

**ANNUAL  
REPORT 2018**

# ANNUAL REPORT 2018

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**“Creating bridges with society is an integral part of what we do and is fundamental to shaping our identity”**

**MARIA A. BLASCO**  
**Director**

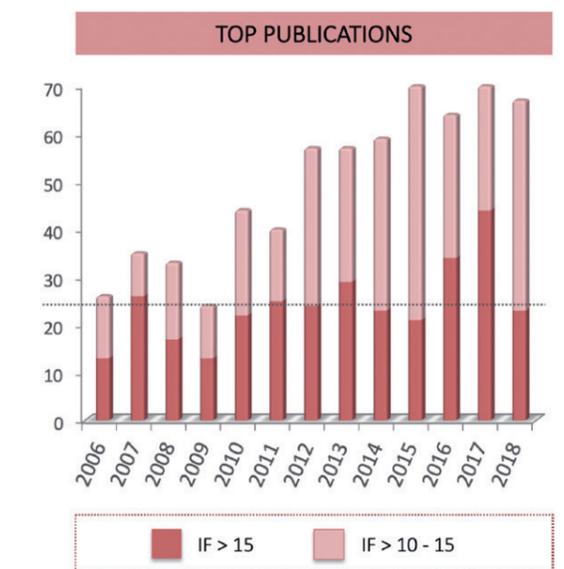
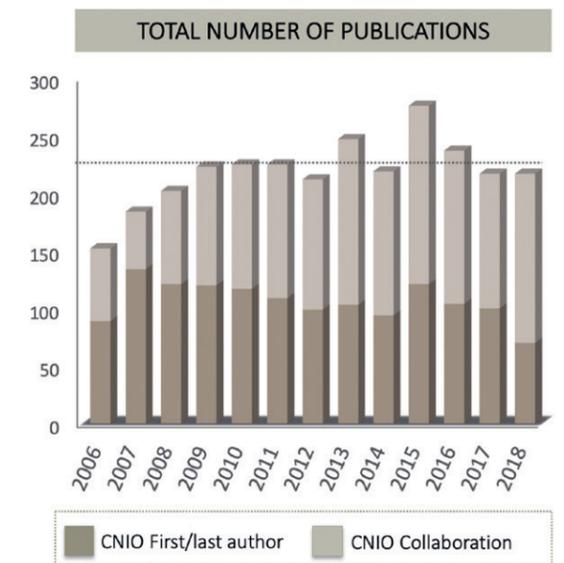
## FOREWORD

**Maria A. Blasco** Director

With 2018 already behind us, we take this opportunity to reflect on the many achievements that the CNIO and its people accomplished throughout the year. We have continued to consolidate our strategy, reinforcing those institutional core values on which our Centre of Excellence rests. One of our main accomplishments continues to be our scientific output, which continues to excel and places the CNIO firmly on the map as a flagship research centre in Europe. This year, according to the *Nature Index* considering our scientific contributions in the life sciences and healthcare field, we moved up the ladder to reach the top position among cancer-focused institutions in Europe. In 2018, the CNIO authored a total of 217 papers, 44 of which were published in journals with impact factor between 10 and 15, and 23 publications in journals with impact factor greater than 15. This achievement unquestionably portrays our international competitiveness and our leadership in cancer research.

Numerous collaborations are taking place with scientists throughout the world and the goal of the Centre is to continue fostering CNIO's participation in international consortia and forums so that we can actively engage in and promote the dissemination of research advances. In line with these efforts of international outreach, we continued to nurture our alliance in 2018 with the Weizmann Institute of Science (WIS) in collaboration with the *Ramon Areces* Foundation. As a result, the first call for collaborative projects for CNIO-WIS went out in 2018; this will materialise in outstanding projects that will cross the boundaries of land and science in 2019.

Our commitment to Spanish science and to bolstering the future of new generations of researchers in Spain perseveres; in 2018, we showed our support and involvement in SOMMA, the *Severo Ochoa* and *Maria de Maetzu* Alliance born in 2017. The first 100xCiencia, organised in the context of the Alliance, took place at the CNIO and successfully brought together various stakeholders, including scientists, science reporters, politicians from all main political parties in Spain and members of the society, in order to put together existing opportunities



and challenges with the aim to forge a bridge between science and society. In 2018, I have come forward to assume the Vice-Presidency of SOMMa, and from that position I am eager to further contribute towards the goals of the Alliance from the CNIO.

Science evolves, and so do we as a Centre. In 2018, we have said goodbye to several esteemed members of the CNIO who have contributed to CNIO's excellence during their time at our Institution. Thus, we pay tribute to our colleagues Manuel Morente, Erwin Wagner, Maria J. Barrero, and Daniel Lietha for their contributions and the years they dedicated to the CNIO: we wish them all the best for their future personal and professional endeavours. This year, we have also reinforced our basic research arm by merging the Cancer Cell Biology Programme with our Molecular Oncology Programme. The promotion of Nabil Djouder to senior group leader also adds to the renewed muscle of our basic research programme. Moreover, Óscar Fernández-Capetillo, one of our more seasoned investigators, has been appointed as new Director of the Programme to steer the wheel and keep heading towards excellence in research and innovation. In 2018, we had the honour to host Scott Lowe from the Memorial Sloan Kettering Cancer Center in NY, as visiting scientist. We also established an agreement with Raúl Rabadán, renowned expert in cancer systems biology and full professor at Columbia University, to strengthen our position in this field and keep abreast of new technologies and developments in cancer genomics. We expect to continue consolidating this relationship next year. 2018 has been an important year to start the recruitment of 2 new senior groups and 2 new junior groups to expand the Molecular Oncology and Structural Biology Programmes that will join our Centre in 2019.

It is by supporting our excellent science, stemming from both our basic, translational and clinical programmes, that we can achieve remarkable numbers that stand testament to our innovation activities. This is another core value at the CNIO, as we believe in contributing to society and our industry ecosystem by turning new discoveries into tangible beneficial products in biomedicine. Our Experimental Therapeutics Programme continues to develop new drug candidates to close the valley of death in drug discovery, bringing CNIO discoveries closer to the patient. This year, we made substantial progress in the development of novel TRF1 inhibitors for treating brain tumours, a project that has been awarded a *CaixaImpulse* grant. Another example of our work in biomedical innovation is another project also supported by *CaixaImpulse*, which exemplifies how leveraging gene editing can become a novel approach for treating cancer. More technologies are growing and developing in our pipeline, which is open to collaborations with investigators both at CNIO and abroad. Our efforts in drug discovery always reach beyond our borders, and we continue to be part of the international initiative Milner

Therapeutics Institute at Cambridge, with our active participation in 2018 in the second symposium organised by the alliance. We continuously measure our innovation impact as we actively strive to improve with every year. In 2018, we obtained more than 580,000 euros in royalties, a 5% increase compared with 2017; this return feeds back into our research organisation, providing us with motivation, incentive and important support to help achieve our institutional goals.

Our training programmes are the main vessel that feeds into our investigators and groups. Creating highly prepared generations of researchers is a priority for the CNIO and we strongly thank our sponsors as they contribute to support our collaborations with investigators abroad. During 2018, we continued to participate in the “Science by Women” programme to host African researchers and contribute to the progress of science abroad. Thanks to this programme, Hayet Rafa from the University of Science and Technology Houari Boumediene in Algiers, will join the CNIO's Melanoma Group for a 6-month stay as visiting scientist.

Society has become an integral part of our strategy and 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. In 2018, our media coverage increased by 46% over the previous year, featuring stories that have a strong impact worldwide. But we also want to create science and research awareness among our citizens through actions such as our event on the “Present and Future of Cancer Research”, co-organised with the *Atresmedia* media group and *Fundación AXA*, that was held at the *Cibeles* Palace with the support of the City of Madrid. We invited the Nobel Laureate Elizabeth Blackburn to be part of a discussion panel to spread the message that research is one of the pillars on which the fight against cancer rests. A special mention is reserved for our platform for scientific education and STEM support, CNIO & The City, which has again proven to be a success among participants as well as volunteers. In 2018, the project was again awarded by the FECYT, consolidating this initiative at the CNIO and in the educational community. In 2018, we aimed to reach out beyond the Region of Madrid; a commitment that will continue in future editions of the project.

Our efforts to reinforce our initiatives to engage with society have translated into a new “Office of Institutional Image and Outreach”; this office 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. The very first step was the launch of our new website, a portal between the scientific community and society with the goal of opening up the CNIO and its discoveries to the world as well as making more CNIO Friends to support our goal to stop cancer. In fact, in 2018 our CNIO Arte project, which explored the common territories

between scientific research and artistic creation, was exhibited in February and brought together the Spanish molecular biologist Margarita Salas and the artist Eva Lootz. This is an unparalleled project that we shared with the world via different forums, including ARCO. The funds obtained from the works of art totalled 100,000 euros, which contributes directly to our CNIO Friends philanthropic initiative. CNIO Friends has provided the funds and means to launch one pre-doctoral researcher contract and six CNIO Friends Postdoctoral Contracts, one of them supported by *Juegaterapia* Foundation to develop a project in brain tumours in children. The growing amount of donations, totalling over 370,000 euros, surpassed our record since the launch of the initiative in 2014. We enthusiastically thank all of our friends for their support and commitment to cancer research.

Finally, as an integral part of our alignment with the values of Responsible Research & Innovation, the CNIO demonstrates its full-blown commitment to gender equality. The work of the CNIO Women and Science (WISE) Office is instrumental to undertake the different initiatives that support women in science and to coordinate efforts with other departments and societal groups involved in closing the gender divide.

During 2018, we had the pleasure of listening to many exceptional women in different fields who shared with us their experiences and wisdom. We are indebted to all of them for their support to women and our cause.

Continuing with our mission to stop cancer will always require a robust team effort — a team of scientists, administrative support personnel and societal partners — who all help to establish a solid foundation to further build upon.



Visual artist Eva Lootz in our CNIO Arte 2018 exhibition.



**OSCAR FERNÁNDEZ-CAPETILLO**  
Vice-Director

**“During 2018, our scientists made significant advances in understanding how cancer originates, develops and progresses into a metastatic disease.”**

The scientists at CNIO have one more year made many important contributions that keep us at the forefront of biomedical research. We have discovered barriers that limit brain metastasis, which could potentially be exploited to limit this phenomenon. We have also identified a novel connection between cancer and differentiation, opening up new avenues for the treatment of pancreatic cancer. We have identified genes that can drive carcinogenesis or suppress it depending on the context, and have revealed how some cancer drivers might be involved in the development of melanoma. We have identified new biomarkers of prognosis for breast cancer, and confirmed the relevance of DNA repair deficiencies in prostate cancer. We have created new tools to model cancer in cells or animals, and obtained important

insights as to how our genome is spatially organised. We now also know that a gene therapy based on telomerase expression does not promote tumorigenesis, an important safety check on the development of this technology for the treatment of age-associated pathologies. While necessarily incomplete, this snapshot provides a quick panoramic view that illustrates the top-quality science that is constantly being produced by our scientists. This success is the combined outcome of the hard work carried out by all our research groups, with the instrumental help of the Biotechnology Units and all the personnel that supports our daily activities. My sincere thanks to all of you.

# ORGANISATION OF RESEARCH

**MARIA A. BLASCO** DIRECTOR

**ÓSCAR FERNÁNDEZ-CAPETILLO** VICE-DIRECTOR

## BASIC RESEARCH

MOLECULAR ONCOLOGY PROGRAMME

**Maria A. Blasco, Oscar Fernández-Capetillo** Acting Programme Directors

**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

**Ana Losada**  
Chromosome Dynamics Group

**Juan Méndez**  
DNA Replication Group

**María S. Soengas**  
Melanoma Group

**Héctor Peinado**  
Microenvironment and Metastasis Junior Group

**Manuel Valiente**  
Brain Metastasis Junior Group

**Alejo Efeyan**  
Metabolism and Cell Signalling Junior Group

CANCER CELL BIOLOGY PROGRAMME

**Erwin F. Wagner** Programme Director

**Erwin F. Wagner**  
Genes, Development and Disease Group

**Francisco X. Real**  
Epithelial Carcinogenesis Group

**Nabil Djouder**  
Growth Factors, Nutrients and Cancer Group

**Massimo Squatrito**  
Seve Ballesteros Foundation-CNIO Brain Tumour Junior Group

STRUCTURAL BIOLOGY PROGRAMME

**Óscar Llorca** Programme Director

**Óscar Llorca**  
Macromolecular Complexes in DNA Damage Response Group

**Daniel Lietha** (until September)  
Cell Signalling and Adhesion Junior Group

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

**Rafael Fernández Leiro**  
Genome Integrity and Structural Biology 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

**Martin Krallinger**  
Biological Text Mining Unit

## TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

**Javier Benítez** Programme Director

**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

**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**  
H120-CNIO Haematological Malignancies Clinical Research Unit

**Luis Paz-Ares**  
H120-CNIO Lung Cancer Clinical Research Unit

BIOBANK

**Manuel M. Morente** (until July) Director

**Miguel Quintela-Fandino** (since August) Acting Programme Director

## INNOVATION

CAROLINA POLA DIRECTOR OF INNOVATION

BIOTECHNOLOGY PROGRAMME

**Fernando Peláez** Programme Director

**Orlando Domínguez**  
Genomics Core Unit

**Sagrario Ortega**  
Transgenic Mice 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

**Sonia Martínez**  
Medicinal Chemistry Section

**Carmen Blanco**  
Biology Section

**Susana Velasco**  
CNIO-Lilly Cell Signalling Therapies Section

**María José Barrero** (until May)  
CNIO-Lilly Epigenetics Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

**Anabel Sanz** Director

# Basic Research

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## MOLECULAR ONCOLOGY PROGRAMME

The Molecular Oncology Programme (MOP) is the largest research programme at the CNIO, hosting 9 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. To do so, MOP research groups use a wide range of technologies including molecular and cellular biology, mouse models, and genetic and chemical screens. The Programme encompasses expertise related to some of the most active areas of research in molecular oncology, including DNA and chromosome stability (María A. Blasco, Óscar Fernández-Capetillo, and Ana Losada), oncogenes and cell cycle kinases (Mariano Barbacid), DNA replication (Juan Méndez), mitosis (Marcos Malumbres), melanoma (María S. Soengas), metabolism and cell signalling (Alejo Efeyan), and metastasis (Manuel Valiente and Héctor Peinado). In addition, starting in 2019, the MOP will also host the Groups of Epithelial Carcinogenesis (Francisco X. Real), Growth Factors, Nutrients and Cancer (Nabil Djouder) and Brain Tumours (Massimo Squatrito), which will broaden our areas of interest and significantly strengthen the Programme.

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

the research conducted by each of these Groups is exemplified through 9 papers published in *Nature* journals (*Nature Medicine*, *Nature Reviews Cancer*, *Nature Cell Biology*, *Nature Communications*, *Nature Structural and Molecular Biology*), 4 papers in *Cell* Journals (*Cancer Cell*, *Cancer Discovery*) and 1 paper in a *Science* Journal (*Science Translational Medicine*); this in addition to the excellent contributions in *Circulation Research*, *Current Opinion in Cell Biology*, *Journal of Clinical Investigation*, *Journal of Experimental Medicine* and *Nucleic Acids Research*.

Besides from publications, scientists at the MOP have continued to be leaders in their respective areas of research and have made important contributions in several areas such as generating patents, organising conferences or bridging the gap with the clinical world.

**María A. Blasco**, *Director*  
**Óscar Fernández-Capetillo**, *Vice Director*

## TELOMERES AND TELOMERASE GROUP

Maria A. Blasco  
Group Leader

Staff Scientists  
Isabel López de Silanes, Rosa M. Marión, Paula Martínez, Marinela Méndez (until April)



Post-Doctoral Fellows  
Giuseppe Bosso (since June), Sergio Piñeiro (since June), Sarita Saraswati (since October), Kurt Whittemore

Graduate Students  
Leire Bejarano, Iole Ferrara, José Carlos González (since November), Jessica Louzame (since September), Juan José Montero, Miguel Ángel Muñoz, Raúl Sánchez

Technicians  
Aksinya Derevyanko (since February), Rosa M. Serrano

Visiting Students  
Pawel Kordowitzki (May-September)

(Polish Academy of Sciences), Jessica Louzame (until August) (Univ. of Amsterdam, The Netherlands), Lydia T. Poluha (until August) (Univ. of Salford, UK), Tommaso Vicano (since October) (Sapienza Univ. of Rome, Italy)

### 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 is absent in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes and are essential for chromosome protection and genomic stability. Progressive shortening of telomeres associated with organism ageing leads to ageing. When telomeres are altered, adult stem cells have a maimed regenerative capacity.

**“We have demonstrated that telomerase activation in mouse models of pulmonary fibrosis can stop the progression of this fatal disease in mice.”**

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.

## RESEARCH HIGHLIGHTS

**Telomerase gene therapy to cure pulmonary fibrosis in mice**

Pulmonary fibrosis is a fatal lung disease that currently lacks effective treatment and is characterised by fibrotic foci and inflammatory infiltrates. Short telomeres can impair tissue regeneration and are found both in hereditary and sporadic cases. We have shown the therapeutic effects of AAV9-telomerase gene therapy in a mouse model of pulmonary fibrosis from a combination of bleomycin-induced lung damage short telomeres. AAV9 targets preferentially regenerative alveolar type II cells (ATII). Treated mice show improved lung function and lower inflammation and fibrosis at 1-3 weeks after treatment, and improvement or disappearance of the fibrosis at 8 weeks post treatment. Treatment results in longer telomeres and increased proliferation of ATII cells, as well as lower DNA damage, apoptosis, and senescence. We have provided a proof-of-principle that telomerase activation may represent an effective treatment for pulmonary fibrosis provoked by or associated to short telomeres.

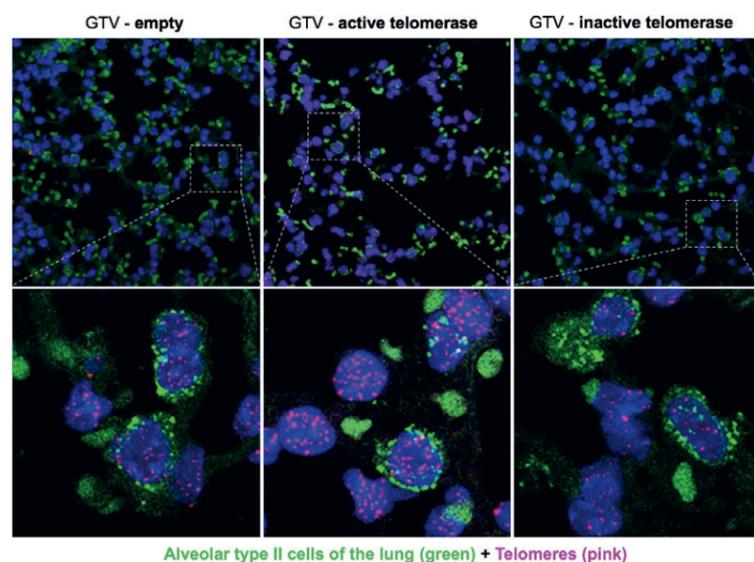
**Telomerase gene therapy does not increase risk of cancer in cancer-prone models**

Short and dysfunctional telomeres result in various age-related conditions, including a group of diseases collectively known as “telomere syndromes” that are provoked by extremely short telomeres arising from germline mutations in telomere genes.

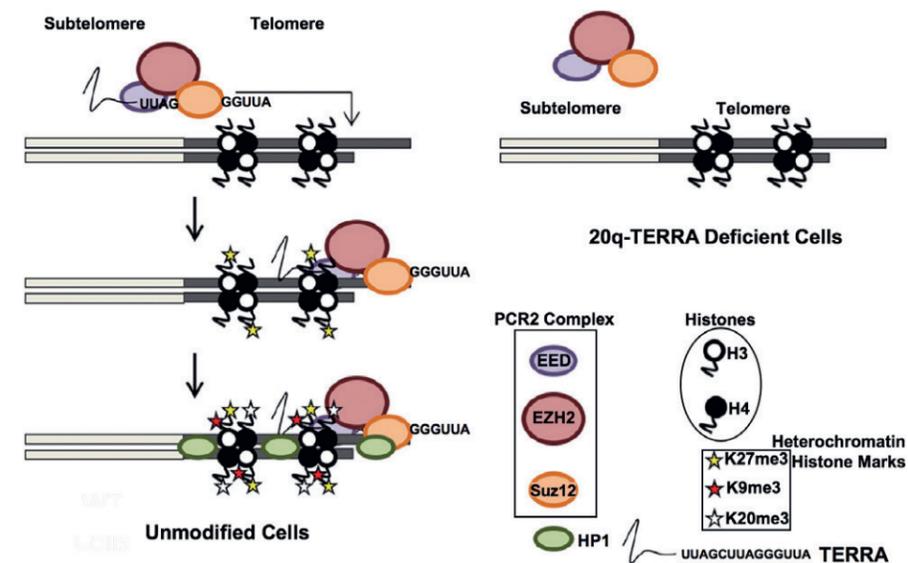
This opens the possibility of using telomerase activation as a potential therapeutic strategy to rescue short telomeres, maintaining tissue homeostasis and ameliorating these diseases. In 2012, we designed a highly innovative strategy: a gene therapy that reactivates the telomerase gene using adeno-associated viruses (AAV9). We have since shown its therapeutic efficacy in mouse models of cardiac infarct, aplastic anaemia, and pulmonary fibrosis. Although we did not observe, in any of the former models, an increased cancer incidence as a consequence of telomerase overexpression in any of those models, the potential medical use of telomerase still clashes with fears surrounding a possible increased cancer risk. We now tested the safety of AAV9-telomerase gene therapy in the context of a cancer-prone mouse model owing to expression of oncogenic *K-ras*. We found that telomerase overexpression resulted in longer telomeres in the targeted tissue (lungs; FIGURE 1) but does not accelerate carcinoma onset or progression. Telomerase activation by using AAV9-mediated telomerase gene therapy has no detectable cancer-prone effects in the context of oncogene-induced mouse tumours.

**TERRAs are important epigenetic regulators**

TERRAs are long non-coding RNAs that protect the telomeres. Our Group had already identified chromosome 20q as one of the main origins of human TERRAs and demonstrated that, by generating the first 20q-TERRA knockout models, they are



**Figure 1** Representative images of lungs treated with gene therapy vectors (GTV). Nuclei are in blue, alveolar type II cells in green and telomeres in red. Lung cells treated with telomerase present the most intense telomeres, indicating that they are the longest of all three scenarios.



**Figure 2** TERRAs regulate the status of telomeric chromatin. After binding to the PRC2 complex TERRAs bring PRC2 to the telomere. Binding of PRC2 makes possible the deposition of heterochromatin marks. These events cannot take place when TERRAs are absent.

essential for telomere length maintenance and protection. Using 20q-TERRA knockout cells we have now addressed the direct role of TERRAs in telomeric heterochromatin formation. We discovered that TERRAs interact with components of the polycomb complex (PRC2), an important epigenetic regulator of gene expression, thus facilitating the assembly of telomeric heterochromatin. We analysed telomere heterochromatin marks in cells deficient for 20q-TERRA and observed that their telomeres had decreased heterochromatic marks; we

discovered that they had lost a histone mark not previously recognised at telomeres (H3K27 trimethylation) that is catalysed by PRC2, a master regulator of gene silencing, which we found locates to the telomere in a TERRA-dependent fashion. Establishment of trimethylation marks in other histones (H3K9 and H4K20) and HP1 binding at telomeres required PRC2-dependent H3K27me3 at telomeres. Our findings demonstrated an important role for TERRAs in telomeric heterochromatin assembly (FIGURE 2). ■

## PUBLICATIONS

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## AWARDS AND RECOGNITION

- Doctorate Honoris Causa, Universidad de Murcia, Murcia, Spain.
- Member of the Board of Trustees, *Príncipe Felipe* Research Centre, Valencia, Spain.
- Member of the Board of Trustees, *Victor Grifols i Lucas* Foundation, Barcelona, Spain.
- Member of the Advisory Board for Research Centres of the Regional Government of Galicia, Spain.
- Member of the Scientific Committee of “Telos” magazine (published by *Fundación Telefónica*), Madrid, Spain.
- Editorial Board Member, *Mechanisms of Ageing and Development*.

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### OVERVIEW

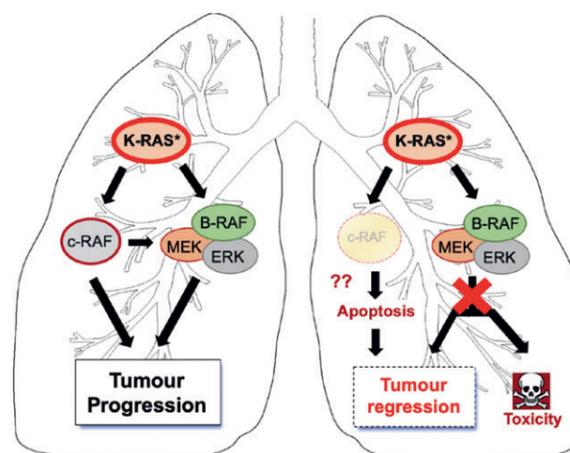
*KRAS* oncogenes have been identified in at least one fifth of all human cancers. In spite of recent successes with checkpoint inhibitors, most *KRAS* mutant tumours, including lung adenocarcinomas, are still treated with cytotoxic compounds approved over 2 decades ago. Moreover, attempts to block *KRAS* oncogenic activity with selective inhibitors of the MEK kinase, a downstream effector, have turned out to be major failures. Two MEK inhibitors, Trametinib and Selumetinib, have failed to show significant anti-tumour activity in large phase III clinical trials due to unacceptable toxicities. In our laboratory, we have continued our quest to validate therapeutic targets 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 c-RAF as a target capable of inducing significant tumour regressions in advanced *KRAS*/*TRP53* mutant lung tumours without inducing major toxicities. These observations suggest that forthcoming c-RAF inhibitors may provide significant therapeutic benefits in the clinic.

**“Systemic ablation of c-RAF induces regression of a significant percentage of advanced *K-Ras*/*Trp53* mutant lung adenocarcinomas by a mechanism independent of MAPK signalling that results in the induction of acceptable toxicities.”**

## RESEARCH HIGHLIGHTS

## Regression of advanced K-Ras/Trp53 mutant lung adenocarcinomas upon systemic ablation of c-RAF expression

Almost a quarter of all solid tumours harbour K-RAS oncogenes. Yet, more than 30 years after their identification in human cancer, there are no selective therapies to treat these tumours. Inhibitors of K-RAS oncogenes activated by G12C mutations, a mutation frequently identified in lung adenocarcinomas, have already entered clinical trials. Yet, direct targeting of other mutations has proven to be challenging. Genetic interrogation of the MAPK pathway revealed that systemic ablation of Mek or Erk kinases in adult mice prevent tumour development but are unacceptably toxic. This year, we demonstrated that ablation of c-Raf expression in advanced mouse lung tumours driven by K-Ras<sup>G12V</sup>/Trp53 mutations led to significant tumour regression with no detectable appearance of resistance mechanisms (FIGURE 1). Tumour regression results from massive apoptosis. Importantly, systemic abrogation of c-Raf expression does not inhibit canonical MAPK signalling, hence, resulting in limited toxicities. These observations suggest that therapeutic strategies aimed at inhibiting c-RAF kinase activity may not be suitable since they may only block MAP Kinase activation. Indeed, three independent c-RAF kinase inhibitors have shown to be rather toxic even at non-therapeutic doses. Therefore, we need to explore other therapeutic strategies. Drugs capable of degrading the c-RAF protein could be most effective in the clinic. Yet, drugs that promote protein degradation are still at very early stages of development. Inhibition of c-RAF by small interfering RNA is another possible approach, but still faces significant technical challenges. Finally, selective targeting c-RAF effectors regulated through non-kinase mechanisms such as ROKalpha, ASK1 and MST2 will also be challenging since they will require to be activated. As Frank MacCormick put it in a Preview that accompanied our *Cancer Cell* paper (San Clemente *et al.*, 2018) “In the effort to harness c-Raf biology to target KRAS mutant



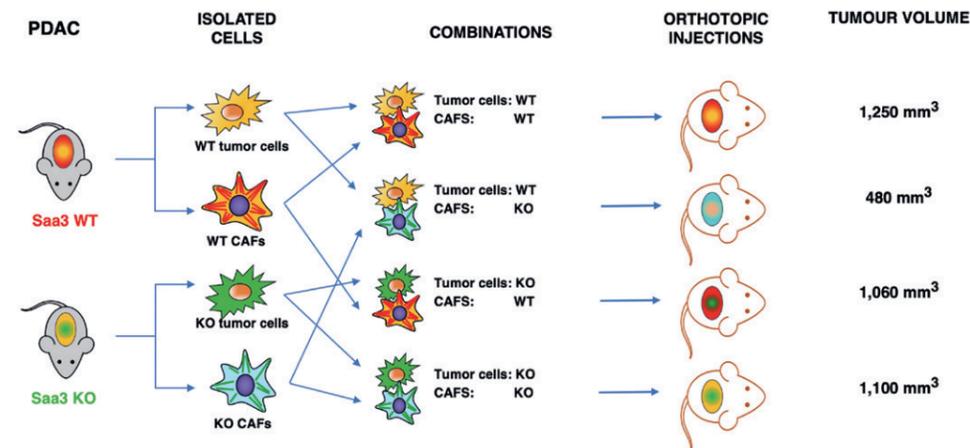
**Figure 1** Systemic ablation of c-RAF expression results in equal or better levels of tumour regression while inducing acceptable toxicities. In contrast, inhibition of the MAP Kinase pathway induces tumour

regression but with unacceptable toxicities both in mice (Blasco *et al.*, *Cancer Cell* 2011) and in patients (Trametinib and Selumetinib failed clinical trials).

cancers, the goal posts seemed to have moved again”. In any case, our results should provide the experimental bases to design novel c-RAF based therapeutics to treat K-RAS mutant cancers.

## SAA3 is a key mediator of the pro-tumorigenic properties of cancer associated fibroblasts in pancreatic ductal adenocarcinomas

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant human tumours for which there are no efficacious therapeutic strategies. This tumour type is characterised by an abundant desmoplastic stroma that promotes tumour



**Figure 2** Crosstalk between pancreatic tumour cells and CAFs in the presence and absence of Saa3. Diagram depicting the *in vivo* orthotopic tumour assays in immunodeficient mice carried out to determine the pro-tumorigenic properties of Saa3 competent (WT) (red) and Saa3 null (KO) (light blue) CAFs on pancreatic tumour cells isolated from Saa3 competent (WT) (yellow) and Saa3 null (KO) (green) tumours.

progression. It is generally accepted that cancer-associated fibroblasts (CAFs) stimulate tumour progression and might be implicated in drug resistance and immunosuppression. Yet, recent studies have shown that physical or genetic elimination of the stroma leads to more aggressive tumour development. In an attempt to clarify the role of the desmoplastic stroma in PDAC development and progression, we decided to reprogram the CAFs that make up the stromal tissue by identifying, and subsequently targeting, genes responsible for their pro-tumorigenic properties. First, we compared the transcriptional profile of PDGFRα+ CAFs isolated from genetically engineered mouse model PDAC tumours with that of normal pancreatic fibroblasts (NPFs) in order to identify genes potentially implicated in their pro-tumorigenic properties. We have observed that the most differentially expressed gene, *Saa3*, a member of the acute-phase Serum Amyloid A (SAA) apolipoprotein family, is a key mediator of the pro-tumorigenic activity of PDGFRα+ CAFs. Whereas

*Saa3* competent CAFs stimulate the growth of PDAC tumour cells in an orthotopic model, *Saa3* null CAFs inhibit tumour growth. *Saa3* also plays a role in the cross-talk between CAFs and tumour cells (FIGURE 2). Ablation of *Saa3* in pancreatic tumour cells makes them insensitive to the inhibitory effect of *Saa3* null CAFs. As a consequence, germline ablation of *Saa3* does not prevent PDAC tumour development in mice (FIGURE 2). The pro-tumorigenic activity of Saa3 in CAFs is mediated by Mpp6, a member of the palmitoylated membrane protein subfamily of the peripheral membrane-associated guanylate kinases. Finally, we interrogated whether these observations could be translated to a human scenario. Indeed, *SAA1*, the orthologue of murine *Saa3*, is overexpressed in human CAFs. Moreover, high levels of *SAA1* in the stromal component correlate with worse survival. These findings support the concept that selective inhibition of *SAA1* in CAFs may provide potential therapeutic benefit to PDAC patients. ■

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  - Cancer Cell: F. McCormick. c-Raf in KRas mutant cancers: a moving target. *Cancer Cell* 33, 158-159.

- Science Translational Medicine: A. Lujambio. A new hope for KRAS mutant cancers. *Sci Transl Med* 10, eaas8964. <http://stm.sciencemag.org/content/10/429/eaas8964.full>. This article was also recommended in *F1000Prime* as being of special significance in its field.
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## PATENT

- Barbacid M, Guerra C, Blasco MT, Navas C (2018). Combined therapy against cancer. *EPI382555.3*.

## AWARDS AND RECOGNITION

- Mariano Barbacid:
  - Premio Fulbright, Madrid, Spain.
  - JC Bose Memorial Lecture, Saha Institute of Nuclear Physics, Kolkata, India.
  - Severo Ochoa Plenary Lecture, XLI Meeting of the Chilean Society for Biochemistry and Molecular Biology, Iquique, Chile.
  - Maimonides Memorial Lecture, Córdoba, Spain.
- Keynote Speaker, AACR Special Conference: Targeting RAS-Driven Cancers, San Diego California, USA.
- Chair, Plenary Symposium on “Genetic Background Matters”, 25th Biennial Congress of the European Association for Cancer Research, Amsterdam, The Netherlands.
- Chair, EMBO Workshop on “Cellular Signalling and Cancer Therapy”, Cavtat, Croatia.
- Carmen Guerra:
  - III Beca Carmen Delgado/Miguel Pérez-Mateo.
  - CNIO Award for Excellence in Research by Staff investigators.

- Manuel San Clemente: Award for PhD-Authorized Publications, CNIO Lab Day.

## CELL DIVISION AND CANCER GROUP

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

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(Kobe University, Japan), Yucheng  
Zhang (Jilin University, China)

### OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated in mammalian cells. During the last years, we have used different mouse models to understand the relevance of cell cycle regulators, including cell cycle kinases and phosphatases, as well as proteins involved in ubiquitin-dependent degradation, in the control of cell division and tissue physiology. Our 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) to find and validate new targets for cancer therapy. As a final goal, we aim to generate information that may be useful to improve therapeutic strategies against cancer cell proliferation.

**“During 2018, we investigated the relevance of PLK1 and MASTL as oncogenes and as therapeutic targets in breast cancer, as well as the effects of MASTL mutations in patients with thrombocytopenia.”**

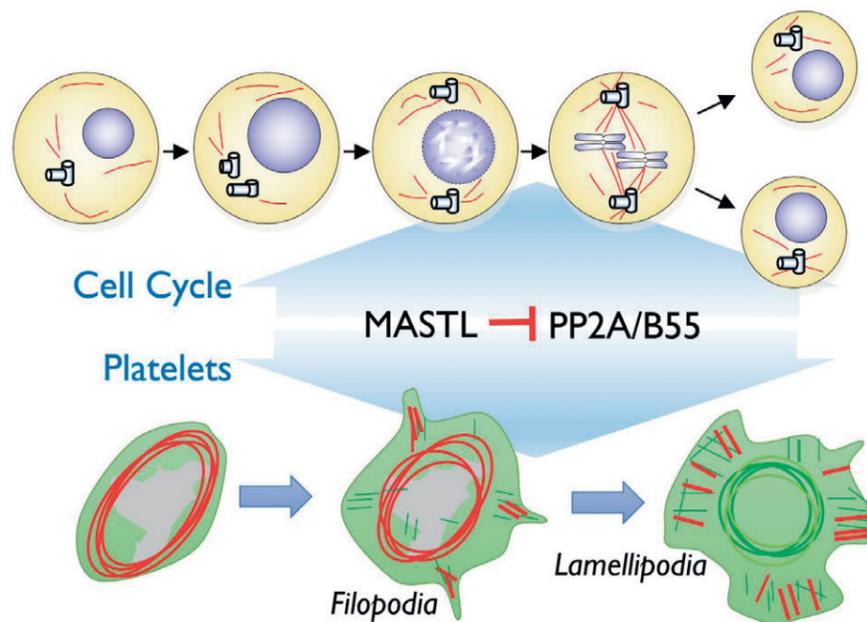
## RESEARCH HIGHLIGHTS

## The MASTL-PP2A axis in the cell cycle and cancer

Cell cycle progression is typically triggered by phosphorylation of a large number of proteins involved in different cellular pathways. Several families of protein kinases involved in cell cycle progression, such as Cyclin-dependent kinases (CDKs) or Polo-like kinases (PLK), have been thoroughly studied over the last few decades. However, the identity and relevance of phosphatases is less well-established. Recently, the cell cycle kinase MASTL (also known as Greatwall) emerged as a key player in cell cycle control by inhibiting the PP2A phosphatase during mitosis. MASTL phosphorylates 2 small

proteins, endosulfine (ENSA) and ARPP19, which in their phosphorylated form bind and inhibit PP2A-B55 complexes, thus contributing to the phosphorylation of mitotic phosphoproteins (FIGURE 1).

However, its physiological relevance in normal tissue homeostasis or disease is less known. After an initial screening in several tumour types, we found that MASTL was upregulated in a significant fraction of breast tumours and correlated with poor prognosis in breast cancer patients (a collaboration with M.A. Quintela, CNIO; and C. Caldas, Cancer Research UK). Importantly, genetic downregulation or ablation of MASTL,



**Figure 1** A dual role for the MASTL-PP2A/B55 pathway in cell cycle progression and actin cytoskeleton dynamics. During the cell cycle, MASTL inhibits PP2A/B55 to prevent dephosphorylation of mitotic phosphoresidues. In postmitotic cells

such as platelets, the same pathway is involved in the control of cytoskeleton dynamics by modulating phosphoresidues in the signalling pathways that control the reorganisation of the actin cytoskeleton.

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rez-Fernández M, Malumbres M, Zubiaga AM (2018). An E2F7-dependent transcriptional program modulates DNA damage repair and genomic stability. *Nucleic Acids Res* 46, 4546-4559.

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either using RNA interference or CRISPR-Cas systems, resulted in defective proliferation of a subset of breast cancer cells, both *in vitro* and *in vivo*, suggesting the therapeutic potential of inhibiting this kinase in breast cancer (a collaboration with the Oncology R&D group at Pfizer; Álvarez-Fernández *et al.*, *Cell Death Differ* 2018).

Before the function of MASTL in mitosis was proposed, the corresponding human gene was found mutated in patients with thrombocytopenia. To understand the effects of this mutation we recently generated a knockin mouse model carrying that mutation (Mastl E166D in the mouse). Mastl E166D mice developed thrombocytopenia but, unexpectedly, this defect was not due to abnormal cell cycle in megakaryocytes but to defective activation of mutant platelets. In the presence of this mutation, PP2A is constitutively inhibited resulting in hyperphosphorylation of proteins involved in actin cytoskeleton signalling during platelet activation. Mastl E166D mutant platelets were prematurely activated and displayed defective morphology and function, as well as decreased survival, thus contributing to thrombocytopenia (Hurtado *et al.*, *J Clin Invest*, in press). These data uncovered a new function of MASTL in the actin cytoskeleton in postmitotic cells, entailing important implications in human disease.

## Plk1: oncogene or tumour suppressor

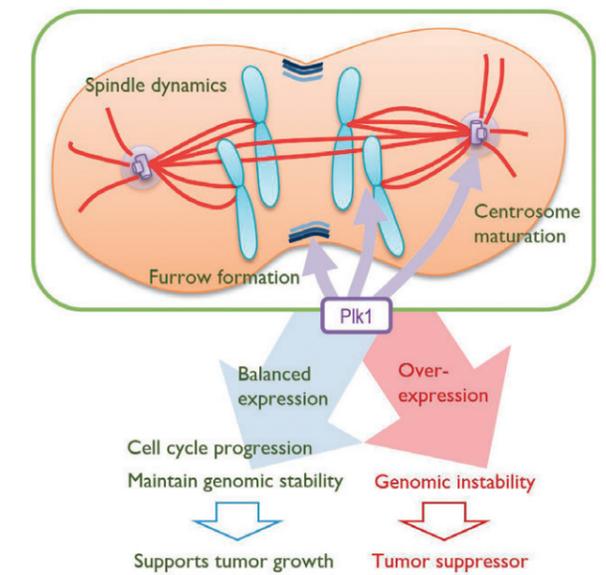
Among the multiple kinases involved in cell cycle progression, PLK1 is considered an attractive cancer target and a few small-molecule inhibitors are currently under evaluation in clinical trials. However, our knowledge about the relevance of this protein in adult mammalian tissues is still limited. In 2017, we described a critical role for the mouse Plk1 in controlling the contraction of smooth muscle cells and blood pressure (de Cárcer *et al.*, *Nat Med* 2017), suggesting possible toxic effects linked to the inhibiting of this kinase that need to be controlled in patients. More recently, we evaluated to what extent the expression levels of Plk1 contribute to tumour development in mouse models. Although Plk1 is frequently considered as an oncogene, we observed that Plk1 overexpression prevented proper cell proliferation by generating genomic aberrations

and AURKC during oocyte meiosis. *Curr Biol* 28, 3458-3468.

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**Figure 2** A central role for Plk1 in controlling genomic stability. Plk1 controls several processes during the cell cycle, including centrosome maturation, spindle dynamics and the formation of the cytokinesis furrow. In normal conditions these functions support cell proliferation and tumour growth, and inhibiting Plk1 may prevent tumour cell proliferation. However, when Plk1 is overexpressed, tumour cells are genomically unstable resulting in defective growth of breast cancers.

in polyploid and aneuploid cells (FIGURE 2). Overexpression of Plk1 impaired breast cancer development induced by Kras or HER2 oncogenes, thereby suggesting a tumour suppressor function for this protein in these models (a collaboration with R. Sotillo, German Cancer Research Center; de Cárcer *et al.*, *Nat Commun* 2018). Specifically, in human breast cancer, PLK1 overexpression correlated with better prognosis. Although these data do not argue against the use of PLK1 inhibitors in the clinic, they add new levels of knowledge that will be critical when optimising the use of mitotic inhibitors in cancer therapy. ■

Biochemical analyses reveal amino acid residues critical for cell cycle-dependent phosphorylation of human Cdc14A phosphatase by cyclin-dependent kinase 1. *Sci Rep* 8, 11871.

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existence of a CDKNIC-E2F1-TP53 axis that is altered in human T-cell lymphoblastic lymphomas. *BMC Cancer* 18, 430.

## PATENT

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## PUBLICATIONS

Bellutti F, Tigan AS, Nebenfuhr S, Dolezal M, Zojer M, Grausenburger R, Hartenberger S, Kollmann S, Doma E, Prchal-Murphy M, Uras IZ, Höllein A, Neuberger DS, Ebert BL, Ringler A, Mueller AC, Loizou JI, Hinds PW, Vogl C, Heller G, Kubicek S, Zuber J, Malumbres M, Farlik M, Villunger A, Kollmann K, Sexl V (2018). CDK6 antagonizes

p53-induced responses during tumorigenesis. *Cancer Discov* 8, 884-897.

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## GENOMIC INSTABILITY GROUP

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Sergio Ruiz (until May)



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### OVERVIEW

The Genomic Instability Group centres its research on understanding how cells respond to DNA damage, in particular to a specific type of harm known as replication stress (RS). Oncogene-induced RS has been confirmed as the main source of genomic rearrangements in cancer cells. In mammals, RS triggers a cellular response initiated by ATR and CHK1 kinases, known as the Replicative Stress Response (RSR). Throughout the years, our laboratory has developed a wide battery of cellular and animal tools for the study of the RSR. Among them, we have mice with enhanced or limited function of ATR and CHK1 kinases, cell lines in which the RSR can be activated at will and chemical inhibitors of ATR. Our studies have enhanced our understanding of the impact of RS on cancer and ageing, and have provided novel drugs with antitumoural potential that exploit the presence of RS in cancer cells. Overall, our goal is to understand the molecular mechanisms governing genome protection and repair – particularly during replication – and to exploit this knowledge as a way to fight against cancer.

**“In 2018 we have, among other achievements, extended our line of research on the mechanisms of resistance to cancer therapies and revealed a novel mechanism by which cells couple DNA replication termination to mitotic entry.”**

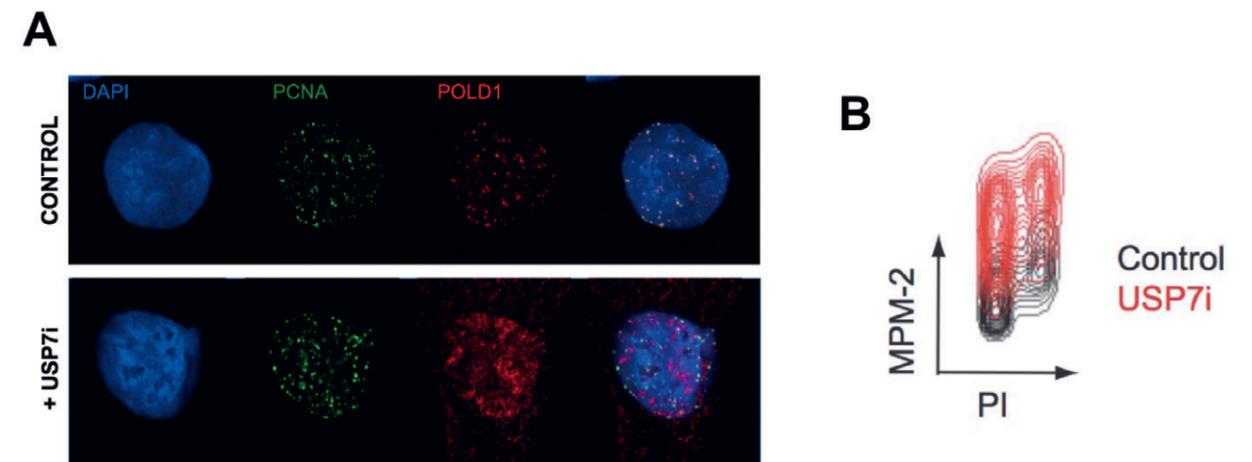
## RESEARCH HIGHLIGHTS

## Coupling DNA replication termination to mitotic entry

The cell cycle consists of a sequence of ordered events leading to the duplication of DNA and ultimately cell division. According to this model, once DNA replication is finished, there is a G2 transition phase that precedes mitosis. However, how and when mitosis is triggered is not yet fully understood. In fact, it has been long speculated that some yet-to-be-discovered checkpoint prevents mitotic entry until DNA replication is completed. We previously described that inhibition of the USP7 deubiquitinase leads to the ubiquitination of replisome components and replication termination. We have now seen that DNA replication triggered by USP7 inhibitors occurs concomitant to a generalised activation of the mitotic kinase CDK1 throughout the entire cell cycle, which impairs chromosome segregation and is toxic for mammalian cells. Accordingly, the toxicity of USP7 inhibitors is alleviated by CDK1 inhibition. Besides from its interest in clarifying how USP7 inhibitors kill cells, this work provides direct evidence for the existence of a ubiquitin-based signalling code that couples DNA replication termination to mitotic entry.

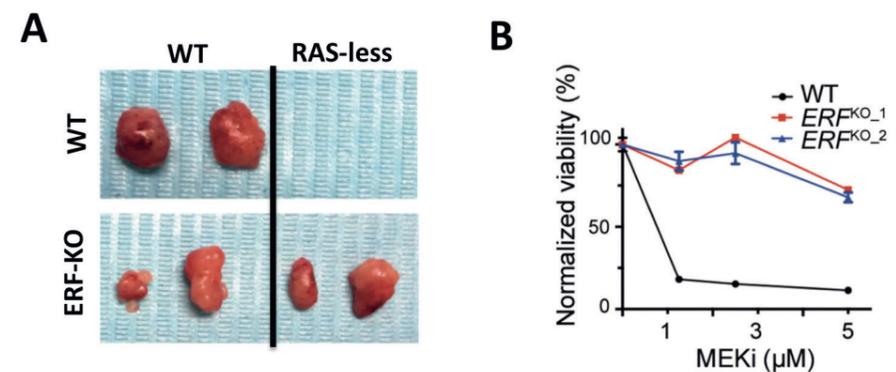
## Rescuing RAS deficiency in mammalian cells

Previous work in our laboratory revealed the mechanisms of resistance to genotoxic anticancer agents such as ATR inhibitors. We have now looked into potential mechanisms of resistance to targeted therapies. In the context of targeted therapies, RAS is assumed to be the 'Holy Grail'. More than 30% of all human cancers are driven by mutations in the RAS family of genes and, if not RAS, another member of the pathway is frequently altered. Since RAS inhibitors have been technically very difficult to develop, most drugs that have reached the clinic actually target some other components of the signalling route such as EGFR, RAF or MEK. However, all of these targeted therapies invariably confront the emergence of resistance. To which extent resistance would also occur to RAS inhibitors remains unknown. In this regard, we have recently identified a mutation – the loss of the ETS-domain factor *ERF* – that enables the growth and differentiation of mouse embryonic stem cells (mESC) lacking all RAS genes (H-, N- and K-*Ras*). Strikingly, *ERF* deficiency supports the generation of RAS-less teratomas, this being the first example of a tumour that can develop in the absence of RAS proteins. We believe this work indicates that, even if potent and selective inhibitors of RAS are finally developed, they might likely confront resistance through mutations of other genes such as *ERF*. We are currently investigating the role of *ERF* and other ETS-domain factors in the context of resistance to targeted therapies in cancer. ■



**Figure 1** USP7 coordinates DNA replication with mitotic entry. (A) Immunofluorescence of PCNA (green) and POLD1 (red) illustrating the effects of USP7 inhibition (USP7i) in replisome disassembly in MEF. DNA was stained with DAPI (blue). (B)

Flow cytometry measuring CDK activity (MPM-2) and DNA content (PI) in cells exposed to USP7i, illustrating the generalised activation of CDK activity throughout the cell cycle in response to the inhibitor.



**Figure 2** *ERF* deficiency is synthetically viable with the absence of RAS signalling. (A) Representative image of teratomas that are observed after injecting nude mice with mESC of the indicated genotypes (RAS-less = deficient in H-, N- and K-*Ras*). (B) Cell-Titer Glo assay in leukaemia-derived KBM7 cells, illustrating the increased resistance of *ERF*-deficient cells to MEK inhibition.

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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 are dissecting the functional specificity of cohesin variant subunits to better understand how their mutation promotes carcinogenesis.”**

## RESEARCH HIGHLIGHTS

Cohesin consists of four core subunits, SMC1, SMC3, RAD21 and SA. There are two versions of the SA subunit in vertebrate somatic cells, SA1 and SA2. Loss of function mutations in the *STAG2* gene encoding SA2 have been identified in bladder cancer, Ewing sarcoma and myeloid malignancies, among others. In cells lacking cohesin-SA2, cohesin-SA1 performs the essential functions of cohesin related to cohesion. We suspect, however, that cohesin-SA1 cannot accomplish other functions of cohesin-SA2 related with chromatin organisation and gene regulation. Importantly, lack of cohesin-SA2 may also generate vulnerabilities that could be exploited in cancer therapy. We aim to identify the specific functions of the two variant complexes in chromatin architecture and gene regulation.

#### Dissecting the role of cohesin-SA1 and cohesin-SA2 in human cells

We analysed the genome-wide distribution of the two variant cohesin complexes in several human cell lines and applied functional genomics to assess their enrichment in different regulatory elements as well as their co-localisation with other factors involved in genome organisation such as CTCF. We then addressed how this distribution changes when one or the other variant is missing and the subsequent alterations in the transcriptome and in chromatin organisation, analysed by Hi-C in collaboration with M. A. Marti-Renom (CNAG-CRG). Our results show that the two complexes fulfil different functions (FIGURE 1). Cohesin-SA1 is important for the organisation of the topological domains or TADs, which make up the global structure of the genome, and works always alongside the CTCF protein. In contrast, cohesin-SA2 is more versatile and is capable of interacting with diverse transcription factors to form local chromatin loops that bring together enhancers and promoters. Cohesin-SA2 is also more dynamic in its chromatin association, and a larger fraction of cohesin-releasing factor Wapl is found associated with SA2 than with SA1.

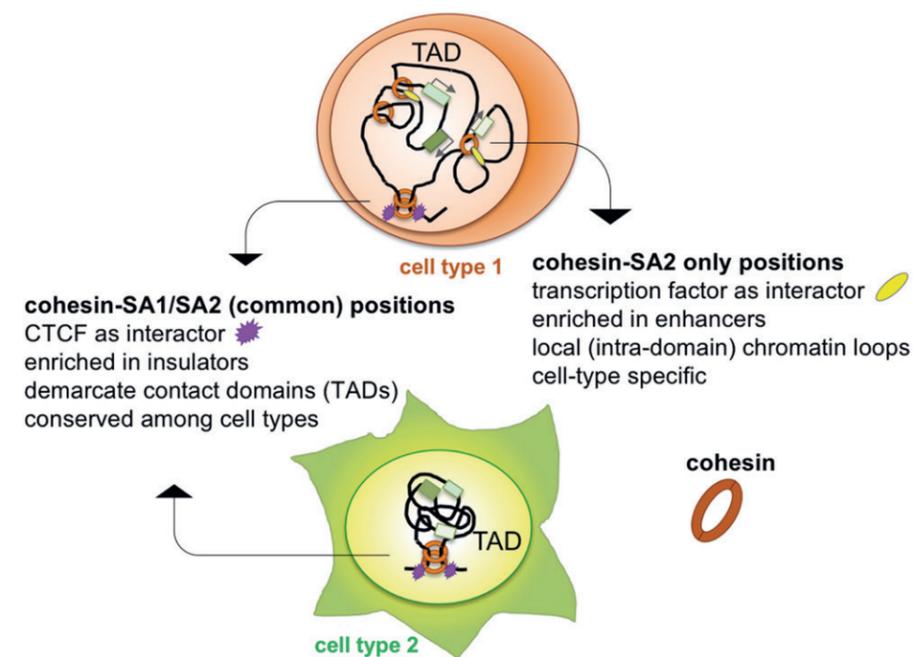
In the absence of cohesin-SA1, cohesin-SA2 can still cooperate with CTCF to demarcate contact domains although border strength is decreased. In the absence of SA2, however, cohesin-SA1 cannot occupy the non-CTCF sites present at many enhancers. As a result, short-range, intra-domain contacts disappear while new, aberrant contacts are formed between neighbouring domains. These changes have more noticeable consequences for gene expression. Moreover, cohesin-SA2 is enriched at superenhancers, regulatory elements that control cell identity. Thus, the absence of this cohesin variant in tumour cells most likely alters their identity and/or affects cell differentiation.

#### Dissecting the role of cohesin-SA1 and cohesin-SA2 in mice

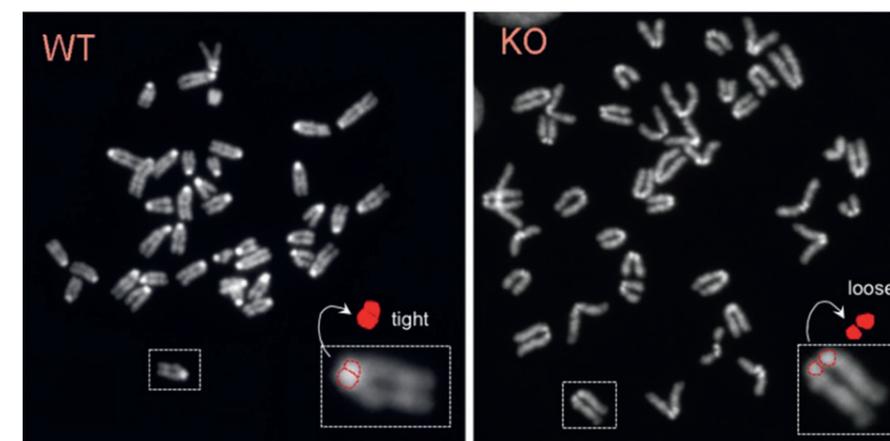
We have generated a conditional *Stag2* knockout allele in collaboration with Francisco X. Real (CNIO). Embryos lacking cohesin-SA2 die by mid-gestation and we are currently addressing the cause of this lethality. We are also using mouse embryo fibroblasts (MEFs) to further understand the specific contribution of cohesin-SA2 to cohesion and genome organisation. We observed loosened centromere cohesion and slower proliferation in the *Stag2* deficient MEFs, consistent with reports in other cell lines (FIGURE 2). However, the defects are milder than expected and are unlikely to be the sole cause of the embryonic lethality. Complementary to previous observations in *Stag1* deficient MEFs, in which the distribution of cohesin changed to include new non-CTCF positions, the number of cohesin binding sites detected in *Stag2* deficient MEFs is restricted to those overlapping with CTCF. This result is in line with the idea that cohesin-SA1 cannot replace cohesin-SA2 at many non-CTCF sites, as described in human cells. Experiments aimed to identify the molecular determinants of the distinct behaviour of the two cohesin variants are underway. ■

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**Figure 1** Graphical summary of the distinct features of cohesin variant distribution and function in genome organization in two cell types. TAD, topological associating domain encompassing several genes and demarcated by CTCF bound sites.



**Figure 2** Metaphase spreads of mouse embryo fibroblasts with (WT) or without (KO) cohesin-SA2 reveal loosened centromere cohesion in the latter. The “tight” and

“loose” configuration of the sister centromeres in terms of cohesion is shown in the magnified chromosome at the bottom right.

## DNA REPLICATION GROUP

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### OVERVIEW

Recent epidemiology studies indicate that up to two thirds of the mutations found in tumours are the consequence of inaccurate DNA replication; the rest are inherited or caused by environmental factors. We study the process of DNA replication and its regulatory pathways, with a particular interest in the phenomenon of replicative stress (RS) caused by the temporal stalling or inhibition of the protein machinery responsible for DNA synthesis. In 2018, we focused on the following areas: (1) the activation of ‘dormant’ replication origins in response to RS; (2) the molecular connection between the speed of replication forks and the frequency of origin activation, the two main parameters affected by RS; and (3) the function of PrimPol primase in ‘replicative tolerance’, i.e. the duplication of chemically damaged DNA molecules in order to facilitate their subsequent repair. We have also applied single-molecule methods to analyse the impact of RS in several biological processes.

**“We have developed a method to determine the primary cause of replicative stress as a necessary step towards the design of methods to restrict it in primary cells and/or enhance it in tumour cells.”**

## RESEARCH HIGHLIGHTS

**Differential activation of replication origins upon replicative stress**

Ten years ago, our laboratory reported that stalled replication forks induce the activation of extra origins as a backup mechanism to complete DNA replication. The genomic characteristics of these ‘dormant’ origins and their mode of activation remained largely unknown. We have now identified, in collaboration with Dr M. Gómez (*Centro de Biología Molecular “Severo Ochoa”, CSIC-UAM, Madrid*) and Dr V. Pancaldi (formerly at CNIO; currently at the Cancer Research Centre of Toulouse, CRCT), the genomic positions and efficiency of activation of thousands of replication origins in mouse embryonic stem cells, in normal growth conditions or under stress to trigger extra origin activation. This comparative analysis has revealed that the vast majority of ‘stress-responsive’ origins are active in a fraction of the control cell population, but their efficiency is significantly increased when stalled forks accumulate. The efficiency of activation of each individual origin correlates with its physical proximity to active or bivalent promoters, CpG islands, and the presence of ‘open chromatin’ epigenetic marks. The integration of linear origin maps into 3D chromatin interaction networks reveals a hierarchical arrangement in which local clusters of origins are brought together by long-range chromatin interactions.

**Cause and effect in replicative stress phenotypes**

Replicative stress (RS) phenotypes are normally identified by specific nuclear patterns of markers  $\gamma$ H2AX and RPA, but their detailed characterisation requires single-molecule analyses of fork speed and frequency of origin activation using DNA fibres. The interpretation of these assays is complicated because primary alterations in fork speed trigger the secondary activation of extra origins, and conversely, primary changes in the number of active origins also affect fork speed. We have designed interventions in which primary effects of RS on fork speed can be distinguished from primary effects on origin firing, and have applied them to our current research on PrimPol protein (FIGURE). Identifying the primary cause of RS may inform us about new methods to enhance it in cancer cells, increasing their susceptibility to chemotherapeutic agents that target DNA repair.

**PrimPol protein and its potential applications in cancer therapy**

Besides Pol $\alpha$ /primase, PrimPol is the only other primase in mammalian cells and it facilitates replication through damaged

DNA templates. In 2018, we used Crispr/Cas9 technology to eliminate PrimPol expression in cancer cells, making them hypersensitive to DNA crosslinking agents. These results open the possibility of inhibiting PrimPol as a coadjuvant in chemotherapy. In collaboration with Dr L. Blanco (*Centro de Biología Molecular “Severo Ochoa”, CSIC-UAM, Madrid*), we have characterised a variant of PrimPol in which amino acid Tyr100 is changed to His, a mutation identified in certain types of lung cancer. Tyr100 mediates the enzyme selection of dNTPs over rNTPs, and Y100H is unusually proficient at using the latter, which may provide a cellular advantage during oncogenic transformation when the dNTP/rNTPs balance is disrupted.

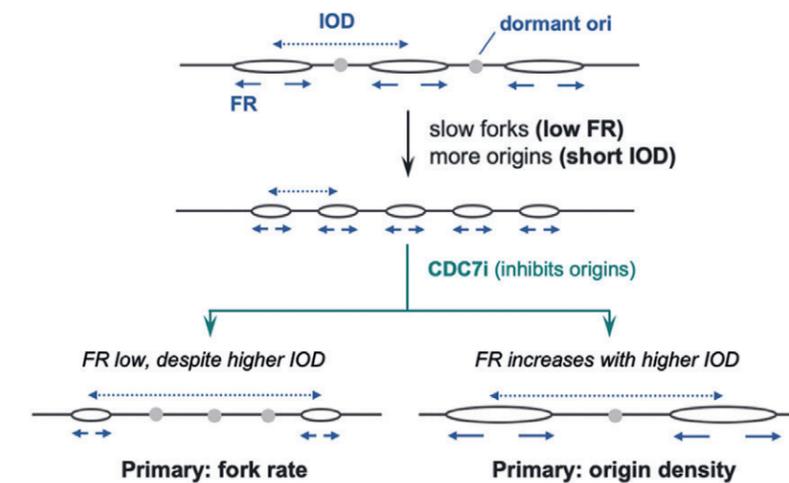
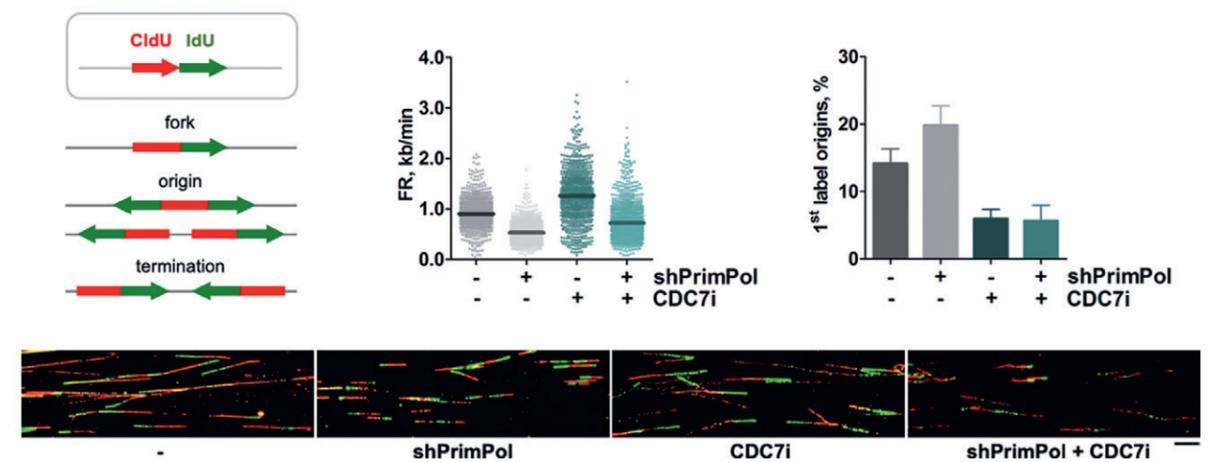
**Single-molecule analysis of DNA replication: shedding light on relevant biological processes**

RS potentially impinges on all biological processes that involve cell proliferation. Over the past year, we participated in two collaborative projects to analyse RS in specific contexts. First, a study led by Dr I. Moreno de Alborán (*Centro Nacional de Biotecnología, CSIC-UAM, Madrid*), has uncovered the replicative defects linked to the loss of transcription factors c-Myc and Max during the differentiation of B lymphocytes. The second study, in collaboration with Dr G. Stoecklin (Heidelberg University) and Dr O. Fernández-Capetillo (CNIO), has led to the functional characterisation of TIAR, an RNA-binding protein that controls mitotic entry and is required for genomic stability. ■

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**A****B**

**Figure** New methods to determine the primary cause of replicative stress. (A) Test based on a CDC7 kinase inhibitor (CDC7i) to separate cause and effect when fork speed is reduced and origin density is increased. A complementary test can be applied in the opposite situation (fork rate increased, origin density reduced;

not shown). (B) CDC7i test applied to U2OS cells undergoing RS after PrimPol downregulation. In this case, RS is due to a primary defect in fork speed. Representative images of DNA fibres used to measure fork speed and origin usage are shown. Bar, 10  $\mu$ m. Adapted from Rodríguez-Acebes et al. (2018).

## MELANOMA GROUP

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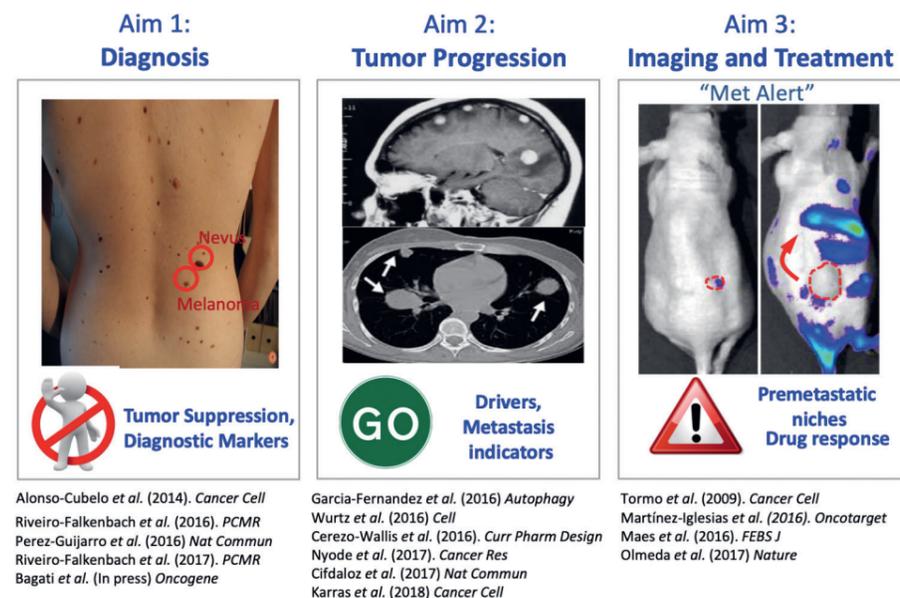


### OVERVIEW

Melanomas are a prime example of how basic and translational research has been translated into improved prognosis for affected patients. Nevertheless, clinical responses are still incomplete. The long-term goals of our Group are to identify new progression biomarkers and therapeutic agents. Focusing on stress response programmes involving apoptosis, autophagy and endosome mobilisation, we have discovered lineage-specific oncogenes that define the melanoma ‘fingerprint’. Transcriptomic and proteomic analyses of the melanoma secretome have enabled us to define how tumour cells remodel the (lymph)angiogenic vasculature and avoid immune recognition. Moreover, we have generated a unique set of animal models for non-invasive imaging of melanoma progression *in vivo*. 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.

**“Combining a series of -omic studies with *in vivo* imaging in mouse models, we have identified a melanoma-associated signature of prometastatic genes that make this tumour uniquely aggressive.”**

## RESEARCH HIGHLIGHTS



**Figure 1** Main objectives of the CNIO Melanoma Group aimed to identify new progression biomarkers and validate more efficient anticancer agents. Indicated are main experimental systems and representative publications.

## CNIO Melanoma Group: objectives and model systems

Melanomas are aggressive solid tumours and provide a prime example of how integrated basic and clinical research has significantly improved patient prognosis. Nevertheless, despite great successes achieved with targeted and immune-based therapies, sustained clinical responses are still limited. Moreover, the field lacks molecular markers of diagnosis, and the knowledge on how melanomas progress and metastasise is largely incomplete. In addition, one of the main hurdles to advance in this disease is the lack of animal models to monitor melanoma initiation and progression *in vivo*. To this end, our Group focuses on 3 main objectives (FIGURE 1):

- **Aim 1.** Oncogenic pathways selectively deregulated in melanoma that may represent new diagnostic indicators.
- **Aim 2.** Risk factors and prognostic markers.
- **Aim 3.** Animal models that allow for non-invasive monitoring of pre-metastatic niches.

## Lineage-specific oncogenic dependencies in melanoma

One of the long-term objectives of the Melanoma Group is to discover **new melanoma drivers**. We have previously identified a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Cubelo *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 because they are largely unexplored in melanoma. Performing a series of genome wide studies, we found novel roles of the RBPs CPEB4 and CUGBP1 in the modulation of mRNA stability, with targets involving master specifiers of the melanocyte lineage (Perez-Guijarro *et al.*, *Nat Commun* 2016; Cifdaloz *et al.*, *Nat Commun* 2017).

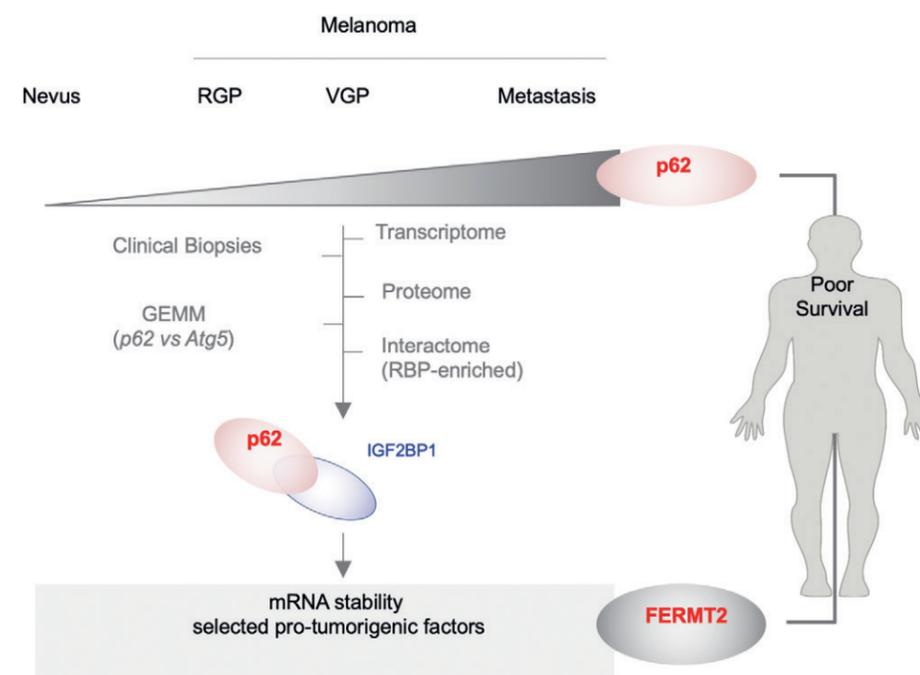
Most recently, we identified additional RBPs in a screen for modulators of melanoma progression. Specifically, we discovered a selected set of RBPs as unexpected binding partners of p62/SQSTM1, a factor we had selected for analysis based on previous reports in the literature linking this protein to autophagy. However, proteomic, transcriptomic and interactomic analyses showed that p62 was largely dispensable for autolysosome maturation in melanoma cells, again differentiating this disease from other tumour types. Instead, we found that p62 acts via a subset of RBPs, exemplified by CUGBP1, as a global coordinator of mRNA half-life of a spectrum of pro-metastatic factors. These include FERMT2 and other transcripts with no previous links to melanoma (FIGURE 2). The relevance of these data is emphasised by follow-up analyses of patient prognosis revealing p62 and FERMT2 as adverse determinants of disease-free survival. These studies were recently published

in *Cancer Cell* (*Cancer Cell*, 2019), and were spotlighted on the cover of the journal.

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

We have also made great progress in regards to one of the most pressing needs in the melanoma field, namely, the mechanisms that enable melanoma cells to disseminate already from lesions of barely 1 mm in depth. Last year, we reported a series of mouse models of melanoma that have the unique feature

of revealing how these cells act 'a distance' from very early stages of tumour development, activating the lymphatic vasculature and preparing metastatic niches before their colonisation (Olmeda *et al.*, *Nature* 2017). These 'MetAlert' animals, together with histological validation in patient biopsies, revealed the growth factor MIDKINE as a new driver of lymphangiogenesis and melanoma metastasis. We have now exploited the MetAlert mice for pharmacological analyses of anticancer agents. These studies revealed the dsRNA-based mimic BO-112 as potent blockers of neolymphangiogenesis and melanoma metastasis (Olmeda *et al.* in preparation). BO-112, a derivative of the polyplex BO-110 generated at the CNIO, is now being tested in Phase I clinical trials. ■



**Figure 2** New functions for pro-metastatic drivers in melanoma. Schematic representation of a set of pro-tumorigenic factors with no previous links to melanoma identified by addressing the expression and functional requirement of p62/SQSTM1 in this disease. Different from roles of p62 in autophagy described in a broad spectrum of types, this protein was found in melanoma to bind a selected set of RNA binding proteins (RBPs), here exemplified by CUGBP1. -Omic studies in cell lines combined with histopathological studies in genetically modified mouse models (GEMM) and histopathological validation in clinical biopsies identified the scaffolding factor FERMT2 as a downstream target of the p62-CUGBP1 axis. Both, p62 and FERMT2 were found overexpressed in advanced melanomas, representing new indicators of poor prognosis.

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## AWARDS AND RECOGNITION

- Coordinator, ASEICA-Mujer (Spanish Association for Research Against Cancer).
- Top100 "Mujeres Líderes de España", *Mujeres & Ciencia*.
- Placa de Honor 2018, *Asociación Española de Científicos*.
- Most influential female scientists and clinical doctors-2018, *Yo Donna/El Mundo*.
- Founding Member, "Influential Woman from Galicia", *WomenTalent*.

## MICROENVIRONMENT & METASTASIS JUNIOR GROUP

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Susana García



### OVERVIEW

Cancer treatment is no longer only focused on tumour cell analysis. The Microenvironment and Metastasis Group studies the communication between tumour and stromal cells along tumour progression. Cancer treatment requires the analysis of the tumour microenvironment to define specific therapies targeting both the tumour and surrounding cells. Data support that combination of therapies against the tumour and its microenvironment are the future of cancer treatment. In our laboratory, we have focused on understanding the message of a novel 'language' between tumour cells and the environment; these are small extracellular vesicles called exosomes. Our data support that tumour-secreted exosomes contain specific signatures of molecules (e.g. proteins, DNA). Exosomes secreted from the tumour can reach metastatic organs and prepare pre-metastatic niche formation, aiding metastatic homing and further metastatic progression.

**“Tumour-secreted exosomes are “the forefront edge” of tumour metastasis, they reach local and distant microenvironments facilitating metastatic spread.”**

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### RESEARCH HIGHLIGHTS

#### Novel factors involved in melanoma progression, the future of liquid biopsies

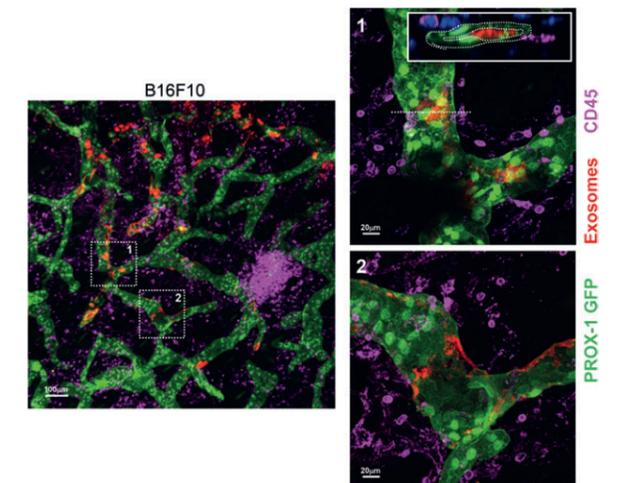
In this project, we study the function of tumour-secreted exosomes in the establishment of pre-metastatic niches within the lymph node (FIGURE). We are analysing the role of the matrix-anchored proteins and neurotrophin receptors in lymph node metastasis and melanoma progression. We are also using novel biofluids (e.g. lymph node exudative seroma obtained post-lymphadenectomy) as a source of biomarkers, analysing protein cargo and BRAF mutations. Our laboratory is also interested in the study of microenvironmental factors influencing melanoma progression such as obesity.

#### Obesity modulates breast cancer behaviour

Obesity has drastically increased to become one of the most serious health problems worldwide and is now recognised as a risk factor for breast cancer incidence, progression, and prognosis. In this project, we aim to understand cellular and molecular mechanisms that underlie inflammation, obesity, and breast cancer metastasis. Furthermore, we are analysing the interaction of cancer cells with immune cells and platelets in metastasis and evasion of immune supervision. Our goal is to understand how obesity modulates breast metastatic behaviour defining novel factors involved and to define new therapies.

#### Defining novel targets in rare diseases

Malignant peripheral nerve sheath tumours (MPNSTs) are highly aggressive and metastatic sarcomas with poor prognosis



**Figure** Analysis of the distribution of tumour-derived exosomes from B16-F10 melanoma cells (red) in GFP-PROX mice (lymphatic endothelial cells in green), demonstrating that tumour-derived exosomes

use the lymphatic vasculature to be transported from the injection site to lymph nodes. Image from collaborators Raghu Kataru and Babak Mehrara at the Memorial Sloan Kettering Cancer Centre.

that are commonly related to neurofibromatosis type 1 (NF1) disease. In this project, we aim to find new biomarkers and novel therapeutic targets to prevent MPNST progression. The data obtained from a multidrug screening on MPNSTs cell lines and the mass spectrometry analysis of their exosomes identified several proteins as top candidates. Thus, we are currently testing the combination of MEK inhibitors, which are already used in the clinic, with novel drugs targeting these two proteins in order to define a new therapeutic window for MPNSTs. ■

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## BRAIN METASTASIS JUNIOR GROUP

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Neibla Priego



Graduate Students  
Laura A. Álvaro (since July), Wendy E. Bindeman (until May), Catia P. Domingues, Pedro García, Lucía Zhu

Technicians  
Lourdes Osuna, Natalia Yebra

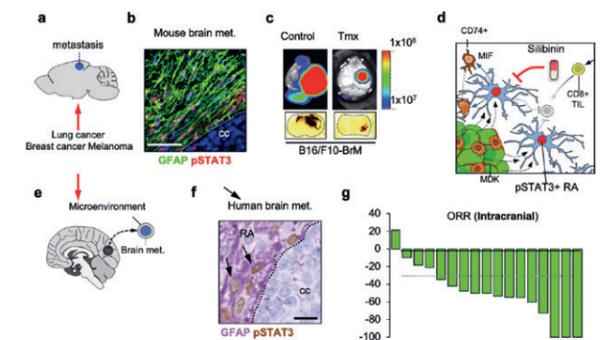
Student in Practice  
Ana de Pablos Aragonese (FUAM Fellow, since September) (Universidad Autónoma de Madrid)

### RESEARCH HIGHLIGHTS

We pioneered the first report proving the importance of glial heterogeneity associated with metastatic brain tumours. As previously shown in other diseases affecting the brain, understanding the contribution of specific glial subpopulations could provide novel therapeutic targets.

The use of genetic and pharmacological approaches has enabled us to discover the critical role of this disease-specific subpopulation of reactive astrocytes in brain metastasis, which is characterised by activation of the STAT3 pathway. Its presence, induced by metastatic cells, involves the establishment of an immunosuppressive local environment that favours tumour growth.

In collaboration with four different national and international clinical institutions we have proved the importance of this finding in patients with brain metastasis. Treatment of stage IV lung adenocarcinoma patients with the STAT3 inhibitor silibinin reduced brain metastasis in 75% of them, which led to an increased survival. This finding involves a proof-of-concept regarding the possibility of developing effective therapies against metastasis by targeting the microenvironment. ■



**Figure** A subpopulation of reactive astrocytes (GFAP), characterised by activated STAT3 (pSTAT3), is present in experimental brain metastasis models (a,b) and human samples (e,f). Targeting this

glial subpopulation in mice (c) and humans (g) impaired the viability of intracranial metastasis. pSTAT3 reactive astrocytes are required to maintain a pro-metastatic niche (d).

### OVERVIEW

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is poor given that local therapies (i.e. surgery and radiation) have limited benefits for patients and the disease inevitably progresses. The rise in the number of patients with brain metastasis is partially due to the increasing number of systemic therapies that work extra-cranially but are unable to provide the same therapeutic benefit in the brain. Consequently, cancer cells present at this secondary site have additional time to evolve and to grow into clinically detectable lesions. In the laboratory, we study why and how cells from different cancer types (breast cancer, lung cancer and melanoma) are able to access the brain, survive and colonise this vital organ. We dissect the biology of these processes *in vivo* using experimental models in order to challenge the current status of this unmet clinical need.

**“We have treated brain metastasis by targeting the microenvironment. We have used a novel therapy both in mice and in patients that reduces established metastasis in the brain and increases survival.”**

#### • PUBLICATIONS

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#### • AWARDS AND RECOGNITION

- Research Award, Royal Academy of Science, University of Zaragoza. Spain.
- Clinic and Laboratory Integration Program (CLIP) Award, Cancer Research Institute, USA.

- Elected Member of the Scientific Committee of the European Association of Neuro Oncology.
- Keynote speaker at the Annual Congress of the European Society of Veterinary Oncology.
- Laura-Álvaro Espinosa was recipient of a *MINECO-Severo Ochoa* PhD Fellowship.
- Neibla Priego received the CNIO Award for Excellence in Research by Postdoctoral/Staff Investigators, CNIO Lab Day.
- Lucía Zhu received the ‘Best Poster’ Award at the CNIO Lab Day.
- *STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis*, by Priego *et al.*, was selected as paper of the month by the Spanish Society for Biochemistry and Molecular Biology.

## METABOLISM AND CELL SIGNALLING JUNIOR GROUP

Alejo Efeyan  
Junior Group Leader

Staff Scientist  
Ana Ortega



### 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 disease states, such as cancer. Together with genetic mutations, environmental perturbations (such as those occurring in obesity) corrupt 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.

**“Mouse models with a very mild genetic activation of nutrient signalling foster cancer, autoimmunity, and ageing; this has profound implications when thinking about the consequences of human nutrient overload.”**

Post-Doctoral Fellow  
Melania Zauri (since October)

Technicians  
Camino Menéndez (until March) (TS), Ana Sagrera (TS), Alba Sanz (since April)

Students in Practice  
Tomislav Kostevc (until June) (Universidad Pompeu Fabra), Leyre Marin (since September) (Universidad Politécnica)

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

\*Titulado Superior (Advanced Degree)

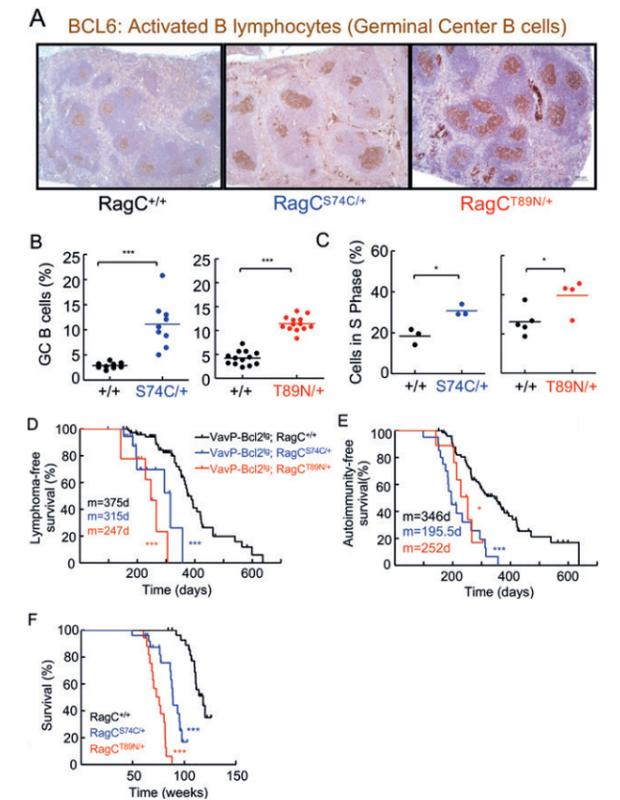
### RESEARCH HIGHLIGHTS

#### 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 (FL), an incurable B lymphocyte tumour. By means of novel strains of mice that express mutant variants of the RagC GTPase, we found that subtle increases in nutrient signalling unleash activation and proliferation of B cells, suppress cell death and drive the development of FL (FIGURE). These results pave our way towards a novel therapeutic strategy against B cell lymphoma, aimed at targeting its corrupted nutrient signalling. In addition, and surprisingly, this mild increase in the signalling of nutrient abundance in B lymphocytes also drives an autoimmune disease.

#### Chronic signalling of elevated nutrients and premature ageing

The study of genetically engineered mice expressing a mildly activating form of RagC revealed that, in the absence of lymphoma, these mice suffer from symptoms and pathologies consistent with premature ageing, including a shortened lifespan (FIGURE). While caloric restriction (CR) and other fasting-like regimes are well-known to delay ageing, as is also the case with the pharmacological inhibition of mTOR with rapamycin in mammalian model organisms, this is the first time that a moderate increase in nutrient signalling in mice shows compromised longevity. We are currently investigating the cellular and molecular alterations responsible for this shortening of the life span. ■



**Figure (A, B & C)** Readouts of enhanced B lymphocyte activation in RagC mutant mice. **(D & E)** Bred to the VavP-BCL2 strain (prone to lymphoma and autoimmunity), RagC mutant mice exhibit accelerated lymphomagenesis and autoimmune disease. **(F)** Lifespan consistent with accelerated ageing of RagC mutant mice.

## CANCER CELL BIOLOGY PROGRAMME

ERWIN F. WAGNER Programme Director



The overall strategic goals of the Cancer Cell Biology Programme are to achieve a better understanding of the events leading to cancer development, progression and metastasis, and to discover molecular mechanisms that could provide a basis for novel therapies. The 4 Groups investigate how tumours grow as ‘external organs’ in close interaction with tumour - associated cells. The spectrum of investigations ranges from epithelial cancers such as liver, pancreas, skin and intestine, to bone and brain tumours. The research covers aspects of tumour cell biology, ranging from tumour stem cells, tumour cell interactions with host cells/environment such as tumour-associated macrophages and fibroblasts, to the role of inflammation, metabolism and metastasis. Powerful state-of-the-art mouse genetic models, human cellular systems, high-throughput genomic/proteomic and biochemical tools, as well as patient-derived materials, are employed. These aspects are successfully covered by the complementary research areas of 3 Senior and 1 Junior Groups.

The Senior Group, led by Francisco X. Real, studies epithelial tumours focusing mainly on pancreatic and bladder cancer. The Group employs an integrative approach to understand the molecular patho-physiology of these tumours and applies this knowledge in the clinical setting. Real’s Group, with contributions from the Wagner lab, made an important discovery demonstrating an inflammatory transcriptional switch in pancreatic cancers involving the nuclear receptor NR5A2 and Jun/AP. Nabil Djouder’s Group aims to dissect the contribution of various environmental stressors, including the nutrient and growth factor signalling pathways, to cancer development and associated diseases, in particular related to the gastro-intestinal tract. Massimo Squatrito’s Group, which is partly supported by the Seve Ballesteros Foundation, studies how brain tumours, mainly glioblastomas, develop and how they respond to therapy. Finally, my own Group focuses on understanding the role of the transcription factor complex AP-1 (Fos/Jun) in physiological and pathological processes, with a strong focus on aspects of inflammation and cancer in liver, lung, skin and bone. We also investigate the role of AP-1 in inflammatory skin diseases, such as psoriasis, and aim to molecularly define the causes leading to lung fibrosis. We have continued to study how the whole organism responds to a locally growing tumour in the context of a complex immune-metabolic impairment in cancer-associated-cachexia.

**“Our main goal is to keep CNIO globally competitive and to ensure that CNIO remains an international institution. Members of 13 different nationalities from 4 continents are represented in our Programme with the goal to perform top-level cancer cell biology as well as to train students and postdocs to become the next-generation of promising scientists.”**

## GENES, DEVELOPMENT AND DISEASE GROUP

Erwin F. Wagner  
Group Leader

Staff Scientists  
Latifa Bakiri, Nuria Gago, María Jiménez, Liliana Mellor

Post-Doctoral Fellows  
Kazuhiko Matsuoka, Álvaro Ucero

Graduate Student  
Lucía T. Díez (until November)

Visiting Graduate Student  
Pia Benedikt (*Karl-Franzens Universität GRAZ*, Austria, April-July)

Undergraduate Student  
Jennifer Cascino (Fulbright Fellow, USA, until June)

Technicians  
Vanessa Bermeo (until August), Ana Guío (TS)\*

\**Titulado Superior (Advanced Degree)*



### OVERVIEW

Our studies aim to analyse gene function in healthy and pathological conditions, e.g. in tumour development, using the mouse as a model organism, but also employing patient-derived samples. Specifically, the functions of the AP-1 (Fos/Jun) transcription factor complex regulating cell proliferation, differentiation and oncogenesis, as well as the cross-talk between organs, are being investigated. The goal is to define molecular pathways leading to disease/cancer development and to identify novel therapeutic targets (FIGURE). We focus on:

- Elucidating a causal link between inflammation, cancer and AP-1 (Fos/Jun) expression, using cell type-specific, switchable genetically engineered mouse models (GEMMs).
- Developing and characterising new GEMMs for cancer and human diseases, such as bone loss, arthritis, fibrosis and psoriasis, and applying these to preclinical studies.
- Using multiple approaches to compare mouse models of disease to human disease and to identify therapeutically relevant targets.

**“Our goal was for the CNIO to remain an international and competitive institution. At present, 3 out of 4 Group Leaders in our department are foreigners, one of whom is partly funded by the Seve Ballesteros Foundation. Thirteen different nationalities from 4 continents are testimony to an international science culture, all focussing on unravelling the mysteries of inflammation, metabolism and cancer.”**

## RESEARCH HIGHLIGHTS

We have developed a powerful technology for switchable, reversible and tissue-specific ectopic gene expression of specific AP-1 monomers/dimers in the liver, lung, skin and bone. We use mouse and human tissue samples for large-scale studies, such as deep sequencing (RNA-Seq, ChIP-Seq) and mass spectrometry analyses. We evaluate possible biomarkers and therapeutic approaches in small-scale preclinical studies based on these screens.

## Bone development, osteosarcomas and arthritis

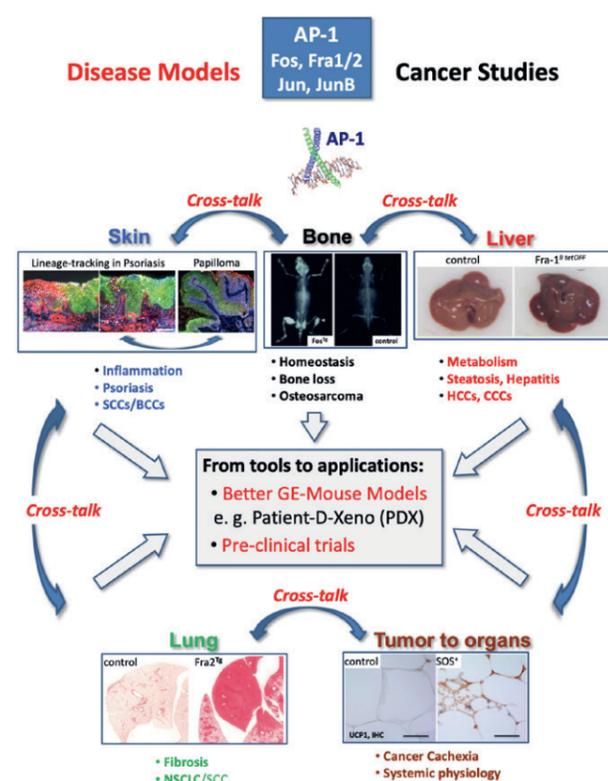
We are studying the function of AP-1 proteins in bone development and disease using loss-of (LOF) and gain-of-function mouse models. In mice, transgenic c-Fos expression leads to osteosarcomas (OSs). Using an inducible bone-specific Wntless LOF GEMM, we found that loss of Wnt signalling delays Fos-induced OS development. Our data also demonstrate that increased Wnt7b and Wnt9a and non-canonical Wnt signalling are causally involved in OS.

Rheumatoid, Psoriatic and Osteoarthritis (OA) are destructive joint pathologies linked to chronic inflammation. Using cell type-specific and inducible AP-1 LOF mouse models, combined with experimental arthritis models, we found that c-Fos is a key regulator of surgery- and age-induced OA.

Using mice with inducible epidermal deletion of JunB and cJun (DKO\*) that develop skin inflammation and a psoriatic-arthritis-like (PsA) disease, we aim to elucidate potential therapeutic targets to alleviate skin and joint inflammation. We previously identified the S100A8/A9 complex as highly elevated in our GEMM as well as in human psoriatic skin samples. We have now generated new DKO\*-GEMM with epidermal and global deletion of S100A9 to determine the specific role of keratinocyte-derived and neutrophil-derived S100A9 in skin or joint inflammation.

## Liver disease—metabolism, fibrosis, inflammation and cancer

AP-1 proteins are important modulators of hepatic lipid metabolism as specific AP-1 dimers can either activate or repress PPAR $\gamma$  transcription. Therefore, fatty liver disease and obesity most likely depend on AP-1 dimer composition. In addition, ectopic expression of specific Fos or Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to liver dysplasia, inflammation, fibrosis and tumours with HCC signatures. Mechanistically, molecular analyses point to the involvement of pathways connected to human



**Figure** Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models to human disease and to identify novel targets. Furthermore, we

are investigating the systemic response of the mouse organism to a growing tumour in cancer cachexia. Preclinical studies are performed using different genetically engineered mouse models (GEMMs) with compounds that target the identified molecules to determine the potential of translating our findings for the treatment of human disease.

hepatocellular carcinoma (HCC), such as the Wnt/ $\beta$ -catenin and Myc pathways and/or to altered cholesterol and bile acids metabolism. A robust connection between c-Fos expression and the activity of the LXR/RXR pathway, an important regulator of cholesterol homeostasis, was unravelled and most likely contributes to the oncogenic function of c-Fos in hepatocytes. We are currently testing whether any of the pathways we discovered can be exploited therapeutically to treat liver cancer in preclinical models.

## Cancer-associated cachexia (CAC)

CAC is a complex wasting syndrome characterised by loss of muscle and fat along with 'browning', a switch from white to brown fat, as previously described. Our aim is to understand the systemic events taking place in CAC and to identify novel biomarkers and therapeutic targets. Systemic inflammation is a consistent event in CAC with innate immune cells, such as neutrophils, as a major cell type. Interestingly, Lipocalin-2, an adipokine important in innate immunity is highly upregulated in CAC and may be a potential new biomarker. We found that CAC is not prevented in a neutropenic situation suggesting that neutrophils may not be the key factor. Ongoing studies show that the Renin-Angiotensin-Aldosterone System (RAAS) is dysregulated in CAC in humans and mice, potentially leading to cardiac dysfunction. We are now dissecting, in mice and in human CAC samples, the involvement of the central and peripheral nervous system, the RAAS as well as the tissue-specific role of Ucp-1 (in collaboration with R. Señaris, Spain, M. Petruzzelli, UK, H. Watzke, M. Poglitsch, P. Benedikt and R. Zechner, Austria).

## Fra-2 in lung fibrosis and cancer

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) lack effective treatments and lead to high mortality. Using GEMMs we found that Fra-2, an AP-1 transcription factor, contributes to both diseases. Fra-2 expression is increased in lung fibrosis patient samples and correlates with poor survival in human NSCLC. In lung fibrosis, Fra-2 is associated with macrophage-specific expression of Type VI collagen in a type2 immune response and mediates disease progression, while in NSCLC, Fra-2 promotes growth in K-Ras-mutated tumours. We aim to find new therapeutic targets and potential disease biomarkers downstream of AP-1. The lung fibrosis studies are conducted in collaboration with Acceleron Pharma (USA), and the cancer studies with Mariano Barbacid's and Luis Paz-Ares' Groups at CNIO and Silvestre Vicent in Pamplona.

## Skin inflammation, cancer and human disease

Characterisation of the systemic inflammatory disease in epidermal-deficient JunB GEMMs indicated a skin inflammation to bone cross-talk by Il-17A-mediated inhibition of Wnt signalling in osteoblasts. These mice also suffer from dysbiosis and chronic *S. aureus* colonisation, which is exacerbated in the absence of adaptive immunity. We have also generated several GEMMs to define the role of the antimicrobial proteins (AMPs), such as S100A8/A9 and Lipocalin-2, in inflammatory skin diseases with a focus on the systemic effects beyond the skin.

Using lineage tracing in the psoriasis-like mouse model, we found that mutant epidermal stem cells (ESCs) initiate epidermal hyperplasia and skin inflammation by priming neighbouring non-mutant epidermal cells to acquire a psoriasis-like phenotype. Mechanistically, TSLP neutralisation reduces non-mutant keratinocytes proliferation and VEGF $\alpha$  expression, an important pro-inflammatory mediator in psoriasis. These findings unravel specific roles of epidermal populations in psoriasis-like disease and provide novel mechanistic insights into epidermal cell interactions under inflammatory conditions.

It has been suggested that psoriatic patients have decreased skin cancer risk. Using our psoriasis-like mouse model and the well-established DMBA/TPA chemical carcinogenesis protocol, we observed that psoriasis-like mice with severe phenotype have a significant decrease in DMBA/TPA-induced skin papillomas compared to controls. Detailed characterisation suggests that in the context of chronic skin inflammation, elevated expression of senescence markers may modulate papilloma formation. ■

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## EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real  
Group Leader

Post-Doctoral Fellows  
Enrique Carrillo (until March),  
Irene Felipe, Eleonora Lapi, Miriam  
Marqués, Sumit Paliwal

Graduate Students  
Isidoro Cobo (until December), Ana  
M. Maldonado, Irene Millán, Catarina  
Pereira, Mónica Pérez

Technicians  
Miguel Deblas, Natalia Del Pozo,  
Jaime Martínez

*\*Titulado Superior (Advanced Degree)*



### OVERVIEW

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

In PDAC, a main hypothesis is that cell differentiation is a potent tumour suppressor mechanism acting early in carcinogenesis. We use the excellent genetic mouse models available because these processes cannot be readily studied in humans. In mice, PDAC can originate in pancreatic progenitors and in adult acinar and ductal cells. Understanding the contribution of early molecular events is crucial to design better strategies for early tumour detection and prevention in subjects at risk.

In UC, 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.

**“We have shown that, in the pancreas, the control of cell differentiation and the suppression of inflammation depend on similar transcriptional regulators indicating that both processes are tightly linked.”**

## 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, but the contribution of precursor lesions and the molecular changes that precede tumour development are less well established. Our lab has pioneered the notion that incomplete acinar cell differentiation 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.

NR5A2 is an orphan nuclear receptor for which putative endogenous ligands as well as pharmacological agonists have recently been identified. In mice, *Nr5a2* germline heterozygosity is associated with a pre-inflammatory state that sensitises the mice to the oncogenic effects of mutant *KRas*. Deletion of one *Nr5a2* allele is sufficient to cause a striking genomic redistribution of the protein in cooperation with AP-1 components. To further explore how this occurs, we have analysed the NR5A2 interactome using immunoprecipitation and mass-spectrometry. We find that reduction of NR5A2 protein levels by 50% (either genetically or during pancreatitis) is also associated with profound effects on the interactome, highlighting the relevance of subtle changes in protein dosage in cells; one of the proteins identified is the ubiquitous transcription factor NFIC (FIGURE 1A,B). At the transcriptomic level, *Nfic*<sup>-/-</sup> pancreata display a mild defect in acinar cell maturation as well as a significant down-regulation of the protein synthesis machinery. NFIC is a novel regulator of acinar differentiation playing an important role in the endoplasmic reticulum stress response. Similar to knockouts of other genes coding for proteins involved in acinar homeostasis, constitutive *Nfic*-null mice developed significantly more PanINs in a mutant *KRAS* context. The function of NFIC in

acinar cells appears to be highly conserved between mice and humans (FIGURE 1C).

## Urothelial carcinoma (UC) genetics, biology, and clinical translation

We are interested in refining our understanding of new genes involved in UC, using organoids 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 two genes and are exploring their role in development and urothelial biology as well as their cooperation with other bladder cancer genes.

*RBM10* somatic mutations occur in several epithelial tumour types, including UC. Germline *RBM10* mutations are associated with TARP syndrome. Our preliminary studies indicate that *Rbm10*-null mice recapitulate facets of this developmental condition. We have generated *Rbm10*-null normal urothelial organoids and are characterising their biological features. In addition, we collaborate with J. Paramio (*CIEMAT*, Madrid) to identify how tumour cells bypass growth requirements in organoid cultures. Also, through single-cell RNA-Seq, we are identifying urothelial cell populations that could shed light on the cell of origin of UC.

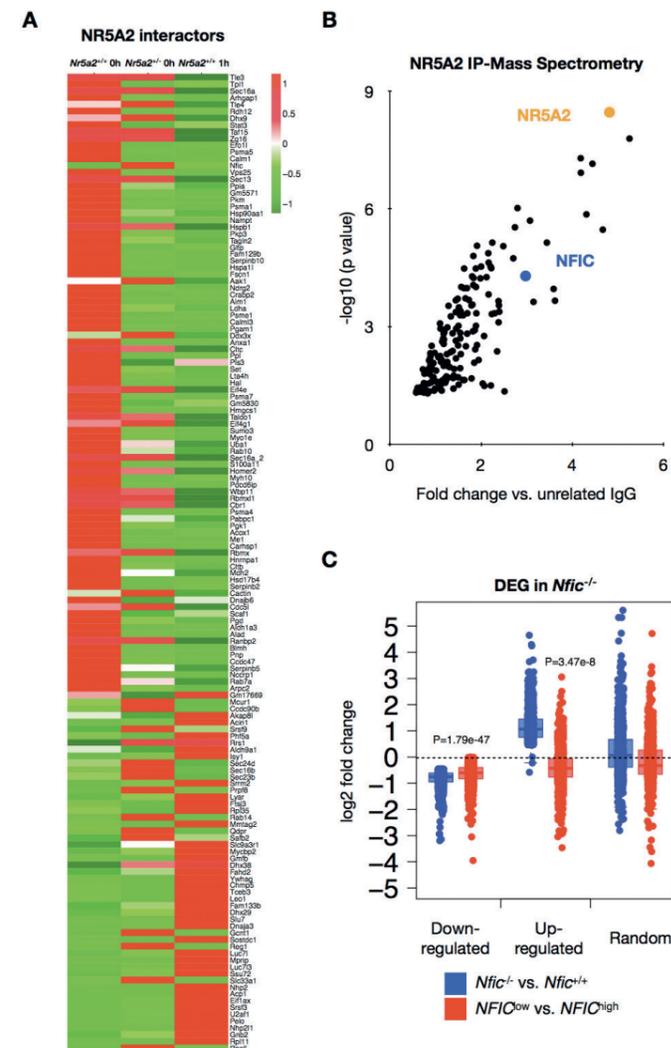
In collaboration with J. Valcárcel (*CRG*, Barcelona), we are analysing the mechanisms through which *RBM10* contributes to UC development using a combination of cellular, molecular and bioinformatics approaches. In addition, we are assessing whether *RBM10*-mutant tumours display specific therapeutic vulnerabilities using a variety of experimental strategies.

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- Molina-Montes E, Gomez-Rubio P, Marquez



**Figure 1** The NR5A2 interactome is dynamic and reveals novel players involved in acinar homeostasis. (A) NR5A2 interactors identified by immunoprecipitation with anti-NR5A2 antibodies and mass spectrometry using pancreatic tissue from wild type mice in basal conditions (*Nr5a2*<sup>+/+</sup> 0h), or 1h after administration of one dose of caerulein (*Nr5a2*<sup>+/+</sup> 1h), and from *Nr5a2* heterozygous mice in basal conditions (*Nr5a2*<sup>+/-</sup> 0h). (B) NFIC is a novel NR5A2 interactor identified in all three analysed settings. (C) Genesets including differentially expressed genes (DEG) in the pancreas of young *Nfic*<sup>-/-</sup> vs. wild type mice: down-regulated genes are expressed at significantly lower levels in normal pancreas from subjects with low levels of *NFIC* (comparison of lower vs top decile of *NFIC* mRNA expression) (in collaboration with F. García and J. Muñoz, CNIO Proteomics Unit).

Our studies with patient samples have provided novel markers predictive of response to cisplatin-based chemotherapy and are guiding the design of novel clinical trials with targeted

therapies and immune checkpoint inhibitors in collaboration with N. Malats, A. Font, D. Castellano, and an extended group of Spanish uro-oncologists. ■

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- AWARDS AND RECOGNITION
- "Constantes y Vitales" Award for the best publication in Biomedicine 2018, *Atresmedia/La Sexta*, Spain.

## GROWTH FACTORS, NUTRIENTS AND CANCER GROUP

Nabil Djouder  
Group Leader

Post-Doctoral Fellows  
Hugo Bernard, Sebastián Thompson  
(until September)

Graduate Students  
Almudena Chaves, Sergio De La Rosa, Amanda Garrido, Tatiana Grazioso, Irene Herrantz (since October), Ana Teijeiro

Technician  
Eva Martínez (until February) (PEJ, CAM) \*

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



### OVERVIEW

Our laboratory devotes effort to understand the molecular mechanisms linking environmental stresses to disease pathogenesis. 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 disorders. However, the exposure to environmental factors, through the regulation and expression of virulent eukaryotic proteins, has often been an ignored permanent challenge for an organism.

Based on the integration of experimental mouse models, combined with the use of state-of-the art technologies and human data, we aim to provide a comprehensive study for a rational approach towards the development of novel mechanism-based therapies to prevent and treat diseases.

**“We aim to understand mechanisms of disease by generating new mouse models that recapitulate pathological features of human syndromes in order to guide early prevention and treatment.”**

## RESEARCH HIGHLIGHTS

Poor diets (under-nutrition, micronutrient deficiencies, over-nutrition, high-fat and low-fibre diets, etc.), alcohol consumption, ionising radiation, bacteria and virus infections, etc., are risk and pathogenic factors for disease development. How these environmental factors can alter the host's eukaryotic epithelial cells to cause various pathologies, potentially progressing to cancer, remains largely unknown. Finding new genes affected by environmental stressors, and understanding their functions and role in disease development, may pave the way for future therapies. In our lab, we therefore focus on the identification and understanding of mechanisms of likely causal links between environmental stresses and pathologies in order to develop new preventive and therapeutic options.

## Unconventional prefoldin RPB5 interactor (URI)

The responses of eukaryotic cells to a variety of environmental stresses involve changes in the expression profile of molecular chaperones. These chaperones are essential to engage protective mechanisms to ensure cellular and protein homeostasis caused by injurious environmental stimuli. In our lab, we focus on studying the roles and functions of the unconventional prefoldin RPB5 interactor (URI), a member of the prefoldin chaperone family, whose expression is modulated by various pathogenic environmental factors. Principally, lessons from genetically engineered URI gain- and loss-of-function mouse models taught us that high URI expression may lead to uncontrolled protein substrate regulation, and decreased URI may induce over-functioning of protein clients – both conditions may lead to various pathologies.

## Microspherule protein 1 (MCRS1)

We also recently discovered MCRS1 (Microspherule protein 1) with scaffolding activities regulating mTORC1 activity in response to amino acids.

## Mechanisms of gastrointestinal tract disease

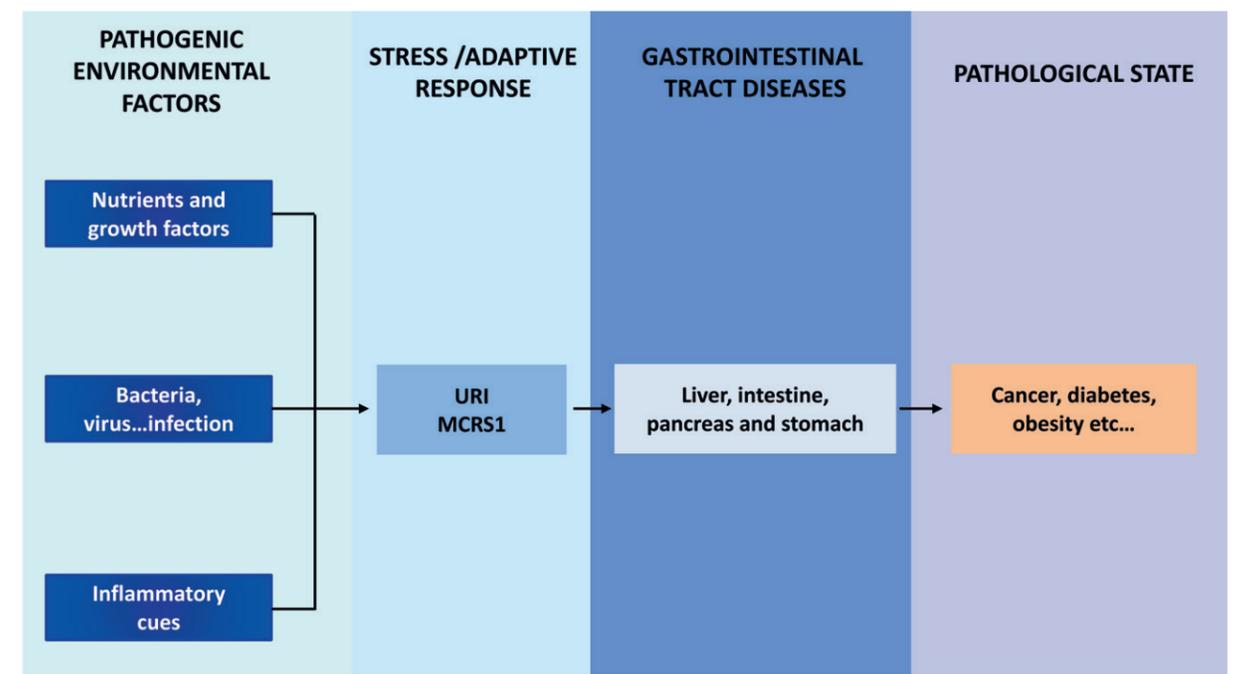
Our interest is therefore driven by the discovery of URI and MCRS1 proteins, both regulated by environmental stressors, which may compromise their functions and activate pleiotropic circuits supporting non-oncogene addiction functions and dependence, provoking severe outcomes. Using URI and MCRS1 mouse models generated in our lab, combined with cutting-edge technologies, we are studying mechanisms of disease predominantly associated to the gastrointestinal tract, often related to pathogenic environmental factors (ionising

radiation, bacteria, viruses and poor diet), with the objective of developing new strategies for treatment. Our research is mainly focussed on the study of intestinal, gastric, pancreatic and liver disorders (FIGURE).

In this regard, we made the following discoveries:

- Inflammatory cues up-regulate hepatic URI, which inhibits *de novo* NAD<sup>+</sup> synthesis causing DNA damage and thereby initiating hepatocellular carcinoma (HCC). Replenishing the pools of NAD<sup>+</sup> by using nicotinamide riboside prevents HCC. Our data suggest that metabolic alterations initiate tumorigenesis prior to genomic instability.
- Nutrient overload increases hepatic URI, which results in NAD<sup>+</sup> deficit-induced DNA damage that activates metabolic inflammation-associated IL-17A to cause non-alcoholic steatohepatitis (NASH) and HCC. Boosting NAD<sup>+</sup> by using nicotinamide riboside or blocking IL-17A axis prevents NASH and HCC.
- Hepatocellular carcinoma originates from transformed hepatocytes, whereas hepatic progenitor cells give rise to benign lesions including regenerative nodules and adenomas.
- Cells exposed to prolonged inadequate glucose concentrations elicit first a protective and adaptive response to optimise glucose utilisation and suppress death, in order to give to the cells an opportunity to recover from metabolic stress. OGT regulation by URI is a sophisticated mechanism conferring c-MYC-dependent survival functions in response to glucose fluctuations.
- MCRS1 has oncogenic and tumour suppressive activities by regulating mTORC1. Inhibition of mTORC1 via MCRS1 deletion in the intestine protects from APC loss-dependent tumorigenesis, whereas it promotes colitis-induced colorectal cancer (CRC). Our work reveals mTORC1 oncogenic and tumour-suppressive roles in intestinal epithelium and avenues to optimised and personalised therapeutic regimens for CRC.

We intend to make significant progress over the next few years in order to elucidate mechanisms of disease associated to the digestive system. This will be made possible thanks to the specific environment at the CNIO providing state-of-the-art facilities and access to key technological platforms with advanced technologies, as well as the availability of various genetically engineered mouse models generated in our lab, patient-derived xenograft models, organoids, cell biological and biochemical techniques, and the large number of omics and human data. ■



**Figure** Representation of our research directive. Our lab is mainly interested in understanding how pathogenic environmental factors lead to disease associated to the gastrointestinal tract. Molecular chaperones are essential to engage protective mechanisms to ensure

cellular and protein homeostasis caused by injurious environmental stimuli. Environmental stress modulates thus URI and MCRS1 expressions to maintain cellular homeostasis or cause disease development.

## PUBLICATIONS

- ▶ Brandt M, Grazioso TP, Fawal MA, Tummla KS, Torres-Ruiz R, Rodriguez-Perales S, Perna C, Djouder N (2018). mTORC1 inactivation promotes colitis-induced colorectal cancer but protects from APC loss-dependent tumorigenesis. *Cell Metab* 27, 118-135.
- ▶ Youssif C, Cubillos-Rojas M, Comalada M,

Llonch E, Perna C, Djouder N, Nebreda AR (2018). Myeloid p38 $\alpha$  signaling promotes intestinal IGF-1 production and inflammation-associated tumorigenesis. *EMBO Mol Med* 10, e8403.

## Book Chapters

- ▶ Djouder N (2018). Prefoldins: the new chaperones. Djouder, N (ed.); Advances

in Experimental Medicine and Biology, vol. 1106. Springer Nature. ISBN 978-3-030-00736-2; ISBN 978-3-030-00737-9 (eBook).

- ▶ Chaves-Pérez A, Thompson S, Djouder N (2018). Roles and Functions of the Unconventional Prefoldin URI, vol. 1106:95-108. In: Advances in Experimental Medicine and Biology. Springer Nature. ISBN 978-3-030-00736-2; ISBN

978-3-030-00737-9 (eBook).

## AWARDS AND RECOGNITION

- ▶ Member of the European Society for Clinical Nutrition and Metabolism (ESPEN).

## SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito  
Junior Group Leader

Staff Scientist  
Barbara Oldrini



### 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 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 current most effective treatment for GBM patients is a combination of radiotherapy and alkylating agents. Increasing the sensitivity of the tumour cells to these therapies will possibly extend the survival of the patients.”**

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 the treatment of brain tumours.

Post-Doctoral Fellow  
Miguel Jiménez (since June)

Technician  
Paula Kroon (since October) (TS) \*

Graduate Students  
Carolina Almeida, Alvaro Curiel (until July), Veronica Matia

\**Titulado Superior* (Advanced Degree)

### RESEARCH HIGHLIGHTS

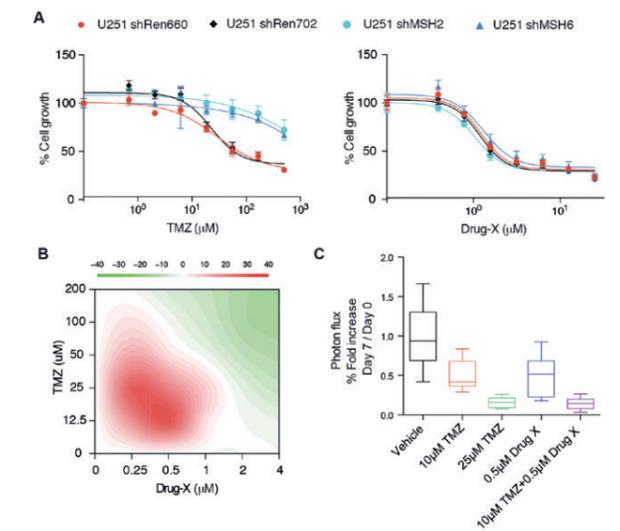
#### Novel therapeutic approaches for therapy-resistant malignant brain tumours

The standard therapies for GBM patients, IR and Temozolomide (TMZ), generate double-strand DNA breaks (DSBs), which are the most deleterious form of DNA damage. The DSBs are then responsible for the initiation of the DNA Damage Response (DDR) and consequently the activation of DNA repair pathways and cell-cycle checkpoints. We have previously presented evidence that alterations in key DNA repair and checkpoint proteins can modulate the GBM treatment response.

The DDR signalling is a very intricate pathway and many of its elements can be altered in a given tumour patient, offering both challenges and opportunities from a treatment perspective. Loss of components of a specific DNA repair pathway might be balanced by the increased activity of other components or pathways. Upregulated DNA repair pathways could lead to resistance to radiotherapy and DNA-damaging chemotherapy, therefore inhibitors of these pathways could potentially increase the sensitivity of the cells to these therapies. By contrast, pathways that are lost represent weaknesses in the DNA repair ability of the tumour cell and they could be exploited by choosing a suitable chemotherapy to induce unreparable (more toxic) DNA damage. It is estimated that the efficacy of radiotherapy and chemotherapy would be improved if tumour cells could be rendered more sensitive without altering the sensitivity of normal tissues.

Through different functional genetic studies, we have observed that defects in components of the Mismatch Repair (MMR) system are significantly associated with resistance to TMZ. Moreover, we have discovered that chromosomal rearrangements of the O-6-methylguanine-DNA methyltransferase (MGMT) lead to overexpression of MGMT and contribute to TMZ resistance, both in high-

grade and low-grade gliomas. Most importantly, we have identified another alkylating agent that is able to overcome these resistance mechanisms and that has a synergistic effect when used in combination with TMZ (FIGURE). ■



**Figure** Overcoming TMZ resistance. (A) Silencing of MMR component (MSH2 and MSH6) leads to resistance to TMZ (left) but not to the novel alkylating drug (right). (B-C) TMZ and Drug-X have synergistic effects both *in vitro* (B) and on brain-tumour slices cultured *ex-vivo* (C).

#### PUBLICATIONS

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Ravisankar S, Haddad B, Ho JSY, Low D, Jin J, Yurchenko V, Prinjha RK, Tarakhovsky A, Squatrito M, Pinto D, Allette K, Byun M, Smith ML, Sebra R, Guccione E, Tumpey T, Krogan N, Greenbaum B, van Bakel H, Garcia-Sastre A, Marazzi I

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## STRUCTURAL BIOLOGY PROGRAMME

ÓSCAR LLORCA Programme Director



### Programme's research areas and strategic goals

The aim of the Structural Biology Programme (SBP) is to provide mechanistic understanding at the molecular level of how proteins and macromolecular complexes related to cancer function. The ultimate goal is to use the new mechanistic insights, and the solved structures, to help guide the search for new compounds and molecules that could interfere with the function of these complexes. Our current research focuses on the study of protein kinases and the DNA damage response. SBP undertakes this thanks to the multiple technologies available via CNIO's Units and Research Groups and through the constant lookout for synergies with other CNIO groups. This year a focus was placed on setting up cryo-electron microscopy (cryo-EM) methods at SBP, a revolutionary technology to observe individual macromolecules at high resolution that is reshaping biological research.

### Summary of milestones & major achievements during 2018

It has been approximately a year now that 3 new Groups started at SBP; many positive changes have taken place since then. Looking back, we can be proud of our many achievements in 2018. The Groups and Units at SBP have made a collective effort to promote collaborations between them, as well as with other groups at the CNIO. The new Groups have set up their labs and new equipment has been acquired. In many cases these resources have been shared between groups, thereby fostering a spirit of scientific interactions, as well as rationalising the use of our resources.

We have made every effort to make our new Programme known around the world. Several top researches in the field of Structural Biology came to the CNIO as speakers, including John L. Rubinstein (University of Toronto, Canada), and Kiyoshi Nagai (LMB-MRC, Cambridge, UK), among others. In addition, we actively advertised the Programme abroad; we visited the LMB-MRC (Cambridge, UK), the Institute of Cancer Research (ICR, London, UK) and the Max Planck Institute of Biochemistry (Munich, Germany). We are also organising a CNIO Frontiers Meeting to be held in the spring of 2019 for which we will be bringing in some of the most renowned experts in Structural Biology of the DNA damage response.

On June 25<sup>th</sup>, SBP was granted a competitive project to access the Electron Bio-Imaging Centre (*eBIC*) at the Diamond Light

**“SBP endeavours to define structures of macromolecules relevant in cancer in order to provide mechanistic understanding, which is a first step towards new therapies. In 2018, SBP determined its first cryo-EM structures.”**

Source (UK). Our project, titled “Stop cancer - structural studies of macromolecular complexes involved in cancer by cryo-EM”, will allow access to this top cryo-EM facility. The Electron Microscopy Unit forms part of this project; the intention is to allow the access to *eBIC* for CNIO groups that are outside the SBP through a collaboration with the Electron Microscopy Unit.

As part of our achievements, the 2 new Junior Groups were awarded ‘Ramon y Cajal’ research contracts and also both obtained grants from the Spanish Government, an excellent boost to start off their independent research. In 2018, SBP published the first atomic structure obtained using cryo-electron microscopy methods. In addition, Daniel Lietha published new insights into the mechanisms involved in the activation of Focal Adhesion Kinase.

Finally, I would like to acknowledge the work of Daniel Lietha, Junior Group Leader at SBP. Over the last few years, Daniel has significantly contributed to the structural and mechanistic understanding of Focal Adhesion Kinase, and other signalling pathways important for proliferation, adhesion and cancer survival. Daniel has also invested considerable effort to look for new ligands to inhibit Focal Adhesion Kinase using structural approaches. On top of all this, Daniel has been an exceptional colleague, always available to help everyone. Daniel Lietha is now moving to CSIC as a ‘distinguished researcher’ and we all wish him and his team the best in this new phase of their scientific career.

In summary, during 2018, SBP has set up the infrastructure, the collaborations and the working environment that we will need in the coming years to push our research, improve our connectivity with CNIO, and to contribute to provide a mechanistic and structural understanding of processes relevant in cancer.

## MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

Óscar Llorca  
Group Leader

Staff Scientists  
Javier Coloma, Andrés López, Ángel  
Rivera, Marina Serna

Post-Doctoral Fellow  
Hugo Muñoz



Graduate Students  
Adrián Del Rincón (since March),  
Carlos Fernández

### OVERVIEW

Activation and assembly of many protein complexes implicated in cancer, such as kinases and polymerases, require the assistance of HSP90, a molecular chaperone. Thus, HSP90 inhibitors are being evaluated as anticancer agents.

HSP90 is needed for the activation and stability of the PI3-kinase-like kinases (PIKKs), including mTOR, ATM and ATR that regulate the DNA damage response and cell growth. Surprisingly, these kinases require the action of HSP90 but working in concert with the R2TP/Prefoldin-like (R2TP/PFDL) complex. R2TP/PFDL is the most complex HSP90 co-chaperone yet described. R2TP/PFDL contains multiple subunits and growing evidence links this complex to cancer.

Yet, how all these processes work is largely unknown. We are using cryo-electron microscopy (cryo-EM) to fully understand the molecular mechanisms of R2TP/PFDL and to bring us a step closer to designing strategies to interfere with PIKK assembly and activation.

**“How kinase complexes implicated in cancer are assembled by HSP90 and R2TP is unclear. The structure of R2TP brings us a step closer to mechanistic understanding and the design of anticancer strategies.”**

## RESEARCH HIGHLIGHTS

**Cryo-EM and structure of macromolecular complexes in cancer**

A defining feature of our Group is our interest in understanding the structural and molecular mechanisms of macromolecular complexes involved in the DNA damage response. For this, we use mostly biochemical and molecular biology tools in combination with cryo-electron microscopy (cryo-EM). Cryo-EM is used to visualise large macromolecular complexes, to observe their flexibility and motions, and to build atomic models. Cryo-EM is especially helpful for complex and flexible assemblies, which are typically difficult to crystallise. The structural and functional information provides mechanistic details to help understand the DNA damage response, and it is an input for the design of new strategies to interfere with these processes.

The Group is currently working on several complexes implicated in the response to DNA damage, but this year our main area of focus was the characterisation and understanding of how HSP90 and the R2TP co-chaperone function to assemble large macromolecular complexes of relevance in cancer.

**How cells build protein interactions in protein kinase complexes**

Assembly, activation and cellular stability of a growing list of macromolecular complexes, many of which are relevant in cancer, require the assistance of molecular chaperones. Among these, the kinases of the PI3-kinase-like family (PIKKs) function as part of large multi-subunit complexes that require HSP90 for assembly.

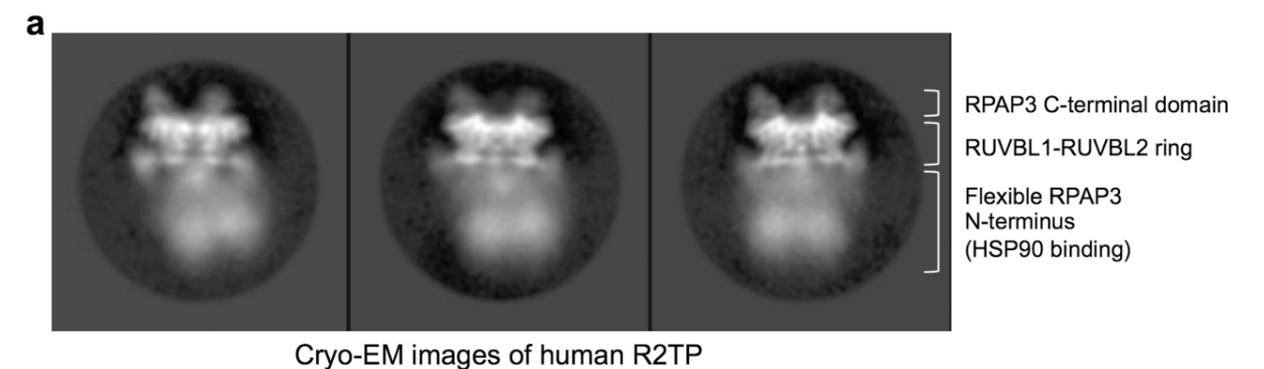
The PIKK family comprises proteins such as ATM, ATR and DNA-PKcs, implicated in DNA repair and DNA damage signalling, and mTOR, which controls cell growth. These kinases interact with other proteins in order to function properly and be active, as in the mTOR complex 1 (mTORC1) or ATR-ATRIP. Building these protein interactions needs the concerted action of the HSP90 chaperone and the R2TP/Prefoldin-like (R2TP/PFDL) co-chaperone. Interestingly,

cells control the level of activation for some of these kinases, such as mTOR, by regulating the building of their functionally active complexes. How all this happens, the molecules involved, the mechanistic details and the implications in cancer remain poorly understood.

Our current aim is to improve our molecular understanding of the structural basis of R2TP-mediated protein complex assembly. In 2018, we reported the 3D structure of the human R2TP complex at a resolution of 3.6Å as part of a collaborative effort between our group and the group of Laurence H. Pearl at the Genome Damage and Stability Centre in the University of Sussex (UK).

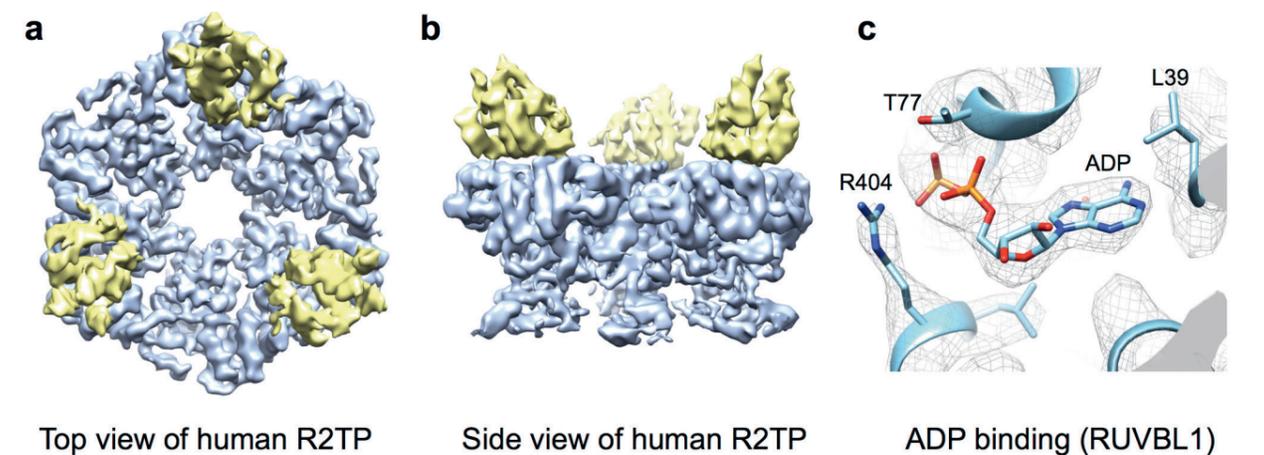
Cryo-EM reveals that a C-terminal domain in RPAP3 (RNA Polymerase Associated Protein 3), one of the components of the human R2TP complex, binds to one hexameric ring of the RUVBL1 and RUVBL2 ATPases. This interaction provides a tight anchor that frees the N-terminal regions of RPAP3 involved in HSP90 binding. Cryo-EM images of R2TP show that HSP90 binding regions are extremely flexible, moving around the core provided by the RUVBL1-RUVBL2 hexameric ring. We propose that such flexible attachment is essential for placing HSP90 in the proximity of the clients, while providing sufficient conformational freedom to interact with a diversity of clients.

Together, our findings provide the first structural view of human R2TP, an essential complex for the HSP90-mediated assembly of mTORC1, ATR-ATRIP and other complexes of the PIKK family. Our structures also highlight important differences between the human complex and the much simpler homologs found in yeast. We have discovered an intricate architecture of the human R2TP complex, providing a flexible tether for HSP90, needed to cope with the assembly of multiple and diverse macromolecular complexes. A structural view of how HSP90 and its co-chaperone assists the assembly of proteins involved in cancer will bring us a step closer to the potential design of new anticancer strategies. ■



**Figure 1** Selected views of the human R2TP complex as observed by cryo-EM. (a) Several domains can

be localised, and the flexibility of the HSP90-binding regions in RPAP3 is detected.



**Figure 2** Cryo-EM map of the RUVBL1-RUVBL2 hexamer (blue colour) bound to the C-terminal domain of RPAP3 (yellow colour),

as seen from the top (a) and side (b). (c) Detail of the ADP binding site. The quality of the cryo-EM density, represented in mesh, is sufficient

to detect the ADP and side chains of residues in the binding site for nucleotides.

## PUBLICATIONS

▶ Martino F, Pal M, Muñoz-Hernández H, Rodríguez CF, Núñez-Ramírez R, Gil-Car-ton D, Degliesposti G, Skehel JM, Roe SM, Prodromou C, Pearl LH, Llorca O (2018). RPAP3 provides a flexible scaf-

fold for coupling HSP90 to the human R2TP co-chaperone complex. *Nat Commun* 9, 1501.

▶ de Jorge EG, Yebenes H, Serna M, Torta-jada A, Llorca O, de Córdoba SR (2018). How novel structures inform understand-ing of complement function. *Semin Im-*

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## Book Chapter

▶ Muñoz-Hernández H, Pal M, Rodríguez CF, Prodromou C, Pearl LH, Llorca O (2018). Advances on the structure of

the R2TP/Prefoldin-like complex. *Adv Exp Med Biol* 1106, 73-83.

## CELL SIGNALLING AND ADHESION JUNIOR GROUP

Daniel Lietha (until September)  
Junior Group Leader

Post-Doctoral Fellows  
Iván Acebrón, Johanne Le Coq

Graduate Student  
Marta Acebrón (until January)

Technician  
Pilar Redondo



### OVERVIEW

Our Group studies regulatory mechanisms of key signalling switches controlling growth and adhesion signals, which regulate important cellular processes such as cell proliferation, migration and survival. We use structural techniques, such as X-ray crystallography and electron microscopy, in combination with biochemical and functional studies to understand these mechanisms at atomic detail and to rationalise how oncogenic events result in their deregulation. The structural understanding allows us to design potential anti-cancer therapeutics that interfere with oncogenic deregulation.

We focus on growth and adhesion signalling systems that interact and are regulated by specific lipids in the plasma membrane. Specifically, we pursue 2 main questions:

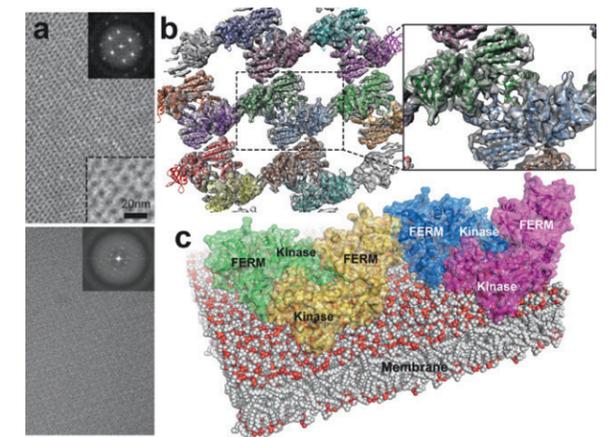
**“Using structure-based design, we generated the first irreversible and sub-nanomolar inhibitor targeting adhesion signals that trigger cancer invasion.”**

- How are adhesion signals in focal adhesion complexes triggered by membrane interactions?
- How are the levels of specific lipids regulated by the SHIP lipid phosphatase to control growth signals?

### RESEARCH HIGHLIGHTS

Focal Adhesion Kinase (FAK) is a key regulator of adhesion signals and localises into a signalling layer on the plasma membrane in focal adhesion complexes. We previously discovered that FAK interacts with  $PIP_2$  lipids in focal adhesions and this triggers its activation by inducing FAK oligomerisation, conformational changes that facilitate its autophosphorylation, Src recruitment and FAK phosphorylation by Src. Currently, we are studying the atomic architecture of FAK oligomers bound to  $PIP_2$  membranes by electron microscopy (EM). We have obtained a 5.9 Å map, which reveals the mode of oligomerisation and large membrane induced rearrangements of FAK's regulatory FERM and kinase domains (FIGURE). The observed conformation suggests that FAK adopts a 'preactivated' primed state when bound to the membrane. We are further investigating how force, induced at focal adhesion sites by actomyosin contraction, can induce changes to these structures to fully activate focal adhesion signalling. We utilise these mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ a fragment based approach to identify allosteric ligands and then use structure based drug design to develop these fragments into inhibitory lead compounds.

SHIP phosphatases remove the 5-phosphate from  $PIP_3$  and thereby, like PTEN, negatively regulate  $PIP_3$  levels in the plasma membrane. Despite their importance, little is known about mechanisms of SHIP regulation. We previously solved a crystal structure containing the catalytic and C2 domains of SHIP2, which, together with extensive biochemistry and cell biology experiments, showed how the C2 domain induces catalytic activation of SHIP2. Currently, we are studying the role of the PH domain flanking the catalytic domain. We find that the domain binds the  $PIP_3$  substrate and  $PIP_2$  product, and that this binding allosterically further activates SHIP. Together, this shows how the C2 and PH domains concertedly act to recruit SHIP to  $PIP_3$  rich membranes in order to adopt a highly active state. ■



**Figure** (a) FAK 2D crystals (with Fourier transforms) formed on  $PIP_2$  membranes imaged by negative stain (upper) or cryo-EM (lower). (b) EM maps at 5.9 Å fitted with FAK domains. Each colour represents one FAK molecule containing a FERM and kinase domain. (c) EM structure of oligomeric FAK on a lipid membrane.

### PUBLICATIONS

- ▶ Toledo RA, Garralda E, Mitsi M, Pons T, Monsech J, Vega E, Otero Á, Albarran MI, Baños N, Durán Y, Bonilla V, Sarno F, Camacho-Artacho M, Sanchez-Perez T, Perea S, Álvarez R, De Martino A, Lietha D, Blanco-Aparicio C, Cubillo A, Domínguez O, Martínez-Torrecuadrada JL, Hidalgo M (2018). Exome sequencing of plasma DNA portrays the mutation landscape of colorectal cancer and discovers mutated VEGFR2 receptors as modulators of antiangiogenic therapies. *Clin Cancer Res* 24, 3550-3559.
- ▶ Bauer MS, Baumann F, Daday C, Redondo P, Durner E, Jobst MA, Milles LF, Mercadante F, Pippig DA, Gaub HE, Gräter F, Lietha D. Structural and mechanistic insights into mechanoactivation of Focal Adhesion Kinase. *Proc Natl Acad Sci USA*, doi:10.1073/pnas.1820567116.
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Lietha D, Frame MC, Coumoul X, Garbay C, Etheve-Quellejeu M, Chen H (2018). Structure-based design, synthesis, and characterization of the first irreversible inhibitor of Focal Adhesion Kinase. *ACS Chem Biol* 13, 2067-2073.

# KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho  
Junior Group Leader

Post-Doctoral Fellow  
Pablo Soriano



## OVERVIEW

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

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

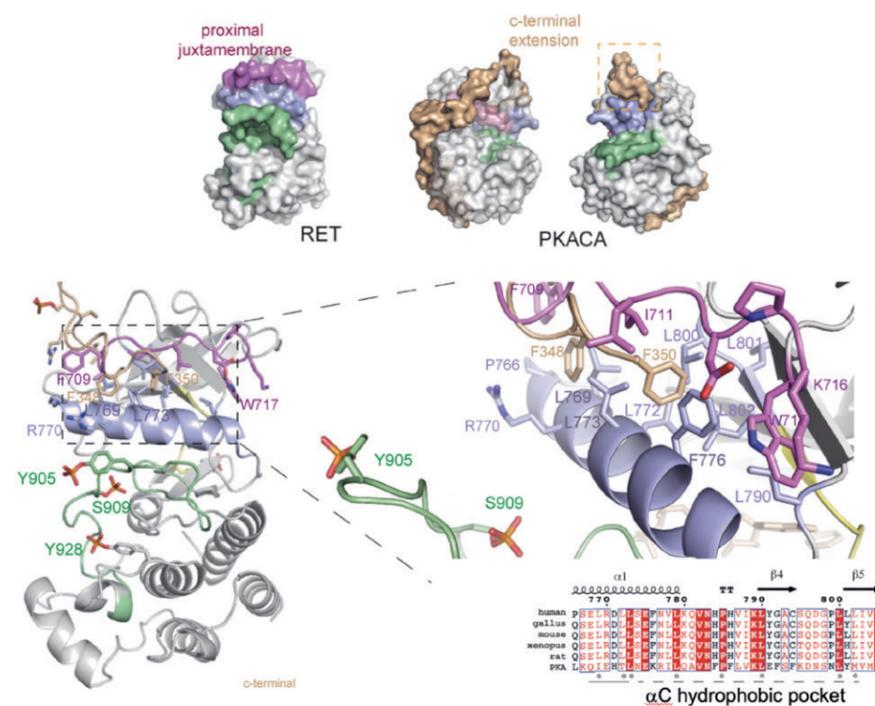
leading to more effective drugs for the treatment of cancer patients.

Graduate Student  
Nicolás Cuesta (since July)

Visiting Master Student  
Moustafa Ahmed Shehata (since July, Cairo University)

Visiting Graduate Student  
Alba Morán (until June, *Universidad Autónoma*)

## RESEARCH HIGHLIGHTS



**Figure** Structural identification of a RET  $\alpha$ C hydrophobic PIF-like allosteric pocket based on the superimposition of the RET (PDB code 5FM3) and PKACA (1ATP) crystal structures, and the resemblance of the c-terminal FxxF hydrophobic motifs of RET (FTRF) and PKACA (FTRF).

During 2018, we have set up the different experimental systems and techniques needed for the adequate functioning of the lab and have established 3 main research lines:

- 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.
- Structure-function studies of RET oncogenic variants, i.e. point mutations targeting the kinase domain and oncogenic fusions generated by DNA-rearrangements.

→ Structure-based drug-discovery of (allosteric) RET inhibitors.

Furthermore, upon invitation by the journal *Endocrine-Related Cancer*, we contributed to a special issue to commemorate the 25th anniversary of the discovery of the RET proto-oncogene as the cause of Multiple Endocrine Neoplasia type 2 (see publication list). ■

## PUBLICATIONS

- Plaza-Menacho I (2018). Structure and function of RET in multiple endocrine neoplasia type 2. *Endocr Relat Cancer* 25, T79-T90.
- Redaelli S, Plaza-Menacho I, Mogni L (2018). Novel targeted therapeutics for MEN2. *Endocr Relat Cancer* 2, T53-T68.

## GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro  
Junior Group Leader

Graduate Student  
Samuel Miguez (since April)



### OVERVIEW

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

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

**“The high-end cryo-electron microscopy setup at the CNIO allows us to look at every detail of the cell’s protein machinery, so that 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 (since July), Araceli Grande (TS)\*

\**Titulado Superior* (Advanced Degree)

### RESEARCH HIGHLIGHTS

#### Mismatch repair

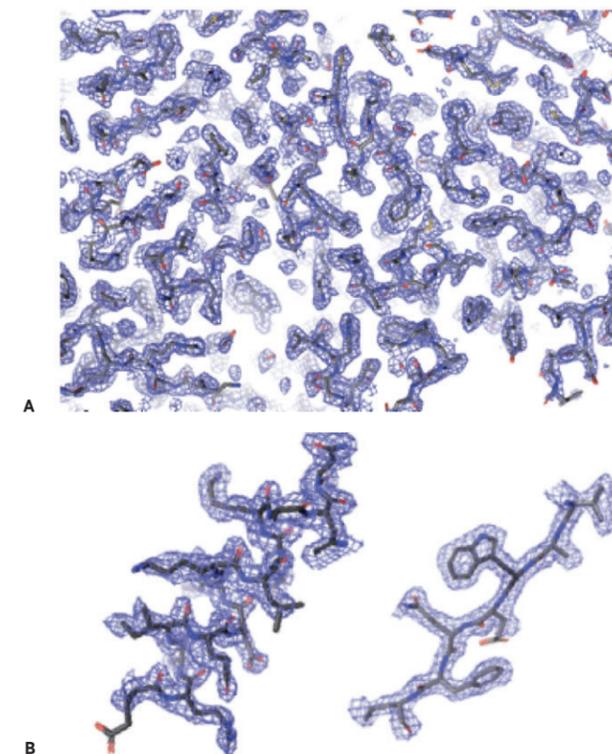
DNA mismatch repair (MMR) is critical for genome stability. The DNA mismatch repair machinery loads onto newly synthesised DNA and searches for mismatches. The recognition of an error in DNA by the MutS protein leads to an ATP-dependent conformational change that transfers MutS into a sliding clamp state. Only this MutS state can activate the MutL ATPase that in turn promotes the cleavage 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 have 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 are carried out in collaboration with Titia Sixma (Netherlands Cancer Institute) and Meindert Lamers (Leiden University).

#### DNA replication & repair - focus on mitochondria

Eukaryotic cells have two genomes: nuclear and mitochondrial. However, how the integrity of the mitochondrial genome is maintained through the equilibrium between DNA replication, repair and degradation, and organelle dynamics, remains unclear. We are interested in understanding these pathways because of their implications for ageing and disease, and in particular, their 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 complexes involved in cancer. Significant 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 the Óscar Llorca Group and the EM Unit to bring the cryo-EM facility at the CNIO to a state-of-the-art level. Moreover, we have been awarded access to high-end microscopes at the Biological Electron



**Figure** High-resolution reconstruction of a protein structure from the lab to 2.1Å resolution (to be published). (A) Shows the overall atomic density of the protein, and (B) shows close-ups of density and model with clear signals for amino acid side-chains.

Bio-Imaging Centre (eBIC) in Oxford (UK). We can now efficiently prepare samples and solve their structures, using the in-house facilities, to a high level of detail. ■

#### AWARDS AND RECOGNITION

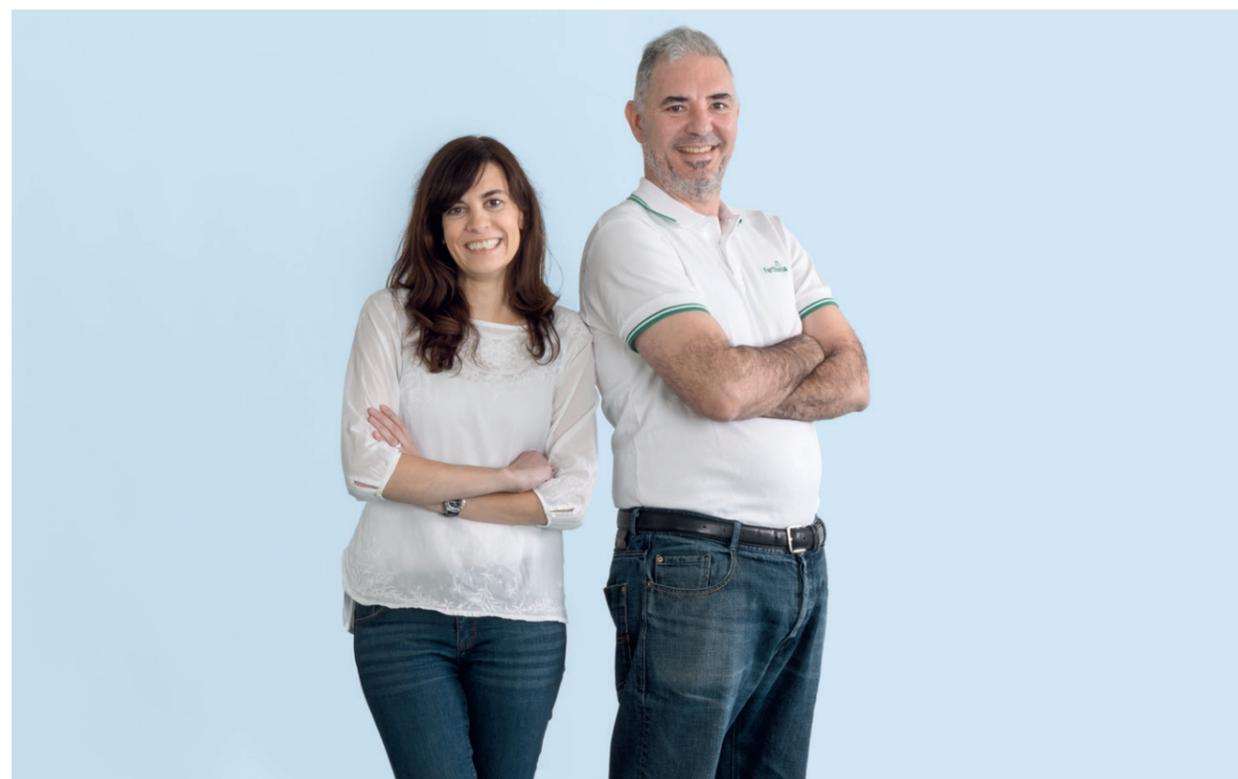
- Awarded with a *Ramón y Cajal* Fellowship, Spanish Ministry of Science, Innovation and Universities.

## SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas  
Head of Unit

Technician  
Clara M. Santiveri (TS)

\**Titulado Superior* (Advanced Degree)



### OVERVIEW

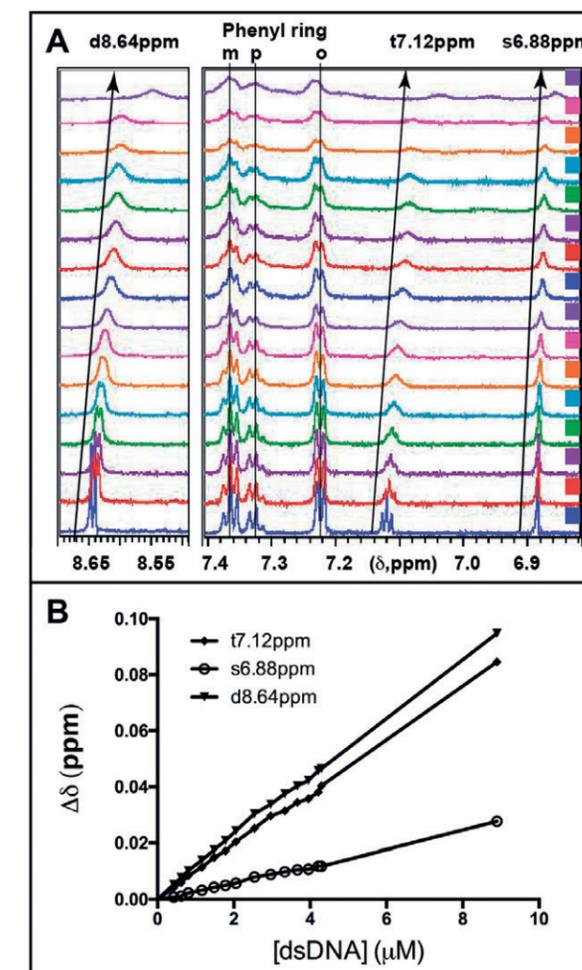
The Unit unifies the technical and scientific management of Nuclear Magnetic Resonance (NMR) Spectroscopy and other molecular biophysics instrumentation available through the Structural Biology Programme. It provides CNIO researchers with equipment and technical support for a variety of techniques used in 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) and their interactions 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 profiles of biofluids, cell growth media and cell and tissue extracts from both animal models of cancer and human samples.

**“In 2018, we identified and quantified interactions of small molecule compounds with tumour-relevant proteins and DNA, thereby contributing to the discovery of possible macromolecular inhibitors, as well as to the understanding of the molecular bases of the cell activity of those compounds.”**

### RESEARCH HIGHLIGHTS

Our Core Unit incorporates 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, the Structural Biology Programme extensively used these technologies throughout 2018. For example, in collaboration with the Experimental Therapeutics -ETP- Programme, we conducted quantitative binding measurements using NMR (see FIGURE) to establish that a cell-active small molecule compound interacts weakly with telomeric double stranded DNA. Thus, telomeric DNA binding appears not to be a significant mode of action of the compound to explain its cellular activity.

The Unit hosts a 700 MHz NMR spectrometer that is well equipped with probes, and a sample changer for running up to 120 samples automatically. This provides the required throughput for the screening of small molecule protein binders (together with the CNIO's Structural Biology and ETP Programmes), as well as for metabonomics measurements that, this year, were performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the ETP), the Cell Division and Cancer Group (-CDC- Group, from the Molecular Oncology Programme), as well as the Growth Factors, Nutrients and Cancer and the Epithelial Carcinogenesis Groups (from the Cancer Cell Biology Programme). For example, in collaboration with the CDC Group, we conducted cell media and intracellular metabolite measurements to characterise the metabolic changes associated to the silencing of the *Mastl* gene. Collectively, with these and other groups, we implemented sample preparation protocols and developed spectroscopic and analytical tools to characterise the metabolites present in different biological samples. ■



**Figure** NMR study of the interaction of a small molecule-compound with a DNA duplex containing 7 telomeric repeats. (A) Superposition of the aromatic region of the  $^1\text{H}$  NMR spectrum of the compound (100  $\mu\text{M}$ ) recorded after addition of increasing amounts of dsDNA (from bottom to top: 0-8.9  $\mu\text{M}$ ). The tilted arrows mark signals changing position upon dsDNA addition, identifying the chemical moieties directly involved in dsDNA binding. In contrast, the 3 signals-from the phenyl ring of the compound only experience broadening as a result of complex formation, but not change of position, as the phenyl moiety does not directly contact the dsDNA. (B) Linear variations in the chemical shift (spectral positions) of three signals as a function of added dsDNA indicate weak binding ( $K_D \gg 10 \mu\text{M}$ ).

## BIOINFORMATICS UNIT

Fátima Al-Shahrour  
Head of Unit

Staff Scientist  
Michael Tress

Post-Doctoral Fellow  
Hector Tejero



### OVERVIEW

Bioinformatics is a key discipline for furthering our understanding of 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

**“VulcanSpot is a novel computational method used to prioritise drugs that can target cancer-specific gene dependencies, unlocking therapeutic options beyond known actionable driver genes.”**

interpretation using computational and statistical methods; and iv) to maintain the CNIO's scientific computing facilities and provide training in bioinformatics tools and methods.

Graduate Students  
Laura Martínez (since February)  
Guillermo Martín-Serrano, Javier Peralas, Fernando Pozo (since February), Kevin Troulé

Technicians  
Andrés Cañada (until July) (TS)\*,  
Diana De La Iglesia (since April) (TS)\*,  
Tomas Di Domenico (TS)\*, Coral Fustero (TS)\*, Gonzalo Gómez(TS)\*,

Osvaldo Graña (TS)\*, Elena Piñeiro (TS)\*

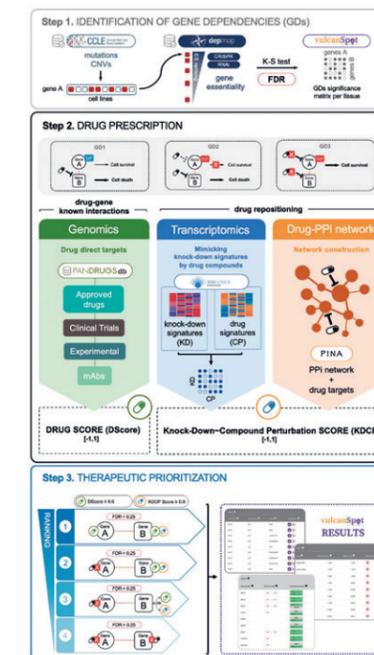
\*Titulado Superior (Advanced Degree)

### RESEARCH HIGHLIGHTS

In 2018, the CNIO Bioinformatics Unit published 16 peer-reviewed articles (see full list on our web site <https://bioinformatics.cnio.es/>) as a result of our ongoing research projects and scientific collaborations with CNIO Research Groups and other national and international research institutions.

During this year, we developed several bioinformatics tools for *in silico* prescription of anticancer drugs: PanDrugs (<https://www.pandrugs.org/>) in collaboration with SING group (*Universidad de Vigo*), and VulcanSpot (<http://vulcanspot.org/>) for detecting and targeting cancer genetic dependencies. All our tools are freely available and have been applied in different genomic studies from our numerous scientific collaborations such as: transcriptomics analysis using our tool nextpresso (Djurec M *et al.*, 2018) or in collaboration with M. Robledo's Group from the Human Cancer Genetics Programme to study pheochromocytoma and paraganglioma (PPGLs) tumours. In addition, the Bioinformatics Unit has published an update of the APPRIS database (<http://appris.bioinfo.cnio.es/>). The principal and alternative isoforms annotated in the APPRIS database are being used to refine and extend the Ensembl/Gencode human reference set. APPRIS annotations have now also been expanded to cover both the human and mouse proteomes in UniProtKB as well as the clinically relevant RefSeq human and mouse gene sets.

The Bioinformatics Unit, as a new node of INB/ELIXIR-ES (<https://inb-elixir.es/>), aims to provide the tools, infrastructure and expertise for the systematic analysis and interpretation of cancer genomes. Importantly, the Bioinformatics Unit is extensively involved in teaching activities – with an important focus on the translational bioinformatics area – to train



**Figure** VulcanSpot workflow: 1) identification of genome-wide vulnerable Gene Dependencies (GD) integrating functional genomics datasets; 2) to propose drugs to target GDs following a dual strategy; and 3) therapeutic prioritisation as a final output targeting GDs detected in the user's gene list.

bioinformatics users and developers. We co-organise the *Master en Bioinformática Aplicada a Medicina Personalizada y Salud* (ISCIII-ENS) as well as advanced Bioinformatics courses for sequencing analysis (visit our web page for a full list of activities). ■

#### SELECTED PUBLICATIONS\*

- Zagorac I *et al.* (incl. Al-Shahrour F ... Quintela-Fandino M) (2018). In vivo phosphoproteomics reveals kinase activity profiles that predict treatment outcome in triple-negative breast cancer. *Nat Commun* 9, 3501.
- Rodríguez JM, Rodríguez-Rivas J, Di Domenico T, Vázquez J, Valencia A, Tress ML (2018). APPRIS 2017: principal isoforms for multiple gene sets. *Nucleic Acids Res* 46, D213-D217.
- Abascal F, Juan D, Jungreis I, Martínez L, Rigau M, Rodríguez JM, Vázquez J, Tress ML (2018). Loose ends: almost one in five human genes still have unresolved coding status. *Nucleic Acids Res* 46, 7070-7084.
- Djurec M, Graña O, Lee A, Troulé K, Espinet E, Cabras L, Navas C, Blasco MT, Martín-Díaz L, Burdiel M, Li J, Liu Z, Vallespinós M, Sanchez-Bueno F, Sprick MR, Trumpp A, Sainz B Jr., Al-Shahrour F, Rabadan R, Guerra C, Barbacid M (2018). Saa3 is a key mediator of the protumorigenic proper-

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Piñeiro-Yáñez E, Reboiro-Jato M, Gómez-López G, Peralas-Patón J, Troulé K, Rodríguez JM, Tejero H, Shimamura T, López-Casas PP, Carretero J, Valencia A, Hidalgo M, Glez-Peña D, Al-Shahrour F (2018). PanDrugs: a novel method to prioritize anticancer drug treatments according to individual genomic data. *Genome Med* 10, 41.

Paumard-Hernández B *et al.* (incl. Al-Shahrour F ... Benítez J) (2018). Whole exome sequencing identifies PLEC, EXO5 and DNAH7 as novel susceptibility genes in testicular cancer. *Int J Cancer* 143, 1954-1962.

López-Nieva P *et al.* (2018). RNA-Seq reveals the existence of a CDKN1C-E2F1-TP53 axis that is altered in human T-cell lymphoblastic lymphomas. *BMC Cancer* 18, 430.

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

## ELECTRON MICROSCOPY UNIT

Jasminka Boskovic  
Head of Unit

Technicians  
Carlos Rodríguez (until July) (TS)\*,  
Carmen García (since August) (TS)\*

\*Titulado Superior (Advanced Degree)



### OVERVIEW

The Electron Microscopy (EM) Unit is a central core facility as well as a research laboratory. It is available to CNIO researchers and the wider research community, providing investigators with instruments and support for Transmission Electron Microscopy analysis. The Unit offers negative staining and cryo-EM specimen preparation techniques for proteins, protein complexes and vesicles. We also give training to regular users on the use of our equipment and provide further guidance regarding specimen preparation.

**“Over the past year, the Electron Microscopy Unit has endeavoured to adapt its facility to better meet the needs of the new members of our Programme, particularly in relation to the cryo-EM technique.”**

### RESEARCH HIGHLIGHTS

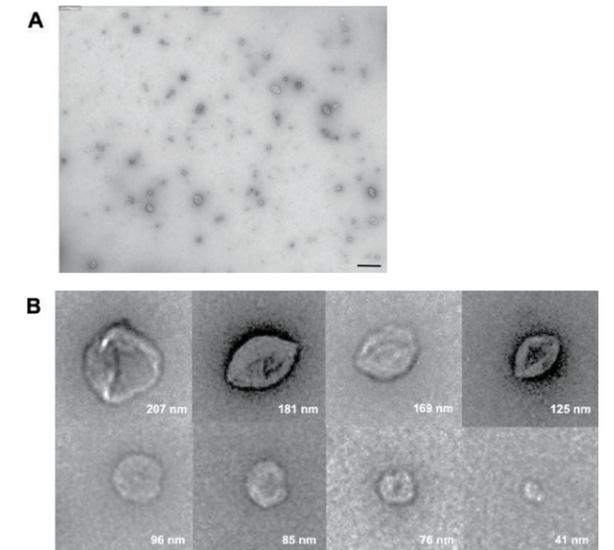
Over the last decade, cryo-EM has emerged as a key technique for studying how biomolecules function and interact. Our 120-kV Spirit G12 EM, equipped with the TVIPS CMOS detector, has cryo-capabilities that enable sample screening and low resolution analysis of standard biological specimens. For high resolution cryo-EM 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 peer-reviewed Block Allocation Group (BAG) access.

Throughout 2018, the Unit has performed EM experiments with all the research groups from the Structural Biology Programme, as well as with several groups from other CNIO Programmes and outside our Centre. For example, in collaboration with CNIO's Microenvironment and Metastasis Group, we have contributed to the characterisation of circulating extracellular vesicles from the lymph and plasma of melanoma patients. Our data supported their analysis of lymph-circulating extracellular vesicles for the detection of residual disease and its reappearance in melanoma. The most frequent mutation in amyotrophic lateral sclerosis and frontotemporal dementia patients involves mutation of the *C9ORF72* gene, resulting, to a certain extent, in the expression of toxic dipeptide arginine repeats (PR). We have evaluated, by electron microscopy, in collaboration with the CNIO Genomic Instability Group, that the presence of (PR)<sub>20</sub> did not affect the *in vitro* assembly of purified 40S and 60S subunits into 80S particles in the absence of mRNA. Moreover, in collaboration with Dr Iván Ventoso – from the *Centro de Biología Molecular ‘Severo Ochoa’ (CSIC-UAM)* and the *Departamento de Biología Molecular of the Universidad Autónoma de Madrid (UAM)* – we used electron microscopy to localise gold-labelled eukaryotic initiation factor-4A (eIF4A) in the ribosomal translation initiation complex 48S. Our results have contributed towards the proposal of a topological model of the scanning ribosomal 43S pre-initiation complex. ■

#### ► PUBLICATIONS

- Toribio R, Díaz-López I, Boskovic J, Ventoso I. (2018). Translation initiation of alphavirus mRNA reveals new insights into the topology of the 48S initiation complex. *Nucleic Acids Res* 46, 4176-4187.
- Lafarga V, Sirozh O, Diaz-Lopez I, Hisaoka M, Zarzuela E, Boskovic J, Jovanovic B,

Fernandez-Leiro R, Munoz J, Stoecklin G, Ventoso I, Fernandez-Capetillo O (2018). DNA and RNA binding mediate the toxicity of arginine-rich peptides encoded by C9ORF72 GGGGCC repeats. *BioRxiv*, doi: <https://doi.org/10.1101/441808>.



**Figure** (A) Representative electron micrograph of negative stained vesicles. Scale bar, 500 nm. (B) Analysis of exosome structure and size after density gradient centrifugation.



## BIOLOGICAL TEXT MINING UNIT

Martin Krallinger  
Head of Unit

Staff Scientists  
José Antonio López (until January),  
Marta Villegas



### OVERVIEW

Biomedical cancer research is a particularly data-heavy discipline, where key information sources are not only limited to genomic information or raw experimental data. Especially unstructured data, such as the scientific literature, clinical texts, medicinal chemistry patents or patient generated content, constitute a valuable resource for a range of scenarios like drug discovery, interpretation of large scale experimental results, drug repurposing or evidence based medicine. Medical big data approaches are only able to efficiently exploit running texts through the use of natural language processing (NLP) techniques relying on deep learning and artificial intelligence strategies. Our Unit is financed through the Plan for the Advancement of Language Technologies; the aim is to generate resources that can improve the exploitation of biomedical data by means of implementing and evaluating the underlying quality of systems

**“Language technologies, together with artificial intelligence, are driving the technological transformation of biomedical and clinical data into actionable information at all levels of cancer research.”**

for automatic recognition of medical concepts, generation of specialised neural machine translation models for the medical domain and the implementation of a medical language technology platform and software components for processing Spanish EHRs.

Technicians  
Aitor González (TS)\*, Ander  
Intxaurreondo (TS)\*, Jesús  
Santamaría (TS)\*

\*Titulado Superior (Advanced Degree)

### RESEARCH HIGHLIGHTS

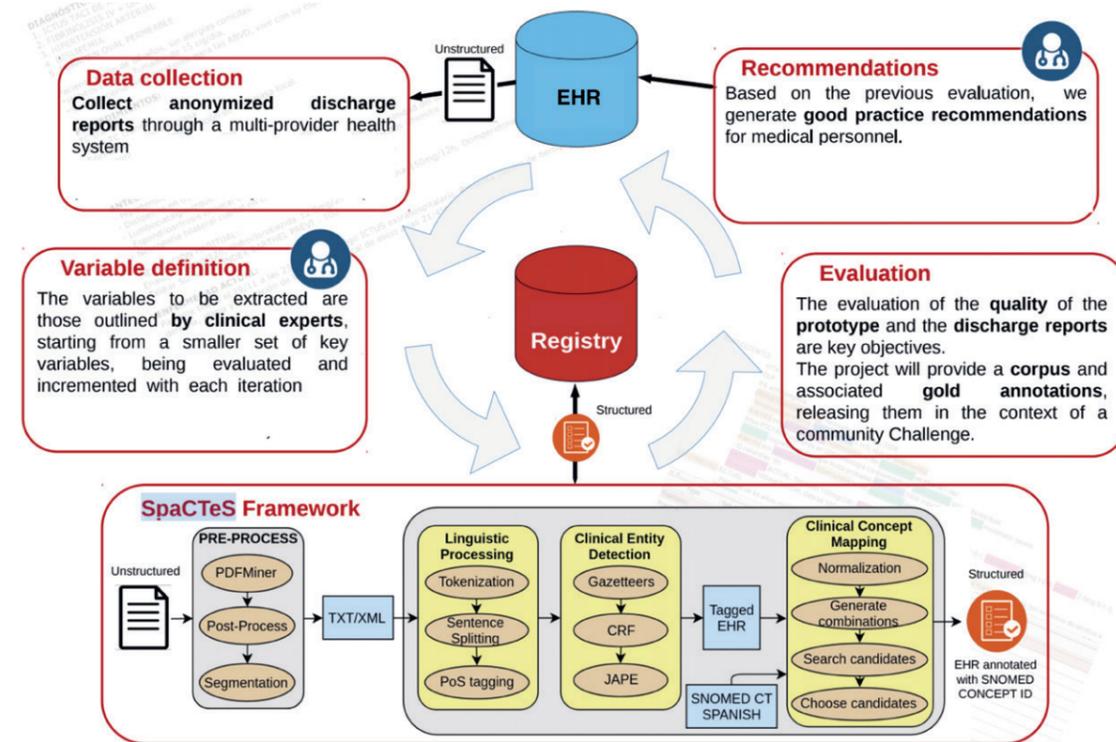


Figure Clinical NLP framework for processing electronic health records in Spanish and Catalan.

The Biological Text Mining Unit has provided consultancy, guidance and technical support for clinical text mining use cases posed by several healthcare institutions (*Hospital Virgen del Rocío, Hospital XII de Octubre, Hospital Son Espases, Hospital Clinic*), national and regional health-related agencies (Spanish Medical Agency, *Instituto Aragonés de Ciencias de la Salud, Servicio Andaluz de Salud, Fundació TIC Salut Social*), and natural language as well as medical informatics academic research groups. The Unit has contributed to benchmarking efforts of clinical text mining systems by organising shared tasks in the context of community challenges organised by the *Sociedad Española para el Procesamiento del Lenguaje Natural (SEPLN-IberEval)* and releasing high quality evaluation datasets. The Unit has published a collection of clinical NLP resources, all freely available at: <https://zenodo.org/>

communities/medicalnlp and <https://github.com/PlanTL>. In addition to annotation guidelines and Gold Standard corpora for developing and evaluating the quality of systems for automatically detecting biomedical and clinical concepts, the Unit has implemented software tools for automatic medical term recognition and normalisation (CUTEXT), an electronic health record sectionizer, a medical sentence boundary recognition system, a medical text tokenizer, lemmatizer and PoS-tagger. Moreover, we have also contributed to the first Protected Health Information (PHI) masker for the Spanish language, a system for medical negation detection, clinical temporal expression detection based on HeidelbergTime, a medical machine translation system and word embeddings. These key constituents are being integrated into the clinical NLP pipeline developed by the Unit. ■

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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.

Currently, the HCGP is composed of three Research Groups and three Units. The Human Genetics Group, led by Javier Benítez, focuses on contributing to the understanding of the genetic bases of some hereditary tumours. Mercedes Robledo leads the Hereditary Endocrine Cancer Group that aims to identify new major susceptibility genes related to hereditary endocrine tumours as well as to define markers associated with differences in anticancer drug response and toxicity. Both Groups are also involved in the search for low susceptibility alleles that explain sporadic cancers. The Genetic and Molecular Epidemiology Group, led by Núria Malats, works not only from a genetic but also from a non-genetic point of view. She analyses exogenous factors that contribute to explain, together with genetic factors (low susceptibility alleles), the susceptibility to pancreatic and bladder cancer. The Genotyping Unit, headed by Anna González-Neira, supports our three research groups from a technical point of view, and provides support to other CNIO groups as well as to external users. They also work in pharmacogenetics within the framework of their own line of research. The Molecular Cytogenetics and Genome Editing Unit, headed by Sandra Rodríguez-Perales, contributes to this provision of support with classical and molecular cytogenetics techniques and with new genome editing technologies. In addition, her research is focused on the design of human stem cell models carrying cytogenetic alterations. Finally, the Familial Cancer Unit coordinates the clinical part of the Programme through the CNIO Familial Cancer Consultancy, which is located at the *Hospital de Fuenlabrada*. Miguel Urioste is responsible for these activities and leads a research line focused on hereditary colorectal cancer.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. This year the Familial Cancer Consultancy attended around 550 consultancies, performed 1,417 genetic diagnoses and carried out 1,290 cytogenetic studies. In addition, the Programme's Groups have hosted 6 resident physicians from different Spanish hospitals who rotated in the Groups and Units for 3-month periods. 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 10

**“We use different omics and epidemiologic studies to achieve our goals; this is combined with functional studies that validate our results. Finally, we translate our conclusions into clinical practice.”**

national visitors were hosted in 2018). In terms of education, 1 foreign and 10 national Master's students and 9 national PhD students have worked on their research projects, 1 of whom has already successfully defended their thesis.

Finally, one of the main objectives of the Programme is to establish research collaborations with national and international groups; 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 international projects. Currently, we collaborate with 14 international Consortia that are representative of the main types of tumours that we focus on. In addition, we participate in 2 international projects from Europe.

Summary of milestones and major achievements during 2018:

- Mercedes Robledo: the identification of DLST as a new pheochromocytoma and/or Paraganglioma (PPGL) susceptibility gene.
- Anna González-Neira: the identification of pharmacogenetic variants predicting response to neoadjuvant single-agent doxorubicin or docetaxel.
- Núria Malats: interaction of FHC and smoking increases pancreatic cancer risk.
- Javier Benítez: the identification of three susceptibility genes *PLEC*, *EXO5* and *DNAH7* as novel susceptibility genes in testicular cancer.
- Sandra Rodríguez-Perales: gene editing cancer therapy project, selected by CaixaImpulse Programme in the 2018 edition.
- Mercedes Robledo: became member of the ENS@T Steering Committee (European Network for the Study of Adrenal Tumours).
- Javier Benítez's Group: was accepted in the international Consortium of Testicular Cancer.

## HUMAN GENETICS GROUP

Javier Benítez  
Group Leader

Staff Scientists  
María José García (since September),  
Ana Osorio

Post-Doctoral Fellow  
Oriol Calvete

Graduate Students  
Juan Miguel Baquero,  
Paloma Martín

Technicians  
Alicia Barroso, Ana Isabel Corao  
(since October), Victoria Fernández  
(TS) \*

\*Titulado Superior (Advanced Degree)



### OVERVIEW

We have continued to decipher the genetic bases of hereditary and sporadic breast cancer. In addition, we participated in a project that combines the genotype and the phenotype in order to stratify and select women at high risk of developing breast cancer. Other families with rare tumours are also the object of our studies, for example, testicular cancer whose genetic bases are unknown. More recently, we started working on a study to elucidate the common genetic origin of different autoimmune-originated pathologies: gastric neuroendocrine tumours or chronic atrophic gastritis plus different immune diseases in other tissues, such as thyroiditis, diabetes or arthritis. We have identified several genes thereby opening up new avenues for new 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 have discovered 3 new genes that confer susceptibility to testicular cancer and a moderate breast cancer susceptibility gene. A whole pathway with several genes associated to gastric neuroendocrine tumours or chronic atrophic gastritis plus several immune diseases, has been identified.”**

## RESEARCH HIGHLIGHTS

## Deciphering the role of rare variants in breast cancer

The European project BRIDGES, in which we participate, has 3 main phases. The first one was the study of 36 candidate genes in 60,000 breast cancer cases and controls in order to determine their role as possible moderate susceptibility genes. This part of the work has been completed and we have confirmed 20% of them. The second phase will involve the whole sequencing of these 60,000 cases, trying to discover new susceptibility genes. The work will start towards the end of year or the beginning of next year; our group will be responsible for coordinating this Work Package.

## Breast cancer susceptibility genes

In a whole-exome sequencing study of 4 BRCA1/2 families we identified a mutation in the moderate susceptibility

gene *ATM* as being responsible for the disease in one of the families (Tavera-Tapia *et al.*, 2017). In a second family, we found a deleterious mutation in an excellent candidate gene *RECQL5* that belongs to a family of DNA helicases that have a role in the Homologous Recombination (HR) DNA repair pathway. Using a combination of targeted next-generation sequencing and genetic and functional assays, we identified 7 deleterious or likely deleterious mutations in the gene in a series of 700 BRCA1/2 cases and only 1 deleterious mutation in 700 controls, suggesting that the gene could actually explain a small percentage of the BRCA1/2 families (Tavera-Tapia *et al.*, submitted).

## SNPs and the BER pathway

We investigated the molecular basis underlying the effect of an SNP in the DNA glycosylase *UNG* as an ovarian cancer

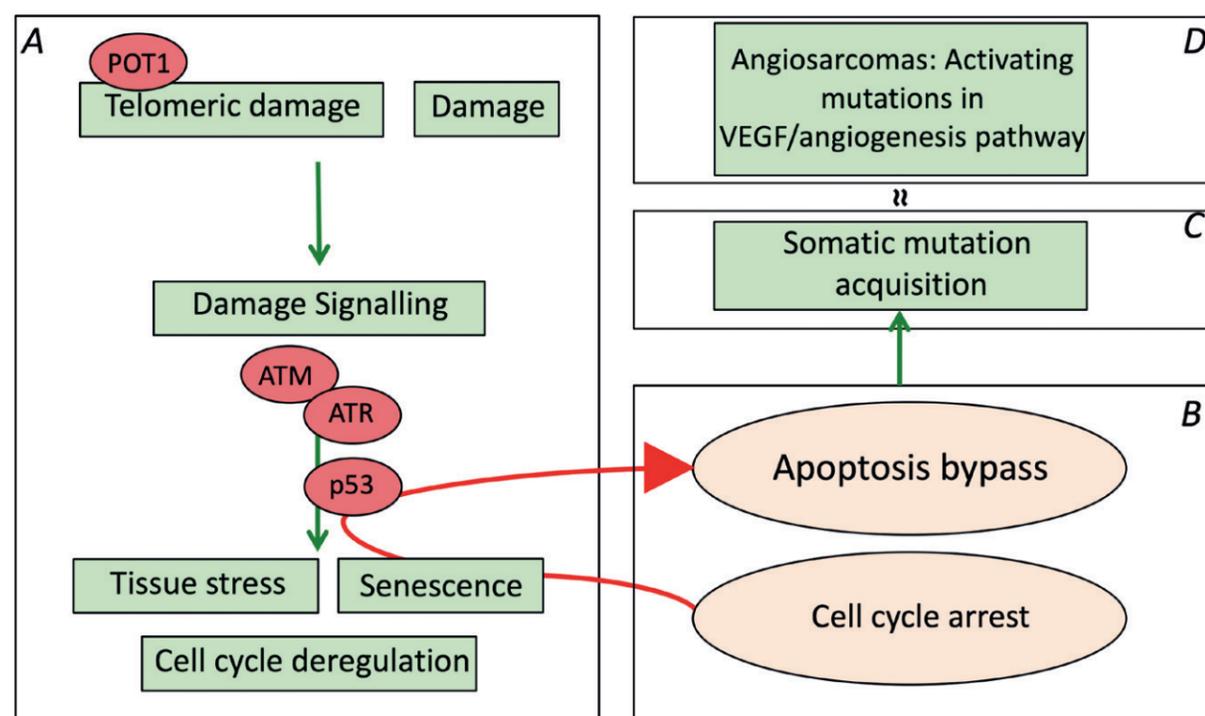
risk modifier in *BRCA2* mutation carriers (Baquero *et al.*, submitted). We found that an SNP rs34259 is associated with significant *UNG* down-regulation and a better performance of the enzyme, measured by a lower accumulation of uracil at the telomeres in *BRCA2* mutation carriers. Our findings could help to explain the association of this variant with a lower cancer risk in *BRCA2* mutation carriers. In addition, we want to study the role of this and 2 other glycosylases previously studied, *OGG1* and *NEIL2*, across the cell cycle.

## Familial cancer exome project

In 2015, we identified a gene responsible for families with cardiac tumours (*POT1*) (Calvete *et al.*, 2015). Recently, we described its relation not only to cardiac tumours but also to other different types of tumours (Calvete *et al.*, 2016). We investigated why the malfunction of this gene involves not only abnormal telomere length but is also able to generate different tumours in different tissues in a similar way as *P53*. We analysed the somatic changes in several cardiac tumours with and without *POT1* mutations and have concluded that the inhibition of *POT1* gene function, and the damage-response malfunction, would activate ATR-dependent DNA damage signalling, which increases cell cycle arrest in asymptomatic tissues and might interfere the apoptosis mechanism; this would permit the further acquisition of somatic mutations in the VEGF/angiogenesis pathway (POT1 deregulation), which drives tumour formation. The same observation was made in sporadic cardiac lesions (Calvete *et al.*, submitted).

In 2015, we published the identification of the *ATP4A* gene as being 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* in a digenic model (Calvete *et al.*, 2017). We further explored the apparent relation of gastric autoimmune disease (gastric neuroendocrine tumour or chronic atrophic gastritis) plus a second immune disease; we found several mutations in new genes involved in homeostasis function (soluble carriers) altering *ATP4A* function. We have designed a panel of 15 genes from this pathway and we are currently performing a screening in a large number of patients carrying these combinations. Several gastroenterologists, pathologists and endocrinologists are collaborating in this project.

During the past few years, we have been collecting families with testicular cancer and also sporadic tumours. We conducted a first study in 17 families with over 71 members and by whole exome sequencing we identified several candidate genes; 3 of them (*PLEC*, *EXO5* and *DNAH7*) were validated in a large case-control association study (Paumard *et al.*, 2018). We then continued the study with more cases and separated 2 main histologic groups, seminomas and non-seminomas. We differentiated several altered pathways and the spermatogenesis pathway was significantly altered. We studied this pathway in depth and discovered a biomarker that differentiated familial, bilateral and sporadic cases, as well as seminomas from non-seminomas. ■



**Figure** Tumorigenic mechanisms for cardiac angiosarcomas (CAS). (A) Mutations found in *POT1* and other genes involved in the damage response signalling (*ATR/ATM* and *TP53*), correlate with the constitutional deregulation of the cell cycle, which triggers senescence and tissue stress due to the depletion of the progenitor cells.

(B) Malfunction of the tissue stress would arise a bypass in the apoptotic regulation. (C) Apoptosis bypass would fuel the acquisition of multiple somatic events. (D) In all the studied CAS (familial – carrying *POT1* mutation – and sporadic CAS), somatic activating mutations were found in the angiogenesis pathway, which drives tumour formation.

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## HEREDITARY ENDOCRINE CANCER GROUP

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Technicians  
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<sup>\*</sup>Titulado Superior (Advanced Degree)

### 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 new susceptibility gene for paraganglioma, discovered predictive markers of mTORi response, and uncovered the Hsa-miR-139-5p/HNRNPF axis as a critical modulator of thyroid tumour virulence.”**

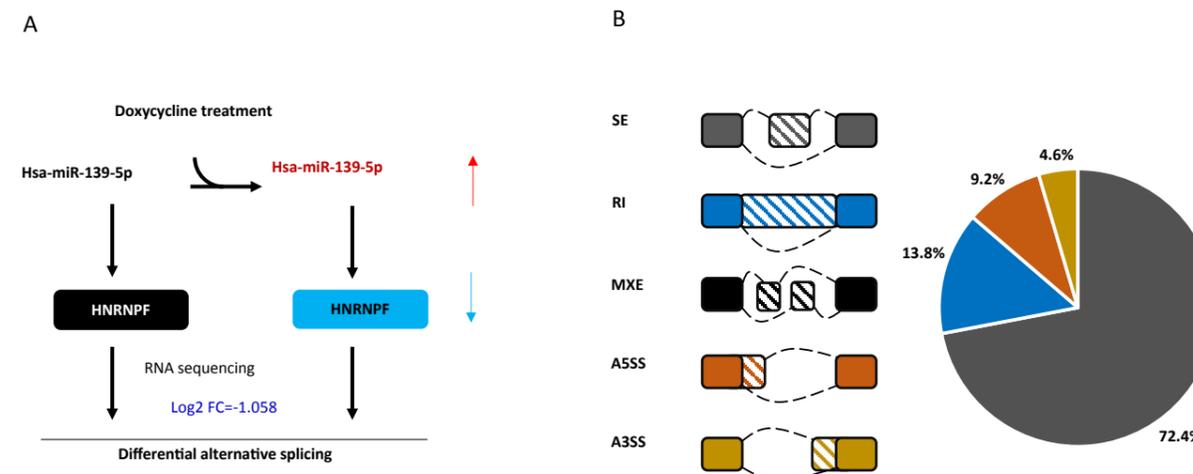
## RESEARCH HIGHLIGHTS

**Recurrent germline *DLST* mutations in patients with multiple pheochromocytomas and paragangliomas (PPGLs).** Taking as a starting point the involvement of the TCA cycle in PPGL development, we aimed to identify novel disease-related genes involved in this key metabolic pathway that could explain additional patients lacking mutations in known susceptibility genes. To this end, targeted sequencing of thirty-seven TCA cycle-related genes was applied to DNA from 104 PPGL patients with no mutations in the major known predisposing genes. In order to decipher the role of the identified variants, omic-based analyses, TCA-related metabolite determination and  $^{13}\text{C}_5$ -glutamate labelling assays were performed. We identified *DLST* germline variants in ~7% of patients. A recurrent mutation, p.Gly374Glu, found in 43% of patients, triggered accumulation of 2-hydroxyglutarate, both in tumours and in a heterologous cell-based assay designed to functionally evaluate *DLST* variants. p.Gly374Glu-*DLST*-mutated tumours exhibited loss of heterozygosity as well as consistent methylation and expression profiles. We also found positive *DLST* immunostaining not only in *DLST*-mutated tumours, but also in other tumours in which the TCA cycle was disrupted. In summary, this study reveals *DLST* as a new PPGL susceptibility gene and further strengthens the relevance of the TCA cycle in PPGL development.

**Mutations leading to extraordinary responses to mTOR inhibitors.** The inhibitors of the mammalian target of rapamycin (mTOR) are key drugs for the treatment of several tumours. However, we lack markers able to identify patients with enhanced treatment sensitivity. To discover molecular determinants of drug response and to contribute to the

definition of predictive biomarkers, we recruited renal cancer patients with extraordinary responses to these drugs and performed a comprehensive genomic, immunochemical and functional characterisation of the tumours. In two young adults with metastatic cancer, a renal epithelioid angiomyolipoma (EAML) and a chromophobe renal cell carcinoma, that upon rapalog treatment had a complete response at metastatic sites and durable responses, we could identify *TSC2* somatic mutations as causative of the extraordinary responses. These findings support a high efficacy of mTOR inhibitors in malignant EAML and in a subset of patients with chromophobe renal cancer, and propose sequencing of mTOR pathway genes to guide therapy with these drugs.

**Deep sequencing of small RNAs reveals a prognosis marker functionally associated with alternative splicing modulation in thyroid cancer.** It is urgent to identify biomarkers and functional networks associated with aggressive thyroid cancer in order to anticipate disease progression and facilitate patient-personalised management. The miRnome sequencing of thyroid tumour series enriched for advanced disease patients uncovered miRnome profiles correlated with tumour-specific histopathological and molecular features, such as stromal-cell infiltration and tumour-driver mutation. Differential analysis considering disease prognosis revealed a consistent hsa-miR139-5p down-expression in primary carcinomas from patients with recurrent/metastatic disease. Exogenous expression of hsa-miR-139-5p significantly reduced migration and proliferation abilities of anaplastic thyroid cancer cells. Proteomics analysis pointed to *RICTOR*, *SMAD2/3* and *HNRNP* as hsa-miR-139-5p putative targets *in vitro*.



**Figure** Hsa-miR-139-5p/HNRNP axis modulates gene-transcripts balance. (A) Alternative splicing analysis experiment. DeSeq2 differential expression analysis showed a reduction of *HNRNP* mRNA abundance (Log<sub>2</sub> FC=-1.058) upon hsa-miR139-5p expression induction. rMATS method identified differences in alternative splicing. (B) Events with a

significant different inclusion level (FDR<0.05) upon hsa-miR-139-5p/HNRNP axis regulation. Analysis considers junction and target exon counts from RNA sequencing data. SE: Skipped exon, MXE: Mutually exclusive, A5SS: Alternative 5' splice site, A3SS: Alternative 3' splice site; RI: Retained intron.

Significantly, mRNA abundance of *HNRNP*, an alternative splicing factor mainly involved in cryptic exon inclusion/exclusion, showed an anti-correlation with hsa-miR-139-5p expression in human tumours. Analysis of alternative splicing from RNA sequencing data revealed 174 events differentially

regulated upon *HNRNP* repression in genes and signalling cascades critical for thyroid cancer (FIGURE). These results point at hsa-miR139-5p/HNRNP/gene-transcripts balance as a novel regulatory axis associated with tumour virulence and modulation of major thyroid cancer signalling pathways. ■

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## AWARDS AND RECOGNITION

- Mercedes Robledo, Group Leader of the 706 Unit, CIBERER (Centro de Investigación Biomédica en Enfermedades Raras), Research Programme on Hereditary Cancer, Haematological and Dermatological diseases.

## GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

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Lola Alonso (TS)\*, Lidia Estudillo,  
Mirari Márquez (until May) (TS)\*  
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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. *Omic*s 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:

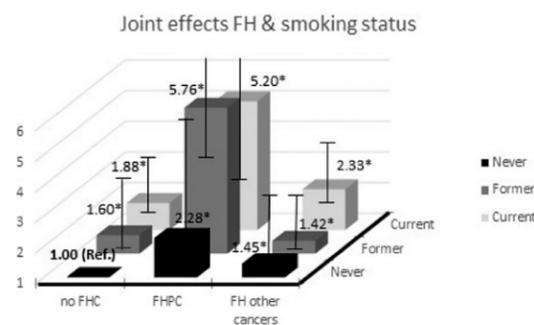
- Identify non-genetic and genetic factors, as well as their interactions, associated with cancer development and progression and with its molecular/*omic*s subphenotypes.
- Develop and apply statistical/informatics tools to model the risk, prediction, and clinical course of patients with cancer by integrating epidemiologic with *omic*s 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 of appropriate analytical strategies. We are contributing to this field towards a personalised prevention of cancer.”**

RESEARCH HIGHLIGHTS

Research findings

In 2018, the Group mainly focussed its research on pancreatic cancer while building resources for bladder cancer research. For **pancreatic cancer (PC)**, we continued exploiting the data generated by the PanGenEU Study to further characterise pancreatic cancer risk. Two main articles exemplify our contributions to this domain. First, by applying complementary analytical approaches we reported that, regardless of non-genetic risk factors, the risk of PC was 2.5 higher among family members with more than 2 relatives affected with PC, with this risk being stronger in current smokers (FIGURE 1). Furthermore, we confirmed that PC was diagnosed at younger ages among those subjects with a family history of PC who smoked than in non-smokers. In the second article, we reported on the underlying genetic basis behind PC and its associated multimorbidities network through a computational approach using the DisGeNET. This strategy allowed us to identify several autoimmune diseases linked to PC and the shared altered genes (FIGURE 2). These associations were subsequently confirmed at the individual level in the PanGenEU study population of 1,705 PC cases and 1,084 controls that resulted in a reduced risk of PC in subjects having  $\geq 2$  autoimmune diseases. These findings again pointed to the role of the immunological status in PC carcinogenesis. We also continued to participate in international large-scale investigations to further characterise the genetic susceptibility and somatic alteration landscape of PC. For **bladder cancer (BC)**, the Group reported on the inverse association between asthma and BC using the Spanish Bladder Cancer/EPICURO Study resources. This reduced risk of BC was especially observed among aggressive tumours. The Group also participated in the discovery and validation of both urine and tumour prognostic marker combination in large European studies of non-muscle invasive BC. We also

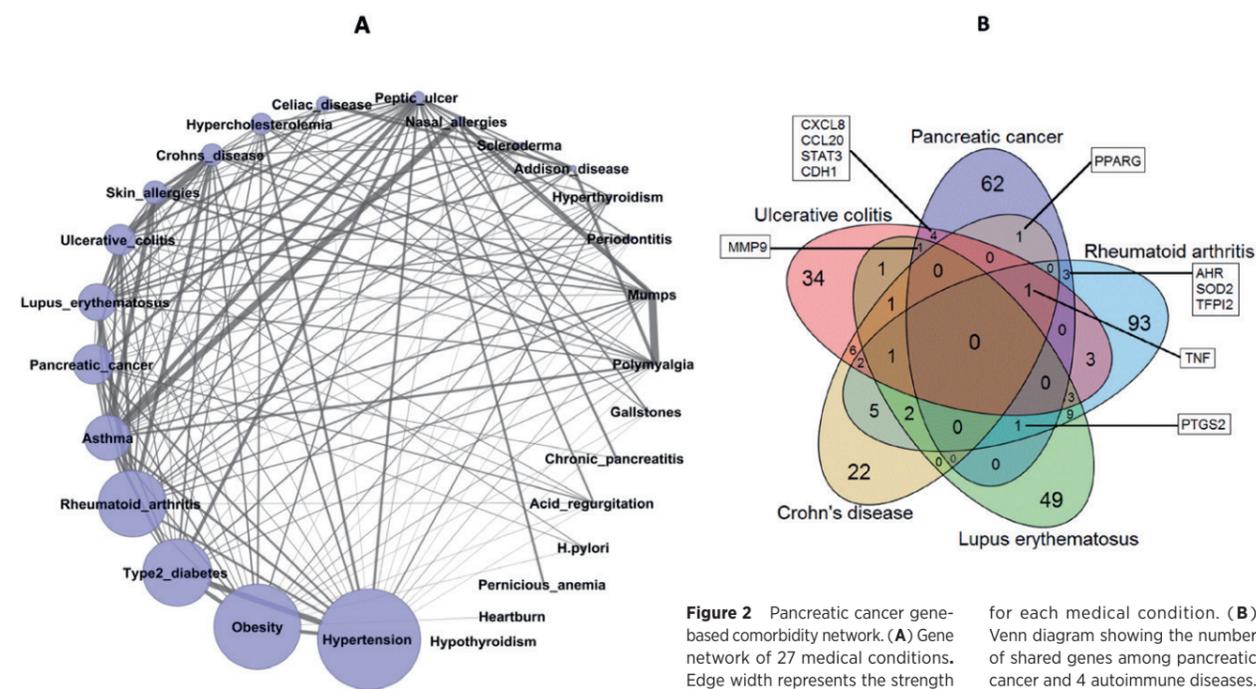


**Figure 1** Odd ratios for the joint effect of family history of cancer (FHC) / Family history of pancreatic cancer (FHPC) and smoking on pancreatic cancer risk. PanGenEU case-control study.

performed a review of the genetic susceptibility to BC risk and progression based on GWAS hits. Most of the variants were common and conferred small risk and, therefore, they were not clinically actionable at the individual level.

Methodological contributions

The Group made contributions to both integrative analytic approaches considering omics and non-omics (OnO) data as well as in the nutrition epidemiological field. Regarding the latter, we compared the antioxidant profiles of 21 a priori-defined Mediterranean diet indexes and reported that the level of dietary antioxidant intake captured through the different indexes differed due to the variation in their construction. As of the data integrative efforts, we observed that only a small number of published studies performed a 'real' integration of OnO data, primarily to predict cancer outcomes. We identified



**Figure 2** Pancreatic cancer gene-based comorbidity network. (A) Gene network of 27 medical conditions. Edge width represents the strength (Jaccard index, JI) for each disease pair. Node size represents the number of genes obtained through DisGeNET for each medical condition. (B) Venn diagram showing the number of shared genes among pancreatic cancer and 4 autoimmune diseases. Squares show the genes shared between pancreatic cancer and autoimmune conditions.

the challenges in OnO data integration and presented, discussed, and proposed integrative analytical strategies towards its integration.

Translational activities

The Group actively provides support in several clinical trials on immunotherapy and vitamin D in bladder cancer at the methodological level. We continue to sustain the Spanish

Familial PC Registry (PanGen-FAM) and the establishment of the European Registry of PC (PancreOS). We lead the Research Work Stream of the Pancreatic Cancer Europe (EPC) multistakeholder platform, with who we hosted a session on PC Liquid Biopsy during the 2018 ESMO GI Meeting. To increase awareness of PC among health policy makers and discuss the urgent need to invest in PC research, we participated and co-organised sessions with MEPs at the European Parliament and with delegates at the Annual Meeting of the European Alliance of Personalized Medicine. ■

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AWARDS AND RECOGNITION

Member of the jury of the Banco Sabadell Award.

## FAMILIAL CANCER CLINICAL UNIT

Miguel Urioste  
Clinical Unit Head

Graduate Student  
Laura Pena

Technicians  
Maika González, Fátima Mercadillo,  
Mario Esteban Muñoz (since August)



### OVERVIEW

Lynch syndrome is a very complex entity associated with high risks for a wide variety of malignancies, including colorectal, endometrial, ovarian, gastric, urinary tract, pancreatic, biliary, small intestinal, prostatic, and brain cancers. Until now, the malignancies developed in people with Lynch syndrome were treated exactly in the same way as their sporadic counterparts. However, recent therapeutic advances in the immunologic effects of microsatellite instability (MSI), the hallmark of Lynch syndrome associated tumours, have resulted in important changes in the treatment of these patients.

MSI, by definition, is characterised by the somatic accumulation of mutations, which subsequently produce potentially antigenic frameshift neopeptides that account for the infiltrating lymphocyte reaction classically observed

in Lynch-associated tumours. The recent emergence of immune checkpoint inhibitors that work on the patients' own immune system has led to the use of this underlying biological characteristic to advance in the treatment of Lynch syndrome-associated tumours.

The Familial Cancer Clinical Unit (FCCU) is not only committed to screening blood samples with the aim of identifying germline mutations, but also to analysing tumour samples to determine their microsatellite status. Both findings play a critical role in the understanding of the molecular drivers of malignancy and the implementation of innovative precision-based therapies.

### CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

The clinical and diagnostic activities carried out by the FCCU through the consultancy in the Medical Oncology Department of Fuenlabrada's University Hospital, have contributed to the selection of patients who are good candidates for targeted therapies. In order to extend the study, we apply a multigene panel test to an increasingly larger number of pathologies. Ovarian cancer (OC) for instance, is a genetically heterogeneous malignancy that is potentially driven by multiple aberrant molecular pathways. Germline *BRCA1/2* mutations account for 65–85% of all hereditary OC, while mutations in Lynch genes (DNA mismatch repair genes) are responsible for 10–15% of these hereditary OC. Germline mutations drive the therapeutic strategy: OC associated to *BRCA1/2* mutations have a demonstrated sensitivity to PARP inhibitors, while immune checkpoint inhibitors are indicated for metastatic solid tumours associated with DNA mismatch repair deficiency.

Our clinical and diagnostic activities this year can be summarised as follows: 550 patients visited our consultancy at HUF (8.69% increase over 2017); and 508 genetic diagnostic studies were performed in the FCCU laboratory (18.69% increase). Among these studies, we identified 25 tumours with MSI, all of them potential candidates to be treated with monoclonal antibodies that target PD-1.

Our research in colorectal cancer (CRC) focuses on early-onset forms and multiple primary tumours. We recently reported the largest series of Synchronous Colorectal Cancers (SCRC), in which clonality was analysed by Single-Nucleotide Polymorphism array, and the subsequent statistical application; we were the first to correlate it with clinical phenotypes. Thirty-six per cent of our SCRC fulfilled clonality features. The existence of clonality within SCRC has important consequences throughout therapeutic management. The stratification in different categories may also serve as a starting point to more selectively analyse the molecular basis of SCRC and its relationship with environmental factors.

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 several aspects such as the high clinical heterogeneity usually result in a late diagnosis. We have studied this pathology at the clinical and molecular level in the largest series of Spanish patients with PHTS (145 probands). Overall, our findings are consistent with the syndrome descriptions in other populations, with a few exceptions such as a higher proportion of carriers of mutations in *PTEN* exon 1. We have also discussed the usefulness of the different diagnostic criteria proposed to date for this disease and have suggested recommendations based on our results. We are currently focusing on the search for phenotype modifiers, as in the case of the *KLLN* gene, as well as for other genetic factors that may explain the disease in *PTEN* wild type patients. For this last purpose, we are using a gene panel to look for mutations on the main pathway antagonised by *PTEN* – the PI3K/AKT/mTOR pathway – and are analysing whole exome sequencing data from selected cases. Our study continues to contribute to a better definition of PHTS and to help accelerate the diagnosis of the patients.

Addressing the functional consequence of germline missense variants involved in cancer genes is very important when prophylactic surgical removal of organs is the only therapeutic option to prevent the development of an aggressive cancer. In this context, we found 3 unrelated families with hereditary diffuse gastric cancer carrying the same germline missense variant in the *CDH1* gene: c.1679C>G. Through genetic and *in vitro* studies, we explored the effect of this variant and finally demonstrated its deleterious effect, suggesting that gastrectomy should be considered in patients harbouring this variant. ■

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## MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

Sandra Rodríguez-Perales  
Unit Head

Staff Scientist  
Raúl Torres



### OVERVIEW

Recurrent chromosomal rearrangements are very common and well-known hallmarks of cancer. One of their main consequences is the creation of new chimeric genes as a result of the fusion of the coding sequences of 2 different genes. The research activity of the Molecular Cytogenetics and Genome Editing Unit (MC&GEU) is focused on increasing the knowledge about the genetics of tumours and the discovery of new therapeutic targets. With the combined use of CRISPR genome editing and cytogenetic technologies, we are creating human *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 technologies used in the fields of molecular cytogenetics and genome editing. The Unit is constantly implementing and developing new technologies in the gene editing field. We

**“We have applied genome engineering approaches for cancer modelling, reproducing chromosome rearrangements and gene alterations. We provide access to the latest Cytogenetic and CRISPR technologies.”**

also participate in collaborative projects with clinical and basic science investigators at the CNIO and other institutes.

Graduate Student  
Pilar Puig (since October)

Technicians  
M. Carmen Martín, Marta Martínez-Lage (TS)\*, Francisco J. Moya (TS)\*, Patricia Moreno (since Feb.) (TS)\*

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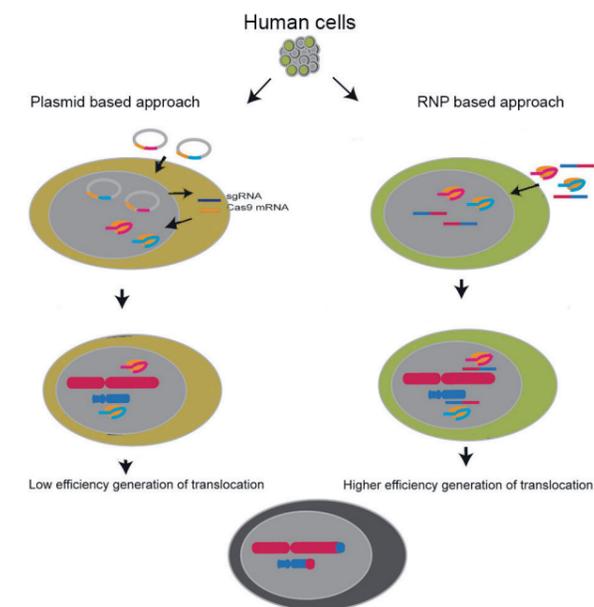
### RESEARCH HIGHLIGHTS

#### 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 have recently demonstrated the feasibility of utilising gRNA/Cas9 ribonucleoprotein (RNP) complexes to model cancers driven by fusion genes generated by chromosomal rearrangements. We have optimised new strategies to enhance the efficiency of the CRISPR-mediated translocation induction in human stem cells, including mesenchymal and induced pluripotent stem cells. We found that the generation of targeted translocation is significantly increased by using a combination of ribonucleoprotein complexes (Cas9 protein+sgRNA) and ssODNs. The CRISPR-Cas9-mediated generation of targeted translocations in human stem cells opens up new avenues to model cancer.

#### Technological and translational activities

We provide state-of-the-art Molecular Cytogenetic and Genome Editing services. The Unit supplies research groups with various techniques that may provide more sensitive and accurate tools to analyse cancer cells, such as chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific PCR-based FISH analysis to detect genome integration sites of small constructs



**Figure** Overview of efficient approaches for recreating cancer-associated chromosome translocations. Usage of RNP and ssODN efficiently recreates human chromosomal translocations.

including LV particles. 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 CRISPR-based cellular platforms offer a rapid, precise and affordable opportunity to functionally interrogate the cancer genome. In 2018, we carried out over 1,500 assays for experimental and clinically-oriented projects. ■

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## HUMAN GENOTYPING-CEGEN UNIT

Anna González Neira  
Unit Head

Graduate Students  
Hugo Tejera, Alejandro Velasco  
(since October)

Technicians  
Charo Alonso, Nùria Álvarez,  
Belén Herráez, Rocío Nuñez (TS)\*,  
Guillermo Pita (TS) \*

Student in Practice  
María Rodrigo (*Universidad  
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\**Titulado Superior (Advanced Degree)*



### 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, epigenetic studies using whole-genome methylation arrays are performed in this Unit. Complementarily, research focused on the identification of predictive biomarkers for precision medicine is also undertaken.

**“Matching cancer patients with treatments that are likely to be more effective and cause fewer side effects is what we strive for.”**

### RESEARCH HIGHLIGHTS

*Pharmacogenetic variants and response to neoadjuvant single-agent doxorubicin or docetaxel: a study in locally advanced breast cancer patients participating in the NCT00123929 phase 2 randomised trial.* Docetaxel and anthracycline are widely used in the treatment of breast cancer despite the benefit being limited to a small proportion of patients, and preoperative biomarkers predictive of clinical outcome remain lacking. We carried out a pharmacogenetic study in 181 patients with locally advanced breast cancer who were previously enrolled in a phase 2 randomised clinical trial (NCT00123929), in which patients were randomly assigned to receive doxorubicin (anthracycline) or docetaxel (taxane) in neoadjuvance. We assessed whether genetic variants in 15 key transport or metabolism genes relevant to doxorubicin and docetaxel drugs could play a role as predictive biomarkers. We identified a genetic variant, located in the promoter of ABCC2, as having the strongest association with tumour response observed in patients treated with doxorubicin ( $P=0.009$ ). We also identified a significant association for an intronic variant, located in CYP1B1, associated with docetaxel tumour response ( $P=2.15 \times 10^{-4}$ ). Our integrated pathway-based approach allows revealing promising genetic biomarkers for treatment outcome in breast cancer patients (Ruiz-Pinto S *et al.*, 2018).

*Genome-wide association study (GWAS) identifies three new loci associated with Ewing sarcoma susceptibility.* Ewing sarcoma (EWS) is a paediatric cancer characterised by the EWSR1-FLI1 fusion. Our previous GWAS identified susceptibility loci at 1p36.22, 10q21 and 15q15. We performed a GWAS of 733 EWS cases and 1346 unaffected individuals of European ancestry. Our study replicates previously reported susceptibility loci at 1p36.22, 10q21.3 and 15q15.1, and identifies new loci at 6p25.1,

20p11.22 and 20p11.23. In the analyses of the new loci, there is evidence of informative eQTLs with nearby biologically plausible candidate genes that could be likely target genes for future functional investigations. It is remarkable that 6 independent susceptibility regions with relatively large effect sizes (estimated OR > 1.7) have been discovered in a sample of 733 EWS cases. In conclusion, our study provides support for a strong inherited genetic component to EWS risk and suggests that interactions between germline variation and somatically acquired EWSR1-FLI1 translocations are important etiologic contributors to EWS risk (Machiela MJ *et al.*, 2018).

*New loci associated with risk to develop tobacco-induced lung cancer: genome-wide association study in heavy smokers.* We genotyped 2.37 million SNPs across the genome in heavy smokers that either developed NSCLC at an early age (extreme cases), or did not present NSCLC at an advanced age (extreme controls), selected from a discovery set ( $n = 3631$ ). We validated significant SNPs in 133 additional subjects with extreme phenotypes selected from databases including >39,000 individuals. Two SNPs were validated: rs12660420 ( $p$  combined =  $5.66 \times 10^{-5}$ ; OR combined = 2.80), mapping to a noncoding transcript exon of PDE10A; and rs6835978 ( $p$  combined =  $1.02 \times 10^{-4}$ ; OR combined = 2.57), an intronic variant in ATP10D. We assessed the relevance of both proteins in early-stage NSCLC. PDE10A and ATP10D mRNA expressions correlated with survival in 821 stage I-II NSCLC patients ( $p = 0.01$  and  $p < 0.0001$ ). PDE10A protein expression correlated with survival in 149 patients with stage I-II NSCLC ( $p = 0.002$ ). In conclusion, we validated 2 novel variants associated with risk of developing tobacco-induced NSCLC in heavy smokers (Fusco JP *et al.*, 2018). ■

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## CLINICAL RESEARCH PROGRAMME

MIGUEL QUINTELA-FANDINO Acting Programme Director



The Clinical Research Programme (CRP) has two 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, the CRP is composed of four Clinical Research Units and one supporting Unit. The Breast Cancer Clinical Research Unit has successfully completed the first kinase-based taxonomy of triple-negative breast cancer. The Prostate Cancer Clinical Research Unit, under David Olmos' supervision, has completed its prospective observational PROCURE study involving >1000 patients, whereby different predictive associations are being explored; two major manuscripts regarding the role of germline and somatic variants in response to antiandrogens or conventional chemotherapy have already been published thanks to this effort. The Lung Cancer Clinical Research Unit, led by Luis Paz Ares, has significantly contributed to the discovery of biomarkers that will impact the selection tools for targeted therapies in advanced lung cancer. They have also led several practice-changing international clinical trials. Finally, the Haematological Malignancies Clinical Research Unit, headed by Joaquín Martínez-López, has developed novel tools for the diagnosis and surveillance of the clinical course of different haematological malignancies. Regarding drug development and novel treatment approaches, an exciting novel line of research based on the *ex vivo* expansion of natural killer cells is currently ongoing. Finally, the Molecular Diagnostics Unit, led by Luis Lombardía, has continued to provide support to hospitals in the diagnosis of haematological malignancies.

Several contracts with 'Big Pharma' were signed during 2018 in order to progress in the development of cancer immunotherapies (Lung Cancer Unit). The Prostate Cancer Unit was awarded with a Department of Defense Grant in 2018. These achievements highlight the relevance of the translational research activities conducted by the CRP during 2018; we hope to further enhance these activities through future alliances with tertiary hospitals and medical societies over the next few years.

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

## BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino  
Junior Clinical Research Unit Head

Staff Scientists  
María José Bueno, Silvana A. Mouron

Clinical Research Fellow  
Juan V. Apala



### OVERVIEW

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

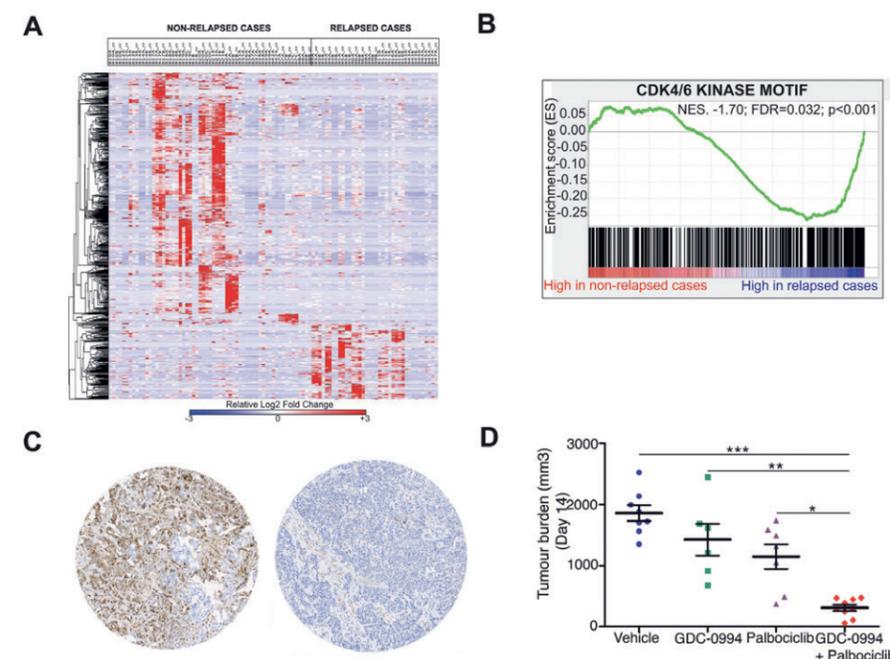
**“In 2018, the BCCRU completed the first study elaborating a kinase-based taxonomy of triple-negative breast cancer. This will enable therapeutic and biomarker-based precision-medicine initiatives.”**

- Study of the mechanisms of resistance against targeted therapies.

Graduate Students  
Elena Arconada (until November),  
Sara Fernández, José Luis Ruiz (since  
November)

Technicians  
Verónica Jiménez, Manuel Muñoz  
\**Titulado Superior* (Advanced Degree)

### RESEARCH HIGHLIGHTS



**Figure (A)** Phosphorylation profiles of whole tumours from TNBC with and without relapse were obtained by mass spectrometry. **(B)** Kinases with increased function accounting for such profiles were solved through an in-house algorithm (“Kinase-set enrichment analysis” or KSEA). **(C)** This information was then translated to immunohistochemistry; the left sample shows a patient with hyperactivated ERK, compared to a hypoactive one. **(D)** Combined treatment with agents targeting the 2 top-hits (CDK4/6 plus ERK inhibitors) in TNBC xenografts achieved a synergistic effect.

This year, we completed the first phosphoproteomic taxonomy of triple-negative breast cancer (TNBC), the most deadly subtype of this disease. Next-generation sequencing studies have failed in the task of finding simple biomarkers for complex phenotypic traits, such as response or resistance to therapeutic agents or disease course outside the context of penetrant oncogenic-addiction drivers. Rather, the TNBC phenotype traits are the result of multiple contributing low-penetrance mutations. We have found that different clusters of mutations collapse into discrete patterns of activation of the proteome in the form of protein phosphorylation, and that such patterns are driven by a small number of hyperactive/hypoactive kinases. Specifically, we found 6 kinases that, when all of them are “switched off”, patients are long-term disease-free after >10 years. However, when 1 or more of those kinases are “on”, the risk of relapse increases 10-fold. More importantly, all 6 kinases are actionable and we have found profound synergy in all 2-by-2 combinations in preclinical models.

On the clinical side of our activities, during 2018, we completed 2 clinical trials that were launched based on our research.

Specifically, one of the trials explored the reversal of immune-tolerance induced by chronic hypoxia observed after prolonged exposure to antiangiogenics. A second trial explored the reversal of the metabolic switch of tumours experiencing vascular normalisation in response to antiangiogenics. Both trials implement targeted agents (a PD-L1 inhibitor or a mitochondrial inhibitor, respectively) directed against the 2 main regulatory nodes in each of the 2 major patterns of angiogenesis inhibitor escape identified during the period 2015-2017. ■

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reveals kinase activity profiles that predict treatment outcome in triple-negative breast cancer. *Nat Commun* 9, 3501-15.

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## PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos  
Junior Clinical Research Unit Head

Clinical Investigator  
Elena Castro

Clinical Research Fellow  
Rebeca Lozano



### OVERVIEW

Prostate cancer remains a major health burden as over a million men around the world are annually diagnosed with prostate cancer. Up to 30% of them may develop metastatic prostate cancer, which is the advanced form of this disease, once it has spread outside the prostate and is no longer curable. This metastatic stage causes about 6,000 deaths every year in Spain alone, whilst in the US over 30,000 men succumb to the disease every year. In recent years, different subtypes of prostate cancer have been identified based on different genomic profiles. We believe that a better understanding of cancer biology, as well as an improved human prostate cancer taxonomy linked to clinical outcomes, could lead to improved patient outcomes through the application of tailored treatment strategies as opposed to the current one-fits-all approach. As an example of one of these clusters, 20-25% of all metastatic prostate cancers have aberrations in DNA repair genes; about half of these aberrations may correspond to inherited mutations.

### RESEARCH HIGHLIGHTS

During 2018, our Group made significant progress in many projects. We finalised the primary analyses of our PROREPAIR-B study and have also completed recruitment in 3 additional studies from our PROCURE platform of prospective biomarker studies: PROSTAC, PROSABI & PRORADIUM. Over 1,000 men with metastatic castration-resistant prostate cancer (mCRPC) have been enrolled in these studies to this day. Our clinical CNIO-IBIMA unit has been consolidated as 1 of the top prostate cancer clinical trials units in Europe, playing a leading role in several early and pivotal clinical trials. We have also reported our first investigator-initiated phase II study. At the preclinical level, we have made some advances in the characterisation of the biological role in prostate cancer of different DDR defects, including Atm loss. We have also made progress in developing representative *in vivo* models of metastatic prostate cancer and patient derived-xenografts.

Post-doctoral Fellow  
Isabel Aragón

Technician  
Carles Moreno (since May)

Graduate Students  
Ylenia Cendón, Lorena Magraner, Paz Nombela

Visiting Scientists  
Teresa Garcés (*Instituto de Investigación Biomédica de Málaga*),

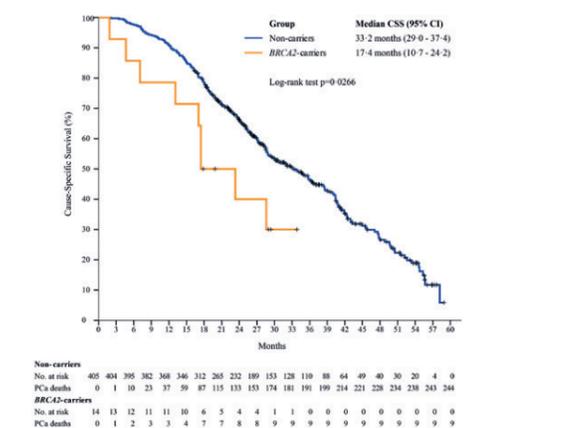
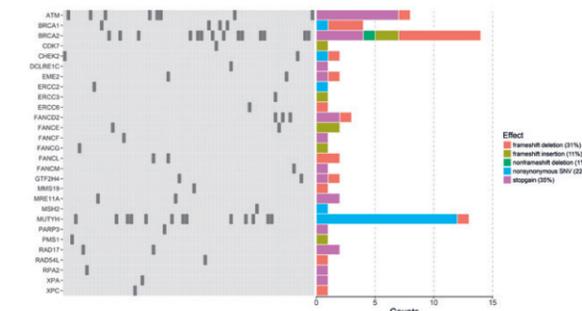
Gala Grau (until March, *Instituto de Investigación Biomédica de Málaga*), Ana M. Gutiérrez-Pecharoman (*Universidad de Móstoles, Madrid*), Fernando López-Campos (until Nov., *Hospital Ramón y Cajal*,

Madrid), M. Isabel Pacheco (*Instituto de Investigación Biomédica de Málaga*), Leticia Rivera (*Instituto de Investigación Biomédica de Málaga*), Benjamín Olmos (until Sept., *Hospital Universitario Virgen de La Victoria, Málaga*)

**PROREPAIR-B study.** The primary analyses of the PROREPAIR-B study will be published early in 2019 in the *Journal of Clinical Oncology*. The highlights of this study can be briefly summarised as follows: up to 16% of mCRPC may carry germline pathogenic mutations in DDR genes; a *BRCA2* germline mutation has twice the risk of progressing to castration-resistant disease and of death from prostate cancer. We have just started analysing the prevalence of somatic aberrations in DDR genes in this study as part of a DoD IMPACT award. In addition, we are completing the genomic and transcriptomic characterisation of a large collection of *BRCA2* mutated prostate cancers in order to identify secondary events that may contribute to the poor prognosis of the affected men.

**AR gain and mCRPC treatment selection.** As part of an ongoing international collaboration with Dr Attard's lab (UCL, London) and Dr di Giorgi's team (IRCSS, Meldola), we have determined that mCRPC, having a normal number of copies of the androgen receptor (AR) gene in ctDNA, have a lower risk of disease progression and a higher life expectancy when they are treated with abiraterone/enzalutamide, with a 50% improvement compared to docetaxel. On the other hand, the patients with more copies of the AR gene respond slightly better to docetaxel.

**SWITCH phase II study.** This study, recently published in the *British Journal of Cancer*, demonstrated that the simple change of the supporting steroid, switched from prednisone to dexamethasone, while maintaining abiraterone, helps to re-induce the response to abiraterone in about 4 out of every 10 patients progressing by PSA criteria. This response does not occur in patients with AR gain detected in plasma ctDNA, while patients with AR normal status benefit the most. ■



**Figure** PROREPAIR-B study. Distribution of the pathogenic mutations in DDR genes identified in the study (top). Kaplan-Meier curves for cause-specific survival from diagnosis of mCRPC: *BRCA2* mutant vs noncarriers (bottom).

### PUBLICATIONS

- Smith MR *et al.* (2018). Apalutamide treatment and metastasis-free survival in prostate cancer. *New Engl J Med* 378, 1408-1418.
- Lawrence MG *et al.* (incl. Lopez-Campos F, Castro E) (2018). Patient-derived models of abiraterone and enzalutamide resistant prostate cancer reveal sensitivity to ribosome-directed therapy. *Eur Urol* 74, 562-572.
- Lorente D, Olmos D *et al.* (2018). Circulating tumour cell increase as a biomarker

of disease progression in metastatic castration-resistant prostate cancer patients with low baseline CTC counts. *Ann Oncol* 29, 1554-1560.

- Mikropoulos C *et al.* (incl. Castro E) (2018). Prostate-specific antigen velocity in a prospective prostate cancer screening study of men with genetic predisposition. *Br J Cancer* 118, 266-276.
- Romero-Laorden N *et al.* (incl. Castro E, Olmos D) (2018). Phase II pilot study of the prednisone to dexamethasone switch in metastatic castration-resistant prostate cancer (mCRPC) patients with limited pro-

gression on abiraterone plus prednisone (SWITCH study). *Br J Cancer* 119, 1052-1059.

- Cassinello J *et al.* (incl. Olmos D) (2018). SEOM clinical guidelines for the treatment of metastatic prostate cancer (2017). *Clin Transl Oncol* 20, 57-68.

### AWARDS AND RECOGNITION

- Member of the Board of Directors, European Organization for Research and Treatment of Cancer (EORTC).
- Impact Award (Partnering PI), US Department of Defense, Congressionally Directed

Medical Research Programs.

- Faculty Board Member, EORTC-EC- CO-AACR-ESMO Methods in Clinical Cancer Research Workshop, Zeist, The Netherlands.
- Elena Castro: Faculty Board Member, ESMO Preceptorships in Prostate Cancer.
- Rebeca Lozano was awarded the 'Merit Award', American Society of Clinical Oncology GU Cancers Symposium, San Francisco; 'Best Communication' Award, 2018 SEOM annual Meeting; and the *Rio Hortega Fellowship* 2018, *Instituto de Salud Carlos III*, Spain.

## MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía  
Unit Head

Technician  
Diana Romero



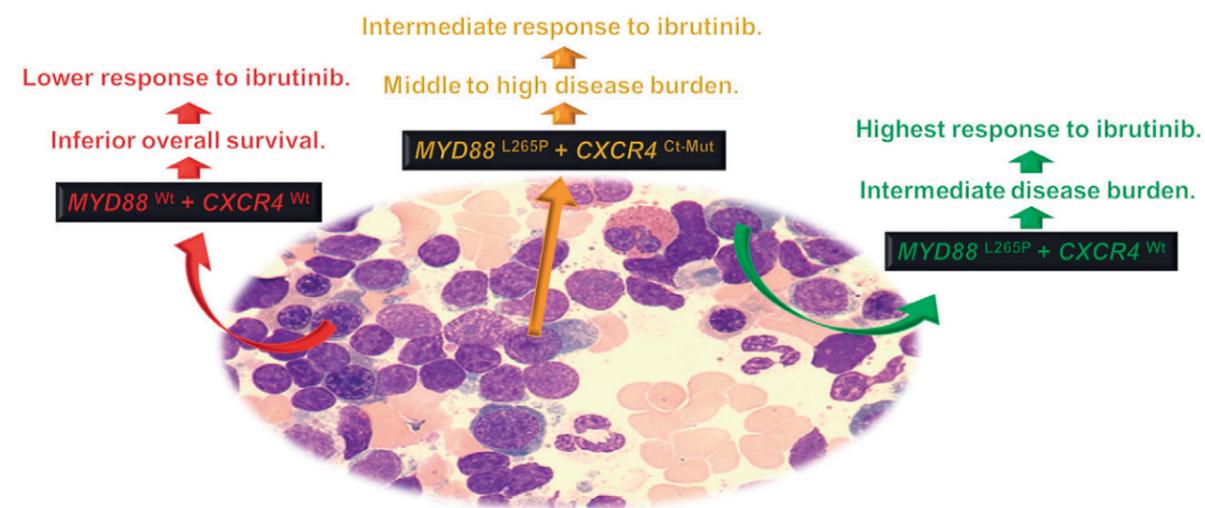
### OVERVIEW

The main objectives of the Molecular Diagnostics Unit (MDU) are directed towards offering quality molecular tests for patients with cancer in order to support the current clinical services and diagnostic laboratories in hospitals of the Spanish National Health System (NHS). In this regard, the Unit provides a wide range of highly sensitive molecular assays to determine changes in the sequence or expression levels of key genes involved in cancer, and to enable the detection of Minimal Residual Disease in patients showing clinical remission as well as to follow-up on their response to therapy. Likewise, MDU is also devoted to implementing recent up-to-date cancer diagnostics solutions, not only to support the NHS but also to assist the Clinical Research Units and Research Groups at the CNIO. In addition, MDU collaborates with international and national groups dedicated to standardising and improving

**“In this new era of precision medicine in cancer, Molecular Diagnostics is playing a fundamental role as demonstrated by the increasing variety of assays requested by haemato-oncologists throughout 2018.”**

molecular diagnostics tests in cancer, and participates in teaching as well as in educational programmes for clinical post-residents, undergraduate and graduate students.

### CORE UNIT HIGHLIGHTS



**Figure** Molecular testing of *MYD88* and *CXCR4* genes in plasmacytoid lymphocytes allows for different prognostic and/or therapeutic options for patients with Waldenström's Macroglobulinemia. (Wt: Wild Type; L265P: Leucine to Proline substitution at position 265; Ct-Mut: C-terminus nonsense/frameshift mutations).

During 2018, we have added and/or expanded 3 diagnostics tests.

First of all, the detection of the fusion gene *BCL1-IgH* by PCR was added to our list of services. Although the genetic translocation t(11;14)(q13; q32) is present in other lymphoproliferative diseases, it occurs mainly in mantle cell lymphomas (50-70%), which are more aggressive and have, in general, a worse prognosis than other low-grade B-cell lymphomas. This assay will be used not only to diagnose patients with a suspected mantle cell lymphoma, but also to monitor and evaluate recurrences of the disease.

We have also complemented the *MYD88* gene testing of patients with Lymphoplasmacytic Lymphoma/Waldenström's Macroglobulinemia (LPL/WM), by implementing a test that enables the detection, by Sanger sequencing, of nonsense and frameshift mutations in the *CXCR4* gene. The protein coded by this gene activates the AKT1/MAPK pathways in B-lineage cells and facilitates cell migration. Mutations in *CXCR4*, commonly found in association with *MYD88 L265P* mutation, are associated with primary resistance and initial lack of response to BTK, PI3K, and mTOR inhibitors. Thus, this assay will be used to aid in the prognosis and therapeutic management of LPL/WM patients (FIGURE).

Additionally, we directed our efforts towards improving the clinical utility of molecular testing based on the *BRAF* gene. In this regard, to complement the detection of the recurrent V600 mutation of BRAF in melanoma patients, we extended the analysis by bi-directional sequencing of exon 11 to enable the management of patients with lung cancer. Mutations in exon 11 are regularly found in lung tumours that are wild type for EGFR, KRAS, ALK, and other driver alterations. Moreover, these patients, with decreased sensitivity to gefitinib, responded to dasatinib with no additional treatment for several years.

Finally, during 2018, in the framework of our training policy, we hosted one medical resident and 2 undergraduate students. ■

#### AWARDS AND RECOGNITION

- Member of the Committee for Ethical Research (CEI; Comité de Ética de la Investigación), Instituto de Salud Carlos III, Madrid, Spain.

## H120-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López  
Clinical Research Unit Head

Staff Scientists  
Lucía V. Fernández, Miguel Gallardo



### Clinical Scientists

Rosa Ayala, Teresa Cedena, María Calbacho, Javier de la Serna, Carlos Grande, Ana Jiménez, Pilar Martínez, Inmaculada Rapado, Antonia Rodríguez, Ricardo Sánchez, Beatriz Sanchez-Vega (until October)

### Post-Doctoral Fellows

Almudena García, Alejandra Leivas, María Linares, Antonio Valeri

### Graduate Students

Sergio Algar (since November), Isabel Cuenca, Jessica Encinas (since

November), Elena Maroto, Rebeca Mateos (February-October), M. Luz Morales, Alejandra Ortíz, Alba Rodríguez, Yanira Ruiz, Laura Sánchez

### Technicians

Pedro Aguilar (since March), Adrián

Fernández (since June), Irene García, Vanesa Garrido, Alexandra Juárez, Laura Moreno, Esther Onecha

### Students in Practice

Laura Carrasco (since November, UAH), Cristina Crespo (since November, UCM)

## OVERVIEW

The Haematological Malignancies Laboratory focuses on investigating novel drivers, biomarkers, diagnostic tools and therapeutic targets and approaches in haematological neoplasms such as myeloma and acute myeloid leukaemia.

Five main lines define our research project:

- Generation of mouse models focused on the molecule hnRNP K, a novel driver of lymphoma and leukaemia.
- Development of novel diagnostic and follow-up tools, such as minimal residual disease analysis in acute myeloid leukaemia (AML).
- Screening of novel drivers, biomarkers and therapeutic targets by next-generation-sequencing (NGS, e.g. exome sequencing of amyloidosis).
- Innovation of immunotherapy approaches. Generation of NK CARs and *in vitro/in vivo* validation.
- Novel therapeutic approaches. Screening of novel compounds (e.g. hnRNP K inhibitors) and pre-clinical trials of new drugs or drug combinations.

**“We have developed a strategy to identify undetectable levels of minimal residual disease using an NGS method, thereby improving the capacity to predict AML outcome over the current technical approaches.”**

## RESEARCH HIGHLIGHTS

## Minimal residual disease monitoring in acute myeloid leukaemia

Assessment of minimal residual disease (MRD) is critical for monitoring patients in morphological remission as well as to inform decisions about further therapy.

We designed and validated a high-throughput sequencing method for MRD assessment of cell clonotypes with 4 typical AML. Our analysis showed better sensitivities ( $10^{-4}$  for SNVs and  $10^{-5}$  for InDels) than current methods or other novel techniques such as dPCR: the sensitivity of dPCR for InDels was similar to that reported in a previously published study ( $10^{-2}$ ). It should be noted that the prediction of survival and progression of AML using MRD-NGS was improved over the other methodologies employed.

In conclusion, we have optimised a new targeted sequencing method with high sensitivity for MRD evaluation and applicability for a high percentage of AML patients, thereby improving the capacity to predict AML outcome over MFC or qPCR in our cohort (work published in *Haematologica*).

## Novel therapeutic combination for primary myelofibrosis

Ruxolitinib is the frontline non-palliative treatment for myelofibrosis; however, a significant number of patients lose or present suboptimal response, are resistant, or have unacceptable toxicity. We found that the combination of ruxolitinib and nilotinib had a synergistic effect against

myelofibrosis. Moreover, the addition of prednisone to the ruxolitinib/nilotinib combination improved the synergistic effect in all myelofibrosis samples studied.

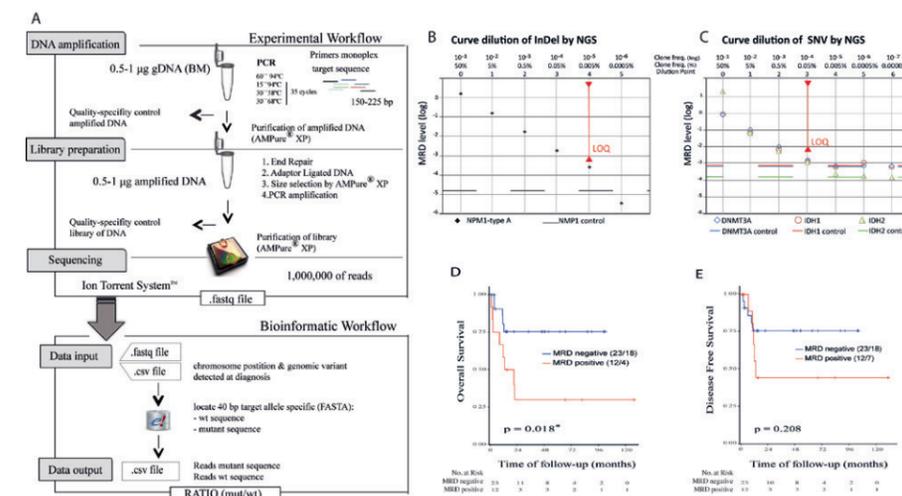
We provide evidence that the ruxolitinib/nilotinib/prednisone combination is a potential therapy for myelofibrosis, possibly through the anti-fibrotic effect of nilotinib, the immunomodulatory effects of ruxolitinib and prednisone, and the anti-proliferative effect of ruxolitinib. This combination will be further investigated in a phase Ib/II clinical trial in myelofibrosis (work accepted with major changes for publication in *Haematologica*).

## Novel therapeutic combination for acute myeloid leukaemia

Different tyrosine kinase inhibitors have been used in FLT3 targeting, but their effects are limited by drug resistance. In order to rationally combine them with other agents, we explored the alternative pathways activated during the development of resistance.

Differentially phosphorylated proteins and resistance mechanisms after tyrosine kinase inhibitor resistance were identified. Efficacy and safety of rational combinational therapies were assayed *in vitro*, *ex vivo* and *in vivo*.

The results suggested activation of the MEK pathway, therefore, we characterised the effect of the MEK inhibitor trametinib *in vitro* and *ex vivo*. Trametinib exerted strong synergy with



**Figure** NSG method for MRD detection. (A) Workflow of NGS-MRD method. (B) InDels calibration curve of MRD in serial dilutions. Top, 10-fold dilution curve for the assessment of sensitivity of sequencing in InDels. (C) SNV calibration curve of MRD in serial dilutions. Top, 10-fold dilution curve for the assessment of sensitivity of sequencing in SNV. (D) Prognosis analysis of OS in AML patients stratified according to MRD levels by Kaplan-Meier conventional methods. (E) Prognosis analysis of DFS in AML patients stratified according to MRD levels by conventional methods. Kaplan-Meier plots.

the tyrosine kinase inhibitor midostaurin, inhibiting different FLT3 downstream pathways.

Our data provide preclinical evidence that combining a tyrosine kinase inhibitor, such as midostaurin, with a MEK inhibitor, such as trametinib, is a rational and efficacious treatment regimen for a wide range of acute myeloid leukaemias (work under review in the *Journal of Experimental & Clinical Cancer Research*).

## DNA methylation mutations predict azacitidine response in myelodysplastic syndromes

Alterations in DNA methylation are involved in the pathogenesis of myelodysplastic syndromes (MDS), however,

whether they can also influence their response to azacitidine/decitabine treatment has not been clearly elucidated.

We analysed frequently mutated regions in 34 genes that are likely candidates to be involved in the pathogenesis of MDS. We have found that the profile of several gene mutations identified at diagnosis may represent a useful predictive biomarker of the response to azacitidine therapy. Meta-analysis identified the *TET2* gene as the strongest biomarker of treatment success. Additionally, the presence of mutations in the DNA methylation pathway and the number of driver mutations are predictors of response to hypomethylating agents in patients with MDS (work published in *Oncotarget*). ■

## PUBLICATIONS

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- Barrio S, Stühmer T, Da-Viá M, Barrio-García C, Lehnert N, Besse A, Cuenca I, Gari-tano-Trojaola A, Fink S, Leich E, Chatterjee M, Driessen C, Martínez-Lopez J, Rosenwald A, Beckmann R, Bargou RC, Braggio E, Stewart AK, Raab MS, Einsele H, Kortüm

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- Arenas Cortés A, Ayala Díaz R, Hernández-Campo P, Gorrochategui J, Primo D, Robles A, Morales ML, Ballesteros J, Rapado I, Gallardo M\*, Linares M\*, Martínez-López J\* (2018). Ruxolitinib in combination with prednisone and nilotinib exhibit synergistic effects in human cells

lines and primary cells from myeloproliferative neoplasms. *Haematologica*. PMID: 30545926.

- Misiewicz-Krzeminska I, Corchete LA, Rojas EA, Martínez-López J, García-Sanz R, Oriol A, Bladé J, Lahuerta JJ, Miguel JS, Mateos MV, Gutiérrez NC (2018). A novel nano-immunoassay method for quantification of proteins from CD138-purified myeloma cells: biological and clinical utility. *Haematologica* 103, 880-889.
- Onecha E, Linares M, Rapado I, Ruiz-Heredia Y, Martínez-Sánchez P, Cedena T, Pratcorona M, Perez Oteyza J, Herrera P, Barragan E, Montesinos P, Garcia Vela JA, Magro E, Anguita E, Figuera A, Rianza R, Martínez-Barranco P, Sanchez-Vega B, Nomdedeu J, Gallardo M, Martínez-Lopez J, Ayala R (2018). A novel deep targeted sequencing method for minimal residual disease monitoring in acute myeloid leukemia. *Haematologica*. PMID: 30093399.

- Ruiz-Heredia Y, Sánchez-Vega B, Onecha E, Barrio S, Alonso R, Martínez-Ávila JC, Cuenca I, Agirre X, Braggio E, Hernández MT, Martínez R, Rosiñol L, Gutiérrez N, Martín-Ramos M, Ocio EM, Echeveste MA, Pérez de Oteyza J, Oriol A, Bargay J, Gironella M, Ayala R, Bladé J, Mateos MV, Kortum KM, Stewart K, García-Sanz R, San Miguel J, Lahuerta JJ, Martínez-Lopez J (2018). Mutational screening of newly diagnosed multiple myeloma patients by deep targeted sequencing. *Haematologica* 103, e544-e548.
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Ayala R, Onecha E, Abaigar M, Such E, Ramos F, Cervera J, Díez-Campelo M, Sanz G, Rivas JH, Lucía A, Martínez-López J (2018). Mutations in the DNA methylation pathway and number of driver mutations predict response to azacitidine in myelodysplastic syndromes. *Oncotarget* 9, 30936.

- Ruiz-Heredia Y, Sanchez-Vega B, Barrio S, Linares M, Rapado I, Braggio E, Stewart K, Folgueira MD, Ramos A, Collado L, Ruiz J, Toldos O, Hernandez-Lain A, Martínez-Lopez J (2018). Concurrent progressive multifocal leukoencephalopathy and central nervous system infiltration by multiple myeloma: A case report. *J Oncol Pharm Pract*. PMID: 29690814.

## Selected publications at other institutions

- Hernández-Boluda JC, Pereira A, Correa JG, Alvarez-Larrán A, Ferrer-Marín F, Raya

JM, Martínez-López J, Velez P, Pérez-Encinas M, Estrada N, García-Gutiérrez V, Fox ML, Payer A, Kerguelen A, Cuevas B, Durán MA, Ramírez MJ, Gómez-Casares MT, Mata-Vázquez MI, Mora E, Gómez M, Cervantes F (2018). Prognostic risk models for transplant decision-making in myelofibrosis. *Ann Hematol* 97, 813-820.

- Macauda A, Castellí E, Buda G, Pelosini M, Butrym A, Watek M, Kruszewski M, Vangstedt AJ, Rymko M, Jamrozak K, Abildgaard N, Haastrup EK, Mazur G, Rios R, Jurczyszyn A, Zawirska D, Dudziński M, Ražny M, Dutka M, Tomczak W, Suska A, Druzd-Sitek A, Marques H, Pettrini M, Markiewicz M, Martínez-Lopez J, Ebbesen LH, Iskierka-Jażdżewska E, Sainz J, Canzian F, Campa D (2018). Inherited variation in the xenobiotic transporter pathway and survival of multiple myeloma patients. *Br J Haematol*. PMID: 30079960.
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Rosiñol L, Oriol A, Teruel AI, Lopez de la Guía A, Bengoechea E, Palomera L, de Arriba F, Hernandez JM, Granell M, Peñalver FJ, García-Sanz R, Besalduch J, Gonzalez Y, Martínez RB, Hernandez MT, Gutierrez NC, Puerta P, Valeri A, Paiva B, Blade J, Mateos MV, San Miguel J, Lahuerta JJ, Martínez-Lopez J; GEM (Grupo Español de MM)/PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) Cooperative Study Group (2018). Prognostic utility of serum free light chain ratios and heavy-light chain ratios in multiple myeloma in three PETHEMA/GEM phase III clinical trials. *PLoS One* 13, e0203392.

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K, Ballesteros J, Ghia P (2018). A novel *ex vivo* high-throughput assay reveals antiproliferative effects of idelalisib and ibrutinib in chronic lymphocytic leukemia. *Oncotarget* 9, 26019-26031.

## AWARDS AND RECOGNITION

- Joaquín Martínez-López:
- Proyectos de Investigación en Salud (PI) 2018 Health Research Project.
- Proyectos de Desarrollo Tecnológico en Salud (DTS) 2018 Healthcare Technology Development Project.
- Miguel Gallardo:
- Proyectos de Investigación en Salud (PI) 2018 Health Research Project.

## H120-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis G. Paz-Ares  
Clinical Research Unit Head

Staff Scientists  
Teresa Argullo, Irene Ferrer, Rocio García, Eva M. Garrido, Santiago Ponce, M. Carmen Riesco



Clinical Investigator  
M. Teresa Muñoz (since October)

Post-Doctoral Fellows  
Pablo Gella (until May), María Pedraza (until April), Beatriz Soldevilla

Graduate Students  
Carlos Carretero, Santiago García, Ángela Marrugal, Ángel Nuñez (since September), Laura Ojeda, Álvaro Quintanal (until February), Javier Ramos, Beatriz Rubio (since

September), Patricia Yagüe

Technicians  
M. Cristina Cirauqui (since July), Patricia Cozar, M. José Durán (until June), Aicha El Bakkali (until August),

Mirella Gallego (since February), Laura García, Beatriz Gil, Rocío Suárez

Students in Practice  
Ana González (until July), Alba Santos (since November)

### 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 that are closer to solving the problems of lung cancer patients. The two main research areas of our Unit involve: the identification of new molecular biomarkers that can be used in the clinic for diagnostic, prognostic and predictive purposes; and the development of novel treatment strategies that include 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. On the other hand, we have developed a patient-derived xenograft (PDX) platform of non-small-cell lung cancers to test new therapeutic strategies. Finally, our Unit has extensive experience in taking new drugs to the clinic (phase I trials), as well as in conducting practice-changing phase II/III trials in the fields of precision oncology 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 (e.g. erlotinib, afatinib, Nivolumab, M7824) as well as combinations of checkpoint inhibitors (e.g. Ipilimumab plus Nivolumab, chemotherapy plus Pembrolizumab, Durvalumab following chemoradiation) in lung cancer that have impacted clinical practice worldwide.”**

## RESEARCH HIGHLIGHTS

## Biomarker discovery and implementation

The Group has deciphered the biological role of FGFR1 and FGFR4 in non-small cell lung cancer (NSCLC) and has developed new biomarkers with a predictive role for anti-FGFR therapy in NSCLC. Currently, we are validating the results on a series of well characterised PDX models, generating a diagnostic kit and carrying out the technical validation of the biomarker, as well as planning a phase II trial proposal with an FGFR inhibitor in NSCLC patients with high expression of the novel biomarker.

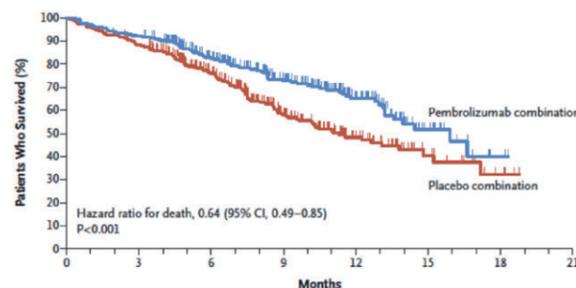
The Group has also validated an NGS-based algorithm for the determination of genomic aberrations (in tumour tissue but also in cfDNA) that may guide treatment for clinical practice. More recently, we have led the first clinical validation of tumour mutational burden as a predictive biomarker for checkpoint inhibitors in lung cancer, and particularly, for Ipilimumab plus Nivolumab.

## Early clinical trials

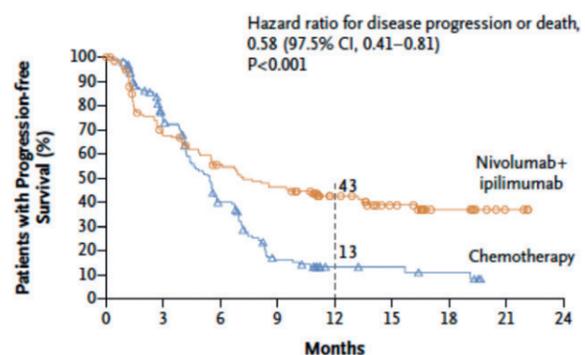
Our Group has significantly expanded its activities regarding the testing of new molecules and combinations in solid tumours, particularly in the field of immune-based approaches; in 2018, we participated in more than 35 projects in this research area. Recently, our Group provided feasibility and encouraging initial data on the anti-tumour activity of M7824, a bifunctional fusion protein targeting PD-L1 and TGF-beta in pretreated NSCLC (response rate in PD-L1 expressing tumours in more than 50% of the cells: 71%). Encouraging tumour-agnostic data of Entrectinib in tumours driven by activated NTRK fusion proteins were presented at the ESMO 2018 Congress (response rate 57.4%; median progression-free survival of 11.4 months).

## 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 with combinations of chemotherapy plus Pembrolizumab or Ipilimumab plus Nivolumab (Hellmann MD *et al.*, *NEJM* 2018; Paz-Ares L *et al.*, *NEJM* 2018). In addition, the Group has actively contributed to the results of a Phase III trial showing a significant improvement in survival for stage III NSCLC patients treated with the anti PD-L1 agent *Durvalumab* following chemoradiation (Antonia S *et al.*, *NEJM* 2018). ■



**Figure 1** Results of the KeyNote 407 randomised clinical trial, showing an improvement in overall survival (OS) of chemotherapy plus Pembrolizumab, as compared to chemotherapy alone (HR 0.64;  $p < 0.001$ ) in stage IV Non-Small-Cell Lung Cancer (NSCLC) patients.



**Figure 2** Results of the Checkmate 227 randomised clinical trial, showing the superiority in progression-free survival (PFS) for the Ipilimumab plus Nivolumab combination, as compared to platinum chemotherapy (HR 0.58;  $p < 0.001$ ) in stage IV NSCLC with high mutational burden (>10 mutations per MB).

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## AWARDS AND RECOGNITION

- Premio 'Best in Class (BIC)' 2018 en *Investigación en Oncología*, Spain.

## BIOBANK

Miguel Quintela-Fandino (since August)  
Acting Director

Manuel M. Morente (until July)  
Director



### OVERVIEW

The 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. Additionally, the CNIO Biobank participates in Biospecimen Science research projects.

Therefore, the 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)\*, Lorena Ayala (since Sept.), Patricia Camacho (until Oct.)(TS)\*,

Francisco de Luna (TS) \*, Maria Herrero (since Nov.)

\**Titulado Superior (Advanced Degree)*

### RESEARCH HIGHLIGHTS

#### Biobanking

In 2018, the CNIO Biobank ceded tissue samples from 675 cases thereby supporting 7 research projects. The accumulated impact of this year’s activity resulted in 6 (Q1) publications acknowledging CNIO’s Biobank contributions. The mean impact factor (IF) of the 6 published articles in 2018 (*Nat Commun, EMBO Mol Med, Cell Metab, Int J Cancer, Proc Natl Acad Sci USA and Oncotarget*), for which our Unit provided support, was 10.409.

#### Other activities

- Sample processing of 107 frozen samples to generate slices for extraction and staining.
- Management of project-driven and diagnostic collections:
  - The Familial Cancer Unit collection: we collaborated with the Familial Cancer Unit in the acquisition of 66 new tissue cases, the histopathological diagnostic revision of 30 cases, and the histopathological evaluation of 80 cases in a research context.
  - Management of 659 human samples for 3 different collections.
  - Histopathology evaluation of 294 cases in collaboration with the Human Cancer Genetics and Clinical Research Programmes.
- Virtual catalogue including 269 images; 139 of them are histological H&E stainings. Work on expanding this catalogue to include whole section paraffin-embedded samples is ongoing.
- CNIO projects were supported for ethical evaluation by the *Instituto de Salud Carlos III (ISCIII)* Research Ethics Committee.

The CNIO Biobank coordinates the extended Spanish Biobank Platform Project funded by the *ISCIII*. CNIO’s Biobank managed 20 projects and 37 requests from basic and clinical researchers, as well as from the industry. It also implemented mobility grants for biobank personnel (10 pers.) and organised 5 specialised Scientific Meetings plus the Spanish Biobank Congress in Oviedo.

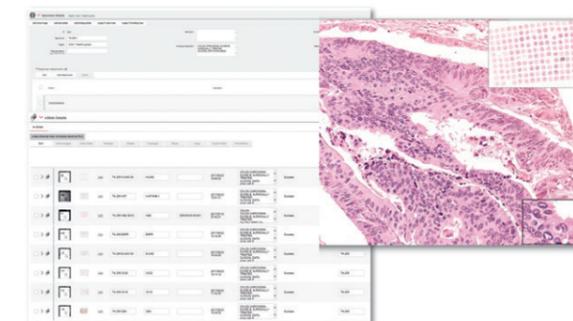


Figure Virtual catalogue of human samples.

The CNIO Biobank participates in 3 multicentre research projects:

- Optimark project (PI16/00946) (led by the CNIO Biobank): focused on the identification of quality markers for tissue samples sensitive to pre-analytical variables.
- Exospore project: 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 2018, we actively participated in 3 school visits and hosted the stays of several internship students: 2 FCT students (Laboratory Training Programme for Technicians), 4 high school students (CNIO & The City Outreach Programme), 2 students from the “4ºESO+Empresa” Programme (*Comunidad de Madrid*), and 1 *Erasmus* predoctoral student (*Erasmus* Internship Programme). The CNIO Biobank also co-organises a Master’s degree in Biobanking at the *Universidad Católica de Valencia*. ■

#### PUBLICATION

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# Innovation

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**CAROLINA POLA**  
Director of Innovation

**“Bringing discoveries into society as new medicines and technologies is a goal that we nurture with an overarching strategy and team effort.”**

Identifying how to leverage our innovation capacities and providing the means and framework to make it happen is the objective of the Direction of Innovation. To turn a scientific discovery into a product requires the identification of those specific scientific results that have the potential to change how a disease, such as cancer, is diagnosed, treated or managed.

The excellent science that emanates from the research carried out by CNIO's investigators is the ideal ground for innovation to thrive. As such, the CNIO has taken part in the Milner Therapeutics Institute since its inception as an affiliate academic institution. In this role, the Centre has had the opportunity to interact with numerous industry partners during the first symposium held in Cambridge in October 2018. This year, the reach out activities of the Direction of Innovation, in close collaboration with the Office of Technology Transfer and Valorisation, also included the marketing of our technologies and capabilities in bio-business meetings, such as BioSpain that was held in Seville, Spain, as well as in other forums. Such efforts have resulted, among others, in meetings with four important venture capitals looking for new opportunities in the biomedical field, and a myriad of interactions with other industry partners, including their open innovation and academic scouting departments.

Our academic drug discovery programme continues to contribute to closing the valley of death and to developing a pipeline that feeds from the invaluable discoveries made by CNIO investigators as novel targets and innovative phenotypic screenings. This year, we have made great strides to increase the openness of the programme in order to become the drug discovery unit of reference for other centres of excellence in Spain, which currently lack our drug discovery capabilities.

In 2018, *CaixaImpulse* awarded two innovation projects led by CNIO researchers: an innovative project to further advance one of our lead drug discovery programmes, led by Maria Blasco; and another innovative advanced therapeutic approach, headed by Sandra Rodríguez-Perales. Moreover, collaborations with industry, along with the licensing activities of our antibodies and sales, underscore co-development opportunities and also represent about 15% of the total income of the CNIO. In 2018, up to 7 discoveries and technologies were identified and protected to be further developed into novel technologies, and conversations continue with a recently created start-up, Senolytic Therapeutics, in order to identify synergies for advancing their products. We expect 2019 to be a year in which we will continue to reap an increasing amount of benefits from our efforts.

## BIOTECHNOLOGY PROGRAMME

FERNANDO PELÁEZ Programme Director



The main mission of the Biotechnology Programme is to provide expert technical support and advice 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 reagents for cancer research. The Programme is currently composed of nine Core Units covering major areas in Biotechnology, namely Genomics, Proteomics, Monoclonal Antibodies, Histopathology, Flow Cytometry, Confocal Microscopy, Molecular Imaging and Transgenic Mice, as well as an Animal Facility. Although the Core Units are mainly focused on meeting the internal demand and collaborating with the CNIO Research Groups, they also provide support and collaborate with groups from other public 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 2018, often in collaboration with CNIO Groups. Among the new technologies introduced at the CNIO this year, it is particularly worth mentioning the acquisition of a 10X Chromium System for cell encapsulation, mainly dedicated to single cell RNAseq. This platform, which has been installed at the Genomics Unit, will allow to implement at the CNIO the technology of single cell gene expression analysis, as well as other applications involving deep sequencing, highly demanded by the CNIO Groups.

In 2018, the Programme and its Core Units were very active in networking activities. Thus, the Programme Director was elected President of the Spanish Society of Biotechnology (SEBiot), which represents the community of researchers working in any of the multiple aspects of biotechnology in Spain. Also, in addition to participating in the activities of the Core Technologies for Life Sciences (CTLS), including the presence of two of our members in the Executive Board of this association, the Unit Heads were very active in participating in other networks and scientific societies from their corresponding fields. As an example, the Proteomics Unit participates in EPIC-XS – a coordinated project that was granted funding in 2018 from the H2020 programme – together with some of the most prominent European Groups and Core Facilities in the field of proteomics.

Also, as an indication of our high commitment with training, education and outreach, the Programme has been deeply involved in the organisation of courses, workshops, student visits, and specialised meetings. We collaborated with the ‘CNIO & the City’ project, organised by the CNIO

**“In order to undertake biomedical research of excellence, it is absolutely essential to have access to cutting-edge technologies and the necessary expertise to put those technologies to the best service of research projects.”**

Communication Department with funding from the FECYT (*Fundación Española para la Ciencia y la Tecnología*). The EuroMabNet network, chaired by the Head of the Monoclonal Antibodies Unit, held two workshops on the validation of antibodies, and specific training courses were put in place by the Flow Cytometry and the Confocal Microscopy Units. Moreover, several members of our staff have participated in an increasing number of Masters 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 several 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 representing a significant funding source for the CNIO, with an increase of 6% 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, 2018 has again been a very productive year scientifically for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in 25 publications co-authored by members of the Units, many of them published in top journals.

## GENOMICS CORE UNIT

Orlando Domínguez  
Core Unit Head

Technicians  
Purificación Arribas, Antonio Iñigo  
(until November), Guadalupe  
Luengo, Jorge Monsech,



### OVERVIEW

The Genomics Unit provides on-demand scientific services to the CNIO research community. Cutting-edge technologies have the capacity to interrogate whole genomes in a single assay, such as next-generation sequencing (NGS). These methodologies reveal the genetic diversity of cancer and contribute to dissect its molecular processes. Structural features, such as mutation landscapes, DNA-binding of protein factors, variations in chromatin structure, as well as functional activation states reflected on changes of transcriptomic profiles (mRNA, miRNA), are being elucidated with these technologies in order to uncover basic mechanisms, therapeutic targets and prognostic biomarkers. We offer a broad range of applications, including powerful solutions such as exome mutational landscapes, protein location analysis by ChIP-seq analysis and transcriptome profiles by RNA-seq technologies, besides

**“The genetic and genomic services provided by the Genomics Unit to assist CNIO’s scientists all help to contribute towards the understanding of the molecular processes of cancer at different levels of biological complexity.”**

from the more traditional microarray platform – suitable for whole genome gene expression, array comparative genomic hybridisation (aCGH) – and capillary DNA sequencing. Among other side activities, we also provide a very active transgenic mouse genotyping service.

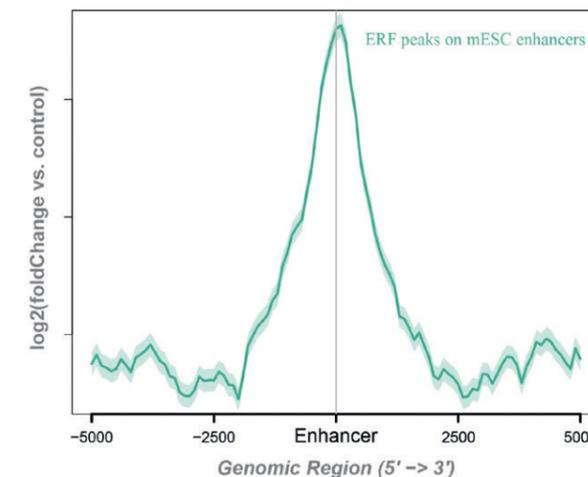
Ángeles Rubio, Delfina Sanguino  
(until September), Marco De Mesa,  
Laura Conde (since November)

### RESEARCH HIGHLIGHTS

The Genomics Unit contributes to the advancement of research projects carried out by multiple CNIO Research Groups. It provides services that survey different levels of complexity. A wide genomic level is addressed mainly by deep-sequencing (NGS) techniques and their applications. NGS permits a variety of different explorations, such as whole genome or whole exome tumour sequencing, transcriptome analyses – long non-coding RNA included – or location of interacting protein factors or RNA binding sites on chromatin. This year, the demand for NGS services has been stable and the number of samples processed has been similar to that of 2017. At a more focused single locus level, other services, such as the traditional DNA capillary sequencing service, are also provided; this service is used to find and confirm mutations in candidate genes, or in the verification of cloned genes or inserts. The Unit also provides a transgenic mouse genotyping service, based on allele-specific quantitative PCR for a quick and efficient turnaround time. The genotyping service has a catalogue of about 120 genetic modifications and 2018 has seen a steady increase in demand over that of former years.

Some of our activities have contributed to two research reports being published in 2018, with the authorship of some of the members of the Unit. When searching for the genetic causes of resistance to antiangiogenic therapies in a metastatic colorectal cancer patient, a collaborator’s project sought to recapitulate the tumour exome from circulating tumour DNA (ctDNA). An L840F somatic mutation in the *KDR/VEGFR2* gene was found as the cause of the resistance. This tumour mutation blocks the angiogenic inhibitors’ binding to VEGFR2. This study demonstrates new opportunities for analyses on ctDNA in order to explain therapy resistance mechanisms and to detect prognostic biomarkers (Toledo RA *et al.*).

RAS proteins are mostly known as oncogenic factors, but they also play a key role in pluripotency. The second report shows how embryonic stem cells devoid of RAS genes are unable to abandon pluripotency (Mayor-Ruiz C *et al.*), a feature that depends on the phosphorylation state and subsequent activation of inhibiting transcription factor ERF. Relative to cancer, this work further suggests the possibility that selective RAS inhibitors, which could eventually be used in therapy, would promote the emergence of resistance mechanisms through the inactivation of mediators such as ERF. ■



**Figure** The enrichment of ERF chromosomal location in Ras<sup>loss</sup> OHT-treated mESCs, at or nearby enhancers, suggests a role for ERF in the global rewiring of the transcriptional gene expression programme associated with pluripotency (Mayor-Ruiz *et al.*, *Genes Dev*, 2018). Figure prepared with ngs.plot (Shen *et al.*, *BMC Genomics*, 2014).

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# TRANSGENIC MICE CORE UNIT

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Graduate Student  
Aleida Pujol



Technicians  
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## RESEARCH HIGHLIGHTS

CRISPR/Cas based tools have revolutionised the way we approach genetic studies. The Unit has incorporated CRISPR/Cas gene editing tools for mouse germ line precise modification, replacing, in many cases, gene targeting in embryonic stem cells (ES cells) for the generation of knockout and knockin mice with high efficiency. CRISPR reagents are introduced directly into mouse zygotes by pronuclear injection or electroporation, avoiding difficult and time-consuming ES cell culture and manipulation. Knockout allele generation by CRISPR is often around 80% to 90% efficient and bi-allelic knockout animals are frequently obtained. Point mutations or small tag insertions are also easily created by CRISPR-induced homologous recombination directly in zygotes, using single stranded oligodeoxynucleotides as donor DNA for repair. We have also developed strategies to increase the efficiency of CRISPR-mediated large (more than 2 Kb) knockin integrations using, in this case, circular plasmids as donor DNA. A high proportion of the pups born after zygote CRISPR microinjection carry targeted knockin inserts. Zygote electroporation is a good alternative to microinjection for gene

knockout generation. Moreover, zygotes obtained by *in vitro* fertilisation can be edited the same day in a fast and efficient way through CRISPR electroporation.

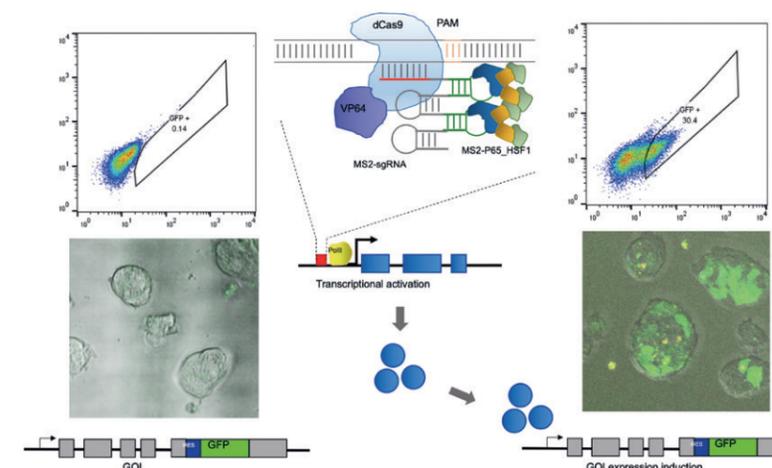
The CRISPR gene editing system may also be used in forward genetics for genome wide screenings of new genes relevant in different aspects of cancer. In the Unit, we have established haploid ES cell lines from genetically modified mice generated at the CNIO, by induction of parthenogenetic division of unfertilised mouse eggs. Haploid ES cells are especially useful for the identification of recessive mutations in genome-wide screenings. We have set up several screenings based on genetic rescue of lethal mutations using lentiviral libraries of gRNAs in combination with the Cas9 endonuclease. We are also using activating CRISPR libraries based on the expression of the dead Cas9-VP64 fusion and modified gRNAs that bind transcription activators, targeted to the promoter regions of coding genes and microRNAs. This approach is being used in a genome-wide search for genes and pathways that activate expression of fluorescent reporters knocked-in in cancer related genes (FIGURE). ■

## OVERVIEW

The laboratory mouse is the most widely used experimental model for genetic studies and preclinical drug development in cancer. The Transgenic Mice Unit is dedicated to the genetic edition of the mouse germ line and to the generation of genetically modified mouse strains. Hundreds of these strains have been created at our Unit. In many cases, they contain modifications that reproduce the genetic alterations found in human cancer and are introduced in the mouse to generate preclinical models of the disease, thereby contributing to the development of more efficient targeted therapies. Genetically modified mice are also created for testing *in vivo*, in a physiological context, hypothesis related to the molecular mechanisms that convert a normal cell to a malignant cell or that contribute to tumour expansion and invasion of distant organs, ultimately causing death. Cancer is an extremely

**“CRISPR/Cas based genome editing technology has improved the generation of genetically edited mice, thereby promoting research progress and accelerating drug discovery in many fields including cancer.”**

complex disease that cannot be sufficiently well studied *in vitro* in a tissue culture plate. The generation of genetically modified mice is one of the basic pillars that sustain cancer research at the CNIO.



**Figure** Example of a genome-wide screening for genes and pathways that induce expression of a gene of interest (GOI) using transcriptional activating CRISPR/Cas gRNA libraries and embryonic stem cells containing a knockin GFP reporter in the GOI. Flow cytometry analysis and confocal images confirm increased expression of GFP upon infection with the CRISPR library.

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## MONOCLONAL ANTIBODIES CORE UNIT

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### OVERVIEW

The application of hybridoma technology for the production of monoclonal antibodies (mAbs) represents one of the most relevant methodological advances in biomedicine of the past decades. The availability of monoclonal antibodies has significantly improved our knowledge about the processes involved in tumour generation and development, opening up a vast array of new possibilities for basic and applied research.

The Monoclonal Antibodies Unit provides CNIO Research Groups with an '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 give added value to their research projects.”**

### RESEARCH HIGHLIGHTS

During the last 18 years, the Monoclonal Antibodies Unit has generated a large number of mAbs, against more than 140 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 90 thoroughly validated high-quality mAbs (accessible at <http://www.cnio.es/ing/servicios/anticuerpos/default.aspx>).

#### Research activities

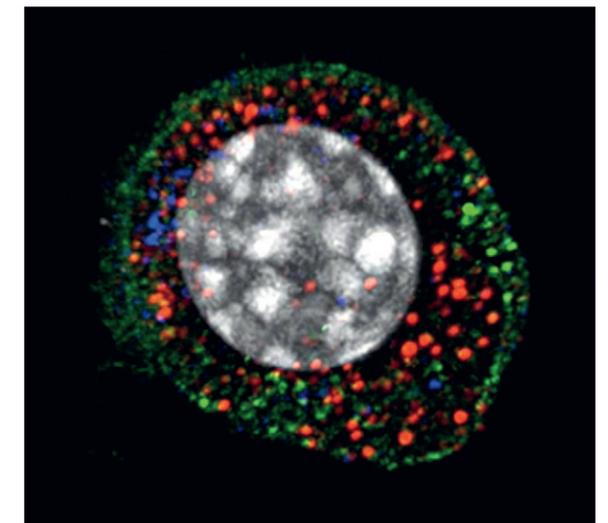
In collaboration with Prof. Pablo Engel from the University of Barcelona, we have produced and characterised a new monoclonal antibody against the protein T-lymphocyte surface antigen Ly-9. Ly9, also known as CD229 or SLAMF3, is one of the 9 members of the immunoglobulin superfamily (SLAM). It is expressed in T and B-lymphocytes and plays an important role in lymphocyte activation and cytotoxicity.

We have investigated the expression of the Ly9 protein in normal and neoplastic lymphoid tissue using a novel rat monoclonal antibody (PIZCU426A) against the Ly9 intracellular domain; this novel mAb recognises its target in paraffin-embedded tissue sections. A large series of normal tissues and B and T-cell lymphomas have been studied using whole sections and tissue microarrays.

In human reactive tissues, we found that Ly9 is restricted to lymphoid tissue, specifically to mature B and T cells. Ly9 was strongly expressed in all cases of myelomas, marginal zone and MALT lymphomas. This new monoclonal antibody may help pathologists in the identification of neoplastic B and T cells in routinely processed tissue samples, and may be used to achieve a better understanding of the pathogenic role of Ly9 in inflammatory and malignant diseases.

#### EuroMAbNet, a European consortium of experts in monoclonal antibody technology

In 2008, in collaboration with Oxford University, we founded EuroMAbNet ([www.euromabnet.com](http://www.euromabnet.com)), a non-profit organisation that currently spans 11 European countries. Members include internationally distinguished academic laboratories that generate and validate monoclonal antibodies. EuroMAbNet is strongly committed to improving the education and training of junior scientists in the field of antibody



**Figure** The confocal image shows the colocalisation of Ly9 (green) and Clathrin (red) proteins in the myeloma cell line U266.

validation. This aim is materialised through the organisation of 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  
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Post-Doctoral Fellow  
Sebastián Thompson (since October)



## OVERVIEW

Molecular Imaging can be defined as the ability to visualise, characterise and quantitatively measure biological and cellular processes and functions *in vivo*. One of the main advantages of *in vivo* molecular imaging is that it enables characterisation of the pathology of tissue diseases without the need of invasive biopsies or surgical procedures; with this information at hand, a more individualised treatment-planning approach can be applied. Molecular imaging encompasses a range of imaging techniques that rely on the utilisation of probes exogenously added to target and detect specific cellular or molecular processes in a living organism.

**“Molecular imaging provides the ability to make visible what would otherwise be invisible, uncovering deeply hidden truths about the mouse/human body.”**

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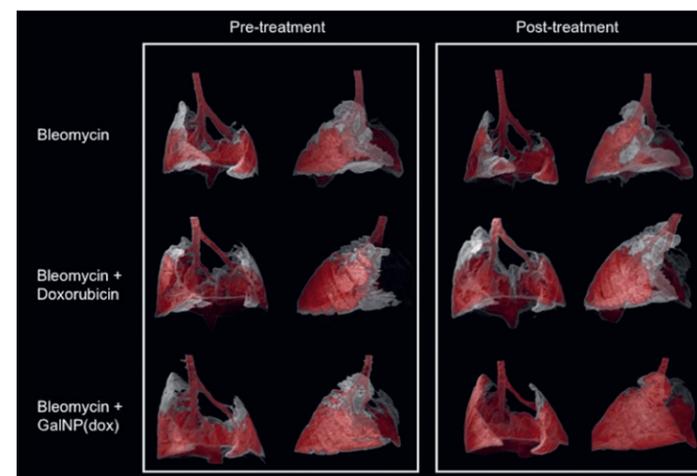
## RESEARCH HIGHLIGHTS

The Molecular Imaging Unit provides CNIO researchers with state-of-the-art molecular imaging equipment and human resources in order to guarantee the highest quality studies, to develop and update protocols and imaging techniques that serve to optimise visualisation of tumours in both the preclinical and clinical fields, and also to assess and advise researchers on the best-suited imaging modality for their research projects (FIGURE).

We continue to test and incorporate new applications to the ImmunoPET strategy in the context of a new collaborative project with CIEMAT group, granted by the BBVA Foundation, in which we will develop a nanobody produced by camelids that is labelled with <sup>68</sup>Ga. By adopting this novel approach, we expect that the antibody, due to its small size, will be able to better cross the

blood-brain barrier and detect brain metastases. The ImmunoPET technique combines the high specificity and selectivity of the antibodies with the high sensitivity and quantitative capabilities of PET. This combination makes this technique suitable to conduct an *in vivo* non-invasive, quantifiable, 3D immunochemistry for the diagnosis and monitoring of tumours.

This year, the Molecular Imaging Unit participated in a Network Programme for Developing Imaging Probes funded by the *Comunidad de Madrid (RENIM-CM)*. This programme is mostly focused on the use of nanoparticles to perform optical imaging and multimodality (optical-MRI or PET-MRI) for the detection of primary tumours and distant metastasis. The results of this research will directly benefit the CNIO scientists who will be able to use and test these new imaging probes. ■



**Figure** CT images of the evolution of lung fibrosis before and after treatment. Healthy lung tissue is shown in red; fibrotic lung lesions are shown in grey. Note the practically complete recovery of the lung after treatment with the new drug delivery system based on Galactose encapsulated nanoparticles. One of these images was featured on the cover of the September issue of *EMBO Molecular Medicine*.

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## AWARDS AND RECOGNITION

- Academic Editor, *PLoS ONE* Journal.

# FLOW CYTOMETRY CORE UNIT

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Technicians  
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## OVERVIEW

Flow Cytometry is an indispensable tool in the oncology field. It enables 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 count with 4 analysers and 3 high-speed cell sorters with different configurations of lasers and detectors, to cater for all our users' needs. We also have an automated magnetic bead separation system (AutoMACS) and 2 automated cell counters.

**“The development and optimisation of immunophenotyping panels by flow cytometry has been a key contributing factor, among others, to help us better understand the role of the immune system in the context of brain metastasis.”**

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 under Biosafety regulations.

## RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in the setting up and optimisation of the flow cytometry techniques of their interest. Some of the applications developed and validated at our Unit are:

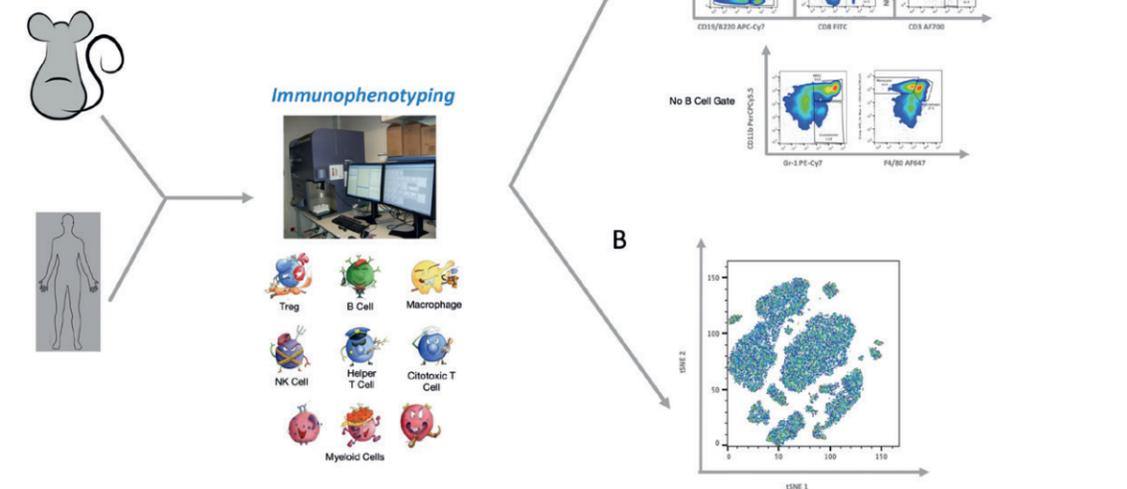
- Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- Apoptosis studies (Annexin V, Mitochondrial Membrane Potential, Caspase 3, etc.).
- Multicolour Immunophenotyping panels (B and T cell development, Tregs, Inflammation, etc.).
- Functional Assays (side population detection, Ca<sup>2+</sup> flux, intracellular pH, etc.).
- Cytometric Bead Arrays to measure several cytokines from cell extracts and plasma.
- Platelet studies.

- Extracellular vesicle detection (microvesicles and exosomes).
- Single cell sorting for OMICs analysis.

We have further optimised our multicolour flow cytometry panels for the characterisation of the immune response in various samples, such as haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Single cell deposition into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We are performing 4-way sorting based on DNA content on live stained samples, and are moving forward 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. ■

**Figure** Multiparametric panels for cancer immunomonitoring are commonly used in research worldwide. As the number of markers increase, so does the complexity of the analysis. Current options are

conventional dot plots analysis to look at the different subsets (A) or the use of mathematical algorithms to look at the relation of all the markers at once (B).



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- **AWARDS AND RECOGNITION**
- Executive Council Member, European Core Technologies for Life Science Association (CTLAS).

## CONFOCAL MICROSCOPY CORE UNIT

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### OVERVIEW

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

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

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

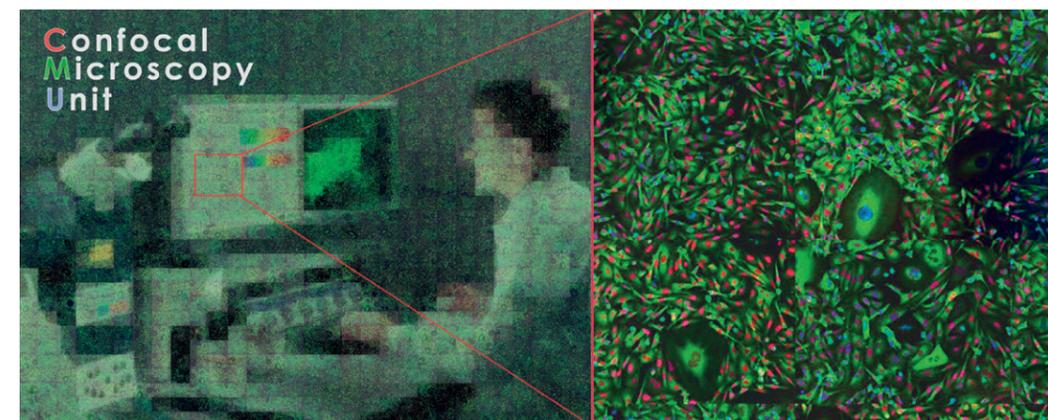
In addition, the Unit has implemented the use of high throughput technologies applied to confocal microscopy using 2 different systems:

- An Opera (Perkin Elmer) High Content Screening (HCS) system, which allows running HCS experiments on fixed and live cells in 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 carry out the automated screening of cell behaviour under different treatments.

The Confocal Microscopy Unit continues to dedicate significant efforts towards the development and implantation of High Content Screening technology at the CNIO. In 2018, we successfully organised the ‘HCS week’, which included the HCS South European meeting, generating a great discussion forum on the latest trends in the field. Additionally, during the same week, we launched the first edition of CNIO’s HCS School with the aim of teaching future experts in HCS the latest applications and informatics tools.

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



**Figure** Confocal microscope mosaic image composed of thousands of fluorescent images acquired at the Unit.

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## PROTEOMICS CORE UNIT

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Graduate Students  
Ana Martínez, Cristina Sayago



### OVERVIEW

Proteins are the molecular effectors of cells and catalyse almost all biological processes. The levels of protein abundance, together with their modification states and interactions, adapt dynamically to external or internal (genetic) stimuli and thus define the cell's functional state and determine its phenotype. Mass spectrometry-based proteomics is the most powerful tool to study the proteome, providing fundamental basic biology information. In addition, recent improvements in sensitivity and throughput now enable the analysis of larger cohorts of samples including biopsies, thereby making proteomics a part of the clinical research toolbox. All these efforts are providing new insights into the molecular mechanisms underlying cancer development as well as the identification of novel biomarkers.

**“The new generation of mass spectrometers enables the analysis of complex samples to unprecedented depth levels, thereby becoming a real alternative to RNAseq for sample profiling.”**

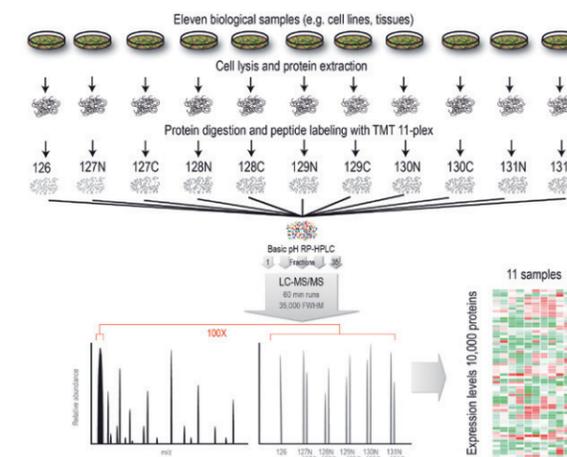
Technicians  
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de Embún (TS) \*, Eduardo Zarzuela (TS)\*

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

### RESEARCH HIGHLIGHTS

Throughout 2018, the Proteomics Unit has acquired 2 novel Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) platforms. The Q Exactive Plus and the Q Exactive HF-X, the latter of which is the fastest instrument on the market, are now part of the equipment available for research projects in our Unit. Both mass spectrometers are coupled to nanoHPLC systems with plug-and-play nESI interfaces. The application of this technology in the CNIO will have a major impact on the characterisation of complex proteomes as well as on the analysis of small protein amounts (e.g. FACS sorted cells, post-translational modifications...). In addition, the high resolution of the Orbitrap detector enables the analysis of TMT11-plex, increasing the range of possibilities during the experimental design with the inclusion of biological replicates and/or different conditions. Furthermore, if coupled to modified pre-fractionation strategies based on basic pH reverse phase HPLC, full proteome coverage can be achieved across 11 samples (FIGURE). The Unit has also continued to work in close collaboration with CNIO Research Groups in several projects. To highlight some of them, together with the Brain Metastasis Group, we analysed the secreted factors of a subpopulation of pSTAT positive astrocytes that mediate brain metastasis. In collaboration with the Cell Division and Cancer Group, we performed a time course phosphoproteome analysis of activating platelets and have identified important defects in the phosphorylation of actin cytoskeleton proteins in a model of thrombocytopenia, which is caused by mutations in MASTL, a cell cycle kinase. Also, in a multi-omic project led by the Melanoma Group, we performed a proteomic analysis to define the interactors of sequestosome, aka p62, which have revealed unexpected roles for this protein in extending the mRNA half-life of several pro-metastatic factors. ■



**Figure** Samples are digested and labelled with new isobaric tagging TMT 11-plex. The pull of peptides is then pre-fractionated by High pH and 35 non-concatenated fractions are analysed on a Q Exactive instrument at high resolution using short gradients. This enables the identification of nearly full proteome coverage.

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## HISTOPATHOLOGY CORE UNIT

Vacant  
Core Unit Head

Staff Scientist  
Eduardo José Caleiras (since March)



### OVERVIEW

Pathology is devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs that underlie disease. By using molecular, immunological and morphological techniques, pathology aims to serve as the bridge between basic science and clinical medicine.

The Histopathology Unit offers assistance and expertise through a full range of services covering from paraffin embedding and tissue sections to histochemical stains, research and diagnostic immunohistochemistry (IHC) testing, antibody validation, and *in situ* hybridisation (ISH), as well as the construction of tissue microarrays. Furthermore, the Unit offers other value added services assisted by a team of highly specialised technicians, such as laser-capture microdissection; slide digitalisation for bright-field, polarisation light and

**“The implementation of multiplexed immunohistochemistry staining to enable the detection of several markers on the same tissue section is an example of the compromise of the Unit with innovation to facilitate the progress of research projects at CNIO.”**

fluorescence; image analysis; and quantification. The Unit collaborates with researchers at any stage of their scientific career in the histological characterisation of phenotypically relevant animal models of disease, thus providing them with the pathology expertise required for the success of their projects.

Technicians  
Nuria Cabrera, María Gómez, Patricia González, Gabino Hernández, Vanessa Pizarro (PEJ, CAM) \*, Irene Roda, Zaira Vega

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

### RESEARCH HIGHLIGHTS

In line with the activity carried out during the last years, the Unit has maintained the portfolio of services demanded by its users in accordance with the needs of their projects. Thus, about 34,000 paraffin blocks of tissue samples were generated, and ca. 35,000 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.

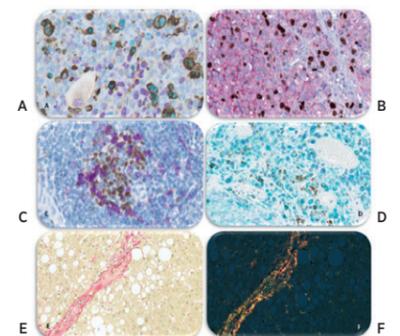
Our main key goals are to guarantee the standardisation and quality of the techniques offered by the Unit, and to focus on the implementation of innovative methodologies. The implementation of new equipment for the routine techniques has been key for the standardisation of the protocols. This has made it possible to introduce new approaches, such as multiplexed IHC staining, which enables the simultaneous visualisation of several markers (up to 4) on the same tissue section. With this new methodology, combined with the slide scanner AxioScan and the ZEN image analysis software available at the Unit, it is now possible to study the distribution and expression levels of different proteins of interest in a single experiment in order to better understand complex pathological processes.

The high quality of the techniques run by the Unit continues being endorsed by External Quality Assessment Schemes. In this respect, it is worth mentioning that our haematoxylin-eosin staining technique was commended in the framework of the evaluation, carried out by UK NEQAS, of our histochemical

techniques. On the other hand, NordiQC 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.

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

**Figure** Co-expression of different proteins in the same tissue section can be assessed by means of multiplexed IHC staining under brightfield. (A) Triple multiplexed staining of Nestin (brown) + phospho-STAT3 (purple) + p21 (light blue) on fibrosarcoma tissue. (B) Dual staining of Ki-67 (brown) + c-MET (red) on melanoma tissue. (C) Dual staining of BCL-6 (brown) + PD-1 (purple) on lymphoid germinal centre tissue. (D) Visualisation of HMB45 expression in melanoma tissue using a new chromogenic substrate from the Roche Discovery Teal HRP kit (light blue).



**Histochemical techniques are often a quick way to analyse the properties of the tissues. Sirius red staining of fibrotic liver under brightfield (E) and polarised light (F) allows visualising collagen and amyloid deposits.**

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## ANIMAL FACILITY

Isabel Blanco  
Core Unit Head

Management  
Vivotecnia Management & Services



**“The Animal Facility provides CNIO researchers with all the support required to work with mouse models, which are critical to understand the processes involved in tumour generation and development, as well as for the validation of new targets and therapeutic approaches.”**

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 International (AAALAC), which granted us full accreditation in October 2016. AAALAC International is a private non-profit organisation that promotes the humane treatment of animals in science through voluntary accreditation and assessment programmes. Nearly 1,000 companies, universities, hospitals, government agencies and other research institutions in 44 countries have earned AAALAC accreditation, which is considered one of the top international recognitions in this field.

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

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

Daily operations and husbandry procedures are highly automated 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/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 Core Unit, which has integrated all its image acquisition instruments within the Animal Facility. Likewise, the work of the Transgenic Mice 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 over 100 specimens of the *Xenopus laevis* frog that are used to obtain eggs for chromosome dynamics studies. Also, in 2018, as new tools in cancer research, we introduced a colony of rats for 2 specific projects: a project involving the generation of monoclonal antibodies directed against mouse antigens, and an ETP project aimed at testing new compounds against cancer.

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 in the possession of the corresponding accreditation as issued by the competent authority. The Animal Facility offers CNIO's new staff a short course focused on the training of personnel performing work with laboratory animals; this is complementary to the online courses that are a requisite to gain access to the facility.

In accordance with our commitment of maintaining 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), which was launched on September 2016. An institutional statement on the use of research animals can be consulted on the CNIO website. ■

## EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director



**“Interrogate biology with chemistry and you will get responses that can trigger unimaginable research and discoveries.”**

The Experimental Therapeutics Programme (ETP) serves as a bridge between basic research results in cancer biology (i.e. novel therapeutic targets and hypothesis) and the development of potential antitumour drugs. This is achieved by the application of early drug discovery phases to obtain advanced compounds with proven *in vivo* Proof of Concept (PoC) results. ETP assists target validation activities by providing high quality chemical probes and participates in the identification of novel targets using its expertise in target deconvolution.

**CDK8 inhibitors.** As previously reported, we selected our first leads, ETP-27 and ETP-93, as dual CDK8/Haspin inhibitors, which have yielded positive results in PoC studies after oral (PO) administration. Now, we have added ETP-18, a highly selective orally bioavailable CDK8 inhibitor, to our set of advanced leads. ETP-18 has demonstrated good biomarker modulation (pSTAT1) after PO administration in pharmacokinetics and pharmacodynamics (PK/PD) studies in MOLM13 xenografts. Currently, we are performing *in vivo* efficacy experiments to complete our PoC studies. Importantly, we have started toxicity studies of our advanced leads in rats to establish their therapeutic index. The results of these experiments will determine the destiny of these series of CDK8 inhibitors regarding their further development.

**Haspin and Mastl Inhibitors** (*in collaboration with Marcos Malumbres' laboratory*). We have generated 2 distinct chemical series of highly potent Haspin inhibitors, which have proven to be very specific after profiling against more than 450 kinases. These molecules have been profiled against a diverse panel of 40 cell lines. Haspin inhibitors showed limited antiproliferative potential as single agents. Now, we are evaluating their effect in combination with clinical antitumour agents; the results of this study will help to define the therapeutic scope for these inhibitors. During 2018, we completed the Structure-Activity-Relationships (SAR) of 2 families of potent Mastl inhibitors; this information has helped to deploy a hit generation campaign to increase the chemical diversity of our Mastl inhibitors. The generation of diverse Mastl inhibitors will increase our chances to identify the most interesting series in terms of potency, cell activity and above all off-target selectivity for pharmacological target validation studies. Interestingly, we have identified the solvent accessible areas in our initial Mastl inhibitors; this information has been used in the design of Mastl-Protacs to target the degradation of this kinase.

**TRF1** (*in collaboration with Maria Blasco's laboratory*). *TRF1 modulators as potential cancer therapeutics.* As reported earlier, we discovered a series of TRF1 inhibitors (ETP-946 series 2) with unknown molecular mechanism of action. Therefore, several target deconvolution strategies were put in place to identify its target. During 2018, we focused our efforts on the preparation of reversible and irreversible affinity probes with proven TRF1 inhibition capacity. As an example, ETP-093, an irreversible probe, has been used in a first pull-down experiment rendering several nuclear proteins as potential target candidates. We are doing replicates of this study in order to have a final selection of candidates for further validation. Furthermore, we have progressed in the SAR generation of series 2 by the synthesis of numerous analogues. These compounds have been included in a recently filed patent application. We have gathered information about the drug-likeness of this series and we are now targeting its optimisation as well as the reinforcement of its intellectual property (IP). These activities are part of an awarded *CaixaImpulse* project (M. Blasco's lab) in which ETP participates. Importantly, we have contributed to the discovery of *novel cellular pathways able to modulate TRF1 binding to telomeres*. As reported earlier, ETP has helped to establish the connection of the PI3K/AKT axis and TRF1. During 2018, we identified other cell signalling pathways involved in TRF1 regulation (currently under detailed investigation at M. Blasco's lab) by the screening of our ETP-antitumour library.

Finally, we have also helped other CNIO groups to carry out screening campaigns, both targeted and phenotypic, and have supported the follow up of the identified hits in several projects.

## MEDICINAL CHEMISTRY SECTION

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Section Head

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Francisco J. García

Carmen Fernández (until April)  
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Technicians  
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Lucía De Andrés (since July) (TS)\*,

\* *Titulado Superior* (Advanced Degree)



### OVERVIEW

The Medicinal Chemistry Section is part of the interdisciplinary Experimental Therapeutics Programme that is dedicated to early Drug Discovery in the oncology field. Our aim is to discover new anticancer agents based on the hypotheses and targets generated by CNIO's Basic Research Groups; this is done in close collaboration with these Groups. Medicinal Chemistry activities start with the identification of hits through High Throughput Screening (HTS) campaigns from targeted or phenotypic assay or hits generated in our Section by applying Rational Drug Design Strategies; these are then optimised to obtain novel lead compounds with *in vivo* activity in different animal models. For hits obtained from phenotypic screenings, we help to decipher the mechanism of action responsible for the observed phenotype, synthesising affinity probes that will be used for cellular localisation (imaging techniques) and extracting the target/s (pull down experiments). We are also developing PROTACs (proteolysis targeting chimeras) as promoters of cell protein degradation to establish their applicability across diverse drug discovery projects.

**“We have successfully designed and synthesised an irreversible affinity chemical probe of ETP-946 that is to be used for imaging and pull-down/proteomics analysis experiments; the aim is to decipher the mechanism of action of this TRF1 modulator.”**

## RESEARCH HIGHLIGHTS

**Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project**

ETP-93, with demonstrated proof of concept studies (PoC) in mouse models, and ETP-18 were identified as potent, selective and orally bioavailable CDK8 inhibitors. We are involved in the multigram scale up of these compounds in order to perform toxicity studies in rats, as well as to determine if the compounds/inhibition of the target is safe enough to progress them to the next phases of drug development. With these results in hand, we will be able to initiate the transfer of our results to companies interested in developing our compounds into drugs. Additionally, we will also perform PoC studies (efficacy and biomarker modulation) with ETP-18.

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

In collaboration with the CNIO Cell Division and Cancer Group, we continue with the exploration around the chemical series identified to obtain potent and selective compounds. Strategies to generate MASTL-PROTACs are also contemplated in order to generate degrader compounds of the protein; the first PROTACs have been synthesised with moderate MASTL activity (FIGURE). We are initiating a hit generation phase to obtain novel Mastl inhibitor hits.

**HASPIN inhibitors**

Highly selective hits identified from 2 previously generated chemical series were scaled up to be characterised *in vivo* in order to determine their pharmacokinetics in mice as well as to be used in the biological characterisation to study the relevance of HASPIN in cancer, including their effect in antiproliferative experiments as single agents and in combination with other antitumour agents. We continue with the chemical exploration around the hits to conclude the SAR activities and to define the scope of their kinase activity.

**Telomeric repeat binding factor 1 (TRF1) inhibitors**

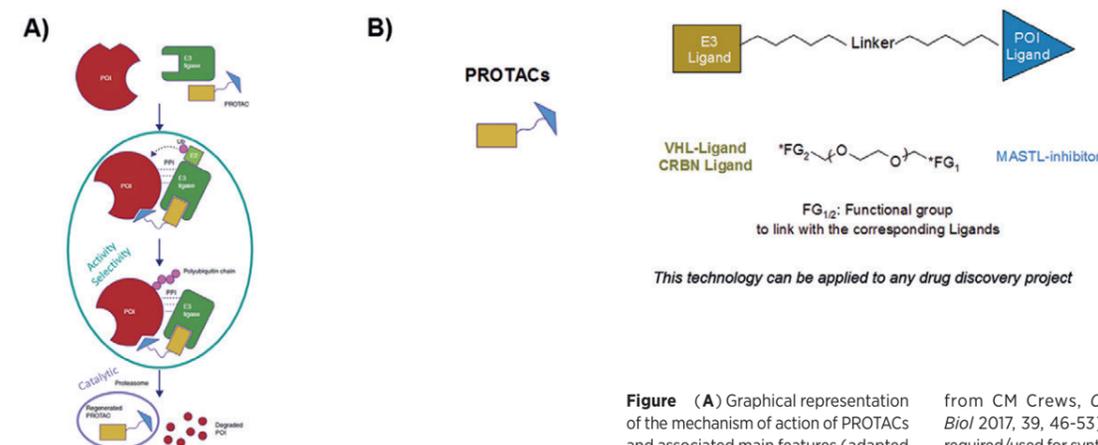
This project is undertaken in collaboration with the CNIO Telomeres and Telomerase Group (TTG). ETP-946 was identified as a TRF1 modulator under screening assay conditions, and we are currently working on deciphering its mechanism of action. One of the approaches that we have taken is to use affinity chemical probes. We generated SAR information from the chemical exploration in the hit-to-lead

phase (approximately 150 compounds were synthesised), and with this information we identified those parts of the hit molecule at which to install linkers and synthesise probes. During this year, we accomplished synthesis of an irreversible affinity chemical probe (ETP-093), which contains photoreactive and reporter groups, made as small as possible to minimise the interference upon binding to the target proteins. The aliphatic diazirine photoreactive group of ETP-093 enables, after incubation with cells, short irradiation to generate the highly reactive carbene species that will react with the binding protein/s. The terminal alkyne reporter of ETP-093 was then used for subsequent target identification by conjugation to suitable reporters (biotin- $N_3$ ) using biorthogonal click chemistry conditions, which enable pull-down experiments. So far, we have performed the first pull down experiment and the proteomic analysis, which will be further repeated 3 more times for a robust interpretation of the results. Pull down experiments with the reversible affinity probe ETP-455 were generated. This chemical probe lacks the photoreactive group, so its binding with affinity protein/s is not covalent and we may lose some relevant information during the washing steps phase. Nevertheless, once we have finalised all the experiments, we will compare results between reversible/irreversible affinity chemical probes. We have filed a patent application to cover the chemical series of ETP-946. This project was recently awarded a grant from the *CaixaImpulse* programme and we are currently working on the optimisation of the drug-like properties of ETP-946 together with the generation of novel chemical space for patent reinforcement.

**Collaborations with other CNIO groups**

We continue our collaborations with other researchers from the Centre, for instance, with Alejo Efeyan (Metabolism and Cell Signalling Group) performing stability, reactivity studies of the hits and synthesis of tools to help decipher their mechanism of action; and with Paco Real (Epithelial Carcinogenesis Group) for the synthesis of reference compounds. ■

PROTACs recruit the Protein of Interest (POI) to the E3 Ubiquitin ligase (available lysines) and subsequent proteosomal degradation. PROTACs activity and specificity is governed by the formation of the ternary complex POI-PROTACs-E3 Ligase. This is a result of the Protein Protein Interactions (PPI) established between POI and E3. PROTACs act catalytically. They degrade super-stoichiometric amounts of the POI.



**Figure** (A) Graphical representation of the mechanism of action of PROTACs and associated main features (adapted from CM Crews, *Curr Opin Chem Biol* 2017, 39, 46-53). (B) Fragments required/used for synthesis of PROTACs.

**PATENT**

Pastor JA, Blasco MA, Martínez S, Blanco-Aparicio C, García AB, Gómez-Casero E, Bejarano, L, Méndez-Pertuz M, Martín-

ez P, García-Beccaria M (2018). Novel TRF1 modulators and analogues thereof. *EP18382659.3*.

## BIOLOGY SECTION

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\**Titulado Superior (Advanced Degree)*

### OVERVIEW

The early Drug Discovery (eDD) process encompasses screening campaigns for hit identification, hit generation and hit to lead and lead optimisation phases, in order to end up with a lead compound able to demonstrate *in vivo* proof-of-concept. ADME – an acronym for absorption, distribution, metabolism and excretion – describes the disposition of a pharmaceutical compound within an organism. ADME properties influence the drug levels and the kinetics of drug exposure to the tissues, and hence influence the performance and pharmacological activity of the compound as a drug. It is fundamental to assess the parameters for ADME properties early on during the discovery stage, since they provide critical information that can help to better interpret the screening results and to design new molecules. Drug-like properties should be optimised in parallel to pharmacological activity against the target. For that reason, we perform ADME characterisation of the compounds during the initial steps of eDD projects and we carry out PK, PK/PD and distribution studies to validate the drug properties of our advanced molecules.

**“The screening of the ETP-antitumour library in the phenotypic TRF1 assay has enabled us to identify new signalling pathways modulating TRF1 levels that will contribute to a better understanding of TRF1 biology.”**

## RESEARCH HIGHLIGHTS

During 2018, our Section was involved in several projects:

**Cyclin-dependent kinase 8 (CDK8)**

We started proof-of-concept studies in mouse models with ETP-18, a selective advanced orally bioavailable lead compound that demonstrated both plasma and tumour levels as well as biomarker modulation (pSTAT1), in a dose dependent manner up to 8 h after oral administration in PK/PD studies in MOLM13 xenografts. Tolerance and efficacy studies will be performed. In parallel, after pharmacokinetics studies of 3 more selective CDK8 inhibitors, we have identified ETP-24 as a backup of ETP-18.

To determine if the inhibition of the target by our compounds is safe enough to progress them to the next phases of drug development, we are running toxicity studies in rats with the leads ETP-93 (dual CDK8/HASPIN-i) and ETP-18 (selective CDK8-i), in comparison with known inhibitors.

**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 85 new compounds in our biochemical assay with active human full length MASTL protein; 11% of them were tested as part of the hit generation phase and we have also tested our ETP-antitumour library to identify novel hits. One drug is under validation as a putative MASTL-inhibitor. For HASPIN, we tested in biochemical and cellular assays, 42 compounds to complete the SAR exploration of the chemical series. We have evaluated the antiproliferative activity of highly selective HASPIN-inhibitors (S(35) of 0.025 and 0.007) from 2 different chemical series in a panel of 40 cell lines covering the more relevant tumour types. Now, we are evaluating their effect in combination treatments. We have also characterised in ADME assays representative compounds for the 2 chemical series. We have performed a preliminary pharmacokinetic study with 1 selected compound with good ADME properties and we are running a distribution study to validate it as a good tool compound for *in vivo* proof-of-concept.

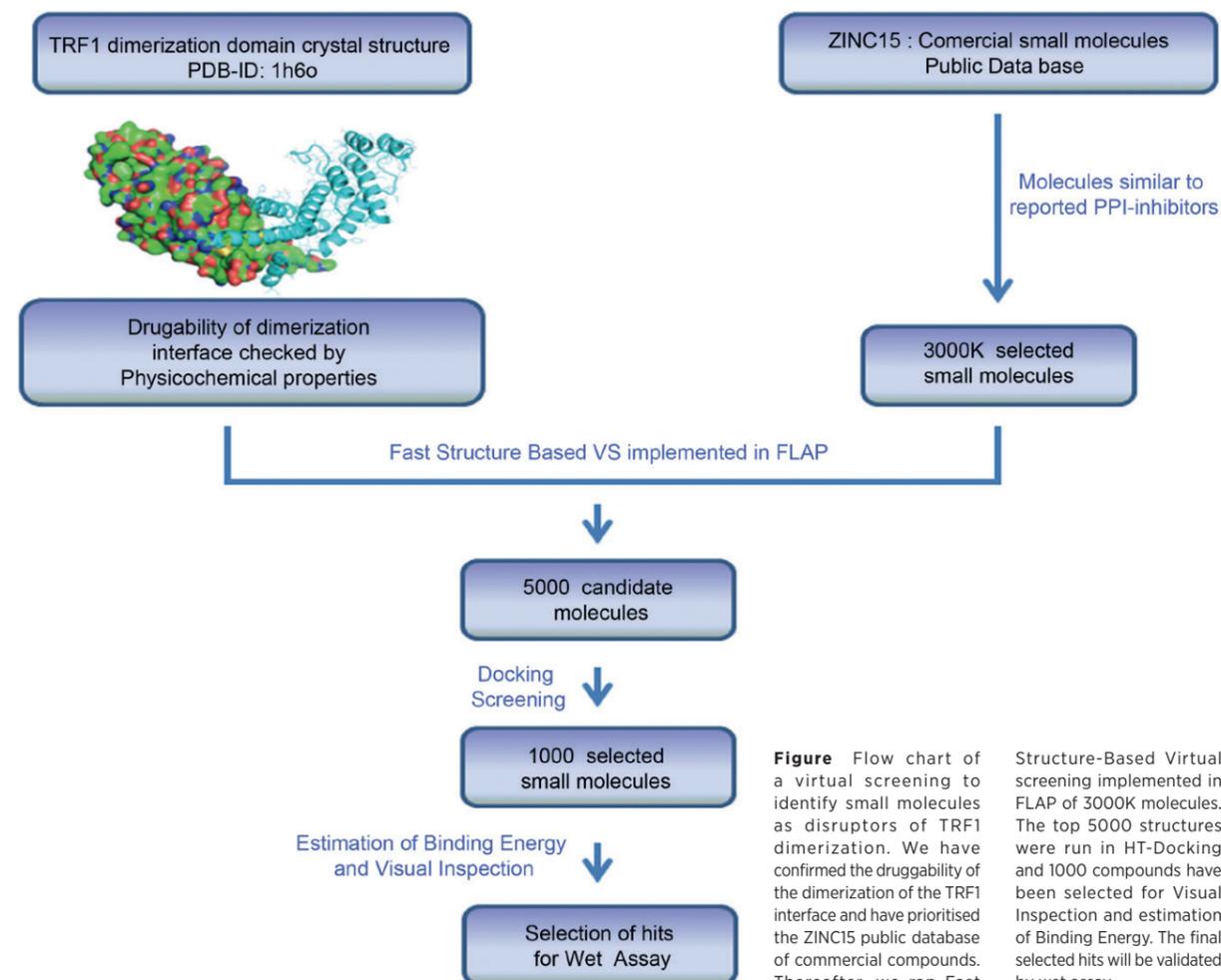
**Telomeric repeat binding factor 1 (TRF1)**

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. A phenotypic assay to measure the association of TRF1 to telomeres has been used to

test 30 compounds, which include ETP-946 analogues and its corresponding irreversible chemical probes. We have identified an active irreversible chemical probe, ETP-093, and we are running pull-down experiments with it. Several nuclear targets have been identified as potential targets and we want to validate them with 2 more pull-down experiments. In the meantime, we have used ETP-455, a reversible chemical probe, to perform pull-down experiments in triplicate. After comparison of the 3 experiments, we have identified 2 putative targets that we are trying to validate by orthogonal assays. We will compare the pull-downs with both reversible and irreversible chemical probes in order to select the best candidates. On the other hand, we have performed distribution studies with ETP-946 and have observed that the compound is distributed in tissues. Furthermore, by using a chemical biology approach, we have validated 3 more signalling pathways that were identified in the screening of the ETP-antitumour library that modulates TRF1 levels at telomeres; Maria Blasco's laboratory is deciphering the molecular mechanism behind this. These results are part of a patent application PCT/EP2018/074832. Finally, we have started a virtual screening with the aim of identifying disruptors of TRF1 dimerization (FIGURE).

**Collaborations with other CNIO Groups**

ETP-Biology continued providing support to follow-up on the results obtained from the screenings performed by the Brain Metastasis Group and the Metabolism and Cell Signalling Group. Moreover, we have provided support by testing and analysing the ETP-antitumour library, either alone or in combination, in order to identify: i) novel treatments of NSCLC mouse cell lines mutant in KRas with and without C-RAF and CDK4, in collaboration with the Experimental Oncology Group; and ii) novel modulators of Midkine expression, in collaboration with the Melanoma Group. ■



**Figure** Flow chart of a virtual screening to identify small molecules as disruptors of TRF1 dimerization. We have confirmed the druggability of the dimerization of the TRF1 interface and have prioritised the ZINC15 public database of commercial compounds. Thereafter, we ran Fast Structure-Based Virtual screening implemented in FLAP of 3000K molecules. The top 5000 structures were run in HT-Docking and 1000 compounds have been selected for Visual Inspection and estimation of Binding Energy. The final selected hits will be validated by wet assay.

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- Priego N, Zhu L, Monteiro C, Mulders M, Wasilewski D, Bindeman W, Doglio L, Martínez L, Martínez-Saez E, Ramón Y Cajal S, Megías D, Hernández-Encinas E, Blanco-Aparicio C, Martínez L, Zarzuela E, Muñoz J, Fustero-Torre C, Piñero-Yáñez E, Hernández-Lain A, Bertero L, Poli V, Sanchez-Martinez M, Menendez JA, Soffietti R, Bosch-Barrera J, Vallente M (2018).

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DNA portrays the mutation landscape of colorectal cancer and discovers mutated VEGFR2 receptors as modulators of antiangiogenic therapies. *Clin Cancer Res* 24, 3550-3559.

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- Pastor JA, Blasco MA, Martínez S, Blanco-Aparicio C, García AB, Gómez-Casero E, Bejarano, L, Méndez-Pertuz M, Martín-

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# CNIO - LILLY CELL SIGNALLING THERAPIES SECTION

Susana Velasco  
Section Head

Staff Scientists  
Ana Cerezo, Eva P. Lospitao, Gloria Martínez del Hoyo, Sonia Hernández

Tiedra (since July), Juan Manuel Funes (until May)

Technicians  
Laura Díezma, 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.

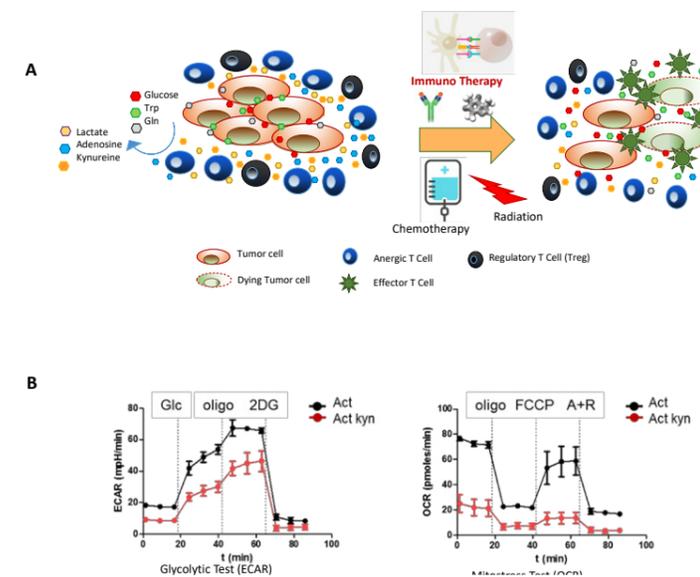
A combination of *in vitro* and *in vivo* approaches is being utilised to obtain a complete understanding of tumour metabolic reprogramming. For this purpose, we have developed a series of biochemical and cell-based assays exploiting advanced

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 T cell response. (B) Extracellular flux analysis for the acidification rate (glycolytic test) and O<sub>2</sub> 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.

# CNIO - LILLY EPIGENETICS SECTION

María José Barrero (until May)  
Section Head

Staff Scientist  
Sergio Ruíz (until March)

## SCOPE OF THE CNIO - ELI LILLY PARTNERSHIP

Eli Lilly and CNIO were collaborating on the identification and validation of novel targets in cancer epigenetics. Our Section was funded through a research contract with Eli Lilly and focuses on the identification of small molecular weight molecules able to modulate the epigenome of malignant cells and ultimately block the growth and spread of tumours. Potential targets were validated *in vitro* and *in vivo* using animal models developed at the CNIO. Furthermore, we set up biochemical and cell-based assays with the aim of understanding the mechanism of action of such targets at the molecular level (FIGURE).

Technicians  
Verónica García (until March) (TS)\*,  
Ana González (until May), Jacinto  
Sarmentero (until May) (TS)\*

\*Titulado Superior (Advanced Degree)

## SCIENTIFIC CONTEXT

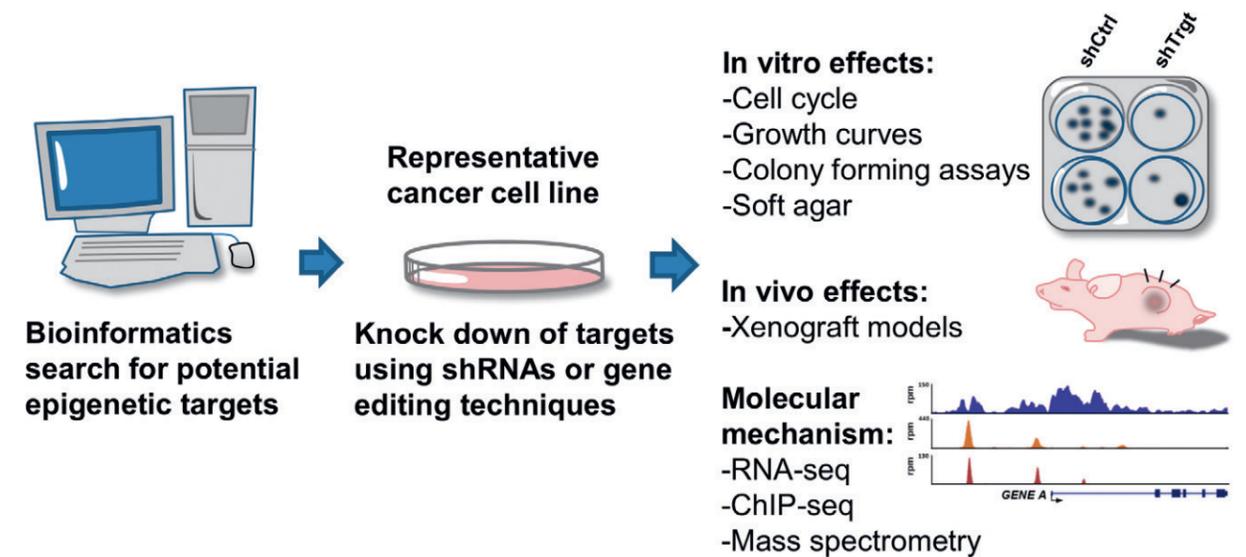


Figure *In vivo* and *in vitro* strategies for target validation.

Recent studies have shown that the alterations that take place in cancer cells not only occur at the DNA sequence but also at the level of the epigenome. Eukaryotic DNA is wrapped around histone proteins to constitute chromatin, which plays fundamental structural and regulatory roles. The epigenome consists of chemical changes in both DNA and histones that can be inherited through cell division and are controlled by the action of a large set of epigenetic regulators that possess enzymatic activity. Ultimately, DNA and histone modifications control the level of chromatin condensation, which in turn regulates the accessibility of transcription factors to the chromatin and, therefore, gene expression.

During the past few years several studies, including our own, have suggested that the deregulation of the chromatin-modifying machineries can lead to aberrant gene expression causing cancer and other human diseases. The epigenome is regulated in a highly dynamic fashion by the coordinated action of regulators able to write, erase and read histone and DNA modifications. Thus, contrary to genetic mutations,

epigenetic aberrations can be reversed through the targeting of the appropriate epigenetic regulators. Indeed, drugs targeting DNA methyltransferases and histone deacetylases have successfully demonstrated anticancer properties and are currently used in the clinic. Therefore, identifying the molecular function of critical epigenetic regulators and their complex relationship with the cancer epigenome (FIGURE), as well as the development of small molecular inhibitors of their activities holds great promise for the therapeutics of cancer. ■

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García-Carpizo v, Ruiz-Llorente S, Sarmentero J, Graña-Castro O, Pisano DG, Barrero MJ (2018). CREBBP/EP300 bromodomains are critical to sustain the GATA1/MYC regulatory axis in proliferation. *Epigenetics Chromatin* 11, 30.

García-Carpizo v, Ruiz-Llorente S, Sarmentero J, Barrero MJ (2018). Therapeutic potential of TAF1 bromodomains for cancer treatment. *bioRxiv*. doi: <https://doi.org/10.1101/394254>.

## TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Anabel Sanz  
Director

Technology Transfer Manager  
M. Cruz Marin



Technician  
Rocio Manzano (until October) (TS)\*

\* *Titulado Superior* (Advanced Degree)

Discoveries made by CNIO scientists result in the development of useful novel biomedical solutions, including diagnostic and therapeutic applications, all for the benefit of cancer patients and the health system in general. The Technology Transfer and Valorisation Office (TTVO) plays an integral part of the conversion of discoveries into technologies that are ready to be taken up by companies and entrepreneurs.

The TTVO carries out the all-round management of all aspects needed to ensure the appropriate intellectual property protection and commercial viability of the research results generated by CNIO's scientist. Additionally, the TTVO Office provides advice and guidance to CNIO scientists regarding the development of research projects that have the potential to create social and economic value and, thereby, also helps to valorise their research.

To this end, the TTVO Office proactively monitors the progress of scientific activity at the CNIO in order to identify projects with high transfer potential. In 2018, a total of 13 new ideas were incorporated to the Technology Transfer portfolio, of which 7 turned into patent applications. These cover a wide range of products, including novel TRF1 inhibitors and their use to treat brain tumours, oligonucleotides for the treatment of lung cancer and other proliferative diseases, combined therapies for the treatment of pancreatic tumours driven by *Kras/Trp53* mutations, gene editing based therapies to treat tumours driven by genomic rearrangements, biomarkers for predicting resistance to immunotherapy, and biomarkers for the prediction of residual disease and relapse in melanoma.

Several new valorisation projects have been launched in 2018. To highlight some of them: a project, in collaboration with a national company, was developed for the screening of compounds of natural origin useful in ageing and cancer treatment; a senescence project based on a CNIO patent aims at the characterisation of antagonists of a novel modulator of cellular senescence; the FuGe project is directed towards the development of a cancer therapy based on CRISPR; and a drug discovery project aims at the characterisation and development of TRF1 inhibitors as a means to modulate telomere activity. The latter 2 projects have been awarded a grant under the *CaixaImpulse* initiative and thereby benefit from funding and mentoring by experts of the national bio-ecosystem.

Additionally, the TTVO identifies appropriate commercial partners for a timely development of technologies, negotiates technology transfer agreements, and manages the relationship with licensees including the payment of royalty fees. In 2018,

**“Discoveries by CNIO scientists result in the development of useful biomedical solutions, including diagnostic and therapeutic applications, all for the benefit of cancer patients and the health system.”**

the TTVO managed 291 technology transfer records related to industrial and intellectual property generated by CNIO's researchers; 171 correspond to agreements (MTAs, CDAs, Research Collaborations, licences, etc.). The majority of these agreements (65%) 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 2.9M euros were secured for research activities. Moreover, 5% of the agreements are licences to commercial partners. Patents and unpatented research tools are licensed in order to increase their availability to the scientific community, as well as to create opportunities for our business partners and to provide a financial return on public investment. The net income derived from licences in 2018 increased to 582,500 euros. Altogether, the net royalty income obtained from the exploitation of industrial and intellectual property assets in the last 5 years has exceeded 17M. This income reverts back to CNIO research activities as well as to the inventors themselves. A total of 36 inventors have contributed towards and benefited from this achievement.

Fostering an innovation culture among our scientists is one of our priorities. With the support of the *Fundación Banco Santander*, we uphold our collaboration with the prestigious IE Business School, through which many of our investigators – 2 new ones in 2018 – have already obtained training in market-oriented innovation strategies. Additionally, the TTVO organised events and seminars directed towards the promotion of the innovation culture among CNIO's scientist.

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

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# Communication

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## Communications

Press Clippings  
2018 Social Network Data  
Social Events

174  
176  
179  
180

# COMMUNICATIONS

**NURIA NORIEGA** Head of Communications

Communications Officers  
Cristina de Martos (until July) (TS)  
Vanessa Pombo (since September)  
(TS)

\**Titulado Superior* (Advanced Degree)



The mission of CNIO's Communications Department is to bring the Centre's research activity closer to those who can benefit the most from it, namely patients and their families as well as society at large. In order to fulfil this objective, the Communications Department uses all the information channels available, both traditional and digital.

In 2018, our news items got extensive media coverage with over 3,400 press appearances, either online or in print (29 % more than in 2016, with heightened impact), and 225 mentions on radio and television. Overall, media coverage increased by 46 % over the previous year. Throughout the year, the featured stories received nearly 71,733 hits (EurekAlert! news service) from around the world.

In an increasingly competitive information environment, some of the scientific news items produced at CNIO made the headlines in the leading media outlets in Spain and other countries: *BBC*, *Clarín* and *El Universal*, among others. For instance, the findings on the lengthening of telomeres to cure lung fibrosis in mice made by Maria A. Blasco and published in January in *eLife*; the protective effects of silibinin against brain metastases made by the team led by Manuel Valiente, published in *Nature Medicine* in June; or the article published in August in *Nature Communications* on the discovery of a protein panel as an early indicator for the prognosis of the most aggressive type of breast cancer by the team led by Miguel Ángel Quintela.

In 2018, strategic work was undertaken in order to better publicise scientific events like the CNIO Distinguished Seminars, offering lectures by leading researchers from all over the world, or the CNIO-"la Caixa" Foundation Frontiers Meetings, gathering the foremost experts on ground-breaking topics in cancer research, such as immunotherapy or ageing. These events were covered by many different media channels, including general newspapers, radio stations and TV channels.

In an effort to reach out to the community, a new edition of the "Present and Future of Cancer Research" with the *Atresmedia* media group and *Fundación AXA* was organised. The event, held at the Cibeles Palace with the support of the City of Madrid, featured Nobel laureate Elizabeth Blackburn, Laura García Estévez (MD Anderson Cancer Center), María José Alonso Fernández (University of *Santiago de Compostela*), Luz Casal (singer, musician, writer and cancer survivor) and CNIO Director Maria A. Blasco. Hosted by

**"Spreading knowledge to valorise science."**

Mamen Medizábal, from *La Sexta* TV, it once again spread the message that research is one of the pillars on which the fight against cancer rests.

Once again, the activities associated with the philanthropic initiative 'CNIO Friends' drew the attention of the media. Among them was CNIO Arte, a ground-breaking project in Spain carried out with the collaboration of *Fundación Banco Santander*, exploring the common ground of scientific research and artistic creation. This first edition called 'Binomio, a Dialogue between Art and Science', which brought together pioneering molecular biologist Margarita Salas and visual artist Eva Lootz, made quite an impact on the press and got coverage in *El País Semanal*, *Mujer Hoy*, and *Atención Obras (La 2)*, among others.

Last but not least, the Communications Department kept working on strengthening ties with patients' associations, participating in events such as the 13<sup>th</sup> *GEPAC* Conference, which drew 2300 attendees and was organised by the Spanish Group of Cancer Patients (*GEPAC*), which comprises 87 cancer patients' associations. Once again, CNIO researchers gathered over the weekend to publicise CNIO's scientific activity – our teams' findings and their impact on the future of cancer prevention and treatment.

PRESS CLIPPINGS



- |  |  |  |  |  |  |  |                              |
|--|--|--|--|--|--|--|------------------------------|
| 1 Revista PAPEL, El Mundo (front page), February 4, 2018 | 3 Diario Médico, February 12, 2018       | 6 Atención Obras, La 2, March 21, 2018       | 8 Pronto, April 17, 2018                   | 10 La Razón (front page), May 5, 2018    | 12 El Cultural, El Mundo, June 8, 2018 | 15 El País Semanal, El País, July 22, 2018 | 18 Clarín, August 29, 2018   |
| 2 Telemadrid Informativos, February 4, 2018              | 4 Mujer Hoy, February 17, 2018           | 7 Enviado Especial, La Sexta, April 12, 2018 | 9 El País Semanal, El País, April 29, 2018 | 11 La Entrevista Canal 24h, May 14, 2018 | 14 El Mundo, June 12, 2018             | 16 Agencia SINC, August 1, 2018            | 19 La Razón, August 31, 2018 |
|  | 5 Correo Farmacéutico, February 19, 2018 |  |  |  |  | 17 Faro de Vigo, August 24, 2018           |                              |



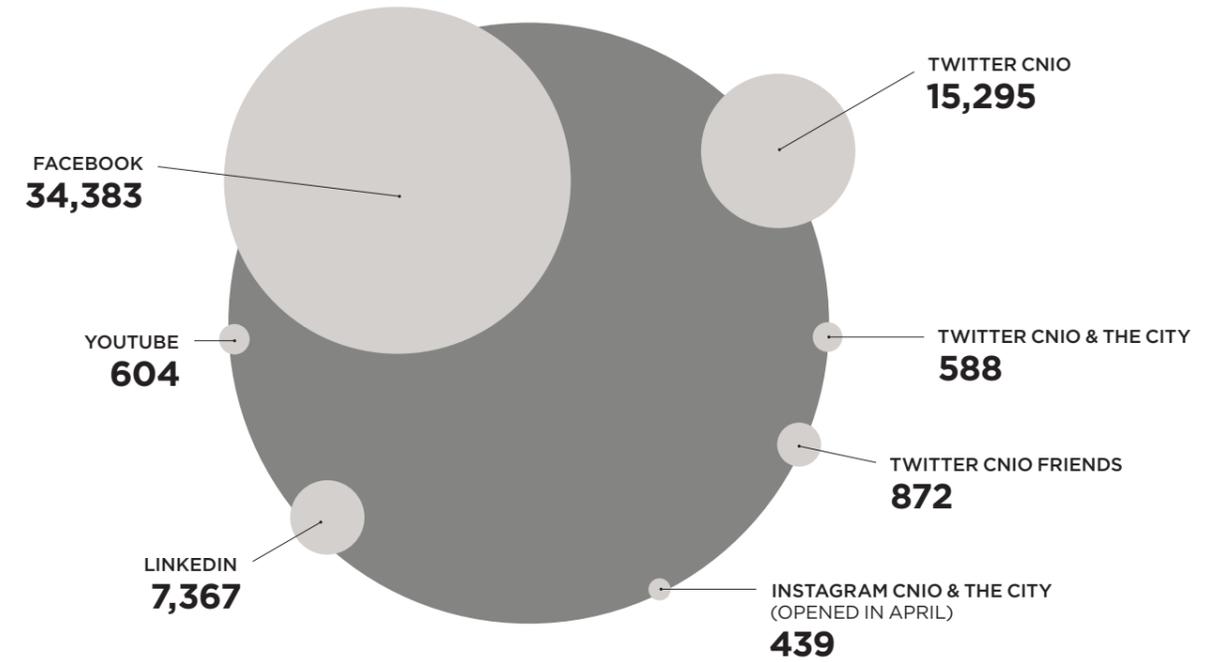
20 Más Vale Tarde, La Sexta, September 24, 2018

21 El País, November 15, 2018

22 ABC, November 18, 2018  
23 El Diario Montañés, November 19, 2018

24 La Voz de Galicia, December 4, 2018  
25 Movistar\*, December 4, 2018

2018 SOCIAL NETWORK DATA FOLLOWERS



LINKEDIN

IMPRESSIONS	233,530
INTERACTIONS	9,650
LINK CLICKS	6,303
INTERACTION RATE (AVERAGE)	4.19 %

YOUTUBE

UPLOADED VIDEOS	91
UPLOADED IN 2018	18
VIEWS IN 2018	26,200
WATCH TIME (IN MINUTES)	80,920
AVERAGE VIEW DURATION (IN MINUTES)	3:05

FACEBOOK

IMPRESSIONS	1,127,383
REACH	780,481
LINK CLICKS	25,202
INTERACTIONS	32,102

TWITTER CNIO

LIKES	8,300
RETWEETS	4,624
IMPRESSIONS	1,896,200
MENTIONS	4,391
VISITS TO THE PROFILE	72,695

The topics that most attracted the attention of our followers on our social media accounts, besides from the scientific news generated by CNIO's research groups and units, were the stories and interviews depicting the more human side of our research and of the people involved, such as *Juegaterapia's* visit with the singer David Bisbal to support 'CNIO Friends' (April), two

interviews with Maria A. Blasco and musician Luz Casal in *El Mundo* (February) and *La Razón* (October), the announcement that Manuel Valiente was elected EMBO Young Investigator (November), and the interview with Giovanna Roncador in *El País* (December), to name a few.

SOCIAL EVENTS



Pedro Duque visited the CNIO a month after having been appointed as the Spanish Minister for Science, Innovation and Universities. Photo by *Europa Press*. July 16, 2018.



CNIO remained faithful to its standing appointment with society during the European Researchers' Night, which is funded by the EU Framework Programme for Research & Innovation, Horizon 2020 — Marie Skłodowska-Curie actions. In the Madrid region, this event is promoted by the Department of Education and Research and coordinated by *Fundación madri+d*. Up to 64 CNIO volunteers and 200 visitors attended this event that has the objective of demonstrating the reality of a collective — the science community — that is key in the development of society. September 28, 2018.



Together with the *Atresmedia* Corporation and the AXA Foundation, we celebrated World Cancer Research Day at the City Council of Madrid with an event entitled "Present and Future of Cancer Research". Elizabeth Blackburn, Nobel Prize winner in Medicine in 2009, held a press conference, gave the keynote speech "Resolving Paradoxes in Telomere Biology and Cancers", and participated in a roundtable discussion with CNIO Director María A. Blasco, Laura García Estévez (MD Anderson Cancer Center), María José Alonso Fernández (University of Santiago de Compostela), and Luz Casal (musician, singer, author, songwriter and former cancer patient). September 24, 2018.



During the 10th edition of the Visiting Researcher Programme, supported by the *Jesús Serra* Foundation (part of the *Catalana Occidente* Group), the Foundation and CNIO announced that the Centre will host a sabbatical stay for Scott Lowe, Chair of the Cancer Biology and Genetics Program at the Memorial Sloan Kettering Cancer Center. Lowe will develop new collaborations with CNIO faculty and initiate mutually beneficial collaborations. December 4, 2018.

↑ Representatives from member institutions of the *Severo Ochoa* and *María de Maetzu* Alliance, *SOMMa* — which encompasses "Severo Ochoa" and "María de Maetzu" Centres and Units of Excellence — gathered at the CNIO for the *100xCiencia.3: Bridging science and society* Congress. In its third edition — the first since the establishment of this Alliance — *100xCiencia* focused on the importance of the participation of society in science. During the event, representatives from the science commission of the Spanish Congress of Deputies debated on the strong and weak points of the science policy in our country. November 15, 2018.

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# International Affairs

## INTERNATIONAL AFFAIRS

CAROLINA POLA Director of International Affairs



In 2018, we succeeded at consolidating our key alliances, thus adding to the goal of the Centre to reinforce CNIO's scientific leadership. We started off the year by implementing a strategy to focus on the promotion, participation in, and coordination of international scientific consortia; the execution of this strategy has created a considerable momentum that we will continue to push forward in light of the upcoming European framework programme. The active engagement of the Department of International Affairs (IAs) with the Spanish Ministry of Science, Innovation and Universities (MICIU), the Centre for the Development of Industrial Technology (CDTI), the Foundation for Science and Technology (FECYT), the European Commission and other funding actors is crucial to better understand where the opportunities lie and how to leverage our strengths and opportunities.

As 'Severo Ochoa' Centre of Excellence and member of the *Severo Ochoa* and *Maria de Maetzu* Alliance (SOMMa), IAs keeps leading the Work package of Outreach and provides a bridge between the activities of SOMMa and the CNIO. In particular, in November, the CNIO co-organised with SOMMa the first '100xCiencia' conference taking place within the alliance's framework. CNIO hosted the meeting, bringing together more than 140 people, both from SOMMa as well as from centres interested in sharing projects and views on Science Education and Public Engagement initiatives. The programme, which grasped the attention of various media outlets, hosted a roundtable with science representatives from various political parties and provided a forum for discussion with different societal actors. The impact and dissemination of the event was aligned with the objectives of SOMMa and CNIO to reach out to society and spread the knowledge obtained by the Spanish scientific community.

We have not ceased efforts to expand our reach, and this year—with the invaluable and instrumental collaboration of the *Ramón Areces* Foundation—the partnership with the prestigious Weizmann Institute of Science (WIS) is taking shape and growing into a fruitful alliance. The first call for CNIO-WIS joint projects was launched in June and the awarded projects will kick off in 2019. The Alliance has agreed that a second CNIO-WIS joint symposium on Cancer Discovery will take place in Israel for further strengthening the partnership and sowing the seeds for future collaborations. There is full commitment from all the partners in this tripartite alliance to achieve a model of international collaboration that will soon show a strong impact on science.

**“Establishing and consolidating international partnerships remains an overarching goal to promote CNIO institutional leadership. We strive to foster collaborations and participation in H2020 and focus on preparing the Centre to excel in the upcoming Horizon Europe.”**

The CNIO has paid special attention to establishing a cancer research consortium in drug discovery for the Spanish Association Against Cancer (AECC) Accelerator Awards call, which we believe can exert a great impact on the field of cancer and contribute to speed up progress in oncology. The rippling effect of such consortia, that comprise top scientists from Spain, UK and Italy, will serve the international cancer community at large. In 2018, the CNIO also actively participated in the first symposium organised by the Milner Therapeutics Institute in Cambridge, an international drug discovery-focused alliance in which the CNIO is an affiliate academic member. This alliance is key to place the CNIO at the heart of drug discovery in Europe and to boost collaborations with the big industry players in the field of biomedicine.

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# Institutional Image & Outreach to Society

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**Institutional Image & Outreach to Society**  
CNIO & The City

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## INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY

AMPARO GARRIDO (since October) Coordinator



The Office of Institutional Image & Outreach to Society was created in October 2018. Although all our paths to knowledge may differ, it is fair to say that all of us at the CNIO are united by the experience, effort, creativity and care that we dedicate towards strengthening links between our Centre and the citizenry which it serves. We hope that our efforts will enable our message to be successfully transmitted to society and that we impress upon people our Centre's reality of being a place where state-of-the-art scientific research is carried out intensively with the aim of defeating a disease that, one way or another, affects each and every one of us.

At the same time, we are at pains to foster an ever-deepening relationship between our teams of scientists and the public at large by organising and publicising various educational, recreational and other activities to heighten public awareness of the current state of play in advanced cancer research. Among those many activities is the initiative known as CNIO Arte.

CNIO Arte is an initiative created by CNIO, with support from the *Banco de Santander* Foundation, in conjunction with the Spanish Foundation for Science and Technology-Ministry of Science, Innovation and Universities, through CNIO & the City. Its goal is to bring together scientists and artists so that they can explore the uncharted territory where scientific research and artistic creation overlap.

We took our cue from CNIO's recently published book entitled 'Excelentes' that includes photographs by Amparo Garrido and texts by Monica G. Salomone, highlighting close-up portraits and biographical background sketches of several notable women and men who have been hosted at the CNIO over the past few years. Each year, CNIO Arte will invite one of these distinguished scientists to Madrid to take part in an inspirational dialogue with a respected artist who will then create one (or more!) works of art based on their interaction.

Our inaugural encounter was called 'Binomio, a Dialogue between Art and Science'. It was curated by Mireia Puigventós and proved to be a thought-provoking platform for its participants: pioneering molecular biologist Margarita Salas and renowned artist Eva Lootz, who was awarded Spain's National Prize for the Plastic Arts. The result was a series of 59 drawings and an audio-visual presentation that remained on display at CNIO headquarters for two and a half months. The artwork also really attracted lots of attention at ARCO, Spain's biggest and best known contemporary art fair. Sharing

**“We do not communicate through words alone. In order for a message to get across properly it has to surmount the complexities of stimuli that surround us. Our office takes care of creating and delivering a message that can prevail over everything else.”**

the credit for its success are Maria A. Blasco (Director of the CNIO), Susana Gómez (*Banco Santander* Foundation), Eva Lootz (Plastic Artist), Estrella de Diego (Distinguished Professor of Art), Amparo Garrido (Artistic Director) and Mireia A. Puigventós (Curator).

Artwork created by the artists who take part in CNIO Arte will be available to the public for viewing and 100% of the proceeds from their sales are earmarked by 'CNIO Friends' for the financing of cancer research at the Centre.

The year 2018 marks the launching of the new CNIO website, an important milestone for which we worked, and continue to do so, with dedication and motivation. One other major goal we have set for ourselves at the CNIO's Office of Institutional Image and Outreach to Society, is to encourage and assist our researchers as they familiarise themselves with our 'corporate identity manual' in order to build an institutional brand that is eye-catching in its visuals and memorable in its content. Those criteria ensure that everyone's creative endeavours, whether it be the design of a T-shirt with the CNIO logo or a presentation made before a congress of professional researchers, are all synergistically linked. Both criteria are of inestimable value in bolstering CNIO as a global brand. With so much ocular stimulation impacting on our daily lives, an effective and consistent visual identity can be particularly helpful in seeing that our message gets through to the public.

## CNIO & THE CITY

**CAROLINA POLA** Director of International Affairs

Science Outreach Officer  
Pablo Camacho (TS)\*

Communications Officer  
Cristina de Martos (until July) (TS)\*

\*Titulado Superior (Advanced Degree)



**“CNIO & The City is our instrument to reach out to the educational community and catalyse interaction between educators and CNIO scientists. We hope to promote knowledge co-creation and value in collaboration with different societal groups.”**

The success of the CNIO & The City’s first edition (May 2017 – March 2018) set a strong precedent and has motivated us to continue with our outreach efforts; these are undertaken through educational activities for pre-university students and by supporting STEM careers to empower society as a whole, hereby emphasizing the full and growing commitment to science outreach. In April 2018, the Spanish Foundation for Science and Technology (FECYT) – Ministry of Science, Innovation and Universities, awarded funding to the CNIO to continue with the project for another year, this time focusing on strengthening the bridges between CNIO scientists, the educational community and society by means of co-creation.

The openness of research centres to society through outreach events, citizen science and public engagement is an aspect of increasing interest and importance for European institutions, funding bodies and evaluators. It is essential that research projects as well as institutions acknowledge the importance of this kind of outreach for achieving new standards of scientific excellence in the future. The CNIO has always celebrated special occasions with science outreach activities, such as the European Researchers’ Night, the Science Week or the guided visits, and has always encouraged the staff to be actively involved. Thanks to CNIO & The City, this commitment can be upheld throughout the whole year.

This new project kicked off in May 2018, maintaining the two main gears of the project (EDCUACNIO for students, and FORMACNIO devoted to teachers), and since then even more students and teachers have ended up swelling the ranks of CNIO & The City participants: ‘Lab Immersion’ and ‘My First Science Project’ (88 Secondary students and 3 classrooms, 70% women) and the ‘Stop Cancer Training Course for

Secondary Teachers’ (50 teachers, 80% women). In 2018, CNIO & The City included new science workshops aimed at primary school students in order to start impacting on younger generations as a stepping stone for accomplishing our objective to foster STEM careers. In addition, the project has focused on supporting the CNIO Arte initiative, and facilitating educational talks at schools and inspirational and vocational material (blog posts, videos...), which are available to the public and disseminated through our social channels.

As the above numbers reveal, this project is also committed to closing the gender divide in science. In CNIO & The City, we regard gender equity values and the importance of women in science as a transversal theme and we thereby strongly encourage the participation of female students in all the activities; strategies to this regards are developed and implemented in collaboration with the CNIO WISE Office. In February, we marked the International Day of Women and Girls in Science by sending some of our female scientists to the schools and celebrating the event ‘Mom, I want to be an artist...and a scientist!’; we benefitted from the special collaboration of the pioneering molecular biology scientist Margarita Salas and the renowned visual artist Eva Lootz .

All CNIO & The City activities represent a team effort and they would not be possible without the commitment and collaboration of the more than 70 CNIO volunteers, ranging from Principal Investigators to technicians and other administrative staff. The integration of science in societal culture is a long-standing demand in Spain, as the 9th Social Perception of Science Survey (FECYT) reveals, and we eagerly join other research centres in leading science outreach projects and meeting society’s needs.



History of Cancer (watch video at <https://www.youtube.com/watch?v=wFAO7fDhZX8>).



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# CNIO Offices

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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, David Olmeda, Adrián del Rincón

### Staff/Faculty:

Ana Losada, Jorge Martínez, Lola Martínez, Francisco X. Real, Manuel Valiente



The CNIO is recognised for the relevance and international projection of its Scientific Programmes. This success would not be possible without the hard work of our personnel in training. Indeed, over 60% of the CNIO's workforce is comprised of PhD students, predoctoral and postdoctoral fellows, medical residents and a broad spectrum of visiting scientists. The CNIO places particular emphasis on career development and to that end, our Centre's core mission is to maximise the chances of success of our personnel in training.

In addition to ensuring proper academic supervision, we acknowledge that scientists need soft skills beyond the bench. Therefore, we pay special attention to areas of public communication, management of intellectual property, and the creation of *start-ups* or *spin offs*. These activities are performed in concert with CNIO's Training Programmes, as well as with the Innovation and Communication Offices. We are most grateful to the *Fundación Jesús Serra* for their

continuous support to strengthen career development programmes at the CNIO.

We believe that an informed society is better prepared to understand (and if needed, face) the diseases that constitute human cancer. Therefore, we are actively involved in knowledge dissemination. For example, over 70 CNIO PhD students and postdoctoral fellow volunteers this year took part in the sixth edition of our '*Meet a Scientist, Become a Scientist*' event. This is an open doors activity that we hold as part of the *Marie Skłodowska Curie European Researchers' Night* and was attended by over 250 participants of all ages who learned about daily life at our Centre and had the opportunity to run an experiment side by side with our researchers.

A particularly inspirational event this year was our Annual CNIO Lab Day. We were fortunate to host **Roland Rad**, Director of the Institute of Molecular Oncology and Functional

Genomics at the Technical University of Munich (Germany), who spoke about his experience in the field of data analysis based on a large volume of patient samples. **Marisol Quintero**, CEO of *Bioncotech Therapeutics*, presented a powerful example of how to transfer intellectual property from a research centre to a clinical trial. In addition, **Gustavo Fúster Olaguibel**, from Hoffmann Eitle, discussed his personal view on how it became possible for a pharmacist like himself to end up working in a law firm specialising in intellectual property and patents. We also had 15 outstanding talks given by CNIO trainees that covered exciting discoveries in the fields of epidemiology, epigenetics, proteomics, metastasis and drug development. Progress made in other basic and translational aspects of cancer were presented in over 60 posters, which together emphasised the breadth of research of our different Scientific Programmes.

Another main highlight of the Lab Day was the announcement of the recipients of our '*Director's List Awards*'. These are recognitions to 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 for Excellence in Research by Predoctoral Fellows

We are grateful again to the Agüera-Nieto family for their 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 **Isidoro Cobo**, from the Epithelial Carcinogenesis Group, for the finding that a gene that increases the risk of pancreatic cancer also controls inflammation in normal tissue (published in *Nature*).

Recipients of other awards in the PhD category were **Panagiotis Karras** (for an article in *Cancer Cell*), **Manuel Sanclemente** (*Cancer Cell*), **Alexandar Kojic** (*Nature Structural and Molecular Biology*) and **Juan José Montero** (*Nature Communications*).

**“PhD students, postdoctoral fellows, and in general all personnel in training are key assets for the CNIO. A main objective of our Centre is to train and empower them to fulfil their potential as future influential leaders.”**

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

This award was shared *ex aequo* by **Neibla Priego** (Brain Metastasis Group), for mechanistic and pharmacological findings in brain metastases published in *Nature Medicine*, and by **Carmen Guerra** (Experimental Oncology Group), for her contribution in the training of researchers and her multiple accomplishments in the field of pancreatic cancer.

### 3. Outstanding Contribution to Outreach and Awareness

**Isabel López de Silanes**, from the Telomeres and Telomerase Group, received this award to honour her generous and altruistic contribution to CNIO's Women in Science Office, organising seminars and lectures, inviting schools and colleges to attend these events, and securing funding for these activities.

In summary, we are as proud as ever of the achievements of our vibrant community of young investigators at the CNIO. We thank all those public and private contributors that have helped support and fuel their efforts, and we will make sure that the coming years will be even more successful in moving the cancer field forward.

## CNIO WOMEN IN SCIENCE OFFICE

Isabel López de Silanes  
(since June)  
Lola Martínez (until June)  
Coordinator

Members  
Marinela Mendez (*Work-Life Balance Coordinator*), Isabel López de Silanes (*Seminars and Events Coordinator*), María A. Blasco, María Jesús Alcamí, Cristina de Martos (until July),



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, 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 represented at the Centre and also includes the Director. All of us share the belief that women are still underrepresented in leadership positions in science and we are committed to ensuring gender equality within the research domain.

Several studies and statistics from different organisations within Spain and the European Union still display the ‘scissors’ graphic when it comes to gender distribution and career ladder positions. There is a clear lack of female talent in leadership

positions, which, together with an average 20% salary gap between men and women, are clear indicators that there remains a lot of work to be done in terms of promoting female talent and facilitating access to areas that are still not traditionally open to women. Still to this day, women are underrepresented in academia. Although data from Universities here in Spain and other European countries show that the percentage of women undertaking university studies is over 50%, and the representation of women at the pre-doctoral and post-doctoral stages is similar, those percentages diminish significantly as we move up the scientific career ladder: a poor 25% of women are represented at the Principal Investigator level, and it is even lower at the levels of department directors and beyond. Moreover, the acknowledgement of women when it comes to prizes continues to be very low and, in particular, when we consider the most prestigious ones; is there simply not enough female talent present in the scientific fields, or is it still just harder for them

Raquel García-Medina, Diego Megías,  
Francisca Mulero, Fernando Peláez,  
Carolina Pola, Sandra Rodríguez

to be recognised for their achievements? We believe it is mainly due to the second cause and so we continue with our endeavours that take the form of a successful seminar series where we give all CNIO members and others (including students from different high schools in Spain) the opportunity to listen to and meet women who embody roles and positions that are traditionally not held by women. With this initiative, it is our mission to promote scientific vocations among girls – it is worrying to see the lack of female students in the so-called STEM careers – as well as to positively change the cultural and gender stereotypes that exist among teenagers. A real change is needed, and we are convinced that it needs to come through the education of our younger generations.

The Office counts two main working groups: 1) *Life/Work Balance* – aimed to promote and support initiatives to help improve the delicate balance between professional and personal life at CNIO; 2) *Seminars and Events* – aimed to raise and stimulate institutional awareness of gender issues, and to provide networking opportunities to all CNIO researchers.

In 2018, the WISE office seminar series continued to host several top female leaders from different areas. Some of the talks given during 2018 include:

- Laura Ferrero Carballo, writer, freelance editor and literary advisor Instituto Vasco Etxepare. Title: ‘Decisiones racionales e irracionales: una breve historia de cómo llegué a ser escritora’. 30/01/2018.
- Elisa Martín Garijo, Chief Technology Officer for IBM, Madrid, Spain. Title: ‘Yes, we can’. 20/02/2018.
- Fátima Bosch, Director of the Centre of Animal Biotechnology and Gene Therapy at the Autònoma University of Barcelona, Spain. Title: ‘Translational Gene Therapy Approaches to Treat Metabolic and Neurodegenerative Diseases’. 06/03/2018.
- Victoria Camps, Philosopher, Professor of Ethics Universidad Autònoma de Barcelona, Spain. Title: ‘La filosofía como instrumento de emancipación’. 24/04/2018.
- Laura González Molero, Ibex35-company Board member and ex-President of Merck-Serono and Bayer HealthCare. Title: ‘Atrévete a ser el rector de tu propia vida’. 05/06/2018.
- Pepe Verdes, from Librotea, interviewed the writer Elvira Sastre. Title: ‘Escritoras, más allá del género y la biografía’. 25/09/2018.
- Eulalia Pérez Sedeño, research professor at the CSIC Institute of Philosophy. Title: ‘Conocimiento y estereotipos de género’. 23/10/2018.

**Here at the WISE Office, we share the view of the American journalist and social political activist Gloria Steinem: “a feminist is anyone who recognises the equality and full humanity of women and men.” To this end, we endeavour to build a fair society for all where stereotypes, unconscious bias and gender barriers are eliminated, as we firmly believe that working together as equals, is the only way to reach true excellence.**

- Marta Macho, professor of mathematics at the Universidad del País Vasco. Title: ‘Entre el suelo pegajoso y el techo de cristal: la realidad de las mujeres en el ámbito laboral’. 11/12/2018.

The WISE Office signed an agreement with the ASTI Foundation to be a strategic partner for their project STEM Talent Girl Madrid. The goal of this project is to promote STEM careers among 13-14 year-old students. Eight Master classes given by top professional women from the STEM field have been organised to be held in the CNIO Auditorium. The first 2 already took place and were given by Rosa García, president of Siemens Spain (November, 2018) and Marta Martínez, president of IBM Spain (December, 2018). 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 carried out in collaboration with ‘CNIO and The City’, a science outreach project, co-financed by the *FECYT (Fundación Española para la Ciencia y Tecnología)* and *Obra Social “la Caixa”*, 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 (since May)



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.

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, manages the ethical certification for projects involving animal experimentation or human samples, 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.

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 article requests for journals that the CNIO is not subscribed to. The Library also provides information regarding reference management software, manages the open-access repository *REPISALUD* and organises the CNIO guided visits.

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

The Scientific Publications Office is responsible for the preparation of institutional scientific publications, including the CNIO Annual Report, booklets of the Scientific Advisory Board meeting and those of other symposia, as well as scientific dissemination leaflets. The Office also provides support for the scientific editing of press notes and other publications of scientific divulgation to a non-specialised audience.

## COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies and is used not only to finance the Centre's outstanding R&D activities, but also strategic actions in Innovation together with Industry partners. The funding is also used to support other relevant activities related to dissemination and scientific outreach; these activities are aimed at promoting public awareness. In 2018, researchers at the CNIO were involved in 134 projects that received extramural funding.

In 2018, the CNIO actively participated in a total of 45 collaborative projects: 14 were international collaborative projects (3 of which are coordinated by the CNIO) and 31 were collaborative projects at the national level (12 of them are coordinated by the CNIO). The international collaborative projects were funded by institutions such as the European Commission through the 7<sup>th</sup> 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 (MRA), the Paradifference Foundation and Worldwide Cancer

Research. At the national level, collaborative projects received important public funding through grants from the Strategic Health Action that is managed by the Institute of Health *Carlos III (ISCIII)*, the State Research Agency of the Spanish Ministry of Science, Innovation and Universities (*AEI/MICIU*), and the R&D Activities Programmes of the Community of Madrid; most of the projects were cofunded 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*).

In addition to these collaborative projects, researchers at the CNIO attracted funding for projects carried out by individual groups. In 2018, 24 of these projects received international funds while 65 of them received national funding (mainly from the *AEI/MICIU*, the *ISCIII* and private foundations). The international individual projects are funded by the European Commission (3 ERC grants and 8 Marie Curie Actions), Worldwide Cancer Research, the Howard Hughes Medical Institute (HHMI), the Prostate Cancer Foundation, the US DoD and the MRA.

## INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

### EUROPEAN COMMISSION



#### 7<sup>TH</sup> FRAMEWORK PROGRAMME (2007-2013)

##### ERA-NET ON TRANSLATIONAL CANCER RESEARCH (TRANSCAN)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria	Bio-PaC: Biomarkers of tumor recurrence in pancreatic cancer (financed by ISCIII, Ref.: AC14/00025)

##### 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)

##### MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	ITN IMMUTRAIN: Training network for the immunotherapy of cancer (Ref.: 641549)

##### SOCIETAL CHALLENGE 1: HEALTH, DEMOGRAPHIC CHANGE AND WELLBEING

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

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Muñoz Fernández, 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)

### INTERREG SUDOE PROGRAMME<sup>1</sup>



### US NATIONAL INSTITUTES OF HEALTH (NIH)



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



**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)
Peinado, Héctor	Organ-tropic metastatic secretomes and exosomes in breast cancer (Ref.: W81XWH-13-1-0427)

**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
Robledo, Mercedes	SDHB-related metastatic paraganglioma: search for the cure

**WORLDWIDE CANCER  
RESEARCH (WCR,  
FORMERLY AICR)**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria (coordinator)	Oral microbiotic profiles and its association with risk of pancreatic ductal adenocarcinoma (Ref.: 15-0391)

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


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

**INTERNATIONAL GRANTS** INDIVIDUAL PROJECTS

**EUROPEAN COMMISSION**

**7<sup>TH</sup> 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)

**MARIE CURIE ACTIONS (MCA)**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Peinado, Héctor	WHRI COFUND ADIPOMET: Analyzing the crosstalk of tumor and adipose tissue during metastasis (Ref.: 608765 WHRI-309)
Plaza, Iván	WHRI COFUND: Structure-Function studies of oncogenic RET kinase Fusions in non-small cell Lung Cancer (NSCLC): from structure to targeted therapy (Ref.: 608765 WHRI-612)
Ramón, Santiago Moreno, María	WHRI COFUND CAD_FL: Revealing the functional mechanism of CAD and its potential as a therapeutic target (Ref.: 608765 WHRI-222)
Real, Francisco X Paliwal, Sumit	WHRI COFUND: NFIC as a novel regulator of pancreatic acinar differentiation and homeostasis (Ref.: 608765 WHRI-609)
Squatrito, Massimo	GLIDD: DNA Damage Response (DDR) signaling in tumor formation and therapeutic resistance of gliomas (Ref.: 618751)
Wagner, Erwin F. Gago, Nuria	WHRI COFUND STEM-PSO: Unraveling the contribution of Epidermal and Non-Epidermal Progenitor (Ref.: 608765 WHRI-319)

**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)
Efeyan, Alejo	ERC Starting Grant NutrientSensingVivo: The Physiology of Nutrient Sensing by mTOR (Ref.: 638891)

**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)

**HOWARD HUGHES MEDICAL  
INSTITUTE (HHMI)**


PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	Exploring the role of replicative stress in cancer and ageing (Ref.: 55007417)

**MELANOMA RESEARCH ALLIANCE (MRA)**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	Prognostic and therapeutic impact of lymphovascular niches in melanoma (Ref.: 348673)
Valiente Cortes, Manuel	Blocking melanoma brain metastasis by targeting the environment (REF.: 498103)

**PROSTATE CANCER FOUNDATION**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	Integration of clinical, molecular and biological characteristics to define an aggressive subtype of prostate cancer based on deficient homologous recombination
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, María	Targeting telomeres in cancer (Ref.: 16-1177)
Lietha, Daniel	Targeting regulatory mechanisms for allosteric cancer drug discovery (Ref.: 15-1177)
Malumbres, Marcos	New therapeutic strategies by inhibiting Mastl in breast tumors (Ref.: 15-0278)
Peinado, Héctor	Evaluation of obesity as a novel risk factor in metastasis (Ref.: 16-1244)
Soengas, María S.	Harnessing endo/exocytosis for a coordinated targeting of melanoma cells, their vasculature and the immune system (Ref.: 15-1374)

**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

**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 SALUD<sup>2</sup>**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Artiga, M <sup>º</sup> Jesús (Coordinator), Morente, Manuel (until August 2018)	<i>Plataforma de Biobancos</i> (Coordination node and Group, Ref: PT13/0010/0001)
Benitez, Javier	<i>Plataforma de recursos biomoleculares y bioinformáticos</i> , PRB <sup>2</sup> (Group, Ref: PT13/0001/0005)
Muñoz, Javier	<i>Plataforma de recursos biomoleculares y bioinformáticos</i> , PRB <sup>2</sup> (Group, Ref: PT13/001/0010)
Al-Shahrou, Fátima	<i>Plataforma de Bioinformática. Instituto Nacional de Bioinformática</i> (Group Ref: PT17/0009/0011)
Artiga, M <sup>º</sup> Jesús Morente, Manuel (until August 2018)	<i>Plataforma de Biobancos</i> (Group, Ref: PT17/0015/0004)
Benitez, Javier	<i>Plataforma de proteómica, genotipado y líneas celulares. Plataforma de recursos biomoleculares</i> , PRB <sup>3</sup> (Group, Ref: PT17/0019/0020)
Muñoz, Javier	<i>Plataforma de proteómica, genotipado y líneas celulares. Plataforma de recursos biomoleculares</i> , PRB <sup>3</sup> (Group, Ref: PT17/0019/0005)

**RESEARCH PROJECTS IN HEALTH/PROYECTOS DE INVESTIGACIÓN EN SALUD<sup>3</sup>**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Artiga, M <sup>º</sup> Jesús (Coordinator) Morente, Manuel (until August 2018)	OPTIMARK project: Optimization of tissue samples for the development and validation of disease biomarkers (Ref.: PI16/00946)
Malats, Núria (Coordinator)	Building and validation of risk prediction models for pancreas cancer. The application of a multi-omics approach (Ref.: PI15/01573)
Olmos, David (Coordinator)	THERATLAS Project: integration of early and adaptive genetic events to establish therapeutic subgroups in Castration-Resistant Prostate Cancer (Ref.: PI16/01565)
Robledo, Mercedes (Coordinator)	Prognostic profiles in endocrine tumours identified by next generation sequencing, and definition of markers with clinical utility (Ref.: PI14/00240)

**TECHNOLOGICAL DEVELOPMENT PROJECTS/PROYECTOS DE DESARROLLO TECNOLÓGICO<sup>4</sup>**

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

2, 3, 4. This Programme is cofunded by the European Regional Development Fund (ERDF)



**STATE RESEARCH AGENCY, MINISTRY OF SCIENCE, INNOVATION AND UNIVERSITIES / AGENCIA ESTATAL DE INVESTIGACIÓN. MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES**



**NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION ( 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-COLABORACIÓN\***

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-Torrecaudrada, 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 MADRID**



Comunidad de Madrid

**R&D ACTIVITIES PROGRAMME IN BIOMEDICINE<sup>5</sup>:**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour Núñez, 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 M., 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 )

**MADRI+D FOUNDATION / FUNDACIÓN PARA EL CONOCIMIENTO MADRI+D**



**SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)**



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 GA 818528
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano (Coordinator)	A multifaceted approach to target pancreatic cancer (Ref.: GC16173694BARB)
Malats, Núria Real, Francisco X. (Coordinator)	Invasive bladder cancer: towards precision medicine (Ref.: GCB14142293REAL)
Gómez, Gonzalo Peinado, Héctor Soengas, María S. (Coordinator)	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.: GCTRA160155EOA)

5. This Programme is cofunded by the European Regional Development Fund (ERDF)



6. These Programmes are cofunded by the European Regional Development Fund (ERDF) and European Social Fund (ESF)

## NATIONAL GRANTS INDIVIDUAL PROJECTS

**INSTITUTE OF HEALTH  
CARLOS III / INSTITUTO DE  
SALUD CARLOS III (ISCIII)  
STRATEGIC HEALTH ACTION  
/ ACCIÓN ESTRATÉGICA EN  
SALUD (AES)**


RESEARCH PROJECTS IN HEALTH <sup>7</sup>	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	Massive sequencing contributes to decipher the genetic bases of families with rare tumors (Ref.: PI16/00440)
Cascón, Alberto	Next-generation sequencing of genes directly and indirectly involved in the Krebs cycle, applied to pheochromocytomas/paragangliomas with hypermethylated phenotype (Ref.: PI15/00783)
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	Ewing Sarcoma Model: induction of the t(11;22) translocation in human mesenchymal stem and iPS cells by the CRISPR-Cas9 system and study of the cellular context and other secondary events role (Ref.: PI14/01884)
Rodríguez, Sandra	Study of the role of epigenetic modifications in the development of Ewing sarcoma: High-throughput screening of epigenetic genes using CRISPR libraries in human (11; 22) + t cells (Ref.: PI17/02303)
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,  
INNOVATION AND  
UNIVERSITIES / AGENCIA  
ESTATAL DE INVESTIGACIÓN.  
MINISTERIO DE CIENCIA,  
INNOVACIÓN Y  
UNIVERSIDADES**


NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION ( 2013-2016)	
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, María	Centre of Excellence "Severo Ochoa" (Ref.: SEV-2015-0510)

R&D EXCELLENCE PROJECTS/PROYECTOS DE I+D EXCELENCIA <sup>8</sup>	
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)
Murga, Matilde Ruiz, Sergio (until April 2018)	Role of RAS signaling in pluripotency and totipotency (Ref.: SAF2016-80874-P)

CHALLENGES-RESEARCH PROJECTS/PROYECTOS RETOS-INVESTIGACIÓN <sup>9</sup>	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	PANTHER: A three prong strategy to fight pancreatic ductal adenocarcinoma (Ref.: SAF2014-59864-R)
Blasco, Maria	TELOHEALTH: Telomeres and Disease (Ref.: SAF2017-82623-R)

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



Djouder, Nabil	URIPAT: URI loss in intestinal pathologies (Ref.: SAF2016-76598-R)
Efeyan, Alejo	NUTRIENTOR: Physiology of nutrient sensing and signaling by the mTOR complex 1 (Ref.: SAF2015-67538-R)
Fernández-Capetillo, Óscar	BREAKINGRAD: Exploring the limits of radioresistance in mammals (Ref.: SAF2014-59498-R)
Lietha, Daniel	FAKACT: Structural studies elucidating the activation mechanism of Focal Adhesion Kinase (Ref.: BFU2016-77665-R)
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)
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)
Ortega, Sagrario	HaploEScancer: Haploid ES cells for cancer research (Ref.: SAF2013-44866-R)
Osorio, Ana	IPAGEN: Exploring the mechanism of action of PARP inhibitors in breast and ovarian cancer patients. Identification of new genetic predictors of response (Ref.: SAF2014-57680-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)
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)
Soengas, María S.	MEL-STOP: Vesicular trafficking in melanoma progression and treatment response (Ref.: SAF2014-56868-R)
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	ReACTIVE BrainMET: Dissecting the role of reactive astrocytes in brain metastasis (Ref.: SAF2014-57243-R)
Valiente, Manuel	Stat3 ReACTIVE: Biology of Stat3+ reactive astrocytes in brain metastasis (Ref.: SAF2017-89643-R)
Wagner, Erwin F.	CANPSOR: Investigating Cancer Risk in Psoriasis (Ref.: SAF2015-70857-R)
EXPLORA PROJECTS/PROYECTOS EXPLORA	
PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	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)
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**NETWORKS AND SCIENTIFIC MANAGERS-EUROPE/EUROPA REDES Y GESTORES**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	CNIO in Horizon 2020: support for proposal preparation and project management (Ref.: EUC2014-51617)

**YOUNG RESEARCHERS PROGRAMME/PROGRAMA JÓVENES INVESTIGADORES<sup>10</sup>**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Álvarez, Mónica	GPGenCan: Functional relevance of Greatwall/PP2A pathway in the maintenance of genomic stability: therapeutic implications in cancer (Ref.: SAF2014-60442-JIN)
Lecona, Emilio (until September 2018)	UBQREP: Modulation of DNA Replication by ubiquitination of chromatin proteins (Ref.: BFU2014-55168-JIN)

**SCIENTIFIC INFRASTRUCTURES/INFRAESTRUCTURAS CIENTÍFICO-TECNOLÓGICAS<sup>11</sup>**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Muñoz, Javier	Sistema cromatográfico UHPLC acoplado a Espectrómetro de Masas de alta resolución para estudios de proteómica avanzada (Ref.: CNIO15-EE-2855)
Al-Shahrour, Fátima	Clúster SMP de Análisis HPC (Ref.: CNIO15-EE-3845)

**SPANISH FOUNDATION FOR SCIENCE AND TECHNOLOGY / FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y TECNOLOGÍA (FECYT)**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Pola, Carolina	'CNIO and the City' - <i>Construyendo un Puente a la Sociedad</i> (Ref.: FCT-16-11115)
Pola, Carolina	'CNIO & the City' - <i>Co-creando conocimiento con la sociedad</i> (Ref.: FCT-17-12743)

**"LA CAIXA" BANKING FOUNDATION AND CAIXA CAPITAL RISK**

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)

**SCIENTIFIC FOUNDATION OF THE SPANISH ASSOCIATION AGAINST CANCER / FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	Grant for projects in childhood cancer: Targeting telomeres in neuroblastoma (Ref.: CICPF18004BLAS)
de Cárcer, Guillermo (until September 2018)	Grant for Emerging Groups: Identification of new biomarkers for breast cancer: mechanisms of sensitivity and resistance to cell cycle drugs (Ref.: LABAE16017DECÁ)
Efeyan, Alejo	Grant for Emerging Groups: Nutrient signaling in the pathogenesis and treatment of B cell Lymphoma (Ref.: LABAE16001EFY)
Squatrito, Massimo	Grant for emerging Groups: Novel therapeutic approaches for therapy-resistant malignant brain tumors (Ref.: LABAE16015SQUA)
Squatrito, Massimo	"Idea Semilla" Grant: Identification of biomarkers of tumor treating fields (TTFields) in glioblastoma (Ref.: IDEAS185SQUA)

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

**FUNDACIÓN INOCENTE**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, María	Validation of the TRF1 telomere protective protein as a novel anticancer target in pediatric glioma and ependymoma

**ASOCIACIÓN DE CÁNCER DE PÁNCREAS-ASOCIACIÓN ESPAÑOLA DE PANCREATOLOGÍA**

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>

**LEUKEMIA AND LYMPHOMA FOUNDATION / FUNDACIÓN LEUCEMIA Y LINFOMA**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Ortega, Ana	"Marcos Fernández" Grant: Functional characterization of RagC mutations in Follicular Lymphoma pathogenesis

**SPANISH ASSOCIATION OF GASTROENTEROLOGY / ASOCIACIÓN ESPAÑOLA DE GASTROENTEROLOGÍA**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Molina, M <sup>ª</sup> Esther	<i>Evaluación del valor pronóstico de diabetes mellitus tipo II (DM2) en pacientes con cáncer 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

**ATRESMEDIA CORPORATION / ATRESMEDIA CORPORACIÓN**

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>

**BBVA FOUNDATION / FUNDACIÓN BBVA**

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmeda, David	Leonardo Grant: <i>Nuevas estrategias para el tratamiento preventivo de la enfermedad metastásica en Melanoma</i> (Ref.: IN18_BBM_TRA_0293)
Squatrito, Massimo	Leonardo Grant: Precision glioma mouse models by somatic genome editing with the RCAS-CRISPR-Cas9 system (Ref.: IN17_BBM_TRA_0366)

**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

## 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 2018, the CNIO signed several new agreements with Spanish Universities and other institutions, namely with the

*Universidad de Francisco de Vitoria, Universidad Alfonso X el Sabio, University Sapienza di Roma, Universidad Operta de Cataluña, Universidad San Pablo CEU; IES Jose Luis San Pedro, IES Las Musas, Rozona Centro de Formación, Centro Educativo Maria Inmaculada and Fundación Juegaterapia.*

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2014	2015	2016	2017	2018
Training of PhD students	108	105	110	112	<b>109</b>
Post-doctoral training	55	48	51	44	<b>50</b>
Training for MDs	14	25	17	21	<b>12</b>
Laboratory training for MSc/BSc students	73	80	95	99	<b>128</b>
Laboratory training for technicians	21	27	26	20	<b>13</b>

### 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 2018, 5 students from 2 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, 128 students participated in these programmes, of whom 8 ended up joining the CNIO as pre-doctoral students.

### TRAINING OF PHD STUDENTS

The training of PhD students in cutting-edge cancer research is of key importance to the CNIO. The Centre offers many opportunities for bright and dynamic university graduates, of all nationalities, to pursue an ambitious PhD project. To attest this, 8 students obtained their PhD degrees in 2018 and 21 others joined the CNIO in the same year. Over 15% of the 109 students working at the CNIO in 2018 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. The CNIO was chosen, as one of such centres, to launch a programme for outstanding young pre-doctoral students, from all over the world, who have an interest in pursuing an ambitious PhD project. Since 2013, the former Spanish Ministry of Economy, Industry and Competitiveness (now Ministry of Science, Innovation and Universities) has undertaken efforts to link the "la Caixa"/CNIO International PhD Programme to distinguished research centres accredited as "Severo Ochoa" Centres of Excellence. In 2018, a new call for the doctoral fellowship programme of the "la Caixa" Foundation, named INPhINIT, was launched to recruit outstanding international students: 1 pre-doctoral student received one of these 3-year contracts. The *Fundación "la Caixa"* also launched another call to carry out a doctorate at Spanish universities and research centres; the CNIO was chosen as a host institution. During 2018, 1 pre-doctoral student received this fellowship.

The distribution of students across the CNIO's Research Programmes in 2018 was as follows: 48% of students worked in the Molecular Oncology Programme, 13% in the Structural Biology Programme, 18% in the Cancer Cell Biology Programme, 13% in the Human Cancer Genetics Programme, 1% in the Experimental Therapeutics Programme, 2% 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 Maria Oliva Contract Programme that offered one position to carry out a thesis at the CNIO. This call is expected to have only this one single edition.

**INPhINIT**  
The Caixa Fellowship Programme

**MANY PEOPLE FORM PART OF HISTORY. ONLY A FEW MANAGE TO CHANGE IT.**

We're offering **57 fellowships** to talented researchers of any nationality to carry out a doctorate in the disciplines of **Bio and Health Sciences, Technology, Physics, Engineering and Mathematics.**

► For more information and applications: [www.inphinitlacaixa.org](http://www.inphinitlacaixa.org)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 713673.

**"la Caixa" Foundation**

FUNDING OF PHD TRAINING	NO.
<b>SPANISH ORGANISATIONS</b>	<b>89</b>
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities / <i>Ministerio de Ciencia, Innovación y Universidades (MCIU)</i> (Predoctoral Fellowships)	34
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities / <i>Ministerio de Ciencia, Innovación y Universidades (MCIU)</i> (I+D Projects)	16
Ministry of Education and Vocational Training / <i>Ministerio de Educación y Formación Profesional</i> (Predoctoral Fellowships)	6
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i>	8
"la Caixa" Banking Foundation / <i>Fundación Bancaria "la Caixa"</i> (Predoctoral Fellowships)	11
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (I+D Projects)	2
Community of Madrid / <i>Comunidad de Madrid</i>	3
Cris Foundation / <i>Fundación Cris</i>	4
A3Media Foundation	1
CNIO	2
Banco Santander Foundation / <i>Fundación Banco Santander</i>	1
Inocente Inocente Foundation	1
<b>INTERNATIONAL ORGANISATIONS</b>	<b>20</b>
Celgene	1
China Scholarship Council (CSC)	2
Consejo Nacional de Ciencia y Tecnología (Mexico)	2
European Research Council	6
GENCODE	2
Human Frontier Science Program Foundation	1
Marie Skłodowska-Curie actions of the European Commission	2
National Institutes of Health	1
Pfizer	1
Portuguese Foundation for Science and Technology (FCT)	1
Roche	1
<b>TOTAL</b>	<b>109</b>

## 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 2018, 50 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 2018, the *Fundación Banco Santander* signed a new annual 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. One young scientist from the Memorial Sloan Kettering Cancer Center (USA) was awarded the *Banco Santander* Foundation-CNIO Fellowship in early 2018.

Thanks to the donations received through the 'CNIO Friends' platform launched in 2016, the third call of the 'CNIO Friends' Postdoctoral Contract Programme, in 2018, resulted in the recruitment of 4 scientists for a 2-year period each. Also, thanks to a 'Juegaterapia-CNIO Friends' Postdoctoral Contract, in 2018, one scientist was able to continue with her project related to paediatric oncology.

FUNDING SOURCES OF POST-DOCTORAL RESEARCHERS	NO.
<b>SPANISH ORGANISATIONS</b>	<b>34</b>
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities / <i>Ministerio de Ciencia, Innovación y Universidades (MCIU)</i> (Postdoctoral Fellowships)	3
State Research Agency / <i>Agencia Estatal de Investigación (AEI)</i> . Ministry of Science, Innovation and Universities / <i>Ministerio de Ciencia, Innovación y Universidades (MCIU)</i> (I+D Projects)	9
Institute of Health Carlos III / <i>Instituto de Salud Carlos III (ISCIII)</i> (Postdoctoral Fellowships)	1
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (Fellowships)	3
Spanish Association Against Cancer (AECC) / <i>Fundación Científica de la AECC</i> (I+D Projects)	2
Spanish Society of Haematology and Haemotherapy	1
Worldwide Cancer Research UK	1
La Marató TV3 Foundation / <i>Fundació La Marató TV3</i>	1
Melanoma Research Alliance	1
CNIO	9
Centre for Molecular Biology "Severo Ochoa" / <i>Centro de Biología Molecular Severo Ochoa (CBMSO)</i>	1
Banco Santander Foundation / <i>Fundación Banco Santander</i>	2
<b>INTERNATIONAL ORGANISATIONS</b>	<b>16</b>
European Commission Framework Programme / H2020	1
Marie Skłodowska-Curie actions of the European Commission	3
Pfizer	1
Worldwide Cancer Research UK	5
AstraZeneca- MedImmune	1
Daiichi Sankyo	1
European Research Council	2
The Paradifference Foundation	1
US Department of Defense	1
<b>TOTAL</b>	<b>50</b>

## 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.



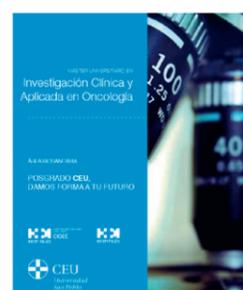
### Master's Degree in Biocomputing Applied to Personalised Medicine and Health

The Master's in *Bioinformática Aplicada a Medicina Personalizada y Salud* is organised together with the National School of Health of the National Institute of Health Carlos III (*Escuela Nacional de Sanidad del Instituto de Salud Carlos III, ENS-ISCiii*).



### Official Master's Degree in Clinical and Applied Cancer Research

The CNIO and the CEU-San Pablo University in Madrid (USP-CEU) co-organise a Postgraduate Training Programme in Clinical and Applied Cancer Research: the *Máster Universitario en Investigación Clínica y Aplicada en Oncología*.



### Official Master's Degree in Therapeutic Targets of Cell Signalling: Research and Development

The CNIO collaborates with the Biochemistry and Molecular Biology Department at the University of Alcalá de Henares (UAH) for the *Máster Oficial en Dianas Terapéuticas en Señalización Celular: Investigación y Desarrollo*.



## LABORATORY TRAINING FOR TECHNICIANS

This training programme has been developed for students in Anatomical Pathology, Clinical Diagnostic Laboratory, and Archiving/Recording; it is organised through agreements with 19 institutions that provide secondary education for laboratory technicians in Spain. It provides students with

hands-on knowledge in cellular and molecular biology techniques. The programme consists of 14 weeks (370-400 hours) of laboratory training for students. Of the 13 students who participated in this programme in 2018, 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 2018, 12 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 2018, 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. Eight other scientists were funded by similar programmes, including the *Juan de la Cierva* programme (Spanish Ministry of Science, Innovation and Universities, 2 contracts); *Miguel Servet* programme (1 contract) of the Institute of Health *Carlos III*; and the Spanish Association Against Cancer (*AECC*, 5 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 2018, Scott Lowe, from the Memorial Sloan Kettering Cancer Centre in New York (USA) was beneficiary 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 has selected Hayet Rafa, from the University of Science and Technology Houari Boumediene in Algiers (Algeria), to carry out a 6-month stay at the CNIO during 2019.



## SCIENTIFIC EVENTS

### CNIO-“LA CAIXA” BANKING FOUNDATION FRONTIERS MEETINGS

The “CNIO-“la Caixa” Banking Foundation Frontiers Meetings” are the main international conferences co-organised by the CNIO and the “la Caixa” Banking 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 two and a half days. The provided learning environment encourages delegates to: exchange

experiences, ideas and practices upheld at their companies; network to 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.

### MOLECULAR, CELLULAR AND ORGANISMAL HALLMARKS OF AGING 7-9 MAY 2018

#### ORGANISERS

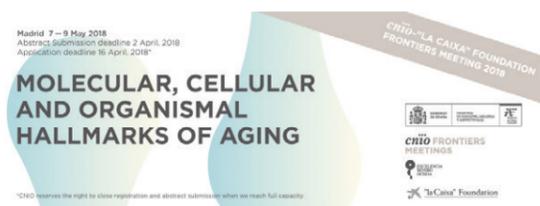
- **Maria A. Blasco**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Kathleen Collins**, University of California at Berkeley, US
- **Alejo Efeyan**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Thomas Rando**, Stanford University, US

#### SESSIONS

- STEM CELLS AND REGENERATION
- GENOME INSTABILITY
- NUTRIENTS
- SENESCENCE
- ENERGY AND MITOCHONDRIA

#### SPEAKERS

- **Johan Auwerx**, Ecole Polytechnique Fédérale de Lausanne, Switzerland
- **Salvador Aznar Benitah**, Institute for Research in Biomedicine, Barcelona, Spain
- **Shelley Berger**, The Perelman School of Medicine at the University of Pennsylvania, US
- **Maria A. Blasco**, Spanish National Cancer Research Centre, Madrid, Spain
- **Anne Brunet**, Stanford University, US
- **Judith Campisi**, Buck Institute for Research on Aging, Novato, US



- **Kathleen Collins**, University of California at Berkeley, US
- **Alejo Efeyan**, Spanish National Cancer Research Centre, Madrid, Spain
- **José Antonio Enríquez**, Spanish National Center for Cardiovascular Research, Madrid, Spain
- **Manel Esteller**, The Bellvitge Biomedical Research Institute, Barcelona, Spain
- **Óscar Fernández Capetillo**, Spanish National Cancer Research Centre, Madrid, Spain
- **Marcia Haigis**, Harvard Medical School, Boston, US
- **Jan Hoeijmakers**, Erasmus MC Rotterdam, Netherlands
- **Steve Horvath**, UCLA David Geffen School of Medicine, Los Angeles, US
- **Juan Carlos Izpisua Belmonte**, Salk Institute for Biomedical Studies, La Jolla, US
- **Matt Kaeberlein**, University of Washington, Seattle, US

- **Cynthia Kenyon**, University of California at San Francisco; Calico Laboratories, US
- **Thomas Rando**, Stanford University, US
- **Manuel Serrano**, Institute for Research in Biomedicine, Barcelona, Spain

- **David Sinclair**, Harvard Medical School, US
- **Jan van Deursen**, Mayo Clinic, Rochester, US

In addition, 8 short talks were selected among participants' contributions and 26 posters were presented.

### FRONTIERS IN IMMUNOMODULATION AND CANCER THERAPY 9-11 JULY 2018

#### ORGANISERS

- **Victoria Aranda**, Senior Editor, Nature, New York, US
- **Nabil Djouder**, Growth Factors, Nutrients and Cancer Group, CNIO, Madrid, Spain
- **Joao Monteiro**, Chief Editor at Nature Medicine, New York, US
- **Marisol Soengas**, Melanoma Group, CNIO, Madrid, Spain
- **Laurence Zitvogel**, Gustave Roussy Institute, Paris, France

#### SESSIONS

- TUMOR-STROMA-IMMUNE SYSTEM CROSSTALK
- IMMUNOMODULATION AND IMMUNOTOLERANCE 1
- IMMUNOMODULATION AND IMMUNOTOLERANCE 2
- MICROBIOTA, IMMUNE SYSTEM AND TUMOR PROGRESSION
- TARGETING IMMUNE SYSTEM: IMMUNOTHERAPY

#### SPEAKERS

- **Yasmine Belkaid**, The National Institute of Health (NIH), Bethesda, US
- **Nina Bhardwaj**, Icahn School of Medicine at Mount Sinai, New York, US
- **Marcus W. Bosenberg**, Yale Cancer Center, New Haven, US
- **Peter Carmeliet**, VIB-KU Leuven Center for Cancer Biology, Belgium
- **Thomas Gajewski**, The University of Chicago, US
- **Carola García de Vinuesa**, John Curtin School of Medical Research, The Australian National Univ., Australia
- **Nicholas W. Haining**, Dana-Farber Cancer Institute & Broad Institute, US
- **Guido Kroemer**, The Cordeliers Research Centre (CRC), Paris, France
- **Dan R. Littman**, The Helen L. and Martin S. Kimmel Center for Stem Cell Biology, NYU Langone, US
- **Randy Longman**, Weill Cornell Medicine, US
- **Alberto Mantovani**, Humanitas Clinical and Research Center, Milan, Italy
- **Glenn Merlino**, Center for Cancer Research, NCI, Bethesda, US
- **Graham Pawelec**, The University of Tübingen, Germany



- **Mercedes Rincon**, University of Vermont Medical Center, Burlington, US
- **Andrea Schietinger**, Memorial Sloan Kettering Cancer Center, NY, US
- **Ton Schumacher**, The Netherlands Cancer Institute (NIK), Amsterdam, Netherlands
- **Melody Swartz**, Institute for Molecular Engineering, University of Chicago, US
- **Erwin Wagner**, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- **Jennifer Wargo**, The University of Texas MD Anderson Cancer Center, Houston, US
- **Laurence Zitvogel**, Gustave Roussy Institute, Villejuif, France

In addition, 10 short talks were selected among participants' contributions and 35 posters were presented.

## OTHER MEETINGS &amp; CONFERENCES

The CNIO annually hosts various international meetings and conferences.

**RETOS DE LA MEDICINA PERSONALIZADA DE PRECISI3N EN ESPAÑA**  
 4 JUNE 2018

## ORGANISERS

- **Fátima Al-Shahrour**, Jefa de la Unidad de Bioinformática, Centro Nacional de Investigaciones Oncológicas (CNIO)
- **Enrique Carrillo de Santa Pau**, Jefe del Grupo de Biología Computacional, IMDEA Alimentación
- **Alfonso Valencia**, Director del Departamento de Ciencias de la Vida, Centro de Supercomputación de Barcelona


**CNIO: MINI SYMPOSIUM - MOLECULAR ONCOLOGY PROGRAMME**  
 18 OCTOBER 2018

## ORGANISER

- CNIO

**RED BIOBANCOS-CIBER-CNIO: JORNADA DE COMITÉS DE ÉTICA DE LOS BIOBANCOS; TRABAJO EN RED**  
 24 OCTOBER 2018

## ORGANISERS

- *Plataforma de la Red Nacional de Biobancos*
- *Centro Nacional de Investigaciones Oncológicas (CNIO)*
- *Consortio Centro de Investigación Biomédica en Red, M.P. (CIBER)*


**CNIO: MINI SYMPOSIUM - CANCER SYSTEMS BIOLOGY**  
 31 OCTOBER 2018

## ORGANISER

- CNIO

**CNIO-SOMMA: 100XCIENCIA.3 BRIDGING SCIENCE AND SOCIETY**  
 15 NOVEMBER 2018

## ORGANISER

The twelve selected 10-minute flash talks presented by both SOMMA and external partners showcased ongoing projects revolving around science education and public engagement. The 100xciencia.3 welcomed **Prof. Robert Huber, 1988 Chemistry Nobel Prize winner**, as the keynote speaker; he delivered an exciting talk on “The century of vision: Protein structures for drug research”.

The challenges and opportunities resulting from citizen science and science education were discussed over three different round tables:

- The media as a channelling agent for science
- Scientists and science policy united for society
- Scientific empowerment of society

**100XCIENCIA.3  
BRIDGING SCIENCE  
AND SOCIETY**

15 NOV 2018 MADRID



## 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 professionals 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.

**WORKSHOP: TRANSCRIPTOME ANALYSIS OF TUMOURS**  
 30 JANUARY 2018

## ORGANISERS

- **Núria Malats**, Genetic and Molecular Epidemiology Group, CNIO
- **Francisco X. Real**, Epithelial Carcinogenesis Group, CNIO
- **Invited Speaker: Aurélien De Reyniès**, CIT Program, Ligue Nationale Contre le Cancer, Paris, France.

The event mainly addressed researchers with an interest in the genomic analysis of tumours.

**STATISTICAL METHODS FOR MICROBIOME STUDIES**  
 9 MARCH 2018

## ORGANISERS

- **Núria Malats**, Genetic & Molecular Epidemiology Group, CNIO
- **CIBERONC ISCIII**
- **Invited Speaker: M. Luz Calle**, Biostatistics and Bioinformatics at the Systems Biology Department, University of Vic, Spain.



### COURSE OF ANIMAL LABORATORY FROM FUNCTION C TO D

12 - 16 MARCH 2018

#### ORGANISERS

- CNIO
- ANIMALARIA

### CNIO-JLABS: INNOVATION DAY WITH JOHNSON & JOHNSON INNOVATION

13 SEPTEMBER 2018

#### ORGANISERS

- CNIO
- JLABS

The session was geared towards scientists, entrepreneurs, researchers and business development professionals of healthcare start-ups. In addition, there was a half-day workshop where attendees learned pitching basics, business development skills and received information about funding opportunities in Spain.



### TALLER CEGEN-PRB2: ESTUDIOS DE ASOCIACIÓN: DISEÑO Y ANÁLISIS DE DATOS

24 SEPTEMBER 2018

#### ORGANISERS

- **Javier Benítez**, Spanish National Cancer Research Centre, CNIO
- **Angel Carracedo**, The University of Santiago de Compostela USC
- **Anna González-Neira**, Spanish National Cancer Research Centre, CNIO
- **Inés Quintela**, The University of Santiago de Compostela USC
- **Maria Torres**, The University of Santiago de Compostela USC



### FLOW CYTOMETRY COURSE

9 - 10 OCTOBER 2018

#### ORGANISERS

- **Lola Martínez**, Head of the Flow Cytometry Unit. CNIO. Madrid. Spain

#### SESSIONS

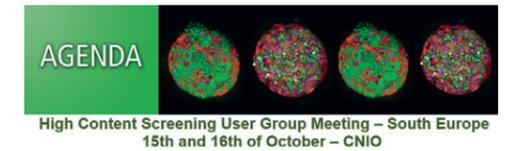
- Intro to Flow! Fundamentals & to collect accurate data in the cytometer
- Main Cytometry Applications – from cell cycle to single cell sorting

### CNIO'S HIGH CONTENT SCREENING USER GROUP MEETING – SOUTH EUROPE

15 - 16 OCTOBER 2018

#### ORGANISERS

- **Diego Megías**, Spanish National Cancer Research Centre, CNIO
- **PerkinElmer**



### HOW TO SUCCESSFULLY PERFORM & ANALYSE A MULTICOLOR FLOW EXPERIMENT WORKSHOP

24 - 26 OCTOBER 2018

#### ORGANISERS

- **Lola Martínez**, Head of the Flow Cytometry Unit. CNIO. Madrid. Spain
- **Andrea Valle**, Application Specialist FCS Express, DeNovo Software. Milano. Italy

#### SESSIONS

- Multicolour Flow: tricks and pitfalls
- Multicolour Flow panel design exercise
- Multicolour Flow hands-on practical exercise
- High-Dimensional analysis using FCS Express 6

### WORKSHOP DE VESÍCULAS EXTRACELULARES -EXOMAS: BIOLOGÍA Y APLICACIONES EN EL CAMPO DE LA BIOMEDICINA

7 - 9 NOVEMBER 2018

#### ORGANISERS

- BIOBANCOS-CIBERES-GEIVEX-CIBERONC

#### SESSIONS

- *Taller A1: "Recogida de Muestras Sólidas"*
- *Taller B1: "Análisis de Riesgos y Oportunidades"*
- *Taller B2: "Recogida de Muestras Prospectivas"*
- *Taller A2: "¿Qué es un Biobanco?"*
- *Taller B3: "Cesión de Muestras y/o Colaboración Científica"*
- *Taller B4: "Poblaciones de Referencia"*
- *MESA DE DEBATE "Interacciones con stakeholders externos: Plataformas ISCIII/Biotecs/Pacientes/Instituciones/Salud Pública"*
- *Conferencia de Clausura "QUALITY MATTERS: INTERNATIONAL STANDARDS FOR BIOBANKING"*



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 general 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.

The idea behind this international seminar series is not simply to host outstanding cancer researchers to the CNIO, but also

serves to, annually, invite 3 to 4 opinion leaders from other areas of science, technology, and literature; the overarching goal is to enable the CNIO to present its know-how as well as its vision on contemporary and future technological, societal and cultural challenges. These “out-of-the-box” seminars are sponsored by the *Fundación Banco Sabadell* and the French Embassy. The breadth of expertise and topics covered creates a multidisciplinary and intellectually challenging environment that goes far beyond the frontiers of cancer research.

In total, the CNIO hosted 18 distinguished speakers in 2018.



DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
19/01/2018	<b>Antoni Castells</b>	Hospital Clinic of Barcelona, Spain	Clinical management of polyposis and non-polyposis colorectal cancer syndromes
26/01/2018	<b>Andrés Aguilera</b>	Andalusian Center for Molecular Biology and Regenerative Medicine, CABIMER, CSIC, Sevilla, Spain	Interplay between RNA and chromatin in the maintenance of genome integrity
FEBRUARY			
02/02/2018	<b>Raúl Méndez</b>	Institute for Research in Biomedicine (IRB Barcelona), Spain	The CPEB-family of RNA-binding proteins, mechanisms of action and new functions in cell cycle and cancer
16/02/2018	<b>Jörg Hoheisel</b>	DKFZ German Cancer Research Center, Heidelberg, Germany	Pancreatic cancer: mechanistic insights, personalised diagnostics, and novel therapy options
23/02/2018	<b>John Rubinstein</b>	The Hospital for Sick Children Research Institute, Toronto, Canada	Electron cryomicroscopy of rotary ATPases
26/02/2018	<b>Shirley Kutner</b>	Hebrew University of Jerusalem, Israel	The women of the Start-up Nation
MARCH			
23/03/2018	<b>Kiyoshi Nagai</b>	MRC Laboratory of Molecular Biology, Cambridge, UK	CryoEM snapshots of the spliceosome provide insights into the molecular mechanism of pre-mRNA splicing
APRIL			
06/04/2018	<b>Stefan Kubicek</b>	CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria	Targeting chromatin for cancer cell synthetic lethality
13/04/2018	<b>Arlene Sharpe</b>	Harvard Medical School, Boston, US	Biology of PD-1 Checkpoint Blockade
20/04/2018	<b>Adrian R. Krainer</b>	Cold Spring Harbor Laboratory, Watson School of Biological Sciences, NY, US	Antisense Therapy for Spinal Muscular Atrophy: Harnessing the Power of a Backup Gene
MAY			
25/05/2018	<b>Edith Heard</b>	Professor at Collège de France / Institut Curie, Paris, France	Exploring epigenetic dynamics in development and disease using the paradigm of X inactivation
JUNE			
01/06/2018	<b>Kun-Liang Guan</b>	Sanford Consortium for Regenerative Medicine (SCRIM) The University California, San Diego, US	The Hippo pathway in cell growth, organ size, and cancer
15/06/2018	<b>Roger Lo</b>	Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, US	Strategies to overcome therapeutic resistance in melanoma

SEPTEMBER			
21/09/2018	<b>Karim Labib</b>	Sir James Black Centre, School of Life Sciences, University of Dundee, Scotland	Destroying the eukaryotic replisome
OCTOBER			
05/10/2018	<b>Rafael Yuste</b>	The NeuroTechnology Center at Columbia University Biological Sciences, US	Reading the neural code: emergent properties of neural circuits
			
NOVEMBER			
23/11/2018	<b>Nicolas Winssinger</b>	University of Geneva, Switzerland	DNA-templated assemblies and reaction in chemical biology
30/11/2018	<b>Caetano Reis e Sousa</b>	The Francis Crick Institute, London, UK	Dendritic cells in immunity to infection and cancer
DECEMBER			
21/12/2018	<b>Jonathan Kipnis</b>	Center for Brain Immunology and Glia (BIG), University of Virginia, Charlottesville, US	Meningeal lymphatics in brain function (and dysfunction)
			

### 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 to socialising with colleagues from other institutions. A total of 45 *ad-hoc* seminars were organised by CNIO researchers in 2018.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
29/01/2018	<b>Salvador Aznar Benitah</b>	Institute for Research in Biomedicine (IRB Barcelona), Spain	Adult stem cells in health and disease: interplay between time, diet and epigenetics
31/01/2018	<b>Antoni Montserrat Moliner</b>	Active Senior Adviser on Public Health European Commission, Luxembourg	30 years of public health action from the European Commission in the field of cancer: From the European Cancer Code 1986 to the iPAAC Joint Action in 2018
FEBRUARY			
07/02/2018	<b>Cédric Blanpain</b>	Université Libre de Bruxelles (ULB), Belgium	Cancer cell of origin and tumor heterogeneity
20/02/2018	<b>Marco Cordani</b>	Faculty of Medicine, Autonomous University of Madrid (UAM), Spain	Mutant p53 proteins alter signaling pathways involved in autophagy and redox regulation of cancer cells
23/02/2018	<b>Kyohei Oyama</b>	Asahikawa Medical University, Japan	Challenge to stable cell cycle exit in cardiac myocytes
27/02/2018	<b>Pablo Baquero</b>	Wolfson Wohl Cancer Research Centre, University of Glasgow, UK	Targeting Autophagy and Mitochondrial Metabolism to Eliminate Leukaemic Stem Cells

MARCH			
07/03/2018	<b>Pasi Janne</b>	Dana Farber Cancer Institute and Harvard University, Boston, US	Strategies to prevent and overcome resistance to EGFR inhibitors in lung cancer
APRIL			
05/04/2018	<b>Doryen Bubeck</b>	Imperial College, London, UK	Cryo-EM of the membrane attack complex reveals a flexible nanopore
05/04/2018	<b>Giuseppe Bosso</b>	SAPIENZA University of Rome, Italy	Insights into the epigenetic maintenance of Drosophila telomeres as revealed by the Hpl $\alpha$ - Effete/UbcD1 relationship
12/04/2018	<b>Mario Rossi</b>	National Scientific and Technological Research Council (CONICET); Max Planck Partner Institute of Biomedicine of Buenos Aires (IBioBA-MSPS), Argentina	Role of protein Ubiquitylation in tumor-cell migration and invasion
16/04/2018	<b>Catharina von-Nicolai</b>	Division of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden	A second DNA binding site in BRCA2 promotes homologous recombination
25/04/2018	<b>Sander van den Heuvel</b>	Institute of Biodynamics and Biocomplexity (IBB); Faculty of Science, Utrecht University, The Netherlands	Cell division, differentiation and tumor formation; insights from <i>C. elegans</i>
25/04/2018	<b>Manuel Saldivia Concepcion</b>	Centre for Immunology & Infection, The University of York, UK	Lighting up parasite development: How I met your kinase
MAY			
03/05/2018	<b>Guillaume Belthier</b>	Centre National de la Recherche Scientifique (CNRS), Montpellier, France	Innovative strategies to isolate CTCs with stemness properties
10/05/2018	<b>Rubén Fernández Busnadiego</b>	Max-Planck-Institute of Biochemistry. Dept of Molecular Structural Biology, Martinsried, Germany	Cryo-electron tomography: The cell biology that came in from the cold
10/05/2018	<b>Eri Sakata</b>	Max-Planck-Institute of Biochemistry. Dept of Molecular Structural Biology, Martinsried, Germany	Conformational landscape of the 26S proteasome gives insights into the gate-opening
16/05/2018	<b>Heinz Neumann</b>	Max Planck Institute of Molecular Physiology Dortmund, Germany	Studying chromatin with neo-functionalized proteins
22/05/2018	<b>Miguel Jimenez Alcázar</b>	University Medical Center Hamburg-Eppendorf, Germany	The Role of Neutrophil Extracellular Traps in Thrombosis
24/05/2018	<b>Florian Karreth</b>	H. Lee Moffitt Cancer Center, Florida. USA	Maximizing melanoma modeling in the mouse
28/05/2018	<b>Ilse Rومان</b>	Vrije Ujnviversiteit Brussel, Belgium	Pancreatic cancer - where did it go wrong?
JUNE			
04/06/2018	<b>Daniel Herranz</b>	Rutgers Cancer Institute of New Jersey, USA	Dissecting NOTCH1-controlled transcriptional and metabolic oncogenic programs in T-ALL
05/06/2018	<b>Melania Sauri</b>	Karolinska Institute, Stockholm, Sweden	Finding cancer Achilles's heels in metabolic pathways
11/06/2018	<b>Luis Álvarez-Vallina</b>	Aarhus University, Denmark	Engineering the Immune System for Enhanced Cancer Immunotherapy

14/06/2018	<b>Paweł Kordowitzki</b>	Institute of Animal Reproduction and Food Research PAS (IARFR) of the Polish Academy of Sciences, Olsztyn, Poland	<i>In vivo veritas?</i> A large animal model for human reproductive aging
19/06/2018	<b>Maria Ibarra Dauden</b>	EMBL Heidelberg - The European Molecular Biology Laboratory, Heidelberg, Germany	Structure basis of the tRNA binding on the Elongator sub-complex
JULY			
04/07/2018	<b>René Medema</b>	The Netherlands Cancer Institute, Amsterdam, Netherlands	Cell fate decisions after DNA damage: location, location, location?
13/07/2018	<b>Daniel Rico</b>	Institute of Cellular Medicine, Newcastle University, UK	Short and long distance relationships (in chromosomes)
16/07/2018	<b>Bruno Conti</b>	Dorris Neuroscience Center, The Scripps Research Institute La Jolla, California	Mechanisms of Aging and Age-Associated Diseases: Neuroimmunology of Parkinson's Disease
17/07/2018	<b>Martin Leeb</b>	University of Vienna, MFPL, Vienna, Austria	Dissecting the genetic complexity of embryonic stem cell differentiation
AUGUST			
23/08/2018	<b>Joana Nunes</b>	Meyer Cancer Center, Weill Cornell Medicine, New York, USA	The Metabolic Needs of Epithelial to Mesenchymal Transition
SEPTEMBER			
07/09/2018	<b>Israel Sanchez Fernandez</b>	Biochemistry and Molecular Biophysics, Columbia University, New York, USA	What do crickets and ribosomes have to do with cancer?
18/09/2018	<b>Sofia Merajver</b>	Breast and Ovarian Cancer Risk Evaluation Program, University of Michigan Comprehensive Cancer Center, US	Personalized Oncology for primary tumors and brain metastasis: from the clinic to the lab and back in 7 days
25/09/2018	<b>Fernando Martín-Sánchez</b>	Institute of Health Carlos III, Madrid, Spain	CHARACTERIZING THE HUMAN EXPOSOME: a key step for Precision Medicine
27/09/2018	<b>Marcus Bosenberg</b>	Yale Cancer Center, Yale University, New Haven, US	Defining Genetic Drivers of Melanoma and Mechanisms of Anti-Cancer Immune Responses
OCTOBER			
02/10/2018	<b>Fernando Martín Belmonte</b>	Department of Developmental Biology and Differentiation at the Centro de Biología Molecular Severo Ochoa (CBMSO), Madrid, Spain	Epithelial tube organization and patterning in development and disease
09/10/2018	<b>Carlos Fernandez Tornero</b>	The Biological Research Center ( <i>Centro de Investigaciones Biológicas - CIB</i> ), CSIC, Madrid	The role of RNA polymerase I in the protection against UV light-induced DNA damage
17/10/2018	<b>Sonia Lain</b>	Professor in Molecular Cancer Pharmacology at Karolinska Institutet, Stockholm, Sweden	From phenotypic screens to the structure of a small molecule bound to its target, and the challenges beyond

NOVEMBER			
06/11/2018	<b>Oriol Gallego</b>	Experimental and Health Sciences (DCEXS) Pompeu Fabra University (UPF), Barcelona, Spain	Cell engineering to implement live-cell structural biology: towards novel mechanisms that control exocytosis
20/11/2018	<b>Steve C. Ley</b>	Imperial College London, UK	Regulation of inflammation by the TPL-2 complex
27/11/2018	<b>Timm Maier</b>	University of Basel, Switzerland	The structural basis for regulation of acetyl-CoA carboxylase 1
29/11/2018	<b>Victor Borrell</b>	The Institute of Neurosciences, Alicante, Spain	Genetic evolution of cerebral cortex size determinants
29/11/2018	<b>Aoife McGinley</b>	School of Biochemistry and Immunology at Trinity College Dublin, Ireland	IL-17A is pathogenic in CNS autoimmunity by promoting IL-1 $\beta$ production that drives encephalitogenic T cells
DECEMBER			
03/12/2018	<b>Aline Bozec</b>	Universitätsklinikum Erlangen, Germany	HIF1 $\alpha$ expression in Immune Cells regulates Autoimmune and Infection Diseases'
12/12/2018	<b>Yacine Kharraz</b>	Cytek Biosciences Inc. Fremont, CA. US	Full-spectrum flow cytometry: How new technologies may drive changes in multicolor flow cytometry
20/12/2018	<b>Antonio Maraver</b>	Oncogenic Pathways in Lung Cancer; Institut de Recherche en Cancérologie de Montpellier (IRCM), France	Role of the Notch pathway in lung adenocarcinoma: beyond the KrasG12V mouse model

## WOMEN IN SCIENCE SEMINARS

30/01/2018	<b>Laura Ferrero Carballo</b>	Writer, freelance editor and literary advisor Instituto Vasco Etxepare. Barcelona	<i>Decisiones racionales e irracionales: una breve historia de cómo llegué a ser escritora</i>
20/02/2018	<b>Elisa Martín Garijo</b>	Chief Technology Officer for IBM, Madrid, Spain	Yes, we can
06/03/2018	<b>Fátima Bosch</b>	Autonomous University of Barcelona, Spain	Translational Gene Therapy Approaches to Treat Metabolic and Neurodegenerative Diseases
24/04/2018	<b>Victoria Camps</b>	Philosopher, Professor of Ethics <i>Universidad Autónoma de Barcelona</i> , Spain	<i>La filosofía como instrumento de emancipación</i>
05/06/2018	<b>Laura González Molero</b>	<i>Consejero Independiente de Acerinox y Ezentis</i>	<i>Atrévete a ser el rector de tu propia vida</i>
25/09/2018	<b>Elvira Sastre</b>	<i>Escritora y traductora literaria</i> ; Writer and literary translator	<i>Escritoras, más allá del género y la biografía</i>
23/10/2018	<b>Eulalia Pérez Sedeño</b>	Department Science, Technology and Society, IFS-CCHS CSIC, Madrid, Spain	<i>Conocimiento y estereotipos de género</i>
11/12/2018	<b>Marta Macho</b>	UPV (University of the Basque Country), Spain	<i>Entre el suelo pegajoso y el techo de cristal: la realidad de las mujeres en el ámbito laboral</i>

## SCIENCE OUTREACH EVENTS

CNIO ARTE  
7 FEBRUARY 2018

## ORGANISERS

- CNIO
- Banco Santander Foundation

The first edition of CNIO Arte, “Binomio: a dialogue between art and science”, curated by Mireia A. Puigventós, was born from the dialogue between two brilliant women from totally different fields of action: the scientist Margarita Salas and the visual artist Eva Lootz. For the occasion, Eva Lootz made an audio-visual piece and a series of 59 drawings representing insights and reflections on Margarita Salas’ main lines of research. The artworks were exhibited at the CNIO from February to April.

OPEN DOORS DAY: 'CNIO FRIENDS'  
20 JUNE 2018

The purpose of this event is to welcome all our donors in order to give them the opportunity to visit our facilities, as well as for us to showcase some of our Research Programmes. Maria

A. Blasco, Marcos Malumbres and Nabil Djouder gave a talk in the Auditorium.

WORLD CANCER RESEARCH DAY: 'PRESENT AND FUTURE OF CANCER RESEARCH'  
EXCLUSIVE MEETING WITH NOBEL LAUREATE PROF. ELIZABETH BLACKBURN  
24 SEPTEMBER 2018

## ORGANISERS

- Constantes y Vitales
- Spanish National Cancer Research Centre (CNIO)
- Fundación AXA

Our guest of honour was Nobel Prize winner Prof. Elizabeth Blackburn, who gave an inspiring talk entitled: “Resolving Paradoxes in Telomere Biology and Cancers”. After her talk, there was a panel discussion in which the following speakers took part:

## SPEAKERS

- Prof. Elizabeth Blackburn, Nobel Prize of Physiology or Medicine 2009
- Maria A. Blasco, Director of the Spanish National Cancer Research Centre (CNIO)



- María José Alonso Fernández, Professor of Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela (USC)
- Laura García Estévez, MD Anderson Cancer Center, Head of the Breast Tumors Section
- Luz Casal, musician, singer, author songwriter and former cancer patient.

RESEARCHERS' NIGHT  
28 SEPTEMBER 2018

This year, the CNIO participated in Researchers' Night; an activity aimed at bringing researchers closer to the general public and concerned families in order to give them the opportunity to learn more about what researchers do for society. Each year, more than 300 European cities participate in parallel in what is ultimately a great night for science. During the activities – promoted by the European Commission and coordinated by the Madrid Regional Government and the madri+d Foundation – a total of 220 people came to the Spanish National Cancer Research Centre (CNIO) to attend Researchers' Night (September 28, 2018) to learn about cancer research. The activities were entirely organised and held thanks to the voluntary efforts of 64 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.

OPEN DOORS DAY: INVESTIGATING TO DISARM CANCER  
5-18 NOVEMBER 2018

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 (XVII Semana de la Ciencia, 5-18 November 2018). In 2018, 57 people participated in the guided visit to the Centre's facilities.



## STEM TALENT GIRL - MADRID

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, organised by ASTI Foundation and CNIO, offers 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.



## 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 2018, more than 830

people participated in such guided visits; most of them were ESO and Bachillerato student groups, but also professionals in the health sector.

# ADMINISTRATION

## BOARD OF TRUSTEES

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- **Pedro Francisco Duque Duque**  
Minister of Science, Innovation and Universities  
*Ministro de Ciencia, Innovación y Universidades*

### → President

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*Secretario General de Coordinación de Política Científica del Ministerio de Ciencia, Innovación y Universidades*

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*Directora del Instituto de Salud Carlos III*

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*Subdirectora General de Redes y Centros de Investigación Cooperativa del Instituto de Salud Carlos III*

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*Jefe de Gabinete de la Consejera de Sanidad del Gobierno de Cantabria*

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*Directora de la Agencia Gallega del Conocimiento en Salud - ACIS*

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- **Sandra García Armesto**  
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*Directora Gerente del Instituto Aragonés de Ciencias de la Salud*

### → Elected Members

- **"la Caixa" Banking Foundation Caixa d'Estalvis i Pensions de Barcelona**  
Representatives: Angel Font Vidal, Director / Jaume Giró Ribas, General Director

- **BBVA Foundation**  
Representatives: Rafael Pardo Avellaneda, General Director / Francisco González Rodríguez, Chairman

- **Grupo PRISA**  
Representative: Ignacio Polanco Moreno, Chairman

### → Secretary

- **Margarita Blázquez Herranz**  
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III  
*Subdirectora General de Redes y Centros de Investigación Cooperativa, Instituto de Salud Carlos III*

### → Legal Advisor

- **Fernando Arenas Escribano**  
Chief State's Attorney of the Spanish Ministry of Health, Consumer Affairs and Social Welfare  
*Abogado del Estado-Jefe en el Ministerio de Sanidad, Consumo y Bienestar Social*

\* In accordance with the Spanish Transparency Legislation (Spanish Royal Decree 451/2012, of March 5), the following information is hereby provided:

- At the close of the financial year, the accumulated remuneration received by the Top Management of the Foundation — the CNIO's Director plus the Managing Director - has amounted to a total of 272,471 euros (251,488 euros in 2017).
- Members of the CNIO Board of Trustees are not remunerated.

## SCIENTIFIC ADVISORY BOARD

· **Mariann Bienz, PhD, FRS, FMedSci (Chair)**  
Joint Divisional Head  
Division of Protein and Nucleic Acid Chemistry  
Medical Research Council Laboratory of Molecular  
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Helsinki, Finland

· **Genevieve Almouzni, PhD**  
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Head of Nuclear Dynamics & Genome Plasticity Unit  
*Institut Curie*, Paris, France

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San Francisco, USA

· **José Costa, MD, FACP**  
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New Haven, USA

· **Sara Courtneidge, PhD, DSc (hc)**  
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· **Denise Galloway, PhD**  
Associate Division Director, Human Biology Division at  
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Research Professor of Microbiology at the University of  
Washington  
Seattle, USA

· **Scott W. Lowe, PhD**  
Chair, Cancer Biology and Genetics Program, SKI  
Chair, Geoffrey Beene Cancer Research Center  
Memorial Sloan-Kettering Cancer Center  
New York, USA

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Neuroscience Institute of Alicante (CSIC-UMH)  
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University Hospital  
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Department of Structural Biology  
Max Planck Institute for Molecular Physiology (MPI)  
Dortmund, Germany

· **Ada E. Yonath, PhD**  
Director, the Helen and Milton A. Kimmelman Center for  
Biomolecular Structure and Assembly  
Martin S. and Helen Kimmel Professor of Structural  
Biology  
Weizmann Institute of Science  
Rehovot, Israel

## MANAGEMENT

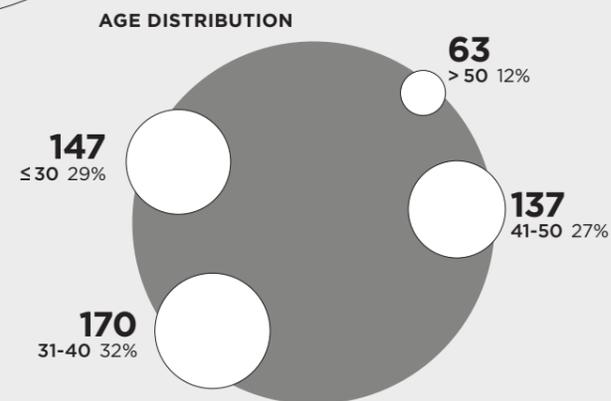
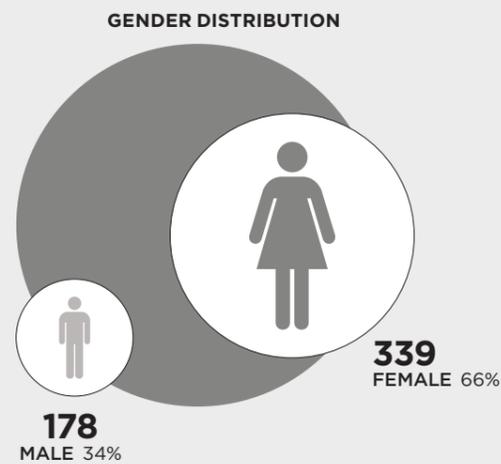
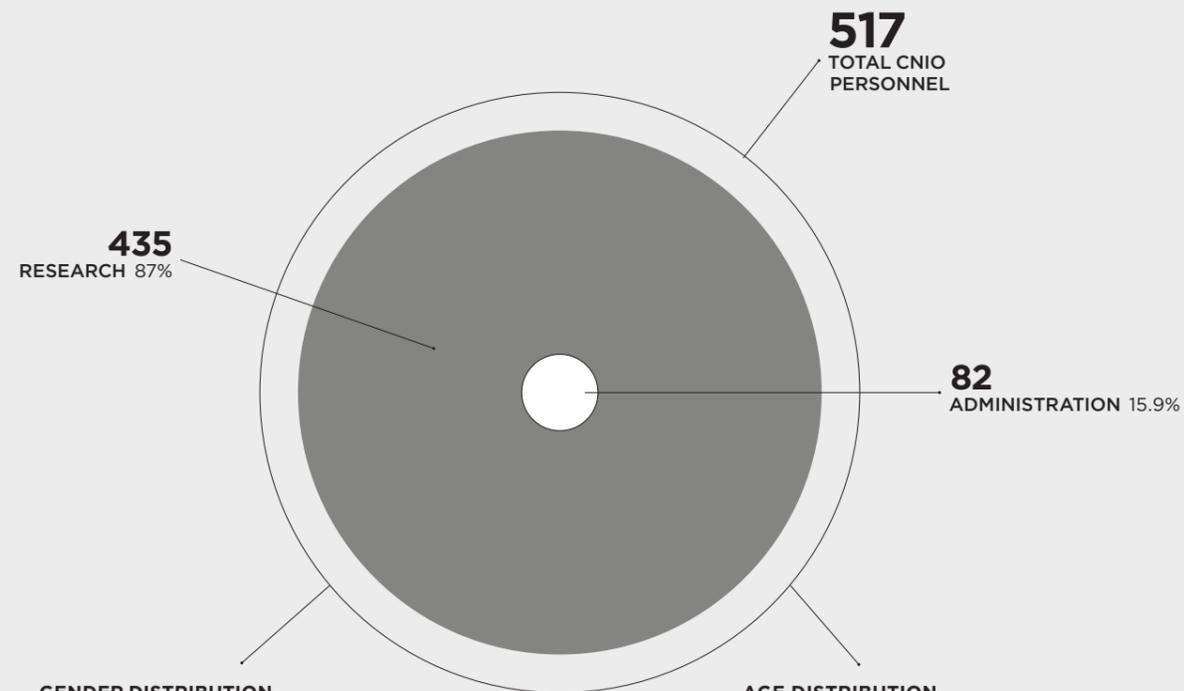
DIRECTOR	<b>Blasco, Maria A.</b>		
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VICE-DIRECTOR	<b>Fernández-Capetillo, Óscar</b>		
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EXTRAMURAL CLINICAL RESEARCH	<b>López, Antonio</b> Director		

CNIO PERSONNEL 2018

SCIENTIFIC PERSONNEL 2018

TOTAL SCIENTIFIC PERSONNEL **435**



**GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS**

Position	Female %	Female Count	Male %	Male Count
GROUP LEADERS, HEADS OF CLINICAL RESEARCH UNIT/SECTION	30%	8	70%	19
HEADS OF UNIT/BIOBANK	59%	10	41%	7
SCIENTIFIC DIRECTION: DIRECTORS, HEADS OF AREA	53%	8	47%	7
MANAGEMENT: DIRECTORS, HEADS OF AREA	29%	4	71%	10

**DISTRIBUTION BY PROGRAMMES**

STRUCTURAL BIOLOGY 11%	<b>47</b>	
BIOTECHNOLOGY 20%	<b>88</b>	
CANCER CELL BIOLOGY 8%	<b>35</b>	
HUMAN CANCER GENETICS 10%	<b>45</b>	
CLINICAL RESEARCH 17%	<b>74</b>	
MOLECULAR ONCOLOGY 27%	<b>117</b>	
EXPERIMENTAL THERAPEUTICS 7%	<b>29</b>	

**DISTRIBUTION BY PROFESSIONAL CATEGORY**

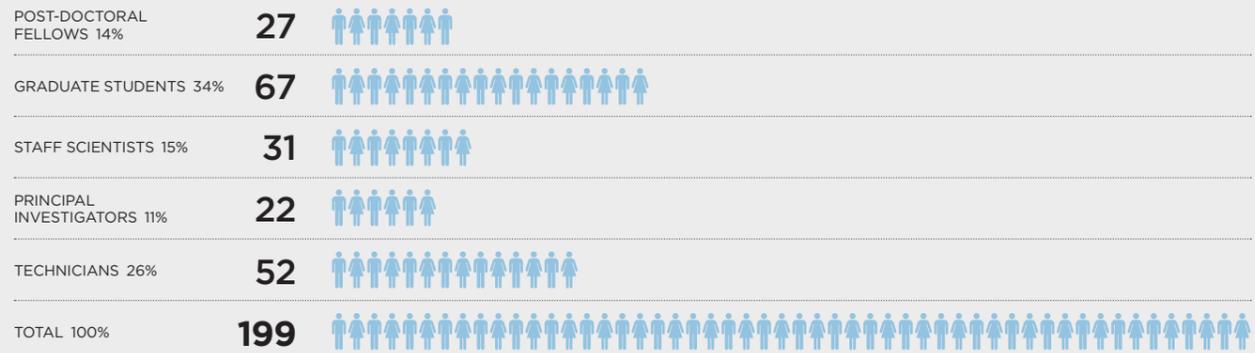
POST-DOCTORAL FELLOWS 9%	<b>37</b>	
GRADUATE STUDENTS 24%	<b>103</b>	
STAFF SCIENTISTS 16%	<b>69</b>	
PRINCIPAL INVESTIGATORS 11%	<b>46</b>	
TECHNICIANS 40%	<b>180</b>	

**GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY**

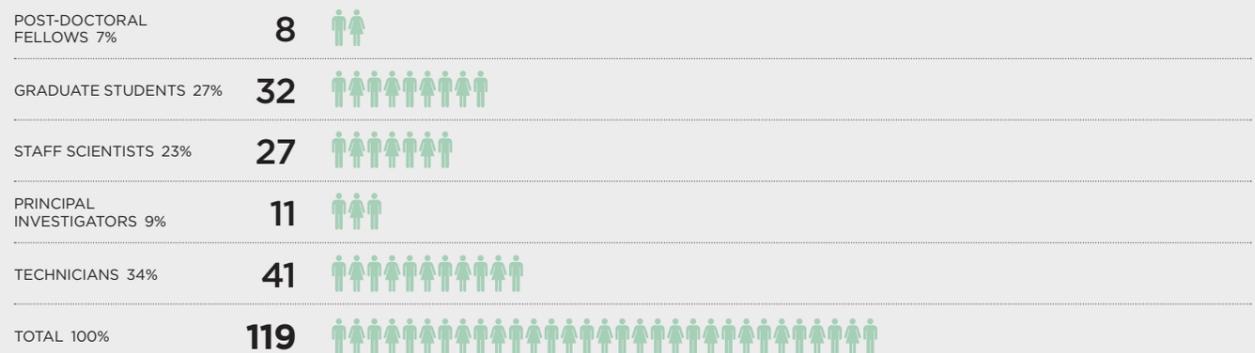
POST-DOCTORAL FELLOWS	FEMALE 57%	<b>21</b>		MALE 43%	<b>16</b>	
GRADUATE STUDENTS	FEMALE 68%	<b>70</b>		MALE 32%	<b>33</b>	
STAFF SCIENTISTS	FEMALE 75%	<b>52</b>		MALE 25%	<b>17</b>	
PRINCIPAL INVESTIGATORS	FEMALE 39%	<b>18</b>		MALE 61%	<b>28</b>	
TECHNICIANS	FEMALE 77%	<b>139</b>		MALE 23%	<b>41</b>	
<b>TOTAL SCIENTIFIC PERSONNEL</b>	FEMALE	<b>300</b>		MALE	<b>135</b>	

**DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH**

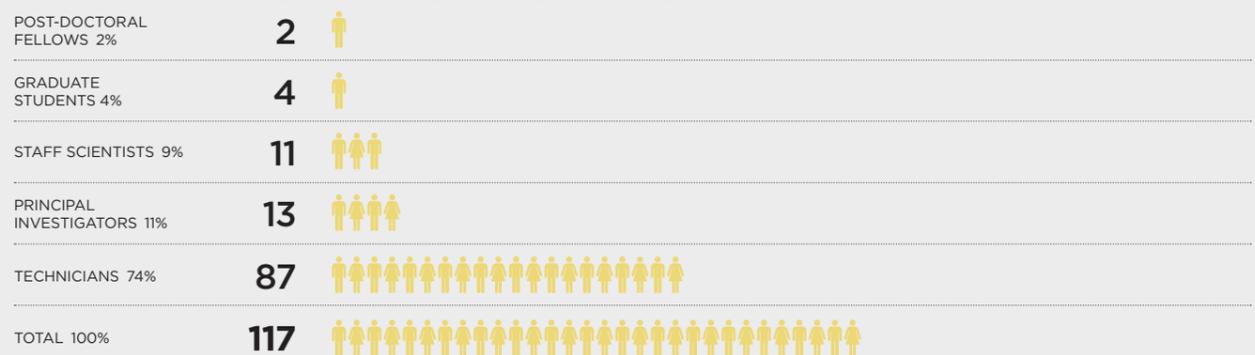
TOTAL SCIENTIFIC PERSONNEL **435**



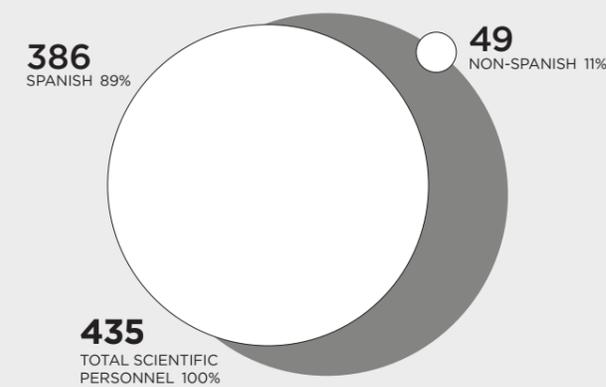
**DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH**



**DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION**



**SCIENTIFIC PERSONNEL: NATIONAL ORIGIN**

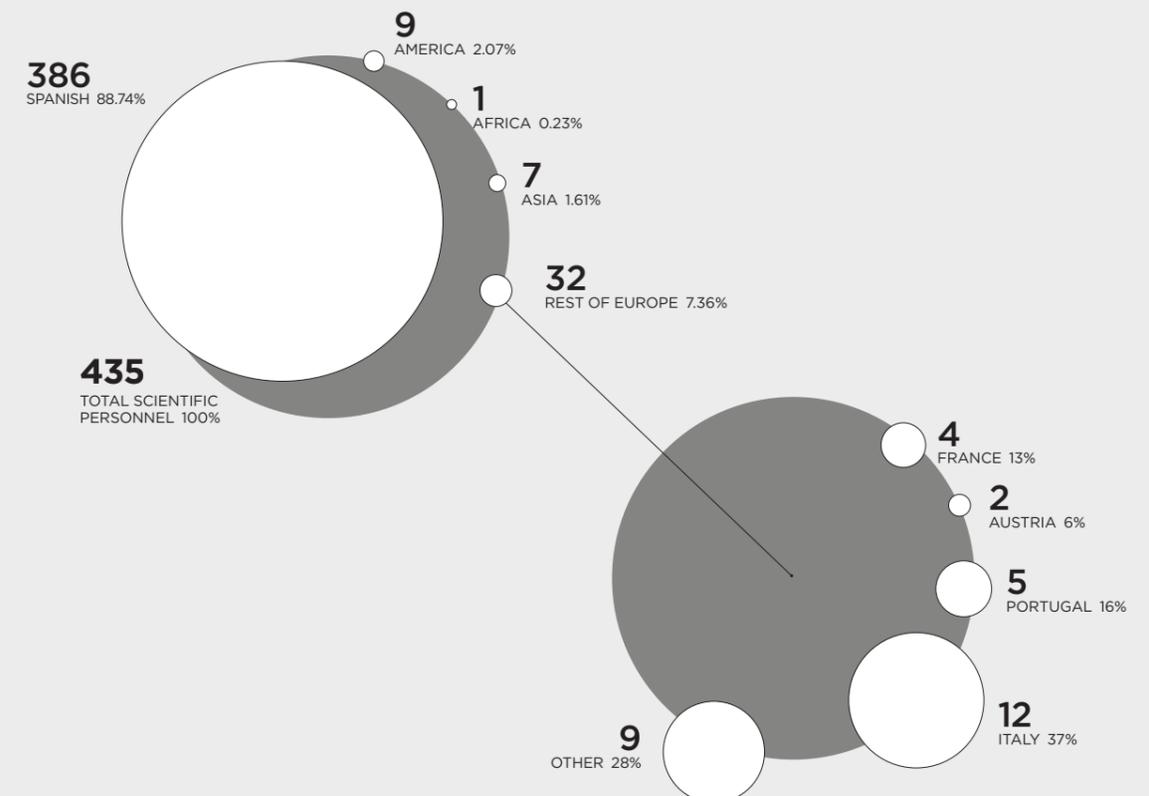


**FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY**



**Total foreign scientific personnel 49**  
Percent values represent percentages of foreign employees of the total CNIO personnel in each category

**DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN**



## PRIVATE SPONSORS

“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 2018. 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. Since 2017, the CNIO participates in the new doctoral fellowship programme of the "la Caixa" Foundation, INPhINIT.



*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 H120-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 2018. Additionally, thanks to the support of the *Fundación Banco Santander*, a group of 2 young researchers received training on managerial and entrepreneurial skills, in collaboration with the IE Business School.



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* supports 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 Researcher Programme that was established to support prestigious international professors for short stays at the CNIO. The recipient of the *Jesús Serra* Foundation's Visiting Researcher Award in 2018 was Scott W. Lowe, Chair of the Cancer Biology and Genetics Program and the Geoffrey Beene Cancer Research Center at Memorial Sloan Kettering Cancer Center (MSKCC) in New York (USA).



The *AXA Research Fund* (ARF) – a global initiative of scientific philanthropy run by the insurance group AXA – awarded an AXA-CNIO Endowed Permanent Chair position in Molecular Oncology to Mariano Barbacid as part of its 2011 call.

## OTHER SPONSORS



Our activities are also supported by individual donations – citizens who wish to contribute personally to the battle against cancer – donations from companies and foundations, as well as via external fundraising from local associations that are equally dedicated to the battle against cancer. During 2018, our research activities and seminars were supported by:

*Juegaterapia, Fundación Inocente Inocente, Fundación Española de Hematología y Hematoterapia, Fundación Investigación Biomédica Hospital Universitario 12 de Octubre, Fundación PFIZER, Asociación Bandera Rosa, Asociación de Mujeres Afectadas de Cáncer de Mama ROSAE, Fressia Group, Colectivo de afectados "El árbol de la Vida", Santa Lucía Seguros, Petroplast, and the Fundación Banco Sabadell*, among others.



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, have ensured the continuation of our research endeavours throughout 2018.

# CNIO Friends

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## CNIO FRIENDS

‘CNIO Friends’ has just celebrated its four-year anniversary with a clean bill of health, closing 2018 with close to 1,200 donors and about 880,000€ collected since the beginning of the initiative. During these first few years, this philanthropic association has been working hard to put CNIO on society’s visible spectrum and has become a meeting place for the charitable initiatives of a society that is increasingly aware of the importance of biomedical research.

Since the launch of the ‘CNIO Friends’ Postdoctoral Contracts Programme, six scientists — in addition to one pre-doctoral researcher — have been recruited to investigate new avenues for advancement in the treatment and diagnosis of the disease. In addition, in 2018, calls for applications were launched for further contracts that will come into effect in 2019; these will open up new avenues of innovative research against cancer. Also in 2018, we continued to receive donations in the form of legacies and bequests.

One of the most rewarding experiences organised in the framework of ‘CNIO Friends’ was the launch of CNIO Arte; an initiative launched by the Centre with the support of the *Banco Santander* Foundation, which connects leading international scientists and artists and explores the common territories shared by scientific research and artistic creation. In 2018, our inaugural encounter was called ‘Binomio, a Dialogue between Art and Science’. This dialogue between researcher and artist has resulted in the creation of unique artwork from which the proceeds will help to fund cancer research.

The project, inspired by the book *Excelentes* — which compiles portraits by Amparo Garrido and texts by Mónica G. Salomone about eminent figures who have visited CNIO in recent years — featured two exceptional individuals in this first edition: Margarita Salas, a global pioneer in molecular biology, and Eva Lootz, winner of the National Visual Arts Prize. Based on their conversations and meetings, Lootz created an audiovisual piece and a series of 59 drawings, conceived of as thoughts or ‘illuminations’ that reflect on Salas’ main lines of research. The exhibition was on display at the CNIO between February and May 2018, and was presented at the prestigious ARCO art fair in Madrid.

In April, we were visited by the *Juegaterapia* Foundation and its Honorary Ambassador, the singer David Bisbal, to celebrate a new donation of 100,000 euros received from the Foundation. Thanks to this collaboration with CNIO, a new *Juegaterapia*-

**“‘CNIO Friends’ places us on society’s visible spectrum.”**

CNIO Friends postdoctoral contract was funded, leading to the incorporation of a young researcher, Miguel Jiménez Alcázar, to the Seve-Ballesteros Foundation Brain Tumours Group in order to develop a project focused on searching for new therapeutic strategies for gliomas, the most common type of brain tumour in children and adolescents.

In addition, throughout the year, we received support from all around Spain: in February, the *ROSAE* association, composed of breast cancer patients or ex-patients, collaborated with ‘CNIO Friends’ and invited us to their sisterhood lunch held in Valdepeñas (Ciudad Real). In June, the *Bandera Rosa* association donated the money they had raised in El Campo de Gibraltar (Cádiz), where they support breast cancer patients and their families. Later in the year, in October, the collective *El Árbol de la Vida*, from Las Pedroñeras (Cuenca), held its III Charity Race Against Cancer, which was attended by almost 3,000 people, donating some of the money raised to ‘CNIO Friends’. The year came to a close with a *San Silvestre* charity race in Soto del Real (Madrid) in support of our initiative, in which hundreds of runners donned their trainers for this good cause and ran to the beats dropped by leading international DJs.

In June, we celebrated one of the most eagerly anticipated events of the year: the ‘CNIO Friends’ Open Doors Day event. On this day, our *Friends* came to CNIO to meet our researchers and explore our labs. With them were the director of CNIO, Maria A. Blasco, and other researchers such as Carolina Maestre and Sebastián Thompson, the latest two recipients of the ‘CNIO Friends’ postdoctoral contracts to investigate cellular proliferation in tumours and the use of nanoparticles against cancer.

We would like to take this opportunity to thank our community of donors as well as all supporters in general for their encouragement and support. Now that we have more than 1,000 *Friends*, we feel responsible for the trust they have placed in us and we are responding in the best way we know: through cancer research.

## CNIO ARTE



Margarita Salas, worldwide pioneer in molecular biology, and Eva Lootz, National Prize Winner for Plastic Arts, both starred together in the first edition of CNIO Arte with the project entitled 'Binomio, a Dialogue between Art and Science'. This CNIO initiative, with the support of the *Banco Santander* Foundation, aims to bring together leading international scientists and artists in order for them to jointly explore the common territories of scientific research and artistic creation.

On February 23, *Binomio* was unveiled at the prestigious ARCO Art Fair with a roundtable on the synergies between art and science. Participants included Eva Lootz, CNIO Director Maria A. Blasco, visual artist Amparo Garrido, Susana Gómez from the *Fundación Banco Santander*, Art Professor Estrella de Diego, and project curator Mireia A. Puigventós.

## 'JUEGATERAPIA-CNIO FRIENDS' POSTDOCTORAL CONTRACT



The *Juegaterapia* Foundation donated 100,000 euros to 'CNIO Friends' thanks to the successful sales of the *Baby Pelones* dolls. *Juegaterapia* visited the Centre with the singer David Bisbal who designed one of these dolls. Those appearing in the picture are from left to right: Mónica Esteban (Chair of *Juegaterapia*), Maria A. Blasco (CNIO Director), David Bisbal (Honorary Ambassador), Valle Sallés (Vice-chair) and Pablo Ibáñez (Honorary Chair).

This donation will support a new 'Juegaterapia-CNIO Friends' postdoctoral contract, through which Miguel Jiménez Alcázar, from the University Medical Center in Hamburg, Germany, will spend two years at CNIO's Seve-Ballesteros Foundation Brain Tumour Group. In his project, he will focus his research on gliomas, the most common type of brain tumours in children and adolescents.

## A MEETING STARRED BY OUR 'FRIENDS'



On June 20th, we celebrated the CNIO Friends Day, an event designed to welcome some of the hundreds of CNIO's most loyal supporters and to bring our science closer to them. They could learn about the research projects that were made possible thanks to our 'CNIO Friends' and they also had the opportunity to visit the Centre's labs and facilities. This year, in addition, we also celebrated the fact that 'CNIO Friends' surpassed the mark of 1,000 supporters.

## BENEFACTOR FRIENDS/SPONSOR FRIENDS

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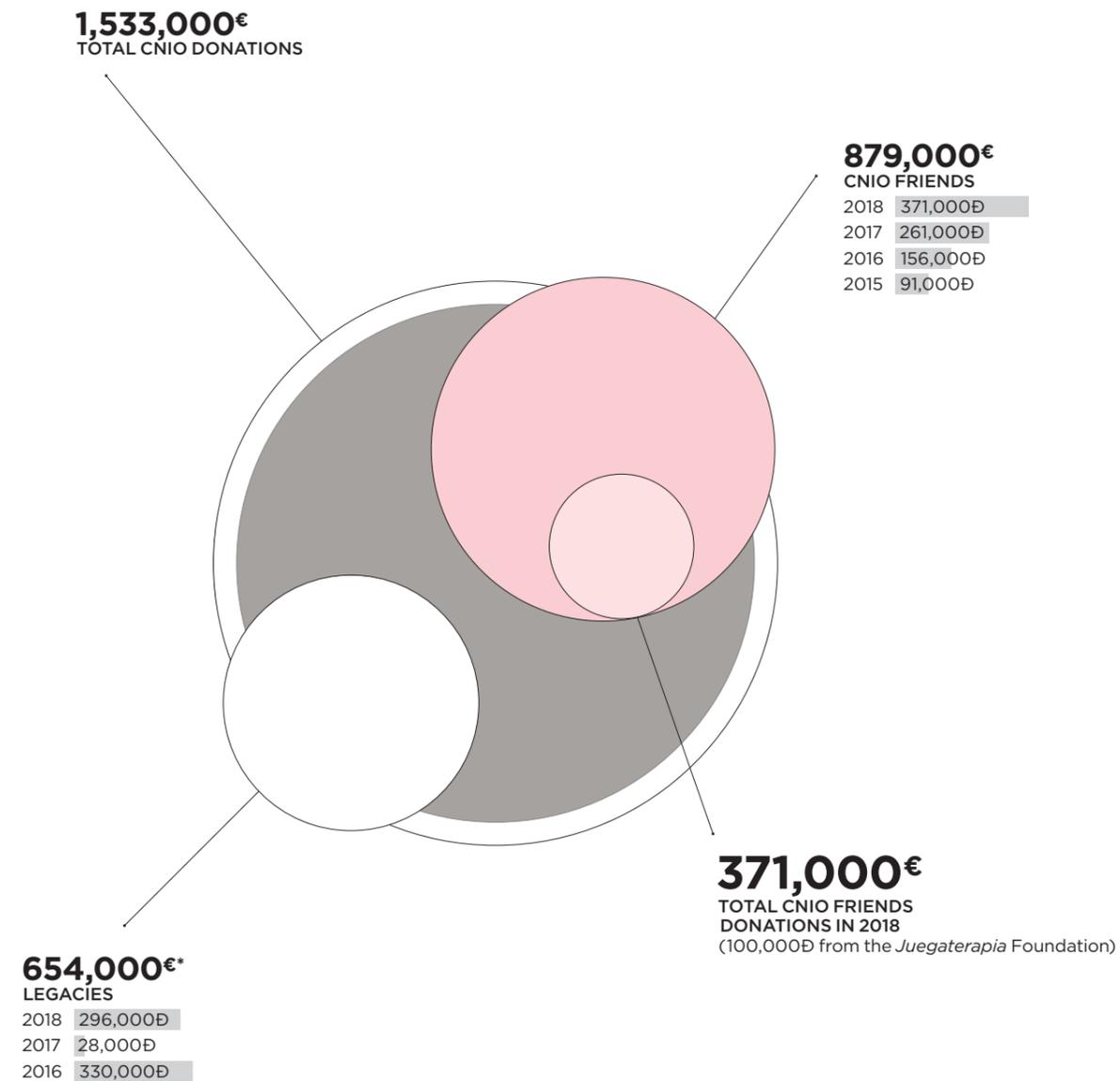
- **Alberto Heras Hermida**  
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Last but not least, we would also like to extend our heartfelt thanks to all the anonymous benefactors who have donated their legacies to support cancer research at the CNIO; in doing so they have contributed to society for generations to come.

DONATIONS TO THE CNIO



\* 463,000€ 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 'Types and Trends on the Threshold of the 21st Century' Alcobendas Collection, among many others. Amparo's most recent solo exhibitions in Spain were shown at the *Sala Robayera de Miengo*, Cantabria 2017, *Galería Trinta*, Santiago de Compostela 2015, and the *Museo del Romanticismo*, Madrid 2012. Her first feature film 'El silencio que queda' was selected to be part of the Documentary Feature Film section of the 22<sup>nd</sup> Málaga Film Festival 2019.

### UNDERBAU DESIGN



Underbau is a design studio that emerged in 2008 from professional designers with 15 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*, *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.

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