma usually develop resistance to treatments. We had previously shown that genetic and chemical inhibition of the shelterin protein TRF1 impairs tumour growth in mouse and patient-derived xenograft models of lung cancer and glioblastoma. Resistance to TRF1 chemical inhibitors eventually occurs, resulting in TRF1 re-expression and tumour growth. New therapeutic approaches are needed to block glioblastoma growth. We have now screened a collection of FDA-approved drugs and drugs in clinical trials. We found that inhibition of several kinases of the RAS cancer pathway, including ERK and MEK, deactivates TRF1 (FIGURE 1).

This highlights the potential of combination therapies based on TRF1 inhibition as a promising therapeutic strategy to overcome drug resistance and effectively block glioblastoma growth.

Regulation of the transcriptional landscape of pluripotent cells

Pluripotent cells can give rise to all cells of the body; harnessing pluripotency may therefore open the door to regenerative medicine and organ culture for transplants. The genetic and epigenetic mechanisms that regulate pluripotency remain unknown. We have now uncovered one of the epigenetic signals controlling pluripotency that explains the observed powerful connection between pluripotency and telomeres. We found that the shelterin protein TRF1 regulates the genome-wide binding of polycomb and polycomb H3K27me3 repressive marks to pluripotency genes, thereby exerting epigenetic changes that contribute to the maintenance of mouse embryonic stem

Figure 1 Telomeres (red) in mouse embryonic fibroblasts; DNA damage (green) caused by ERK inhibition. ERK is an enzyme in the RAS pathway whose inhibition leads to TRF1 deactivation. Arrow shows telomere damage.

**PUBLICATIONS**

- ▶ Callen E, Zong D, Wu W, Wong N, Stanlie A, Ishikawa M, Pavani R, Dumitrache LC, Byrum AK, Mendez-Dorantes C, Martinez P, Canela A, Maman Y, Day A, Kruhlak MJ, Blasco MA, Stark JM, Mosammaparast N, McKinnon PJ, Nussenzweig A (2019). 53BP1 enforces distinct pre- and post-resection blocks on homologous recombination. *Mol Cell*. PMID: 31653568.
- ▶ Muñoz-Lorente MA, Cano-Martin AC, Blasco MA (2019). Mice with hyper-long

telomeres show less metabolic aging and longer lifespans. *Nat Commun* 10, 4723.

- ▶ Bejarano L, Bosso G, Louzame J, Serrano R, Gómez-Casero E, Martínez-Torrecuadrada J, Martínez S, Blanco-Aparicio C, Pastor J, Blasco MA (2019). Multiple cancer pathways regulate telomere protection. *EMBO Mol Med* 11, e10292.
- ▶ Whittemore K, Vera E, Martínez-Nevado E, Sanpera C, Blasco MA (2019). Telomere shortening rate predicts species life span. *Proc Natl Acad Sci U S A* 116, 15122-15127.
- ▶ Marión RM, Montero JJ, López de Silanes

I, Graña-Castro O, Martínez P, Schoeffer S, Palacios-Fábrega JA, Blasco MA (2019). TERRA regulate the transcriptional landscape of pluripotent cells through TRF1-dependent recruitment of PRC2. *Elife* 8, pii: e44656.

- ▶ Whittemore K, Derevyanko A, Martínez P, Serrano R, Pumarola M, Bosch F, Blasco MA (2019). Telomerase gene therapy ameliorates the effects of neurodegeneration associated to short telomeres in mice. *Aging (Albany NY)* 11, 2916-2948.
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cells (ES) in a naïve state. TRF1 mediates these effects by regulating TERRA, the telomeric RNAs. We propose a model in which TRF1-dependent changes in TERRA levels modulate polycomb recruitment to pluripotency and differentiation genes, explaining why TRF1 is essential for the induction and maintenance of pluripotency (FIGURE 2).

Telomere rate of shortening predicts species life span

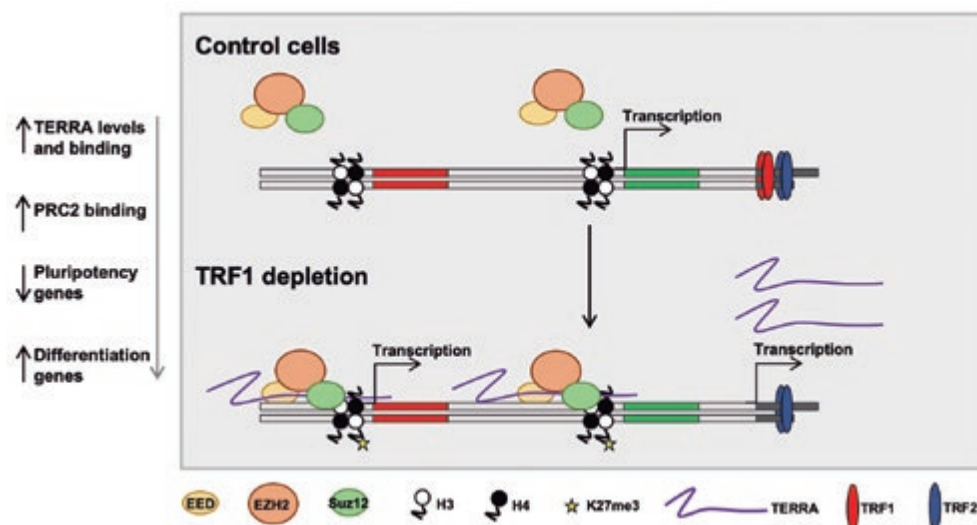
The shortening of telomeres below a critical length can trigger ageing and shorter life spans in mice and humans by induction of a persistent DNA damage response at the telomeres and loss of cellular viability. We set to determine whether telomere shortening can be a single parameter to predict species longevities. To this end, we measured in parallel the telomere length of a broad variety of species (birds and mammals) of very different life spans and body sizes, including mouse, goat, Audouin's gull, reindeer, griffon vulture, bottlenose dolphin, American flamingo, and Sumatran elephant. We found that the telomere shortening rate, and not the initial telomere length alone, is a powerful predictor of species life span. Critical telomere shortening and the consequent onset of

telomeric DNA damage and cellular senescence are a general determinant of species life span.

Less metabolic ageing and longer life spans in mice with hyper-long telomeres

Short telomeres in mice and humans trigger age-related pathologies and shorter life spans. We generated mouse ES cells with longer telomeres than normal (hyper-long telomeres) in the absence of genetic manipulations, which contributed to all mouse tissues. We have now obtained mice in which 100% of their cells derive from hyper-long telomere ES cells and observed that they have longer telomeres and less DNA damage with ageing. Hyper-long telomere mice are lean, show low cholesterol and LDL levels, improved glucose and insulin tolerance, and have less cancer and increased longevity. Thus, longer telomeres than normal in a given species are not deleterious, showing instead beneficial effects such as increased longevity, delayed metabolic ageing and less cancer. ■

Figure 2 In normal induced pluripotent stem cells TRF1 is highly expressed, the Polycomb (PRC2) complex (EED, EZH2 and Suz12) binds weakly to the genome and pluripotency genes are expressed. After TRF1 depletion, TERRA expression increases leading to PRC2 recruitment to genes involved in pluripotency and differentiation control and establishment of the K27me3 epigenetic mark, altering their expression.



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AWARDS AND RECOGNITION

- ▶ Member of the Advisory Board of the Spanish Ministry of Health and Social Services, Madrid, Spain.
- ▶ Vice-President of SOMMa ("Severo Ochoa" Centres and "María de Maeztu" Units of Excellence Alliance), Spain.
- ▶ Member of the Board of Trustees of the Museo Nacional del Prado, Madrid, Spain.

EXPERIMENTAL ONCOLOGY GROUP

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Musteanu



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Carolina Navas, Guillem Paniagua

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Fernández, Jing Li, Vasiliki Liaki,

Lucía Morales, Marina Salmón,
Manuel Sanclemente

Visiting Graduate Student

Xiao-Xu Zhu (*Sun Yat-sun
University, Guangzhou, China*) (until
September)

Technicians

Irene de Diego (since March), M.
Carmen González (TS)*, Silvia
Jiménez, Marta San Román, Raquel
Villar

**Titulado Superior* (Advanced Degree)

External Associates

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(*Universidad Autónoma de Madrid*),
Juan Velasco (*Lilly Spain*)

OVERVIEW

KRAS oncogenes have been identified in one fifth of all human cancers. Yet, no selective inhibitors have been approved so far. Moreover, attempts to block *KRAS* oncogenic activity with selective inhibitors of *KRAS* signalling pathways have failed so far due to unacceptable toxicities. In our laboratory, we have continued our quest to validate therapeutic targets for *KRAS* driven lung and pancreatic tumours using a new generation of genetically engineered mouse tumour models that allow us to evaluate their anti-tumour properties as well as their potential toxic effects in tumour-bearing mice. These studies have allowed the identification of RAF1 as the only target within the MAPK signalling pathway capable of inducing significant tumour regressions in advanced *KRAS*/TRP53 mutant tumours without inducing major toxicities. We are now focusing our research interests on: (i) devising therapeutic strategies to selectively targeting RAF1 and (ii) identifying additional targets that may cooperate with RAF1 inhibition, with the ultimate goal of translating these results to the clinic.

“We have achieved, for the first time, the complete regression of a significant percentage of advanced *K-Ras*/*Trp53* mutant pancreatic ductal adenocarcinomas by inducing the systemic ablation of combined RAF1 and EGFR expression.”

RESEARCH HIGHLIGHTS

Complete regression of advanced pancreatic ductal adenocarcinomas upon combined inhibition of EGFR and RAF1 expression

We have developed a new GEM strain that separates temporally and spatially tumour development from target ablation/inhibition. This strain, *K-Ras^{+/FSPG 12V};Trp53^{flr/flr};Elas-tTA/TetO-FlpO*;Tg.h*UBC*-CreERT2, designated as KPeFC, incorporates 2 distinct recombinases, FlpO and CreERT2. FlpO is responsible for tumour induction, whereas expression of the tamoxifen (TMX)-inducible CreERT2 recombinase is driven by the human Ubiquitin C promoter (h*UBC*). Thus, exposure of KPeFC mice to a TMX-containing diet allows the systemic recombination of any conditional floxed allele added to this strain. We used this strain to interrogate the therapeutic properties of ablating RAF1 and EGFR based on previous observations using initiation models. Tumour-bearing KPeFC;*Egfr^{lox/lox};c-Raf^{lox/lox}* mice were exposed to a TMX-containing diet. Eight of 12 KPeFC;*Egfr^{lox/lox};c-Raf^{lox/lox}* mice displayed a rapid decrease in tumour volume upon TMX exposure. Six of these mice became tumour-free by micro-ultrasound analysis after 6 weeks of TMX exposure. Four mice were sacrificed after 6 weeks of TMX exposure, whereas the remaining animals were allowed to survive for 10 additional weeks. No tumour reappearance was observed during this time period. Moreover, detailed histological examination of their pancreata revealed normal tissue architecture, indicating that these tumours had completely regressed. Many therapies fail in the clinic due to unacceptable toxic effects. However, systemic deletion of EGFR and RAF1 expression led to acceptable toxicities, all induced by loss of EGFR expression, mainly involving skin alterations such as hyperplasia and disorganisation of the epidermis, hyperkeratosis, folliculitis and inflammation with increased numbers of mast cells and significant hair loss. These skin defects are highly reminiscent of the acneiform rash and folliculitis observed in human patients treated with EGFR inhibitors.

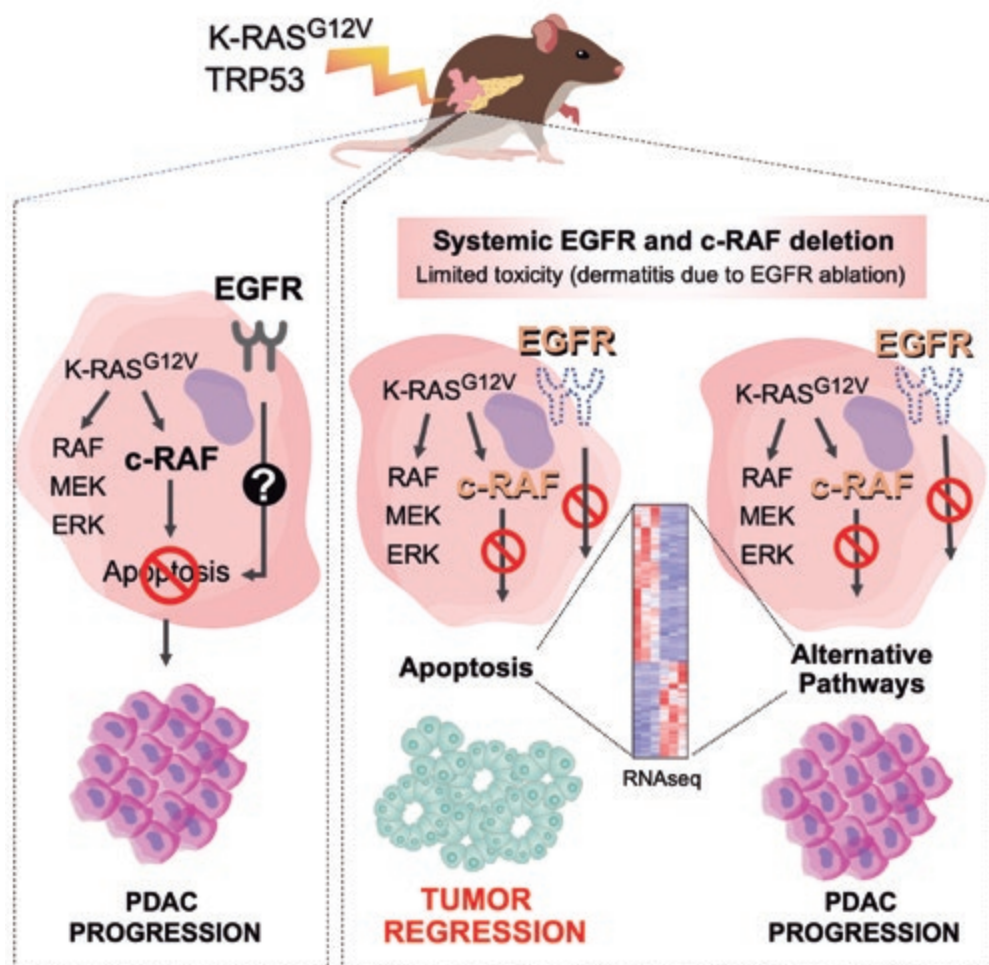
Tumours present in 2 mice that initially regressed started to grow rapidly after 6 to 8 weeks of TMX exposure, killing them 4 to 5 weeks later. Western blot analysis of tumour tissue revealed the absence of EGFR and c-RAF expression, indicating that tumour progression was not due to incomplete recombination but to additional changes that made them resistant to RAF1 and EGFR ablation. Tumours present in 4 KPeFC;*Egfr^{lox/lox};c-Raf^{lox/lox}* mice did not respond to the TMX diet. Histopathological analyses did not reveal significant differences with tumours present in control animals. Surprisingly, these tumours retained active MAPK and PI3K/AKT signalling pathways even in the absence of EGFR or RAF1 expression. Transcriptional analysis of tumour cells derived from tumours that did not

respond to EGFR and RAF1 ablation revealed very distinct transcriptional profiles with more than 2,000 differentially expressed genes when compared with those tumours that regressed upon RAF1 and EGFR ablation.

Gene Set Enrichment Analysis (GSEA) identified several pathways enriched in the resistant tumour cells, including those corresponding to 'E2F targets', 'EMT' and 'MYC targets'. Other enriched pathways included the 'PI3K/AKT/mTOR' and 'IL6/JAK/STAT3' signalling pathways. Comparison of data obtained by RNAseq analysis with a transcriptional classification of human PDACs (Bailey *et al.*, 2016) revealed that NC cells displayed a transcriptional profile most similar to the 'squamous subtype'.

Finally, we determined whether combined inhibition of EGFR and c-RAF signalling could provide therapeutic benefit to PDAC patients. To this end, we knocked down their expression in cells derived from 10 PDX tumour models harbouring *K-RAS* and *TRP53* mutations. Individual knockdown of EGFR or c-RAF expression reduced their proliferative properties to various extents. More importantly, combined knockdown of EGFR and c-RAF expression completely interfered with the proliferative capacity of those cells derived from 9 out of the 10 PDX tumour models. Four of the PDX-derived tumour cells that fully responded to EGFR or c-RAF knockdown were injected into immunocompromised mice. Again, only the combined knockdown of EGFR and c-RAF effectively inhibited growth of these human PDAC tumour cells *in vivo*. These observations suggest that combined inhibition of EGFR and c-RAF expression may have significant therapeutic activity in human PDAC tumours. ■

Figure Schematic representation of the therapeutic strategy used to induce complete regression of a significant percentage of K-RAS/TP53 driven pancreatic ductal adenocarcinomas.



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- Cancer Discovery 9: 623 (2019).
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- Barbacid M, Guerra C, Blasco MT, Navas C. Combined therapy against cancer. *PCT/EP2019/069908*.

AWARDS AND RECOGNITION

- Honorary Member, Iberoamerican Academy of Pharmacy, Granada.

- Honorary Member, Spanish Society of Biochemistry and Molecular Biology (SEBBM), Madrid.
- Averroes Gold Award, Córdoba.
- ECO Award to a Career in Oncology Research, ECO Foundation, Madrid.
- Health Award (*Premio a la Salud*), Fundación Gala, Madrid.
- Best researcher of 2019 (*Investigador del año*), *La Razón*, Madrid.
- Keynote speaker, BioCity Turku Frontiers of Science, Turku, Finland.
- Chair, RAS-Targeted Drug Discovery Summit, Boston, USA.
- Opening Lecture, NCRI Cancer Conference, Glasgow, UK.
- Keynote Lecture, CIBERONC workshop on Preclinical Models for Translational Research, Barcelona.

CELL DIVISION AND CANCER GROUP

Marcos Malumbres
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(until January) (PEJ, CAM)**,
Elisabet Zapatero (TS)*

**Titulado Superior* (Advanced Degree)

***Plan de Empleo Joven de la Comunidad
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Community of Madrid)

(*Imagine - Institut des Maladies
Génétiques*, France), Senn
Wakahashi (*Kobe University*, Japan)

Visiting Scientists

Andrzej Cwetsch (May-July)

OVERVIEW

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

“We have investigated the implication of cell cycle kinases such as CDK4/6 and MASTL in cell proliferation and metabolism, and their relevance as therapeutic targets in breast and pancreatic cancer.”

RESEARCH HIGHLIGHTS

New applications of inhibiting CDK4/6 kinases in cancer therapy

CDK4/6 inhibitors have recently been approved for the treatment of advanced breast cancers, and inhibiting CDK4/6 activity is considered an attractive therapeutic intervention for multiple malignancies. However, it is generally assumed that these inhibitors should not be used in combination with classical chemotherapy seeing as 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. This is a major problem for cancer patients, as the standard of care for most patients with advanced disease is 'classical' chemotherapy (DNA damaging agents, topoisomerase inhibitors, taxanes etc.).

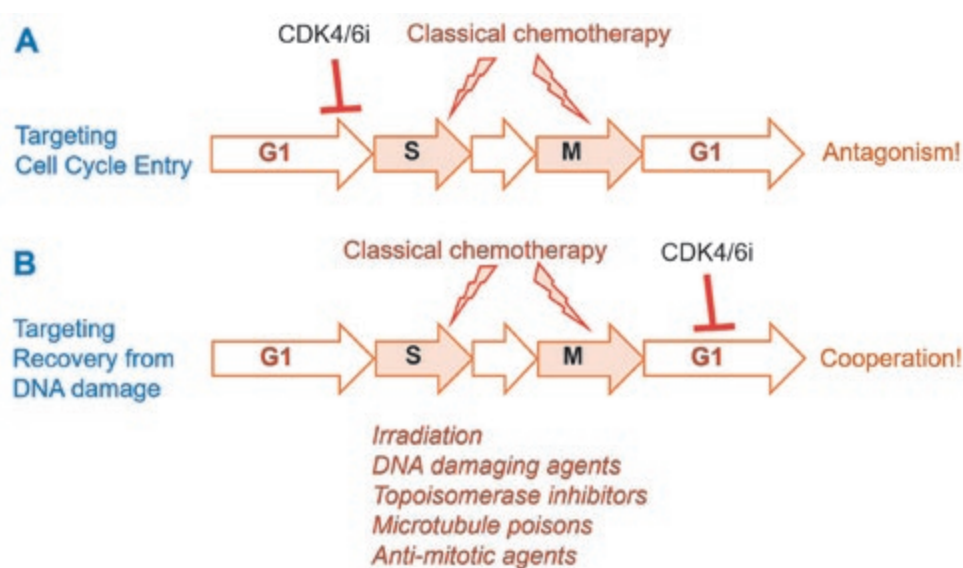
Using pancreatic adenocarcinoma (PDAC) as a model, in collaboration with M. Hidalgo (CNIO and Cornell University, NY, USA) and D. Shields (Pfizer, USA), we generated data suggesting that, both *in vitro* and *in vivo*, applying CDK4/6 inhibitors right after taxanes strongly cooperates to prevent tumour cell proliferation. We have also demonstrated that the mechanism behind these observations is the requirement for CDK4/6 activity in homologous recombination, which is required for recovery from the chromosomal damage imposed by taxanes. 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 cancer patients.

MASTL: a cell cycle kinase with new roles in the control of metabolism

Work in the last few years has established a new cell cycle regulatory module involving the protein kinase MASTL, also known as Greatwall in flies or *Xenopus*, in the control of PP2A phosphatase activity. This regulation has been shown to be critical for maintaining CDK1-mediated phosphorylation of mitotic substrates, and MASTL is now considered an essential cell cycle kinase of relevance in cancer therapy. Our recent data suggest an unexpected connection between MASTL and the feedback loop that controls AKT activity in response to maintained mTOR/S6K signalling. This feedback loop is an essential component of mTOR signalling to prevent excessive AKT activity upon continuous nutrient signalling and has major implications in multiple AKT-mTOR-downstream processes. MASTL ablation impairs the proper regulation of this feedback loop, resulting in hyperactivation of AKT. This work not only adds a new parallel pathway that restricts phosphatase activity upon mTOR/S6K signalling, but also provides a molecular mechanism for our *in vivo* data showing increased glucose tolerance in MASTL-deficient mice. The possibility of modulating MASTL-PP2A activity in some

Figure 1 CDK4/6 inhibitors (CDK4/6i) impair entry into the cell cycle thus antagonising the cytotoxic effect of classical chemotherapy (A). However, sequential use of CDK4/6i after chemotherapy (B) potentiates the cytotoxic effect of many drugs that are the standard-of-care in a wide variety of metastatic cancers.



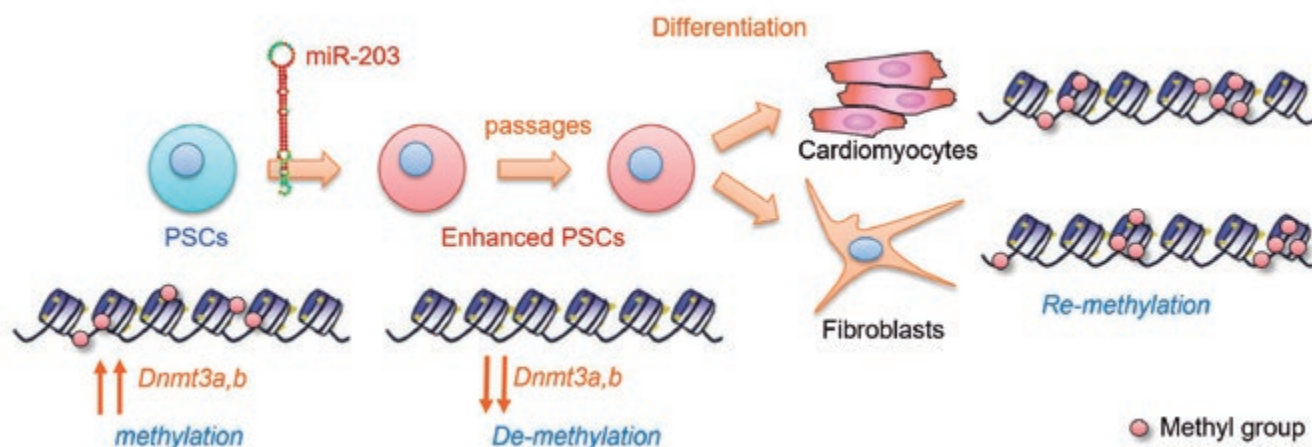


Figure 2 Expression of miR-203 leads to direct repression of Dnmt3a and Dnmt3b and genome-wide demethylation of DNA in pluripotent

stem cells (PSCs), thereby favouring the generation of differentiated cells of interest in regenerative medicine.

metabolic disorders like diabetes or obesity will be studied in the lab during the upcoming months.

Understanding pluripotency and improving the use of pluripotent cells in regenerative medicine

How pluripotent cells control their self-renewal and differentiation potential is a major topic of research in our lab. Our recent work suggests that a microRNA expressed in early development, miR-203, is able to induce naive pluripotency in both murine and human iPSCs and ESCs, 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. The miR-203-mediated resetting of the epigenetic memory improves the developmental potential of both iPSCs and ESCs, mouse and human, with important potential implications in regenerative medicine. ■

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PATENT

- Malumbres M, Salazar-Roa M, Trakala M, Álvarez-Fernández M. Method for expanding Stemness and Differentiation potential of pluripotent cells. *WO2018/215662. National Phase Entry.*

AWARDS AND RECOGNITION

- Editorial Board Member, *PLoS Biology*.

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Pablo Valledor

Technicians

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(TS)*, Sara Rodrigo

**Titulado Superior (Advanced Degree)*

Visiting Master Student

Judith Mateos (January-June)
(Universidad Autónoma de Madrid)

OVERVIEW

In the Genomic Instability Group, our aim is to obtain a mechanistic understanding of cancer and other age-related diseases, and then use this information to develop new therapies. To this end, we combine molecular and cellular biology approaches with chemical and genetic screens. In addition, we also develop mouse models of disease, which we can later use as platforms for testing new treatments. Using these tools, in recent years, we have made exciting discoveries in several areas such as mechanisms of resistance to cancer therapies, targeting nucleolar stress in cancer, drug development and neurodegeneration. Ultimately, our objective is to find new or better treatments for human disease.

“In 2019, we have further invested in the technologies to conduct forward genetic screens in mammalian cells and revealed a new mechanism of action for USP7 inhibitors that are being developed as anticancer agents.”

RESEARCH HIGHLIGHTS

Targeting cells with higher ploidy

Haploid organisms, such as yeast, have for the majority of the 20th century been the model of choice to conduct forward genetic screenings, the reason being that mutations in 1 allele frequently suffice to reveal a phenotype. Because many biological questions are intrinsic to mammals, attempts to isolate and grow haploid mammalian cells in culture initiated in the 1960s, and were finally developed in the last decade. However, despite their usefulness, haploidy remains an unstable state in mammalian somatic cells as these cultures rapidly become enriched in diploids. A few years ago, we revealed that the so-called ‘diploidization’ was rather a selective loss of haploid cells due to the activation of a p53-dependent response. Based on this property, we have now performed a chemical screen in human haploid cells aiming to identify compounds that facilitate the maintenance of haploid cells in culture. Our search identified that 10-Deacetyl-baccatin-III, a precursor in the synthesis of taxol, could do so. Remarkably, this compound not only selects for haploidy, but also presents a general capacity to select for diploid cells in mixed cultures of diploid and tetraploid cells (FIGURE 1). Altogether, this study has identified a compound that solves the problem of ‘diploidization’ of haploid mammalian cell lines and has revealed a general strategy to select for cells with lower ploidy in mixed cultures of mammalian cells.

USP7 counteracts the activation of the mitotic programme

Chemical inhibitors of the deubiquitinase USP7 are currently being developed as anticancer agents due to their capacity to upregulate the tumour suppressor P53. However, besides from this activity, USP7 inhibitors also generate DNA damage in a p53-independent manner, and the actual molecular mechanism underlying its genotoxicity remains unknown. We previously reported that USP7 has an essential role to ensure replication progression, and thus USP7 inhibitors prevent the completion of this process. Our more recent results illustrate that this effect occurs concomitantly with a premature activation of mitotic kinases, such as CDK1, throughout the cell cycle, which impairs chromosome condensation and is toxic for mammalian cells (FIGURE 2). This year, we have delved into the molecular mechanisms triggering this premature process and found that USP7 deubiquitinase works through the interaction with the phosphatase PP2A, which, in turn, is one of the proteins involved in the control of the mitotic activation programme. Supporting our model, the toxicity of USP7 inhibitors is alleviated by lowering CDK1 kinase activity or activating PP2A phosphatase. Our results provide new information regarding the regulation of the mitotic activation programme and are the first example of triggering premature mitosis through the perturbation of ubiquitin signalling. Additionally, these studies reveal that the anticancer properties of USP7 inhibitors are actually related to the premature activation of the mitotic machinery in cells that have not yet fully replicated their genome. ■

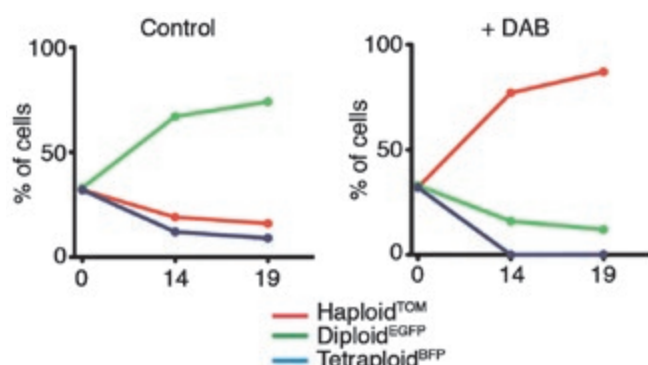


Figure 1 DAB selects for cells with lower ploidy. The figure represents the effects of 10-Deacetyl-baccatin-III (DAB) in mixed cultures of human HAP1 cells of different ploidy. In control conditions (left),

haploid and tetraploids grow less than diploids. However, a treatment with DAB (right) exerts ploidy dependent toxicity so that in its presence selection of haploid cells occurs.

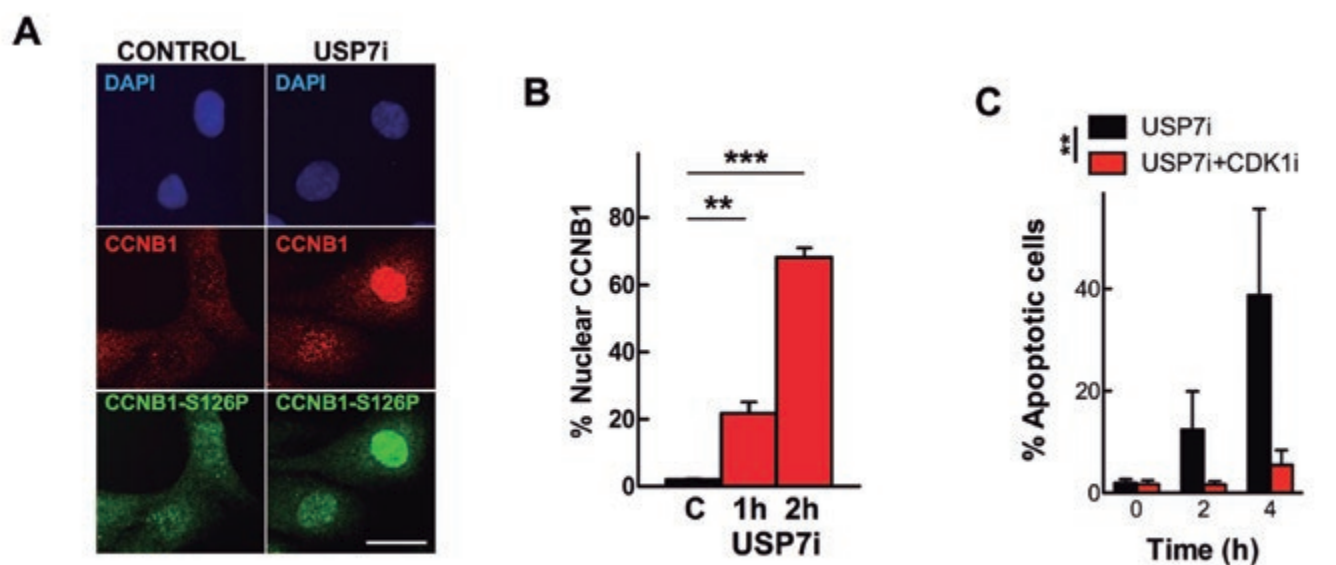


Figure 2 CDK1 mediates the toxicity of USP7 inhibitors. USP7 inhibitors lead to a generalised activation of CDK1 exemplified, for instance, by the nuclear accumulation of cyclin B1

(CCNB1), phosphorylated in a CDK1-dependent site (**A**, **B**). Consistently, the toxicity of USP7 inhibition is alleviated by the concomitant inhibition of CDK1.

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TOPOLOGY AND DNA BREAKS GROUP

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Ernesto López (since October)



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Marta Muñoz (since November)
(TS)*

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**Titulado Superior* (Advanced Degree)

OVERVIEW

DNA topoisomerases have a dual relationship with the genome. They are essential to solving 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: (i) how DNA topoisomerase activity is regulated to integrate different aspects of genome dynamics; (ii) how an imbalance in these processes can lead to the appearance of pathological DNA breaks; and (iii) 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 cancer development, especially in cancer predisposition human genetic syndromes such as Ataxia Telangiectasia.”

RESEARCH HIGHLIGHTS

Work of the Group at the CNIO in these last few months since starting in September 2019 has focused on finishing data analysis to establish a link between a deficient response to topoisomerase II (TOP2)-induced DNA double strand breaks (DSBs) and spontaneous cancer predisposition. Specifically, we have worked with animal models of Ataxia Telangiectasia (AT), a human genetic syndrome that results from loss-of-function mutations in *ATM*.

The ATM kinase is a master regulator of the DNA damage response to DSBs and a well-established tumour suppressor. Loss-of-function mutations in the gene are not only found frequently in many types of cancer, but also constitute the underlying cause of the neurodegenerative and cancer-prone genetic syndrome Ataxia Telangiectasia. AT patients are particularly predisposed to develop lymphoid cancers, which are thought to arise from inefficient signalling and inaccurate repair of RAG-induced DSBs during V(D)J recombination, and which the *Atm*^{-/-} mouse models recapitulate in the form of very aggressive T-cell malignancies.

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 in 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 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. ■

Figure 1 TDP2 deficiency increases thymic cancer predisposition of *Atm*^{-/-} mice. Kaplan-Meier survival curve (A) and cumulative occurrence of thymic tumours (B) of mice with the indicated *Tdp2* and *Atm* genotypes. Adapted from Álvarez-Quilón *et al.* (Nat Commun, 2020).

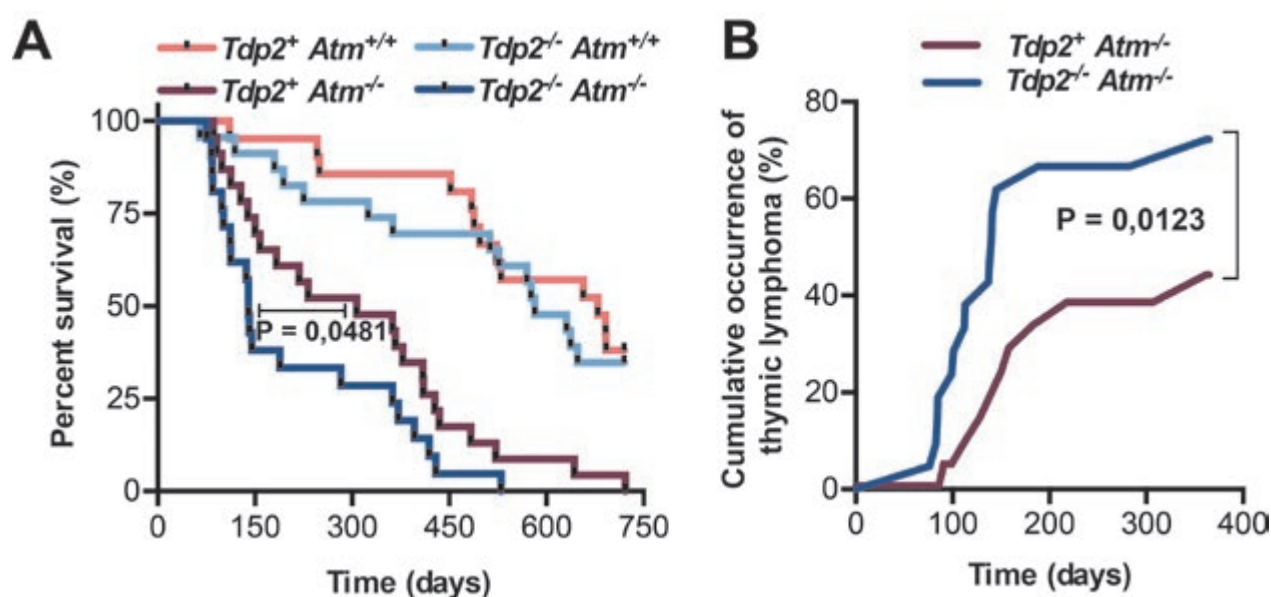
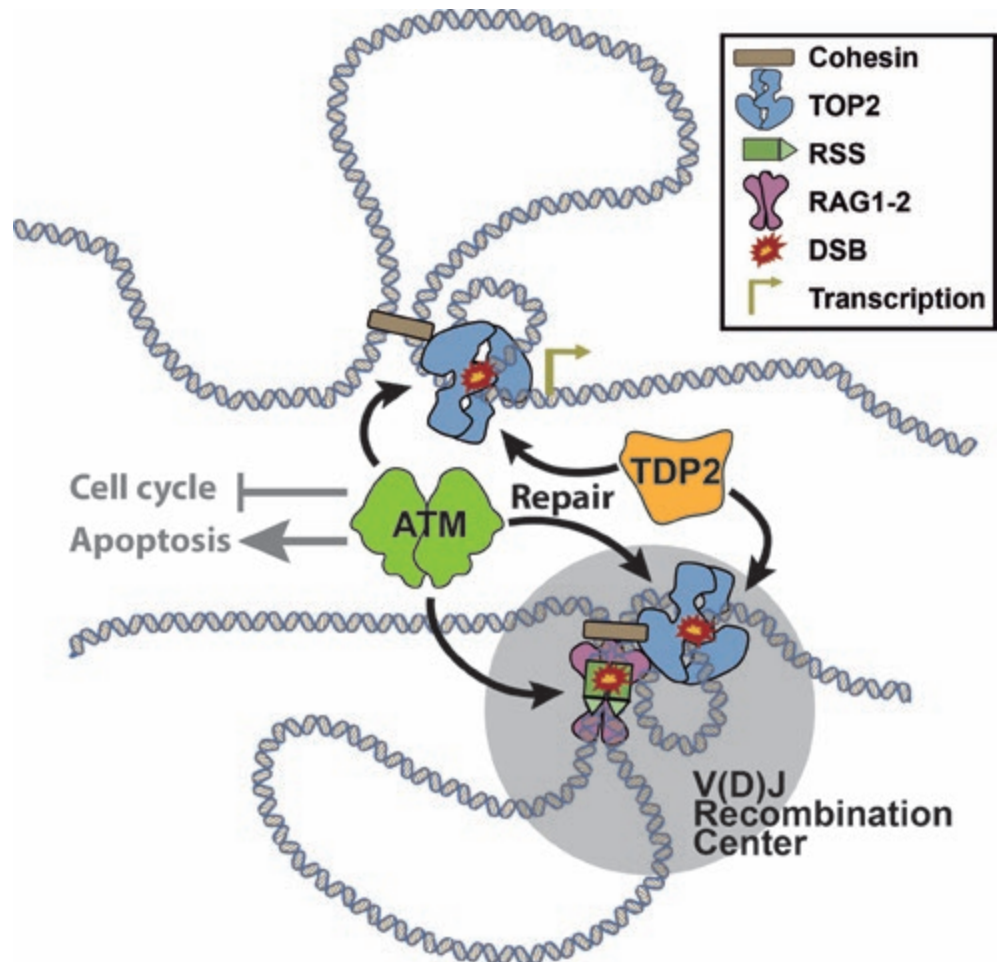


Figure 2 Model to explain aberrant TOP2 activity as a driver of ATM-deficient thymic tumours. TOP2 activity accidentally results in DSBs throughout the genome (top). Additionally, TOP2-DSBs arise associated to V(D)J genome reorganisations (bottom), concurring with RAG-mediated DSBs. TDP2 and ATM limit the oncogenic potential of these lesions (Álvarez-Quilón *et al.*, *Nat Commun*, 2020).



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CHROMOSOME DYNAMICS GROUP

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OVERVIEW

Our research focuses on a protein complex named cohesin that embraces DNA to mediate sister chromatid cohesion, a process essential for chromosome segregation and faithful DNA repair by homologous recombination. Cohesin also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation. Mutations in cohesin have been found in several tumour types, most prominently in bladder cancer, Ewing sarcoma and acute myeloid leukaemia. Germ line 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 complexes carrying SA1 or SA2 make distinct contributions to the spatial organisation of the embryonic stem cell genome, and their loss impacts differently on gene expression.”

RESEARCH HIGHLIGHTS

Dissecting the role of cohesin-SA1 and cohesin-SA2 in mouse embryonic stem cells

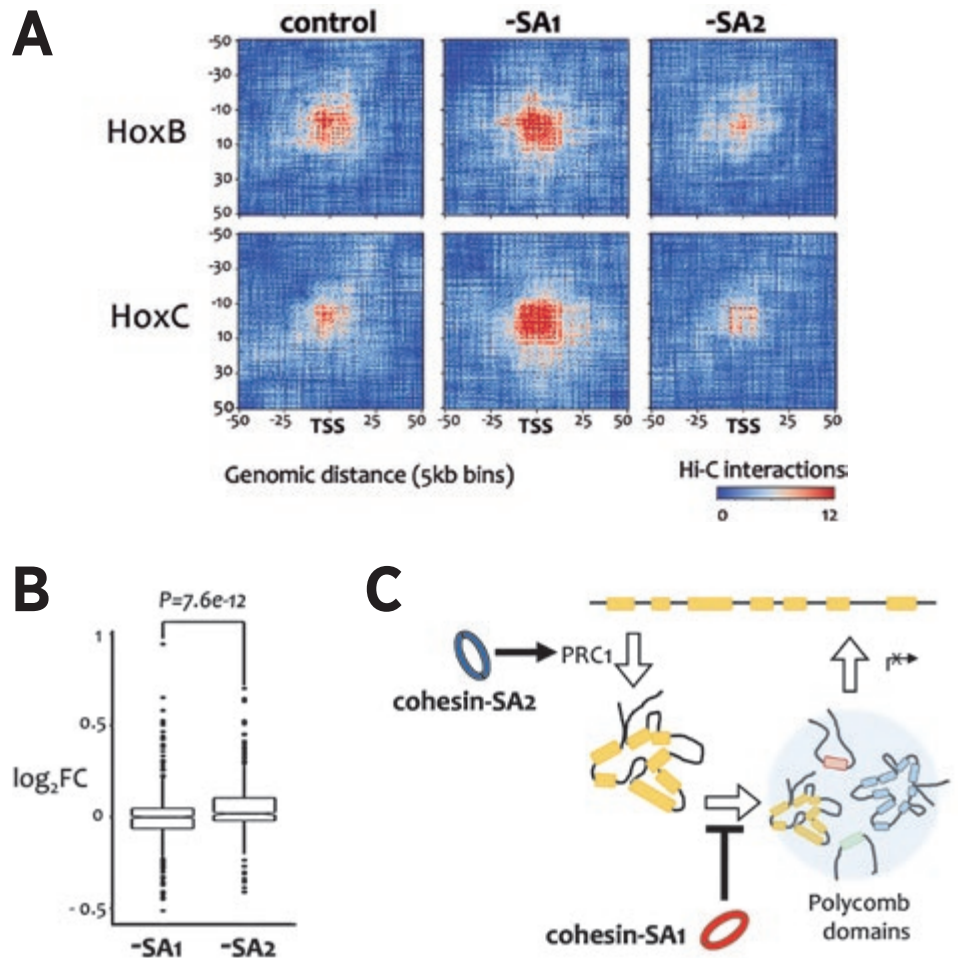
Cohesin consists of 4 core subunits, SMC1, SMC3, RAD21 and SA/STAG. There are 2 versions of the SA subunit in all vertebrate somatic cells, SA1 and SA2. Our recent studies in human cells analysing the genomic distribution of cohesin, gene expression and 3D chromatin organisation in the absence of either variant showed that cohesin-SA1 is important for the organisation of the topological associated domains (TADs) that make up the global structure of the genome and works always alongside the CTCF protein. In contrast, cohesin-SA2 promotes more local chromatin contacts that are relevant for tissue-specific transcription independently of CTCF. In the absence of SA2, a situation that occurs in several tumours, cohesin-SA1 cannot occupy the non-CTCF cohesin sites normally bound by cohesin-SA2, and gene expression is altered.

To confirm these findings, we have now carried out similar analyses in mouse embryonic stem cells (mESCs), as they provide a powerful experimental system to address the functional cross-talk between genome organisation and gene regulation and differentiation. Maintenance of stem cell properties requires a careful balance between self-renewal and differentiation, which is achieved by the active transcription of pluripotency genes under the control of super-enhancers and the repression of lineage specification genes by Polycomb Repressing Complex (PRC)1 and PRC2. Genes and gene clusters occupied by PRC1 in mESCs, most prominently the *Hox loci*, define a new class of self-interacting domains of compacted chromatin that is different from TADs, the Polycomb domains. We have found that cohesin-SA2 facilitates Polycomb domain compaction through PRC1 recruitment and in this way promotes the establishment of long-range interaction networks between distant Polycomb-bound promoters and contributes to gene repression (FIGURE). Cohesin-SA1, in contrast, disrupts these networks, while preserving TAD borders. Also in super-enhancers, cohesin-SA2 mediates local, short-range interactions while cohesin-SA1 plays a more important role in defining domain borders. The diverse effects of both complexes on genome topology may reflect two modes of action of cohesin that depend on their dynamic association with chromatin. Fluorescence recovery after photobleaching (FRAP) experiments in mouse cells show that cohesin-SA1 binding to chromatin is more stable than the binding of cohesin-SA2. This is probably due to the lower affinity of SA1 for Wapl, a cohesin releasing factor, and its higher affinity for CTCF. We are currently testing these hypotheses and further exploring the consequences of SA1 or SA2 loss on cell differentiation.

Cohesin dynamics and DNA replication

How the transcription and replication machineries deal with the presence of cohesin on chromatin remains unclear. The dynamic association of cohesin with chromatin depends on Wapl and Pds5, which exists in two versions in vertebrate cells, Pds5A and Pds5B. Using genetic deletion in mouse embryo fibroblasts and a combination of gene editing by CRISPR and RNA interference in human cells, we have analysed the consequences of Pds5 depletion for DNA replication. We found that either Pds5A or Pds5B is sufficient to allow proper cohesin dynamics while their simultaneous removal increases cohesin's residence time on chromatin and slows down DNA replication. A similar phenotype is observed in Wapl-depleted cells. Normal fork rates are restored in Pds5 deficient cells by cohesin downregulation, suggesting that chromatin-bound cohesin hinders the advance of the replisome. We further showed that Pds5 proteins are also required to recruit Wrip1, Rad51 and Brca2 to stalled forks and, in their absence, nascent DNA strands at unprotected forks are degraded by the Mre11 nuclease. These findings provide new hints as to how cohesin and its regulators contribute to the response to replication stress, a common feature of cancer cells. ■

Figure Cohesin variants regulate distinctly the spatial organisation of mESCs. **(A)** Aggregate analyses of Hi-C interactions between promoters within the HoxB or HoxC loci and any other Polycomb-bound promoter in cells with (control) or without SA1 or SA2. **(B)** As a consequence of decreased interactions between Polycomb domains in the absence of SA2, Polycomb repressed genes are de-repressed, as shown in this box plot. **(C)** A model for the roles of cohesin-SA1 and cohesin-SA2 in intra- and inter-Polycomb domain interactions, which ultimately affect gene repression.



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DNA REPLICATION GROUP

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

OVERVIEW

Our laboratory studies the process of genome duplication, which is responsible for many of the mutations and genomic alterations found in human cancer. While the protein machinery responsible for DNA replication is normally very accurate, it becomes error-prone when the DNA displays chemical alterations caused by endogenous reactive species, external drugs or ionising radiation. We are interested in the phenomenon of replicative tolerance, i.e. the mechanisms that allow the progression of the ‘replisome’ proteins through damaged DNA, a step that normally precedes the activation of specific DNA repair pathways. In 2019, we focused on two major areas: (1) studying the efficiency of replication origins in response to replication stress in pluripotent cells, in the context of their three-dimensional positions in the nuclear chromatin; and (2) evaluating the function of DNA primase PrimPol in the tolerance of cytotoxic DNA lesions in normal and cancer cells with different genetic backgrounds.

“We have identified that PrimPol primase counteracts the effect of cisplatin and other DNA crosslinking agents and mediates an adaptive response in cancer cells that could be exploited in chemotherapy.”

RESEARCH HIGHLIGHTS

In previous years, we identified 2 cellular responses to replicative stress (RS), a phenomenon defined by the slowdown of DNA synthesis caused by damaged DNA templates or special DNA secondary structures that are difficult to replicate. The first response involves the activation of extra replication origins, and the second one involves specialised enzymes that promote re-initiation of DNA synthesis downstream of the lesion, leaving it behind and triggering a signal to initiate its subsequent repair. In 2019, we have continued to characterise both pathways.

Mapping of ‘dormant’ origins that react to replicative stress

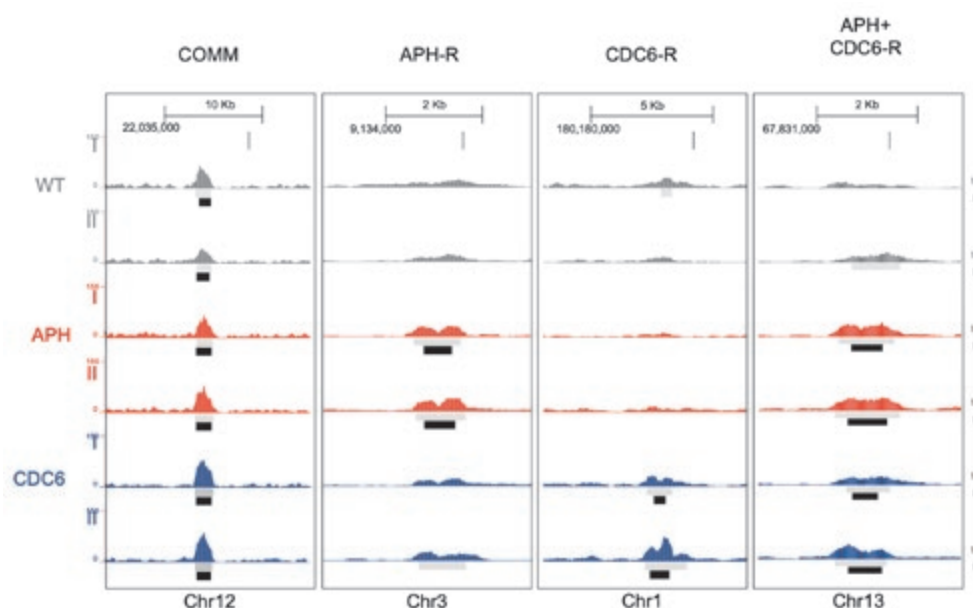
As one of the early proponents of the role of ‘dormant origins’ in counteracting RS, we are interested in their characteristics as well as their localisation and mode of activation. We led a study to map active origins in embryonic stem cells under normal growth conditions or in the presence of RS (FIGURE 1), in collaboration with M. Gómez (*Centro de Biología Molecular “Severo Ochoa”* in Madrid), Alfonso Valencia (Barcelona Supercomputing Center) and V. Pancaldi (Cancer Research Centre of Toulouse). Because origins are not ‘fired’ in every cell in the population, they can be classified according to their efficiency, i.e. the proportion of cells in which they are activated. The most efficient origins tend to be located at regions displaying ‘open chromatin’ epigenetic marks, frequently overlapping

with CpG islands and transcriptional start sites. In contrast, RS-responsive origins correspond to less efficient initiation sites and are not enriched in these genomic elements. RS triggers a global increase in origin efficiency, maximising the activation of low-usage origins as a backup for replication. To identify structural determinants of origin efficiency, we integrated origin maps into 3D networks of chromatin contacts, finding that origin efficiency is proportional to their connectivity with other origins (FIGURE 2). Interconnected origins tend to display similar efficiency and replicate at the same time in S phase, providing a logical framework for the concept of chromosomal ‘replication factories’ (Jodkowska *et al.*, 2019). ■

Replicative tolerance mediated by PrimPol protein

PrimPol is the only primase in mammalian cells besides the canonical Pol α /primase involved in the initiation of DNA replication. In previous work, we reported that PrimPol facilitates replication through UV-induced DNA adducts by synthesising DNA primers downstream of the damaged nucleotides. In 2019, we collaborated with the laboratory of A. Vindigni (Washington University, St Louis, MO, USA) to show that PrimPol mediates an adaptive cellular response to the administration of multiple doses of cisplatin in a regime that mimics chemotherapy. This role of PrimPol becomes very important in BRCA-deficient cells, which have already lost an alternative mechanism to counteract RS (Quinet *et al.*, 2019).

Figure 1 Identification of stress-responsive origins. Genome browser regions containing origins mapped by SNS-Seq in two replicates (I-II) of mESCs (WT), or the same cells subjected to RS with aphidicolin (APH) or CDC6 overexpression (CDC6). One origin active in all conditions (COMM) and 3 stress-responsive origins (APH-R, CDC6-R, APH+CDC6-R) are shown. Chromosome number and genomic coordinates are indicated. M (MACS) and P (Picard) refer to the 2 separate algorithms used for peak calling. Called peaks are shown by grey and black boxes. Adapted from Jodkowska *et al.* (2019).



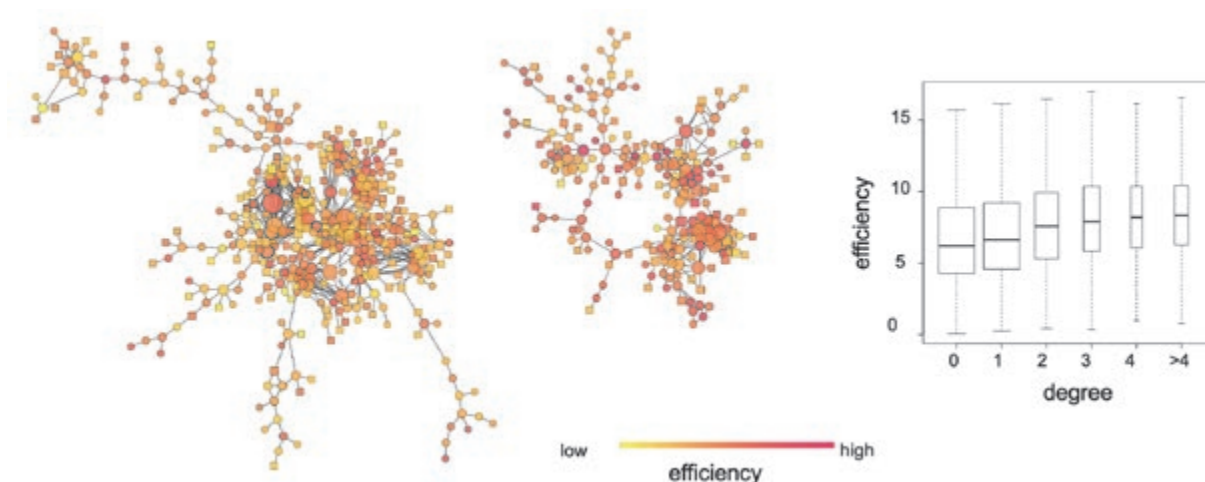


Figure 2 Origin connectivity is linked to efficiency. Representation of a subset of the total network of origins located in a 3D chromatin contact map generated by promoter-capture Hi-C. Origin efficiency, indicated by a yellow-to-red colour

gradient, is directly proportional to the number of connections (degree) established by each node with other nodes in the network. This correlation is reflected in the box plots shown in the right panel. Adapted from Jodkowska *et al.* (2019).

In parallel, together with L. Blanco (*Centro de Biología Molecular “Severo Ochoa”, CSIC-UAM, Madrid*), we characterised a variant of PrimPol found in some lung cancers, in which a single point mutation (Y100H) changes the enzyme specificity of nucleotide sugar selection from dNTPs to rNTPs (Díaz-Talavera *et al.*, 2019).

These and other recent results from our Group imply that PrimPol counteracts the effect of DNA-damaging agents. Therefore, PrimPol inhibition may increase the efficacy of chemotherapy treatments. We have started a screening for potential PrimPol small molecule inhibitors, in collaboration with the CNIO Experimental Therapeutics Programme.

DNA replication in other biological contexts

We contributed to the characterisation of TIAR, an RNA-binding protein required for genomic stability that restricts CDK1 activity under RS (Lafarga *et al.*, 2019). We also started a collaboration with Scott Lowe (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) to perform CRISPR-based genetic screenings related to the effect of uncontrolled DNA replication in the process of gene amplification.

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AWARDS AND RECOGNITION

- Visiting Scientist, Memorial Sloan-Kettering Cancer Center in New York (USA). Sponsored by the Fulbright Commission and the ‘Salvador de Madariaga’ Programme (Ministerio de Ciencia, Innovación y Universidades, Spain).
- Coordinator of the “Molecular and Cellular Biology” area, *Agencia Estatal de Investigación*, Spain.

MELANOMA GROUP

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Tonantzin Calvo, Estela Cañón (TS)*, Cynthia Mucientes (since October) (TS)*

**Titulado Superior* (Advanced Degree)

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OVERVIEW

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

“The long-term goal of the Soengas Laboratory is to translate basic research in melanoma into the clinic, by identifying novel tumour markers and drug targets.”

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 achieved 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. These are main unmet needs, as melanomas are the only tumours where lesions barely over one millimetre in depth can be at risk for metastasis. One of the main objectives of our Group is to define drivers of this lineage-specific behaviour. In addition, we are interested in imaging and targeting (pre)metastatic niches *in vivo*. Specifically, we have elected to visualise the tumour-induced expansion of the lymphangiogenic vasculature (neo-lymphangiogenesis), as this is a process that is activated at early stages of melanoma initiation. However, the specific contribution of lymphatic endothelial cells (LECs) to melanoma is unclear. LECs can secrete a variety of cytokines, which, depending on the context, may favour or inhibit immune surveillance. Moreover, sentinel lymph node removal does not necessarily extend the overall survival of melanoma patients, suggesting that melanoma cells ‘escape’ very early to distal (pre)metastatic niches, but how this occurs remains poorly defined.

The main aims of our Group are (FIGURE 1):

1. To define when and how melanomas act ‘at a distance’ (on stromal and immune compartments) before tumour cell dissemination.
2. To determine how melanoma cells evade the immune system, and whether distinct mechanisms may be activated at different anatomical sites.
3. To dissect the impact of the microenvironment, particularly alarmins and bacterial response factors.
4. To develop anticancer agents to prevent and eliminate metastatic sites.

Lineage-specific oncogenic dependencies in melanoma

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 unique features of autophagy genes (ATG5) in melanoma (García-Fernández *et al.*, *Autophagy* 2016).

Other melanoma-enriched regulatory mechanisms were identified by focusing on RNA binding proteins (RBPs). We selected RBPs (a family of over 1500 members) because they are largely unexplored in melanoma, although this is a tumour characteristically associated with a plethora of changes in mRNA gene expression profiles. Performing a series of genome-wide studies (i.e. genomic, transcriptomic, proteomic and interactomic analyses), we uncovered new roles of the RBPs CPEB4 and CUGBP1 in the modulation of mRNA stability, with unexpected targets involving master specifiers of the melanocyte lineage (Perez-Guijarro *et al.*, *Nat Commun* 2016; Cifdaloz *et al.*, *Nat Commun* 2017). We have now identified new roles of the RNA binding protein IGF2BP1 in the control of the half-life of a large set of pro-metastatic factors. These included FERTM2 and a series of tumour drivers with no previous links to melanoma. Transcriptomic, proteomic and interactomic analyses, combined with mouse models, identified p62/SQSTM1 as a key binding partner of IGF2BP1. This was unexpected, as p62 was considered a key modulator of autophagy or oxidative stress, but had not been reported as an RBP-binding factor. The relevance of these data was emphasised by clinical analyses of patient prognosis revealing p62 and FERMT2 as adverse determinants of disease-free survival (Karras *et al.*, *Cancer Cell* 2019; with the cover highlighting the new signalling cascades identified).

‘MetAlert’ mice for the visualisation of premetastatic niches in melanoma and as a platform for gene discovery and target validation

We previously published lymphoreporter melanoma models to visualise metastatic niches before their colonisation (Olmeda *et al.*, *Nature* 2017). ‘MetAlert’ animals, in combination with human tissue specimens, revealed growth factor MIDKINE as a new driver of lymphangiogenesis and melanoma metastasis. We have now exploited the MetAlert mice to further define immunomodulatory roles of MDK, and to screen for anticancer agents. In particular, we identified an unexpected ability of dsRNA-based mimic BO-110 to blunt neolymphangiogenesis and the associated melanoma metastases (Olmeda *et al.*, *BioRxv* 2019; see comparative analyses with respect to targeted agents or immunomodulatory antibodies in FIGURE 2). The MetAlert and its potential for gene discovery and pharmacological testing has been recognised through various awards conferred to María S Soengas, such as the prestigious 2018 *Estela Medrano Award* by the Society for Melanoma Research and the 2019 *Fritz Anders Medal* by the European Society for Pigment Cell Research (ESPCR), among others. ■

Figure 1 Main objectives of the CNIO Melanoma Group aimed to identify new progression biomarkers and validate more efficient anticancer agents.

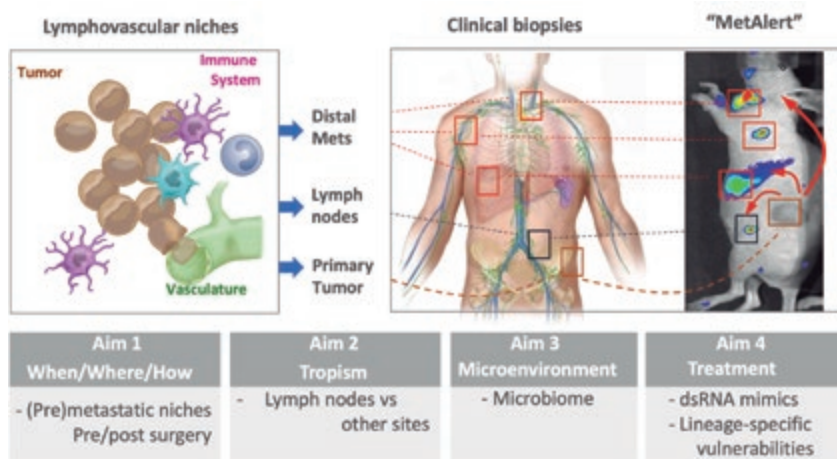
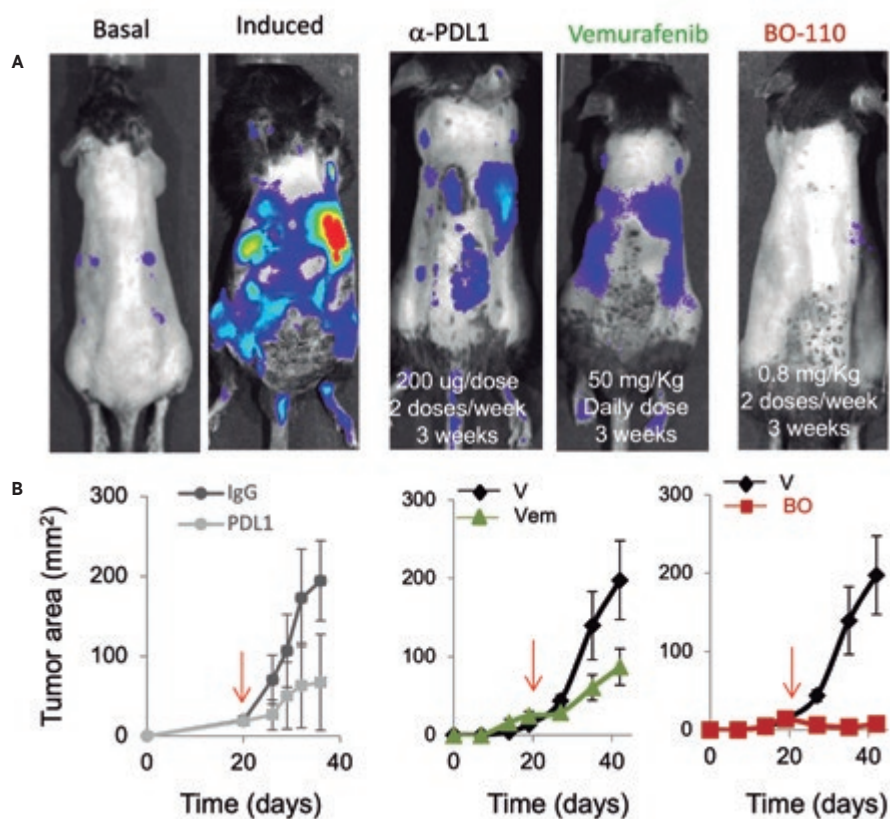


Figure 2 MetAlert mice for pharmacological testing of anticancer agents. Luciferase-based imaging of drug response in MetAlert mice that recapitulate malignant melanoma driven by oncogenic *Braf^{V600E}* in the context of *Pten* loss (*Vegfr3^{Luc}; Tyr:CreERT2; Braf^{V600E}; Pten^{flax/flax}*). (A) Representative examples of mice with no tumours (not induced), and mice in which melanomas were induced by tamoxifen, but were left either untreated or treated as indicated. (B) Growth curves of treatments as in (A).



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Antimetastatic dsRNA mimics identified by live imaging of pathogenic neolymphangiogenesis. *bioRxiv*, doi: <https://doi.org/10.1101/2019.12.26.887943>.

AWARDS AND RECOGNITION

- Fritz Anders Medal, European Society for Pigment Cell Research (ESPCR).
- Sesderma Award for Influential Women in Science and Innovation.
- Yo Dona-Poder Femenino 2019 (Top 500

Influential Spanish Women).

- Elected Member, *Academia Gallega de Farmacia*; *Real Academia Nacional de Farmacia*.
- Elected Treasurer, *Confederación de Sociedades Científicas de España (COSCE)*.
- Special mention *Ciencia y Mujer* 2019, WomenCEO. *Jóvenes Investigadores Gallegos en el Extranjero* Event.
- Top100 *Mujeres Líderes de España* 2019 (Top100 Women Leaders in Spain), *Mujeres & Cía*.

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Elena del Pilar Andrada, Irene Felipe,
Eleonora Lapi, Sumit Paliwal, Mark
Kalisz (until June), Gabriel Piedrafita
(since April)



Graduate Students

Sonia Corral (since September), Ana M. Maldonado, Irene Millán, Catarina Pereira (until September), Mónica Pérez

Technicians

Miguel Deblas, Natalia del Pozo, Jaime Martínez (TS)*

**Titulado Superior* (Advanced Degree)

Visiting Scientists

Luis César Fernández Díaz (since October) (*Universidad Europea Madrid*), Mark Kalisz (since July) (*CIBER, Madrid*)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial bladder carcinoma (UBC), adopting 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.

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

“Our Group was the initiator of the International Bladder Cancer Molecular Taxonomy Group, which, in 2019, released the Second Consensus Classification.”

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

The genetic/genomic changes associated with PDAC have been extensively described over the last few years by the genome consortia, and there is increasing interest in defining the contribution of precursor lesions and the molecular changes that precede tumour development. Our lab has pioneered the notion that cell differentiation is the first tumour suppressor process in the pancreas. Focusing on acinar cells, we have identified several novel players – and mechanisms – including GATA6, GATA4, NR5A2, HNF1A, and NFIC. Dysregulation of these transcription factors is associated with a scenario of pre-inflammation or inflammation and with predisposition to PDAC development using mutant *KRas*-driven genetic mouse models. These studies provide the basis for the pharmacological – or genetic – manipulation of acinar differentiation as a tumour preventative strategy.

We have found that while loss of either GATA6 or GATA4 favour PDAC development/progression in mice, these proteins play distinct roles in acinar differentiation and inflammation, and they contribute differently to tumour initiation. We are now focusing on understanding their specific functions and deciphering their transcriptional programmes using a combination of mouse models and genomic approaches (i.e. RNA-Seq and ChIP-Seq). In collaboration with J. Ferrer (CRG, Barcelona), we have developed conditional knockout mouse models of *Hnf1a* that are providing new evidence of the tumour suppressor role of this gene in PDAC initiation. Using a dual recombinase system, we are exploring whether HNF1, and its partner NR5A2, play similar roles in tumour maintenance. Our overarching goal is to establish the rules governing the transcriptional control of acinar differentiation and its contribution to preneoplasia and cancer.

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

We are interested in refining our understanding of new genes involved in UBC, using organoids and genetic mouse models to unravel their function, and to apply this knowledge in the clinical setting.

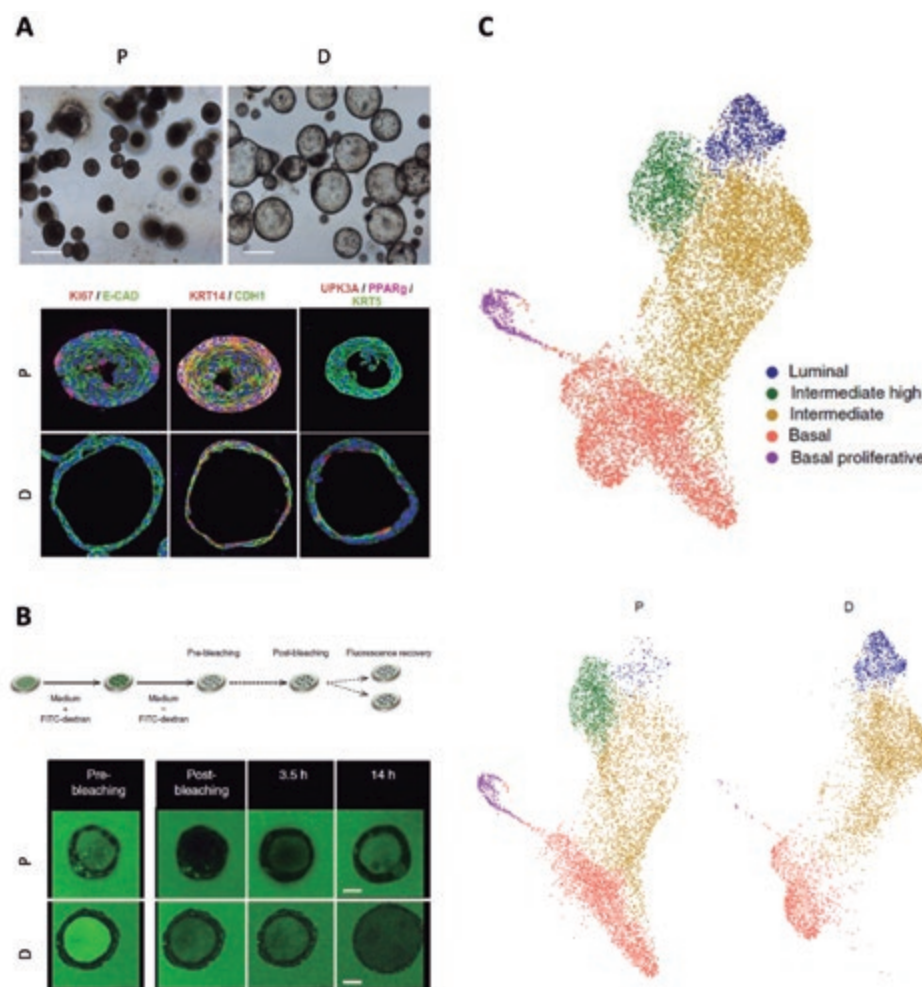
Through exome sequencing we identified mutations in *STAG2*, coding for a cohesin subunit, and in *RBM10*, coding for a splicing regulator, as new UC genes that are more broadly involved in human cancer. We have generated conditional mouse models for these 2 genes and are exploring their role in development and urothelial biology as well as their cooperation with other bladder cancer genes.

STAG2 is a cohesin complex component. Increasing evidence shows that it acts as a tumour suppressor through rather unique mechanisms, largely unrelated to the canonical role of cohesin in chromosome segregation. Using normal cultured urothelial cells, we have found that the genomic effects of *STAG2* loss are largely dependent on the differentiation state of the cells. These findings support the strong association of *STAG2* inactivation with a luminal/urothelial phenotype and pave the way for the discovery of new mechanisms leading to UBC.

Our recent effort to develop organoids from normal urothelial cells has culminated in the first single cell RNA-Seq analysis of these cells, which provided novel evidence on the role of the NOTCH pathway in urothelial differentiation, and uncovered new genes associated with this process (FIGURE 1). The organoid cultures provide key clues to understanding urothelial stem cell biology and the control of cell proliferation and regeneration in homeostasis and upon damage.

Our translational studies focus on the prediction of response to cisplatin-based chemotherapy and to immune checkpoint blockade (ICB). In collaboration with an extended group of Spanish uro-oncologists and Núria Malats, 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. ■

Figure 1 Organoids derived from normal mouse urothelium provide a unique tool to understand tissue biology. **(A)** Urothelial organoids can be perpetuated for more than 1 year under proliferative conditions (P) and can be induced to differentiate (D), as shown by the down-regulation of basal keratins and the up-regulation of uroplakins. **(B)** Differentiated organoids, but not their proliferative counterparts, acquire barrier function characteristic of the normal urothelium. This is evidenced using FITC-dextran loading and fluorescence recovery after photobleaching (FRAP). **(C)** Single cell RNA-Seq has allowed the identification of the features of normal proliferative urothelial cells as well as the role of the Notch pathway in urothelial differentiation. Upper panel shows the UMAP plots visualising integrated analysis of cells from P and D organoids as a joint plot (top) or as separate plots (bottom).



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GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

Nabil Djouder
Group Leader

Post-Doctoral Fellows
Hugo Bernard (until November),
Eunjeong Kim (since May)

Graduate Students
Almudena Chaves (until July), Sergio



de La Rosa, Amanda Garrido, Tatiana Grazioso, Irene Herranz, Maria del Mar Rigual (since October), Paula Sánchez (since October), Beatriz Tarrafa (until April), Ana Isaura Teijeiro

Students in Practice
Ana Ferre (January-April) (*École Normale Supérieure Université Paris-Saclay, France*), Aude Jalón (June-July) (*École Normale Supérieure Université Paris-Saclay, France*)

OVERVIEW

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. The identification of likely causal links between environmental stresses and diseases progressing to cancer will help to elucidate mechanisms of diseases and to identify, and functionally validate, targets with preventive and therapeutic values to treat frequent lethal human disorders with increased worldwide incidence and unmet medical needs.

“We put lots of effort to understand mechanisms of diseases by generating and using new mouse models that recapitulate pathological features of human syndromes in order to guide the design of novel medicines.”

RESEARCH HIGHLIGHTS

Our laboratory is devoted to the understanding of the molecular mechanisms that link environmental stresses to disease pathogenesis affecting organs of the digestive system, including the liver, intestine, pancreas and stomach (FIGURE).

Based on the integration of experimental mouse models, combined with the use of state-of-the art technologies and human data, our interest is mainly driven by the discovery of the proteins URI (Unconventional prefoldin RPB5 Interactor) and MCRS1 (Microspherule protein 1), whose expressions are regulated by various environmental factors – radiation, bacteria, viruses, diet, etc. (Djouder *et al.*, 2007; Fawal *et al.*, 2015; Chaves-Perez *et al.*, 2019) – compromising their functions and activating pleiotropic circuits to support non-oncogene addiction functions and dependence, thereby provoking severe outcomes.

In this regard, in the past, we conceptually demonstrated that metabolic alterations initiate tumorigenesis prior to genomic instability (Tummala *et al.*, 2014; Brandt *et al.*, 2017) and thus, are driving forces in cancer development. Our findings indicate that hallmarks of cancer are part of different time-dependent events arising dependently during tumorigenesis.

Moreover, 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-Perez *et al.*, 2019).

By employing an interdisciplinary approach, which includes the use of various genetically engineered mouse models (GEMMs) and human data combined with state-of-the art technologies, including cell biology with organoid culture and quantitative imaging, biochemistry and functional genomic methodologies, we intend in the near future to continue deconstructing mechanisms of pathologies associated to the digestive system in response to environmental stressors, with a special focus on understanding the mechanisms of colorectal cancer and liver diseases.

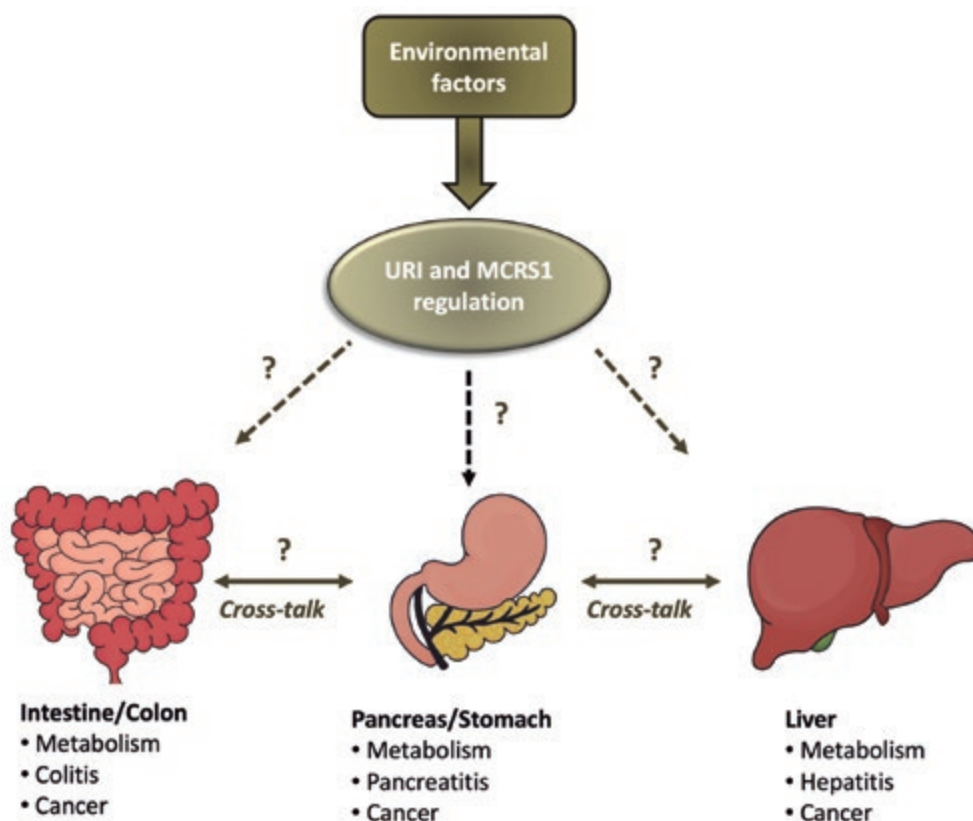
1. Mechanisms of colorectal cancer

By using gain- and loss-of-function GEMMs for URI, we aim to understand the mechanisms of colorectal cancer development and to determine the cells at origin of tumours. Our goal is to track LR cells during tumorigenesis through the use of sophisticated mouse genetic experiments.

2. Mechanisms of liver diseases

We aim to elucidate mechanisms of liver cirrhosis and its role in hepatocellular carcinoma, a frequent lethal liver neoplasm. Our final goal is to identify and functionally validate targets with potential preventive and therapeutic values to treat two deadly human disorders with increased worldwide incidence and unmet medical needs. We will put effort to fulfil the following objectives: (1) to find out what goes wrong in diseased and cancerous tissues; (2) to understand how the organ can regenerate and how regeneration in chronic injury can impact liver cancer development; and (3) to promote healthy liver regeneration and reduce cirrhosis and liver cancer. Special effort will also be put on developing new approaches for the quantitative assessment and analysis of single cell RNA sequencing. Moreover, we have developed an interest in nanotechnology-based theranostics, and plan to combine conceptual advances arising from my laboratory with this exploding technology in order to develop new therapeutic approaches for the prevention and treatment of liver cancer. ■

Figure Representation of our future research directive. By using mouse models generated in our lab, we mainly focus on deconstructing mechanisms of pathologies associated to the digestive system in response to environmental stressors.



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TRANSFORMATION AND METASTASIS GROUP

Eva González Suárez
(since August)
Group Leader

Staff Scientists
Patricia González (since October),
María Jiménez (since August), Gema
Pérez (since October)



Graduate Students

Alexandra Barranco (since October),
Marina Císcar (since September),
Alejandro Collado (since
September), Jaime Redondo (since
October)

OVERVIEW

Current therapies for breast cancer and other epithelial tumours are in many cases not effective because of intrinsic or acquired resistance, and tumours often relapse, leading to metastatic disease.

Research in the Transformation and Metastasis Group aims to identify novel therapeutic targets for cancer prevention and treatment, and to elucidate resistance mechanisms to drugs currently available. Tumours exploit and manipulate for their benefit the same mechanisms that work correctly in healthy tissue. Thus, we first aim to understand normal development and identify the key events that lead to tumour initiation, progression and metastasis to avoid and combat them. We work with primary cell cultures (including 3D cultures), genetically modified mouse models, patient derived xenografts and clinical samples.

The main contributions of the Group are within the field of mammary gland biology and breast cancer. Our goal is to translate our basic research findings into relevant clinical results.

“Using a collection of patient-derived breast cancer xenografts and multi-OMIC approaches, we have identified mechanisms of chemoresistance and propose novel strategies to guide the use of the right chemotherapy.”

RESEARCH HIGHLIGHTS

Amplification of chromosome 12p in triple negative breast cancer is associated with emergent docetaxel resistance and carboplatin sensitivity

Taxanes are the mainstay of treatment in triple-negative breast cancer (TNBC), with *de novo* and acquired resistance limiting patient survival. To investigate the genetic basis of docetaxel resistance in TNBC, exome sequencing was performed on matched TNBC patient-derived xenografts (PDXs) sensitive to docetaxel, and their counterparts that developed resistance *in vivo* upon continuous drug exposure. Most mutations, small insertions/deletions and copy number alterations detected in the initial TNBC human metastatic samples were maintained after serial passages in mice and emergence of resistance. We identified a chromosomal amplification of chr12p in a human *BRCA1*-mutated metastatic sample and the derived chemoresistant PDX, but not in the matched docetaxel-sensitive PDX tumour. Chr12p amplification was validated in a second pair of docetaxel-sensitive/resistant *BRCA1*-mutated PDXs and after short-term docetaxel treatment in several TNBC/*BRCA1*-mutated PDXs and cell lines, as well as, during metastatic recurrence in a *BRCA1*-mutated breast cancer patient who had progressed on docetaxel treatment. Analysis of clinical data indicates an association between chr12p amplification and TNBC/basal-like breast cancer patients, a *BRCA1* mutational signature, as well as poor survival after chemotherapy. Detection of chr12p amplification in a cohort of TNBC PDX models was associated with an improved response to carboplatin. Our findings reveal tumour clonal dynamics during chemotherapy treatments and suggest that a pre-existing population harbouring chr12p amplification is associated with the emergence of docetaxel resistance, but carboplatin responsiveness, in TNBC/*BRCA1*-mutated tumours. These findings indicate that sequential treatment with docetaxel and carboplatin could be more efficient in this subset of TNBC patients than the combination or single treatments. FIGURE 1.

The altered transcriptome and DNA methylation profiles of docetaxel resistance in breast cancer PDX models

Taxanes are standard therapy in clinical practice for metastatic breast cancer; however, primary or acquired chemoresistance are a common cause of mortality. Breast cancer patient-derived xenografts (PDXs) are powerful tools for the study of cancer biology and drug treatment response. Specific DNA methylation patterns have been associated to different breast cancer subtypes, but their association with chemoresistance remains unstudied. Aiming to elucidate docetaxel resistance

mechanisms, we performed genome-wide DNA methylation in breast cancer PDX models, including luminal and triple-negative breast cancer (TNBC) models sensitive to docetaxel, their matched models after emergence of chemoresistance, and residual disease after short-term docetaxel treatment. We found that DNA methylation profiles from breast cancer PDX models maintain the subtype-specific methylation patterns of clinical samples. Two main DNA methylation clusters were found in TNBC PDX and remained stable during the emergence of docetaxel resistance; however, some genes/pathways were differentially methylated according to docetaxel response. A DNA methylation signature of resistance able to segregate TNBC based on chemotherapy response was identified. Transcriptomic profiling of selected sensitive/resistant pairs and integrative analysis with methylation data demonstrated correlation between some differentially methylated and expressed genes in docetaxel-resistant TNBC PDX models. Multiple gene expression changes were found after the emergence of docetaxel resistance in TNBC. DNA methylation and transcriptional changes identified in docetaxel-sensitive and -resistant TNBC PDX models or residual disease may have predictive value for chemotherapy response in TNBC. IMPLICATIONS: Subtype-specific DNA methylation patterns are maintained in breast cancer PDX models. While no global methylation changes were found, we uncovered differentially DNA methylated and expressed genes/pathways associated with the emergence of docetaxel resistance in TNBC. FIGURE 2. ■

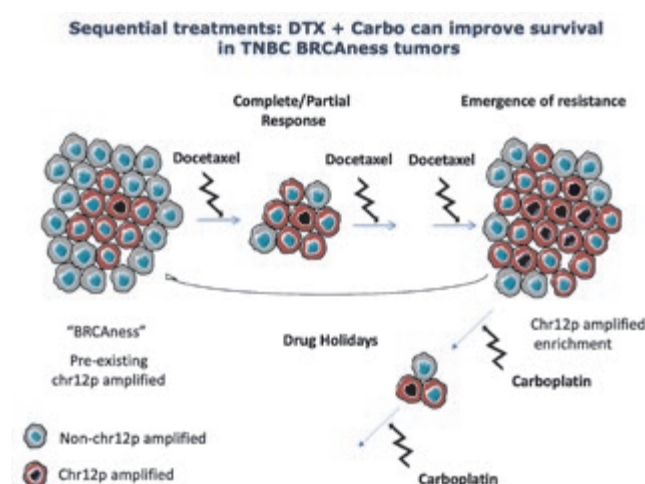
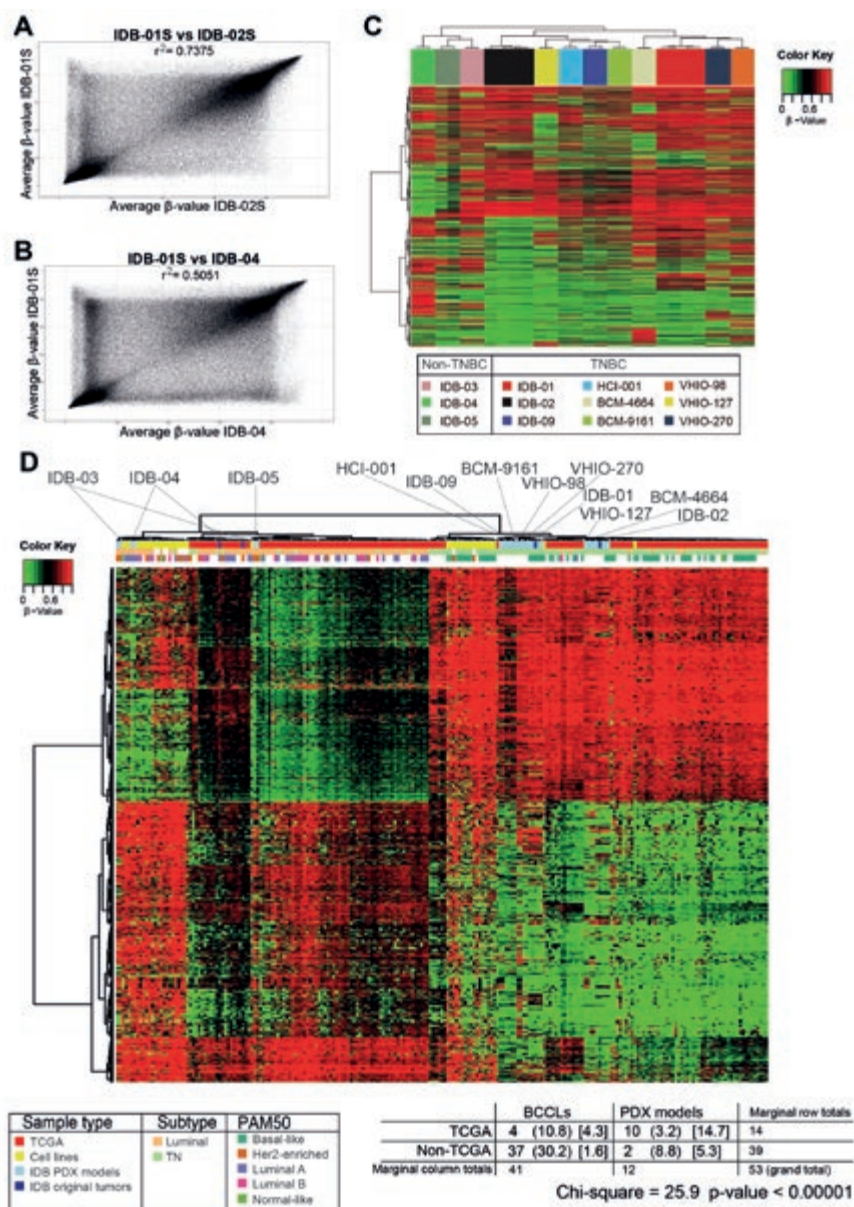


Figure 1 A pre-existing population harbouring chr12p amplification is associated with the emergence of docetaxel resistance, but carboplatin responsiveness in TNBC/*BRCA1*-mutated tumours. These findings

indicate that sequential treatment with docetaxel and carboplatin could be more efficient in this subset of TNBC patients than the combination or single treatments.

Figure 2 Global DNA methylation patterns of breast cancer PDX resemble breast cancer human samples. **(A, B)** Scatter plot of groupwise mean genome-wide DNA methylation levels analysed by GenomeStudio between two sensitive TNBC PDX models **(A)** or between a TNBC and a luminal PDX model **(B)**. Correlations are indicated as r^2 . **(C)** Unsupervised hierarchical clustering using 32,264 most differentially methylated CpGs between breast cancer PDX models. Methylation difference between at least one of the groups is equal or higher to 75%, p -value < 0.01 . **(D)** Supervised hierarchical clustering applying 743 selected CpGs that discriminate TNBC vs luminal subtype from TCGA breast cancer to BCCLs, breast cancer PDX models and human tumour samples of origin and TCGA clinical samples. Breast cancer PDX models are indicated. Chi-square and corresponding p -value indicating association between TCGA breast tumours and PDX models or BCCLs from the same subtype are indicated below.



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AWARDS AND RECOGNITION

- Eva González-Suárez:
- Ideas Semilla AECC 2019* Research Grant (PI), the Spanish Association Against Cancer (AECC).
- La Marató TV3* Grant (PI), *La Marató TV3* Foundation, Spain.
- Alexandra Barranco was awarded the pre-doctoral contract PRE2018-086522 (MCIU).
- Alejandro Collado received the pre-doctoral fellowship FPU18/00546 (MEFP) and an FI-AGAUR 2019 PhD contract.
- Jaime Redondo was the recipient of a Severo Ochoa PhD contract (PRE2018-086522, MCIU).

MICROENVIRONMENT & METASTASIS JUNIOR GROUP

Héctor Peinado
Junior Group Leader

Staff Scientist
Susana García

Post-Doctoral Fellows
Marta Hergueta, Laura Nogués



OVERVIEW

Our laboratory aims to understand how the microenvironment impacts metastatic behaviour. Most of the efforts in the past focused on defining intrinsic factors involved in tumour progression and metastasis. However, our interpretation of tumour evolution and metastasis has shifted and currently it is widely accepted that extrinsic factors are actively involved in cancer progression. To tackle this question, our laboratory uses melanoma models to define the role of secreted exosomes in pre-metastatic niche formation and metastasis in the lymph node. In addition, we are exploring the influence of obesity on the metastatic behaviour of melanoma and breast cancer. Finally, we are using novel therapeutic approaches to prevent metastasis in melanoma, lymphoma and malignant peripheral nerve sheath tumour metastasis by targeting both intrinsic and extrinsic factors.

“Our interpretation of how the tumour microenvironment impacts on cancer progression has changed over the past few years. Now we know that the surrounding microenvironment is one of the driving forces of metastasis.”

Graduate Students

Ana I. Amor, Elena Castellano (since October), Teresa González, Alberto Hernández, Lucía Robado, Alicia Teijeira (until July)

Technicians

Marina Mazariegos, Sara Sánchez-Redondo

Students in Practice

Alberto Carretero (since October) (*Hospital Universitario 12 de Octubre*), Raúl Pérez (January-May) (*Universidad Complutense de Madrid*)

RESEARCH HIGHLIGHTS

Novel strategies for diagnosis and treatment of metastatic melanoma

Liquid biopsies provide information about cancers in a non-invasive way. In this context, we explore the use of exosomes circulating in biofluids from melanoma patients for minimal residual disease detection and treatment monitoring. Since an important number of patients are refractory to therapy, we additionally study the tumour-immune system cross-talk to find new therapeutic strategies to defeat melanoma metastasis focused on restoring the sensitivity to targeted therapies and/or anti-tumour immunity. We are also interested in the analysis of matrix remodelling processes and microenvironmental factors that influence melanoma progression and could open avenues for new treatment strategies.

How does obesity impact metastasis?

Obesity is a chronic inflammatory condition associated with enhanced cancer incidence and mortality. In fact, obesity is thought to be responsible for up to 20% of cancer-related deaths in adults. We are currently analysing how obesity induces systemic changes favouring metastasis (e.g., increased vascular permeability, platelet recruitment and immune cell impairment). Furthermore, we are visualizing pre-metastatic niches *in vivo* by intravital imaging, in collaboration with the CNIO Confocal Microscopy Unit, to understand how breast tumour cells home in metastatic lungs in normal and high fat diet-fed mice, and the mechanisms underlying this process.

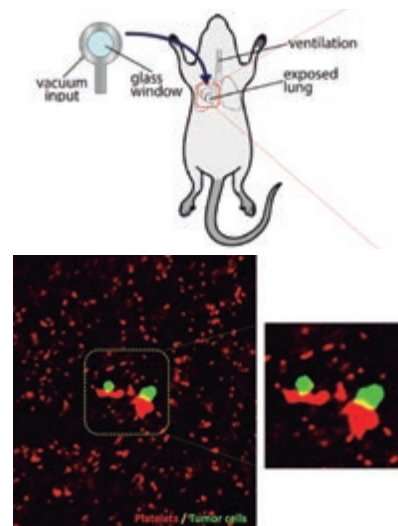


Figure Intravital imaging of metastatic niches. Upper panel: schematic representation of the setup for lung intravital imaging. Lower

panel (left and right): representative images of breast cancer tumor cells (green) and platelets (red) interacting *in vivo*.

Defining novel targets in rare diseases

In this setting we are investigating how lymph node stroma dysregulation affects the tightly controlled physiology of immune cell populations and gives rise to lymphoproliferative disorders. Finally, we are analysing novel therapeutic targets in malignant peripheral nerve sheath tumours (MPNSTs). We are currently testing treatment strategies combining FDA-approved drugs and anti-angiogenic antibodies to improve therapeutic efficacy in MPNSTs. ■

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dondo S, Hernández-Barranco A, Ximénez-Embún P, Nogués L, Mazariegos MS, Amor López A, Rodríguez C, García-Martín C, Boskovic J, Letón R, Montero C, Robledo M, Muñoz J, Peinado H) (2019). Use of extracellular vesicles from lymphatic drainage as surrogate markers of melanoma progression and BRAF V600E mutation. *J Exp Med* 216, 1061.

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BRAIN METASTASIS JUNIOR GROUP

Manuel Valiente
Junior Group Leader

Post-Doctoral Fellows
Mariam Al-Masmudi (since July),
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 that 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 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.

“Our ongoing projects are showing us that the untreatable nature of brain metastasis could be questioned by the identification of vulnerabilities that could be exploited therapeutically.”

Graduate Students

Laura A. Álvaro, Ana de Pablos Aragoneses (since November), Catia P. Domingues (until April), Pedro García, Lucía Zhu

Technicians

Lourdes Osuna (until November), Natalia Yebra (until August), Diana Patricia Retana (since August)

Students in Practice

Ana de Pablos Aragoneses (until October) (*Universidad Autónoma de Madrid*), Pablo Sanz Martínez (since July) (*Universidad Autónoma de Madrid*)

RESEARCH HIGHLIGHTS

We reported the possibility to challenge brain metastasis by targeting altered molecular patterns present in pro-metastatic components of the microenvironment. During this year, we expanded our knowledge about the functional reprogramming affecting this metastasis-associated cell type (reactive astrocytes) as well as the benefits that it provides to cancer cells. This particular aspect has brought our attention to additional cell types in the microenvironment including microglia/macrophages and T cells that infiltrate the brain when affected by metastasis. The newly established networks among different components of the metastasis-associated microenvironment represent an active area of investigation in the laboratory since they are a novel source for therapeutic targets.

Given our unprecedented success obtained with a recent therapeutic strategy targeting the microenvironment both in experimental models and in patients with brain metastasis, we further explored the possibility of applying this rationale

earlier in order to prevent brain metastasis instead of just treating it. We found a potential mechanism that could be inhibited right after metastatic cells crossed the blood-brain barrier and that has a major impact on experimental animals by preventing the formation of metastasis.

In addition, our newly developed drug-screen platform (METPlatform) has enabled us to identify new compounds with anti-metastatic activity that have been validated *in vivo* in experimental models and in patient-derived organotypic cultures.

Not only are we interested in developing new treatments, but we also try to understand why classic treatments, such as radiotherapy, do not provide greater benefits to patients. In this sense, we have identified a mechanism that could explain the low responses to this therapy *in vivo* and an inhibitor that could target it, thus increasing the anti-tumour effects with reduced radiation doses. ■

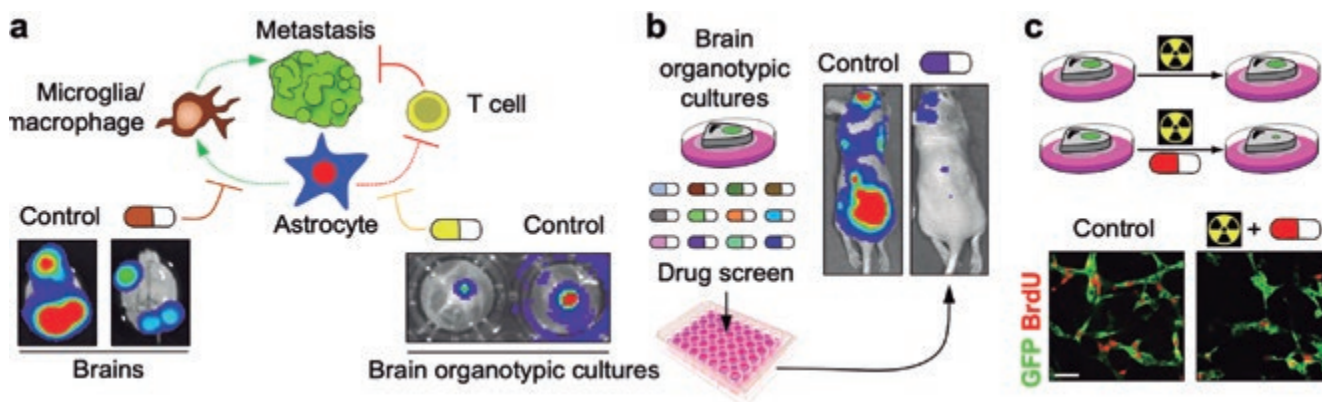


Figure (a) Targeting altered components of the microenvironment (astrocytes) and their related networks with other cellular components such as microglia/macrophages or T cells. (b)

METPlatform uses organotypic cultures to identify new potential anti-metastatic drugs. (c) Resistance to radiation could be reversed by a blood-brain barrier permeable compound.

PUBLICATIONS

- Wingrove E, Liu ZZ, Patel KD, Arnal-Estapé A, Melnick M-A, Politi K, Monteiro C, Zhu L, Valiente M, Chiang VL, Nguyen DX (2019). Transcriptomic hallmarks of

tumor plasticity and stromal interactions in brain metastasis. *Cell Rep* 27, 1277-1292.

- Priego N, Valiente M (2019). The potential of astrocytes as immune modulators in brain tumors. *Front Immunol* 10, 1314.

AWARDS AND RECOGNITION

- EMBO Young Investigator Award.
- Ana de Pablos Aragoneses was recipient of a "la Caixa" INPhINIT PhD Fellowship.
- Neibla Priego received an AECC Postdoc-

toral Fellowship and a Spanish Society for Biochemistry and Molecular Biology (SEBBM) Award for the best publication of the year (2nd position).

- Lucía Zhu was awarded an EMBO Short-Term Fellowship.

METABOLISM AND CELL SIGNALLING JUNIOR GROUP

Alejo Efeyan
Junior Group Leader

Staff Scientist
Ana Ortega

Post-Doctoral Fellow
Melania Zauri



OVERVIEW

In the Metabolism and Cell Signalling Lab we study the interplay of nutrients, metabolism and cancer. Every cell in the organism integrates signals emanating from the abundance of intracellular nutrients and from the nutritional state of the organism as a whole. Integration of cellular and systemic nutrient abundance cues is key for adequate cellular and organismal functions, and importantly, the components of these signalling cascades are generally corrupted in cancer cells. Together with genetic mutations, environmental perturbations, such as those occurring in obesity, affect the cellular signalling cascades that control the responses to nutrients and hormones. 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.

“A large fraction of tumours hijacks the normal responses to the fluctuations in nutrient levels to satiate the need for growth and proliferation, but these alterations also impose exploitable vulnerabilities.”

Graduate Students
Celia de La Calle, Nerea Deleyto, Ana Belén Plata

Technicians
Cristina Lebrero (since April) (TS)*,

Ana Sagrera (TS)*, Alba Sanz
*Titulado Superior (Advanced Degree)

Students in Practice
Marina Gladkova (June-August)
(Lomonosov Moscow State

University), Leyre Marín (until June)
(Universidad Politécnica de Madrid),
Javier Vicente (July-August)
(Universidad de Alcalá de Henares)

Visiting Scientist
Sebastian Thompson (since
February) (Instituto Imdea
Nanociencia)

RESEARCH HIGHLIGHTS

Nutrient signalling in B cell lymphoma

One of the most rapid proliferation bursts in mammalian cells is that of B lymphocytes upon encountering certain pathogens or antigens. This proliferation suddenly multiplies the energetic and metabolic demands of the activated B cell and, accordingly, precise nutrient sensing and signalling are key to successfully accomplish the energetically onerous rounds of growth and division. Recently, components of the Rag GTPase pathway, a key nutrient signalling pathway that enables the anabolic capacity of the cell for rapid proliferation, were found mutated in follicular lymphoma, an incurable B lymphocyte tumour. By means of novel strains of mice that express mutant variants of the RagC GTPase, we found that these mutations drive B cell activation and development of lymphomas, but underlie an exquisite sensitivity to pharmacological inhibition of the mTORC1 pathway (FIGURE A). Such treatment may be particularly efficacious for 1 out of 6 follicular lymphoma patients with RagC mutations, and also for patients without genetic mutations but with functional deregulation in the nutrient signalling pathway.

Chronic signalling of elevated nutrients and premature ageing

In the absence of lymphoma, mice with elevated nutrient signalling show multiple features of premature ageing (FIGURE B), including the thinning of dermal and subcutaneous fat layers of the skin (FIGURE C) and increased senescence-associated β -galactosidase activity in multiple organs (FIGURE D). These mice are the first genetic mammalian system to interrogate the mechanisms that link elevated nutrient intake and accelerated ageing. We are currently investigating

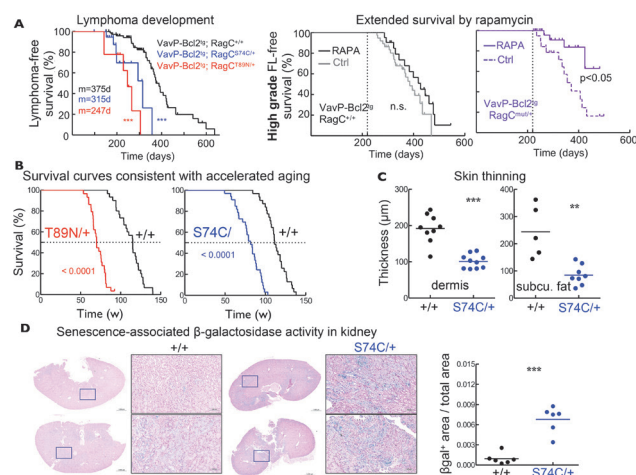


Figure (A) Left: RagC mutations accelerate FL when bred to the VavP-Bcl2 strain. Right: Rapamycin extends survival of mice bearing RagC^{mut} FL. (B) Survival curves of RagC^{mut} mice. (C) Thinning of the skin of RagC^{mut} mice. (D) Increased SA β -galactosidase activity in kidneys from old RagC^{mut} mice.

the cellular and molecular alterations responsible for this shortening of the life span, and the organ-to-organ crosstalk that may contribute to the acceleration of ageing. ■

PUBLICATIONS

- Ortega-Molina A, Deleyto-Seldas N, Carreras J, Sanz A, Lebrero-Fernández C, Menéndez C, Vandenberg A, Fernández-Ruiz B, Marín-Arraiza L, de la Calle Arregui C, Belén Plata-Gómez A, Caleiras E, de Martino A, Martínez-Martín N, Troulé K, Piñeiro-Yáñez E, Nakamura N, Araf S, Victoria GD, Okosun J, Fitzgibbon J, Efeyan A (2019). Oncogenic Rag GTPase signaling enhances B cell activation and drives follicular lymphoma sensitive to pharmacological inhibition of mTOR. *Nat Metab* 1, 775-789.
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PUBLICATIONS AT OTHER INSTITUTIONS

- Do MH, Wang X, Zhang X, Chou C, Nixon BG, Capistrano KJ, Peng M, Efeyan A, Sabatini DM, Li MO. Nutrient mTORC1 signaling underpins regulatory T cell control of immune tolerance (2019). *J Exp Med*. PMID: 31649036.

SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

Staff Scientist
Barbara Oldrini

Post-Doctoral Fellow
Miguel Jiménez



OVERVIEW

Glioblastoma (GBM) is the most common and lethal primary central nervous system tumour in adults. Despite the recent advances in treatment modalities, GBM patients generally respond poorly to all therapeutic approaches and prognosis remains dismal. Radiation and chemo-resistance are characteristic of various cancer types, however it is not clear if this therapy resistance is a consequence of tumour progression or if it is intrinsically associated with the genetic events that lead to the tumour formation in the first place. Gaining insights into the pathways that determine this poor treatment response will be instrumental for the development of new therapeutic modalities.

In our laboratory, we use a variety of approaches – both genetic and small molecule drug screenings – coupled with in vivo

“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.”

GBM mouse models in order to identify genes involved in therapy resistance of gliomas. We reason that these studies will help to define new therapeutic targets for treatment of brain tumours.

Graduate Students
Carolina Almeida (until March),
María Del Mar Gardeazabal (since
September), Verónica Matía

Technician
Paula Kroon (TS)*
*Titulado Superior (Advanced Degree)

Master Student in Practice
Marina Gómez Rosel (since

September) (*Universidad Francisco
de Vitoria*)

Visiting Students
Marta Marciniak (until April)
(*Cardinal Stefan Wyszyński*

University, Poland), Lucas Kissling
(February-September) (*Swiss
Federal Institute of Technology
Zurich*)

RESEARCH HIGHLIGHTS

MGMT genomic rearrangements contribute to chemotherapy resistance in gliomas

Temozolomide (TMZ) is an oral alkylating agent used for the treatment of glioblastoma and is now becoming a chemotherapeutic option for patients diagnosed with high-risk, low-grade gliomas. The therapeutic benefits of TMZ depend on its ability to methylate DNA, which takes place at the N-7 and O-6 positions of guanine and the N-3 position of adenine. Although the minor product O-6-methylguanine (O6-meG) accounts for less than 10% of the total alkylation, it exerts the greatest potential to induce apoptosis. The O-6-methylguanine-DNA methyltransferase (MGMT) is responsible for the direct repair of the O6-meG lesion by transferring the alkyl group from guanine to a cysteine residue. Epigenetic silencing, due to promoter methylation of the MGMT gene, prevents the synthesis of this enzyme and consequently increases tumour sensitivity to the cytotoxic effects induced by TMZ and other alkylating compounds. Still

today, *MGMT* promoter hypermethylation is the only known biomarker for TMZ response. However, the discordance between promoter methylation and protein expression detected in a subset of patients limits the prognostic value of methylation assessment. This evidence suggests that other mechanisms, in addition to promoter methylation, could contribute to MGMT upregulation in recurrent tumours.

By analysing a large cohort of IDH wild-type and mutant recurrent gliomas treated with TMZ, we have discovered that a subset of patients carries distinct *MGMT* genomic rearrangements. By leveraging CRISPR/Cas9 technology, we generated some of these *MGMT* rearrangements in glioma cells and demonstrated that the *MGMT* genomic rearrangements contribute to TMZ resistance, both *in vitro* and *in vivo*. Lastly, we showed that such fusions can be detected in tumour-derived exosomes and could potentially represent an early detection marker of tumour recurrence in a subset of patients treated with TMZ. ■

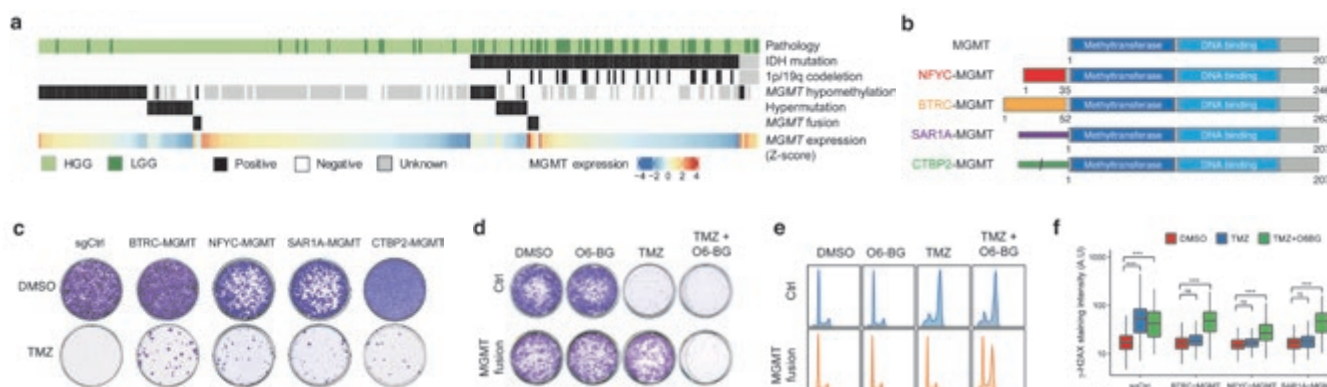


Figure (a) MGMT fusions are mutually exclusive with other known TMZ resistance mechanisms. (b) Schematic representation of the identified fusions.

(c-f) MGMT fusions protect from TMZ-induced DNA damage and cell cycle arrest. Resistance can be reverted by the MGMT-inhibitor O6-BG.

PUBLICATION

Pascual-García M, Bonfill-Teixidor E, Planas-Rigol E, Rubio-Perez C, Iurlaro R, Arias A, Cuartas I, Sala-Hojman A, Es-

cudero L, Martínez-Ricarte F, Huber-Ruano I, Nuciforo P, Pedrosa L, Marques C, Braña I, Garraza E, Vieito M, Squatrito M, Pineda E, Graus F, Espejo C, Sahuquillo J, Tabernero J, Seoane J. LIF regulates

CXCL9 in tumor-associated macrophages and prevents CD8(+) T cell tumor-infiltration impairing anti-PD1 therapy. *Nat Commun* 10, 2416.

AWARDS AND RECOGNITION

Miguel Jiménez Alcázar has been awarded an EMBO long-term fellowship.

STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA Programme Director



Programme's research areas and strategic goals

The goal of the Structural Biology Programme (SBP) is to provide mechanistic insights at the molecular level of proteins and macromolecular complexes that contribute to cancer progression. A better understanding of how these macromolecules work, together with knowledge of their three-dimensional structures, provide information to guide the design of new strategies against cancer. The groups at SBP are currently focused on the study of protein kinases as well as complexes involved in the DNA damage response. A special emphasis has been placed on setting up high-resolution cryo-electron microscopy (cryo-EM) methods, a powerful technique for high-resolution structural characterisation of individual molecules that is reshaping biological research.

SBP is composed of 1 Senior Group, 2 Junior Groups and 4 Units. During 2019, 2 new Junior Groups joined the Programme to reinforce the computational studies applied to cancer research.

Summary of milestones & major achievements during 2019

It has been roughly two years since the Structural Biology Programme was restructured to incorporate 3 new Groups and high-resolution cryo-EM technologies. 2019 was the year when these changes were firmly consolidated. The new cryo-EM microscope was installed and started to generate high-resolution data; we also set up all the computational resources and methodologies essential for high-resolution image processing. The new Groups are fully operational having published remarkable results in *Nature*, *ACS Chemical Biology*, *Journal of the American Chemical Society* and *Proceedings of the National Academy of Sciences of the USA*. In addition, the on-going activity of the Units contributed to the research conducted at CNIO, resulting in publications in *Nucleic Acids Research*, *Journal of Experimental Medicine*, *Bioinformatics* and *Cancer Cell*, among other journals. During 2019, the Groups and Units at SBP also secured access for data collection, based on competitive applications, to the eBIC Biological Cryo-Imaging - Diamond Light Source (UK) and the ALBA synchrotron Light Source (Spain).

In 2019, SBP was deeply engaged in organising scientific meetings. Groups and Units at SBP participated in the organisation of four meetings: two CNIO "la Caixa" Frontiers

"SBP provides structural and mechanistic understanding of macromolecules relevant in cancer, which is a first step towards developing new therapies. To achieve this goal, cryo-EM technologies were made fully operative in 2019."

Meetings (on 'Structural and Molecular Biology of the DNA Damage Response' and 'Heterogeneity and Evolution in Cancer'), the 'CCP-EM High Resolution EM Model Building and Validation Workshop' and the 'Workshop in Advances in the R2TP/URI-Prefoldin Complex in Cancer'. These meetings were an excellent opportunity to discuss the latest advances in these areas, but also to advertise the good science performed at SBP and the CNIO as a whole. In addition, excellent speakers were invited to our seminar series on topics connecting Structural Biology and cancer research; of particular interest was the visit of Sjors Scheres (MRC-Laboratory of Molecular Biology, Cambridge, UK), one of the main references in cryo-EM applied to biomedical research.

Finally, 2 new Groups, led by Solip Park and by Geoff Macintyre respectively, recently joined the CNIO to strengthen computational science in SBP. They will combine high-throughput technologies, big data and computational modelling to characterise the complexity of tumours and to develop better diagnostic and therapeutic tools for personalised medicine.

MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Óscar Llorca
Group Leader

Staff Scientists
Javier Coloma, Ana Isabel Hernández
(since May), María Ibarra (since
March), Andrés López, Ángel Rivera,
Marina Serna



Post-Doctoral Fellows
Sofía Cabeza (since June), Alba Ruiz (since September)

Graduate Students
Natalia Cuervo (since November),

Adrián del Rincón (until August), Carlos Fernández

Technician
Ana González (since September)

Students in practice
Cristina de La Vega (until July) (*Universidad Autónoma de Madrid*),
Nayim González (January-July) (*Universidad Autónoma de Madrid*)

Visiting Scientist
Mohinder Pal (until October) (University of Sussex)

OVERVIEW

Our key mission is to provide in-depth structural and molecular understanding of how macromolecular complexes implicated in cancer work. This information is essential to comprehend why and how some proteins are involved in the development of cancer. This fundamental knowledge is the basis to start the search for potential strategies to interfere with the function of these macromolecules. To accomplish this, we make use of several biochemical and molecular biology tools in combination with cryo-electron microscopy (cryo-EM). Cryo-EM is used to visualise large macromolecular complexes, and to observe their motions. Two main objectives drive our current research: (i) the study of macromolecular complexes that function in the cellular response to DNA damage; and (ii) an HSP90 co-chaperone network implicated in the assembly, activation and regulation of several complexes that are essential for cancer progression. In addition, we also address other relevant questions about human disease, in collaboration with other groups.

“R2TP/URI Prefoldin-like complex is a molecular chaperone that contributes to cancer by poorly understood mechanisms. In 2019, we showed how 2 essential ATPases in this complex are regulated.”

RESEARCH HIGHLIGHTS

Regulation of RUVBL1-RUVBL2 ATPases in the R2TP co-chaperone

Activation and assembly of several protein complexes implicated in cancer require the assistance of Heat Shock Protein 90 (HSP90), a molecular chaperone; thus, HSP90 inhibitors are being evaluated as potential anticancer agents. HSP90, working in concert with the R2TP/URI Prefoldin-like complex, is needed for the activation and stability of the PI3-kinase-like family of kinases (PIKKs), regulating the DNA damage response and cell growth. R2TP/URI Prefoldin-like complex is the most complex HSP90 co-chaperone yet described; it is involved in cancer progression and is the focus of several studies looking for potential inhibitors.

We are using cryo-EM to fully understand the structure of this co-chaperone complex and how it works. R2TP forms a core sub-complex containing 2 ATPases, RuvB-like protein 1 (RUVBL1) and RuvB-like protein 2 (RUVBL2). These ATPases form a hexameric ring that interacts with RNA polymerase-associated protein 3 (RPAP3) and PIH1 domain-containing protein 1 (PIH1D1) to assemble the R2TP complex. Several studies have demonstrated that the ATPase activity of RUVBL1 and RUVBL2 is required for their biological functions *in vivo*, but how this is controlled and regulated is still mysterious.

In our new study, cryo-EM is used to reveal one of the mechanisms regulating these ATPases. RUVBL1 and RUVBL2 cannot efficiently hydrolyse ATP because the access to their nucleotide-binding site is obstructed by hexamerization, making ATP/ADP exchange more difficult. Cryo-EM of RUVBL1 and RUVBL2 in complex with PIH1D1, one of the components of the R2TP complex implicated in client recruitment, reveals that the interaction of PIH1D1 with RUVBL2 induces large conformational rearrangements that lead to the destabilization of an N-terminal segment of RUVBL2 acting as a gatekeeper to nucleotide exchange (FIGURE 1). These results identify PIH1D1 as a factor that regulates the accessibility to the nucleotide-binding site in RUVBL2, thereby facilitating nucleotide exchange and activation.

Architecture of the Type VII secretion system in mycobacterium

Tuberculosis is an infectious disease and one of the 10 leading causes of death worldwide. The mycobacterium causing this disease uses a membrane-embedded secretion system (Type VII secretion system, T7SS) to inject virulence factors required for infection into the host immune cells, some of which block the defensive response. Targeting the function of this secretion mechanism has been proposed as a way to stop infection, but the lack of information on how T7SS works at the atomic level has prevented progress in designing these new therapeutic strategies. In a joint effort between our group and the group headed by Sebastian Geibel at the University of Würzburg, we managed to describe in detail the structure of T7SS at the atomic level.

We used *M. smegmatis* as a model to study *M. tuberculosis*, a bacteria that shares the same secretion system. Our work showed that T7SS is a sophisticated nanomachine in which several proteins cooperate to inject the virulence factors produced by the bacterium into the cells of the immune system (FIGURE 2). Most interestingly, EccC3, one of the proteins in the cytosolic side of the membrane, comprises 4 linked but flexible ATPase domains involved specifically in recruiting the virulence factors and energising secretion. Our structure shows that this ATPase protein makes direct contact with an alpha helix from the periplasmic pore protein, which inserts all the way into the cytoplasm. The architecture of T7SS and the experiments performed suggest that conformational changes induced by ATP hydrolysis most likely regulate the opening and closing of the secretion pore. ■

PUBLICATIONS

- ▶ Famelis N[§], Rivera-Calzada A[§], Degliesposti G, Wingender M, Mietrach N, Skehel JM, Fernandez-Leiro R, Böttcher B, Schlosser A, Llorca O^{*}, Geibel S^{*} (2019). Architecture of the mycobacterial type VII secretion system. *Nature*. 576, 321-325. (†co-first authors; * co-corresponding authors).
- ▶ Muñoz-Hernández H, Pal M, Rodríguez CF, Fernandez-Leiro R, Prodromou C, Pearl LH, Llorca O (2019). Structural mechanism for regulation of the AAA-ATPases RUVBL1-RUVBL2 in the R2TP co-chaperone revealed by cryo-EM. *Sci Adv* 5, eaaw1616.
- ▶ Remacha L, Pirman D, Mahoney CE, Coloma J, Calsina B, Currás-Freixes M, Letón R, Torres-Pérez R, Richter S, Pita G, Herráez B, Cianchetta G, Honrado E, Maestre L, Urioste M, Aller J, García-Uriarte Ó, Gálvez MÁ, Luque RM, Lahera M, Moreno-Rengel C, Eisenhofer G, Montero-Conde C, Rodríguez-Antona C, Llorca Ó, Smolen GA, Robledo M, Cascón A (2019). Recurrent germline DLST mutations in individuals with multiple pheochromocytomas and paragangliomas. *Am J Hum Genet* 104, 1008-1010.
- ▶ Rivera-Calzada A, Carroni M (2019). Editorial: technical advances in cryo-electron microscopy. *Front Mol Biosci* 6, 72.
- ▶ Serna M (2019). Hands on methods for high resolution cryo-electron microscopy structures of heterogeneous macromolecular complexes. *Front Mol Biosci* 6, 33.

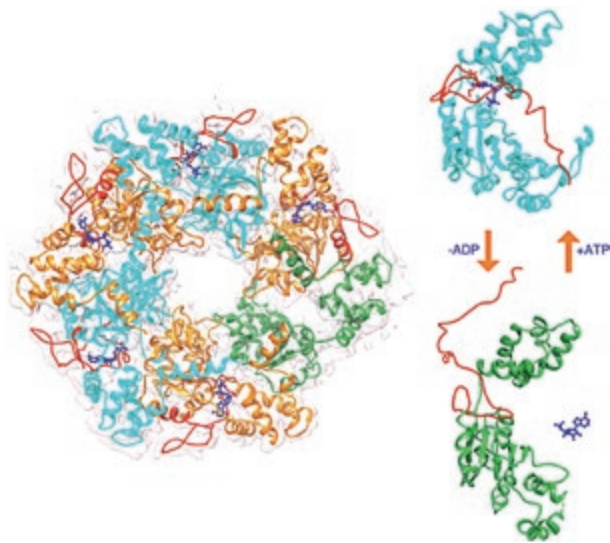


Figure 1 Cryo-EM structure of the RUVBL1-RUVBL2 hexameric ring, revealing conformational changes in RUVBL2 after PIH1D1 binding (left panel). Structures of one RUVBL2 subunit in the ADP-bound conformation (cyan) and an open

ADP-empty conformation (green) are shown in the right panels. ADP is shown in blue. The N-terminal segment of RUVBL2 (residues 8 to 49) is coloured in red (adapted from Muñoz-Hernández *et al.*, 2019).

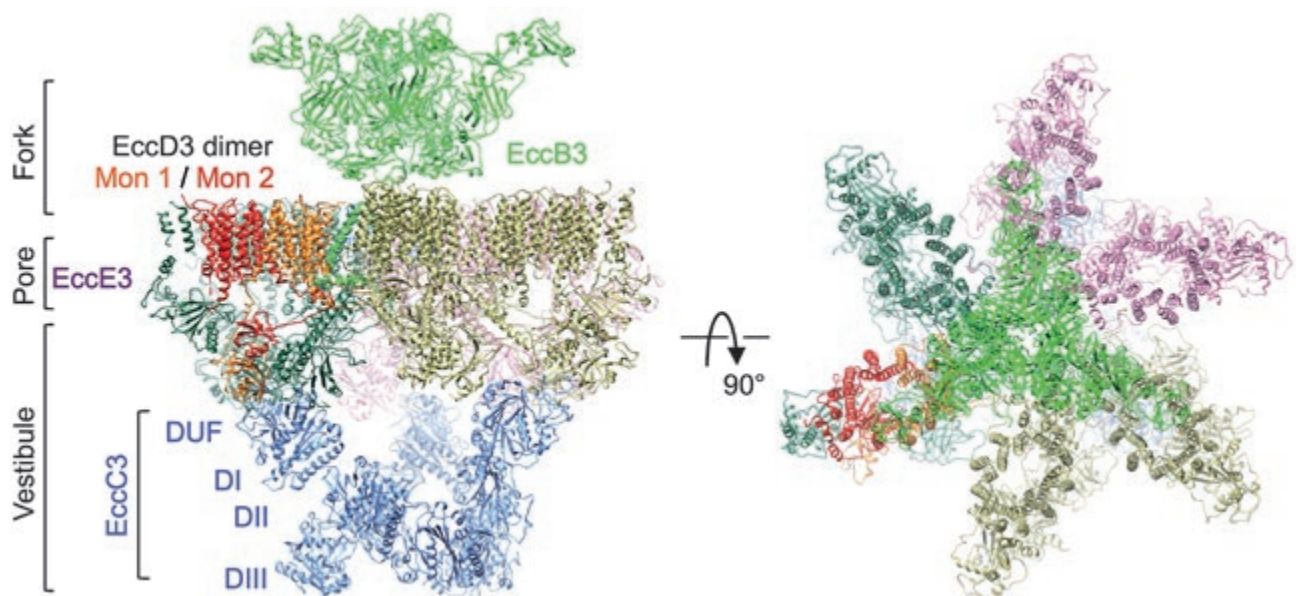


Figure 2 Side and top views of the hexameric model for T7SS built from 3 dimers, each coloured differently. One monomer in the hexamer is selected to colour each of the constituent proteins. EccB3 protein is periplasmic, and its conformational changes can regulate the opening and

closing of the pore. EccC3 is a flexible cytoplasmic ATPase that engages close to the membrane with a helix from EccB3 to possibly regulate opening and closing (adapted from Famelis, Rivera-Calzada *et al.*, 2019).

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellows
Pablo Soriano, Rubén Julio Martínez
(since December)



OVERVIEW

Rational and precise targeting of oncogene-driven signalling is a crucial and, yet today, outstanding challenge in cancer research. Understanding the structural and molecular bases of oncogene activation and signalling is key for the design and development of better therapeutics. Our research focuses on the structural and molecular understanding of protein kinase function: (i) how protein kinases are activated and regulated by posttranslational modifications and allosteric inputs, and (ii) how they assemble into macromolecular protein complexes to transmit signals inside the cell. We put a special emphasis on how these mechanisms are corrupted in cancer and disease 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, eventually leading to more effective drugs for the treatment of cancer patients.

“Understanding protein kinase function and inhibition for better cancer therapeutics.”

Graduate Students
Nicolás Cuesta, Moustafa Ahmed
Shehata

Students in Practice
Carolina Hernández (June-August,
Universidad Católica de Valencia),
Fabiola Piscopo (March-September,
*Università degli Studi Napoli Federico
II*), David Sánchez (until June,

Universidad Autónoma de Madrid),
María Valdés (until June, *Universidad
Autónoma de Madrid*)

RESEARCH HIGHLIGHTS

We established 4 main research lines in 2019, and at the same time the lab expanded with the arrival of 2 'CNIO Friends' fellowship recipients: Rubén Julio Martínez Torres (postdoctoral contract) and Moustafa Ahmed Shehata (Carmen Gloria Bonnet predoctoral contract).

- Structural and molecular determinants of RET catalytic activity and signalling, both *in cis* by intrinsic elements and *in trans* by effector kinases and adaptor proteins. We paid special attention to the crosstalk between RET and non-receptor tyrosine kinases (NRTKs).
- Structure-function studies of RET oncogenic variants, i.e. point mutations targeting the kinase domain and oncogenic fusions generated by DNA-rearrangements, with a special emphasis on the latter in the context of aggressive types of cancers.
- Structure-based drug-discovery for new (allosteric) RET inhibitors.
- Histidine phosphorylation and structure-function studies of histidine kinases.

We also focused on less known phospho-specific modifications such as histidine phosphorylation and the regulation of histidine kinases. The current understanding of this type of phosphorylation is poor. Together with T. Schirmer's group at the Biozentrum (University of Basel), we directly contributed to the first crystal structure of a full-length hybrid histidine kinase and the molecular dissection of its full catalytic cycle (FIGURE). This work was recently published in *PNAS* (Dubey *et al.*, 2020). ■

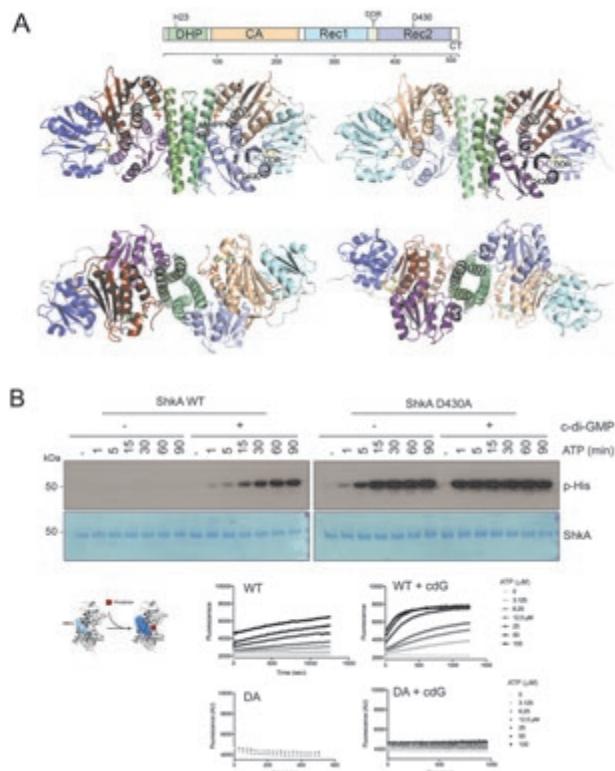


Figure Crystal structure of full-length hybrid histidine kinase ShkA. (A) Domain organisation (colour coded) of hybrid histidine kinase ShkA. Crystal structure representations, front-back and top-bottom views of AMP-PNP bound protein, depict a compact dimeric and catalytic incompetent arrangement of the multidomain protein, from Dubey *et al.* *PNAS* 2020. (B) Functional evaluation of

dual histidine kinase and phosphatase activities. Western blots (WBs) of ShkA wild-type (WT) and phospho-transfer deficient mutant (D430A) in the presence or absence of second messenger c-di-GMP using histidine phospho-specific antibodies, upper panel. Phosphate sensor analyses capturing phosphatase activity (i.e. phosphate release) of ShkA WT and D430A, lower panel, from Plaza-Menacho *et al.* unpublished.

► PUBLICATION

- Dubey BN, Agustoni E, Böhm R, Kaczmarczyk A, Mangia F, von Arx C, Jenal U, Hiller S, Plaza-Menacho I, Schirmer T (2019). Hybrid histidine kinase activation by cyclic di-GMP-mediated domain liberation. *Proc Natl Acad Sci USA*. PMID 31882446.

► AWARDS AND RECOGNITION

- Rubén Julio Martínez Torres was the recipient of a 'CNIO Friends' Postdoctoral Contract.
- Moustafa Ahmed Shehata was awarded the 'CNIO Friends' Carmen Gloria Bonnet Predoctoral Contract.

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno (since May)

Graduate Students
Ester Casajús (since July), 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 we can act to correct it and prevent it from happening.

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

“The high-end cryo-Electron Microscopy setup at the CNIO enables us to look at every detail of the protein machinery of the cell, so we can understand how it works and intervene.”

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

Technicians

Ana González (until September),
Araceli Grande (TS)*

**Titulado Superior* (Advanced Degree)

Students in Practice

Alba de Haro (since October)
(*Universidad Autónoma de Madrid*), Anna Lippert (until June)
(*Universidad Complutense de Madrid*), Alberto López-Francos

(July-September) (*Universidad Autónoma de Madrid*), Marta Seijo
(October-December) (*Universidad Complutense de Madrid*)

RESEARCH HIGHLIGHTS

Mismatch repair

DNA mismatch repair (MMR) is critical for genome stability. The DNA mismatch repair machinery loads onto newly synthesised DNA and searches for mismatches. The recognition of an error in DNA by the MutS protein leads to an ATP-dependent conformational change that transfers MutS into a sliding clamp state. Only this MutS state can activate the MutL ATPase that in turn promotes the removal of the DNA for repair. These protein complexes are incredibly dynamic and flexible, and many steps of the cycle have remained elusive to structural analysis. Using cryo-EM, we captured multiple functional steps and we have studied the conformational changes that these proteins undergo in order to recognise the mismatch and license 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 integrity of the mitochondrial genome is maintained through the equilibrium between DNA replication, repair and degradation, as well as organelle dynamics, remains unclear. We are interested in understanding these pathways because of their implications for ageing and disease and, in particular, their relation to cancer.

Cryo-electron microscopy (cryo-EM)

Combined with many other approaches already established at the CNIO, we use cryo-EM to study diverse macromolecular

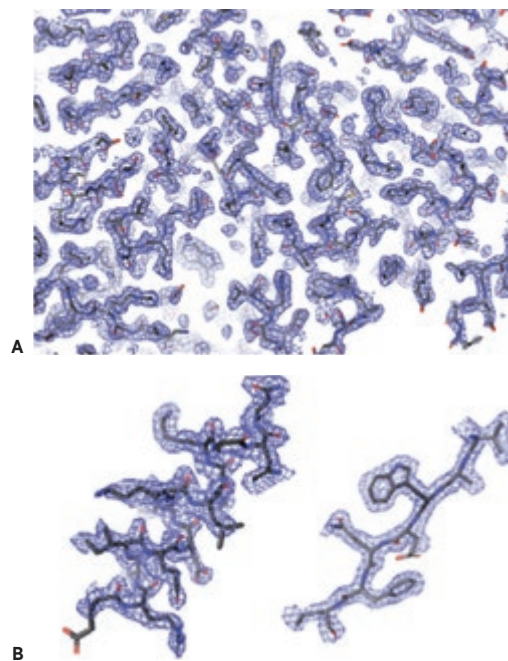


Figure High-resolution reconstruction of a protein structure from the lab to 2.0 Å resolution (in press). (**A**) Shows the overall atomic density of the protein, and (**B**) shows close-ups of density and model with clear signal for amino-acid side-chains.

complexes involved in cancer. Recent technological developments in microscopes, detectors and image processing tools have significantly improved the resolution of the technique, enabling the structural analysis of many elusive macromolecules to an unprecedented level of detail. Last year, we worked together with Oscar Llorca's Group and the EM Unit to bring the cryo-EM facility at the CNIO to a state-of-the-art level. Moreover, we were awarded access to high-end microscopes at the Biological Electron Bio-Imaging Centre (eBIC) in Oxford (UK). ■

PUBLICATIONS

- Famelis N *et al.* (incl. Rivera-Calzada A, Fernandez-Leiro R, Llorca O) (2019). Architecture of the mycobacterial type VII secretion system. *Nature* 576, 321-325.
- Barrio JD *et al.* (incl. Fernández-Leiro R) (2019). Emerging two-dimensional crystallization of cucurbit[8]uril complexes: from supramolecular polymers to nanofibers. *J Am Chem Soc* 141, 14021-14025.
- Muñoz-Hernández H *et al.* (incl. Rodríguez CF, Fernandez-Leiro R, Llorca O) (2019). Structural mechanism for regulation of the AAA-ATPases RUVBL1-RUVBL2 in the R2TP co-chaperone revealed by cryo-EM. *Sci Adv* 5, eaaw1616.
- Flydal MI *et al.* (incl. Fernandez-Leiro R)

- (2019). Structure of full-length human phenylalanine hydroxylase in complex with tetrahydrobiopterin. *Proc Natl Acad Sci USA* 116, 11229-11234.
- Míguez Amil S *et al.* (incl. Fernandez-Leiro R) (2019). The cryo-EM structure of *Thermotoga maritima* β -galactosidase: quaternary structure guides protein engineering. *ACS Chem Biol*. PMID: 31874027.

AWARDS AND RECOGNITION

- Maria Dolores Moreno was the recipient of a 'CNIO Friends' Postdoctoral Contract.
- Alberto López-Francos López-Romero received a fellowship from the summer training programme of the Spanish Association Against Cancer (AECC).

COMPUTATIONAL CANCER GENOMICS JUNIOR GROUP

Solip Park (since September)
Junior Group Leader



OVERVIEW

Cancer is one of the most complex human diseases, involving genetic, environmental and even unknown factors. Over the past several decades, large-scale genomics analyses of cancer patients have been made in order to understand this complex disease. One of the most striking findings of large-scale cancer genomics is the remarkable heterogeneity in cancer driver (oncogene or tumour suppressor gene) alterations across different patients and cancer types. However, even though various important biological characteristics are commonly measured in cancer patients, little is currently known about the cancer type- or context-specific tumour progression for each gene in each cancer type. Furthermore, by analysing large-scale cancer genomics data, many exceptions have been observed, including haploinsufficiency in tumour suppressor genes, or amplification-linked mutations in oncogenes and even

“Large-scale public cancer genomics has provided new insights to understand the contribution of inherited mutations to cancer risk.”

in dual-functional genes. Clearly, activity levels of genomic alterations in cancer genes are disparate across cancer types, and their optimal models for tumour progression may also vary depending on contexts or cancer types.

RESEARCH HIGHLIGHTS

During 2019, we: (1) set up 3 collaborations to identify novel cancer predisposition genes based on our own developed method; and (2) established a new research line for understanding the context-dependent cancer fitness landscape.

Finding novel cancer predisposition genes

We established 3 collaborations (2 in Spain and 1 in South Korea) to find novel cancer predisposition genes using our previous method (Park *et al.*, 2018, *Nature Communications*). We applied it to 3 different cancer studies and this will increase the scientific impact of our method.

Cancer-type specific fitness landscape

To understand the comprehensive cancer fitness landscape, we established the dosage (by copy-number changes)-fitness function when it is mutated (either somatic or germline), for each gene in each cancer type. We applied it to all possible human genes (~ 20,000 genes) across more than 30 cancer types. Based on this analysis, we could classify many different classes of cancer genes, including unexpected, novel cancer types. We expect to validate our observations by computational (independent dataset) and experimental analysis in 2020. ■

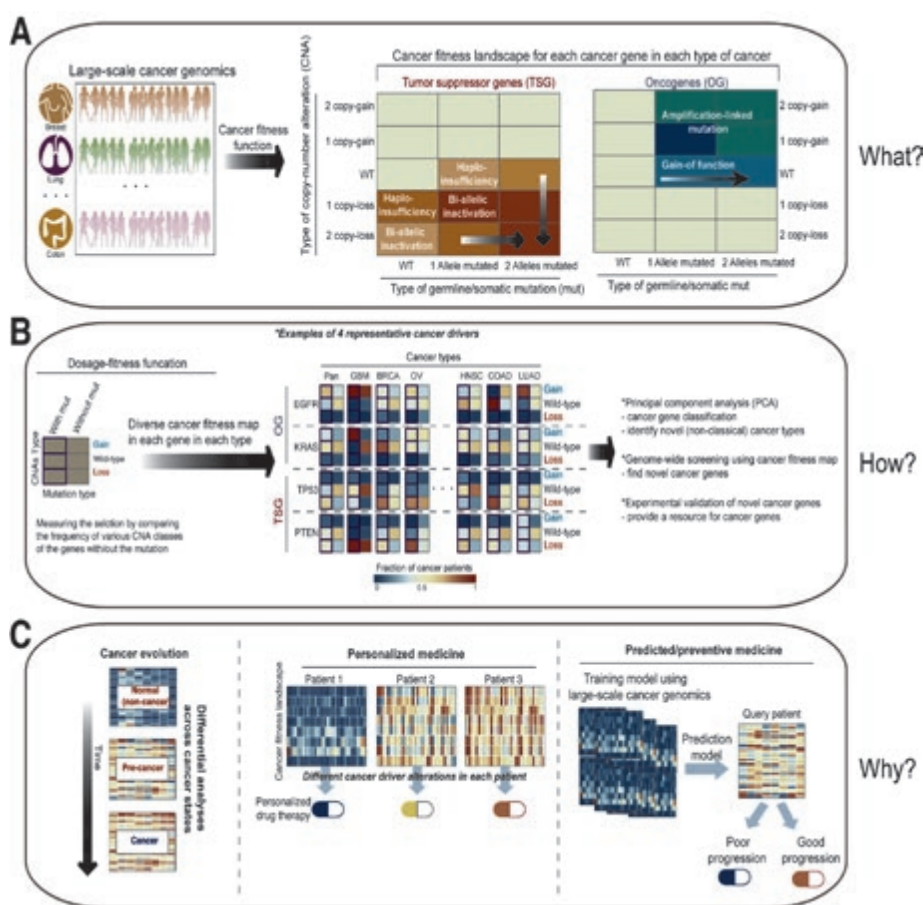


Figure The phenotypic outcome of a reduction (tumour suppressor genes) or induction (oncogenes) in gene expression is differentially manifested depending on the cancer type. **(A)** Large-scale cancer genomics data will be analysed to understand the cancer fitness landscape in each cancer gene across different cancer types. **(B)** All possible tumour progression mechanisms by using genomics data. **(C)** Multi-dimensional cancer fitness landscapes will be measured by many different phenotypic factors.

PUBLICATION AT OTHER INSTITUTIONS

- Díaz-Gay M, Franch-Expósito S, Arnau-Collell C, Park S, Supek F, Muñoz J, Bonjoch L, Gratacós-Mulleras A, Sánchez-Rojas PA, Esteban-Jurado C, Ocaña T, Cuatrecasas M, Vila-Casadesús

M, Lozano JJ, Parra G, Laurie S, Beltran S, Consortium E, Castells A, Bujanda L, Cubiella J, Balaguer F, Castellví-Bel S (2019). Integrated analysis of germline and tumor DNA identifies new candidate genes involved in familial colorectal cancer. *Cancers (Basel)* 11, E362.

COMPUTATIONAL ONCOLOGY JUNIOR GROUP

Geoffrey John Macintyre
(since December)
Junior Group Leader



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 the lowest survival rates, but they also share a common trait which we can exploit – extreme chromosomal instability (CIN). By therapeutically targeting CIN, we aim to improve outcomes in these tumours.

Our main research areas include:

- Using tumour organoids to develop therapeutic strategies to target CIN.
- Predicting therapy response using genomic signatures of CIN in patient biopsies.

“Tackling some of the deadliest cancers by targeting the causes of chromosomal instability.”

- 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

Different types of chromosomal instability leave distinct “scars” in the DNA of a tumour which we can detect using whole-genome sequencing. These genetic scars, or “signatures of CIN”, represent a way to detect and quantify different causes of CIN in a tumour.

Using tumour organoids to target CIN

Tumour organoids can be treated *in vitro* providing a way to link genomic features with drug response. By determining the activity of different types of CIN and linking them to drug response we are building a rational framework for therapy selection in the clinic using CIN as a biomarker.

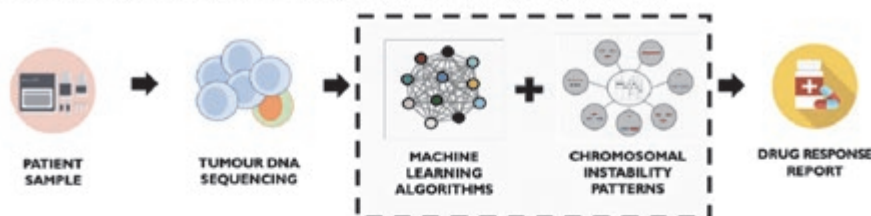
Predicting therapy response in patients

Our lab is performing a series of retrospective clinical studies looking at predicting response to therapies using signatures of CIN (FIGURE A). The long-term goal is to predict response in prospective clinical trials in order to improve patient stratification and trial success.

Single cell DNA sequencing to detect ongoing CIN

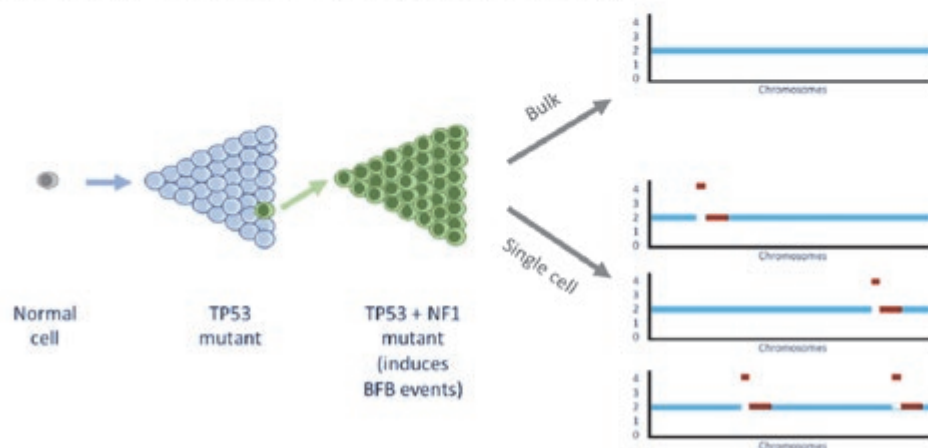
Standard “bulk” whole-genome sequencing does not allow us to separate CIN that is ongoing in a tumour, from CIN that is historical (occurred during the evolutionary history of the tumour). We are using single cell DNA sequencing to interrogate the changes unique to each cell, which enables us to separate ongoing CIN from historical CIN (FIGURE B). Our lab is applying this technology to premalignant lesions allowing us to observe CIN at its earliest stages in tumour development, before it causes aggressive, resistant tumours, with the goal of developing chemo-preventive treatment strategies. ■

A) Predicting therapy response using signatures of CIN



B) Detecting ongoing CIN using single cell sequencing

Figure (A) An overview of the workflow for predicting drug response from clinical samples. (B) A schematic outlining how a normal cell can acquire ongoing CIN through TP53 mutation and NF1 deletion. The plots on the right show genome-wide copy number profiles obtained from bulk or single cell sequencing approaches. As the bulk is an average across cells, the newly acquired CIN cannot be detected.



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 for biophysical studies of molecules involved in cancer. This includes the application of NMR to the *in vitro* characterisation of the structure and dynamics of biomolecules (proteins in particular). Together with NMR, diverse biophysical techniques are used to study the affinity and kinetics of the interactions of proteins with other biopolymers, as well as with small molecules that could represent initial hits in the drug discovery process or research compounds for biophysical and functional studies. Furthermore, we use NMR to characterise the metabolic

“In 2019, we conducted NMR experiments in cell media and extracts to quantify metabolite changes induced by gene silencing or by chemical inhibitors, thereby contributing to the elucidation of the cellular function of the silenced genes and the specificity and mode of action of the inhibitors.”

profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples.

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 a surface plasmon resonance (SPR) instrument. Research groups mostly from, but not limited to (i.e. the Haematological Malignancies Clinical Research Unit, the Epithelial Carcinogenesis Group and the Experimental Therapeutics Programme – ETP), the Structural Biology Programme extensively used these technologies throughout 2019.

The Unit hosts a 700 MHz NMR spectrometer that is equipped with probes and a sample changer to run up to 120 samples automatically. This provides medium throughput for the screening of small molecule protein binders (together with the CNIO's Structural Biology and ETP Programmes), as well as for metabolite quantification that in 2019 was done in collaboration with the CNIO-Lilly Cell Signalling and Immunometabolism Section, from the ETP, and the Cell Division and Cancer and the Metabolism and Cell Signalling – MCS – Groups, from the Molecular Oncology Programme. As an example, in collaboration with the MCS Group, we conducted ^{13}C tracing intracellular metabolite measurements by NMR to characterise the metabolic changes associated with exposure to a small molecule chemical inhibitor (FIGURE). Collectively, with this and other groups, we continue to implement sample preparation protocols and to develop spectroscopic and analytical tools to characterise the metabolites present in different biological samples. ■

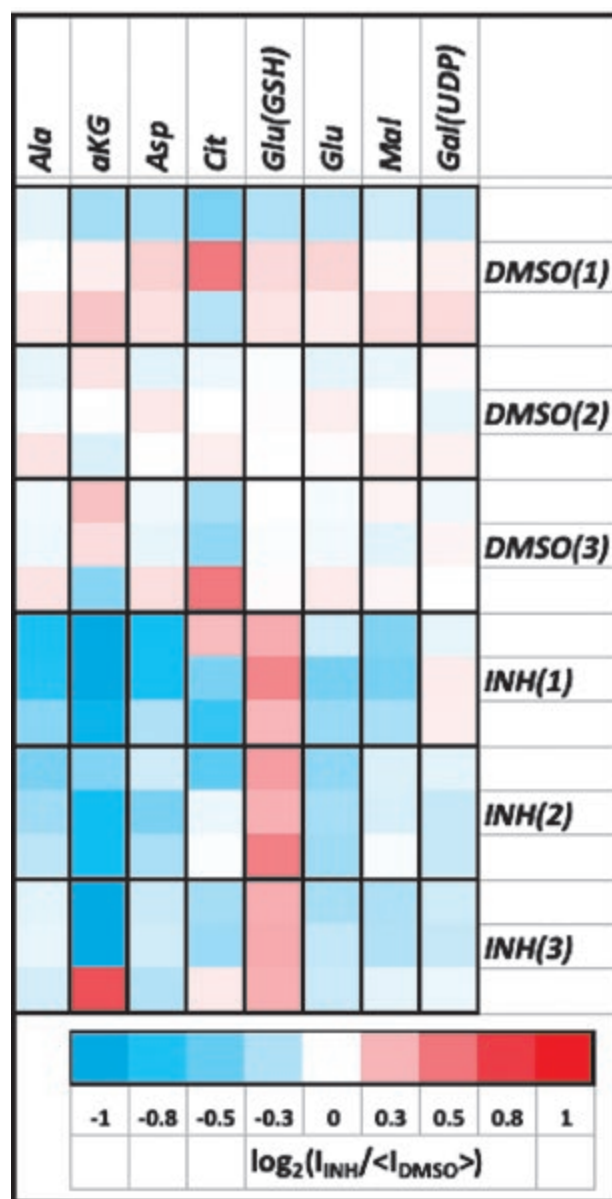


Figure NMR quantification of inhibitor-affected ^{13}C -labelled polar intracellular metabolites derived from U- ^{13}C -glucose in MDA-MB468 breast cancer cells. Each colour-coded square corresponds to the relative variation of each metabolite in a particular sample upon inhibitor treatment (INH) relative to the average value of the 3 independent control (DMSO) experiments. The putative metabolic enzyme

inhibitor was discovered in a cell phenotypic screening performed by MCS and ETP. With the exception of the glutamate in glutathione, the compound produces a decrease of the metabolic flux from glucose to most metabolites, including: the Krebs cycle intermediates citrate, α -ketoglutarate (αKG) and malate; and the glutamic and aspartic acids that are proxies for αKG and oxalacetate, respectively.

BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Staff Scientist
Michael Tress

Post-Doctoral Fellow
Hector Tejero (until June)



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 huge amount of biological data into comprehensive models that provide an in-depth understanding of cancer disease and the complex relationships among genotype and phenotype that are needed to identify cancer driver molecular alterations and new therapeutic targets.

The CNIO Bioinformatics Unit (BU) has several goals: (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 in order to identify new biomarkers and mechanisms of drug response; (iii) to provide bioinformatics support with data analysis and

“PanDrugs predictions can be correlated with clinical outcome and can be useful to manage intratumour heterogeneity in patients while increasing therapeutic options and demonstrating their clinical utility.”

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.

Graduate Students

María José Jiménez (since June),
Laura Martínez, Guillermo Martín-
Serrano (until February), Fernando
Pozo, Kevin Troulé

Technicians

Daniel Cerdán (since November)
(TS)*, Diana de La Iglesia (until
June) (TS)*, Tomas Di Domenico
(TS)*, Coral Fustero (TS)*, Gonzalo
Gómez (TS)*, Osvaldo Graña (TS)*,

Elena Piñeiro (TS)*, Thomas Anthony
Walsh (since December) (TS)*

**Titulado Superior* (Advanced Degree)

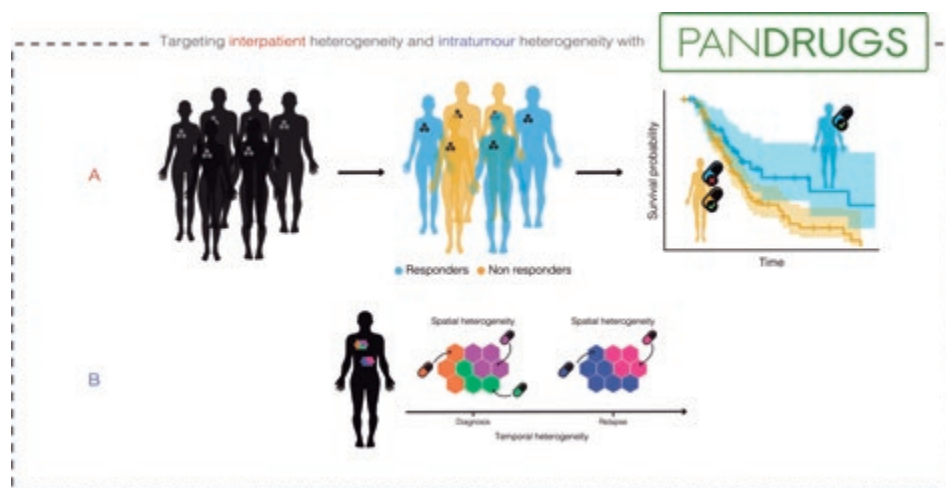
RESEARCH HIGHLIGHTS

In 2019, the CNIO Bioinformatics Unit published 19 peer-reviewed articles (see full list on our web site <https://bioinformatics.cnio.es/>) generated from our ongoing research projects and scientific collaborations. We studied the impact of inter- and intra-tumour heterogeneity using our tool PanDrugs and its predictive power of clinical outcome (Piñeiro-Yáñez *et al.*, 2019). Our results showed that patients labelled as responders according to PanDrugs predictions had significantly increased overall survival compared to non-responders. Additionally, PanDrugs' usefulness was assessed considering spatial and temporal intratumor heterogeneity and showed that it can propose drugs or combinations to target clonal diversity. Additionally, we published vulcanSpot (Perales-Patón *et al.*, 2019) for detecting and targeting cancer genetic dependencies. All our tools are freely available and have been used in our scientific collaborations, such as the use of PanDrugs in a case of T-ALL (Fernández-Navarro *et al.*, 2019) or mutational analysis during tumour clonal evolution (González-Rincón *et al.*, 2019).

Additionally, the Unit extended its analysis of misannotated coding genes (Frankish *et al.*, 2019) from the human genome to the mouse genome, and manual annotators are currently revising the annotations of coding genes in Ensembl/GENCODE for both genomes. Finally, the Unit analysed the importance of SINE Alu transposable elements in the human genome and found that these elements are preferentially incorporated into human coding genes.

The Bioinformatics Unit, as a node of INB/ELIXIR-ES, is also involved in the organisation of events and training activities. During 2019, we organised the ONCONET-SUDOE Workshop on Innovative IT for Healthcare as well as a Software Carpentry workshop. With regard to training, we co-organised the *Master en Bioinformática Aplicada a Medicina Personalizada y Salud (ISCHII-ENS)* (visit our web page for a full list of activities). ■

Figure PanDrugs is able to: (A) predict potential responder and non-responder patients to antitumour therapies; and (B) perform an in-silico drug prescription for targeting cancer patient heterogeneity. It is a promising strategy to design more effective treatments in combinatorial (or sequential) regimens.



► SELECTED PUBLICATIONS*

► Fernández-Navarro P *et al.* (incl. Piñeiro-Yáñez E, Al-Shahrour F) (2019). The use of PanDrugs to prioritize anticancer drug treatments in a case of T-ALL based on individual genomic data. *Bmc Cancer* 19, 1005.

► Perales-Patón J, Domenico TD, Fustero-Torre C, Piñeiro-Yáñez E, Carretero-Puche C, Tejero H, Valencia A, Gómez-López G, Al-Shahrour F (2019). vulcanSpot: a tool to prioritize therapeutic vulnerabilities in cancer. *Bioinformatics*. PMID: 31173067.
► Piñeiro-Yáñez E, Jiménez-Santos MJ, Gómez-López G, Al-Shahrour F (2019).

In silico drug prescription for targeting cancer patient heterogeneity and prediction of clinical outcome. *Cancers (Basel)* 11, E1361.

► Frankish A *et al.* (incl. Di Domenico T, Tress ML) (2019). GENCODE reference annotation for the human and mouse genomes. *Nucleic Acids Res* 47, D766-D773.

► González-Rincón J, Troulé K, Perales-Patón J, Al-Shahrour F JA (2019). Clonal dynamics monitoring during clinical evolution in chronic lymphocytic leukaemia. *Sci Rep* 9, 975.

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

ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Technicians
Carmen García (TS)* (PEJ)**,
Pilar Redondo

**Titulado Superior (Advanced Degree)*

***Plan de Empleo Joven (since October,
Youth Employment Plan)*



OVERVIEW

The aim of the Electron Microscopy (EM) Unit is to help researchers to solve their biological questions using various transmission electron microscopy techniques. We have extensive knowledge in sample preparation, negative staining and cryo-electron microscopy, as well as experience in image processing, 2D analysis and 3D reconstruction. We offer guidance for selecting suitable techniques, specimen preparation and training on the use of our microscopes and support equipment. More advanced studies are usually provided through research collaboration.

“Over the past year, we devoted our efforts to provide technical and scientific support to the users of the Unit in addition to fine-tuning the recently installed high-resolution cryo-electron microscope.”

RESEARCH HIGHLIGHTS

Nowadays, cryo-electron microscopy (cryo-EM) has become a main structural biology method to study macromolecules in more ‘native,’ i.e. biochemically functional, buffer conditions. At the CNIO, we use our 120 kV Tecnai G2 Spirit microscope, equipped with the TVIPS CMOS detector, to screen cryo-samples and to carry out low resolution analysis of different biological specimens. For high-resolution data collection, CNIO’s Structural Biology Programme has been granted access to high-end cryo-EM microscopes at the Electron Bio-Imaging Centre (eBIC) (Oxford, UK) through a peer-reviewed Block Allocation Group (BAG). In addition, we are performing the final set-up of our new cryo-electron microscope (JEM-2200FS) equipped with a K3 direct electron detector for medium-high resolution data collection.

During 2019, our scientific activity was developed through collaborations with all the research groups of the Structural Biology Programme, as well as with several groups from other Programmes and with scientists outside the CNIO. For instance, in collaboration with the Kinases, Protein Phosphorylation and Cancer Group (Structural Biology Programme), we worked on the structural characterisation of an oncogenic fusion of RET tyrosine kinase; in collaboration with the Macromolecular Complexes in DNA Damage Response Group (Structural Biology Programme), we were involved in the structural characterisation of several protein complexes (e.g. DNA repair and RUVBL1/2 complexes); together with M. Palacín’s Group (IRB Barcelona), we collaborated on the high-resolution structural characterisation of heteromeric amino acid transporter complexes; with the Genome Integrity and Structural Biology Group (Structural Biology Programme), we collaborated to set up a pipeline to use cryo-EM as a tool for drug discovery; with the Experimental Oncology Group (Molecular Oncology Programme), we worked on optimising, for cryo-EM, the purification of c-Raf’s protein complex; and, lastly, together with the Cell Division and Cancer Group, we analysed how the mutations in a centrosomal protein affect the architecture of centrioles. ■

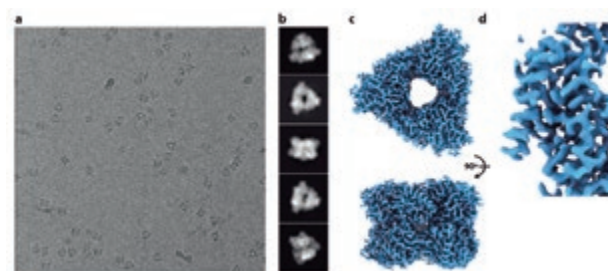


Figure Proof-of-concept for single-particle cryo-EM as a drug discovery tool. (a) Representative micrograph; (b) Reference-free 2D averages.

Scale bar represents 10 nm; (c) Preliminary 3D reconstruction at 2.6 Å resolution; (d) Zoom-in detail of cryo-EM density.

► PUBLICATION

- García-Silva S, Benito-Martín A, Sánchez-Redondo S, Hernández-Barrañco A, Ximénez-Embún P, Nogués L, Mazariegos MS, Brinkmann K, Amor López A, Meyer L, Rodríguez C, García-Martín C, Boskovic J, Letón R, Montero C, Robledo M, Santambrogio L, Sue Brady

M, Szumera-Cieckiewicz A, Kalinowska I, Skog J, Noerholm M, Muñoz J, Ortiz-Romero PL, Ruano Y, Rodríguez-Peralto JL, Rutkowski P, Peinado H (2019). Use of extracellular vesicles from lymphatic drainage as surrogate markers of melanoma progression and BRAF V600E mutation. *J Exp Med* 216, 1061-1070.

CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

Inés Muñoz
Unit Head

Staff Scientist
Jorge L. Martínez

Postdoctoral Fellows
Raquel S. Correia (since April),
Johanne Le Coq



OVERVIEW

The Crystallography and Protein Engineering Unit (XTPEUnit) is a core facility that provides on-demand services at different levels, from protein cloning to the determination of its 3D structure, with the purpose to fulfil the demands of our users and to comprehend how their protein targets work. Thus, we produce proteins for different types of biochemical/biophysical/*in vitro*/*in vivo* assays, for monoclonal antibody generation, and we also offer macromolecular structural determination at low resolution by small-angle X-ray scattering (SAXS) or at atomic resolution by X-ray crystallography. The latter includes protein co-crystallisation in the presence of inhibitors or small fragments – a method that we routinely combine in parallel with the quantification of protein thermal stability (thermofluor assay) – during the guided drug discovery process.

“Fragment screening based on crystallography can be used to map the interactions of small molecules with proteins, allowing rapid, specific and efficient progress in drug discovery projects.”

Technicians

Aida Contreras (since October) (PEJ)*, Leticia Martín (until January), Miguel A. Navarro (until January) (PEJ, CAM)**, Álvaro Otero (since June) (TS)***,

Javier Querol (since May), Eduardo Rebollo (since October) (PEJ)*, Federico Ruiz (until March) (TS)***

*Plan de Empleo Joven (Youth Employment Plan)

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

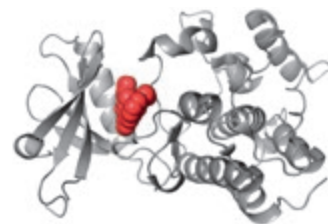
***Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

Fragment-based screening service was recently included in our portfolio. It is a well-established and powerful approach to early drug discovery. The purpose of this method is to expose protein to libraries of fragments and to solve the crystal structures of the complexes. Our first target was the dimerization domain of TRF1, a project in which we joined efforts with the Telomeres and Telomerase Group. The work was financially supported after approval of an XChem proposal by the synchrotron Diamond Light Source (UK) in the context of an H2020 iNEXT European project. As in previous years, we worked closely with the Experimental Therapeutics Programme on several projects that have since led to the production of recombinant proteins (full-length human MASTL, tyrosine kinase domain of human DDR1, full-length mouse TRF1 and human TRF1 dimerization domain) for biochemical and structural analyses. Also, in support of drug discovery projects, we performed several thermal shift assays (thermofluor) in the presence of compounds developed in the Medicinal Chemistry Section.

We have also continued our work on the production of proteins for the generation of antibodies by the Monoclonal Antibody Unit (Biotechnology Programme), including several cancer-related proteins such as POT1A, HIF1A, Syncytin1, CDK16, TRIB2, TRIB3, and FNIP2, among others. Furthermore, we have combined efforts with the Haematological Malignancies Clinical Research Unit in the structural and biophysical characterisation of hnRNPK. Additionally, we undertake a number of internal collaborations with other CNIO Groups and Units, providing them with recombinant proteins for biochemical and/or cell-based functional assays, 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 Lung Cancer Clinical Research Unit, and the Macromolecular Complexes in DNA Damage Response Group.

Figure 3-D crystal structure of the complex of DDR1 kinase domain (in grey) with the drug ETP-078 (in red).



The Unit also has ongoing collaborations with external groups such as the Environmental Biology Department (*CIB-CSIC*), 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 Químico-Físico Rocasolano, CSIC*), the Protein Tools Unit (*CNB-CSIC*), the Biomedical Application of Radioisotopes Unit (*CIEMAT*), the Department of Molecular Engineering (*Århus University, Denmark*) and the Division of Pulmonary and Critical Care Medicine, Fibrosis Research Center, and the Center for Immunology and Inflammatory Diseases (Harvard Medical School, USA).

During 2019, the Unit also continued with its own scientific projects. We worked on targeting the function of the Mdm2-MdmX E3 complex activity, in the context of an NIH-funded collaboration with the Department of Pharmacology and Therapeutics at Roswell Park Cancer Institute. Moreover, we solved by SAXS the structure in-solution of 2 new bispecific mAbs. Our collaborators (*Århus University*) verified their high inhibition potency against tumours *in vivo*. Finally, the Unit is taking part in a collaborative project with the Biomedical Application of Radioisotopes Unit of *CIEMAT* and the Molecular Imaging Unit to develop new antibody-based positron emission tomography (immunoPET) imaging tools for tumour visualisation; this work is supported by a grant from the BBVA Foundation. ■

PUBLICATIONS

- ▶ de Salas F *et al.* (incl. Virseda-Jerez A, Muñoz IG) (2019). Structural and biochemical insights into an engineered high-redox potential laccase overproduced in *Aspergillus*. *Int J Biol Macromol* 141, 855-866.
- ▶ Garaulet G *et al.* (incl. Mulero F, Martínez-Torrecuadrada J) (2019). Intratumoral expression using a NFκB-based promoter enhances IL12 antitumor efficacy. *Cancer Gene Ther* 26, 216-233.
- ▶ Lennon S *et al.* (incl. Martínez-Torrecuadrada JL) (2019). Pancreatic tumor microenvironment modulation by EphB4-

ephrinB2 inhibition and radiation combination. *Clin Cancer Res* 25, 3352-3365.

- ▶ Bejarano L, Bosso G, Louzame J, Serrano R, Gómez-Casero E, Martínez-Torrecuadrada J, Martínez S, Blanco-Aparicio C, Pastor J, Blasco MA (2019). Multiple cancer pathways regulate telomere protection. *EMBO Mol Med* 11, e10292.

AWARDS AND RECOGNITION

- ▶ Member of the Board of Directors, *Asociación de Usuarios de Sincrotrón de España*.

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HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ Programme Director



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. Our main objectives are to: (i) discover genes responsible for familial cancer; (ii) decipher the genetic bases of sporadic cancer; (iii) analyse the role of modifier factors (genetic and non-genetic) in cancer development; (iv) understand the relationship between genes and drug response; (v) implement new strategies for the cure of genetic disorders, *e.g.*, genome editing; and (vi) translate this knowledge into clinical practice through genetic diagnostic studies and genetic counselling.

Currently, the HCGP is composed of 3 Research Groups and 3 Units: the Human Genetics Group, led by Javier Benítez; the Hereditary Endocrine Cancer Group, led by Mercedes Robledo; and the Genetic and Molecular Epidemiology Group, led by Núria Malats. The Genotyping Unit, headed by Anna González-Neira, supports our 3 research groups from a technical point of view, and provides support to other CNIO groups as well as to external users. The Molecular Cytogenetics and Genome Editing Unit, headed by Sandra Rodríguez-Perales, contributes to this provision of technical support. Finally, the Familial Cancer Unit coordinates the clinical part of the HCGP through the CNIO Familial Cancer Consultancy, which is located at the *Hospital de Fuenlabrada*. Miguel Urioste is responsible for these activities. In addition, the 3 Units conduct their own research activities.

The HCGP collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. In 2019 the Familial Cancer Consultancy carried out around 570 consultancies, and the HCGP performed 1,410 genetic diagnoses and 1,230 cytogenetic studies. In addition, the HCGP's Groups hosted 10 medical residents from different Spanish hospitals, who rotated between the Groups and Units for 3-month intervals. We also offer professionals from different national and international research centres the opportunity to join us, either as visitors or for training visits consisting of short-term stays of 1-3 months; a total of 6 international and 14 national visitors and students were hosted in 2019. In terms of education, 14 national PhD students worked on their research projects, 2 of whom already successfully defended their theses.

Finally, one of the main objectives of the HCGP is to establish research collaborations with national and international groups;

“We are entering a new era of medicine: the study of healthy people based on risk algorithms, in order to identify groups at high-risk of developing cancer and to perform individualised follow-up.”

this is well demonstrated by our publication record as well as the key roles held by several of the Programme's members in consortia and projects, both nationally and internationally. Currently, we collaborate with 14 international consortia representative of the main tumour types on which we focus. In addition, we lead or participate in 15 national and 3 international European projects.

Milestones and major achievements of the HCGP in 2019 include:

- Javier Benitez: the discovery of a group of solute carrier genes that explain several autoimmune pathologies.
- Mercedes Robledo: Group Leader of the 706 Unit, CIBERER (*Centro de Investigación Biomédica en Enfermedades Raras*).
- Sandra Rodriguez-Perales: filed Patent EP18382746.8 (gene editing based cancer treatment).
- Javier Benitez: became member of the International Consortium of Testicular Cancer.

HUMAN GENETICS GROUP

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OVERVIEW

We continue to decipher the genetic bases of hereditary and sporadic breast and ovarian cancers. In addition, we have participated in a project that combines the genotype and phenotype in order to stratify and select women at high risk of developing breast cancer (BC). Other families with rare tumours are also object of our studies, for example, testicular cancer whose genetic bases are unknown. More recently, we started a study to elucidate the common genetic origin of different autoimmune-polyendocrinopathies (APs), such as chronic gastritis atrophy, thyroiditis, diabetes or arthritis. We have identified several genes (most of them solute carriers) opening the way for new diagnoses and treatments. Finally, we have progressed in understanding the role of glycosylase genes as modifiers of hereditary breast cancer and their role along the cell cycle.

“We continue working to solve new challenges associated with hereditary BC diagnosis and treatment, but in parallel have extended our studies to discover new genes that explain families with rare tumours. In this regard, a whole pathway, including solute carrier genes associated with APs, has been identified.”

RESEARCH HIGHLIGHTS

Looking for new breast cancer susceptibility genes

Deciphering the role of rare variants in breast cancer

The European project BRIDGES, aims to build a panel of high and moderate susceptibility genes to identify families with breast cancer having a genetic aetiology. For this purpose, we have analysed 36 candidate genes in 60,000 breast cancer cases and controls and have confirmed 20% of them as susceptibility genes. The second step comprises the whole exome sequencing of these 60,000 cases, aiming to discover new susceptibility genes. This part of the work was conducted throughout the year. We will translate this knowledge – the new genes plus the previous (already confirmed) genes – to several cancer consultancies throughout Europe in order to analyse their value in clinical practice.

Identification of RECQL5 as a new candidate moderate-risk gene in breast cancer

As a complementary approach to the BRIDGES project, we conducted another study using next-generation sequencing (NGS) technologies to identify new BC susceptibility genes in a few, very well selected families. This approach led to our identifying *RECQL5*, a member of the RECQL-helicases family, as a new breast cancer susceptibility candidate deserving further study. We used a combination of whole exome sequencing in a family negative for mutations in *BRCA1/2* (BRCAX) – in which we found a probably deleterious variant in *RECQL5* – as well as targeted NGS of the complete coding regions and exon-intron boundaries of the candidate gene in 699 BC Spanish families with BRCAX mutations and 665 controls. Functional characterisation and *in silico* inference of pathogenicity were performed to evaluate the deleterious effect of detected variants. These results prompted us to propose *RECQL5* as a gene that would be worth analysing in larger studies in order to explore its possible implication in BC susceptibility (Tavera-Tapia *et al.*, 2019).

Modifier genes in BRCA1/2 genes

Single nucleotide polymorphisms (SNPs) in DNA glycosylase genes involved in the base excision repair (BER) pathway can modify breast and ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers. We previously found that SNP rs34259 in the uracil-DNA glycosylase gene (*UNG*) might decrease ovarian cancer risk in *BRCA2* mutation carriers. Now, we have validated this finding in a larger series of patients with familial breast and ovarian cancer and gained insight into how this *UNG* variant exerts its protective effect. We found that rs34259 is associated with significant *UNG* downregulation

and with lower levels of DNA damage at telomeres. In addition, we found that this SNP is associated with significantly lower oxidative stress susceptibility and lower uracil accumulation at telomeres in *BRCA2* mutation carriers (Baquero *et al.*, 2019).

Familial cancer exome project

Autoimmune polyneuroendocrine syndrome (APS)

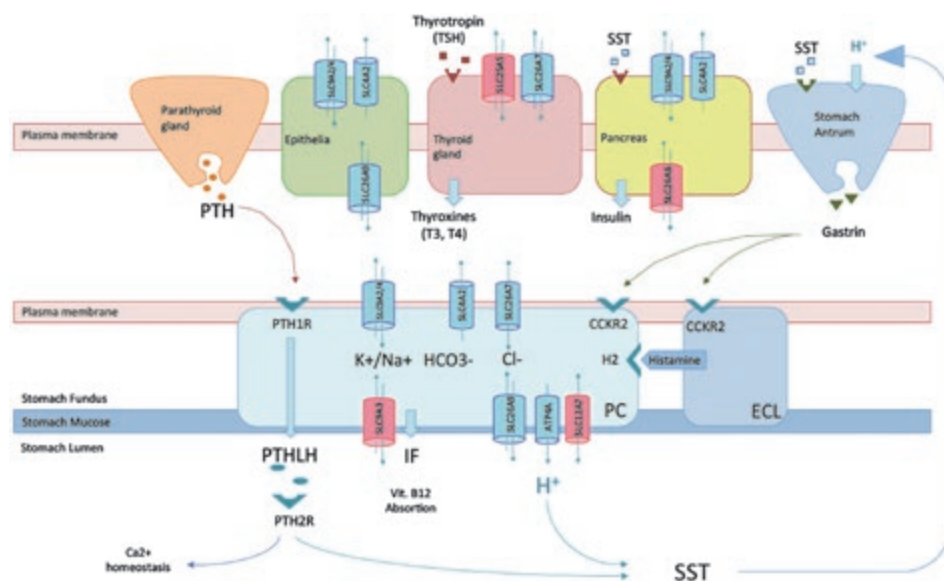
In 2015, we published research identifying the *ATP4A* gene as responsible for families with gastric neuroendocrine tumours (Calvete *et al.*, 2015). In 2017, we extended this study to a new family that presented the same lesion along with hypothyroidism and arthritis. The family presented 2 mutations in *ATP4A* and *PTHRI* following a digenic model that explained the 3 concurrent autoimmune pathologies (Calvete *et al.*, 2017). In 2019, we published a model that involves the uncovered genetic landscape with the acid-base balance malfunction and explained the autoimmune response activation that triggers malignant progression (Benitez *et al.*, 2019).

We are further investigating the apparent relation of gastric autoimmune disease (gastric neuroendocrine tumour or chronic atrophic gastritis) with other autoimmune diseases such as thyroid pathology, arthritis, diabetes type 1, vitiligo, etc. Through targeted sequencing, using a custom panel containing 12 solute carrier genes and some receptors involved in the previously described mechanism, we found several mutations in new genes involved in homeostasis function (solute carriers) – not only in stomach parietal cells but also in other tissues (epithelium, pancreas, thyroid) – altering the acid-base balance and explaining the presence of different autoimmune pathologies (apparently unrelated) in a family or an individual. Several gastroenterologists, pathologists and endocrinologists are collaborating in this project (FIGURE).

Testicular Germ Cell Tumour (TGCT)

During the past year, we completed the first part of our study by performing whole exome sequencing (WES) in familial testicular cancer and described 3 new low susceptibility genes (*PLEC*, *EXO5* and *DNAH7*) that contribute to cancer development (Paumard *et al.*, 2018). We have revisited the data using different selection criteria and filtering of the variants in order to look for moderate-high susceptibility genes. Additionally, we are exploring the genetic landscape and somatic contribution that drives different tumour histologies (seminomas and non-seminomas) in different hereditary models (familial, bilateral and sporadic TGCT). The

Figure Different solute carriers are mutated in a large number of autoimmune polyendocrinopathies. Some of them coincide in thyroid, skin or parietal cells, among others, because the solute carriers are present not only in parietal cells (stomach) but also in other tissues, altering the acid-base balance that drives achlorhydria and explaining the different, apparently unrelated, pathologies in families or individuals.



preliminary results are being validated in newly recruited cases. Finally, we have also joined The Testicular Cancer Consortium (TECAC), led by Dr Katherine Nathanson, to contribute with our data and compare our results in a bigger cohort.

Ovarian cancer

Endometrioid (EOC) and clear cell (CCOC) ovarian carcinomas are considered refractory to chemotherapy and present a poor outcome once disseminated. Defects in mismatch repair (MMR) and microsatellite instability (MSI) are predictors of immunotherapy response. During 2019, we compiled 180 EOC

and CCOC that are being characterised using a comprehensive approach that integrates assessment of global microsatellite instability (MSI), mutation burden and tumour-infiltrating lymphocytes. In order to maximise the potential translatability of our results, we designed an ‘ad hoc’ NGS ovarian cancer panel (OvaSeq-MSI) that specifically includes a large set of MS sequences to improve MSI determination and that generates information about mutations in ovarian carcinogenesis genes, ovarian cancer susceptibility genes or therapeutic targets. We have already sequenced 50 tumours of our series. Altogether, we expect to find markers to enable more rational therapeutic decision-making for patients with EOC and CCOC. ■

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HEREDITARY ENDOCRINE CANCER GROUP

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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-21-3p/TSC2/mTOR axis with predictive value for mTOR inhibitor sensitivity in pheochromocytoma and showed that PTEN expression and TSC1/TSC2/MTOR mutations predict response to rapalogs in renal cancer.”

RESEARCH HIGHLIGHTS

Integrative multi-omics analysis identifies a prognostic miRNA signature and a targetable miR-21-3p/TSC2/mTOR axis in metastatic pheochromocytoma

Metastatic pheochromocytoma and paraganglioma (mPPGL) represent major clinical challenges due to the limitations in accurate diagnosis and effective treatments. We aimed to identify reliable metastatic risk miRNAs for mPPGL, and to decipher their role in progression through an integrative multi-omic data analysis. For this purpose, we carried out a comprehensive analysis of miRNomes in tumours from 443 patients, the largest discovery cohort explored so far thanks to the participation of an International Consortium of Reference Centres with expertise on this disease.

First, we succeeded in identifying in tumour tissues a miRNA signature related to metastatic risk and to shorter time to progression. Higher levels of these miRNAs were also detected in mPPGL patients' liquid biopsies compared with controls. Using a prognostic predictive model, we built a miRNA-based classifier, which was further studied in an *in vitro* model, concluding that the classifier's miRNA overexpression induces aberrant levels of mesenchymal and neuroendocrine markers and enhances cell migration capacity.

The miRNA/mRNA data integration of the same tumours revealed a link between miR-21-3p and TSC2. Moreover, a pan-cancer TCGA integrative study, also including proteomics data, further elucidated TSC2 as a potential target for miRNA-21-3p, being able to uncover a non-previously identified regulatory miR-21-3p/TSC2/mTOR axis, which turned out to have a relevant role not only in metastatic pheochromocytoma,

but also in other cancer types, such as low-grade glioma. We observed that miR-21-3p levels correlate with TSC2 expression, mTOR pathway activation, and a predictive signature for mTOR inhibitor-sensitivity. This observation was further confirmed *in vitro*, where higher rapamycin sensitivity was exhibited by cells with ectopic miR-21-3p expression.

Altogether, the coordinated effort of multidisciplinary teams and a large consortium resulted in a comprehensive and integrative omic study, providing insights into the biological significance of the miRNA data. Through this analysis we have been able to identify PPGL at risk of metastatic progression as well as to select patients who may benefit from therapies with mTOR inhibitors (FIGURE).

PTEN expression and MTOR, TSC1 and TSC2 mutations predict response to rapalogs in renal cancer

The mammalian target of rapamycin (mTOR) pathway inhibitors, also known as rapalogs, are key drugs for the treatment of many tumour types; however, there are no predictive biomarkers in clinical use. To address this challenge, we performed a molecular and immunohistochemical characterisation of key mTOR pathway components in a series of 105 renal cell carcinoma patients treated with rapalogs, and recruited by the Spanish Oncology Genitourinary Group (SOGUG) and the University Hospitals Leuven. We demonstrated that response to these drugs occurred more frequently in cases with mTOR pathway mutations than in those without mutations ($p=0.030$). Negative PTEN immunohistochemistry staining was detected in 58% of the

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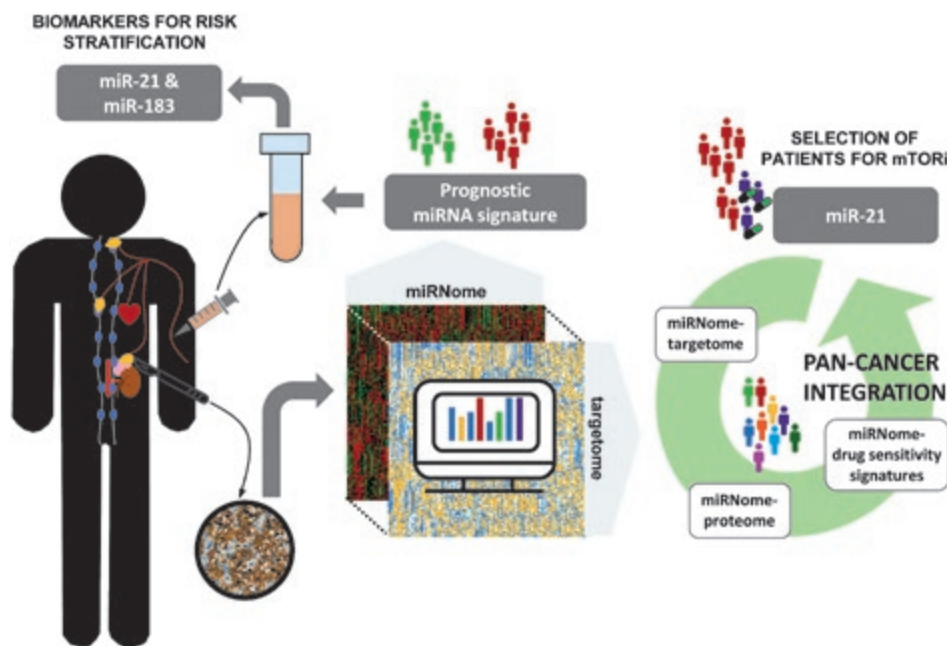
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tumours, and it was more frequent in rapalog responder patients ($p=0.029$). Furthermore, mutations and negative PTEN protein expression were not mutually exclusive events, and their combination improved response prediction ($p=0.008$). In conclusion, our findings suggest that mTOR

pathway mutations, negative PTEN immunohistochemistry staining, and their combination, are potential markers of rapalog response. These results provide a step forward with regard to guiding treatment with mTOR inhibitors and personalising renal cancer treatment. ■

Figure Schematic representation of the integrative omic analysis. MiRNA profiles found in primary tumours were analysed to define a prognostic miRNA signature. The integration with transcriptomes revealed a targetome, which was further integrated with proteomic data. This latter identified a microRNA, whose overexpression could be a potential marker to select patients for treatment with mTOR inhibitors. This prognostic signature, also detected in patients' serum, shed light upon how to improve the disease follow-up.



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OVERVIEW

The scope of the research carried out by our Group ranges from the identification of aetiological agents and mechanisms, 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 to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. Omics data provide a unique opportunity in this regard and the Group explores its integration 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 current genomic tests and data.

“The Integration of omics and non-omics data in the same risk models poses several challenges and demands appropriate analytical strategies. We are contributing to this field towards the personalised prevention of cancer.”

RESEARCH HIGHLIGHTS

Research findings

In 2019, the Group focused its research on pancreatic and bladder cancers. Regarding pancreatic cancer (PC), we progressed in the characterisation of pancreatic cancer risk factors by investigating the common genetic background of PC and different morbidities through a computational approach, and further evaluated the less explored association between PC and autoimmune diseases (AIDs) through an epidemiological analysis. Fifteen morbidities shared at least 1 gene with PC in the DisGeNET database. Based on common genes, several autoimmune diseases were genetically associated with PC, pointing to a potential link between them. Gene-disease associations were further analysed in the PanGenEU case-control study population of 1,705 PC cases and 1,084 controls. This analysis confirmed that having any of the 9 autoimmune diseases studied was significantly associated with a reduced risk of PC, which further decreased in subjects having ≥ 2 AIDs. Several inflammatory-related morbidities shared a common genetic component with PC based on public databases. These molecular links could shed light onto the molecular mechanisms underlying PC development and simultaneously generate novel hypotheses (FIGURE 1). Furthermore, we pursue the characterisation of the genetic susceptibility and somatic alteration landscape of PC by participating in

international large-scale investigations. Regarding bladder cancer (BC), the Group participated in an international consensus initiative on the molecular classification of muscle-invasive BC based on transcriptomics data that enabled us to identify of 6 subgroups, namely, basal-squamous, luminal papillary, luminal unstable, luminal non-specified, stroma-rich, and neuroendocrine. This work paves the way for future analysis on the specific aetiology of each BC subtype. The Group was also involved in the risk assessment of BC associated with air pollution. Finally, we contributed to the discovery and validation of both urine- and tumour-predictive and prognostic markers in large Spanish and European studies of both non-muscle and muscle invasive BC.

Methodological contributions

The Group continues to explore analytic strategies and tools to integrate both omics and non-omics (OnO) data. In this regard, we reported that the efforts to integrate OnO data are scarce, having been done mainly in the epidemiologic field. We identified and listed the challenges in OnO data integration and proposed integrative analytical strategies towards its integration (FIGURE 2).

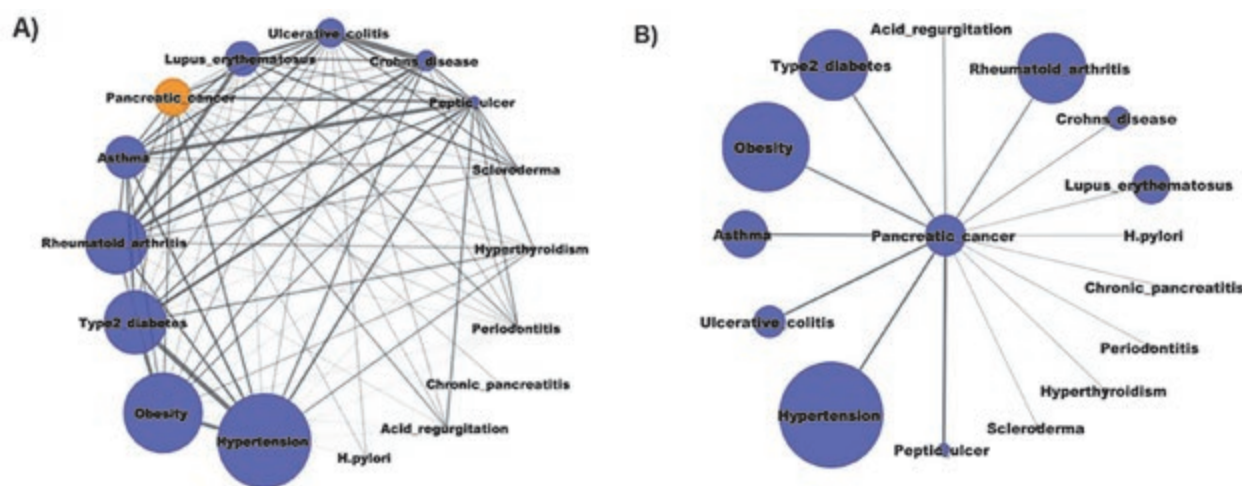


Figure 1 Gene network of medical conditions associated with pancreatic cancer through common genes. (A) Network of diseases that share genes with pancreatic cancer and all corresponding connections. (B) Network of diseases that share genes with pancreatic cancer, only connections with pancreatic cancer

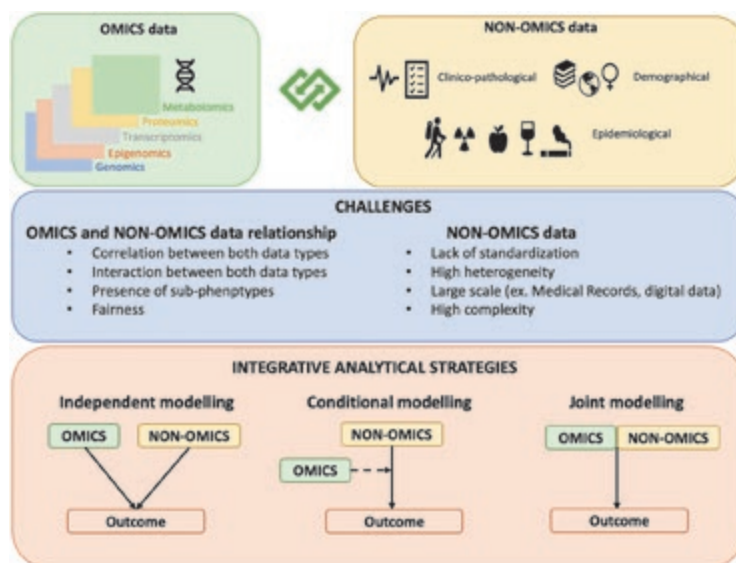
are shown. Edge width represents the Jaccard index for each disease pair; Jaccard indexes were multiplied by 100 in order to allow better visualisation. Node size represents the number of genes obtained through DisGeNET for each medical condition.

Translational activities

The Group actively provides methodological support to several clinical trials on immunotherapy and vitamin D in BC. We continue to sustain the Spanish Familial PC Registry (PanGen-FAM) and the European Registry of PC (PancreOS). We also

lead the Research Work Stream of the Pancreatic Cancer Europe (EPC) multistakeholder platform. By joining efforts and participating in the European Alliance of Personalized Medicine Annual Meeting, we also made advances in increasing awareness of PC among health policy makers and in discussing the urgent need to invest in PC research. ■

Figure 2 Challenges found in the integration of omics and non-omics (OnO) data and analytical designs for building hybrid models containing OnO data.



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AWARDS AND RECOGNITION

- Stand Up To Cancer (SU2C)/Lustgarten Foundation – Pancreatic Cancer Collective Grant, \$1 million over 2 years (2019-2021), awarded to Núria Malats and Raúl Rabadán (Columbia University, USA). *Identification of Genomic and Immune Factors in High-Risk Populations for Pancreatic Cancer*.
- External Scientific Advisor of the AECC funded GenRisk Cancer Study (Genetic and metabolomics screening: towards precision medicine in cancer prevention).
- Scientific External Advisor of the H2020-funded VISION Project.

FAMILIAL CANCER CLINICAL UNIT

Miguel Urioste
Clinical Unit Head

Graduate Student
Laura Pena

Technicians
Verónica García (since June), Maika
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OVERVIEW

CRC is the third most frequent type of cancer and the third cause of cancer-related deaths in most developed countries. Age is the main risk factor. The median age at diagnosis is 68 years in men and 72 in women. Since the mid-2000's, CRC global incidence and mortality in the USA and Europe have been decreasing at an annual rate of 2-3% for both sexes. This decrease is probably related to the extended use of the faecal occult blood test and colonoscopy, which facilitates the removal of precursor lesions, and to the increased awareness among the general population of the preventive and risk factors.

Recent epidemiological studies indicate that CRC incidence in people under 50 is increasing, which is the opposite situation for individuals over 50 years of age. The greater increase was observed in the age range of 40 to 49 years, in which the incidence changed from 18.2 cases per 100,000 population

in 1992, to a rate of 26.5 per 100,000 in 2015. This caught the attention of researchers and the general media. Several causal hypotheses were contemplated – new exogenous factors, epigenetic modifications, low-penetrance gene variants and their interactions – and there is a proposal to launch a research agenda to advance knowledge about the aetiological factors and diagnostic methods of early-onset CRC (EOCRC). Since 2010 the Familial Cancer Clinical Unit (FCCU), together with the Surgery Department of the *Fundación Jiménez Díaz* University Hospital and the Institute for Biomedical Research of Salamanca, has been committed to investigating EOCRC. The aim is to: (i) accelerate research to address unanswered questions about the causes of the increase in EOCRC; and (ii) increase the adoption of evidenced-based practices to identify and manage younger adults at risk for CRC and to facilitate early diagnosis.

CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

The FCCU's clinical and diagnostic activities contribute to the subclassification of CRC according to its molecular basis, age-of-onset and tumour location. The analysis of main susceptibility genes enables the selection of those cases of special interest: EOCRC not associated with germline mutations in known genes; or CRC of very early onset (<29 years-old) with microsatellite stability. Another considerable group of patients with EOCRC have a family history of cancer, usually tumours of the Lynch syndrome spectrum, but no germline mutations in the mismatch repair genes or in other CRC susceptibility genes have been identified. Many of these tumours are MACS (microsatellite and chromosome stable) that fall into the "Familial CRC type X" category, described years ago, and which constitutes a special interest group for the search of new susceptibility genes.

Our clinical and diagnostic activities in 2019 can be summarised as: 538 patients visited our consultancy at HUF (6.00% increase over 2018); and 572 genetic diagnostic studies were performed in the FCCU laboratory (12.59% increase). Among these studies, we identified 17 cases of CRC in individuals younger than 45, 2 of whom were patients younger than 30 (14 and 28 years old). The FCCU also focuses its research efforts on less frequent cancer predisposition syndromes. One of these is the PTEN hamartoma tumour syndrome (PHTS), in which the high clinical heterogeneity is a major obstacle to establishing an early diagnosis. We studied this pathology at the clinical and molecular level in the largest series of Spanish patients with PHTS (145 probands). Our findings are consistent with the syndrome descriptions in other populations, with few exceptions such as a higher proportion of carriers of mutations in *PTEN* exon 1 who apparently have an increased risk of developing renal cancer. We discussed the usefulness of the different diagnostic criteria proposed to date and made recommendations based on our results (FIGURE).

Noteworthy is that we highlight a novel risk for patients with PHTS, namely the development of cancer at young ages, for

which we suggest to anticipate cancer screening in these individuals. Moreover, we demonstrated that the presentation of cancer types within the PHTS spectrum criterion alone is not sufficient to refer patients for *PTEN* screening, at least as a first measure. In collaboration with the groups of Dr Pulido (*Ikerbasque*) and Dr Cid (*UCM*), we functionally demonstrated the deleterious effect of several *PTEN* variants of unknown significance. However, about half of our PHTS patients could not be explained by alterations in *PTEN*, and therefore we also focused our efforts on the search for other genes possibly involved in this disease, using a gene panel (including PI3K/AKT/mTOR pathway genes) and whole exome sequencing. Future directions will focus on unravelling the relevance of these findings. Our study continues to contribute to a better definition of PHTS and to accelerate its diagnosis. ■

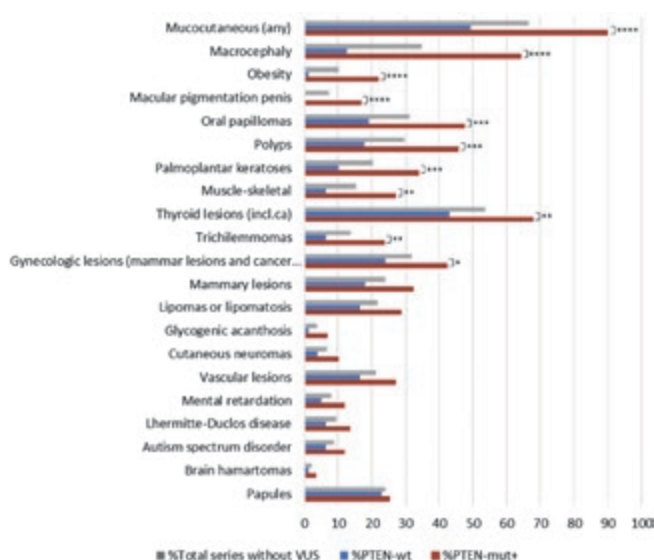


Figure Proportion of individuals in our PHTS series, excluding carriers of variants of unknown significance showing each clinical manifestation

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MOLECULAR CYTOGENETICS AND GENOME EDITING 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. Recent technological advances have improved our ability to detect and understand these rearrangements. A better understanding of these cancer-causing mechanisms will lead to novel therapeutic regimens to fight cancer. The research activity of the Molecular Cytogenetics and Genome Editing Unit focuses on increasing the knowledge about the role of chromosomal rearrangements in cancer development and progression and the discovery of new therapeutic targets. With the combined use of CRISPR genome editing and cytogenetic technologies, we are creating *in vitro* models that recapitulate chromosomal, genetic, and epigenetic cancer alterations. The goal of the Unit is to provide the CNIO and external researchers with the latest

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

technologies used in the fields of molecular cytogenetics and genome engineering. The Unit is continuously implementing and developing new technologies in those fields. We also participate in collaborative projects with clinical and basic science investigators at the CNIO and other institutions.

Technicians

M. Carmen Martín, Marta Martínez-Lage (TS)*, Patricia Moreno (since October) (TS)*, Francisco J. Moya (TS)*(PEJ)**

*Titulado Superior (Advanced Degree)

**Plan de Empleo Joven (Youth Employment Plan)

RESEARCH HIGHLIGHTS

Modelling cancer using CRISPR/Cas9 genome editing technology

Efficient methodologies for recreating cancer-associated chromosome aberrations and gene mutations are in high demand as tools for investigating how such events initiate cancer. We recently demonstrated, by generating chromosomal rearrangements, the feasibility of utilising gRNA/Cas9 ribonucleoprotein (RNP) complexes to model cancers driven by fusion genes. We optimised new strategies to enhance the efficiency of CRISPR-mediated translocation induction in human stem cells, including mesenchymal and induced pluripotent stem cells. The CRISPR-Cas9-mediated generation of targeted translocations in human stem cells opens up new avenues to modelling cancer. We are also working on an efficient approach to selectively eliminating cancer-associated fusion oncogenes.

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

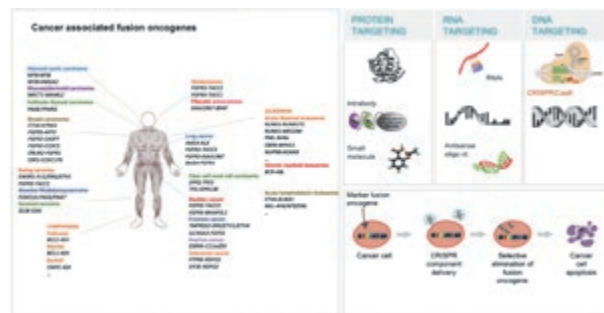


Figure Representation of cancer-associated fusion oncogenes and developed targeted therapies. Development of an innovative

CRISPR gene-editing strategy against fusion oncogenes essential for tumour viability.

cytogenetics technology analysis of human and mouse chromosomes, including conventional karyotyping, FISH, SKY and CGH array. As the field of cancer cytogenomics moves forward with the identification and cataloguing of recurrent chromosomal aberrations and gene mutations in a variety of human cancers, our Unit offers rapid, precise and affordable technologies to analyse cancer cells at the chromosome level and to functionally interrogate the cancer genome. In 2019, we carried out over 1,200 assays for experimental and clinically-oriented projects. ■

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HUMAN GENOTYPING- CEGEN UNIT

Anna González-Neira
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Graduate Students
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OVERVIEW

The most abundant types of genetic variation are single nucleotide variants (SNVs) and copy number variants (CNVs). Association studies involving the large-scale analysis of both SNVs and CNVs in thousands of patients can help to identify genes underlying complex diseases such as cancer and drug responses. In this Unit we implement different high-throughput and cost-effective methods to measure from one to millions of SNVs and CNVs. In addition, we also perform epigenetic studies using whole-genome methylation arrays. Complementarily, research focused on the identification of predictive biomarkers for precision medicine is also undertaken.

“Advances in our understanding of patients’ responses to treatment will help to personalise cancer patient care.”

Technicians

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Student in Practice

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RESEARCH HIGHLIGHTS

Searching for predictive biomarkers of anthracycline-induced cardiotoxicity (AIC). Cardiotoxicity can become a leading cause of death, even surpassing metastases and relapses. For that reason, and with the aim of identifying genetic factors associated with AIC, we recruited a cohort of 877 breast cancer patients treated with epirubicin (a type of anthracycline). We carried out whole exome sequencing in 28 patients that presented extreme phenotypes. Thanks to that approach, we identified several rare variants associated with a higher risk to develop cardiotoxicity in two genes TTN ($P=9.29 \times 10^{-4}$) and HCN2 ($P=1.1 \times 10^{-4}$). After replication in an independent cohort, the implementation of these biomarkers in the clinic may allow to discriminate those breast cancer patients with a high risk of developing AIC.

Genetic factors underlying the risk of persistent chemotherapy-induced alopecia in patients treated with docetaxel. Persistent chemotherapy-induced alopecia (pCIA) appears in its more severe grade (grade 2) in up to 10% of breast cancer patients treated with docetaxel-based therapies, and has severe psychological impact on these patients. We conducted a genome-wide association study (GWAS) to identify variants associated with the risk to develop this adverse effect. A regulatory variant located in an enhancer element that interacts with the ABCB1 promoter was found to be associated with pCIA appearance; this finding was validated in the replication cohort (OR_{combined} 4.05; 95% IQR, 2.46- 6.67; $P=3.946 \times 10^{-8}$). This

variant affects ABCB1 mRNA expression and is the risk allele associated with decreased ABCB1 expression levels ($P=1.64 \times 10^{-20}$). To our knowledge, this is the first study to identify a genetic factor related to this adverse effect.

Mutational characterisation of glioblastomas for the identification of prognostic markers and therapeutic alternatives. Glioblastoma (GBM) is the most common and aggressive malignant brain tumour in adults. Despite the advances in surgical resection, the prognosis for patients with GBM remains poor, with a median survival of 15 months, and tumours generally recur after standard multimodal treatments. We have performed the genomic characterisation of 89 primary tumours from GBM patients recruited from the *Hospital Clínico San Carlos* and *Hospital 12 de Octubre*, using a custom NGS panel with 1313 amplicons of 48 genes including 26 genes significantly mutated in GBMs in the Cancer Genome Atlas Project, and also 22 actionable genes to identify clinically relevant mutations in these patients. We identified a total of 339 pathogenic variants having an average of 4.3 (1-26) mutations per tumour. We found that *PTEN* mutations are strongly associated with shorter survival in patients with GBM ($P=0.00413$). We also found mutations in several actionable genes, such as *PARP* and *BRAF* genes. Our results demonstrate that the molecular characterisation of GBM tumours using NGS multigene panels could be a good strategy to improve the management of these patients. ■

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CLINICAL RESEARCH PROGRAMME

MIGUEL QUINTELA-FANDINO Acting Programme Director



The Clinical Research Programme (CRP) has 2 main aims: 1) to translate preclinical research into novel clinical care standards; and 2) to address novel clinical oncology challenges with preclinical research. The specific areas of work include: 1) development of novel agents; 2) study of mechanisms of action of novel compounds and tackling drug resistance; and 3), moving forward in the field of biomarkers, functional taxonomy and precision medicine.

Currently, 2 functional objectives summarise the CRP's new operating model: i) generating synergies with ongoing research lines in the basic research programmes; ii) constituting a bi-directional bridge to facilitate closer interactions between the CNIO and tertiary cancer hospitals. There are 4 agreements in place with tertiary hospitals (*Hospital 12 de Octubre*, *Hospital de Málaga*, *Hospital de Fuenlabrada* and *Hospital Quirón Pozuelo*), where the clinical activity of the CRP's Clinical Units takes place. These agreements foster the interaction between clinicians and scientists, and allow scientists from all CNIO Programmes to participate in translational research studies. The number of ongoing collaborations between the CRP Units and CNIO Research Groups have increased to 39 projects and 3 coordinated grants, which account for the high translational research activity of the institution. Nine medical oncology residents from different Spanish hospitals completed their 3-month optional stays at CNIO during 2019.

The Breast Cancer Clinical Research Unit, led by Miguel Quintela-Fandino, published a major finding about the role of fatty acid synthase as a key factor eliciting transformation of the breast epithelium. This is of importance for the development of prevention strategies for populations at high risk of developing breast cancer. The Lung Cancer Clinical Research Unit, led by Luis Paz-Ares, completed a pivotal clinical trial to register the immunotherapy combination nivolumab and ipilimumab for lung cancers with high mutational burden, where the risk for disease progression was almost halved compared to standard chemotherapy. The Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez-López, enhanced our understanding of hnRNP K as a *bona fide* oncogene. The Prostate Cancer Clinical Research Unit, under David Olmos' supervision, expanded the PROCURE network and confirmed the role of ATM in hereditary prostate cancer, which led to the publication of a seminal manuscript in the field; the Unit was recently awarded the prestigious 'Proyectos AECC Grant' to strengthen this pioneering line of research. Finally, the Molecular Diagnostics Unit, led by Luis Lombardía,

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

continued to provide support to hospitals in the diagnosis of haematological malignancies. With the large number of ongoing translational research collaborations, the arrival of novel immuno-oncology drugs, and the search for novel groups for the CRP, we face an exciting year 2020 for patient-oriented oncology research at CNIO.

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Staff Scientists
María José Bueno, Silvana Andrea Mouron



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 they range from preclinical models to the sponsoring of multicentric clinical trials. Specifically, our research areas are:

- Discovery of new targets for breast cancer prevention.
- Breast cancer functional taxonomy: by using a systems biology approach, we are clustering the disease into subtypes defined by biological features that constitute therapeutic targets.
- Study of the mechanisms of resistance against targeted therapies.

“In 2019, the BCCRU finalised its characterisation of fatty acid synthase as a key mediator of breast epithelium transformation. This opens up an unprecedented opportunity for cancer prevention.”

Clinical Research Fellow
Juan Víctor Apala (until August)

Post-Doctoral Fellow
Rebeca Gema Jimeno (since May)

Technicians
Verónica Jiménez, Manuel Muñoz

Graduate Students
Sara Fernández, José Luis Ruiz

*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

Contrary to what was previously known, we have found that fatty acid synthase (FASN, an enzyme with low expression in healthy tissue but high expression in epithelial malignancies) exerts a key role during the early steps of cancer initiation, but not when the cancer is established. *In vitro* and *in vivo* models of breast carcinogenesis cannot undergo transformation in the absence of FASN; however, its deletion after the cancer is established has little effect. Mechanistically, this preventive role is independent of its biosynthetic product. FASN consumes acetyl-CoA, which unlocks reductive isocitrate dehydrogenase-dependent carboxylation. This allows the production of the reductive power necessary to quench the reactive oxygen species (ROS) produced during the 2D-to-3D growth transition. This necessary hallmark of cancer is abrogated in the absence of FASN due to intramitochondrial ROS accumulation, which disrupts the mitochondrial respiratory supercomplexes and results in cell death. These findings open up therapeutic opportunities in the preventive phase of breast cancer.

Our previous findings about the metabolic adaptation of tumours in response to metabolic-normalising antiangiogenics were confirmed in a clinical trial, where patients with early HER2-negative breast cancer were treated with bevacizumab alone or bevacizumab plus a mitochondrial inhibitor. The latter patients experienced a 3-fold decrease in the Ki67 replicative fraction.

Finally, we worked on the search for predictive markers of activity of anti-PD-L1 agents. In patients with advanced breast cancer, we found that those with a baseline higher quotient of T-effector/T-memory populations had a higher chance of response to durvalumab. ■

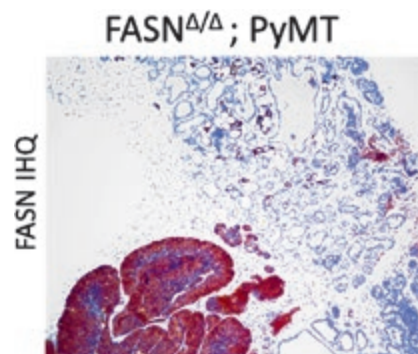


Figure IHC staining of FASN of the mammary gland of an MMTV-PyMT mouse with incomplete systemic KO for FASN. This system provides an ideal context for appreciating the role of FASN: FASN-positive areas can form tumours (characterised by invasive growth, distorting acini architecture and breaking basement membrane), as opposed to FASN-negative areas.

PUBLICATIONS

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- Quintela-Fandino M, Apala JV, Malon D, Mouron S, Hornedo J, Gonzalez-Cortijo L, Colomer R, Guerra J (2019). Nintedanib plus letrozole in early breast cancer: a phase 0/1 pharmacodynamic, pharmacokinetic and safety clinical trial of combined FGFR1 and aromatase inhibition. *Breast Cancer Res* 21, 69.
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PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Clinical Investigator
Elena Castro (until March)

Staff Scientist
Pedro P. Lopez-Casas (since July)



OVERVIEW

Prostate cancer (PrCa) is one of the most heritable human cancers. Inherited mutations in several genes involved in DNA damage response and repair have been reported to predispose men to PrCa, including mutations in *BRCA2*. Seminal work from our Group has established that these mutations are an independent prognostic factor for the disease.

The Prostate Cancer Clinical Research Unit remains a major contributor to CNIO's scientific productivity, with a total IF > 90 in 2019, and an accumulated IF > 500 since the Unit's creation in 2012. These figures include 15 manuscripts led or co-led by the team in first-decile journals, and 3 major contributions to clinical practice included in the NCCN, EAU, ASCO, and/or ESMO PrCa management and treatment guidelines. Our associated CNIO-IBIMA CRU has consolidated as the biggest disease-specific CNIO clinical unit in numbers of CNIO sponsored

studies and patients enrolled in these studies. The Unit has contributed significantly to major clinical trials in the field, such as SPARTAN or PROFOUND (both published in *NEJM* with D. Olmos as author), that led to the approvals of the novel anti-androgen apalutamide and the PARP inhibitor olaparib, respectively. In 2019, this Unit enrolled a record of 132 patients in prostate cancer trials.

Our Group continues to be successful in securing competitive national and international grants. 2019 was a record year in terms of funding: we received the 'Excellence in Research' CRIS Foundation Grant, the prestigious 'Proyectos AECC Grant', as well as 1 of the Top 3 AES-ISCIII research grants (both in scoring and financial support). Overall this positions our Unit as one of top performing units at CNIO, contributing significantly to the excellence profile of the institution.

Clinical Research Fellow
Rebeca Lozano

Post-Doctoral Fellows
María Isabel Pacheco (since March),
Isabel Aragón, Alejandro Sanz (since
September)

Graduate Students
Lorena Magraner, Ylenia Cendón

Technicians
Carles Moreno, Leticia Rivera
(since February), Mónica Balsells
(since February)

Administrator
Carmen Carpio (since February)

Visiting Student
Carlo Catrini (since October)
(*Università degli Studi di Genova*,
Italy)

Visiting Scientists
Teresa Garcés del Rey (until May)
(*Instituto de Investigación Biomédica
de Málaga*), Francesca Vitrone (since
January) (*FIMABIS, Málaga*)

RESEARCH HIGHLIGHTS

During 2019, our Group made significant progress in its multiple projects, with the following highlights:

- We demonstrated that *ATM* gene aberrations associate with a higher frequency of advanced disease and metastatic spread. Similarly, in TRAMP^{T/+} GEMMs and prostate cancer xenografts, *ATM* knock-out or silencing translated into increased aggressiveness and a higher rate of metastasis.
- Previously, we had shown that germline *BRCA2* mutations are associated with worse prognosis in different stages of prostate cancer and may be a predictors of response to certain standard therapies. In 2019 we demonstrated that somatic *BRCA2* and *RB1* co-deletion as well as *MYC* amplification occur more frequently in *gBRCA2* mutant tumours. The addition of these events progressively increases the risk of metastasis and death from prostate cancer in both sporadic and *gBRCA2* mutant associated prostate cancer.
- We established a methodology for developing prostate cancer metastatic xenograft models using both cell lines and patient derived xenografts, in which we can trace biomarkers of treatment sensitivity and resistance (using circulating tumour cells –CTCs– and metastasis). As proof of concept, we identified a biomarker of both docetaxel

efficacy and resistance. First, we observed an early (post-24h) increase of pHH3 (mitosis arrest) in CTCs derived from tumours that responded to docetaxel and, then, an increase in the aneuploidy index in tumours that did not respond to docetaxel. The early increase of pHH3 in CTCs was associated with docetaxel response in advanced prostate cancer patients undergoing treatment with docetaxel. ■

PUBLICATIONS

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- Naldaiz-Gastesi N, Goicoechea M, Aragón IM, Pérez-López V, Fuertes-Alvarez S, Herrera-Imbroda B, López de Munain A, de Luna-Díaz R, Baptista PM, Fernández MA, Lara MF, Izeta A (2019). Isolation and characterization of myogenic precursor cells from human cremaster muscle. *Sci Reports* 9, 3454.

AWARDS AND RECOGNITION

- David Olmos:
- Member of the Board of Directors, European Organisation for Research and Treatment of Cancer (EORTC).
- CRIS Foundation Excellence Award 2019.
- Elena Castro: Faculty Board Member, ESMO Preceptorships in Prostate Cancer.
- Juan Rodés Fellowship 2019, *Instituto de Salud Carlos III*.
- Rebeca Lozano: awarded the 'Merit Award', European Society of Medical Oncology, Barcelona, 2019.
- Carlo Catrini: awarded an 'ESMO research fellowship' 2019, European Society of Medical Oncology.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero



OVERVIEW

The activity of the Molecular Diagnostics Unit (MDU) focuses primarily on providing a wide range of reliable and time/cost-efficient molecular diagnostic assays to clinicians working in hospitals of the Spanish National Health System, with the aim of helping them manage their patients with different malignancies. Therefore, we work to strengthen, update and expand the assays that are currently offered by implementing the latest diagnostic tests available, or by upgrading the most established ones. In parallel, MDU is also devoted to providing support to the Clinical Research Units and Research Groups at the CNIO by collaborating with them through our technical expertise in the field, for example, by developing novel solutions for their research needs. Furthermore, MDU collaborates with international and national associations dedicated to standardising and improving molecular diagnostics in cancer.

“The implementation of assays with new molecular diagnostics biomarkers expands the range of therapeutic options that haematologists and oncologists can offer to their patients with cancer.”

Finally, our Unit remains committed towards its policy of training and mentoring students, technicians and medical residents in our techniques and methods.

CORE UNIT HIGHLIGHTS

Expanding our support

During 2019, we expanded our catalogue by adding one new molecular diagnostic test based on bi-directional Sanger sequencing. This assay detects activating mutations in exons 4, 5, 6 and 8 of the runt-related transcription factor 1 (*RUNX1*), a key regulator gene required for the differentiation of myeloid progenitor cells to granulocytes. The *RUNX1* gene is the most frequent target for chromosomal translocations associated with human leukaemias. However, loss-of-function *RUNX1* mutations have also been identified in patients with *de novo* AML. These alterations could coexist, or be mutually exclusive, with mutations in other genes included in the assays already available in our portfolio (*i.e.* *FLT3*, *TP53*, *IDH1*, *IDH2*, *TET2*, *NPM1*, *CEBPA*, *etc.*). Consequently, even if patients with *RUNX1* mutations were classified as high-risk group due to adverse prognostic outcomes (*i.e.* shorter relapse-free survival), depending on coexisting detected mutations, they could benefit from specific targeted therapies (FIGURE).

Additionally, MDU started several project collaborations with the CNIO Microenvironment and Metastasis Group. Thus, for the purpose of evaluating the clinical utility of liquid biopsies for molecular testing, we designed allelic discrimination assays that make qRT-PCR detection of *BRAF*^{V600E} and *GNAQ*^{Q209L/P} mutations possible in different biological fluids (plasma, circulating exosomes, lymphatic fluids) from patients with, respectively, cutaneous or uveal melanomas. Another collaboration with this Group aims to analyse, by whole exome sequencing, primary tumours and lymph nodes from melanoma patients to understand the genomic evolution of nodule metastasising tumours. The objective is to establish a mutational signature that could help to determine risk groups that have residual disease using plasma samples and/or post-lymphadenectomy lymphatic fluids.

Tutoring

In 2019, we hosted one secondary school student, and a postgraduate student carried out her Master's degree project at MDU. ■

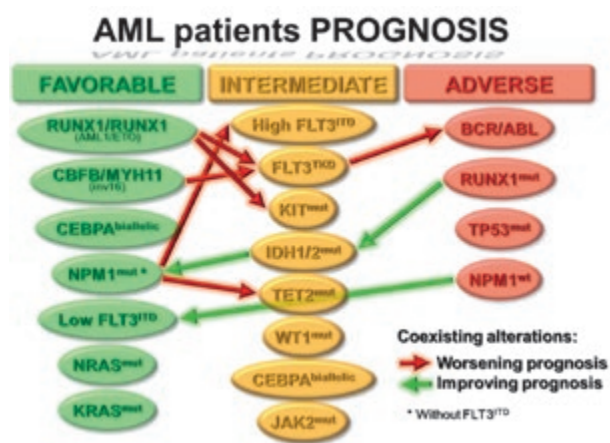


Figure Detection of concurrent mutations can help haematologists to establish an optimal approach to manage AML patients whose prior therapeutic options might have been

limited (e.g., *RUNX1* and *IDH1/2*). (Wt: Wild Type; mut: mutated; ITD: Internal Tandem Duplication; TKD: Tyrosine Kinase Domain).

► PUBLICATION

► D'Errico G, Alonso-Nocelo M, Vallespinos M, Hermann PC, Alcalá S, García CP, Martín-Hijano L, Valle S, Earl J, Cassiano C, Lombardia L, Feliu J, Monti MC, Seufferlein

T, García-Bermejo L, Martinelli P, Carrato A, Sainz B Jr (2019). Tumor-associated macrophage-secreted 14-3-3 ζ signals via AXL to promote pancreatic cancer chemoresistance. *Oncogene* 38, 5469-5485.

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López
Clinical Research Unit Head

Staff Scientists
Santiago Barrio (since June), Lucía V. Fernández, Miguel
Gallardo, María Linares (until November)



Clinical Investigators

Rosa Ayala, María Calbacho, Teresa Cedena, Javier de La Serna, Carlos Grande, Ana Jiménez, Pilar Martínez, Inmaculada Rapado, Antonia Rodríguez, Ricardo Sánchez

Post-Doctoral Fellows

María del Carmen Fiuza (since May), Almudena García, Alejandra Leivas, María Asunción Martín (since May), Yanira Ruíz, Antonio Valeri

Graduate Students

Javier Alcázar (since May), Sergio

Algar, Isabel Cuenca, Jessica Encinas, Elena Maroto, M. Luz Morales, Alejandra Ortiz, Alba Rodríguez, Laura Sánchez

Technicians

Pedro Aguilar, Paula Calvo (since November), Adrián Fernández, Vanesa

Garrido, Alexandra Juárez (until April), Laura Moreno, Esther Onecha (until June), Laura Rufián (since May)

Students in Practice

Laura Carrasco-Rubio (January-June) (UAH), Cristina Crespo-García (January-May) (UCM)

OVERVIEW

Haematological malignancies include a myriad of heterogeneous diseases with high frequency, such as non-Hodgkin's lymphoma, or with high mortality rates, such as multiple myeloma and acute myeloid leukaemia. In the laboratory, we dissect the biology of haematological cancers by investigating: (i) novel diagnosis and prognosis biomarkers; (ii) innovative tools and techniques to identify and monitor the disease; (iii) original therapeutic targets and treatments against haematological malignancies; and (iv) how to decipher the biological processes underlying the diseases and characterise the drivers, oncogenes and tumour suppressors.

The following main lines of research define our laboratory:

- Role of hnRNP K, a novel driver of lymphoma and leukaemia in tumorigenesis.
- Molecular fingerprint in clonal evolution, heterogeneity and drug resistance.
- Liquid biopsy and next-generation sequencing.
- Immunotherapy: NK-CAR and T-CAR in haematological and paediatric cancers.

“We have identified a novel master regulator of cancer, hnRNP K, a tumour suppressor now characterised as an oncogene. Lymphoma patients might benefit from more personalised therapies based on targeting hnRNP K.”

RESEARCH HIGHLIGHTS

Uncovering the oncogenic role of hnRNP K in B-cell lymphomas

Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is an RNA-binding protein that is aberrantly expressed in cancer. We, and others, have previously shown that reduced hnRNP K expression downmodulates tumor-suppressive programmes. However, overexpression of hnRNP K is the more commonly observed clinical phenomenon, yet its functional consequences and clinical significance remain unknown. hnRNP K is overexpressed in patients with diffuse large B-cell lymphoma and in mouse models without *MYC* genomic alterations. Clinical samples, mouse models, global screening assays, and biochemical studies have revealed that hnRNP K's oncogenic potential stems from its ability to post-transcriptionally and translationally regulate *MYC*. Our findings indicate that hnRNP K is a bona fide oncogene when overexpressed and represents a novel mechanism for c-Myc activation in the absence of *MYC* lesions (work published in *JNCT*).

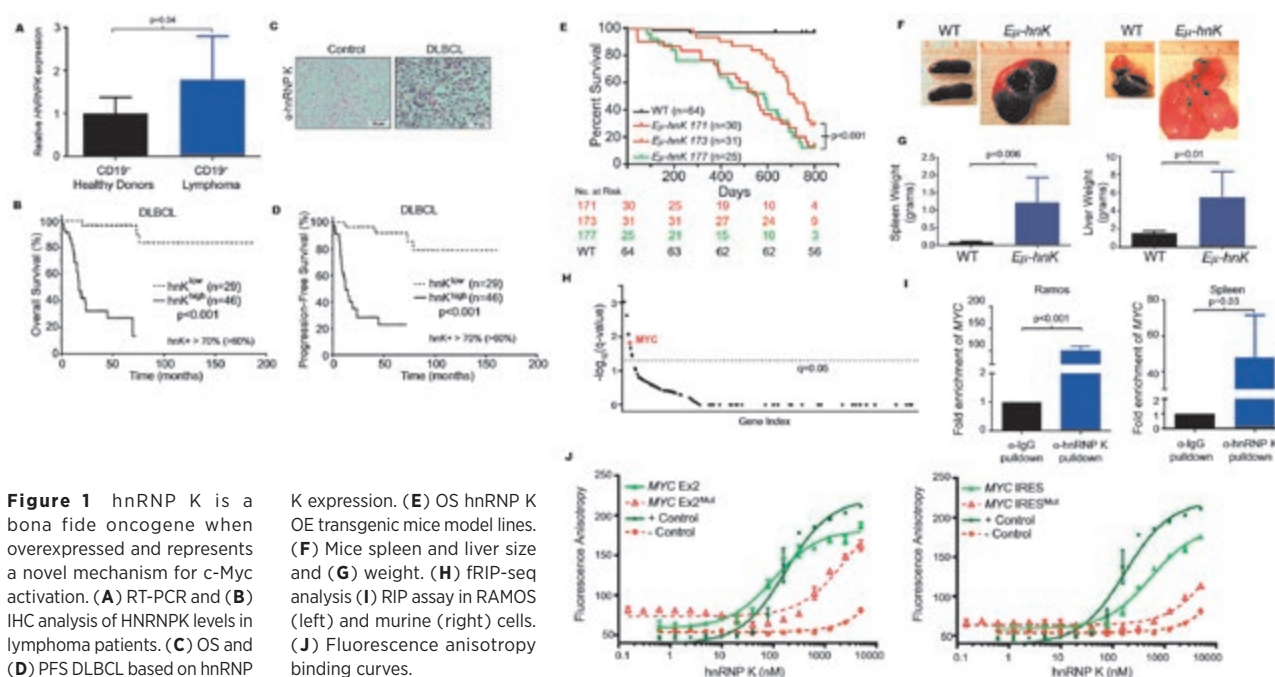
Hierarchy of TP53 alterations in multiple myeloma cell fitness

Recently, biallelic ("double hit") *TP53* inactivation, occurring in 2% to 4% of newly diagnosed multiple myeloma (MM) patients, was identified as an ultimate high-risk feature of MM, being associated with a median survival of <2 years. The

implications of monoallelic *TP53* lesions for the clinical outcome remain controversial, but clonal selection and evolution is a common feature of myeloma progression, and patients with *TP53* wild-type (WT) or monoallelic inactivation may present a double hit on relapse. Here, we addressed the hypothesis that sequential acquisition of *TP53* hits lead to a gain in the proliferative fitness of MM cancer cells, inducing the expansion and domination of the affected clones within a patient's bone marrow. To test this hypothesis, we established fluorescence-marked isogenic AMO-1 MM sublines with WT, mono-, and biallelic *TP53* events, and co-cultivated these cells in different *in vitro* competition assays. In our model, we were able to observe clonal evolution and estimate competitive advantages of both mono- and biallelic *TP53* variants. Strikingly, we demonstrated that subclones with *TP53* double hits outcompete and overgrow other *TP53* variants. Reflecting these results, a meta-analysis, including publicly available data sets, confirmed single- and double-hit myelomas to be significantly enriched in patients who relapsed (work published in *Blood*).

Mitochondrial activity plays a key role in multiple myeloma

Mitochondria control several key biological pathways involving cell proliferation and apoptosis. Many studies have implicated a functional role for mitochondria in tumour formation and development; however, their impact on the pathogenesis of



multiple myeloma (MM) remains largely unexplored. We investigated the impact of mitochondrial load and activity on the progression and relapse of MM. RNAseq data from 770 newly diagnosed patients with MM revealed overexpression of mitochondrial activity-related genes correlating with poor outcome. The expression of mitochondrial genes and proteins were elevated in patients who relapsed with bortezomib compared with previous stages, concomitant with an increase in mitochondrial activity. In proteasome inhibitor-relapsed MM patients, an elevation in c-Myc and CD38 expression, both involved in metabolic activation, could explain the consequent increase in mitochondrial activation triggered by proteasome inhibitor treatment. *In vitro* and *in vivo* studies with primary MM cells and the JJN3-Luc-GFP cell line showed the efficacy of the mitochondrial inhibitor tigecycline, alone and in combination with the frontline treatment bortezomib, reversing the bortezomib resistance induced by mitochondrial activation. Our findings provide a strong rationale for investigating tigecycline and other mitochondrial inhibitors in combination with current MM therapies (work under review in *Blood*). ■

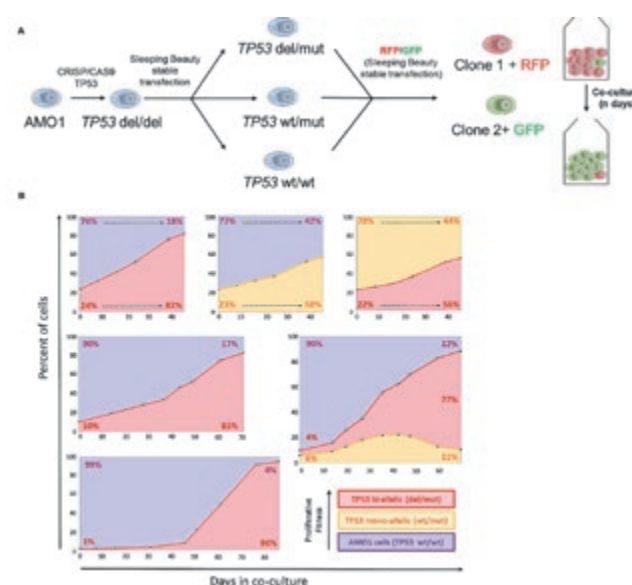


Figure 2 Clonal evolution and competitive advantages of TP53 variants. (A) Experimental flow-chart. (B) Clonal competition assays

of TP53bi-allelic (red), TP53mono-allelic (yellow) and TP53WT/AMO1 cells (purple).

PUBLICATIONS

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- Gallardo M *et al.* (incl. Martínez-López J) (2019). Uncovering the role of hnRNP K, an RNA-binding protein, in B-cell lymphomas. *J Natl Cancer Inst.* PMID: 31077320.
- Pertesi M *et al.* (incl. Martínez-López J) (2019). Exome sequencing identifies germline variants in DIS3 in familial multiple myeloma. *Leukemia* 33, 2324-2330.
- Puig N *et al.* (incl. Martínez-López J, Lahuerta JJ) (2019). Flow cytometry for fast screening and automated risk assessment in systemic light-chain amyloidosis. *Leukemia* 33, 1256-1267.
- Li N *et al.* (incl. Martínez-López J) (2019). Dual PAK4-NAMPT inhibition impacts growth and survival, and increases sensitivity to DNA-damaging agents in Waldenstrom Macroglobulinemia. *Clin Cancer Res* 25, 369-377.
- Cortés AA *et al.* (incl. Gallardo M, Martínez-López J) (2019). Ruxolitinib in combination with prednisone and nilotinib exhibit synergistic effects in human cells lines and primary cells from myeloproliferative neoplasms. *Haematologica* 104, 937-946.
- Durrant ST *et al.* (incl. Martínez-López J) (2019). Results from HARMONY: an open-label, multicentre, 2-arm, phase Ib, dose-finding study assessing the safety and efficacy of the oral combination of ruxolitinib and buparlisib in patients with myelofibrosis. *Haematologica*. PMID: 31073072.
- Alonso R *et al.* (incl. Cedena MT, Valeri A, Jiménez A, Sánchez R, Lahuerta JJ, Martínez-López J) (2019). Imaging and bone marrow assessments improve minimal residual disease prediction in multiple myeloma. *Am J Hematol* 94, 853-861.
- Fernández I *et al.* (incl. Fernández A, Leivas A, Gallardo M, Martínez-López J) (2019). GMP-compliant manufacturing of NKG2D CAR memory T cells using CliniMACS Prodigy. *Front Immunol*, doi: <https://doi.org/10.3389/fimmu.2019.02361>.
- Vela M *et al.* (incl. Fernández L) (2019). Anti-CXCR4 antibody combined with activated and expanded natural killer cells for sarcoma immunotherapy. *Front Immunol* 10, 1814.
- Sánchez R, Ayala R, Martínez-López J (2019). Minimal residual disease monitoring with next-generation sequencing methodologies in hematological malignancies. *Int J Mol Sci* 20, pii: E2832.
- Linares M *et al.* (2019). Collaborative intel-

ligence and gamification for on-line malaria species differentiation. *Malar J* 18, 21.

- Carreño-Tarragona G, Cedena T, Montejano L, Alonso R, Miras F, Valeri A, Rivero A, Lahuerta JJ, Martínez-López J (2019). Papain-treated panels are a simple method for the identification of alloantibodies in multiple myeloma patients treated with anti-CD38-based therapies. *Transfus Med* 29, 193-196.

PUBLICATIONS AT OTHER INSTITUTIONS

- Lahuerta JJ *et al.* (incl. Martínez-López J) (2019). Role of urine immunofixation in the complete response assessment of MM patients other than light-chain-only disease. *Blood* 133, 2664-2668.
- Jiménez-Ubieto A *et al.* (incl. Grande C, Rodríguez A, Lorza L, Lahuerta J, GELTAMO (Grupo Español de Linfomas y Trasplantes de Médula Ósea) Cooperative Study Group (2019). Autologous stem cell transplantation may be curative for patients with follicular lymphoma with early therapy failure without the need for immunotherapy. *Hematol Oncol Stem Cell Ther.* PMID: 31319058.

PATENT

- Barrio García s, Ayala Díaz RM, Martínez López J, Onecha de la Fuente ME, Rapado

Martínez MI. Method for determining the presence or absence of minimal residual disease (MRD) in a subject who has been treated for a disease. *EPI9382730.0*.

AWARDS AND RECOGNITION

- Miguel Gallardo was awarded AECC Investigator 2019 (PI). Project: the new master switch of cancer, hnRNP K.
- Lucía Fernández obtained an AECC Co-ordinated Groups 2019 grant (co-PI). Project: *ensayo clínico en fase I con células T memoria expresando un receptor químico antigénico especificidad NKG2D en niños, adolescentes y adultos jóvenes con sarcoma avanzado*.
- Esther Onecha was awarded with the 2019 Primer Premio de Investigación en Medicina Personalizada de Precisión UCM-Fundación Instituto Roche for the best scientific publication (*Haematologica* 2019).
- Elena Maroto was recipient of a Young Investigator Award, XVII IMW International Myeloma Workshop 2019.
- Rosa María Ayala Díaz received an AES 2019 research grant (PI). Project: *medicina de precisión en Leucemia Mieloide Aguda: detección de pacientes resistentes a drogas y desarrollo de nuevas aproximaciones terapéuticas*.

H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis G. Paz-Ares
Clinical Research Unit Head

Staff Scientists
Teresa Argullo, Irene Ferrer, Eva M.
Garrido

Clinical Investigators
Rocio García-Carbonero, M. Teresa



Muñoz, Santiago Ponce, M. Carmen Riesco, José Luis Solórzano (since July)

Post-Doctoral Fellows
Juan Manuel Coya (since February), Beatriz Soldevilla

Graduate Students

Carlos Carretero, Santiago García, Ángela Marrugal, Ángel Nuñez, Laura Ojeda, Javier Ramos, Beatriz Rubio, Patricia Yagüe

Technicians

M. Cristina Cirauqui, Patricia Cozar, Mirella Gallego, Laura García, Beatriz Gil, Alicia Luengo (since March), Arantxa Rosado (since July), Lorena Ruiz (July-October), Rocio Suárez

Students in Practice

Alba Santos (*Universidad Complutense de Madrid*)
Guiomar Casado (July-August) (*Universidad de Alcalá de Henares*)

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: (i) the identification of new molecular biomarkers for diagnostic, prognostic and predictive purposes; and (ii) 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 developed an extensive platform of patient-derived xenografts of non-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 has significantly contributed to the development of novel biomarkers that have impacted the currently available selection of targeted therapies (e.g. EGFR mutation in the clinic) and novel immunotherapeutics (e.g. tumour mutational burden). We have led randomised clinical trials with novel agents as well as combinations of targeted therapies (e.g. Ramucirumab plus Erlotinib) or checkpoint inhibitors (e.g. chemotherapy plus Pembrolizumab or Nivolumab plus Ipilimumab) in lung cancer that have impacted clinical practice worldwide.”

RESEARCH HIGHLIGHTS

Biomarker discovery and implementation

We currently own an extensive PDX platform that has led to deciphering the role of the tyrosine kinase receptors FGFR1 and FGFR4 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.*, *JTO* 2019). In addition, this PDX platform has contributed to the discovery of novel therapeutic targets, such as Yes1 (Garmendia I *et al.*, *AJRCCM* 2019) or Notch (Bousquet Mur E *et al.*, *JCI* 2019), as well as to the development of novel therapies based on targeting the cardiotrophin-like cytokine factor 1 (CLCF1)–ciliary neurotrophic factor receptor (CNTFR) signalling axis (Kim JW *et al.*, *Nat Med* 2019).

We performed a harmonisation study to determine tumour mutational burden (TMB) in a clinically well-annotated cohort of NSCLC patients. We calculated the TMB of these samples (n=100) with 2 in-house NGS-based panels and correlated it with the TMB obtained with the gold-standard method (Foundation One CDx), demonstrating a strong correlation among them. Additionally, we adjusted the cut-offs for each of the in-house panels (Garrido-Martin EM *et al.*, *ESMO* 2019). We performed multiparametric molecular and immune profiling of NSCLC tumours (n=200 early stage), after which a genomic and transcriptomic study of the tumours was carried out. This allowed us to describe novel subgroups of tumours based on multiparametric signatures with putative predictive value as biomarkers of response to therapy with immune checkpoint inhibitors.

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 2019, we participated in more than 40 projects in this research area, including 9 new trials. Recently, we published a phase II trial of Lurbenectidin, a novel transcription inhibitor, in small cell lung cancer. The results showed encouraging activity in the second-third line setting (Response rate: 34%), particularly in patients with sensitive relapse (median survival: 11.8 months) (Trigo JM *et al.*, *Lancet* 2020). Based on encouraging activity, the drug is now being tested in a phase III registrational study.

Changing standard-of-care treatments in clinical practice

The Lung Cancer Clinical Research Unit has led phase III trials whose results have significantly impacted the clinical practice in the context of stage IV lung cancer, such as the combination

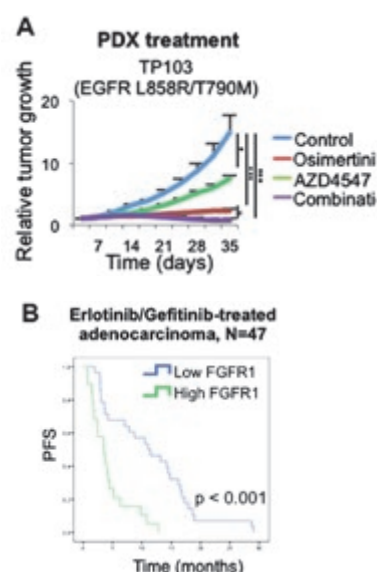


Figure 1 (A) Effect of osimertinib and AZD4547 (FGFR inhibitor) on tumour growth of the lung adenocarcinoma PDX model; (B) Effect of FGFR1 mRNA expression on progression-free survival (PFS) of patients with erlotinib- or gefitinib-treated lung adenocarcinoma, showing that patients with high FGFR1 expression had a shorter PFS period.

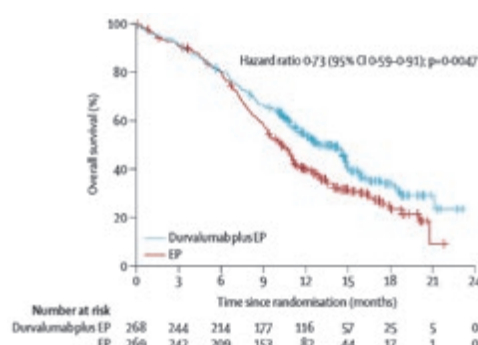


Figure 2 Results of the CASPIAN randomised clinical trial, showing improved overall survival (OS) of durvalumab plus platinum-etoposide, when compared to platinum-etoposide (HR 0.73, p=0.0047), in treatment-naïve patients with extensive-stage small-cell lung cancer (SCLC).

of chemotherapy plus Durvalumab in small cell lung cancer (SCLC) patients (Paz-Ares L *et al.*, *Lancet* 2019). The novel regimen was shown to significantly increase survival when compared to chemotherapy alone (HR 0.73, p<0.0047; median survival 13 vs 10.3 months). In addition, the mature results of the Checkmate 227 trials validated the Ipilimumab-Nivolumab combination strategy, impacting on the proportion of long-term responders and survival of stage IV NSCLC patients, regardless of the expression of PD-L1 in their tumours (Hellmann M *et al.*, *NEJM* 2018; Hellmann M *et al.*, *NEJM* 2019). ■

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► AWARDS AND RECOGNITION

- Premio 'Best in Class (BIC)' 2019 in *Investigación en Oncología*, Spain.

Innovation

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CAROLINA POLA
Director of Innovation

“Through our innovation activities we boost our impact and foster public-private alliances that contribute to bringing value and novel health solutions to society.”

From the identification of an idea to the transference of our product to the market, the Department of Innovation aims to improve the process every step of the way. Our close collaboration with investigators and to protect their research interests and assets are the main drivers to obtain results and boosts our indicators of innovation.

This optimised process has allowed us to identify four main areas of innovation potential that we have supported in 2019: drug discovery capabilities, biotechnological potential and novel basic discoveries in cancer, ageing and regenerative medicine, and computational biology. Boosting these areas has been a main objective of the Innovation Department and in this regard, we focused on identifying synergies and potential translational projects that can move forward by channeling collaborations between CNIO investigators and other institutions. Consequently, the CNIO obtained a substantial net return in royalties derived from licenses totalling 674,894 €, a growing amount compared to the last two years.

Our collaborations with the University Hospital H120 have started to yield important results with great impact for the health and biomedical ecosystem. In 2019, we closed an agreement with a spin-off company from the H120 called Altum

for the diagnosis of residual disease and we co-developed an antibody against one type of cancer. This antibody promises to have a great impact at a societal and clinical level. Additionally, two patent applications were submitted in collaboration with industry, which highlights our partnerships with the private sector to advance translational research.

Maintaining our contact with the national and international industry and life sciences investors is crucial for the development of long-term agreements and to keep up to date on new developments and companies that are important players in the field of small molecules and advanced therapies. Our presence in the Milner Therapeutics Symposium held in Cambridge every year is testament of our international activities to stay in contact with the biopharmaceutical industry.

In 2019, a *Caixaimpulse* was awarded to an exciting project on cell therapy in diabetes regeneration led by Maria Salazar and Marcos Malumbres, and we worked on a proposal for the development of telomerase gene therapy in lung and heart disease. As CNIO positions itself in the competitive field of gene and cell therapy with novel approaches, our Department will keep exploring potential partners and providing understanding and input to contribute to development plans of our assets.

BIOTECHNOLOGY PROGRAMME

FERNANDO PELÁEZ Programme Director



The main mission of the Biotechnology Programme Core Units is to provide expert technical and scientific support to CNIO Research Groups in a number of disciplines and technologies widely used in biomedical research, as well as to implement and develop state-of-the-art biotechnological tools and protocols. The Programme consists of 9 Core Units covering major areas in Biotechnology, namely Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Mouse Genome Editing, as well as an Animal Facility. Although the Core Units are mainly focused on providing support and collaborating with the CNIO Research Groups, they also work with groups from other public research institutions as well as with private companies.

Faithful to its mission, a number of different technological innovations have been explored or implemented by the Core Units during 2019, often in collaboration with CNIO Groups. Among the new technologies introduced at the CNIO this year, it is particularly worth mentioning the Histopathology Unit's implementation of the RNAScope system for the detection and visualisation of specific mRNAs by microscopy directly on tissue samples. Likewise, this year the Genomics Unit set up a new technology for single cell gene expression analysis (scRNAseq), based on a cell encapsulation system (Chromium 10X) acquired in late 2018. Moreover, the technological capabilities of the Units have been upgraded during 2019, with the acquisition of an Illumina NextSeq550 instrument for deep sequencing at the Genomics Unit, and a new ultrasound VisualSonics Vevo 3100 system at the Molecular Imaging Unit, among other equipment. Finally, the purchase process of a STED platform for super-resolution microscopy, co-funded with support from a call for scientific infrastructures from the Ministry of Science, Innovation and Universities (*MCIU*), has been initiated. The system will be deployed in 2020.

As an indication of our strong commitment to training, education and outreach, the Programme has been deeply involved in the organisation of courses, workshops, student visits, and meetings. We collaborated with the 'CNIO & the City' project, coordinated by the CNIO Institutional Image and Outreach to Society Office. The EuroMabNet network, chaired by the Head of the Monoclonal Antibodies Unit, held a workshop on the validation of antibodies, and specific training courses and workshops were organised by diverse Units (Confocal Microscopy, Flow Cytometry, Molecular Imaging, Animal Facility). Moreover, several members of

“The capability to maintain and upgrade state-of-the-art core facilities is one of the key elements behind the success of CNIO and its outstanding track record of scientific achievements.”

our staff participated in various Masters courses and other training activities at the CNIO and elsewhere.

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. Also, 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, with an increase of 10% compared to the previous year; in addition, several new agreements have been signed with different companies to sell and distribute those antibodies.

Last but not least, 2019 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 nearly 30 publications co-authored by members of the Units, many of them in top journals.

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head



OVERVIEW

The Genomics Unit provides on-demand services in the genetics/genomics fields to CNIO researchers and the wider research community. Technologies are put in place with the capacity to interrogate genomes and their activities in a single assay. These methodologies contribute to dissecting molecular hierarchies and pathways. Processes such as functional activation states, as delineated by transcriptomic profiles (mRNA, miRNA) or protein factor interplays at the gene level, and structural features, such as mutation landscapes or variations in chromatin structure, can all be examined to study the quick-paced lives of cancer tumours. We cover a broad range of applications, including solutions such as exome mutational landscapes, protein location analysis by ChIP-seq analysis and transcriptome profiles by RNA-seq technologies, complemented with more traditional characterisations by

“The services in the genetics-genomics fields provided by the Genomics Unit contribute towards the understanding of molecular processes in homeostasis and disease at different levels of biological complexity.”

capillary Sanger DNA sequencing. Among other side activities, we also manage a rather active transgenic mouse genotyping service.

Technicians

Purificación Arribas, Guadalupe Luengo, Jorge Monsech, Ángeles Rubio, Marco de Mesa, Laura Conde

José Luis Espadas (since December) (PEJ)*

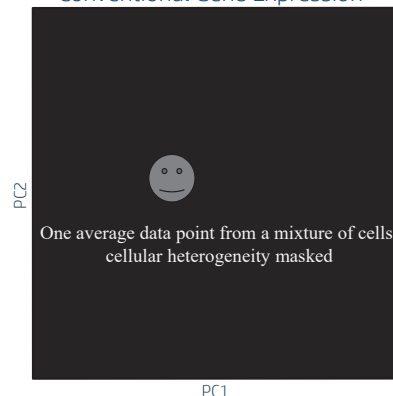
**Plan de Empleo Joven* (Youth Employment Plan)

RESEARCH HIGHLIGHTS

With its portfolio of services that survey different levels of biological complexity, the Genomics Unit contributes to the research projects of multiple CNIO groups. A wide genomic level is addressed by deep-sequencing technologies (NGS) and their applications. NGS permits a variety of different explorations, such as whole genome and whole exome tumour characterisation, transcriptomic analyses and location of chromatin interacting protein factors or RNA binding. This year we acquired and installed 2 pieces of equipment that enrich our capacity in this field: a Chromium Controller from 10x Genomics, suitable to perform single-cell genomics studies; and a sequencer (NextSeq, Illumina), a necessary element for the readout of NGS applications.

Some of our contributions led to the two following research reports being published in 2019, with the co-authorship of some of the Unit's members: Fernández-Barral *et al.* report the presence of the vitamin D nuclear receptor (VDR) and stem cell markers (LGR5) in the human intestinal mucosa. Transcriptomics and ChIP-seq data support a direct effect of calcitriol, the active metabolite of vitamin D, in colon mucosa crypt stem cells. Vitamin D was found to display both pro-stemness and antiproliferative effects on the intestinal mucosa and is therefore proposed to contribute to the homeostasis of healthy intestine. The other report by Santos *et al.* describes organoids that recapitulate urothelial features *in vivo*. 90% of bladder cancer cases originate in the urothelium, and its growth and differentiation characteristics are poorly understood. The single-cell transcriptomics platform of 10x Genomics revealed cellular heterogeneity in different organoid culture conditions, and common and distinct cellular programmes under differentiation or proliferative responses. In addition, the study uncovered the involvement of Notch signalling in urothelial differentiation, which is consistent with the reported findings of mutated Notch pathway components in bladder tumours, and with the down regulation of NOTCH1 transcript levels in transformed bladder cells. ■

Conventional Gene Expression



Single Cell Gene Expression

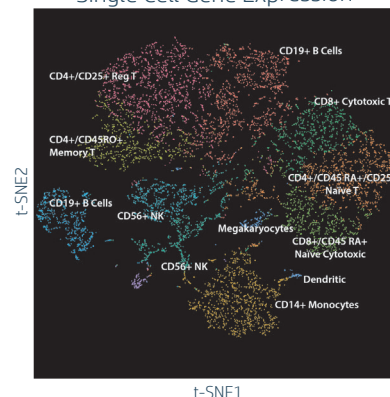


Figure Conventional gene expression analysis is performed on RNA extracts from multicellular tissues or samples. Since an average expression value per gene is obtained, the cellular heterogeneity of complex

cell populations is masked. On the other hand, the analysis of thousands of individual cells at the single cell level exposes the unique reality of complex cell populations.

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MOUSE GENOME EDITING CORE UNIT

Sagrario Ortega
Core Unit Head

Graduate Student
Aleida Pujol

Technicians
Estefania Ayala, Marina Cabrerizo
(since November) (PEJ)*,



OVERVIEW

Genetically modified mice represent one of the basic pillars that sustain cancer research at the CNIO. The term ‘cancer’ includes a variety of extremely complex diseases in which malignant cells communicate with different body systems, such as immune cells or blood vessels, that modulate tumour growth, expansion and invasion. Such complexity cannot be sufficiently well studied by using *in vitro* systems alone. The Mouse Genome Editing Unit is dedicated to the design, generation and cryopreservation of genetically modified mouse models of cancer, using state-of-the-art technology for the controlled modification of the mouse germ line. In collaboration with CNIO groups, we have created hundreds of genetically engineered mouse strains that are crucial for understanding the molecular basis of tumour development and for the preclinical validation of new and more efficient

“The Mouse Genome Editing Unit is developing new CRISPR/Cas based technologies to optimise the generation of genetic mouse models and the use of mouse genetics to promote the advancement of cancer research.”

cancer therapies. The Unit currently maintains a collection of more than 1000 cryopreserved mouse strains from which the entire scientific community may benefit for the advancement of Science in many different research disciplines.

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

*Plan de Empleo Joven (Youth Employment
Plan)

**Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

CRISPR/Cas based gene editing tools have revolutionised the way we approach genetic studies both in cells and in animals. The Unit has incorporated the CRISPR/Cas gene editing system for mouse germ line precise modification, replacing, in many cases, gene targeting in embryonic stem cells (ES cells) to generate knockout and knockin alleles with high efficiency. CRISPR reagents, introduced directly in mouse zygotes by pronuclear injection or electroporation, replace, in many cases, difficult and time-consuming ES cell culture and manipulation. The efficiency of knockout allele generation with CRISPR is often around 80-90% and bi-allelic knockout animals are frequently obtained. CRISPR-mediated homologous recombination directly in mouse embryos, using single stranded oligodeoxynucleotides as donor DNA for repair, leads to the efficient generation of point mutations or small tag insertions, thereby allowing precise and reliable genome edition. We have also developed strategies to increase the efficiency of CRISPR-mediated large (more than 3 Kb size) knockin integrations using, in this case, circular plasmids as donor DNA. As many as 40% of the pups born after zygote

CRISPR microinjection carry targeted knockin inserts (FIGURE).

Zygote electroporation is a good alternative to microinjection for gene knockout generation. It is a much faster and easier process than embryo microinjection. Moreover, zygotes may also be obtained by *in vitro* fertilisation (IVF) and edited, on the same day, by CRISPR electroporation, increasing the chance of having a large number of fertilised mouse embryos. As many as 40 zygotes can be electroporated simultaneously in a single pulse and no embryo alteration (removing the zona pellucida) before electroporation is required. However, not all embryos are equally tolerant to the electroporation process. C57Bl6 zygotes exhibit a much lower viability than embryos of other genetic backgrounds, such as hybrid F1(B6. CBA) embryos, upon electroporation. We have also optimised protocols for electroporation of pure C57Bl6 embryos without compromising embryo viability. ■

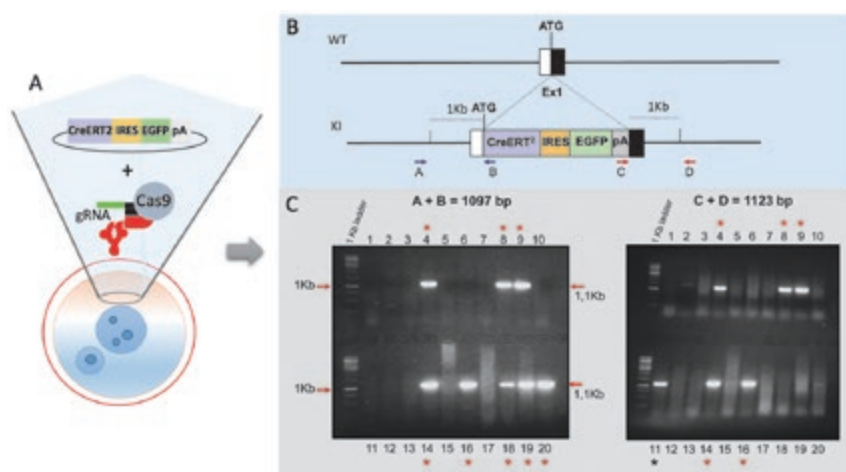


Figure Efficient targeted integration of large inserts by CRISPR-mediated DNA homologous repair in mouse embryos. (A) CRISPR reagents (Cas9 + gRNA) and circular plasmid DNA, as donor DNA for repair, are injected in the pronucleus of fertilised mouse embryos. (B) Structure of the knockin allele generated by integration of the CreERT2-IRES-EGFP-pA cassette in the target gene. (C) PCR analysis of mouse tail DNA of pups born from CRISPR injected embryos. Red asterisks indicate pups that are positive for correct targeted cassette integration (40%).

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MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

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

Reyes, Andrea Belén Romero (since November) (PEJ)**

**Titulado Superior* (Advanced Degree)

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



OVERVIEW

The production of monoclonal antibodies has had a profound impact on multiple branches of biomedical research, and has driven a fundamental shift in the analysis of biological problems. Monoclonal antibodies allow a better understanding of life processes, and can help in the discovery and elucidation of new pathways for the diagnosis, prevention and treatment of cancer.

The Monoclonal Antibodies Unit provides CNIO Research Groups with *à la carte* generation of mAbs. 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 service of *Mycoplasma* testing for the cell culture facility.

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

RESEARCH HIGHLIGHTS

During the last 19 years, the Monoclonal Antibodies Unit has generated a large number of mAbs, directed against more than 150 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 that are looking for licensing opportunities.

Research activities

In collaboration with P. Engel from the *Universidad de Barcelona*, we have produced and characterised several new mAbs against the leukocyte immunoglobulin-like receptor family (LILR, LIR, ILT, CD85). LILRs are widely expressed in haematopoietic-lineage cells and mediate activation or inhibition of the functions of various immune cells, primarily myeloid cells. It is becoming clear that LILRs, with their capacity to regulate immune responses and mediate protumour functions, represent a new class of receptors that can be targeted for the treatment of a variety of immunologic disorders and cancer.

Targeting one member of the LILR family with mAbs, however, is extremely complex due to the high homology shown among family members. The use of nonspecific antibodies might trigger the function of other members, which may complicate the interpretation of the biologic effects. The study of the functional role of LILRs in cancer is challenged by the lack of suitable mAbs able to specifically recognise each family member. For this reason, we developed and extensively validated novel mAbs specific for CD85A and CD85G that will help to study how LILRs regulate myeloid function and tumour progression, as well as to test the therapeutic efficacy of targeting LILRs for the treatment of malignant, autoimmune, and inflammatory diseases.

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 11 European countries.

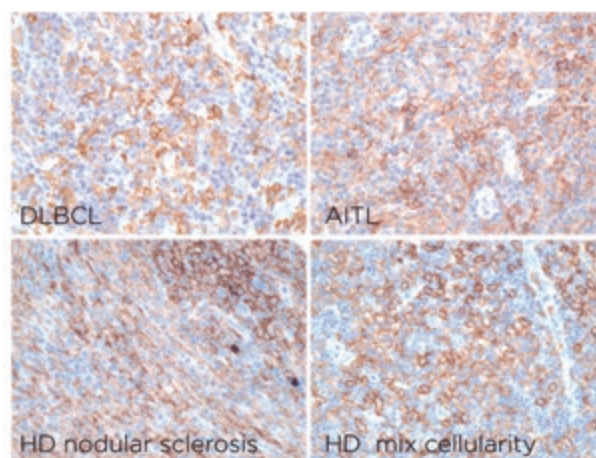


Figure Expression of CD85A mAb in diffuse large B cell lymphoma (DLBCL), angioimmunoblastic T cell lymphoma (AITL), nodular sclerosis and mix cellularity Hodgkin lymphoma (HD).

Members include internationally distinguished academic laboratories that generate and validate mAbs. EuroMAbNet is strongly committed to improving the education and training of junior scientists in the field of antibody validation. We achieve this aim by organising annual Antibody Validation Workshops in different venues across Europe.

The final goal of EuroMAbNet is to strengthen European leadership in mAb technology, improve education in the field on an international level, and actively engage with industrial partners to ensure the optimum benefits from using mAb technology to improve human health. ■

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MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Tatiana Álvarez, Guillermo Garaulet
(TS)*, Silvia Leal, Sergio Mateos
(since October) (PEJ)**, Guillermo



OVERVIEW

Molecular imaging is defined as the *in vivo* measurement of biological processes at the cellular and molecular levels. These techniques can visualise pathophysiological processes noninvasively in real time, with the potential for serial monitoring, and provide information about specific molecular alterations underlying the disease status of individual subjects. By complementing conventional ‘anatomical or physiological’ imaging, molecular imaging enables early detection of disease, disease staging, and quantitative assessment of therapeutic response.

“Molecular imaging with tumour-specific probes acts as a virtual biopsy, providing biological characterisation in a non-invasive way.”

Medrano (since May), Cristina Penalba (until March) (TS)*, Elka Jesarela San Martín, Gloria Visdomine

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

In 2019, we installed a new ultrasound system with better resolution to perform diagnosis and follow-up of tumours, as well as to phenotype different models and organs. The system improves the quality of ultrasound diagnosis by increasing the image resolution and signal-to-noise ratio (FIGURE).

The Molecular Imaging Unit continues to provide CNIO researchers with state-of-the-art molecular imaging equipment and human resources in order to guarantee the highest quality studies and to develop and update protocols and imaging techniques that 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.

As a result of a collaboration with the CNIO Breast Cancer Clinical Research Unit, we contributed to establishing the

clinical usefulness of ^{18}F -FDG PET as a tool to assess patients' tumour responses in clinical trials.

The Molecular Imaging Unit continued to work on 2 main projects. Granted in collaboration with the *CIEMAT* group, one focuses on developing and labelling nanobodies produced by camelids based on the ImmunoPET strategy; 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. Our project focuses mostly on developing nanoparticles for optical and multimodality (optical-MRI or PET-MRI) imaging to detect primary tumours and distant metastasis. The results of this research will directly benefit CNIO scientists, who will be able to use and test these new imaging tools. ■

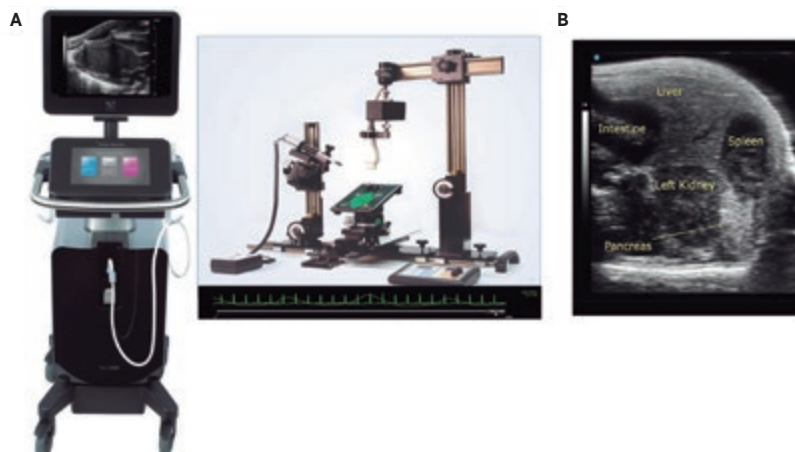


Figure (A) New Ultrasounds System Vevo 3100. (B) Abdominal ultrasound showing the high resolution of the new device (B mode).

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diet on ^{18}F -FDG-PET imaging in a mouse model of lung cancer. *Mol Imaging Biol* 21, 279-2085.

► AWARDS & RECOGNITION

- Project evaluator of the *Junta de Andalucía and Generalitat Valenciana. Investigación, Desarrollo e Innovación Biomédica y en Ciencias de la Salud* 2019.

FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Renan Antonialli (until March) (TS)*,
Julia García (TS)*, Sara García (since

November) (PEJ-L)**, Tania López
(until April) (TS)*

**Titulado Superior* (Advanced Degree)

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



OVERVIEW

Flow Cytometry is an indispensable tool in the oncology field. It allows multiparametric analysis for the identification, quantification and isolation of defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other.

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.

We have 4 analysers and 3 high-speed cell sorters, with different configurations of lasers and detectors, to cater to all our users' needs. We also have an automated magnetic bead separation system (AutoMACS), 2 automated cell counters and a tissue

“To further push the development and optimisation of immunophenotyping panels, we have installed a fifth laser line into our LSR Fortessa. We also developed a 16-colour panel to characterise immune subsets in human blood samples.”

homogeniser (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. We can accept human samples to sort according to Biosafety 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 of their interest. Some of the applications developed and validated at our Unit are:

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

- Extracellular vesicles detection (microvesicles and exosomes)
- Single cell sorting for omics analysis.

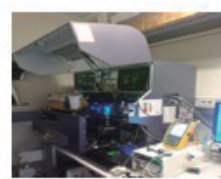
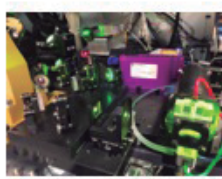
We further optimised our multicolour flow cytometry panels to characterise immune response in various samples from haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Single cell deposition into 96 or 384 PCR plates to perform single omics techniques is now part of our routine portfolio. We perform 4-way sorting based on DNA content on live stained samples and are advancing to separate even further to isolate 6 different fractions of DNA content. Additionally, we are also pushing the power of our analytical tools by moving towards high dimensional analysis, performing 'unsupervised' clustering analysis on our multiparametric panel assays. ■

CNIO LSR Fortessa X20

Laser - Optical Filter	Typical Fluorochromes
355 – 375/28	BUV395
355 – 450/50	DAPI/Hoechst
355 – 585/30	BUV563
355 – 670/50	BUV661
355 – 730/40	BUV737
408 – 450/50	BV421
408 – 515/30	BV510
408 – 710/50	BV711
408 – 780/60	BV786
488 – 525/50	GFP/FITC/AF488
488 – 695/40	PerCP/PerCP-Cy5.5
561 – 586/15	PE/Cy3/mRFP
561 – 610/20	AF594/PECF594/mCherry
561 – 710/50	PECy5.5/Katuskha
561 – 780/60	PECy7/PEeF7880
640 – 670/30	APC/Cy5/AF647
640 – 730/40	AF700/APC-Cy5.5
640 – 780/60	APC-H7/APCeF780

5 laser (UV, Violet, Blue, YG and Red)
20 parameters

Deep Multicolor Immunophenotyping



Human 18 marker
Immune panel



Murine 20 marker
Immune panel

Figure Upgrade of CNIO's LSR Fortessa. We have optimised an 18-marker immunophenotyping panel in human blood samples and

we are characterising different T and Myeloid subsets and developing a 20-marker one in murine tissues.

AWARDS AND RECOGNITION

- ▀ Vice-treasurer of the European Association 'Core Technologies for Life Sciences (CTLS)'.
- ▀ Re-elected Member of the Board of Directors of the Iberian Cytometry Society (S/C).

CONFOCAL MICROSCOPY CORE UNIT

Diego Megías
Core Unit Head

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

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

***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 the Research Groups at the CNIO. Training activities are also an essential component of our mission.

“The Confocal Microscopy Unit is fully committed to disseminating advanced microscopy methodologies that are useful for cancer research and society at large; we 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

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.

In addition, the Unit has implemented high throughput technologies applied to confocal microscopy using 2 different systems:

- An Opera (Perkin Elmer) High Content Screening (HCS) system, which allows HCS experiments to be run on fixed and live cells in multiwell plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence.
- A Matrix Screening Application integrated into the SP5 confocal systems, enabling high throughput feeding of the instrument, not only in multiwell 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 effort towards developing and implementing High Content Screening technology at the CNIO. In 2019, we further developed new advanced Machine Learning solutions oriented towards data mining and classification, thus allowing us to manage multiple object features applied to, for example, HCS multi-parametric treatment activity classification. Our activity in this field has been recognised and has led to the publication of several articles in journals as well as scientific book chapters.

Expansion microscopy

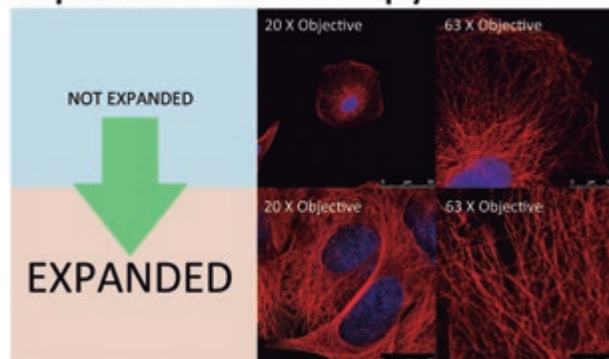


Figure Example of an Expansion Microscopy protocol. The upper panel shows alpha tubulin (red) and DNA (blue staining) with different

magnifications compared to an expanded sample (lower panel) of the same cell type with the same staining.

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, the demand for the performance of Microfluidics, used for live cell assays in perfusion chambers, has increased greatly. Experiments in the field of intra-vital microscopy are available and we are now running several projects for studies of metastasis, skin alterations and immune system response.

In 2019 the Unit was awarded an infrastructure grant for the acquisition of new equipment. ■

PUBLICATIONS

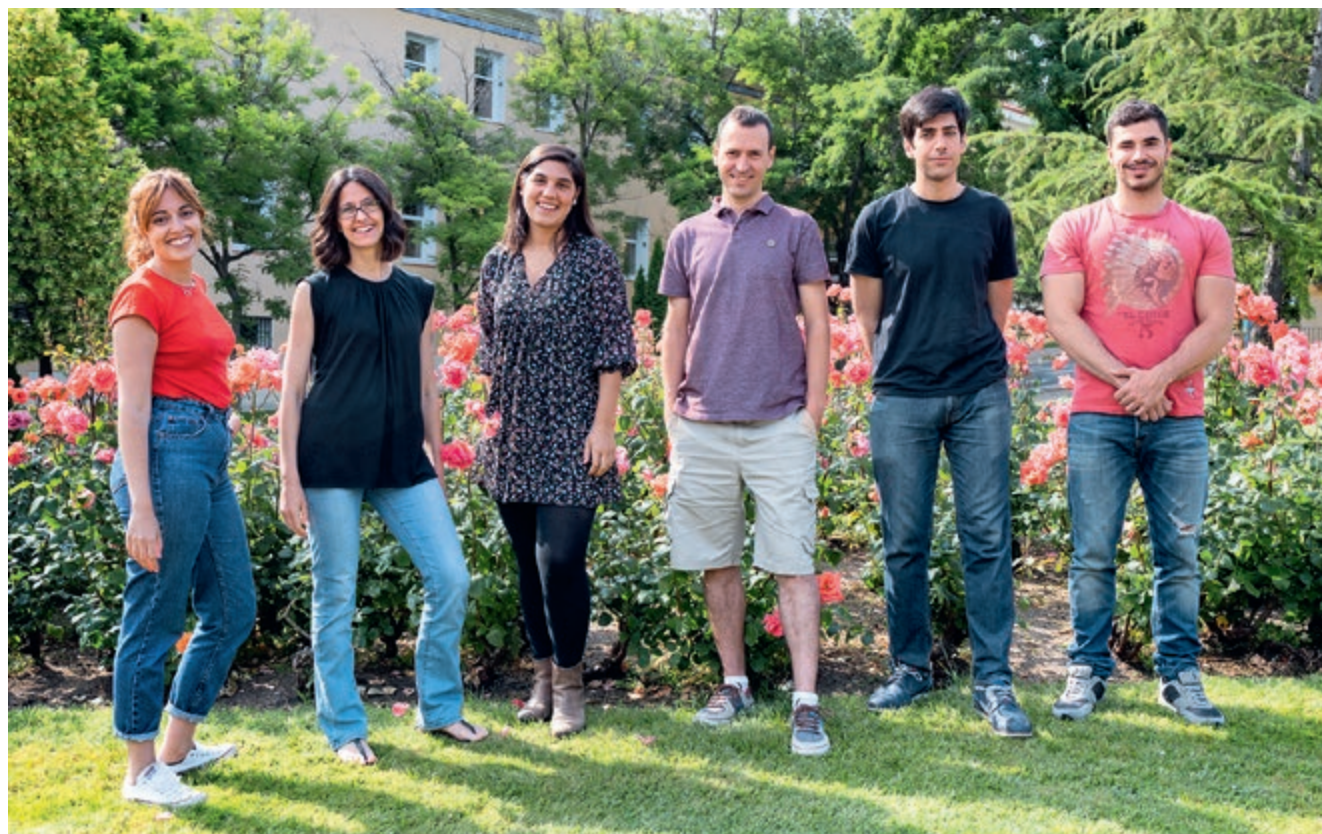
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- ▶ Barradas M, Link W, Megías D, Fernandez-Marcos PJ (2019). High-throughput image-based screening to identify chemical compounds capable of activating FOXO. *Methods Mol Biol* 1890, 151-161.

PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Post-Doctoral Fellow
Francisco J. Sánchez (since October)

Graduate Students
Ana Martínez (until February),
Cristina Sayago



OVERVIEW

Proteins act as the molecular effectors of cells and catalyse virtually all biological processes. In this regard, proteomics aims to characterise the complete repertoire of proteins to better understand how cells function at the molecular level. Global analysis of proteins is challenging, owing to their high complexity (>12,000 genes transcriptionally active in mammalian cells) and high dynamic range (9 orders of magnitude between high- and low-expressed proteins). Furthermore, proteins are post-translationally modified (e.g. phosphorylation) and interact with each other to form complexes; both processes are highly divergent in time and space. To tackle these analytical challenges, proteomics uses a combination of sample preparation, mass spectrometry (MS) and bioinformatics. The CNIO Proteomics Core Unit provides MS-based proteomics methodologies to research

“In the last decade, mass spectrometry-based proteomics has emerged as the most powerful technique to explore proteome structure and function.”

groups in order to better understand, at the proteome level, the underlying molecular basis of cancer.

Technicians

Elvira Fernández-Vigo (until November) (TS)*, Fernando García (TS)*, Nuria Ibarz (until September) (TS)*, Jana Sánchez (since July) (TS)*, Álvaro

Soriano (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 2019, the Proteomics Unit continued working on the application of proteomic approaches to answer biological questions relevant to our understanding of cancer biology. State-of-the-art MS technology coupled to quantitative multiplexing approaches enable profiling the entire proteome across multiple biological conditions (11 in a single experiment). In collaboration with the Hereditary Endocrine Cancer Group, this technology was applied to identify potential targets of the miRNA hsa-miR-139-5p, which has been associated with thyroid cancer through its involvement in alternative splicing. Furthermore, exosomes and extracellular vesicles have gained great interest in the medical community as possible sources of biomarkers in non-invasive liquid biopsies. In this regard, in collaboration with the Microenvironment & Metastasis Group, we have explored the protein content of extracellular vesicles extracted from lymphatic drainage as surrogate markers of melanoma progression. We found that seroma-derived exosomes are enriched in proteins mimicking melanoma progression. Along the same lines, in collaboration with the Genes, Development and Disease Group, we applied this strategy to perform proteomic analysis of exosomes in a mouse lung fibrosis model and found that they are enriched in collagen-related proteins secreted by macrophages. Finally, the Unit continues to implement new technologies to the catalogue of available services. Throughout 2019, we optimised protocols for the global analysis of protein methylation, including mono- di- and tri- methylation of lysines as well as mono- and di- (symmetric and asymmetric) methylation of arginines. ■

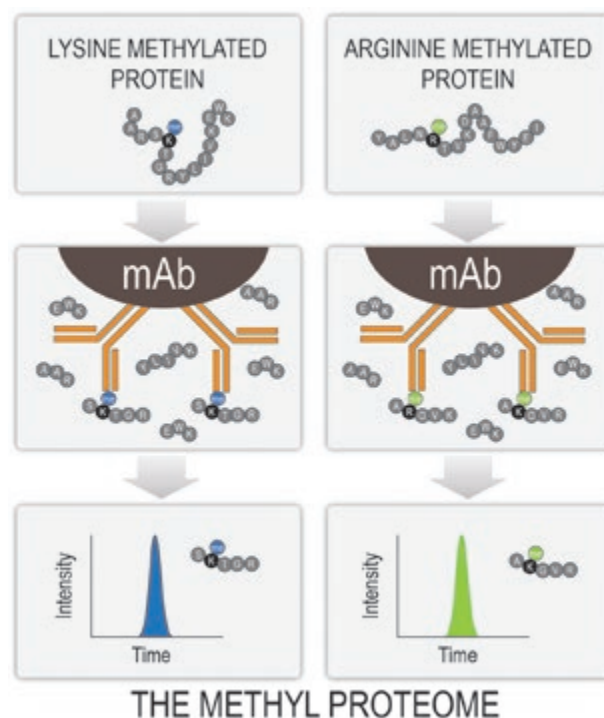


Figure Immunopurification of methylated peptides coupled to high-resolution mass spectrometry analysis enables the identification of thousands of lysine and arginine

methylated proteins from cell samples. Currently, all forms of methylation can be purified through highly specific monoclonal antibodies.

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HISTOPATHOLOGY CORE UNIT

Vacant
Core Unit Head

Staff Scientist
Eduardo José Caleiras

Technicians
Nuria Cabrera, María Gómez,
Patricia González, Gabino



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 throughout a full range of services covering 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, assisted by a team of highly specialised technicians, offers other value-added services, 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

“The implementation of the *in situ* hybridisation technology RNAScope and multiplexed immunohistochemistry staining, to enable the detection of several protein markers and mRNAs on the same tissue section, is an example of the Unit’s commitment to innovation to facilitate the progress of research projects at CNIO.”

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

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RESEARCH HIGHLIGHTS

In line with the activity carried out over the last few years, the Unit has maintained the portfolio of services demanded by its users in accordance with the needs of their projects. Thus, about 30,000 paraffin blocks of tissue samples were generated, and *ca.* 25,000 techniques performed, including histological and IHC techniques, *in-situ* chromogenic hybridisation, tissue microarrays, slide scanning, etc. Also, during this time we introduced new IHC markers useful for the study of tumour development, as well as new chromogenic substrates for the visualisation of those markers.

During 2019, the Unit implemented RNAScope technology for *in situ* hybridisation, using the Ventana-Roche automatic platform for IHC stains. This new technique allows the efficient detection of specific mRNAs directly on sections from formalin-fixed paraffin-embedded (FFPE) tissues, thus providing a spatial dimension to gene expression analysis. The applications of this new technology are expected to be 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. Also, sometimes it is feasible to combine this technique with IHC, allowing for double stains to detect both mRNA and protein, or any other marker of interest.

The high quality of the techniques run by the Unit continues to be endorsed by External Quality Assessment Schemes. Thus, our histochemical techniques were evaluated by UK NEQAS. On the other hand, NordiQC and SEAP has evaluated a subset of our IHC techniques under different modules, including general markers, breast cancer markers and PD-L1; these all obtained very high scores.

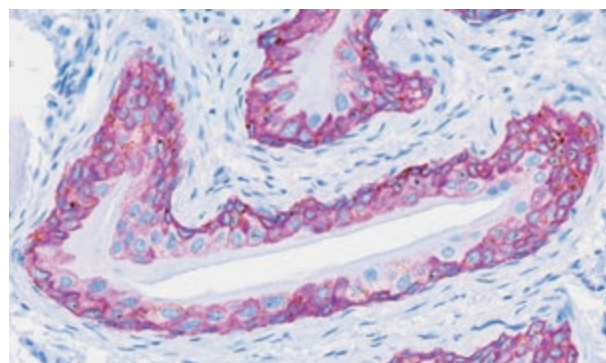


Figure Detection of cyokeratin 5 in bladder tissue by both mRNA *in situ* hybridisation (RNAScope) and IHC: mRNA is shown in brown (DAB), protein in purple (in collaboration with the CNIO Epithelial Carcinogenesis Group).

Training and outreach activities are also a critical component of the Unit's activity. This includes our participation in modules of *Formación Profesional* for pathology technicians, mentoring of high school students during short-term stays at the Unit, conducting guided visits to the laboratories for students and other audiences, as well as offering practice sessions on the different technologies run by the Unit in Masters and other courses, among other activities. ■

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

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

“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.”

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 27 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 in order to safe-guard our personnel from any associated risks; robotic devices perform the potentially hazardous tasks such as the processing of dirty bedding, the washing and filling of cages and bottles, etc. These automated systems maximise the productivity and ensure the quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility currently harbours more than 40,000 mice representing more than 3,000 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying close to 400 gene targeted alleles and more than 200 transgenic integrations. The Facility also provides access to more than 50 tool strains, including constitutive and inducible Cre strains, FLP strains, reporter strains, Tet transactivator strains and others.

The Animal Facility offers the possibility of running a broad number of experimental procedures in the premises, including the use of gamma irradiation, UV light and volatile carcinogenic agents, as well as surgical procedures, some behavioural studies, a non-invasive blood pressure system, and a lab animal monitoring system (Oxylet) that enables measuring a number of physiological parameters for metabolic profiling and phenotyping of mouse models.

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 are to be carried out by qualified people with the corresponding accreditation issued by the competent authority. The Animal Facility offers CNIO's new staff a short course, focused on the work with laboratory animals, complementary to the online courses that are a requisite to gain access to the facility.

In accordance with our commitment to maintain the highest possible standards in relation to animal research issues, the CNIO has 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.

In 2019, the Animal Facility's Head was elected as President of the Spanish Society for Laboratory Animal Sciences (*SECAL*), for a 2-year period. *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 first PROTAC has reached the clinic in 2019. In drug discovery projects, it is time to consider this avenue as an upfront option and not as a ‘nice to have’ complement to classical inhibitors.”

The following highlights summarise some of the achievements of the Experimental Therapeutics Programme (ETP) during 2019.

CDK8 inhibitors. ETP-18, a highly selective orally bioavailable CDK8 inhibitor, demonstrated good results after PO administration in pharmacokinetics and pharmacodynamics (PK/PD) studies in MOLM13 xenografts. ETP-18 is well tolerated and efficacious in this model. The lead compounds ETP-93 and ETP-18 were scaled up for toxicity studies in rats. The corresponding PK experiments were carried out to identify the dosing for those studies.

Haspin and MASTL Inhibitors. (In collaboration with Marcos Malumbres, CNIO Cell Division and Cancer Group). We previously generated 2 distinct chemical series of highly potent and selective Haspin inhibitors. In 2019, we evaluated combinations of our Haspin inhibitors with a library of 114 antitumour drugs. Additionally, we performed RNAseq experiments with both inhibitors, detecting the upregulation of certain genes. The results of both approaches could position Haspin inhibition as an interesting antitumour therapeutic option in combination treatments (confidential).

We also designed 2 additional series of potent MASTL inhibitors with higher commercialisation potential due to their chemical novelty. Currently, we are assessing their kinase selectivity to trigger and guide their further optimisation. Importantly, we undertook the development of PROTACs against MASTL kinase. We have already identified some molecules with low nanomolar affinity for the protein, and are performing MASTL degradation studies in cells.

TRF1. (In collaboration with Maria Blasco, CNIO Telomeres and Telomerase Group). We are still embarked on the target

deconvolution of ETP-946. Previous pull-down experiments with reversible and irreversible affinity probes, using ‘click chemistry’ for their conjugation to a biotin-based tag, did not identify any validated target for ETP-946. This was due to inconsistent results between biological replicates. We have now designed new affinity probes that will not require the click chemistry step, a potential interference in our previous assays. We continued the SAR (Structure Activity Relationship) exploration of ETP-946. Several analogues blocking metabolism hotspots have demonstrated good TRF1 activity together with better metabolic stability. Selected analogues are being evaluated in PK and distribution studies. Furthermore, we contributed to identifying additional signalling pathways involved in TRF1 modulation. One of them, the RAF/MEK/ERK pathway, was further validated as a key regulator of TRF1 in M. Blasco’s laboratory.

We also set up two ‘proximity assays’ to identify small molecules that disrupt homodimerization of TRF1 or binding of TRF1 to ds-Telomeric DNA. Identifying such molecules by directly targeting 2 of the reported features of TRF1 association to telomeres will be highly innovative in the field of TRF1 inhibition.

During the year, the ETP also helped other CNIO groups with several research activities such as screening campaigns and the synthesis of key tool compounds.

Importantly, the Singapore-based biotech company *AUM Biosciences* acquired from *Inflection Biosciences* an original ETP-CNIO multi-kinase inhibitor series. We are hopeful that *AUM Biosciences* will facilitate their clinical development.

MEDICINAL CHEMISTRY SECTION

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Section Head

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February) (TS)*

Technicians
Lucía de Andrés (TS)* (PEJ)**,
Miriam Pastor (until February) (TS)*,

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

OVERVIEW

The Medicinal Chemistry Section is part of the interdisciplinary Experimental Therapeutics Programme dedicated to early Drug Discovery. Our activities consist of the design and synthesis of potential drugs with a therapeutic use in the field of cancer. Other activities integrated in our Section include the synthesis of high-quality chemical probes – potent, selective and cell-permeable compounds that are essential for target validation activities and the early stages of the drug discovery process. Additionally, we help CNIO's basic research groups to decipher the mechanism of action that mediates an observed phenotype in cancer cells after screening for small organic molecules. Identifying the molecular target is essential to increase our knowledge about cancer and is a great aid to start drug discovery activities. To do this, we synthesise affinity chemical probes that enable cellular localisation of the target through imaging techniques and target identification through pull-down experiments coupled with proteomics.

“We implement PROTAC-target degradation strategies in our projects. The first PROTAC-like molecules have been synthesised that will be used to search for MASTL protein degraders.”

RESEARCH HIGHLIGHTS

During 2019, we were involved in several different projects:

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

We scaled-up our 2 lead compounds, ETP-93 and ETP-18, for additional biological characterisation. Proof-of-concept studies in MOLM13 xenografts with ETP-93 and ETP-18 were completed, showing positive results. Additionally, pharmacokinetics (PK) studies in rats with both compounds were carried out. These compounds will be used in further toxicity studies in rats.

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

In collaboration with the CNIO Cell Division and Cancer Group, we continued the exploration of the chemical series identified to obtain potent and selective compounds. We identified 2 chemical series of novel inhibitors, in a new chemical space, and in the low nanomolar range. We are currently performing selectivity studies against a panel of 468 kinases (KINOMEscan) in order to determine the selectivity profile, an important aspect for a compound. As a complementary strategy to target inhibition, we are also exploring the field of target degradation. More than 20 PROTAC-like molecules have been synthesised, among which some have been identified as having low nanomolar activity at the biochemical level. We are currently performing degradation studies with them.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This project is undertaken in collaboration with the CNIO Telomeres and Telomerase group (TTG). We continued our efforts around the ETP-946 chemical series to expand Structure-Activity-Relationships (SAR). On the one hand, we wanted to improve the bioavailability parameters of our lead compound. For this purpose, we explored the molecule by blocking all the potential unstable positions through introduction of halogens or other stable fragments. The new compounds proved to be TRF1 inhibitors, and *in vitro* ADMET (absorption, distribution, metabolism, excretion and toxicity) studies were carried out. PK studies with those compounds showing a positive ADME profile will be performed. A second approach in this chemical series is the exploration of different scaffolds and linkers to generate new chemical space and novel chemical series. So far, we have identified some modifications that retain TRF1 activity.

As part of the target deconvolution activities, our previous affinity probes synthesized using click chemistry did not allow us to identify a target. Now, we are applying several strategies in order to get new affinity probes while avoiding the click chemistry step. One of the first approaches that we took is to synthesise biotinylated compounds that preserve TRF1 activity and that will be used in pull-down experiments by direct binding to streptavidin beads (FIGURE). Finally, in order to get molecules that disrupt dimerization of TRF1 or binding of TRF1 to double-stranded (ds) telomeric DNA, the corresponding assays were set up and validated in the Biology Section, and small screenings are being run.

Discoidin domain receptor (DDR) 1/2 inhibitors

As a result of a 4-year PhD project, we worked on searching for DDR1/2 inhibitors. Discoidin domain receptors, DDR1 and DDR2, are 2 members of the collagen receptor family that belong to the tyrosine kinase receptor subgroup and are potential targets for human cancer and inflammation-related diseases. During 2019, we generated a novel chemical series of potent inhibitors in the low nanomolar range, at the biochemical and cellular levels. The chemical series presents an interesting selectivity profile and promising *in vitro* ADME data. The next step will be to perform PK studies to evaluate the *in vivo* levels after oral and IV administration with the most advanced compounds, in order to determine their use in efficacy studies.

Collaborations with other CNIO Groups

We gave support to different groups by synthesising tools or reference compounds, for example, to Miguel Gallardo, from the H12-CNIO Haematological Malignancies Clinical Research Unit; to the Metabolism and Cell signalling Group; to Hector Peinado, from the Microenvironment and Metastasis Group; and to Vanesa Lafarga, from the Genomic Instability Group. ■

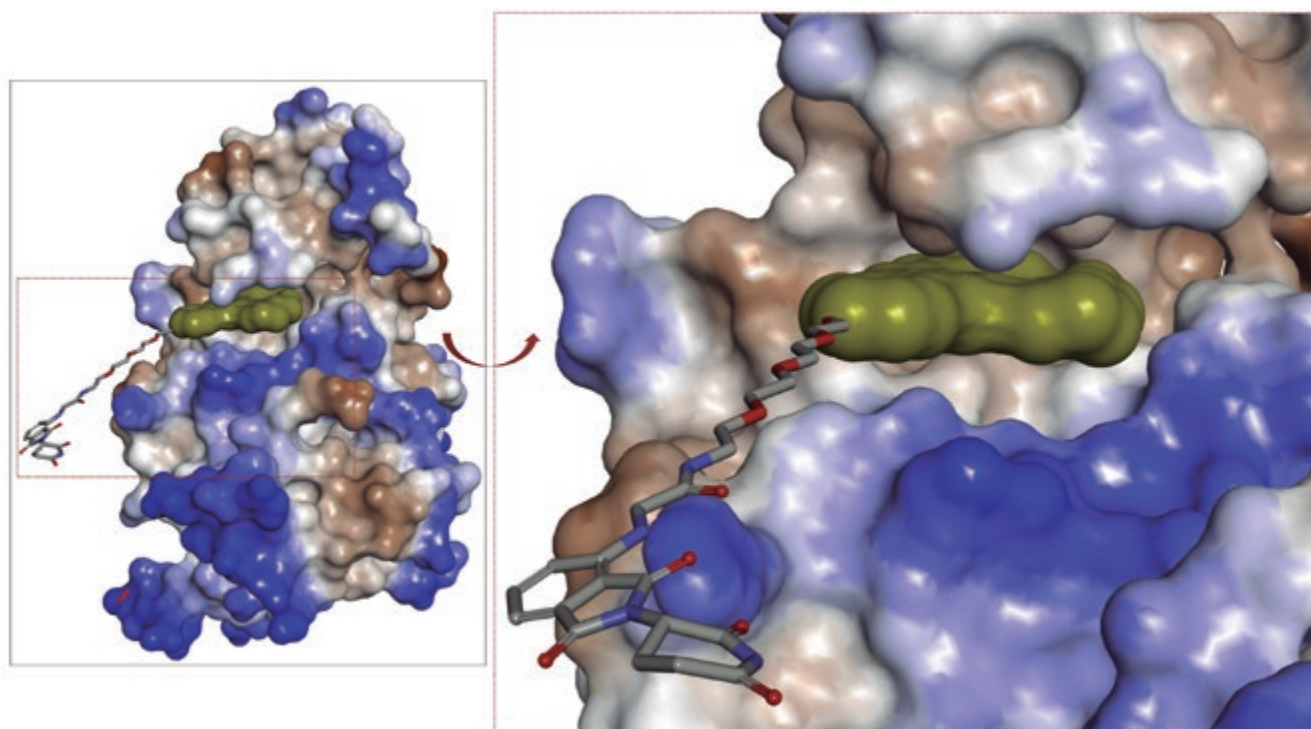


Figure Molecular docking of one of our first PROTAC-like molecules with Mastl protein using the reported X-ray crystal structure of the hGWL-kinase domain in complex with STU (PDB: 5olh). Our molecule fits in the

catalytic site of the protein (surface in green colour; the chemical structure is confidential), and the linker and CRBN moieties are extended into the solvent area to be able to interact with the E3 ligase.

PUBLICATIONS

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- ▶ Utero AC, Bakiri L, Roediger B, Suzuki M, Jimenez M, Mandal P, Braghetta P, Bonaldo P, Paz-Ares L, Fustero-Torre C,

Ximenez-Embun P, Hernandez AI, Megias D, Wagner EF (2019). Fra-2-expressing macrophages promote lung fibrosis in mice. *J Clin Invest* 129, 3293-3309.

- ▶ Bejarano L, Bosso G, Louzame J, Serrano R, Gómez-Casero E, Martínez-Torreceda J, Martínez S, Blanco-Aparicio C, Pastor J, Blasco MA. (2019). Multiple cancer pathways regulate telomere protection. *EMBO Mol Med* 11, e10292.
- ▶ Mohlin S, Hansson K, Radke K, Martinez S, Blanco-Aparicio C, Garcia-Ruiz C, Welinder C, Esfandiyari J, O'Neill M, Pastor J, von Stedingk K, Bexell D (2019). Anti-tumor effects of PIM/PI3K/mTOR triple kinase

inhibitor IBL-302 in neuroblastoma. *EMBO Mol Med* 11, e10058.

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- ▶ Klett J, Gómez-Casero E, Méndez-Pertuz M, Urbano-Cuadrado M, Megias D, Blasco

MA, Martínez S, Pastor J, Blanco-Aparicio C (2019). Screening protocol for the identification of modulators by immunofluorescent cell-based assay. *Chem Biol Drug Des*. PMID: 31469231.

PATENT

- ▶ Pastor JA, Blasco MA, Martinez S, Blanco-Aparicio C, García AB, Gómez-Casero E, Bejarano, L, Méndez-Pertuz M, Martínez P, García-Beccaria M. Novel TRF1 modulators and analogues thereof. *PCT/EP2019/074459*.

BIOLOGY SECTION

Carmen Blanco
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Cebriá (TS)*, Jennifer García (until



April), Adrián Amezcua (May-September), Elena Gómez-Casero (TS)*, Javier Klett (TS)*, María Martín (since September) (PEJ)**, M. Carmen Rodríguez de Miguel (TS)*,

José A. Torres (since September) (PEJ)**

**Titulado Superior* (Advanced Degree)

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

OVERVIEW

In the Experimental Therapeutics Programme, we perform both phenotypic and targeted-based drug discovery. The Biology Section is devoted to the biochemical, cellular, and *in vitro/in vivo* pharmacological characterisation of the compounds synthesised within the Programme.

At the biochemical assay level, we have developed a panel of different biochemical assays and optimised them for the targets used in our screening campaigns. We have currently established more than 30 different types of biochemical assays, mainly focused on kinase activities and covering a broad range of sensitivities and technologies (FI, FP, FRET, TR-FRET, chemiluminescence, coupled enzymatic reactions, binding assays, etc.) adapted to the targets that we have been working on.

Recently, in order to identify inhibitors of relevant cancer targets with no enzymatic activity, we have started to develop biochemical assays to measure protein/protein interaction and protein/DNA interactions. These assays are based on AlphaScreen and AlphaLISA technology.

“HASPIN inhibitors could have potential antitumour activity in combination with different approved drugs.”

RESEARCH HIGHLIGHTS

During 2019, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

We finished proof of concept studies in mouse models with ETP-18, a selective, advanced, orally bioavailable lead compound that was safe in mice and has shown efficacy in MOLM13 xenografts.

We also performed pharmacokinetic (PK) studies in rats with ETP-93 (dual CDK8/HASPIN-i) and ETP-18 (selective CDK8-i) compounds compared with known inhibitors at efficacious doses in mice. The results of the toxicity studies will be reported accordingly.

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

These projects are undertaken in collaboration with the CNIO Cell Division and Cancer Group. For MASTL, we tested in our biochemical assay with active human full-length MASTL protein, around 150 new compounds, both MASTL-i and MASTL PROTAC like molecules. We are characterising those with nanomolar biochemical activity at the cellular level, evaluating both the modulation of P-ENSA, a direct substrate of MASTL, and the degradation of MASTL with MASTL PROTAC-like molecules. For HASPIN, we obtained 2 highly selective chemical probes with nanomolar biochemical and cellular modulation of HASPIN. After testing them in a panel of 38 tumour cell lines, we evaluated their potential in combination with a library of 114 antitumour drugs and validated the results both by determination of the combination index and in dose response experiments (FIGURE). Moreover, we performed RNAseq experiments with both chemical probes in order to predict possible combinations. At that point, the results were confidential.

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. We have continued our efforts to deconvolute the molecular target of our hit ETP-946. After triplicate pull-down experiments with reversible and irreversible chemical probes of ETP-946, we could not validate any target. Interestingly, we identified a molecule with a single change in one atom with respect to ETP-946 that was not able to modulate TRF1 and that is being used to help in deconvolution studies. The results of RNAseq experiments

with ETP-946 and its inactive analogue are being analysed to try to identify the molecular target of ETP-946. Moreover, we tested in a phenotypic assay to measure the association of TRF1 to telomeres, 77 compounds that include analogues of ETP-946 to improve its bioavailability and to try to expand chemical diversity. The metabolic stability of the active compounds has been determined and we are performing PK and distribution studies with stable compounds. Furthermore, by using a chemical biology approach, we validated that the RAF/MEK/ERK pathway modulates TRF1 levels at telomeres. Finally, we set up 2 assays based on AlphaScreen and AlphaLISA technology to identify small molecules that disrupt dimerization of TRF1 or binding of TRF1 to ds telomeric DNA; in parallel, we established their corresponding counterscreen. After virtual/wet screening and analogue searching, we identified several low micromolar hits that disrupt TRF1 dimerization and that are being validated. Furthermore, we started virtual screening to identify disruptors of TRF1 binding to ds telomeric DNA that will be tested in our wet assay.

Collaborations with other CNIO Groups

ETP-Biology provided ongoing support to follow-up on the results obtained from the screenings performed by the Brain Metastasis Group and the Metabolism and Cell Signalling Group. We also provided support for *in vivo* studies performed by the Microenvironment and Metastasis and the Experimental Oncology Groups, and by setting up and carrying out a screening to identify PrimPol inhibitors in collaboration with the DNA Replication Group. We conducted the analyses to determine compound levels in several biological samples from the Telomeres and Telomerase Group. Moreover, we collaborated with Experimental Oncology Group, testing and analysing the ETP-antitumour library to identify novel treatments of mutant KRas NSCLC mouse cell lines that regrow after knocking down mutant KRas. ■

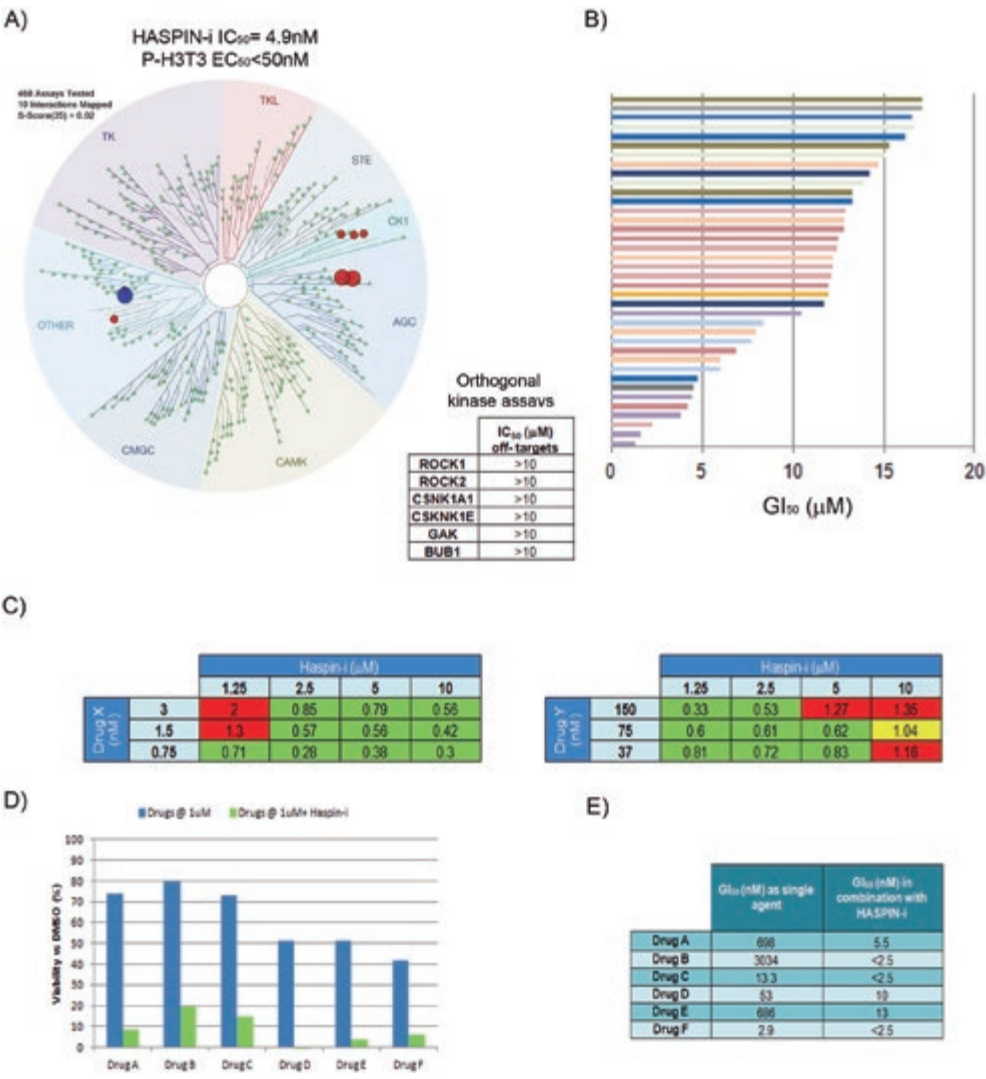


Figure Antiproliferative activity of HASPIN-i as a single agent and in combination. **(A)** Biochemical and cellular inhibition of HASPIN-i and overall selectivity (Kinomescan™). **(B)** Growth inhibition 50 (GI_{50}) of HASPIN-i in a panel of 38 tumour cell lines (each bar represents a cell line, and each colour represents a different tumour type). **(C)** Combination index of HASPIN-i with two different antimitotic drugs. **(D)** Selected drugs from the ETP-antitumour library that were more effective in combination with HASPIN-i at a single dose. **(E)** Validation of the decrease in GI_{50} of the identified drugs in combination with HASPIN-i.

► **PUBLICATIONS**

► Trigg RM, Lee LC, Prokoph N, Jahangiri L, Reynolds CP, Amos Burke GA, Probst NA, Han M, Matthews JD, Kai Lim H, Manners E, Martinez S, Pastor J, Blanco-Aparicio C, Merkel O, de Los Fayos Alonso IG, Kodajova P, Tangermann S, Höglér S, Luo J, Kenner L, Turner SD. (2019). The targetable kinase PIM1 drives ALK inhibitor resistance in high-risk neuroblastoma independent of MYCN status. *Nat Commun* 10, 5428.

► Bejarano L, Bosso G, Louzame J, Serrano R, Gómez-Casero E, Martínez-Torrecuadrada J, Martínez S, Blanco-Aparicio C, Pastor J, Blasco MA. (2019). Multiple cancer pathways regulate telomere protection. *EMBO Mol Med* 11, e10292.

► Mohlin S, Hansson K, Radke K, Martinez S, Blanco-Aparicio C, Garcia-Ruiz C, Welinder C, Esfandyari J, O'Neill M, Pastor J, von Stedingk K, Bexell D (2019). Anti-tumor effects of PIM/PI3K/mTOR triple kinase inhibitor IBL-302 in neuroblastoma. *EMBO Mol Med* 11, e10058.

► Llanos S, Megias D, Blanco-Aparicio C, Hernández-Encinas E, Rovira M, Pietrocola F, Serrano M (2019). Lysosomal trapping of palbociclib and its functional implications. *Oncogene* 38, 3886-3902.

► Martínez-González S, Rodríguez-Arístegui S, Gómez de la Oliva CA, Hernández AI,

González Cantalapiedra E, Varela C, García AB, Rabal O, Oyarzabal J, Bischoff JR, Klett J, Albarrán MI, Cebriá A, Ajenjo N, García-Serelde B, Gómez-Casero E, Cuadrado-Urbano M, Cebrián D, Blanco-Aparicio C, Pastor J (2019). Discovery of novel triazolo[4,3-b]pyridazin-3-yl-quinoline derivatives as PIM inhibitors. *Eur J Med Chem* 168, 87-109.

► Godoy CA, Klett J, Di Geronimo B, Hermoso JA, Guisán JM, Carrasco-López C (2019). Disulfide engineered lipase to enhance the catalytic activity: a structure-based approach on BTL2. *Int J Mol Sci* 20, E5245.

► Klett J, Gómez-Casero E, Méndez-Pertuz

M, Urbano-Cuadrado M, Megias D, Blasco MA, Martínez S, Pastor J, Blanco-Aparicio C (2019). Screening protocol for the identification of modulators by immunofluorescent cell-based assay. *Chem Biol Drug Des*. PMID: 31469231.

► **PATENT**

► Pastor JA, Blasco MA, Martinez S, Blanco-Aparicio C, García AB, Gómez-Casero E, Bejarano L, Méndez-Pertuz M, Martínez P, García-Beccaria M. Novel TRF1 modulators and analogues thereof. *PCT/EP2019/074459*.

CNIO - LILLY CELL SIGNALLING AND IMMUNOMETABOLISM SECTION

Susana Velasco
Section Head

Staff Scientists

Ana Cerezo, Sonia Hernández Tiedra,
Eva P. Lospitao, Gloria Martínez del
Hoyo, Camino Menéndez (since
September)

Technicians

Laura Diezma, Roberto Gómez (since
September) (TS)*, Borja Jiménez
(since May) (TS)*, Guiomar Jiménez
(until April), Tamara Mondejar (TS)*,
Sandra Peregrina (TS)*

**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 anti-tumour agents, or activating the anti-tumour immune response. Exploring how to better target these mechanisms would lead to better and more efficient therapeutic options.

A combination of *in vitro* and *in vivo* approaches is being utilised to obtain a complete understanding of tumour metabolic reprogramming. 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 that includes the use of non-invasive *in vivo* imaging technologies, and 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. Otto Warburg proposed in the early XX century that, 'this altered metabolic state was the underlying cause for cancer' (Warburg 1956). The past decade has been a period of very active research in the area of tumour metabolic reprogramming, and major molecular mechanisms involved in the process have been identified and characterised. It was found that both oncogenes (Ras, Myc) and tumour suppressor genes (p53, RB, LKB1) impart an altered metabolic phenotype in cancer cells 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, 2017). All these alterations have led tumours to rely heavily on specific metabolic pathways to obtain their energy, while using other pathways to grow in order to give tumour cells a growth advantage. This situation may leave tumour cells in a frail position under certain treatments or circumstances, 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 A, B). Thus, the mechanistic understanding of cancer metabolism has led to renew interest in developing therapeutics that target key enzymes involved in this process. Checkpoint-blockade immunotherapy has been one of the most exciting advances made in cancer treatment in recent years. Metabolic interplay in the local microenvironment can mediate T cell differentiation and function. 'Checkpoint-blockade' antibodies can also influence cellular metabolism. Finally, recent clinical trials have shown that combination immunotherapy, based on immune checkpoints blockade and targeted and non-targeted therapies, provides even higher response rates than either approach alone. Several clinical trials are currently using this approach, however, not all patients respond to immunotherapy and it is, therefore, necessary to determine which patients would be good candidates for the treatment. It has been found that an inflammatory tumour microenvironment – 'hot' tumours – greatly increases patient survival. One of the objectives of our laboratory has been to identify, and characterise the expression of novel and known tumour markers that may enable a better patient stratification for future therapies. This approach has shown that, in addition to the levels of expression of an immunotherapy target, the type of cells that express the marker may also be a feature to consider. ■

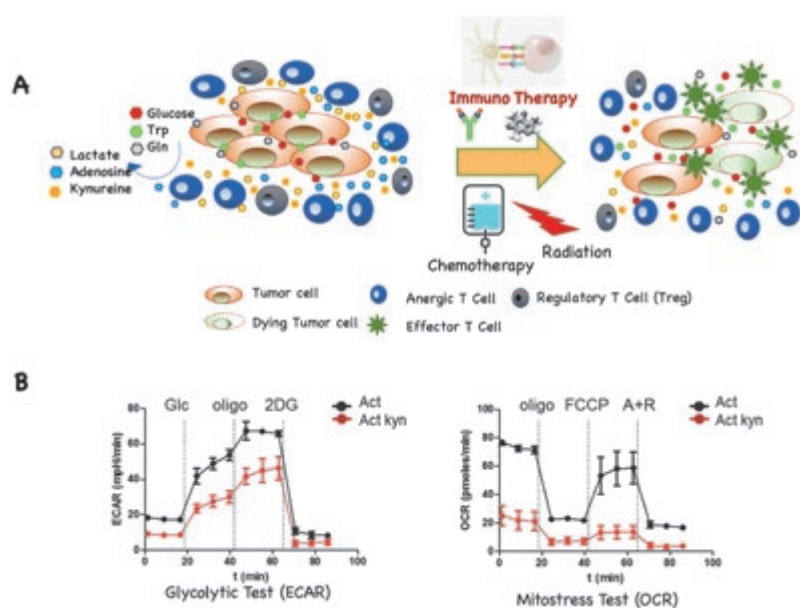


Figure Targeting cancer metabolic immune suppression. (A) Tumour cells produce a battery of immunosuppressive metabolites such as lactic acid, kynurenine or adenosine that result in an anergic T cell phenotype, while consuming key metabolites such as glucose or tryptophan necessary for a proper Teff (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) of the tumour and/or the tumour microenvironment, would diminish the production

of immune suppressive metabolites and increase the levels of metabolites such as glucose, or tryptophan necessary for a proper anti-tumour T cell response. (B) Extracellular flux analysis for the acidification rate (glycolytic test) and O₂ consumption OCR (mitochondrial test). Fully active effector T cells require an activated glycolytic and an oxidative metabolism in order to synthesize cytokines and other molecules necessary for their cytotoxic activity. Immune suppressive metabolites, like kynurenine, suppress the metabolic activity of effector T cells inhibiting their cytotoxic activity.

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Irene Herrera (since June)
Anabel Sanz (until April)
Director

Technology Transfer Senior Manager
M. Cruz Marín (until December)

Technology Transfer Manager
Julia Mendoza (since October) (PEJ)*

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



“Our office is strongly committed to translating new discoveries in cancer research 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 in order 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 2019, 15 new ideas were incorporated into the technology transfer portfolio, of which 2 turned into priority patent applications and 9 are under patentability analysis. These cover a wide range of products, including a method for determining the presence or absence of minimal residual disease in patients treated for a proliferative disease, a combination of an antibody with activated and expanded natural killer cells for cancer immunotherapy, drug inhibitors, new biomarkers, a monoclonal therapeutic antibody, a cell therapy, and an approach to screening of molecules.

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, filing and prosecution process. Four PCT (Patent Cooperation Treaty) applications for international extension were filed in 2019, and 2 patents with proven commercial interest entered the national phase. Licensed patents make up a remarkable 37% of the CNIO portfolio. Among the licences signed in 2019, a license agreement with the company Altum Sequencing represents a milestone for the associated CNIO-H120 Clinical Research Units.

To ensure that scientific ideas and results are transferred to the private sector, a proof-of-concept phase is usually necessary to validate its potential application in the market. The TTVO supports the preparation, coordination and advice of CNIO scientists so that their ideas reach the point of development necessary for potential companies to decide to invest and co-develop.

This is the case of calls aimed at technological development projects such as *CaixaImpulse* and *FET-OPEN*, among others. Besides from the 2 *CaixaImpulse* projects launched in 2018, a new cell therapy project for type 1 diabetes was awarded a *CaixaImpulse* grant in 2019, and thereby benefits from funding and mentoring by experts of the national bio-ecosystem. A *FET-Open* grant was awarded to a Consortium in which CNIO researchers will focus on the use of probes for discriminating between different types of cancer and heat generation in order to increase the permeability of the blood-brain barrier.

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 2019, the TTVO managed 281 technology transfer records related to industrial and intellectual property generated by CNIO's researchers; 231 correspond to agreements (MTAs, CDAs, Research Collaborations, licenses, etc.). Among these industrial partnerships is worth noting the collaboration with Lilly, which has been extended for 3 more years to incorporate new scientific studies that include work on immuno-metabolism, oncogenic drivers, resistance, platforms, and knowledge of resistance in immuno-oncology. Other collaborations include: the *IRONMAN-ES* study funded by the Movember Foundation, in which 10 to 15 hospitals participate and in which the CNIO and Institute of Oncological Research (VHIO) are co-promoters; the extension of the collaboration contract with the Spanish company Lipottrue; and a collaboration contract with the *Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori*.

The majority of these agreements (68%) were established with international entities, which is an indicator of the internationalisation of the CNIO's research activity. Through research collaborations with industry, up to 1.7 million euro were secured for research activities. Moreover, 4% of the agreements are licences to commercial partners. Patents and unpatented research tools are licensed. The net income derived from licences in 2019 increased to 674,900 euro. This income reverts back to CNIO research activities as well as to the inventors themselves. A total of 40 inventors and 10 research groups have contributed towards and benefited from this achievement.

Effective transfer of research results to the productive environment requires intensive networking and asset promotion. In addition to attending international forums on advanced therapies in cancer and regenerative medicine, in 2019 TTVO participated in the ASEBIO Investors Day in Madrid and the Milner Therapeutics Symposium in Cambridge.

All the above-mentioned achievements stand testament to the excellence and hard work of the CNIO scientists and to the CNIO's unwavering encouragement of innovation and technology transfer activities.

Biobank

BIOBANK

Eva Ortega-Paño
(since September)
Director

Miguel Quintela-Fandino (Acting
Director, until August)



OVERVIEW

CNIO Biobank – authorised by the Health Authorities of the *Comunidad Autónoma de Madrid (CAM)* and registered in the National Registry of Biobanks with reference B.000848 – is a ‘biobank for biomedical research purposes’, as defined by the Spanish Law 14/2007 and the Royal Decree RD 1716/2011. It is therefore defined as a public, non-profit organisation that hosts several collections of human biological samples for biomedical research, specifically in cancer and related diseases. The main objective of the CNIO Biobank is to facilitate access to human samples for researchers, ensuring that both the acquisition and use of human samples complies with all the legal and ethical principles that protect donors’ rights.

In addition to this biobanking activity, a number of services have been implemented, both for sample processing and for supporting different aspects of the management of human

samples for biomedical research, in order to facilitate the use of human samples for CNIO researchers.

CNIO Biobank is a founder member of the Spanish Biobank Network, a project funded by the *Instituto de Salud Carlos III*, *ISCIII*, currently through the *Acción Estratégica en Salud – AES* 2017 supportive platforms for research programmes. CNIO Biobank leads and/or participates in many different projects such as: centralised request management system (design and development); ethical, legal and social issues (ELSI); harmonisation; biospecimen science; and marketing plans.

Therefore, CNIO Biobank is a cross-service platform for CNIO researchers, as well as the general scientific community, and is geared towards the promotion of biomedical research in cancer and related diseases.

Technicians

Nuria Ajenjo (TS)*, Inmaculada Almenara, M. Jesús Artiga (TS)*, Francisco de Luna (TS)*

**Titulado Superior* (Advanced Degree)

Student in Practice

Daniel Alba (September-October) (*Universidad A Coruña*)

RESEARCH HIGHLIGHTS

Biobanking

In 2019, CNIO Biobank ceded tissue samples from 468 cases to support 7 research projects. The return in impact related to this activity resulted in 5 (Q1) publications acknowledging CNIO's Biobank contributions, with a mean impact factor (IF) of 9.577.

- Management of project-driven and diagnostic collections:
 - CNIO Biobank holds responsibility for management of 3 different collections. This year's activity accounted for a turnover rate of 659 human samples.
 - CNIO Biobank collaborated with the Familial Cancer Unit in the acquisition of 37 new cases.
- The CNIO Biobank's Virtual Catalogue includes 269 images, 139 of them histological H&E staining. We continue expanding this catalogue to include whole section paraffin-embedded samples.

Ethical and legal services

CNIO Biobank supported 3 CNIO project submissions for ethical evaluation by the *Instituto de Salud Carlos III (ISCIII)* Research Ethics Committee.

CNIO Biobank is participating in 3 **multicentre research projects**:

- Optimark (PI16/00946) (led by the CNIO Biobank): focused on identifying quality markers for tissue samples sensitive to pre-analytical variables.

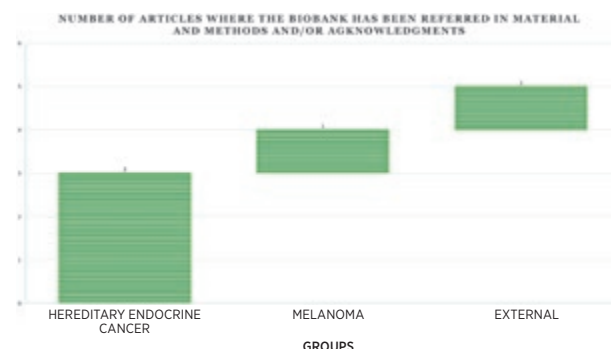


Figure Number of journals where CNIO Biobank has been acknowledged. The main task of a biobank is to provide quality human samples to researchers. The impact of this service is therefore measured through several activity indicators such as a unique reference of the biobank in acknowledgements.

- Exospore: aimed at defining the optimal procedures for collecting human samples for micro-vesicle studies.
- Histological images banking: a repository for digital images.

Teaching activities

During 2019, we actively participated in 3 guided school visits (as part of CNIO's educational activities) and hosted a graduate student for a short-term stay.

CNIO Biobank in collaboration with Lund University (Sweden), University of Copenhagen and the Danish National Biobank (DNB) organised and led a PhD course and Symposium in November entitled "*The Future of Biobanking*" in Copenhagen and Lund (Sweden).

PUBLICATIONS

- ▶ Martínez-González S, Rodríguez-Arístegui S, Gómez de la Oliva CA, Hernández AI, González Cantalapiedra E, Varela C, García AB, Rabal O, Oyarzabal J, Bischoff JR, Klett J, Albarrán MI, Cebriá A, Ajenjo N, García-Serelde B, Gómez-Casero E, Cuadrado-Urbano M, Cebrián D, Blanco-Aparicio C, Pastor J (2019). Discovery of novel triazolo[4,3-b]pyridazin-3-yl-quinoline derivatives as PIM inhibitors. *Eur J Med Chem* 168, 87-109.
- ▶ Esteve-Socias M, Artiga MJ, Bahamonde O, Belar O, Bermudo R, Castro E, Escámez T, Fraga M, Jauregui-Mosquera L, Novoa I, Peiró-Chova L, Rejón JD, Ruiz-Miró M, Vieiro-Balo P, Villar-Campo V, Zazo S, Rábano A, Villena C (2019). In search of an evidence-based strategy for quality assessment of human tissue samples: report of the tissue biospecimen research

working group of the Spanish biobank network. *J Transl Med* 17,370.

- ▶ Devereux L, Watson PH, Mes-Masson AM, Luna-Crespo F, Thomas G, Pitman H, Speirs V, Hall AG, Bollinger N, Posada M, Lochmüller H, Thorne H, Eng CB, Riegman PHJ, Ng W, Parry-Jones A (2019). A review of international biobanks and networks: success factors and key benchmarks-a 10-year retrospective review. *Biopreserv Biobank* 17, 512-519.

Book Chapter

- ▶ Ortega-Paino E, Tupasela AM (2019). Biobank and biobank networks. In book: global genes, local concerns. Publisher: Edward Elgar, pp.229-241. DOI: 10.4337/9781788116190.00022.

Communication

Communications

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COMMUNICATIONS

NURIA NORIEGA Head of Communications

Science Communication
and Social Media
Vanessa Pombo



For yet another year, the CNIO's Communications Department brought the research activity of the Centre closer to those who benefit most – patients, their families and society in general – using the available communication channels. This is an essential task, for our society to be more and better informed on science issues, especially in the current information environment in which fake news often prevail.

Media coverage of CNIO activities and discoveries continues to grow each year. In 2019, we appeared more than 4,400 times in the press, both on paper and online (34% more than in 2018), and we were mentioned 340 times on radio and television. In general, media coverage increased by 28% compared to the previous year.

Scientific news of the CNIO attracted the interest of the main Spanish media and those of other countries, such as the BBC and *Clarín*, and also made it to the front page of local and regional national newspapers, such as *El Mundo* and *La Razón*. News items included the confirmation of the link between the most aggressive prostate cancer and hereditary breast cancer (David Olmos, *Journal of Clinical Oncology*); the elimination of some types of pancreatic cancer in animal models (Mariano Barbacid, *Cancer Cell*); the discovery of a new way to protect against the side effects of radiotherapy (Nabil Djouder, *Science*); the finding that the rate of telomere shortening predicts species' lifespans (Maria Blasco, *PNAS*); a new immunotherapy treatment that improves survival in a type of aggressive lung cancer (Luis Paz-Ares, *The Lancet*); and the resolution of the 3D structure of the molecular mechanism that gives virulence to tuberculosis (Óscar Llorca, *Nature*).

Around February 11 (the International Day of Women and Girls in Science), the *#YoRompoTechoCristal* [IBreakTheGlassCeiling] social media campaign was run to make the obstacles faced by professional women scientists visible using the video 'Women and Science' by CNIO & The City as a starting point. With this activity, 2 proposed objectives were exceeded: on the one hand, in terms of video views – it was the most-watched video of 2019 and the all-time 5th of our YouTube channel – and, on the other hand, in terms of use of the hashtag beyond the campaign: many users used it, mainly on Twitter, in their demands for female visibility in science. Moreover, the initiative was picked up by media outlets such as *TVE*, *El País*, *Voz Pópuli* and *Yo Dona*.

As part of our efforts to reach society, in September we again kept our commitment to International Cancer Research Day. With the support of "la Caixa", we brought scientists and the general public together at *CaixaForum*, Madrid, for the conference 'New horizons in cancer research: from bench to bedside'. The event was attended by researcher and theoretical physicist Raúl Rabadán, Professor of the Department of Systems Biology and Director of the Program

"Only a well-informed public will defend scientific knowledge as key to address global challenges."

for Mathematical Genomics at Columbia University (USA); he gave the keynote lecture 'The Genomic Revolution in Cancer'. Afterwards, a round table, moderated by journalist and author Cristina Villanueva, was held in which Maria Blasco (CNIO Director), Manuel Valiente (Head of the Centre's Brain Metastasis Group), Yolanda Fernández (Head of the Breast Cancer Section of the Central University Hospital of Asturias, *HUCA*), and former patient and 'CNIO Friend' Mila García Calvo participated.

Also during 2019, a collaboration agreement with L'Oréal Spain was formed to launch, together with La Roche Posay, the *#InvestigaciónEsVida* [ResearchIsLife] campaign on the occasion of World Cancer Day. The two-week campaign was aimed at raising awareness of the 'CNIO Friends' initiative to seek new supporters for cancer research at the CNIO. The campaign was present in pharmacies throughout Spain, to which activities in social networks and informational mailings to customers, consumers and the media were added.

In addition, the CNIO Arte project that, with the collaboration of the *Banco Santander* Foundation, explores the common areas of scientific research and artistic creation, brought together in its second edition the quantum physicist Ignacio Cirac and the photographer Chema Madoz, winner of the National Photography Award. Once again, the reception of the project in the media was exceptional, attracting the interest of cultural programmes such as 'Atención, obras!' of the Spanish TV channel *La 2*, the news programme of the Spanish national television channel *TVE*, National Geographic, the magazines 'El Cultural' and 'GQ' and *Canal Metro*, of the Madrid metro – to mention just a few.

In our effort to bring science to spaces where it is not usually present, in November CNIO Director Maria Blasco was interviewed in the programme 'El Intermedio' of the Spanish TV channel *La Sexta*. Also at this time, researchers Maria Blasco and Mariano Barbacid participated in the advertising campaign *#ValoremosAlosCientíficos* [Let'sValueOurScientists] through which 'Constantes y Vitales', the corporate responsibility campaign of *la Sexta* and AXA, wanted to recognise the work and image of Spanish scientists.

These are some of the initiatives in which the CNIO participated that give value to the researchers' work, and to the importance of science and research as a driving force to build a better society.

PRESS CLIPPINGS



1 BBC News Mundo, January 14, 2019
2 La Mañana, La 1, February 4, 2019

3 La Razón, February 4, 2019
4 La Mañana, La 1, February 11, 2019

5 iAtención obras!, La 2, March 4, 2019
6 ABC, March 6, 2019

7 El País, March 29, 2019
8 El Mundo, April 4, 2019



9 La Sexta Noticias, La Sexta, April 11, 2019

10 El Mundo, April 27, 2019

11 Telediario, La 1, May 31, 2019

12 La Razón (front page), July 9, 2019

13 SINC, August 22, 2019

14 Gaceta Médica, September 9, 2019

15 El Correo Gallego, September 14, 2019

16 Diario Médico, October 7, 2019

17 Diario 24 Horas, Canal 24 Horas, October 10, 2019



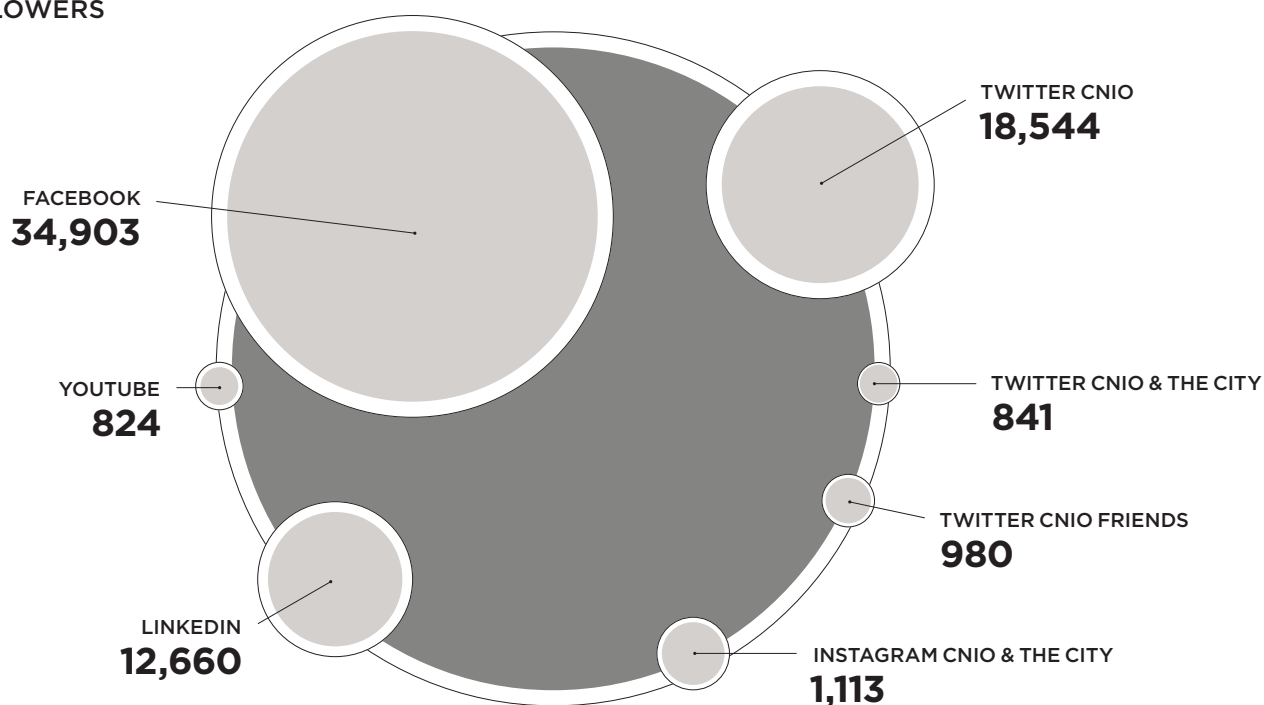
18 ABC, October 18, 2019
19 El Mundo (front page),
October 30, 2019

20 El País Semanal, November 3,
2019
21 Faro de Vigo, November 5, 2019

22 El Intermedio, La Sexta,
November 14, 2019
23 Diario Médico (front page),
November 25, 2019

24 El Mundo (front page),
November 26, 2019
25 El País, December 20, 2019

2019 SOCIAL NETWORK DATA FOLLOWERS



LINKEDIN

IMPRESSIONS	804,388
INTERACTIONS	43,034
LINK CLICKS	28,626
INTERACTION RATE (AVERAGE)	4.50%

YOUTUBE

UPLOADED VIDEOS (TOTAL)	112
UPLOADED IN 2019	21
VIEWS IN 2019	36,692
WATCH TIME (IN MINUTES)	122,346
AVERAGE VIEW DURATION (IN MINUTES)	3:20

TWITTER CNIO

LIKES	10,047
RETWEETS	4,324
IMPRESSIONS	2,425,000
MENTIONS	5,842
VISITS TO THE PROFILE	55,041

FACEBOOK

IMPRESSIONS	2,066,256
REACH	1,308,280
LINK CLICKS	28,789
INTERACTION (REACTIONS, COMMENTS, SHARES)	40,044

Our most shared and commented news on the CNIO social network channels offer a good overview of the issues that most interest society with regard to cancer research, but also with regard to science in general.

In 2019, some of the highlighted topics were the work of the teams of Mariano Barbacid about pancreatic cancer (*Cancer Cell*, April), Nabil Djouder on the side effects of radiotherapy

(*Science*, May), and Maria Blasco about the longevity of species (*PNAS*, July); interviews such as Maria Blasco in 'El Intermedio' of *La Sexta*, Sandra Rodríguez in *El Mundo*, and Manuel Valiente in *El País*; the awards and recognitions to our scientists; the outstanding position of the CNIO in the international rankings of research centres; and events such as the CNIO's 'Frontier Meeting on Heterogeneity and Evolution in Cancer', and the 'European Researchers' Night'.

SOCIAL EVENTS



CNIO and the Moross Integrated Cancer Center (MICC) of the Weizmann Institute of Science held in Rehovot, Israel, an international symposium on the newest approaches in cancer research and the development of new joint projects. This was the second of the biannual events that take place alternately in Spain and Israel following a cooperation strategy agreed upon in 2017 by CNIO and the Weizmann Institute of Science – two of the world's top cancer research centres –, and *Ramón Areces* Foundation. *September 4-5, 2019.*

Scientists from CNIO and the Spanish National Centre for Cardiovascular Research (CNIC), both affiliated with the National Institute of Health Carlos III (ISCIII), organised a Joint Meeting at CNIO headquarters to share knowledge and promote synergies and future collaborations. Raquel Yotti, Director of the ISCIII, opened the meeting, and Maria Blasco and Valentín Fuster, Directors of CNIO and CNIC, respectively, presented an overview of some of the most innovative research lines of each of their institutions and underscored the vital importance of collaborative research. *September 20, 2019.*



With the support of “la Caixa” Foundation, we celebrated the World Cancer Research Day at *CaixaForum* Madrid, with an event entitled ‘New Horizons in Cancer Research: from Bench to Bed’. Raúl Rabadán, from Columbia University, gave the keynote speech ‘The Genomic Revolution in Cancer’. Afterwards, a roundtable was held with the participation of Maria Blasco, Manuel Valiente, Yolanda Fernández (Central University Hospital of Asturias), and Mila García Calvo (former cancer patient and CNIO Friend). *September 24, 2019.*

CNIO renewed its commitment with the European Researchers' Night, which is funded by the EU Framework Programme for Research & Innovation, Horizon 2020 - Marie Skłodowska-Curie actions. In the region of Madrid, it is promoted by the Department of Education and Research and coordinated by *Fundación madri+d*. More than 40 CNIO volunteers and 200 visitors participated in the event, aimed at showing the reality of a collective - the science community - that is key for the development of society. *September 27, 2019.*



The SOMM Alliance held its first meeting on gender equality policies at CNIO. Directors of SOMMa Centres discussed the network's best practices in gender equality, to implement a change of culture that allows gender equality to be integrated into strategic science decisions. Invited speaker Cheryl Smythe, gender equality expert from the Babraham Institute, talked about the gender policies implemented by her centre. The British Embassy in Spain took part in the event, which was closed by Ana Puy, Director of the Women and Science Unit at the Ministry of Science, Innovation and Universities. *October 29, 2019.*



CNIO addressed the ethical and philosophical aspects of the revolution that could extend the biological limits of human beings in a workshop entitled 'CNIO Workshop on Philosophy & Biomedical Sciences: Debates on Conceptual and Social Issues' with the support of the *Banc Sabadell* Foundation. Scientists Maria Blasco, Lluís Montoliú, Alfonso Valencia and Henrik Vogt exchanged views with philosophers María Cerezo, Antonio Diéguez, Íñigo de Miguel Beriain, Arantza Etxeberria and Michael Hauskeller. Discussion topics illustrated the social impact of current molecular biology: longevity, human enhancement and transhumanism, personalised medicine, and gene editing. *November 19, 2019.*

International Affairs

INTERNATIONAL AFFAIRS

CAROLINA POLA Director of International Affairs



The combined efforts of different departments spearheaded by the Department of International Affairs (IAs) translate into outputs for the institution that have an impact at a science and institutional level. In collaboration with our Projects Office, we continue to explore funding opportunities to propel the research impact of our investigators and the CNIO. In 2019, we continued participating in strategic working groups organised by the Spanish Ministry of Science, Innovation and Universities (MICIU) and the Centre for the Development of Industrial Technology (CDTI), as a fundamental action to maintain our engagement with funding and influencing institutions at the National and European level. Our institutional strategy to boost the coordination of H2020 consortia projects by CNIO investigators has resulted in an increase in 67% in proposals submissions for coordinated projects.

Since its launch in 2017, the CNIO has been a member of the *Severo Ochoa* and *Maria de Maetzu* Alliance (SOMMa). This year, CNIO Director Maria Blasco held the position of Vice-Chair of the Alliance, directly contributing to its performance and governance. The IAs led the WP of Outreach and actively participated in the organisation of the second ‘100xCiencia’ conference held in November at the Tabakalera in San Sebastian that focused on how science can bring value to society. During this event, the CNIO Director Maria Blasco shared with the lay audience the advancements in gene therapy for age-related diseases, and AIs brought a stand in which we gamified different biomedical innovation projects from CNIO.

Continuing with our approach to promote cooperation with international research institutions, in 2019 we led a proposal for an interdisciplinary project in collaboration with institutions from three different countries. The Accelerator Awards call from CRUK and AECC is a key framework for developing a competitive consortium in alliance with 3 European countries. These and other opportunities have been leveraged to boost our visibility and cooperation beyond our borders.

One of our main successful alliances is the one we keep nurturing with the Weizmann Institute of Science and the *Ramon Areces* Foundation. In 2019, this successful partnership has translated into a collaborative project for the development of new immunotherapies and knowledge in the field of melanoma, and a cancer research symposium held in Rehovot, Israel. This partnership is a true example of collaboration and commitment between international institutions devoted to the advancement of science.

“We align our scientific and institutional strengths with Europe’s vision for research and innovation to maximise our outputs through collaborative projects and public-private alliances.”

As the new European framework programme becomes a reality, the IAs has been actively involved in the scrutiny of the working documents for the upcoming Horizon Europe. This aims to facilitate the involvement, participation and influence of CNIO in future calls in order to harness our expertise and leadership in key fields in cancer and ageing-related diseases. We expect 2020 will help us achieve more outputs from the groundwork that we strive to build and strengthen every year.

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INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY

AMPARO GARRIDO Coordinator

Science Outreach Officer
Pablo Camacho (TS)*

**Titulado Superior (Advanced Degree)*



CNIO Arte 2019 exhibition. In this photo, from left to right: visual artists Eva Lootz, Amparo Garrido and Chema Madoz, and historian and art critic Carlos Jiménez.

In 2019, the CNIO Cancer Research Centre was transformed into a venue for mutually beneficial encounters between the worlds of science and art. Its walls were given over to multimedia works curated by visual artist Amparo Garrido, who also serves as Coordinator of Institutional Image and Outreach to Society at the CNIO. Prominent among the participants was Ignacio Cirac, who heads the Max Planck Institute of Quantum Optics in Garching (Germany). He was paired off with Chema Madoz, winner of Spain's National Prize for Photography and the sparks given off materialised in an edition of 30 signed, limited copies of a print by Madoz, the proceeds of which were earmarked for projects funded by the 'CNIO Friends' philanthropic initiative.

The photographs by Chema Madoz remained on display from February to April. During that time, Madoz's work was also exhibited at the ARCO and JUSTMAD contemporary art fairs, while the CNIO also kept its profile high during the 'Quantum' exhibition at the Centre for Contemporary Culture in Barcelona (CCCCB). Begoña Gómez, co-director of the Masters Programme in Fundraising Management at Madrid's Complutense University, was present for the Barcelona event as was Carlos Jiménez, emeritus professor at the European University of Madrid, a specialist in art history and critical theory, along with Maria A. Blasco, Director of the CNIO, and Amparo Garrido, project curator and presenter of the event. CNIO Arte is an initiative created by CNIO, with support from the *Banco Santander* Foundation.

It was the CNIO's turn to host some of the big players in philosophy and ethics on occasion of the Centre's annual Symposium on Science and Philosophy. The scientists involved included Maria A. Blasco, Director of the Centre, Lluís Montoliú, Head of the Department of Molecular and Cellular Biology at the Biotech Center (CNB-CNIC) in Barcelona, and Alfonso Valencia, Head of the Bioinformatics and Life Sciences programme at the Barcelona Supercomputing Center. A formidable array of thinkers was mustered to keep the arguments lively, including Antonio Diéguez, distinguished Professor of Logic and Philosophy of Science at the University of Málaga, author of *Transhumanism: The Technological Quest for Human Improvement*. Also present was Iñigo de Miguel, inter-university research Professor of Legal Aspects of the Human Genome at the University of the Basque Country; Arantza Etxeberria, Professor of Philosophy and Science at the same institution UPV/EHU; Henrik Vogt, Physician and Philosopher at the Centre for Medical Ethics at the University of Oslo (Norway); and Maria Cerezo, Professor of Logic and

"CNIO Arte allows scientists and artists to explore the common ground on which the act of creation takes place. That's where you'll find the true sources of inspiration."

Philosophy of Science at the University of Murcia. Finally there was Michel Hauskeller, Head of the Philosophy Department at the University of Liverpool (UK).

The initiative continued to pick up momentum throughout the spring of 2019. In April of that year, CNIO teamed up with "la Caixa" Foundation for a panel discussion centred on Mary Shelley's novel *Frankenstein*. Was Victor Frankenstein the prototype of the heroic scientist? Or just a mad one? Sharply different opinions were advanced, defended and discussed by Maria A. Blasco, Director of the CNIO, along with novelist/essayist Lorenzo Luengo, and Cristina Higuera, novelist, actress, and theatrical producer. The controversial clash between confluence of science and philosophy was at the core of the follow-up discussion moderated by writer Fernando Marías.

In 2019, our redesigned institutional web received an honorary mention in the *Prisma Prize for the Advancement of Science*, the most prestigious award of its kind in Spain. In their citation, the judges singled out CNIO's "innovative proposal for presenting the content and activities of a major research institution directly to the public in a format that is both modern and accessible to all." All told, the CNIO produced a series of custom videos and held numerous science outreach events throughout the year.

Thanks in part to our new website launched in 2018, the CNIO has been able to consolidate its profile. The roughly 190 events scheduled last year were open to the public, and it was a particular satisfaction to be involved in the 'International Day of Women and Girls in Science' on February 11 and other diversity initiatives highlighting the obstacles facing young women who have demonstrated their qualifications to study and build a career in the traditional WISE (women in science and engineering) disciplines.

CNIO & THE CITY

CAROLINA POLA Director
of International Affairs

Science Outreach Officer
Pablo Camacho (TS)*

**Titulado Superior (Advanced Degree)*



**“CNIO & The City wants
to present science as an
attractive profession.
Despite its difficulties,
Science is an option for
the future!”**

During one's school years, there are doubts and important decisions to think about: which career to choose, what to work on... CNIO & The City is an educational and science outreach project established in 2017 to strengthen the bridges between CNIO scientists, the educational community, and society as a whole. It was released to impact and inspire them with our science!

After the success of CNIO & The City's first editions (May 2017 - March 2018; May 2018 - June 2019), funded by the Spanish Foundation for Science and Technology (FECYT) – Ministry of Science, Innovation and Universities, the CNIO decided to incorporate this project and its values as an essential core of the CNIO outreach strategy.

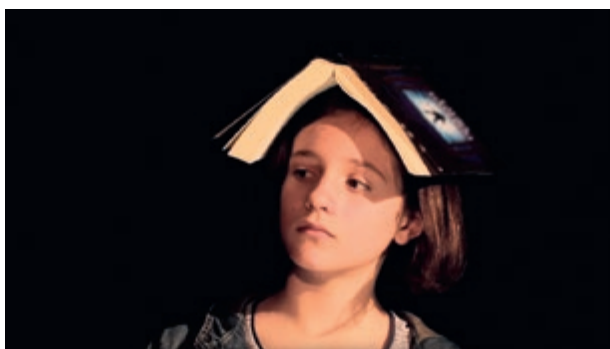
New standards of scientific excellence are reached thanks to these initiatives. This is widely known here at the CNIO, and over 140 CNIO scientists are involved in our activities: principal investigators (32%), staff scientists (15%), post-doctoral fellows (17%), graduate students (7%) and technicians (29%). Thanks to them, we organised lab immersions and scientific projects with secondary and high school students (EDUCACNIO); training courses for teachers (FORMACNIO); and scientific workshops in classrooms (DIVULGACNIO), which were a unique opportunity for the more than 1,200 participants (students and teachers) over the last 2 years.

We also want to go one step further and get closer to the whole of society. This is why we dedicate time to think about and create educational videos, which can be played everywhere: in class, in a large auditorium and even on smartphones! In 2019 we launched 2 cartoon videos about general topics in cancer science ('History of Cancer' and 'Cancer Risk Factors').

Another video related to the 'Women in Science' issues was released to empower our CNIO & The City female participants (78%) and raise awareness about gender balance in science and breaking the glass ceiling. This video was very well received by the media thanks to our February 11th #YoRompoTechoCristal [IBreaktheGlassCeiling] Challenge (International Day of Women and Girls in Science) and was one of the 'Science Film Festival' #LabMeCrazy! finalist videos.

CNIO & The City must be a stimulus for young generations and their families too. The inspirational video 'Changing the World', which was directed by Amparo Garrido, invited CNIO scientists and their kids to talk about future scientific careers and passions. "I have seen monkey cells!", explained Diego Megías, Head of the Confocal Microscopy Core Unit, to his son. Science is not only about discovering, but also about getting fascinated and trying new things!

All these activities have transformed 'CNIO & The City' into an innovative, inclusive and STEAM education committed project with gender issues as a transversal theme. We have also found that our participants' knowledge and perceptions about cancer, research and innovation are evolving in a positive way, which suggests that we are helping new generations grow up with a more critical, reflective and no-barriers view of the world around them. This is our mission, and we hope to accomplish it in the coming years!



Changing the World inspirational video (<https://go.cnio.es/CambiarElMundo>). Photo & video by visual artist Amparo Garrido.



Women in Science educational video (<https://go.cnio.es/YoRompoTechoCristal>).

Development & Philanthropy

DEVELOPMENT & PHILANTHROPY

JESSICA ROSE Director (since December 30)

Development and Philanthropy Officer
Mercedes Antona (since September)
(TS) *

**Titulado Superior (Advanced Degree)*



In the picture: Mercedes Antona.

The Development and Philanthropy Office, established in September 2019, is key to achieving two of CNIO's strategic goals: attracting new sources of funding to ensure financial sustainability and connecting CNIO to society.

The Office was established to enable the CNIO to proactively build a variety of funding sources to support the Centre's strategic goals. Sources of funding will include the establishment of new collaborative partnerships with corporate organisations, foundations and high net worth individuals. It will also build upon our existing programme of charitable bequests and donations from society at large. Diversifying and increasing our funding sources is critical to enable the CNIO to continue to perform in the top tier of cancer research worldwide.

In late December, Jessica Rose, an expert in fundraising, joined the Development and Philanthropy Office as Director of the office, bringing many years of experience in sponsorship and philanthropy from a global perspective. Together with the CNIO Director and Mercedes Antona – a philanthropy professional who established the office in 2019 – Jessica will work on a global fundraising strategy to develop a comprehensive philanthropic programme for the CNIO.

The Office will manage the giving platform 'CNIO Friends', which started in 2014 and until September of this year was collectively administered by different areas of the CNIO. The initiative was a success from the start, growing each year to reach nearly €1.4 million in total donations thus far, which have been channelled into 12 research contracts opening new lines of research across the CNIO. In 2019 alone, CNIO Friends raised €515,000, which will be used to engage more scientific talent in 2020. In addition, the Office will manage the legacy programme which continues to grow and has received a cumulative total of €888,000 since 2015. In 2019, CNIO received charitable bequests of €284,000 with €844,000 pending to be executed.

Since the establishment of the Development and Philanthropy Office, a strategy to raise awareness has been designed to implement in the first half of 2020. Leading figures have been contacted in a variety of spheres to become CNIO ambassadors, sharing the Centre's values and mission with larger sectors of the population. This work will support the 'Outreach to Society' and 'Communications' Offices in creating a bridge between the CNIO and society.

“We want to develop ties of solidarity, making people part of our scientific achievements by walking side by side in this promising and necessary journey.”

Although very new, the Philanthropy and Development Office has already made an impact at the CNIO and has successfully started to build strong alliances with companies and foundations to collaboratively create a positive impact on people and society. In a positive sign for the future of philanthropy for the CNIO, contacts were made with several foundations that are expected to bear fruit in 2020, with at least two €100,000 donations already in solicitation. We look forward to working together with industry and society to enable the CNIO to be the very best it can be.

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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, Fernando Pozo

Staff/Faculty:

Ana Losada, Jorge Martínez, Lola Martínez, Francisco Real, Manuel Valiente, Héctor Peinado, Rafael Fernández-Leiro



One of the elements contributing to the CNIO's international projection is the commitment of our established investigators to foster new generations of scientists. In fact, over 60% of the workforce at our institution are personnel in training, involving undergraduate students, predoctoral and postdoctoral fellows, medical residents and a broad spectrum of visiting scientists supported by competitive grants through various funding agencies. Also very successful are our diverse exchange and visitor programmes. In this context, we are also most grateful to the *Fundación Jesús Serra*, for its continuous support to strengthen career development programmes at the CNIO.

The CNIO Dean's Office oversees the fulfilment of academic requirements to ensure timely and productive PhD dissertations. In addition, we pay attention to soft skills that may increase the chances of success of our personnel, in and beyond academia. Indeed, we are very proud of the altruistic spirit of our young trainees. This year, 50 volunteers

participated in our seventh '*Meet a Scientist, Become a Scientist*' event. This is an open doors activity we hold as part of the *Marie Skłodowska Curie European Researchers' Night*, where over 250 participants of all ages learn about daily life at our Centre and have the opportunity to run an experiment alongside our researchers. Other multiple volunteers participated in the '*Semana de la Ciencia*' and in scientific tours the CNIO offers to interested parties.

A particularly successful event this year was our Annual CNIO Lab Day. We had a total of 55 posters and 15 oral presentations, which highlighted the "best-to-come" in each of the Centre's Scientific Programmes. We learned about start-ups and about different aspects of communication and leadership from distinguished invited speakers. Laura Soucek (*Vall d'Hebron* Institute of Oncology, Barcelona) told us how endurance and self-confidence allowed her to patent a product from her group (Omomye) and create a small biotech

company (Peptomic), which will soon proceed to clinical trials in patients with aggressive cancers. Gerardo Barcia (CEO, BBVA Next Technologies) presented new concepts on flexible team building, to improve creativity and ensure personnel satisfaction, to ultimately promote healthier and more productive work environments. In turn, Diego Marqueta (Diego Marqueta Consulting) discussed new visual tools and presentation strategies for effective presentations. The audience was indeed thrilled!

A main highlight of the Lab Day was the announcement of the recipients of our '*Director's List Awards*'. These recognise 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 acknowledging the PhD student authoring the article with the highest impact in a scientific journal. This year, the '*Antonia Nieto Award*' went to Almudena Chaves-Pérez, for impressive work published in *Science* on new discoveries in the field of secondary effects of radiation therapy. Additional awards in the PhD category went to María T Blasco (*Cancer Cell*), Catarina P Santos (*Nature Communications*), Miguel Ángel Muñoz (*Nature Communications*) and Laura Remacha (*American Journal of Human Genetics*).

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

The awardee was Ángel Rivera Calzada, for the crystal structure of a nanomachine involved in the pathogenesis of tuberculosis (*Nature*).

"At the CNIO we aim high: to carry out the most innovative basic and translational research, and to prepare our trainees 'to think outside the box' so that they can best fulfil their potential as influential leaders."

3. Outstanding Contribution to Outreach and Awareness

The recipient was María J Alcamí, for her tireless efforts and dedication to the CNIO's Women in Science Office (WISE). The award was presented by María Luisa Villafranca from the '*Asociación ROSAE*', a non-profit organisation in support of breast cancer patients that belongs to our growing community of 'CNIO Friends'.

The Lab Day proceeded with 6 additional Awards from the Dean's Office to the *Best Oral Presentations* and to the *Best Posters*. The closing included yet an additional Award for *T-Shirt Design*, which was particularly moving. The most voted entry was the 'Give us a Hand' design that illustrates the concept that to make progress, scientists need the help of multiple hands: their supervisors, academic and clinical institutions, grantees, foundations, and patients and their families. This was a beautiful allegory of our commitment to and for the society.

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

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



WISE Members (from left to right):

-In the group picture*: Diego Megías, María Jesús Alcamí, Isabel López de Silanes, María A. Blasco.

-Portrait photos: Fernando Peláez, Sandra Rodríguez-Perales, Francisca Mulero, Carolina Pola, Eva Ortega, Raquel García-Medina, Celia de la Calle, Lola Martínez.

*This was the last photo taken and for Coronavirus precautions not all members of the office could attend the photo session.



Maria A. Blasco, María Jesús Alcamí,
Celia de la Calle (since November),
Lola Martínez, Diego Megías,
Francisca Mulero, Eva Ortega (since
September), Fernando Peláez,

Carolina Pola (until October), Sandra
Rodríguez-Perales

The CNIO Women in Science Office (WISE) was established in 2012. Our main objectives are to give visibility to women, to raise awareness regarding the importance of gender equality, to help correct imbalances in the career ladder at the CNIO community especially in the leadership positions, to try to promote and support women in their professional careers, as well as to come up with ideas and policies to improve the life/work balance at the CNIO. The WISE Office is composed of CNIO volunteers from across all the areas present in the Centre, including the Director.

In 2019, the WISE Office was involved in making the CNIO a better place to work and to reconcile work and private life. Thanks to the joint efforts of the CNIO Direction and Management, The WISE Office, and the Works Council, we have approved a very innovative Equality Plan, a 'remote working' pilot programme, as well as measures to ensure digital disconnection.

In addition, we continued organising the WISE seminar series, in which we invite several top female leaders from different areas. Some of the talks given during 2019 include:

- Consuelo Madrigal, jurist. Title: '*La mujer profesional: el largo camino a la igualdad*'. 15/01/2019.
- Rosa Montero, journalist and writer. Title: '*Palabra de mujer*'. 12/02/2019.
- Luz Casal, singer and songwriter, was interviewed by the journalist Virginia Díaz. Title: '*Aproximaciones a una biografía*'. 05/03/2019.
- Ruth Vera, President of the Spanish Society of Medical Oncology (SEOM). Title: '*Mujeres en la Oncología*'. 09/04/2019.
- María Luisa de Contes, General Secretary and Board Member of the subsidiaries of the Renault Group in Spain. Title: '*Un proyecto para la igualdad de Género en las Empresas*'. 07/05/2019.
- María Hervás, actress. Title: '*La alquimia de un cuerpo que actúa*'. 20/06/2019.
- Susana Malcorra, Minister of Foreign Affairs of the Argentine Government with President Macri and Chef de Cabinet of the Secretary-General Ban Ki-moon in the United Nations. Title: '*The leadership in times of change*'. 15/10/2019.
- M^a Pilar Allué, General Deputy Director of Human Resources and Training of the National Police Corps. Title: '*40 años de la incorporación de la mujer a la Policía Nacional*'. 26/11/2019.
- M^a José San Román, chef and restaurateur. Title: '*Mujeres en Gastronomía*'. 03/12/2019.

Here at the WISE Office, we share what the American writer, poet and activist Maya Angelou said: "How important it is for us to recognise and celebrate our heroes and she-roses!" Our work efforts aim to build a fair society for all where stereotypes, unconscious bias and gender barriers are gone, as we firmly believe that working together as equals, is the way to reach true excellence.

We also organised and hosted the 1st Gender Equality Event of the SOMMa Research Centres of Excellence (held October 29, 2019). This event aimed to share best practices in the SOMMa (the Alliance of the "*Severo Ochoa*" Centres and *María de Maeztu* Units) to promote a change of culture in a coordinated and institutional manner, and support female talent and gender equality in science and strategic decision-making. The event was organised in collaboration with the British Embassy and different SOMMa Directors, and with the participation of gender officers.

We continued to hold Master classes from the STEM Talent Girl programme (as part of an agreement signed with the ASTI Foundation in 2018). The goal of this project is to promote STEM careers among 13 to 14-year-old students. Eight Master classes given by top professional women from the STEM field were held in the CNIO Auditorium. The last 6 took place in 2019 and were given by Carmen García Matea, Professor in the TSC Department at the University of Vigo (January, 2019); Pilar López Álvarez, President of Microsoft Spain (February, 2019); María Martínón-Torres, Director of CENIEH (March, 2019); Helena Herrero, President and CEO of HP for Spain and Portugal (April, 2019); Verónica Pascual Boé, CEO of ASTI TechGroup (May, 2019); and Yaiza Canosa, CEO and Founder of Goi (June, 2019).

We also participated in other educational initiatives, through the *11defebrero.org* platform, to promote scientific careers among students from the *Comunidad de Madrid*. This initiative was done in collaboration with the 'CNIO and The City' project, which was funded by the Spanish Foundation for Science and Technology (FECYT) – Ministry of Science, Innovation and Universities – with the aim of creating closer links between society and the education system.

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SCIENTIFIC MANAGEMENT

ISABEL BARTHELEMY Scientific Management Director

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



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 the 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 student's 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 science outreach events.

The Library administers the electronic subscriptions of over 300 scientific journals at the CNIO and manages journal

"All our efforts are dedicated towards building a strong and flexible framework to support our scientists and to help them achieve excellence."

article requests for journals that the CNIO is not subscribed to. The Library also provides information regarding reference management software 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 a non-specialised audience.

COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies and is used not only to finance the Centre's outstanding R&D activities, but also strategic actions in Innovation together with Industry partners. The funding is also used to support other relevant activities related to dissemination and scientific outreach; these activities are aimed at promoting public awareness. In 2019, researchers at the CNIO were involved in 136 projects that received extramural funding.

CNIO actively participates in a total of 53 collaborative projects: 18 were international collaborative projects (4 of which are coordinated by the CNIO) and 35 were collaborative projects at the national level (13 of them are coordinated by the CNIO). The international collaborative projects were funded by institutions such as the European Commission through the 7th Framework Programme and Horizon 2020, the Interreg SUDOE Programme, 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, and the Lustgarten Foundation - Stand-up 2 Cancer Initiative. At national level, collaborative

projects received important public funding through grants from 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 the projects were co-funded by European Structural and Investment Funds (European Regional Development Fund and European Social Fund). Private funders and charities also recognised the excellence of our scientific projects, among them, the Scientific Foundation of the Spanish Association Against Cancer (Fundación AECC), the Ramón Areces Foundation and "La Caixa" Banking Foundation.

In addition to these collaborative projects, researchers at the CNIO attracted funding for projects carried out by individual groups. In 2019, 15 of these projects received international funds while 68 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 (5 ERC grants and 5 Marie 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 and the Melanoma Research Alliance.

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

EUROPEAN COMMISSION



7TH FRAMEWORK PROGRAMME (2007-2013)

ERA NET NEURON II: NETWORK OF EUROPEAN FUNDING FOR NEUROSCIENCE RESEARCH

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malumbres, Marcos	MicroKin: Deciphering the multifaceted pathways underlying MCPH pathogenesis in the mouse and human (financed by MEIC, Ref.: PCIN-2015-007)

HORIZON 2020 (2014-2020)

SOCIETAL CHALLENGE 1: HEALTH, DEMOGRAPHIC CHANGE AND WELLBEING

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	BRIDGES: Breast cancer risk after diagnostic gene sequencing (Ref.: 634935)

FET OPEN - NOVEL IDEAS FOR RADICALLY NEW TECHNOLOGIES

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

INTEGRATING AND OPENING RESEARCH INFRASTRUCTURES OF EUROPEAN INTEREST

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Muñoz, Javier	EPIC-XS: European Proteomics Infrastructure Consortium providing Access (Ref.: 823839)

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)
Soengas, María S.	ITN IMMUTRAIN: Training Network for the Immunotherapy of Cancer (Ref.: 641549)

INTERREG SUDOE PROGRAMME¹

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	ONCONET: European Network for Translational Research and Innovation in Oncology / <i>Réseau Européen de Recherche translationnelle et d'Innovation en oncologie</i> (Ref.: SOE1/P1/F0082)

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)
Peinado, Héctor	Exosome-mediated transfer of c-MET to bone marrow progenitors promotes metastasis (Ref.: R01CA169416)
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)

MELANOMA RESEARCH ALLIANCE (MRA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S. (Coordinator)	Imaging and targeting dormant and pro-metastatic melanoma lesions <i>in vivo</i> (Ref.: 401181)

THE PARADIFFERENCE FOUNDATION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima Robledo, Mercedes	SDHB-related metastatic paraganglioma: search for the cure

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



**WORLDWIDE CANCER
RESEARCH (WCR,
FORMERLY AICR)**

PRINCIPAL INVESTIGATOR

Valiente, Manuel (Coordinator)

PROJECT TITLE

S100A9-dependent radiation resistance in brain metastasis (Ref.: 19-0177)

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

PRINCIPAL INVESTIGATOR

Llorca, Óscar (Coordinator)

PROJECT TITLE

Photochemical trap and high-resolution imaging of transient chromatin complexes from living cells (Ref.: RGP0031/2017)

**LUSTGARTEN FOUNDATION
& STAND-UP2CANCER**

PRINCIPAL INVESTIGATOR

Malats, Núria

PROJECT TITLE

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

PRINCIPAL INVESTIGATOR

Soengas, Maria S. (Coordinator)

PROJECT TITLE

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)
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)

MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S. Frago, Susana	METMEL: Long range-acting drivers of premetastatic niches in melanoma (Ref.: 753442)
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	PROJECT TITLE
Castro, Elena	Prospective study of lethal prostate cancer clinical and genomic evolution in DNA repair deficient tumours

WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Targeting telomeres in cancer (Ref.: 16-1177)
Peinado, Héctor	Evaluation of obesity as a novel risk factor in metastasis (Ref.: 16-1244)

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Role of exosomes and Endoglin in Neurofibromatosis Progression (Ref.: W81XWH-16-1-0131)

CANCER RESEARCH INSTITUTE

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	Brain-specific strategies to improve responses to immunotherapy (Ref.: 54545)

BEUG FOUNDATION FOR METASTASIS RESEARCH

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valiente, Manuel	Altered brain vessels as a novel target in brain metastasis

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Elucidating mechanisms of epigenetic changes in type 1 diabetes and treatment with DNA demethylating drugs

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 SALUD²**

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	<i>Plataforma de Biobancos</i> (Group Ref.: PT17/0015/0004)
Benítez, Javier	<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 SALUD³

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Artiga, M ^a Jesús (Coordinator)	OPTIMARK project: Optimization of tissue samples for the development and validation of disease biomarkers (Ref.: PI16/00946)
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)
Olmos, David (Coordinator)	THERATLAS Project: integration of early and adaptive genetic events to establish therapeutic subgroups in Castration-Resistant Prostate Cancer (Ref.: PI16/01565)

TECHNOLOGICAL DEVELOPMENT PROJECTS / PROYECTOS DE DESARROLLO TECNOLÓGICO

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)

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



3. This Programme is cofunded by the European Regional Development Fund (ERDF)



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



NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION (2013-2016 AND 2017-2020)

EXCELLENCE NETWORKS/ REDES DE EXCELENCIA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Efeyan, Alejo (Coordinator)	METABOCANCER: Crosstalk between systemic and cellular metabolism in cancer (Ref.: SAF2016-81975-REDT)
Fernández-Capetillo, Óscar (Coordinator)	UBIRed: Ubiquitin like proteins in signalling, proliferation and cancer (Ref.: SAF2017-90900-REDT)

CHALLENGES-COLLABORATION PROJECTS/PROYECTOS RETOS-COLABORACIÓN⁴

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	New approaches for treatment of lung cancer. (Ref.: RTC-2017-6576-1)
Djouder, Nabil	NRCANCER: <i>Desarrollo de nueva terapia antitumoral basada en nicotinamida-ribosido</i> (Ref.: RTC-2016-5431-1)
Muñoz, Inés Martínez-Torrecuadrada, Jorge	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)

**COMMUNITY OF MADRID /
COMUNIDAD AUTÓNOMA DE
MADRID⁵**



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

4. This Programme is cofunded by the European Regional Development Fund (ERDF)



5. These Programmes are cofunded by the European Regional Development Fund (ERDF) and European Social Fund (ESF)

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/BIO4747)

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 2018-2019, organized by Madri+d Foundation and founded by EU-H2020 Programme. Marie Skłodowska-Curie actions (Ref.: 818528)

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)
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.: GCTRA16015SEO)

"LA CAIXA" BANKING FOUNDATION / FUNDACIÓN BANCARIA "LA CAIXA"**HEALTH RESEARCH PROJECTS**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
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)

ASOCIACIÓN ESPAÑOLA DE INVESTIGACIÓN SOBRE EL CÁNCER (ASEICA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	<i>Análisis de la secreción de neoantígenos en exosomas tumorales de pacientes con cáncer microcítico de pulmón metastásico obtenidos de sangre periférica</i>

NATIONAL GRANTS INDIVIDUAL PROJECTS

**INSTITUTE OF HEALTH
CARLOS III / INSTITUTO DE
SALUD CARLOS III (ISCIII)
STRATEGIC HEALTH ACTION
/ ACCIÓN ESTRATÉGICA EN
SALUD (AES)**
**RESEARCH PROJECTS IN HEALTH⁶**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	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)
Ortega, Ana	Targeting dysregulated nutrient-sensing pathway in follicular lymphoma (Ref.: PI18/00816)
Quintela, Miguel Ángel	Tumor-tolerant immune reprogramming secondary to hypoxia-inducing antiangiogenics in breast cancer: physiopathogenic mechanisms and therapeutic utility (Ref.: PI16/00354)
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 (T1; 22) + t cells (Ref.: PI17/02303)
Urioste, Miguel	PTEN-hamartoma tumour syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene (Ref.: PI14/00459)

**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	Centre of Excellence “Severo Ochoa” (Ref.: SEV-2015-0510)

R&D EXCELLENCE PROJECTS/PROYECTOS DE I+D EXCELENCIA⁷

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)

6. This Programme is cofunded by the European Regional Development Fund (ERDF)



7. This Programme is cofunded by the European Regional Development Fund (ERDF)



CHALLENGES-RESEARCH PROJECTS/PROYECTOS RETOS-INVESTIGACIÓN*

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, María	TELOHEALTH: Telomeres and Disease (Ref.: SAF2017-82623-R)
Djouder, Nabil	URIPAT: URI loss in intestinal pathologies (Ref.: SAF2016-76598-R)
Djouder, Nabil	HEPATOCAR: Studying the Role and Function of MCRS1 in Hepatocellular Carcinoma Development (Ref.: RTI2018-094834-B-I00)
Efeyan, Alejo	NUTRIENTOR: Physiology of nutrient sensing and signaling by the mTOR complex 1 (Ref.: SAF2015-67538-R)
Fernández-Capetillo, Óscar	RESCATE: Mechanisms of resistance to anticancer therapies (Ref.: RTI2018-102204-B-I00)
Llorca, Óscar	RuvBL1-RuvBL2 ATPases in DNA/RNA surveillance and human diseases: molecular and structural mechanisms (Ref.: SAF2014-52301-R)
Losada, Ana	COHESIN2: Molecular mechanisms of variant cohesin function (Ref.: BFU2016-79841-R)
Malumbres, Marcos	Cyclexit: Physiological and therapeutic relevance of mitotic kinases and phosphatases (Ref.: SAF2015-69920-R)
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)
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)
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.	TRANS-PDAC: Transcriptional control of pancreatic cancer development (Ref.: SAF2015-70553-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)
Squattrito, Massimo	GLIO-TRK: TRKING down oncogenic genetic rearrangements in gliomas (Ref.: RTI2018-102035-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)
Valiente, Manuel	Stat3 ReACTIVE: Biology of Stat3+ reactive astrocytes in brain metastasis (Ref.: SAF2017-89643-R)

8. This Programme is cofunded by the European Regional Development Fund (ERDF)



EXPLORA PROJECTS/PROYECTOS EXPLORA

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Non canonical treatment for neurodegenerative diseases: telomerase gene therapy (Ref.: SAF2015-72455-EXP)
Djouder, Nabil	Thermo-regulation of NAD ⁺ to protect from age-related diseases and cancer (Ref.: SAF2017-92733-EXP)
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-TECNOLÓGICAS⁹

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

9. This Programme is cofunded by the European Regional Development Fund (ERDF)



SPANISH FOUNDATION FOR SCIENCE AND TECHNOLOGY / FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y TECNOLOGÍA (FECYT)



"LA CAIXA" BANKING FOUNDATION



SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)



GRANTS FOR SCIENTIFIC CULTURE DISSEMINATION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Pola, Carolina	'CNIO & The City' – <i>Co-creando conocimiento con la sociedad</i> (Ref.: FCT-17-12743)

PRECIPITA CROWDFUNDING FUNDING

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Salazar, María	"MATCH POINT" <i>contra el cáncer de mama</i> (Ref.: PR242)

HEALTH RESEARCH PROGRAMME

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	Targeting Telomeres in Cancer (Ref.: HR18-00023)

CAIXAIMPULSE PROGRAMME

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	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)
Salazar, María	miRNA-based strategy to expand cell therapy potential for treating diabetes (Ref.: CI19-00001)

GRANTS FOR RESEARCH PROJECTS IN CHILDHOOD CANCER:

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	Targeting telomeres in neuroblastoma (Ref.: CICPF18004BLAS)

GRANTS FOR EMERGING GROUPS:

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)
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
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
Squatrito, Massimo	Identification of biomarkers of tumor treating fields (TTFields) in glioblastoma (Ref.: IDEAS185SQUA)

FUNDACIÓN INOCENTE INOCENTE**ASOCIACIÓN DE CÁNCER DE PÁNCREAS-ASOCIACIÓN ESPAÑOLA DE PANCREATOLOGÍA**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria	Validation of the TRF1 telomere protective protein as a novel anticancer target in pediatric glioma and ependymoma

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
Peinado, Héctor	Liquid biopsy by nanoplasmonic detection of exosomes: predicting response to (immuno- and radio)-therapy
Valiente, Manuel	Predictive biomarkers for brain metastasis in small cell lung cancer

**“LA SEXTA” / AXA
FOUNDATION**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	<i>Premio Constantes y Vitales en la categoría “Joven talento en investigación biomédica” 2015</i>

**FUNDACIÓN PROYECTO
NEUROFIBROMATOSIS**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	<i>Análisis de Hsp90 como una nueva diana en neurofibromatosis</i>
Peinado, Héctor	<i>Combinación de terapias antiangiogénicas e inhibidores de MEK en neurofibromatosis</i>

**BBVA FOUNDATION /
FUNDACIÓN BBVA**

LEONARDO GRANTS	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmeda, David	<i>Nuevas estrategias para el tratamiento preventivo de la enfermedad metastásica en Melanoma (Ref.: IN18_BBM_TRA_0293)</i>
Squatrito, Massimo	Leonardo Grant: Precision glioma mouse models by somatic genome editing with the RCAS-CRISPR-Cas9 system (Ref.: IN17_BBM_TRA_0366)

**RAMÓN ARECES
FOUNDATION / 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

FUNDACIÓN PFIZER


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	Tumour exosome integrins determine organotropic metastasis
Djouder, Nabil; Teijeiro, Ana I.	Metabolic Inflammation-Associated IL-17A Causes Non-alcoholic Steatohepatitis and Hepatocellular Carcinoma

**"L'ORÉAL-UNESCO FOR
WOMEN IN SCIENCE"
PROGRAMME**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Ana	<i>Estudio de la implicación de ruta de señalización de mTORC1 en la patología del Linfoma Folicular y autoinmunidad</i>

**SPANISH ASSOCIATION OF
GASTROENTEROLOGY /
ASOCIACIÓN ESPAÑOLA DE
GASTROENTEROLOGÍA**


TAMARITE - AESPANC - ACANPAN GRANT	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Molina, Esther	<i>Radiomics en cáncer de páncreas para una medicina estratificada y de precisión: un estudio piloto.</i>

EDUCATION AND TRAINING PROGRAMMES

One of the principal goals of the CNIO is to increase its training capacity in order to give students and professionals the opportunity to advance their careers in the healthcare sector. During 2019, the CNIO signed several new agreements with Spanish Universities and other institutions, namely with the *Universidad de Alcalá de Henares, Universidad San Pablo CEU, Universidad de Barcelona, Universidad Operta de Cataluña,*

Universidad Castilla La Mancha, Universidad de Oviedo, IES Félix Rodríguez de la Fuente, IES Villaverde, IES Príncipe Felipe, Rozas Educación SL, Centro Profesional Europeo de Madrid, Escuela Técnica de Enseñanzas Especializadas, Colegio Virgen de Mirasierra and Ministerio de Ciencia, Innovación y Universidades.

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2015	2016	2017	2018	2019
Training of PhD students	105	110	112	109	100
Post-doctoral training	48	51	44	50	49
Training for MDs	25	17	21	12	20
Laboratory training for MSc/BSc students	80	95	99	128	150
Laboratory training for technicians	27	26	20	13	15

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 2019, 6 students from 3 different countries participated in this programme.
- Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO individual Research Groups or Units. This year, 150 students participated in these programmes, of whom 7 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 2019 and 21 others joined the CNIO in the same year. Over 15% of the 100 students working at the CNIO in 2019 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

Since 2008, the *Fundación “la Caixa”* 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. In 2018, a new call for the doctoral fellowship programme of the “la Caixa” Foundation, named INPhINIT, was launched to recruit talented Early-Stage Researchers of any nationality, who wish to pursue doctoral studies in Spanish or Portuguese territory, offering them an attractive and competitive environment for conducting research of excellence. The CNIO was chosen as a host institution. During 2019, 2 pre-doctoral students received this fellowship to join the CNIO.

The distribution of students across the CNIO's Research Programmes in 2019 was as follows: 67 % of students worked in the Molecular Oncology Programme, 13% in the Structural Biology Programme, 11% in the Human Cancer Genetics Programme, 1% in the Experimental Therapeutics Programme, 3% in the Biotechnology Programme, and 5% in the Clinical Research Programme.

Thanks to an individual donation received through the 'CNIO Friends' platform, CNIO created the Predoctoral Carmen Gloria Bonnet Moreno Contract Programme that offered 1 position to carry out a thesis at the CNIO. This call is expected to have only this one single edition.



FUNDING OF PHD TRAINING	NO.
SPANISH ORGANISATIONS	83
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities/ <i>Ministerio de Ciencia, Innovación y Universidades (Predoctoral fellowships)</i>	48
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities/ <i>Ministerio de Ciencia, Innovación y Universidades (I+D Projects)</i>	6
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC (I+D Projects)</i>	4
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i>	2
Cris Foundation / <i>Fundación Cris</i>	2
Community of Madrid / <i>Comunidad de Madrid</i>	3
CNIO	4
Banco Santander Foundation / <i>Fundación Banco Santander</i>	2
"la Caixa" Banking Foundation/ <i>Fundación Bancaria "la Caixa" (I+D Projects)</i>	1
"la Caixa" Banking Foundation/ <i>Fundación Bancaria "la Caixa" (Predoctoral fellowships)</i>	11
INTERNATIONAL ORGANISATIONS	17
BiovelocITA	1
China Scholarship Council (CSC)	2
<i>Consejo Nacional de Ciencia y Tecnología</i> (Mexico)	2
European Society for Clinical Nutrition and Metabolism (ESPEN)	1
European Commission Framework Programme / H2020	1
European Research Council	2
GENCODE	2
Human Frontier Science Program Foundation	1
Lilly Foundation / <i>Fundación Lilly</i>	1
Marie Skłodowska-Curie actions of the European Commission	3
Portuguese Foundation for Science and Technology (FCT)	1
TOTAL	100

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 2019, 49 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 2019, the *Fundación Banco Santander* renewed the agreement with the CNIO to continue the 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 call will be closed in 2020.

Thanks to the donations received through the 'CNIO Friends' platform launched in 2016, the fourth call of the 'CNIO Friends' Postdoctoral Contract Programme, launched in 2019, resulted in the recruitment of 4 scientists for a 2-year period each. Also, thanks to a single donation to 'CNIO Friends', CNIO launched the Postdoctoral 'Eva Plaza/CNIO Friends' Programme that offered a postdoctoral researcher the opportunity to carry out a 2-year postdoctoral stay at the CNIO, to accomplish a research project on triple negative breast cancer. This call is expected to have only this single edition. Additionally, thanks to a 'Juegaterapia-CNIO Friends' Postdoctoral Contract, in 2019, 1 scientist was able to start a project related to paediatric oncology.

FUNDING SOURCES OF POST-DOCTORAL RESEARCHERS	NO.
SPANISH ORGANISATIONS	39
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities / <i>Ministerio de Ciencia, Innovación y Universidades (Postdoctoral fellowships)</i>	6
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities / <i>Ministerio de Ciencia, Innovación y Universidades (I+D Projects)</i>	3
Spanish Society of Haematology and Haemotherapy	1
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC (Fellowships)</i>	5
Community of Madrid / <i>Comunidad de Madrid</i>	4
CNIO	17
Banco Santander Foundation / <i>Fundación Banco Santander</i>	2
"la Caixa" Banking Foundation/ <i>Fundación Bancaria "la Caixa" (Postdoctoral Fellowships)</i>	1
INTERNATIONAL ORGANISATIONS	10
<i>Daiichi Sankyo</i>	1
European Commission Framework Programme / H2020	3
European Research Council	1
European Society for Medical Oncology / <i>Sociedad Europea de Oncología Médica</i>	1
Lilly Foundation / <i>Fundación Lilly</i>	1
Marie Skłodowska-Curie actions of the European Commission	1
Worldwide Cancer Research UK	2
TOTAL	49

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 for students. Of the 15 students who participated in this programme in 2019, 2 were 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 2019, 20 medical residents from 10 different hospitals enjoyed the benefits of rotations within the different Groups and Units at the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2019, the *Ramón y Cajal* Programme supported 7 scientists. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (currently the State Research Agency of the Spanish Ministry of Science, Innovation and Universities) aims to encourage Spanish or foreign scientists working abroad to return to or relocate to Spain. Successful candidates are selected on the basis of their potential capacity to lead independent projects and groups,

or to contribute successfully to the ongoing research in the existing groups. Seven other scientists were funded by similar programmes, including the *Juan de la Cierva* programme (Spanish Ministry of Science, Innovation and Universities, 3 contracts); *Miguel Servet* programme (1 contract) of the Institute of Health *Carlos III*; and the Spanish Association Against Cancer (*AECC*, 3 contracts).

VISITING RESEARCHER 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 2019, 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 Researcher 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.

During 2019, thanks to this Programme, we had the pleasure of hosting Hayet Rafa from the University of Science and Technology Houari Boumediene, Algiers, for a 6-month stay



as a visiting scientist in the CNIO's Melanoma Group.

SCIENTIFIC EVENTS

CNIO-"LA CAIXA" BANKING FOUNDATION FRONTIERS MEETINGS

The CNIO-"la Caixa" Foundation Frontiers Meetings (CFMs) are the main international conferences co-organised by the CNIO and "la Caixa" Foundation. They focus on specific, cutting-edge aspects of cancer research, thus providing a unique platform for an intensive and dynamic exchange and debate on scientific ideas. The invited speakers – 20 internationally renowned leaders in oncology – present their latest findings during 2 and a half days. The provided learning environment encourages delegates to: exchange experiences, ideas and practices upheld at their companies; network and create connections with researchers with similar interests; listen to and meet the keynote speakers; enjoy the extra-curricular conference programme; and hear about the latest developments in the research field. Up to 100 additional

participants are selected – via a widely publicised call for applications – based on their potential to make relevant contributions to the conference by presenting hot topics as posters or short talks.

In 2019 we arranged 2 CFMs: '*Structural and Molecular Biology of the DNA Damage Response*', during which the potential use of novel approaches was analysed for studying DNA damage and naturally occurring DNA-repair failures – a cross-cutting theme in cancer research – and '*Heterogeneity and Evolution in Cancer*'. These brought together more than 130 cancer experts and computational, physical and mathematical biologists from the world's most active groups in the area.

STRUCTURAL AND MOLECULAR BIOLOGY OF THE DNA DAMAGE RESPONSE 20-22 MAY 2019

ORGANISERS

- **Óscar Llorca**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Rafael Fernández Leiro**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Laurence H. Pearl**, University of Sussex, Brighton, UK
- **Titia Sixma**, Netherlands Cancer Institute, Amsterdam, Netherlands

SESSIONS

- **Chromatin and chromatin complexes**
- **DNA Replication and replication stress**
- **DNA transcription**
- **DNA damage and repair**

SPEAKERS

- **James Berger**, Johns Hopkins School of Medicine, Baltimore, US
- **Maria Blasco**, Spanish National Cancer Research Centre, Madrid, Spain
- **Alessandro Costa**, The Francis Crick Institute, London, UK

Madrid 20 – 22 May 2019
Abstract Submission deadline 22 April
Application deadline 6 May

Structural and molecular biology of the DNA damage response

Organisers

- Oscar Llorca**
Macromolecular complexes in DNA damage Response (CNIO), Madrid, Spain
- Rafael Fernández Leiro**
Genome Integrity and Structural Biology (CNIO), Madrid, Spain
- Laurence H. Pearl**
Genome Damage and Stability Centre, University of Sussex, UK
- Titia Sixma**
Netherlands Cancer Institute, NCI, Netherlands

Confirmed Speakers

- James Berger**
Johns Hopkins School of Medicine, USA
- Alessandro Costa**
The Francis Crick Institute, UK
- Patrick Cramer**
Max Planck Institute for Biophysical Chemistry, Germany
- Aidan Doherty**
Genome Damage and Stability Centre, University of Sussex, UK
- Daniel Durocher**
The Lunenburg Foundation Research Institute, Canada
- Rafael Fernández Leiro**
Genome Integrity and Structural Biology (CNIO), Spain
- Karl-Peter Hopfner**
Giles Center Munich, Germany
- Stephen Jackson**
Wellcome Trust, Cancer Research UK, Cancer Institute, UK
- Oscar Llorca**
Macromolecular complexes in DNA damage Response, CNIO, Spain
- Juan Mändez**
DNA Replication Group, CNIO, Spain
- Eva Nogales**
Lawrence Berkeley National Laboratory, Ernest Rutherford Medical Institute, USA
- Lori Passmore**
Laboratory of Molecular Biology, MRC LMB, UK
- Laurence H. Pearl**
Genome Damage and Stability Centre, University of Sussex, UK
- Luca Pellegrini**
University of Cambridge, UK
- Titia Sixma**
Netherlands Cancer Institute, NCI, Netherlands
- Song Tan**
Purdue University, USA
- Nicolas Thomé**
Friedrich Miescher Institute, Switzerland
- Alessandro Vannini**
Institute of Cancer Research, ICR, UK
- Roger Williams**
Laboratory of Molecular Biology, MRC LMB, UK
- Wei Yang**
National Institute of Health, NIH, USA
- Xiaodong Zhang**
Imperial College London, UK

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- **Patrick Cramer**, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
- **Aidan Doherty**, Genome Damage and Stability Centre, Univ. of Sussex, Brighton UK
- **Daniel Durocher**, The Lunenfeld-Tanenbaum Research Institute, Toronto, Canada
- **Karl-Peter Hopfner**, Gene Center Munich, Munich, Germany
- **Meindert Lamers**, Leiden University, The Netherlands
- **Óscar Llorca**, Spanish National Cancer Research Centre, Madrid, Spain
- **Juan Méndez**, Spanish National Cancer Research Centre, Madrid, Spain
- **Eva Nogales**, HHMI/University of California at Berkeley, US
- **Lori Passmore**, MRC Laboratory of Molecular Biology, Cambridge, UK
- **Laurence H. Pearl**, Genome Damage and Stability Centre-University of Sussex, Brighton, UK
- **Luca Pellegrini**, Cambridge University, Cambridge, UK
- **Song Tan**, Penn State University, Pennsylvania, US
- **Nicolas Thomä**, Friedrich Miescher Institute, Basel, Switzerland
- **Alessandro Vannini**, Institute of Cancer Research, ICR, London, UK
- **Roger Williams**, MRC Laboratory of Molecular Biology, Cambridge, UK
- **Wei Yang**, National Institutes of Health, NIH, Bethesda, US
- **Xiaodong Zhang**, Imperial College London, UK

In addition, 9 short talks were selected among participants' contributions and 42 posters were presented.

HETEROGENEITY AND EVOLUTION IN CANCER 23-25 SEPTEMBER 2019

ORGANISERS

- **Fátima Al-Shahrou**, Spanish National Cancer Research Centre, CNIO, Madrid, Spain
- **Arnold Levine**, The Simons Center for Systems Biology, Institute for Advanced Study, Princeton, US
- **Solip Park**, Spanish National Cancer Research Centre, CNIO, Madrid, Spain
- **Raúl Rabadán**, Columbia Systems Biology, Columbia University, New York, US

SPEAKERS

- **Alexander R.A. Anderson**, Moffitt Cancer Center and Research Institute, Tampa, US
- **Niko Beerenwinkel**, ETH Zürich, Switzerland
- **Ivana Bozic**, University of Washington, US
- **Curtis G. Callan**, Princeton University, US
- **Neal G. Copeland**, University of Texas MD Anderson Cancer Center, Houston, US
- **Christina Curtis**, Stanford University, School of Medicine, Stanford, US
- **Adolfo Ferrando**, Institute for Cancer Genetics, Columbia University Medical Center, New York, US
- **Trevor Graham**, Barts Cancer Institute, London, UK
- **Benjamin D. Greenbaum**, Icahn School of Medicine at Mount Sinai, New York, US
- **Holger Heyn**, National Centre for Genomic Analysis, Barcelona, Spain
- **Nancy Jenkins**, University of Texas MD Anderson Cancer Center, Houston, US



- **Tal Korem**, Columbia University Irving Medical Center, New York, US
- **Christina Leslie**, Memorial Sloan Kettering Cancer Center, New York, US
- **Arnold Levine**, The Simons Center for Systems Biology, Institute for Advanced Study, Princeton, US
- **Nuria Lopez-Bigas**, Institute for Research in Biomedicine, Barcelona, Spain
- **Scott W. Lowe**, Memorial Sloan Kettering Cancer Center, New York, US
- **Guillermina Lozano**, University of Texas Anderson Cancer Centre, Houston, US
- **Marta Łuksza**, Icahn School of Medicine, Mount Sinai, New York, US
- **David Posada**, School of Biology, University of Vigo, Spain
- **Carol Prives**, Columbia University, New York, US
- **Benjamin J. Raphael**, Lewis-Sigler Institute for Integrative Genomics, Princeton University, US
- **Darryl Shibata**, Keck School of Medicine of USC, Los Angeles, US
- **Andrea Sottoriva**, The Institute of Cancer Research, London, UK
- **Doug Winton**, Cancer Research UK Cambridge Institute, Cambridge, UK

In addition, 11 short talks were selected among participants' contributions and 22 posters were presented.

OTHER MEETINGS & CONFERENCES

The CNIO annually hosts various international meetings and conferences.

IV TRANSLATIONAL MEETING

17 JANUARY 2019

ORGANISERS:

- **Maria González Cao**, Quiron Dexeus University Hospital, Barcelona, Spain
- **Susanna Puig**, Clinic Hospital, Barcelona, Spain
- **Marisol Soengas**, Spanish National Cancer Research Centre, Madrid, Spain

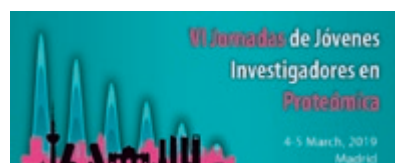


VI JORNADAS DE JÓVENES INVESTIGADORES EN PROTEÓMICA

4-5 MARCH 2019

ORGANISERS:

- **Centro Nacional de Investigaciones Oncológicas, CNIO** Proteomics Unit
- **SEProt**, Sociedad Española de Proteómica



METABOCANCER I CLOSING MEETING 27-28 MAY 2019

NETWORK MEMBERS:

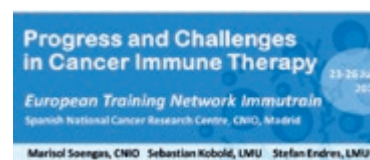
- Alejo Efeyan, CNIO
- Anna Bigas, IMIM
- Arkaitz Carracedo, bioGUNE
- Guadalupe Sabio, CNIC
- José Cuezva, CBMSO
- Marc Claret, IDIBAPS
- María Mittelbrunn, CBMSO
- Mariona Graupera, IDIBELL
- Ruben Nogueiras, CIMUS
- Xosé Bustelo, CIC-USAL



PROGRESS AND CHALLENGES IN CANCER IMMUNE THERAPY EUROPEAN TRAINING NETWORK IMMUTRAIN 23-26 JULY 2019

ORGANISERS:

- María Soengas, CNIO, Madrid, Spain
- Sebastian Kobold, LMU, Munich, Germany
- Stefan Endres, LMU, Munich, Germany



ADVANCES IN CANCER RESEARCH

JOINT MICC - SPANISH NATIONAL CANCER RESEARCH CENTRE (CNIO) SYMPOSIUM
4-5 SEPTEMBER 2019

ORGANISERS:

- Weizmann Institute of Sciences - Moross Integrated Cancer Center (MICC)
- Spanish National Cancer Research Centre, CNIO



CNIO-CNIC JOINT MEETING 19 SEPTEMBER 2019

ORGANISER:

- Spanish National Cancer Research Centre, (CNIO)



FIRST GENDER EQUALITY EVENT OF THE SOMMA RESEARCH CENTRES OF EXCELLENCE

29 OCTOBER 2019

ORGANISERS:

- **British Embassy in Madrid**
- **Spanish National Cancer Research Centre, CNIO**

Whilst most research centres are committed to gender equality, activities are still mostly taken forward by individuals. In addition, the absence of dedicated resources, the lack of the required critical mass that leads to transformation or stability over time, and the absence of a coordinated strategy with other centres, prevent the gender agenda from being embedded into the institution's spirit.

This event aims at sharing best practices in the SOMM Alliance ('Severo Ochoa' Centres and María de Maeztu Units, or SOMMa) to learn and promote a change of culture, and support female talent and gender equality in science and strategic decision-making.

The gender expert Cheryl Smythe, from the Babraham Institute in Cambridge, UK, presented her work on implementing the gender equality measurements at the Babraham, as a result of which the centre received the Athena-SWAN award. Also, presentations from SOMMa Directors included examples of success stories on gender equality, scientific excellence and leadership carried out at their institutions.



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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.

ADVANCE CELL SORTING WORKSHOP 7-8 FEBRUARY 2019

ORGANISER:

- Flow Cytometry Unit, CNIO, Madrid, Spain

SPEAKERS:

- Rui Gardner, Head of the Flow Cytometry Core Facility. Memorial Sloan Kettering Cancer Center, NY, USA
- Lola Martinez, Head of the Flow Cytometry Unit. CNIO, Madrid, Spain

CCP-EM

MODEL BUILDING WORKSHOP CNIO

MODEL BUILDING AND REFINEMENT USING CRYO-EM MAPS: CCPEM, REFMAC, PROSHADE, LOCSCALE, COOT, SCIPION, ISOLDE
14-15 MARCH 2019

This course is aimed at students and postdocs currently in the process of building and refining atomic models into cryo-EM maps. The course covered the theory and principles behind the software and procedures through a series of seminars and

thorough tutorial sessions on the tools widely used by the community. During the workshop there was time to interact with all the speakers and instructors, and participants were welcome to bring their own data to solve specific issues.

ONCONET-SUDOE

WORKSHOP ON INNOVATIVE IT FOR HEALTHCARE

"THE PATIENT JOURNEY: INFORMATION TECHNOLOGIES FOCUSED ON THE CANCER PATIENT"

3-4 APRIL 2019

TOPICS:

- Technical standards for information regarding cancer care throughout the disease course.
- Ensuring patient confidentiality and privacy.
- Patient virtual communities and social media: value in connecting with each other and in mining the online environment for information to help them cope with their disease.
- *In silico* drug prescription and clinical trials design.
- Analysing potential side effects of cancer therapies.
- Improving continuity of care across disease stages.
- Electronic Health Records (EHRs).
- Development of new programmes for digital training of clinical and medical staff.
- Challenges: data interoperability, systems interoperability, usability, adaptation to physicians' workflows.
- Overcoming resistance to digital change in patients and



clinical personnel.

- Financial incentives for digital transformation.
- Transversal topics: Ethics, GDPR, patient rights, access to sensitive clinico-genomics data.

SOFTWARE CARPENTRY WORKSHOP - R FOR REPRODUCIBLE SCIENTIFIC ANALYSIS

6-7 JUNE 2019

TOPICS:

- An introduction to scripting in R and using the language for scientific applications and data handling.
- Effective use of the Unix command line.
- Version control with git and GitHub.

WORKSHOP: ADVANCES IN THE R2TP/URI-PREFOLDIN COMPLEX IN CANCER

3 OCTOBER 2019

ORGANISERS:

- **Nabil Djouder**, Growth Factors, Nutrients and Cancer Group, Molecular Oncology Programme, CNIO
- **Oscar Llorca**, Macromolecular Complexes in DNA Damage Response Group, Structural Biology Programme, CNIO

CNIO WORKSHOP ON PHILOSOPHY & BIOMEDICAL SCIENCES: "DEBATES ON CONCEPTUAL AND SOCIAL ISSUES"

19 NOVEMBER 2019

ORGANISERS:

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

WITH THE SUPPORT OF:

- **Sabadell Foundation**

SPEAKERS:

- **Maria A. Blasco**, Spanish National Cancer Research Centre, Spain
- **Maria Cerezo**, University of Murcia, Spain
- **Iñigo de Miguel Berain**, University of the Basque Country, Spain
- **Antonio Diéguez**, University of Málaga, Spain
- **Arantza Etxeberria**, University of the Basque Country, Spain
- **Michael Hauskeller**, University of Liverpool, UK
- **Lluís Montoliu**, National Centre for Biotechnology, Spain
- **Alfonso Valencia**, Spanish National Bioinformatics Institute (INB-ISCIII), Spain
- **Henrik Vogt**, The University of Oslo, Norway



CNIO DISTINGUISHED SEMINARS

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

In total, the CNIO hosted 15 distinguished speakers in 2019.



2018—19

Sep—Dec 2018	Jan—Dec 2019	
<p><u>Friday 21 Sep</u> Karim Labib Sir James Black Centre, School of Life Sciences, University of Dundee, Dundee, Scotland</p> <p><u>Friday 5 Oct</u> Rafael Yuste  The NeuroTechnology Center at Columbia University Biological Sciences, US</p> <p><u>Friday 23 Nov</u> Nicolas Winssinger Organic Chemistry Department, University of Geneva, Switzerland</p> <p><u>Friday 30 Nov</u> Caetano Reis e Sousa The Francis Crick Institute, London, UK</p> <p><u>Friday 21 Dec</u> Jonathan Kipnis  Center for Brain Immunology and Glia (CBIG) University of Virginia, US</p>	<p><u>Friday 18 Jan</u> Jeremy N. Rich Department of Biomedicine, Division of Regenerative Medicine, Director of Neuro-Oncology & Brain Tumor Institute, University of California, San Diego, US</p> <p><u>Friday 25 Jan</u> Jan H. J. Hoeijmakers Erasmus MC, Rotterdam, Netherlands</p> <p><u>Friday 1 Feb</u> Maite Huarte Icahn School of Medicine at Mount Sinai, University of Navarra, Pamplona, Spain</p> <p><u>Friday 8 Feb</u> Rebecca Fitzgerald University of Cambridge (FitzBio), MRC Research Centre, UK</p> <p><u>Friday 15 Feb</u> Didier Stainier Max Planck Institute, Bad Nauheim, Germany</p> <p><u>Friday 22 Feb</u> Riccardo Dalla Favera Institute for Cancer Genetics, Columbia University, NY, US</p> <p><u>Friday 8 Mar</u> W. Kimryn Rathmell Vanderbilt University Medical Center, Tennessee, US</p>	<p><u>Friday 29 Mar</u> Magdalena Götz German Research Center for Environmental Health, Helmholtz Zentrum München, Germany</p> <p><u>Friday 5 Apr</u> Kathrin Plath UCLA School of Medicine, Los Angeles, US</p> <p><u>Friday 12 Apr</u> Katherine L. Nathanson Perelman School of Medicine, University of Pennsylvania, Philadelphia, US</p> <p><u>Friday 26 Apr</u> Charles M. Perou UNC School of Medicine, Chapel Hill, US</p> <p><u>Friday 17 May</u> Johanna Joyce University of Lausanne, Switzerland</p> <p><u>Friday 24 May</u> Susan Taylor University of California, San Diego, US</p> <p><u>Friday 28 Jun</u> David Sancho Spanish National Center for Cardiovascular Research, Madrid, Spain</p> <p><u>Friday 27 Sep</u> Himisha Beltrán Dana-Farber Cancer Institute, Boston, US</p>

Out of the Box Seminars supported by           

www.cnio.es/events/seminars

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
18/01/2019	Jeremy N. Rich	Brain Tumor Institute University of California, San Diego, US	Brain Tumor Stem Cells
25/01/2019	Jan H. J. Hoeijmakers	Erasmus MC, Rotterdam, Netherlands	DNA damage induced transcriptional stress in aging and the protective effect of nutritional interventions
FEBRUARY			
01/02/2019	Maite Huarte	Center for Applied Medical Research (CIMA). University of Navarra, Pamplona, Spain	Chromatin-dependent and independent functions of lncRNAs in cancer
08/02/2019	Rebecca Fitzgerald	University of Cambridge Hutchison/MRC Research Centre, UK	Applying molecular characterisation of oesophageal cancer and its precursor Barrett's to clinical practice
15/02/2019	Didier Stainier	Max Planck Institute, Bad Nauheim, Germany	Genetic compensation and transcriptional adaptation
22/02/2019	Riccardo Dalla Favera	Institute for Cancer Genetics Columbia University, New York, US	From genomics to targeted therapy in Diffuse Large B cell Lymphoma
MARCH			
08/03/2019	W. Kimryn Rathmell	Vanderbilt University Medical Center, Nashville, US	Linking epigenetics and immune response in renal cell carcinoma
29/03/2019	Magdalena Götz	German Research Center for Environmental Health, Neuherberg, Germany	From mechanisms of neurogenesis towards neuronal repair
APRIL			
05/04/2019	Kathrin Plath	UCLA School of Medicine, Los Angeles, US	A new framework for the function of the lncRNA Xist in X-inactivation
12/04/2019	Katherine L Nathanson	Perelman School of Medicine, University of Pennsylvania, Philadelphia, US	From Soup to Nuts: Inherited Genetics and Testicular Germ Cell Tumors
26/04/2019	Charles M. Perou	UNC school of Medicine, Chapel Hill, US	Quantitative Medicine for Breast Cancer Patients
MAY			
17/05/2019	Johanna Joyce	University of Lausanne, Switzerland	Exploring and Therapeutically Exploiting the Tumor Microenvironment
JUNE			
28/06/2019	David Sancho	Spanish National Center for Cardiovascular Research, Madrid, Spain	Dendritic cells in immunity and inflammation
SEPTEMBER			
26/09/2019	Himisha Beltrán	Dana Farber Cancer Institute, Boston, US	Understanding treatment resistance and the neuroendocrine phenotype in prostate cancer
OCTOBER			
25/10/2019	Susan S. Taylor	University of California, San Diego, US	PKA: from Molecules to Cells

AD-HOC SEMINARS

In addition to the CNIO Distinguished Seminar Series, the CNIO also hosts numerous *ad-hoc* seminars throughout the year. *Ad hoc* seminars are organised for the purpose of academic interactions, academic elevation and enrichment, as well as academic *vis-a-vis* social networking; in addition, they facilitate socialising with colleagues from other institutions. A total of 36 *ad-hoc* seminars were organised by CNIO researchers in 2019.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
14/01/2019	Ramón Alemany	Catalan Institute of Oncology – IDIBELL, Barcelona, Spain	Cancer viro-immunotherapy with oncolytic adenoviruses
FEBRUARY			
05/02/2019	José Javier Fuster Ortuño	Hematovascular Pathophysiology, CNIC, Madrid, Spain	Somatic mutations and clonal hematopoiesis in cardiovascular disease: commonalities with cancer
21/02/2019	Sjors Scheres	MRC laboratory of Molecular Biology, Cambridge, UK	Cryo-EM structures of Tau Filaments from Alzheimer's and Pick's Disease
21/02/2019	Maria Stella Carro	Universitäts Klinikum Freiburg, Germany	ZBTB18 function and regulation in glioblastoma
MARCH			
04/03/2019	Katherine Hoadley	The University of North Carolina at Chapel Hill, US	Integrative molecular analysis of testicular germ cell tumors
11/03/2019	Gabriel Piedrafita	Wellcome Trust Sanger Institute, Hinxton, UK	Cell behavior and clonal competition dynamics in normal murine squamous epithelium
14/03/2019	Luuk Hawinkels	Leiden University Medical Center, Leiden, The Netherlands	Targeting (endoglin in) the tumor stroma in colorectal cancer
18/03/2019	Luis Santin	Structural and Molecular Biology (ISMB), UCL/Birkbeck, London, UK	The complete atomic structure of the essential Tra1 protein by single-particle cryo-EM
20/3/2019	Remi Samain	Cancer Research Center of Toulouse, France	Protein synthesis inhibition in fibroblasts alters macrophage polarization and blocks pancreatic cancer metastasis
22/03/2019	Marta M. Alonso Roldón	Center for Applied Medical Research - CIMA, Pamplona, Spain	Oncolytic Virotherapy for DIPG: from the bench to the clinic
25/03/2019	Silvia Hernández-Ainsa	ARAID, INA-ICMA, University of Zaragoza, CSIC, Spain	Using DNA to build custom-designed nanostructures for biomedical research
APRIL			
11/04/2019	Borja Ibarra	IMDEA Nanociencia, Madrid, Spain	Optical tweezers to study life under tension, one molecule at a time

MAY			
08/05/2019	Alvaro Cortes	Galchimia S.A. for GlaxoSmithKline (GSK), Madrid, Spain	Computational Drug Discovery: from Structure-Based Design to Artificial Intelligence and Beyond
10/05/2019	Gertrudis Perea	Cajal Institute, CSIC, Madrid, Spain	Understanding the role of astrocytes in brain function
14/05/2019	Judith Agudo	Dana-Farber Cancer Institute, Boston, US	Deciphering immune evasion at single cell level
16/05/2019	Kyuson Yun	Houston Methodist Research Institute. Weill Cornell Medical College, Houston, USA	Deciphering immune suppression mechanisms of GBM at the single cell level
30/05/2019	German Rivas	CIB-CSIC, Madrid, Spain	Life in a crowded-phase-separated world: biochemical and physiological consequences
JUNE			
14/06/2019	Massimiliano Mazzone	Institute for Cancer Research and Treatment of Torino, Italy	Podoplanin-expressing macrophages: a "PoEM" on breast cancer metastasis
JULY			
01/07/2019	Konstantina Topouridou	Scientific Officer at the ERC Executive Agency	The European Research Council - All you need to know before applying
02/07/2019	Jennifer Kefauver	The Scripps Research Institute, La Jolla, US	Structure of the human Volume-Regulated Anion Channel
22/07/2019	Kazuhiro Maeshima	National Institute of Genetics, Mishima-shi, Japan	Chromosome organization in living human cells revealed by single nucleosome imaging
26/07/2019	Florencia McAllister	MD Anderson Center, Houston, US	The pancreatic tumor microenvironment and the influence of the gut-tumor bacterial axis
29/07/2019	Antonio Pérez	La Paz Hospital, Madrid, Spain	Optimizing CAR-T cells to kill pediatric cancer
SEPTEMBER			
18/09/2019	Elli Kapyla	Aspect Biosystems Ltd, Vancouver, Canada	Microfluidic 3D bioprinting: Bringing creative control to tissue engineering
26/09/2019	Ana Sastre Perona	NYC Langone Health New York, USA	Transcriptional networks governing self-renewal and differentiation in SCCs
30/09/2019	Leila Akkari	Netherlands Cancer Institute, Amsterdam, Netherlands	Immunomodulation in glioblastoma treatment: Macrophages at play
OCTOBER			
24/10/2019	Hind Medyouf	Georg-Speyer-Haus & Frankfurt Cancer Institute Frankfurt am Main, Germany	Exploring the many facets of TAM receptors (Tyro3, Axl and Mertk) biology in cancer
29/10/2019	Khalil Rawji	University of Cambridge, UK	Factors that impair tissue regeneration in the ageing Central Nervous System

NOVEMBER			
12/11/2019	Alicia González	Autonoma University of Madrid (UAM) Institute for Biomedical Research Alberto Sols (CSIC-UAM), Madrid, Spain	MicroRNA control of immune tolerance, autoimmunity and cancer
14/11/2019	Stefano Stella	Novo Nordisk Foundation Center for Protein Research, Copenhagen, Denmark	CRISPR-Cas12a genome editing tool and biosensor. Can we do more?
21/11/2019	Ana Toste Rego	LMB - Laboratory of Molecular Biology, Cambridge, UK	Cryo-EM of Fully Recombinant Human Proteasomes – A New Tool for Functional and Structural Studies
21/11/2019	Direna Alonso	Memorial Sloan Kettering Cancer Center, New York, USA	The epigenetic control of epithelial – immune crosstalk during pancreatic tumorigenesis
DECEMBER			
02/12/2019	Maria Casanova-Acebes	Mount Sinai School of Medicine, New York, US	Harnessing innate immunity in homeostasis and cancer
17/12/2019	María Guillamot	New York University Medical Center, US	Inflammation, stress hematopoiesis, and the induction and maintenance of leukemia

WOMEN IN SCIENCE SEMINARS

DATE	SPEAKER	ORGANISATION	TITLE
15/01/2019	Consuelo Madrigal	Prosecutor of the Supreme Court Chamber	La mujer profesional: el largo camino a la igualdad
12/02/2019	Rosa Montero	Journalist and writer	Women's Word
05/03/2019	Luz Casal	Musician, singer, author and songwriter	Aproximaciones a una biografía
09/04/2019	Ruth Vera García	Spanish Society of Medical Oncology (SEOM), Chairman, Spain	Mujeres en la Oncología
07/05/2019	María Luisa de Contes	Secretaria General y Consejera de las filiales del Grupo Renault en España, Directora de RSC y de la Fundación Renault para la Inclusión y la Movilidad Sostenible	Un proyecto para la igualdad de Género en las Empresas
20/06/2019	María Hervás	Actress, Madrid, Spain	La alquimia de un cuerpo que actúa
15/10/2019	Susana Malcorra	Former Foreign Minister of Argentina and Chef de Cabinet to the Secretary-General of the United Nations (with Ban Ki-moon)	Leadership in times of change
29/10/2019	Cheryl Smythe	Babraham Institute, Cambridge, UK	Creating an inclusive environment (what, why and how)
26/11/2019	María Pilar Allué Blasco	Deputy Director General of Human Resources and Training of the National Police Corps, Madrid, Spain	40 años de la incorporación de la mujer a la Policía Nacional
03/12/2019	María José San Román	Chef and restaurateur awarded with 1 Michelin star, Restaurant Monastrell, Alicante, Spain	Mujeres en Gastronomía

SCIENCE DISSEMINATION EVENTS

WORLD CANCER RESEARCH DAY: 'NUEVOS HORIZONTES EN LA INVESTIGACIÓN DEL CÁNCER: DEL LABORATORIO AL PACIENTE'
24 SEPTEMBER 2019

ORGANISER:

- **Centro Nacional de Investigaciones Oncológicas (CNIO)**

WITH THE SUPPORT OF:

- **"la Caixa" Foundation**



RESEARCHERS' NIGHT
27 SEPTEMBER 2019

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

Madrid joined 370 other European cities to participate in this initiative that seeks to foster new scientific vocations and demonstrate the reality of researchers, which is very unlike their stereotypical image. The activities were entirely organised and held thanks to the voluntary efforts of 40 researchers. The guests were provided with the opportunity to meet researchers in an interactive and entertaining way, including welcome talks and short talks, hands-on experiments, view of a virtual tour through the facilities via a video project recorded by scientists from CNIO 'CNIO for Kids', and a speed dating session with the researchers. 200 attendees had the opportunity to visit us.



OPEN DOORS DAY: INVESTIGATING TO DISARM CANCER

4-17 NOVEMBER 2019

The CNIO also dedicates considerable efforts 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 (XIX *Semana de la Ciencia y de la Innovación*, 4-17 November 2019). In 2019, 53 people participated in the guided visit.



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 2019, more than 907

people participated in such guided visits; most of them were ESO and *Bachillerato* student groups, but also professionals in the health sector.

STEM TALENT GIRL - MADRID

ORGANISERS:

- ASTI Foundation
- CNIO

The objective of the Stem Talent Girl project is to inspire, educate and empower the next generation of leading women in science and technology.

The Stem Talent Girl project in Madrid offered eight ‘masterclasses’ during the academic year; the classes were given by women of international prestige in the STEM areas and took place between November 2018 and June 2019.



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* In accordance with the Spanish Transparency Legislation (Spanish Royal Decree 451/2012, of March 5), the following information is hereby provided:

- The amount received by the Top Management of the Foundation – the CNIO's Director plus the Managing Director – at the end of the financial year amounted to 359,124 euros in 2019 (272,471 euros in 2018). To this amount, the variable remuneration for directors amounting to 135,954 euros accrued during 2016, 2017 and 2018, must be added. Like every year, there is a provisional amount of 55,605 euros for the variable accrued in 2019.
- Members of the CNIO Board of Trustees are not remunerated.

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

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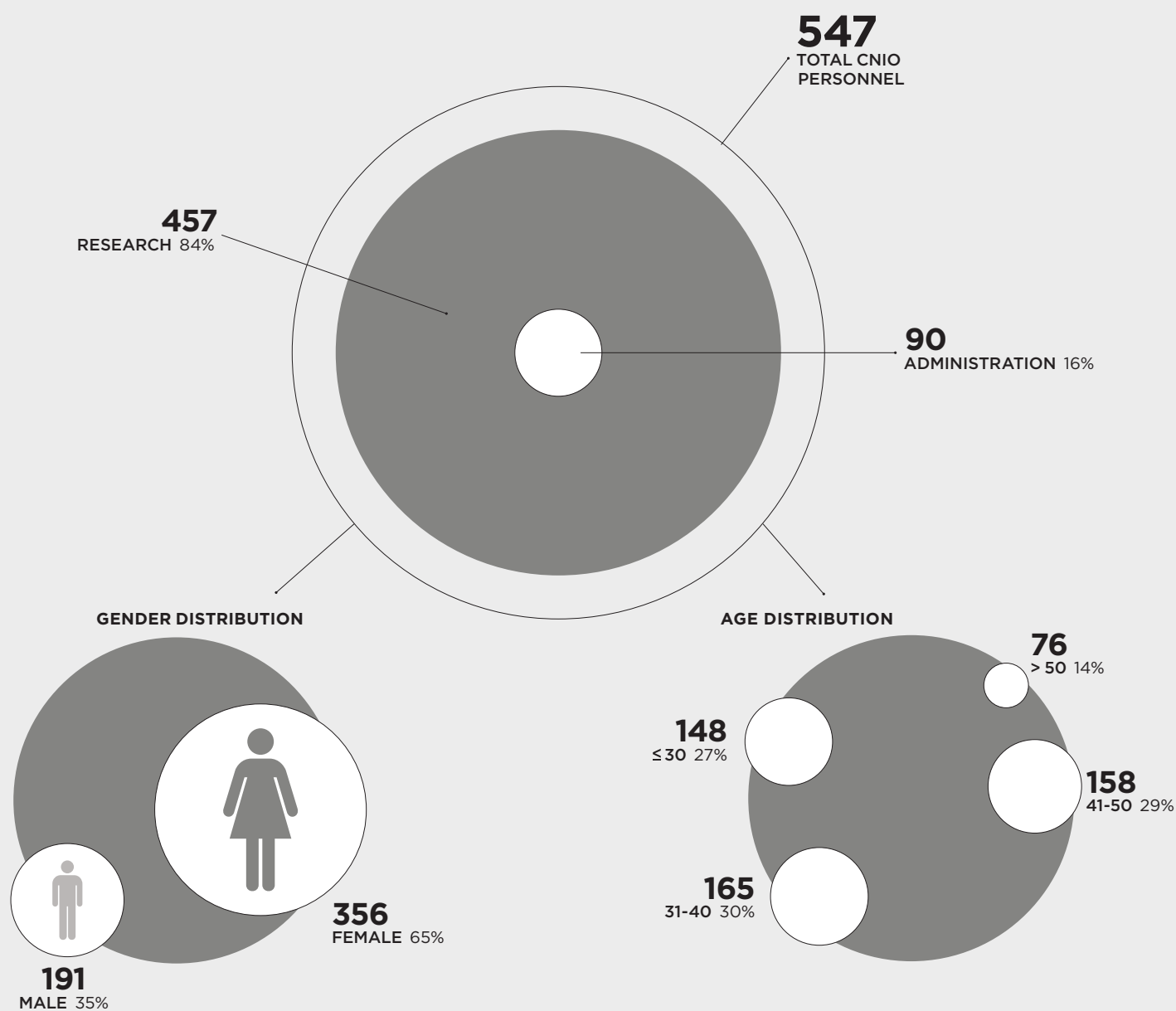
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CNIO PERSONNEL 2019



GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

Category	Gender	Percentage	Count	Visual Representation
GROUP LEADERS, HEADS OF UNIT/SECTION	FEMALE	45%	21	
	MALE	55%	26	
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	FEMALE	57%	8	
	MALE	43%	6	
MANAGEMENT: DIRECTORS, HEADS OF AREA	FEMALE	29%	4	
	MALE	71%	10	

SCIENTIFIC PERSONNEL 2019

TOTAL SCIENTIFIC
PERSONNEL

457

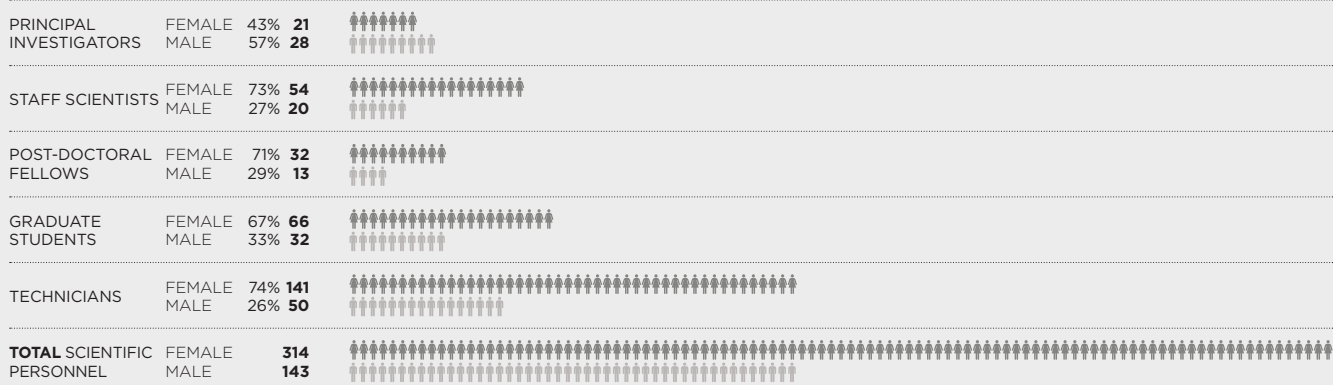
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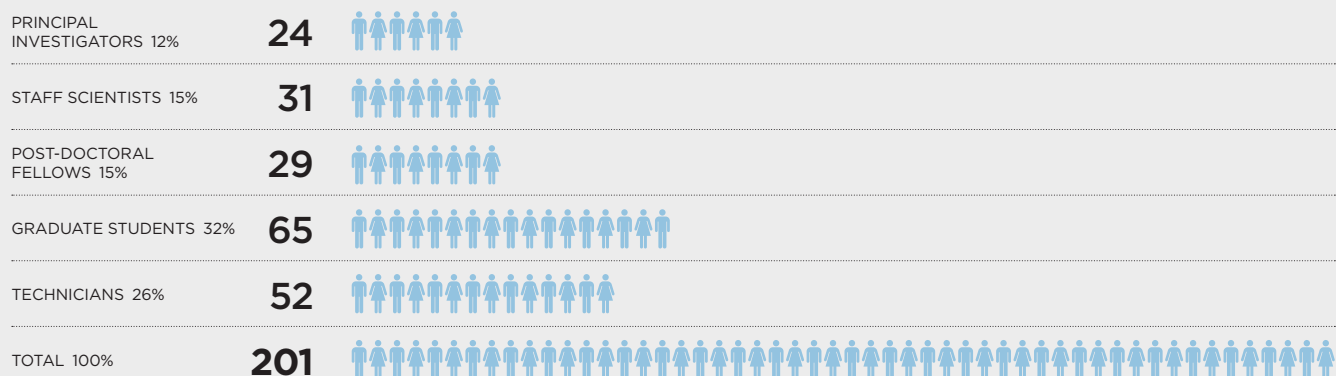
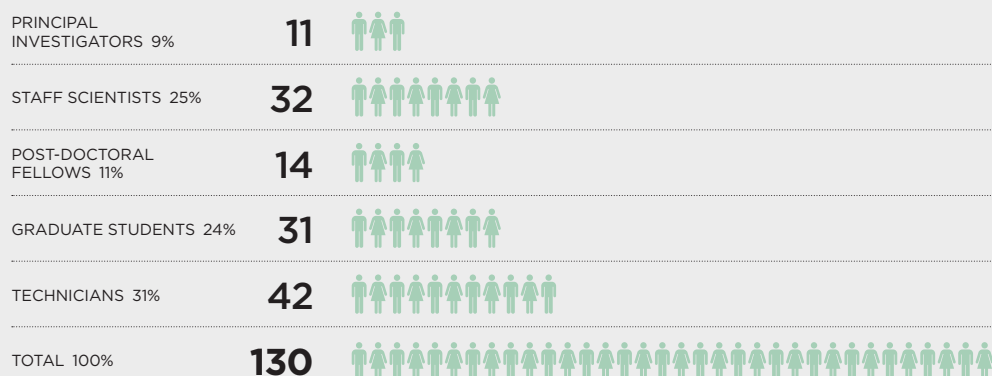
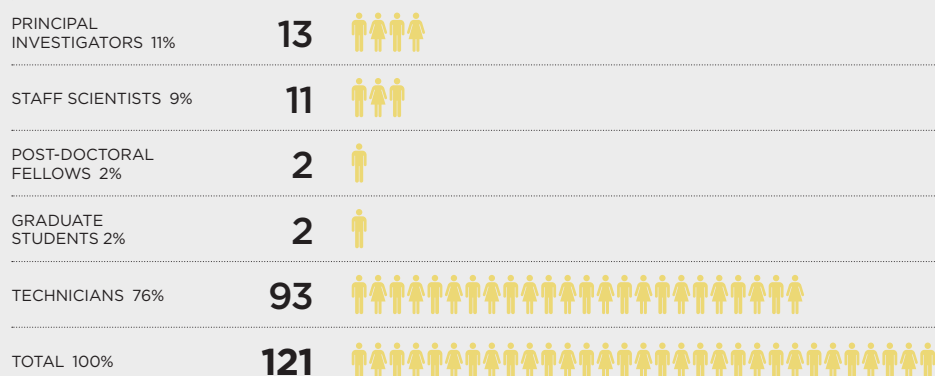
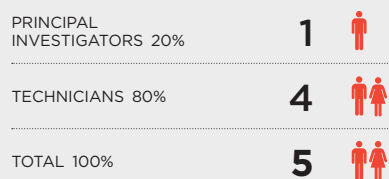


DISTRIBUTION BY PROFESSIONAL CATEGORY

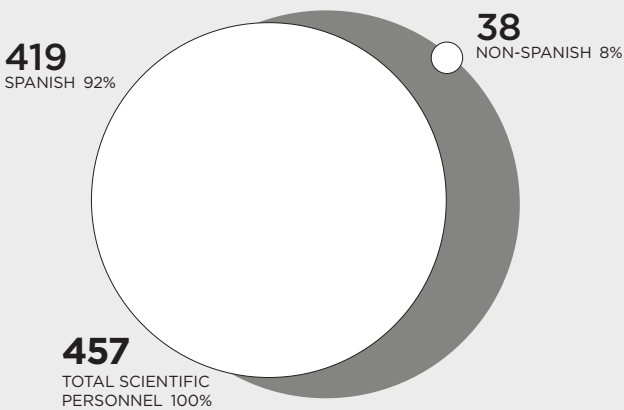


GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY



DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCHTOTAL SCIENTIFIC
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SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

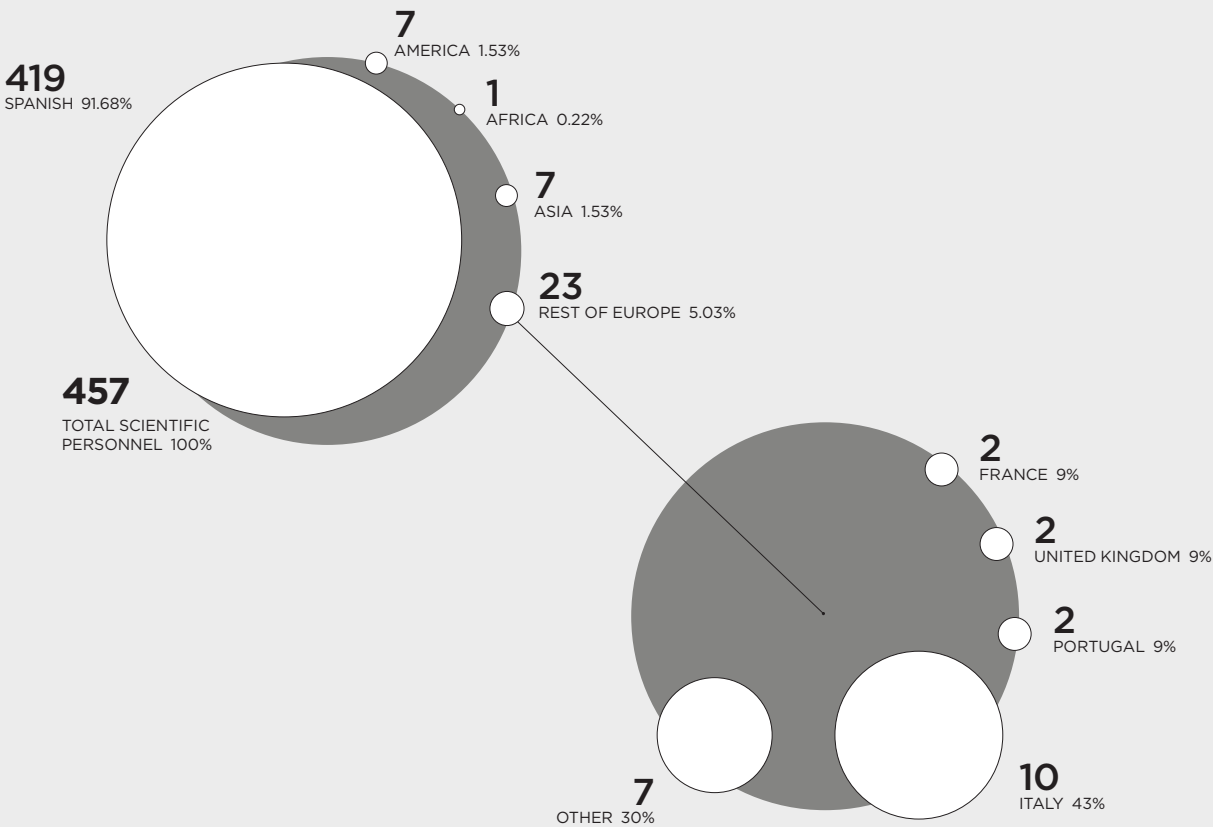


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

PRINCIPAL INVESTIGATORS 12%	6	
STAFF SCIENTISTS 8%	6	
POST-DOCTORAL FELLOWS 20%	9	
GRADUATE STUDENTS 8%	8	
TECHNICIANS 5%	9	

Total foreign scientific personnel 38
Percent values represent percentages of foreign employees of the total CNIO personnel in each category

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN



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“We take this opportunity to express our thanks and appreciation to all our sponsors and donors for the generous support that we received from them in 2019. They play an inherent role in our present and future successes.”



The *Fundación "la Caixa"* helps finance our most prominent international conferences, the CNIO-"la Caixa" Foundation Frontiers Meetings. Another main goal of the "la Caixa" Foundation is to support an innovative programme aimed at fostering international fellowships in order 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 new 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 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 3 research groups at the CNIO: 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 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 groups led by Maria A. Blasco and Óscar Fernández-Capetillo.



The *Fundación Banco Santander* funds the *Banco Santander Foundation* – CNIO Fellowships for Young Researchers. 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, Luis Javier Leandro-García from the Memorial Sloan Kettering Cancer Center in New York, was the recipient of a Santander Foundation-CNIO Fellowship in 2019.



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 the identification of 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 2019 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.

OTHER SPONSORS



Our activities are also supported by individual donations – citizens who wish to contribute personally to the battle against cancer – as well as via external fundraising from local associations that are equally dedicated to the battle against cancer. During 2019, our

research activities and seminars were supported, among others, by *Asociación Bandera Rosa*, *Asociación de Mujeres Afectadas de Cáncer de Mama "ROSAE"*, *Colectivo de afectados "El árbol de la Vida"*, *Freesia Group*, *Fundación Banco Sabadell*, *Fundación Inocente Inocente*, *Fundación Investigación Biomédica Hospital Universitario 12 de Octubre*, *Fundación Juegaterapia*, *L'Oréal España*, *Petroplast*, *Santa Lucía Seguros*, and the *British Embassy*.

**BECOME A
CNIO FRIEND**

more RESEARCH
less CANCER

cnio stop cancer

Lastly, we extend our heartfelt thanks to all '**CNIO Friends**' donors, sponsors and benefactors who, thanks to their generous donations to support cancer research at the CNIO, ensured the continuation of our research endeavours throughout 2019.

CNIO Friends

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CNIO FRIENDS

‘CNIO Friends’ is celebrating its 5th birthday with nearly €1.4 million raised from 1,350 donors over five years. The initiative, which has become a bridge connecting society to cancer research, is ready for a new, more mature stage, marked by the establishment of a Philanthropy Office. This year, ‘CNIO Friends’ raised a total of €515,000 which is our most successful year yet for the programme.

Donations to ‘CNIO Friends’ enables the Centre to hire new predoctoral and postdoctoral talent from around the world via a competitive application process. In 2019, we were delighted to recruit 4 young researchers to explore new ways of diagnosing and treating cancer. Ruben Martínez joined the Kinases, Protein Phosphorylation and Cancer Group; María Moreno joined the Genome Integrity and Structural Biology Group; Neibla Priego will continue working on her projects in the Brain Metastasis Group; and Sarita Saraswati joined the Telomeres and Telomerase Group.

For the second year in a row, we organised CNIO Arte, an initiative supported by *Banco Santander* Foundation to connect internationally renowned artists and scientists and invite them to explore common ground together. Each year, the exploration results in a unique artwork, which is then sold to fund the hiring of scientists through the ‘CNIO Friends’ Programme. In its 2nd edition, CNIO Arte was curated by Amparo Garrido, visual artist and Coordinator of the Office of Institutional Image and Outreach to Society at CNIO. It featured quantum physicist Ignacio Cirac, winner of the Prince of Asturias Award 2006, and photographer Chema Madoz, winner of the National Photography Award 2000. Madoz created a photographic work based on Cirac’s research, which led to a series of 30 numbered and signed photo engravings. The work, presented at the ARCOMadrid Contemporary Art Fair and at the *Centre de Cultura Contemporània de Barcelona (CCCB)*, was on display at CNIO from February to April.

In 2019, the CNIO was delighted to receive support from all over Spain. In addition, CNIO enjoyed working with a number of foundations, companies and associations to both raise awareness and raise funds for cancer research. To highlight a few examples, in February, *ROSAE*, an association of breast cancer patients, invited us to attend their convivial meal in Valdepeñas, Ciudad Real. In June, the *Bandera Rosa* association, supporting breast cancer patients and their families, held a number of activities in Campo de Gibraltar, Cádiz to raise funds for the Centre. In October, *El Árbol de la Vida*, an association based in Pedroñeras, Cuenca, held their 3rd

“‘CNIO Friends’ faces a new, more mature stage, marked by the establishment of the Development and Philanthropy Office.”

Charity Race Against Cancer, drawing 3,000 participants and donating part of the funds raised to the CNIO.

To recognise World Cancer Day, CNIO and La Roche-Posay (L’Oréal Group) carried out the *#InvestigaciónEsVida* [ResearchIsLife] campaign, handing out CNIO leaflets in over 1,000 pharmacies across Spain and thus reaching more than 60,000 users.

In June, we invited all of our donors to join us for our annual ‘CNIO Friends’ Meeting Day. Over 100 Friends and their companions were welcomed by Scientific Director Maria Blasco, to hear the latest news and major developments at CNIO. Researchers Paula Martínez, María Moreno, Neibla Priego and Miguel Jiménez enjoyed the opportunity to present their research projects that were made possible thanks to donations to the CNIO. Afterwards, our visitors took a tour of our labs before sitting down to a shared meal in the late afternoon.

In September 2019, CNIO established a Development and Philanthropy Office aimed at concentrating and optimising the efforts to identify and cultivate new donor relationships and continue to recognise and thank our existing supporters. The newly established office will manage the ‘CNIO Friends’ initiative and seek to build new collaborative partnerships with corporate partners, individuals and philanthropic foundations. The legacy programme continues to grow and has received a cumulative total of €888,000 since 2015. In 2019, CNIO received charitable bequests of €284,000 with €844,000 pending to be executed.

It has been an exciting and successful year for the CNIO Friends and we look forward to a whole host of innovative opportunities to collaborate with society through our new Philanthropy Office in 2020. We would like to take this opportunity to thank our donors once again for their commitment and support. The best way of demonstrating our thanks is by continuing to maintain the quality of cancer research we have been doing for the past 20 years, whilst always striving to do more. Together, let’s stop cancer.

QUANTUM PHYSICS AND PHOTOGRAPHY, BROUGHT TOGETHER BY CNIO ARTE



The 2nd CNIO Arte, a project sponsored by *Banco Santander* Foundation, was curated by Amparo Garrido. It featured quantum physicist Ignacio Cirac, winner of the Prince of Asturias Award 2006, and photographer Chema Madoz, winner of the National Photography Award 2000. Madoz created a photographic work based on Cirac's research, which led to a series of 30 numbered and signed photo engravings. The funds raised from the sale go to CNIO Friends.

MEETING WITH OUR FRIENDS



On June 27, 2019, we opened our doors to the community of CNIO Friends' donors to celebrate the CNIO Friends Day and honour them for their support of cancer research.

More than 100 Friends and their companions were welcomed by Maria Blasco, who told them about the latest CNIO achievements. Researchers Paula Martínez, María Moreno, Neibla Priego and Miguel Jiménez described the four projects they are carrying out thanks to donations from CNIO Friends. Our supporters were given a lab tour and enjoyed a lunch with the researchers. Raquel Yotti, Director of Spanish Institute of Health Carlos III, under whose purview CNIO is ascribed, also attended the presentation.

CNIO-LA ROCHE-POSAY AGREEMENT



LA ROCHE-POSAY
LABORATOIRE DERMATOLOGIQUE

SÚMATE
A LA LUCHA CONTRA EL CÁNCER
#InvestigaciónEsVida
4 DE FEBRERO - DÍA MUNDIAL DEL CÁNCER

cnio stop cancer

EN
TU
PIEL

On the occasion of World Cancer Day, CNIO and La Roche-Posay (L'Oréal Group) carried out the *#InvestigaciónEsVida* [ResearchIsLife] campaign.

Unfolding for 2 weeks in January and February, the campaign consisted of handing out CNIO leaflets in over 1,000 pharmacies across Spain and thus reaching more than 60,000 users, including customers and L'Oréal/La Roche-Posay staff. The campaign was advertised in mass media and in the L'Oréal website and social media.

BENEFACTOR FRIENDS/SPONSOR FRIENDS

→ Benefactor Friends

- **Ajos La Veguilla**
Las Pedroñeras, Cuenca
- **Alberto Heras Hermida**
Majadahonda, Madrid
- **Alfonso Agüera Nieto**
Santa Ana-Cartagena, Murcia
- **Álvaro Gil Conejo**
Mijas, Málaga
- **Andrés Macarro Onieva**
Alcalá de Henares, Madrid
- **Andrés Sánchez Arranz**
Madrid, Madrid
- **Anunciación Calvo Prieto**
Madrid, Madrid
- **Anunciación de los Milagros García Calvo**
Madrid, Madrid
- **Asociación Militar de Guardias Civiles-Profesional AMGC** (Valencia, Valencia)
- **Asociación Oncológica Tierra de Barros**
Almendralejo, Badajoz
- **Asociación Social Los Fuertes**
El Espinar-Segovia
- **Breatel SLU**
Madrid, Madrid
- **Carmen García García**
Madrid, Madrid
- **César Marianetti Netti**
Pozuelo de Alarcón, Madrid
- **Colectivo de Mujeres Afectadas de Cáncer Las Supernenas**
Tomelloso, Ciudad Real
- **Encarnación Fernández Pérez**
Madrid, Madrid
- **Eurolíneas Personales, S.L.**
Catarroja, Valencia
- **Fabián Jesús Toribio Cuadrado**
Cabrerizos, Salamanca
- **Fernando Pascual Carreras**
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- **Francisco Javier Gállego Franco**
Barbastro, Huesca
- **Gema Rubio González**
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- **Guillermo Alonso Borrego**
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San Vicente del Raspeig, Alicante
- **International Road Technology Consulting S.L**
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Castellón de la Plana, Castellón
- **Jesús Labrador Fernández**
Tres Cantos, Madrid
- **Jesús Miguel Iglesias Retuerto**
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- **José Enrique León Santos**
Getafe, Madrid
- **José Limiñana Valero**
Alicante, Alicante
- **Josep Vicente Roig Borrell**
Denia, Alicante
- **Laura Lozano Alberich**
Madrid, Madrid
- **Lydia Limiñana Valero**
Alicante, Alicante
- **Luis David Sanz Navarro**
Madrid, Madrid
- **Manuela Pérez Borrego**
Madrid, Madrid
- **María Begoña Toca Yarto**
Irún, Guipúzcoa
- **María Dolores Barrón Cervera**
Valencia, Valencia
- **María Dolores Díaz Almagro**
Sevilla, Sevilla
- **María Jesús Fernández Ruiz**
Madrid, Madrid

• **María Josefa Azcona Peribáñez**
Madrid, Madrid

• **María Rodríguez López**
Valladolid, Valladolid

• **Mujeres a Seguir**
Madrid, Madrid

• **Nemesio Carro Carro**
León, León

• **Rubén Murillo Franco**
Zaragoza, Zaragoza

• **The Nezwizzy Company, S.L.**
Valencia, Valencia

• **Vicente Belenguer Tarín**
Valencia, Valencia

→ **Sponsor Friends**

• **Asociación Bandera Rosa**
Algeciras, Cádiz

• **Asociación de Mujeres Afectadas de cáncer de mama Rosae**
Valdepeñas, Ciudad Real

• **Colectivo de afectados “El árbol de la Vida”**
Las Pedroñeras, Cuenca

• **Freesia Group**
Salou, Tarragona

• **Fundación Inocente Inocente**
Madrid, Madrid

• **Fundación Juegaterapia**
Madrid, Madrid

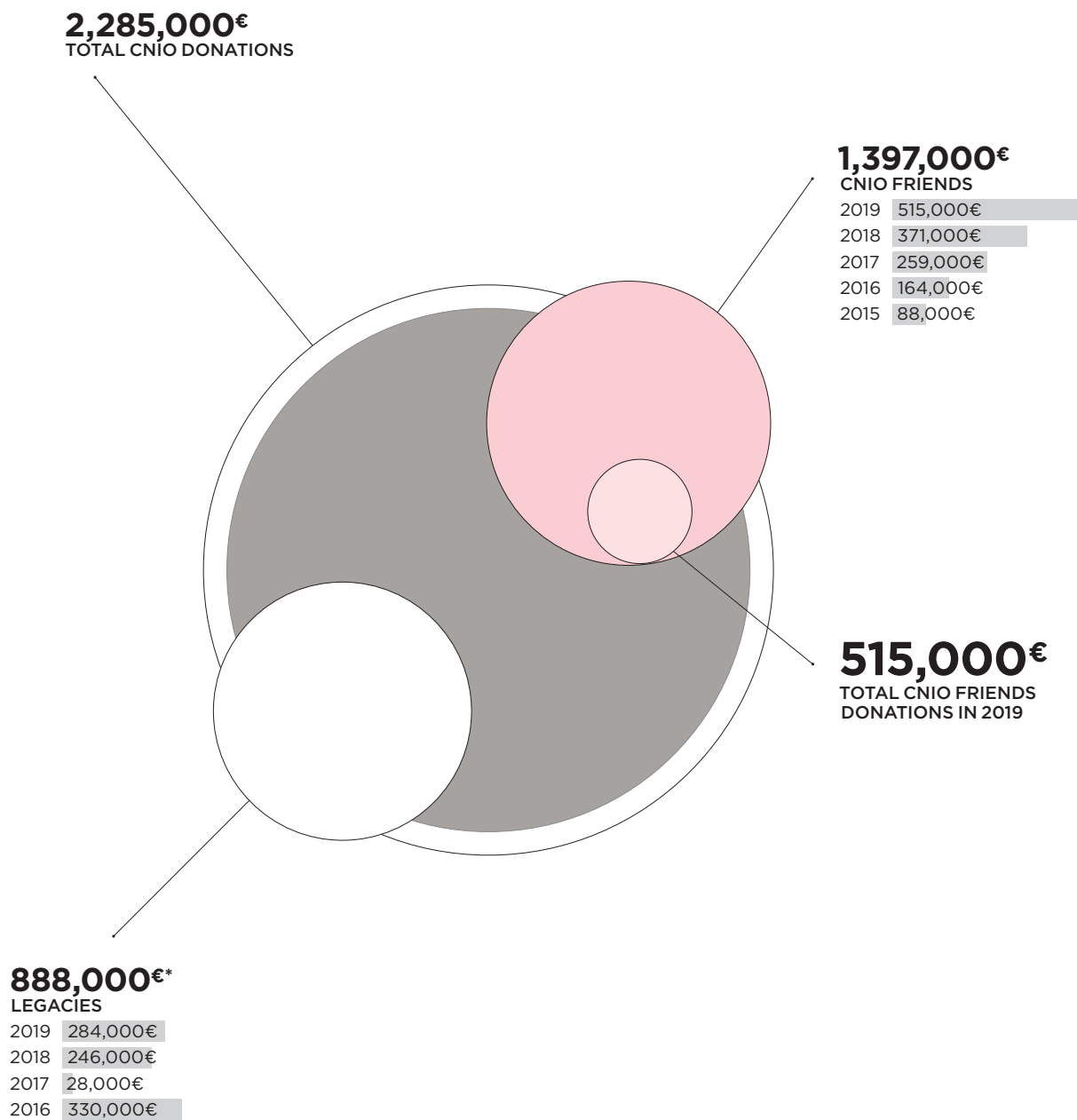
• **L'Oréal España**
Madrid, Madrid

• **Petroplast**
Logroño, La Rioja

• **Santa Lucía Seguros**
Madrid, Madrid

Last but not least, we would also like to extend our heartfelt thanks to all the anonymous benefactors who have donated their legacies to support cancer research at the CNIO; in doing so they have contributed to society for generations to come.

DONATIONS TO THE CNIO



* 844,000€ pending to be executed.

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 (TFFDOC/INTERNAZIONALE) – Torino, Italy 2019, among others that have not yet officially 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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