CONTENTS

FOREWORD

VICE DIRECTOR

ORGANISATION OF RESEARCH

BASIC RESEARCH

Molecular Oncology Programme
- Telomeres and Telomerase Group
- Experimental Oncology Group
- Cell Division and Cancer Group
- Genomic Instability Group
- Chromosome Dynamics Group
- DNA Replication Group
- Melanoma Group
- Microenvironment & Metastasis Junior Group
- Brain Metastasis Junior Group
- Metabolism and Cell Signalling Junior Group

Cancer Cell Biology Programme
- Genes, Development and Disease Group
- Epithelial Carcinogenesis Group
- Growth Factors, Nutrients and Cancer Group
- Seve Ballesteros Foundation-CNIO Brain Tumour Junior Group

Structural Biology Programme
- Macromolecular Complexes in DNA Damage Response Group
- Cell Signalling and Adhesion Junior Group
- Kinases, Protein Phosphorylation and Cancer Junior Group
- Genome Integrity and Structural Biology Junior Group
- Bioinformatics Unit
- Electron Microscopy Unit
- Crystallography and Protein Engineering Unit
- Biological Test Hiring Unit

TRANSLATIONAL RESEARCH

Human Cancer Genetics Programme
- Human Genetics Group
- Hereditary Endocrine Cancer Group
- Genetic and Molecular Epidemiology Group
- Familial Cancer Clinical Unit
- Molecular Cytogenticists and Genome Editing Unit
- Human Genotyping-CEGEN Unit

Clinical Research Programme
- Breast Cancer Junior Clinical Research Unit
- Prostate Cancer Junior Clinical Research Unit
- Molecular Diagnostics Unit
- H2O-CNIO Haematological Malignancies Clinical Research Unit
- H2O-CNIO Lung Cancer Clinical Research Unit

Biobank

COMMUNICATION

Social Events

INTERNATIONAL AFFAIRS

INSTITUTIONAL IMAGE & OUTREACH TO SOCIETY

CNIO OFFICES

FACTS & FIGURES

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

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 Maeztu 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 biomedical science. 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 TRP 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 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 Boumedine 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 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 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 106,000 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 Janssen. CNIO Friends have also agreed 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.
“During 2018, our scientists made significant advances in understanding how cancer originates, develops and progresses into a metastatic disease.”

The scientists at CNIO have once more 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.
TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Javier Benítez  Programme Director

Miguel Urioste  Human Genetics Group

Mercedes Robledo  Hereditary Endocrine Cancer Group

Núria Malats  Genetic and Molecular Epidemiology Group

CLINICAL RESEARCH PROGRAMME

Miguel Quintela-Fandino  Acting Programme Director

Joaquín Martínez-López  Breast Cancer Junior Clinical Research Unit

David Olmos  Prostate Cancer Junior Clinical Research Unit

José J. Lombardía  Molecular Diagnostics 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

Sagrarro Orbega  Transgenic Mice Core Unit

Giovanna Boncador  Monoclonal Antibodies Core Unit

Francisco Mulero  Molecular Imaging Core Unit

Loa Martínez  Flow Cytometry Core Unit

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor  Programme Director

Susana Velasco  CNIO-Lilly Cell Signalling Therapies Section

Carmen Blanco  Biology Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Anabel Sanz  Director
Basic Research

Molecular Oncology Programme
- Telomeres and Telomerase Group
- Molecular Oncology Group
- Cell Division and Cancer Group
- Genomic Instability Group
- Chromosome Dynamics Group
- DNA Replication Group
- Melanoma Group
- Microenvironment & Metastasis Junior Group
- Brain Metastasis Junior Group
- Metabolism and Cell Signalling Junior Group

Cancer Cell Biology Programme
- Genes, Development and Disease Group
- Epithelial Carcinogenesis Group
- Growth Factors, Nutrients and Cancer Group
- Seve Ballesteros Foundation-CNIO Brain Tumour Junior Group

Structural Biology Programme
- Macromolecular Complexes in DNA Damage Response Group
- Cell Signalling and Adhesion Junior Group
- Kinases, Protein Phosphorylation and Cancer Junior Group
- Genome Integrity and Structural Biology Junior Group
- Spectroscopy and Nuclear Magnetic Resonance Unit
- Bioinformatics Unit
- Electron Microscopy Unit
- Crystallography and Protein Engineering Unit
- Biological Text Mining Unit
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 (Maria 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.

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

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 iPSCs.

“We have demonstrated that telomerase activation in mouse models of pulmonary fibrosis can stop the progression of this fatal disease in mice.”
Telomerase gene therapy to cure pulmonary fibrosis in mice

Pulmonary fibrosis is a fatal lung disease that currently lacks effective treatment and is characterized 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

Telomeres 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, 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 (PCF2), an important epigenetic regulator of gene expression, thus facilitating the assembly of telomeric heterochromatin. We analysed telomere heterochromatin marks in cells deficient for 20q-TERRAs and observed that their telomeres had decreased heterochromatic marks; we discovered that they had lost a histone mark not previously recognised at telomeres (H3K27 methylation) that is catalysed by PCF2, 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 H3F3a binding at telomeres required PCF2-dependent H3K27me3 at telomeres. Our findings demonstrated an important role for TERRAs in telomeric heterochromatin assembly (FIGURE 2).

Telomeres are important epigenetic regulators

TERRAs are long non-coding RNAs that protect the telomeres. Telomerase activation does not accelerate tumorigenesis in the context of oncogenic K-ras-induced lung cancer. Using 20q-TERRA knockout cells we have now addressed the direct role of TERRAs in telomeric heterochromatin formation. Telomerase gene therapy does not increase risk of cancer in cancer-prone models.

References

2. Martínez P, Blasco MA (2018). AAV9-mediated telomerase gene therapy in a mouse model of pulmonary fibrosis makes possible the deposition of heterochromatin marks. These events cannot take place when TERRAs are absent.
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.”
Regressive of advanced K-Ras/Trp53 mutant lung adenocarcinomas upon systemic ablation of e-Raf expression

Almost a quarter of all solid tumours harbour K-Ras/Trp53 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 unacceptable toxic. This year, we demonstrated that ablation of e-Raf expression in advanced mouse lung tumours driven by K-Ras/G12V/Trp53 mutations led to significant tumour regression with no detectable appearance of resistance mechanisms (FIGURE 1). Tumour progression results from massive apoptosis. Importantly, systemic abrogation of e-Raf expression does not inhibit canonical MAPK signalling, hence, resulting in limited toxicities. These observations suggest that therapeutic strategies aimed at inhibiting e-Raf kinase activity may not be suitable since they may only block MAPK Kinase activation. Indeed, three independent e-Raf kinase inhibitors have shown to be rather toxic even at non-toxic doses. Therefore, we need to explore other therapeutic strategies. Drugs capable of degrading the e-Raf protein could be more effective in the clinic. Yet, drugs that promote protein degradation are still at very early stages of development. Inhibition of e-Raf by small interfering RNA is an alternative approach, but still faces significant technical challenges. Finally, selective targeting e-Raf effectors regulated through non-kinase mechanisms such as BOKalpaka, Ask1 and MST2 will also be challenging since they will require to be actively acquired. Frank McCormick 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 cancers, the goal posts seemed to have moved again”. In any case, our results should provide the experimental bases to design novel e-Raf-based therapeutics to treat KRAS 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 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 PDGFβR+ CAFs isolated from genetically engineered mouse 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 PDGFβR+ CAFs. Whereas Saa3 competent CAFs stimulate the growth of PDAC tumour cells in an orthotopic model, Saa3 null CAFs inhibit tumour growth. Saa3 null CAFs play 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 tumour cells. As a consequence, gemelinen 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.

**PUBLICATIONS**

**AWARDS AND RECOGNITION**
- Manuel Sánchez: Award for PhD-Authoried Publications, DNB Lab-Diap.
- Keystone Speaker, AACR Special Conference: Targeting RAS-Driven Cancer, 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, EMBD Workshop on “Cellular Signaling and Cancer Therapy”, Cordoba, Spain.
- Sirio Chirori, Chair, Milenio Memorial Lecture, XII Meeting of the Chilean Society for Biochemistry and Molecular Biology, Iquique, Chile.
- Carmen Guerra: III Beca Carmen Delgado/Miguel Alonso.
- CNIO Award for Excellence in Research by Staff Investigators.
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/B55 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 phospho-proteins (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, such as in platelets, the same pathway is involved in the control of cytoskeleton dynamics by modulating phospho-residues in the signalling pathways that control the remodelling of the actin cytoskeleton.

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 phosphoproteases. In postmitotic cells such as platelets, the same pathway is involved in the control of cytoskeleton dynamics.

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 in polypliod 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.

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 cell proliferation. However, when Plk1 is overexpressed, tumour cells are permanently unstable resulting in defective growth of breast cancer.

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 in polypliod 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.

...
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 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 EGRF, 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.
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.”

Coehsin 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 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.

**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 (CN10). 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 Stag2 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.

**PUBLICATIONS**

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 γH2AX 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 Polα/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 J. Moreno de Albornoz (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.

Figure 1: New methods to determine the primary cause of replicative stress. (A) Test based on a CDK1 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 μm. Adapted from Rodríguez-Acebes et al. (2018).
MELANOMA GROUP

Basic Research

Evolution of Melanoma: From Basic Research to Clinical Applications

Molecular Oncology Programme

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 \textit{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 \textit{in vivo} imaging in mouse models, we have identified a melanoma-associated signature of prometastatic genes that make this tumour uniquely aggressive.
ANNUAL REPORT 2018

Aim 1. **Diagnosis**

Group focuses on 3 main objectives (FIGURE 1):
- melanoma initiation and progression

To this end, our advance in this disease is the lack of animal models to monitor the knowledge on how melanomas progress and metastasise. Moreover, the field lacks molecular markers of diagnosis, and based therapies, sustained clinical responses are still limited. Nevertheless, example of how integrated basic and clinical research has significantly improved patient prognosis. Nevertheless, risk factors and prognostic markers.

Aim 2. **Tumor Progression**

Risk factors and prognostic markers.

Aim 3. **Imaging and Treatment**

Metabolic drivers, Metastasis Indicators

**Oncogenic pathways selectively deregulated in melanomas**

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 endosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Curbelo et al., Cancer Cell 2014). Further analyses of lysosomal-dependent pathways also revealed unique features of autophagy genes (ATG9) in melanoma (García-Fernández et al., Autophagy 2016). Further analyses of the RBP s, 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 of autolysosome maturation in melanoma (Gallego-Sánchez 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 dispensable for autolysome maturation in melanoma and as a platform for gene discovery 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 anti-cancer 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.

**RESEARCH HIGHLIGHTS**

- **Drivers, Metastasis Indicators**
- **Premetastatic niches Drug response**

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

**Figure 2** New functions for pro-metastatic drivers in melanoma. Schematic representation of a set of pro-luminal-vascular factors with no previous links to melanoma identified by addressing the expression and functional requirement of p62/SQSTM1 in this disease. Differences 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. MetAlert studies in cell lines combined with histopathological studies in genetically modified mouse models (GEMM) and histopathological validation in clinical biopsies identified the scaffolding factor FERM2 as a downstream target of the p62/CUGBP1 axis. Both p62 and FERM2 were found overexpressed in advanced melanomas, representing new indicators of poor prognosis.

**Figure 3** Schematic representation of a set of RNA binding proteins (RBPs) 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 FERM2 as a downstream target of the p62/CUGBP1 axis. Both p62 and FERM2 were found overexpressed in advanced melanomas, representing new indicators of poor prognosis.
**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 small extracellular vesicles called exosomes. Our data support that tumour-secreted exosomes can reach metastatic organs facilitating metastatic spread.

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

**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 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.
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 79% 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.

**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 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**


- “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.”

**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 79% 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.

**AWARDS AND RECOGNITION**

- 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-Alejandra Espinoza was recipient of a MECO-Soros-Ochsner PhD Fellowship.
- Nebia Priego received the CNIO Award for Excellence in Research by Postdoctoral Staff/Investigators, CNIO Lab-Day.
- Lucía A. Zhu received the “Best Poster” Award at the CNIO Lab Day.
- STAT3 label (a) and increases survival.”

**Figure**

A subpopulation of reactive astrocytes (GFAP), characterised by activated STAT3 (∗), is present in experimental brain metastasis models (a,b) and human samples (e,f). Targeting this STAT3 label (c) and increases survival.”

- 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 79% 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.
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.
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.”
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.”
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 Wnt-mouse, 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 (PAa) disease, we aim to elucidate potential therapeutic targets to alleviate skin and joint inflammation. We previously identified the St004A9 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 St004A9 to determine the specific role of keratinocyte-derived and neutrophil-derived St004A9 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 PPARγ transcription. Therefore, fatty liver disease, inflammation, fibrosis and tumours with HCC signatures. Mechanistically, molecular analyses point to the involvement of pathways connected to human hepatocellular carcinoma (HCC), such as the Wnt/ß-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 unveiled and most likely contributes to the oncogenic function of c-Fos in hepatocarcinogenesis. 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, Lipocatin-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 neutrophil-deficient situation suggesting that neutrophils may not be the key factor. Ongoing studies show that the Benign-Adjointer (SAA) 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. Sørensen, Spain, M. Petruzelli, UK, H. Watzke, M. Poglitsh, P. Benedikt and R. Zechner, Austria).

Fra-2 in lung fibrosis and cancer

Lang 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 V1 collagen in a type II 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 Lipocatin-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 epithelial stem cells (RSCs) initiate epidermal hyperplasia and skin inflammation by priming neighbouring non-mutant epithelial cells to acquire a psoriasis-like phenotype. Mechanistically, TSLP neutralisation reduces non-mutant keratinocytes proliferation and VEGF 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.

REFERENCES

We 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 Wnt-mouse, 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 (PAa) disease, we aim to elucidate potential therapeutic targets to alleviate skin and joint inflammation. We previously identified the St004A9 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 St004A9 to determine the specific role of keratinocyte-derived and neutrophil-derived St004A9 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 PPARγ transcription. Therefore, fatty liver disease, inflammation, fibrosis and tumours with HCC signatures. Mechanistically, molecular analyses point to the involvement of pathways connected to human hepatocellular carcinoma (HCC), such as the Wnt/ß-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 unveiled and most likely contributes to the oncogenic function of c-Fos in hepatocarcinogenesis. 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, Lipocatin-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 neutrophil-deficient situation suggesting that neutrophils may not be the key factor. Ongoing studies show that the Benign-Adjointer (SAA) 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. Sørensen, Spain, M. Petruzelli, UK, H. Watzke, M. Poglitsh, P. Benedikt and R. Zechner, Austria).

Fra-2 in lung fibrosis and cancer

Lang 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 V1 collagen in a type II 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 Lipocatin-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 epithelial stem cells (RSCs) initiate epidermal hyperplasia and skin inflammation by priming neighbouring non-mutant epithelial cells to acquire a psoriasis-like phenotype. Mechanistically, TSLP neutralisation reduces non-mutant keratinocytes proliferation and VEGF 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.

REFERENCES
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.”
Pancreatic cancer molecular pathology

The genetic/genomic changes associated with PDAC have been extensively described over the last few years by the genome consortium, 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 heterozygosity is associated with a pre-inflammatory state that sensitises the mice to the oncogenic effects of mutant KRas. Deletion of one Nr1a2 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 interface using immunoprecipitation and mass-spectrometry. We find that reduction of NR5A2 protein levels by 90% (either genetically or during pancreatitis) is also associated with profound effects on the interface, 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, Nf1c pancreatea 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, endoplasmic reticulum stress response. NFIC is a novel regulator of acinar differentiation.

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

In collaboration with J. Valcarcel (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.

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.

Pancreatic cancer molecular pathology

The genetic/genomic changes associated with PDAC have been extensively described over the last few years by the genome consortium, 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 heterozygosity is associated with a pre-inflammatory state that sensitises the mice to the oncogenic effects of mutant KRas. Deletion of one Nr1a2 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 interface using immunoprecipitation and mass-spectrometry. We find that reduction of NR5A2 protein levels by 90% (either genetically or during pancreatitis) is also associated with profound effects on the interface, 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, Nf1c pancreatea 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, endoplasmic reticulum stress response. NFIC is a novel regulator of acinar differentiation.

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

In collaboration with J. Valcarcel (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.

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.
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

Poore 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 protein1) with scaffolding activities regulating mTORC1 activity in response to amino acids. 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.

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.

Pathogenic environmental factors

- Bacteria, virus...infection
- URI MCRS1
- Inflammatory cues

Stress/Adaptive response

- Nutrients and growth factors
- Live, intestine, pancreas and stomach

Gastrointestinal tract diseases

- Liver, intestine, pancreas and stomach

Pathological state

- Cancer, diabetes, obesity etc...

In this regard, we made the following discoveries:

- Inflammatory cues up-regulate hepatic URI, which inhibits de novo NAD synthesis causing DNA damage and thereby initiating hepatocellular carcinoma (HCC). Replenishing the pools of NAD 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 deficit-induced DNA damage that activates metabolic inflammation-associated IL-17A to cause non-alcoholic steatohepatitis (NASH) and HCC. Boosting NAD 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 mTORC1-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.

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).
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 chemoresistance 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 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 targets for the treatment of these therapies will possibly extend the survival of the patients.

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

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 increased 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.

Novel therapeutic approaches for therapy-resistant malignant brain tumours

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.\footnote{The current most effective treatment for GBM patients is a combination of radiotherapy and alkylating agents.}

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.
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 25th, SBP was granted a competitive project to access the Electron Bio-Imaging Centre (eBIC) at the Diamond Light 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.
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.”
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 assists the assembly of 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 mTORC1 complex 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.

**REFERENCES**


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.

**REFERENCES**


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.

**REFERENCES**


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.

**REFERENCES**

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:

- 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?

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

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 FH 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_2 rich membranes in order to adopt a highly active state.

- 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?

**PUBLICATIONS**

KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

Iván Plaza Menacho
Junior Group Leader

Post-Doctoral Fellow
Pablo Soriano

Graduate Student
Nicolás Cuenda (since July)

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

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

RESEARCH HIGHLIGHTS

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).

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

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

Figure
Structural identification of a RET αC 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).

∞ PUBLICATIONS


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 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.

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

“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.”

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.

Awards and recognition

- Awarded a Ramon y Cajal Fellowship, Spanish Ministry of Science, Innovation and Universities.
- Awarded a Ramon 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 metabolomics 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 1H NMR spectrum of the compound (100 µM) recorded after addition of increasing amounts of dsDNA (from bottom to top: 0-8.9 µM). 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<sub>d</sub> > 10 µM).)
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 genotype 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 interpretation using computational and statistical methods; and iv) to maintain the CNIO’s scientific computing facilities and provide training in bioinformatics tools and methods.

**OVERVIEW**

“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.”

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

**SELECTED PUBLICATIONS**


**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 (Byures M et al., 2018) or in collaboration with M. Böhlebo’s Group from the Human Cancer Genetics Programme to study phaeochromocytoma and paragangioma (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 role in teaching the master’s course in Bioinformatics Applied to Medicine Personalized (Solu8 [BCIU-EN]-ENS) as well as advanced Bioinformatics courses for sequencing analysis (visit our web page for a full list of activities).
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 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 di peptide arginine repeats (PR). We have evaluated, by electron microscopy, in collaboration with the CNIO Genomic Instability Group, that the presence of (PR)_20 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.

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.”

PUBLICATIONS

OVERVIEW

The Crystallography and Protein Engineering Unit (XTPEUnit) is a central core facility as well as a research laboratory whose main goal is to supply the requests of the research groups within the CNIO, as well as external groups. The Unit covers all the services that range from the coding of proteins to the resolution of the 3D structure, whilst always focusing on helping the research groups in reaching a sequence cloning to the resolution of the structure in-solution of two mAbs that correspond to the drug ETP-885. (c,d) X-ray structure of HASPIN in complex with the drug ETP-885. (e,f) SAXS study of two trimers produced for cancer therapy. Fit of the ab initio SAXS structures (envelopes in grey) and the corresponding generated trimers (chains in blue, magenta and cyan).

“...we aim to show how protein information at atomic resolution is crucial for understanding the processes occurring in the biology of cancer.”

with the Department of Pharmacology and Therapeutics at Roswell Park Cancer Institute. Also, we have solved, by SAXS, the structure in-solution of two mAbs that correspond to the first generation 4-1BB agonistic IgG 1D8, and which are specific tumour-targeted trimers (FIGURE). Our collaborators, at the Department of Molecular Engineering (Denmark), have shown their high tumour inhibition potency in vivo. Finally, the Unit is taking part in a project, in collaboration with the Biomedical Application of Radioisotopes Unit at CIEMAT and the CNIO’s Molecular Imaging Unit, to develop new antibody-based positron emission tomography (immunoPET) imaging tools for tumour visualisation.

• PUBLICATIONS

• AWARDS AND RECOGNITION
  • Member of the Board of Directors, Asociación de Universidades de la Región de España.
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 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.

“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.”

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 Rocio, 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 HeidelTime, a medical machine translation system and word embeddings. These key constituents are being integrated into the clinical NLP pipeline developed by the Unit.

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

Human Cancer Genetics Programme
Human Genetics Group 94
Hereditary Endocrine Cancer Group 96
Genetic and Molecular Epidemiology Group 100
Familial Cancer Clinical Unit 104
Molecular Cytogenetics and Genome Editing Unit 108
Human Genotyping-CEGEN Unit 110

Clinical Research Programme
Breast Cancer Junior Clinical Research Unit 114
Prostate Cancer Junior Clinical Research Unit 116
Molecular Diagnostics Unit 118
H12O-CNIO Haematological Malignancies Clinical Research Unit 120
H12O-CNIO Lung Cancer Clinical Research Unit 122

Biobank 126

130
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 consultations, 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 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 Benitez’s Group: was accepted in the international Consortium of Testicular Cancer.

“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.”
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.”

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.”

OVERVIEW
we identified a mutation in the moderate susceptibility Breast cancer susceptibility genes

In a whole-exome sequencing study of 4 BRCAX families Breast cancer susceptibility genes

we found a deleterious or likely deleterious mutations in the gene in a series of 700 BRCA X cases and only 1 deleterious mutation in 700 controls, suggesting that the gene could actually explain a small percentage of the BRCA X families (Tavera-Tapia et al., submitted).

Deciphering the role of rare variants in breast cancer

Breast cancer susceptibility genes

In a whole-exome sequencing study of a BRCA X 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 that an SNP rs1929919 associated with significant UNG down-regulation and a better performance of the enzyme, measured by a lower accumulation of uracil at the telomeres in BRCA X mutation carriers. Our findings could help to explain the association of this variant with a lower cancer risk in BRCA X 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.

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 (Baquerizo et al., submitted). We reported that an SNP rs1454729 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 types of tumours (Calvete et al., 2016). We investigated if the mutation 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 and with and without POT1 mutations and have concluded that the inhibition of POT1 gene function, and the damage-response machinery, would activate ATR-dependent DNA damage signalling, which cells 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) and other angiomasomas (mutations in damage-signalling), which drives tumour formation. The same observation was made in sporadic cardiac lesions (Calvete 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 (Baquerizo et al., submitted). We reported that an SNP rs1454729 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 types of tumours (Calvete et al., 2016). We investigated if the mutation 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 and with and without POT1 mutations and have concluded that the inhibition of POT1 gene function, and the damage-response machinery, would activate ATR-dependent DNA damage signalling, which cells 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) and other angiomasomas (mutations in damage-signalling), 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 ATPI4 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 mutation in ATM and PTEN 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 expressed genes in sporadic tumours (solute carriers) altering ATM4 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 variations. 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 DAX1) were validated in a large case-control association study (Paumand 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.

In 2015, we published the identification of the ATPI4 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 mutation in ATM and PTEN 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 expressed genes in sporadic tumours (solute carriers) altering ATM4 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 variations. 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 DAX1) were validated in a large case-control association study (Paumand 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.
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.”
Deep sequencing of small RNA sequences reveals a prognostic marker functionally associated with alternative splicing modulation in thyroid cancer. It is urgent to identify biomarkers and functional networks associated with aggressive thyroid cancer as well as consistent methylation and expression profiles. We also found positive DLST immunostaining not only in PPGL but also in other tumours in which the TCA cycle is active. Metabolome-guided genomics of patients with paraganglioma showed consistent methylation and expression profiles. We also found positive DLST immunostaining not only in PPGL but also in other tumours in which the TCA cycle is active. Metabolome-guided genomics of patients with paraganglioma showed consistent methylation and expression profiles. These findings support a high efficacy of mTOR inhibitors in malignant EAML and in a subset of patients with chromophobe renal cancer, and propose splicing of mTOR pathway targets to guide therapy with these drugs.

Significantly, many abounds of hsa-miR-139-5p, an alternative splicing factor mainly involved in cryptic exon inclusion/exclusion, showed an anti-correlation with hsa-miR-139-5p in patients with metastatic cancer. A renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer, a renal epithelioid angiomyolipoma with metastatic cancer.

Regrettably germline DLST mutations in patients with multiple pheochromocytomas and paragangliomas (PPGLs) take an important step towards understanding 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, omics-based analyses, TCA-related metabolite determination and 13C-glutamate labelling assays were performed. We identified DLST germline variants in 7% of patients. A recurrent mutation, c.G432T, 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. 

PUBLICATIONS


Figure: hsa-miR-139-5p/NRNP2 axis modulates gene expression. (A) Alternative splicing analysis experiment. (B) Differential expression analysis showed an induction of hsa-miR-139-5p expression in human tumours. Analysis of alternative splicing from RNA sequencing data revealed 174 events differentially regulated upon NRNP2 repression in genes and signaling cascades critical for thyroid cancer (FIGURE). These results point at hsa-miR-139-5p/NRNP2 gene-transcript axis as a novel regulatory axis associated with tumour virulence and modulation of major thyroid cancer signalling pathways.
OVERVIEW

The scope of the research carried out by our Group ranges from the identification of aetiological agents and mechanisms, to the translation of the findings into the clinical and Public Health domains, focusing on bladder, pancreatic, and breast cancers.

We employ a wide variety of biomarkers to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. Omics data provide a unique opportunity in this regard and the Group explores its integration in epidemiologic studies.

The strategic goals of the Group are to:

→ Identify non-genetic and genetic factors, as well as their interactions, associated with cancer development and progression and with its molecular/omics subphenotypes.
→ Develop and apply statistical/informatics tools to model the risk, prediction, and clinical course of patients with cancer by integrating epidemiologic with omics information.
→ Assess clinical and public health strategies for cancer control using current genomic tests and data.

“The integration of omics and non-omics data in the same risk models poses several challenges and demands of appropriate analytical strategies. We are contributing to this field towards a personalised prevention of cancer.”
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 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 in non-smokers. In the second article, we reported on the underlying genetic basis behind PC and its associated multimorphologies 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 ≥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 studies 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 Epidemiology Resource. The reduced risk of BC was especially observed among aggressive tumours. The Group also participated in the discovery process of both autoimmune and tumour prognostic marker combination in large European studies of non-muscle invasive BC. We also 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 combining omics and genetics (OnO) data as well as in the nutrition epidemiological filed. 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 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 vitamins D in bladder cancer at the methodological level. We continue to sustain the Spanish BC research Work Stream of the Pancreatic Cancer Europe (EPC) multisite platform, with whom 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 Association of Personalized Medicine.

Familial PC Registry (PanGen-FAM) and the establishment of the European Registry of PC (PanGen2018). We lead the Research WorkStream of the Pancreatic Cancer Europe (EPC) multisite platform, with whom 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 Association of Personalized Medicine.

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 clinical and diagnostic activities carried out by the FCCU through the consultancy in the Medical Oncology Department of Puente la Reina’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 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 consultation 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-Strand Conformation Polymorphism assay, 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 CRC 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 CRC and its relationship with environmental factors.

The Clinical and Diagnostic Research Highlights

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 (348 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.

Lynch syndrome is a very complex entity associated with a high risk of 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 field 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 neoepitopes that account for the infiltrating lymphocyte reaction classically observed in Lynch-associated tumours. The recent emergence of immune checkpoint inhibitors that work on 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.
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 techniques, 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 models of cancer modelling, reproducing a variety of cancer types up to clinical scenarios.

“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 assay 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 including LV particles. At 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.

\[\text{Figure} \quad \text{Overview of efficient approaches for recreating cancer-associated chromosome translocations.}\]

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 assay 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 including LV particles. At 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.

\[\text{Figure} \quad \text{Overview of efficient approaches for recreating cancer-associated chromosome translocations.}\]
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. Doxorzel 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 183 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 ABCG2, 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 doxorzel tumour response (P=2.15x10-4). 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, 10q21.3 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 = 3681). We validated significant SNPs in 133 additional subjects with extreme phenotypes selected from databases including >99,000 individuals. Two SNPs were validated: rs12660420 (p = 5.66x10-5; OR combined = 2.80), mapping to a noncoding transcript exon of FDE10A; and rs68397978 (p = 1.62x10-4; OR combined = 2.57), an intronic variant in ATP10D. We assessed the relevance of both proteins in early-stage NSCLC. FDE10A and ATP10D mRNA expressions correlated with survival in 821 stage I-I NSCLC patients (p < 0.001). FDE10A 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).

**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 one to millions of SNVs and CNVs. In addition, epigenetic studies using whole-genome methylation arrays are performed in this Unit. Complementary, 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.”
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.”
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. 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 years. However, when 1 or more of those kinases are “on”, the risk of relapse increases 10-fold. More importantly, all 6

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. Specifically, one of the trials explored the reversal of immunosuppression 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 (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.
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, PROSKARI & PRORADUIM. 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 consolidated as 1 of the top prostate cancer clinical treatment centers. 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, PROSKARI & PRORADUIM. 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 consolidated as 1 of the top prostate cancer clinical treatment centers. Our clinical CNIO-IBIMA unit has consolidated as 1 of the top prostate cancer clinical treatment centers. We have further progressed in genetic 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 (IBCSS, 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/enaahmatide, 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 supportive steroid, switched from prednisone to dexamethasone, while maintaining abiraterone, helps to re-induce the response to abiraterone in about 40 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.

* PUBLICATIONS


* 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-CC-AACR-EPSO Methods in Clinical Cancer Research Workshop, Zeist, The Netherlands.

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 in noncarriers (bottom).
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 $CXCR4$ 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.

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

“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.”

*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-terminal nonsense/frameshift mutations).
TRANSLATIONAL RESEARCH

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:

1. Generation of mouse models focused on the molecule hnRNP K, a novel driver of lymphoma and leukaemia.
2. Development of novel diagnostic and follow-up tools, such as minimal residual disease analysis in acute myeloid leukaemia (AML).
3. Screening of novel drivers, biomarkers and therapeutic targets by next-generation sequencing (NGS, e.g. exome sequencing of amyloidosis).
5. 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.”
Minimal residual disease monitoring in acute myeloid leukemia

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 lineotypes with 4 typical AML. Our analysis showed better sensitivities (10-4 for SNVs and 10-6 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 (10-5).

It should be noted that the 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 the tyrosine kinase inhibitor treatment in vivo and ex vivo. Trametinib exerted strong synergy with the tyrosine kinase inhibitor midostaurin, inhibiting different 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 leukemias (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, 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 to be implicated in the pathogenesis of MDS. We have found that the profile of several of these 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 identified. 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**


**Figure 5** shows the synergistic effects of ruxolitinib and nilotinib had a synergistic effect against the tyrosine kinase inhibitor treatment in vivo and ex vivo. Trametinib exerted strong synergy with the tyrosine kinase inhibitor midostaurin, inhibiting different downstream pathways.

**Selected publications at other institutions**


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.”
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 of a series of well-designed 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 NSG-based algorithm for the determination of genomic aberrations (in tumour tissue but also in cDNA that may give guidance for clinical trials). 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 Pembrolizumab plus Nivolumab.

Early clinical trials

Our Group has significantly expanded its activities regarding early clinical trials, plus Nivolumab.

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 Pembrolizumab plus Nivolumab.

Chemotherapy alone (HR: 0.64; p=0.030) in stage IV Non-Small-Cell Lung Cancer (NSCLC) patients.

**Table 1**

<table>
<thead>
<tr>
<th>Publication</th>
<th>Authors</th>
<th>Journal</th>
</tr>
</thead>
</table>

**Figure 2**

Results of the CHECKMATE 027 randomised clinical trial, showing the superiority in progression-free survival (PFS) for the ipilimumab plus nivolumab combination, as compared to platinum-based chemotherapy (HR: 0.56; p=0.009) in stage IV NSCLC with high tumour mutational burden (>10 mutations per Mb).

**Figure 3**

Results of the Checkmate 231 randomised clinical trial, showing the superiority in progression-free survival (PFS) for the ipilimumab plus nivolumab combination, as compared to platinum-based chemotherapy (HR: 0.58; p=0.002) in stage IV non-Small-Cell Lung Cancer (NSCLC) patients.

**Clinical Research Programme**

**Figure 4**

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 BIO00848 – 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.

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 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), 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


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.
Innovation

Biotechnology Programme
Genomics Core Unit
Transgenic Mice Core Unit
Monoclonal Antibodies Core Unit
Molecular Imaging Core Unit
Flow Cytometry Core Unit
Confocal Microscopy Core Unit
Proteomics Core Unit
Histopathology Core Unit
Animal Facility

Experimental Therapeutics Programme
Medicinal Chemistry Section
Biology Section
CNIO - Lilly Cell Signalling Therapies Section
CNIO - Lilly Epigenetics Section

Technology Transfer and Valorisation Office
“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.
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 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

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 features, such as mutation landscapes, DNA-binding of 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.

"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.”

OVERVIEW

GENOMICS CORE UNIT

INNOVATION

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 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.

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.

"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.”
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 knockin 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 knockin-in in cancer related genes (FIGURE) ．

“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.”

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 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.
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. Ly-9, also known as CD329 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), 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 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.

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

**Research highlights**

**Innovations**

**Core Unit Head**

Giovanna Roncador

**Technicians**

Álvaro García, Sherezade Jiménez, Lorena Maestre (TS), Ana I. Reyes (TS) (Advanced Degree)

**Members**

Lorena Maestre (TS), Ana I. Reyes (TS), Álvaro García, Sherezade Jiménez, Giovanna Roncador, Carla Sánchez-Buato, M. Pina MA, Rodríguez-Iturbe A, Vomir M, Sierra CA, Vincze Á, Balogh P, Balog 13, 1177271918757480.

**Publications**


**Outside funding**

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."

**OVERVIEW**

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 the Ciemat group, granted by the BBVA Foundation, in which we will develop a nanobody produced by camelids that is labelled with 68Ga. 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 immunohistology 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.

**PUBLICATIONS**

- AWARD AND RECOGNITION

One of these images was featured on the cover of the September issue of *EMBO Molecular Medicine*. The new drug delivery system based on Galactose encapsulated nanoparticles with the new drug delivery system based on Galactose encapsulated nanoparticles...
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.

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.

“...The development and optimisation of phenotyping 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.

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++ flux, intracellular pH, etc.).
- Cytometric Bead Arrays to measure several cytokines from cell extracts and plasma.
- Platelet studies.

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.

**Core Unit Head**

Lola Martínez

**Technicians**

- Renan Antoanassli (since October) (TS)
- Julia García (since February) (TS)
- Tania López (TS)
- Titulado Superior (Advanced Degree)

**Publications**

The Confocal Microscopy Unit is equipped with: 3 laser scanning confocal systems (Leica SPS) 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 DM6000 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 SPS 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.

**PUBLICATIONS**

  
  
  
  
  
  
  TERRA recruitment of polycomb to telomeres is essential for histone tryptopholmation and telomere heterochromatin. Nat Commun 9, 1548.
ANNUAL REPORT 2018

INNOVATION

PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Graduate Students
Ana Martinez, Cristina Sayago

Overview

Proteins are the molecular effectors of cells and catalyze 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.”

PUBLICATIONS

1. Zhang N, Zhu L, Montoro C, Maldonado M, Wailewski O, Bredenberg W, Driogli L, Martinez L, Marínez G, Barón Y, Cani P, Megías D, Hernández-Echavere E, Blanco-Apascio C, Martínez L, Zahidal E, Muñoz J, Fuentes-Tomé C, Pérez-Velázquez E, Hernández-Lairín A, Berlín L, Prieto S, Vázquez-Martínez M, Henríquez A, Arbelo-B, Rosell-B, Barredo-J, Valiente M, STAT5b targets a subpopulation of reactive astrocytes required for 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 of metastatic tumors to study the molecular drivers of metastasis in triple-negative breast cancer. We have also studied the role of post-translational modifications... and identified important roles for the protein in extending the mRNA half-life of several pro-metastatic factors.

RESEARCH HIGHLIGHTS

Throughout 2018, the Proteomics Unit has acquired 2 novel Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) platforms. The QExactive Plus and the QExactive 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 NSF interfaces. The application of this technology in the CNIO will have a major impact on the characterization of complex proteomes as well as on the analysis of small protein amounts (e.g. FACS sorted cells, post-translational modifications...). In addition, high-resolution Orbitrap detection 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.

PUBLICATIONS


BIOTECHNOLOGY PROGRAMME | PROTEOMICS CORE UNIT

Technicians
Elena Fernández-Vigo (since February), Fernando García (TS), Núria Banz (TS), Álvaro Soriano (since February), Pilar Camacho (CAM), Pilar Ximénez

Título Superior (Advanced Degree) *Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Community of Madrid)
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 histochimical 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 trained technicians. The construction of 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 to be endorsed by External Quality Assessment Schemes. In this respect, it is worth mentioning that our haematolympho-endothelial staining technique was commended in the framework of the evaluation, carried out by UK NEQAS, of our histochimical 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.

BIOTECHNOLOGY PROGRAMME | HISTOPATHOLOGY CORE UNIT

Technicians
Nuria Cabrera, María Gómez, Patricia González, Gabiño Hernández, Vanessa Pizarro (IEI, CAM), Irene Roda, Zaira Vega

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.

The implementation of multiplexed IHC staining techniques was commended in the framework of the evaluation, carried out by UK NEQAS, of our histochimical 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.

Histochemical techniques are often a quick way to analyze the properties of the tissue. Sirius red staining of fibrotic liver under brightfield (A) and polarised light (F) allows visualising collagen and analytic deposits.
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 10,000 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 III 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 redelivery. 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.

In addition to mice, the Animal Facility hosts over 100 species 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 RD 53/2013 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 EUC/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 (COECE) 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.
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.

“Interrogate biology with chemistry and you will get responses that can trigger unimaginable research and discoveries.”
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 antimitotic 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 alkyn reporter of ETP-093 was then used for subsequent target identification by conjugation to suitable reporters (biotin-N₃) 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.
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.”
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-38, 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 (SIC50 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-991, 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 Muskhine expression, in collaboration with the Melanoma Group.

**RESEARCH HIGHLIGHTS**

**Innovation**

measure the association of TRF1 to telomeres has been used to

Telomeres and Telomerase Group. A phenotypic assay to measure the association of TRF1 to telomeres has been used to

*Publications*

Inspection and estimation of Binding Energy. The final

Structure-Based Virtual screening implemented in

**PUBLICATIONS**


**PATENTS**

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, HIF, 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 Gilmore & 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 metabolic imbalance that they are unable to maintain if exposed to 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 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.

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 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.

**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 techniques such as extracellular flux analysis (Seahorse *Ana Cerezo*, *Eva P. Lospitao*, *Gloria Martínez del Río*, *Susana Hernández*

**SCIENTIFIC CONTEXT**

**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 anti-tumour T cell response. (B) Extracellular flux analysis for the acidification rate (glycolytic test) and O₂ consumption OCR (mitochondrial test). Fully active effector T cells require an activated glycolysis and an oxidative metabolism in order to synthesize cytokines and other molecules necessary for their cytolytic activity. Immune suppressive metabolites, like kynurenine, suppress the metabolic activity of effector T cells inhibiting their cytolytic activity.

---

**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 anti-tumour T cell response. (B) Extracellular flux analysis for the acidification rate (glycolytic test) and O₂ consumption OCR (mitochondrial test). Fully active effector T cells require an activated glycolysis and an oxidative metabolism in order to synthesize cytokines and other molecules necessary for their cytolytic activity. Immune suppressive metabolites, like kynurenine, suppress the metabolic activity of effector T cells inhibiting their cytolytic activity.
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.
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, 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 reveres 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.
Communication
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 groundbreaking 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 Atremedia 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 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 groundbreaking 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 13th 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.

“Spreading knowledge to valorise science.”
Luz Casal: “Llegué a recoger el resultado, estaba con dos chicos, los dos se vinieron abajo…” “Te quedas débil, … PRESERVAR TU FERTILIDAD (HOJA 28) CIENCIA ADOLESCENTES Y TUMORES: VIVIR EN TIERRA DE NADIE (HOJA 34)
Scott Lowe, del Memorial Sloan Kettering Cancer Center de Nueva York, en el CNIO.

1,127,383
233,530
91

September 24, 2018
20
November 15, 2018
22
November 19, 2018
23

MADRID

raquelserrano@unidadeditorial.es

enfermedades tumorales no escapa al fenómeno de las resistencias. En inmunoterapia, por beneficio. Los análisis genéticos ofrecen estratificaciones. El manejo de las células del sistema inmune para conseguir respuestas", ha explicado.

DEL 10 AL 16 DE DICIEMBRE DE 2018

Unidos, centro pionero en ensayos sobre resistencias y del que James Allison, último premio Nobel de Medicina, es uno de los principales impulsores. Su objetivo es desarrollar la tecnología "hot" que no responde a otras terapéuticas son la gran esperanza observan con las otras dos terapéuticas que están en estudio.

El pasado mes de marzo, publicaba los resultados de un ensayo clínico con un tumor que suele afectar a niños, donde se obtuvo una tasa de respuesta del 21% en la fase 1. Los análisis genéticos de las células del sistema inmune para conseguir respuestas," ha explicado.

La terapéutica se basa en el uso de una combinación (un corticoide inhalado) que no responde a otras terapéuticas son la gran esperanza observan con las otras dos terapéuticas que están en estudio.

Scott ha realizado una estancia sabática en el Centro Nacional de Investigaciones Oncológicas (CNIO) y en el Instituto de Investigación oncología, es la esperanza para el futuro. "Fácil de enseñar, fácil de aprender, que muchas enfermedades dependen de una combinación (un corticoide inhalado) que no responde a otras terapéuticas son la gran esperanza observan con las otras dos terapéuticas que están en estudio.

—Ahora se están dando los primeros pasos. ¿Presenciarán una revolución en el futuro? —Pregunta más común en esta época. —Eso es algo que se lleva intentando hacer desde hace un siglo y que aún no ha sido posible. —Responde un experto en el campo. —En el sistema nervioso de hidras –unos cien millones de neuronas se comunican en red, distinguimos cómo se forman los circuitos y cómo evoluciona el cerebro. —¿Qué investigaciones están desarrollando técnicas para aprender cómo las neurociencias nos permiten alterar y ver la esencia de nuestra mente. —Debemos protegerlos para evitar que con la evolución se pierdan. «Debemos protegerlos para evitar que con la evolución se pierdan. Eso es algo que se lleva intentando hacer desde hace un siglo y que aún no ha sido posible. —En el sistema nervioso de hidras –unos cien millones de neuronas se comunican en red, distinguiendo el curso de ríos y verteres desde dentro. —¿Qué investigaciones están desarrollando técnicas para aprender cómo las neurociencias nos permiten alterar y ver la esencia de nuestra mente.

Antes o después ocurrirá. Ya estamos llevando a cabo estudios para comprender los sistemas más complejos. —Ahora se están dando los primeros pasos. ¿Presenciarán una revolución en el futuro? —Pregunta más común en esta época. —Eso es algo que se lleva intentando hacer desde hace un siglo y que aún no ha sido posible. —En el sistema nervioso de hidras –unos cien millones de neuronas se comunican en red, distinguiendo el curso de ríos y verteres desde dentro. —¿Qué investigaciones están desarrollando técnicas para aprender cómo las neurociencias nos permiten alterar y ver la esencia de nuestra mente. —Debemos protegerlos para evitar que con la evolución se pierdan. «Debemos protegerlos para evitar que con la evolución se pierdan. Eso es algo que se lleva intentando hacer desde hace un siglo y que aún no ha sido posible. —En el sistema nervioso de hidras –unos cien millones de neuronas se comunican en red, distinguiendo el curso de ríos y verteres desde dentro. —¿Qué investigaciones están desarrollando técnicas para aprender cómo las neurociencias nos permiten alterar y ver la esencia de nuestra mente.

La terapéutica se basa en el uso de una combinación (un corticoide inhalado) que no responde a otras terapéuticas son la gran esperanza observan con las otras dos terapéuticas que están en estudio.

Eso es algo que se lleva intentando hacer desde hace un siglo y que aún no ha sido posible. —En el sistema nervioso de hidras –unos cien millones de neuronas se comunican en red, distinguiendo el curso de ríos y verteres desde dentro. —¿Qué investigaciones están desarrollando técnicas para aprender cómo las neurociencias nos permiten alterar y ver la esencia de nuestra mente. —Debemos protegerlos para evitar que con la evolución se pierdan. «Debemos protegerlos para evitar que con la evolución se pierdan.
COMMUNICATION

COMMUNICATION

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.

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.

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 Maria A. Blasco, Laura García Estévez (MD Anderson Cancer Center), María José Alonso Fernández (University of Santiago de Compostela), and Luc Casal (musician, author, songwriter and former cancer patient). September 24, 2018.

Representatives from member institutions of the Severo Ochoa and María de Maeztu Alliance, SOMMa — which encompasses “Severo Ochoa” and “María de Maeztu” 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.
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.

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.

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.”
Institutional Image & Outreach to Society

CNIO & The City
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 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.

“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 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.

“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.”
CNIO Offices

Dean’s Office
CNIO Women In Science Office
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 Rud, 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 Bionotech Therapeutics, presented a powerful example of how to transfer intellectual property from a research centre to a clinical trial. In addition, Gustavo Fuster Olaguibel, from Hoffmann Eitge, 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 Eiderdo 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).

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

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.

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

This award was shared ex aequo by Neihla 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.

“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.”
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 balance between professional and personal life at 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’ graph 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 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, and 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:

- Elisa Martín Garajo, Chief Technology Officer for IBM, Madrid, Spain. Title: ‘Yes, we can’. 28/02/2018.
- Fátima Bosch, Director of the Centre of Animal Biotechnology and Gene Therapy at the Autonoma 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 Autónoma de Barcelona, Spain. Title: ‘La filosofía como instrumento de emancipación’. 24/04/2018.
- Laura González Molero, Ibec38-company Board member and ex-President of Merck-Serono and Bayer HealthCare. Title: ‘Atrévete a ser el rector de tu propias vida’. 05/06/2018.
- Eudalia 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.
Facts & Figures

Scientific Management
- Competitive Funding
- Education and Training Programmes
- Scientific Events

Administration
- Board of Trustees
- Scientific Advisory Board
- Management
- CNIO Personnel 2018

Private Sponsors
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.

“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 divulgence to a non-specialised audience.
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 to disseminate and public outreach. The funding is also used to support other activities related to dissemination and scientific outreach, including corporate sponsorship and promotional ties. 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 7th Framework Programme and Horizon 2020, the Interreg SUDOE Programme, the US National Institutes of Health (NIH), the US Department of Defense (DoD), the International Human Frontier Science Program Organization, the Melanoma Research Alliance (MRA), the Paradifference Foundation, and the Worldwide Cancer Research Foundation. 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 Programme 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 recognized 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 funding, 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.

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, and the US DoD. The national projects are funded by the Department of Defense (DoD), the National Institutes of Health (NIH), the Melanoma Research Alliance (MRA), the Paradifference Foundation, and the Worldwide Cancer Research Foundation.
### INTERNATIONAL GRANTS

#### INDIVIDUAL PROJECTS

##### EUROPEAN COMMISSION

**7TH FRAMEWORK PROGRAMME (2007-2013)**

**EUROPEAN RESEARCH COUNCIL (ERC)**

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fernández-Capetillo, Óscar</td>
<td>ERC Consolidator Grant RSHEALTH: Investigating the causes and consequences of replication stress in mammalian health (Ref.: 67840)</td>
</tr>
</tbody>
</table>

**MARIE CURIE ACTIONS (MCA)**

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peinado, Héctor</td>
<td>WHRI COFUND ADIPOMET: Analyzing the crosstalk of tumor and adipose tissue during metastasis (Ref.: 608765 WHRI-309)</td>
</tr>
<tr>
<td>Plaza, Iván</td>
<td>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-631)</td>
</tr>
</tbody>
</table>

**HORIZON 2020 (2014-2020)**

**EUROPEAN RESEARCH COUNCIL (ERC)**

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbari, Mariano</td>
<td>ERC Advanced Grant THERACAN: Novel therapeutic strategies to treat pancreatic and lung cancer (Ref.: 695566)</td>
</tr>
<tr>
<td>Efeyan, Alija</td>
<td>ERC Starting Grant NutriSenseCryo: The Physiology of Nutrient Sensing by mTOR (Ref.: 638891)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soengas, María S.</td>
<td>METHEL: Long-range-acting drivers of premetastatic niche in melanoma (Ref.: 75442)</td>
</tr>
<tr>
<td>Efeyan, Alija</td>
<td>METLUNK: Identification of links between cancer cell growth and metabolism genes (Ref.: 79407)</td>
</tr>
</tbody>
</table>

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

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

**PERSONAL INVESTIGATOR | PROJECT TITLE**

Llorea, Óscar (Coordinator) | Photochemical trap and high-resolution imaging of transient chromatin complexes from living cells (Ref.: RGP0031/2017) |
### MELANOMA RESEARCH ALLIANCE (MRA)

- **Principal Investigator**: Soengas, María S.
  - **Project Title**: Prognostic and therapeutic impact of lymphovascular niches in melanoma (Ref.: 348673)

- **Principal Investigator**: Valiente, Manuel
  - **Project Title**: Blocking melanoma brain metastasis by targeting the environment (Ref.: 498053)

### PROSTATE CANCER FOUNDATION

- **Principal Investigator**: Olmos, David
  - **Project Title**: Integration of clinical, molecular and biological characteristics to define an aggressive subtype of prostate cancer based on deficient homologous recombination

- **Principal Investigator**: Castro, Elena
  - **Project Title**: Prospective study of lethal prostate cancer clinical and genomic evolution in DNA repair deficient tumours

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

- **Principal Investigator**: Soengas, María S.
  - **Project Title**: Prognostic and therapeutic impact of lymphovascular niches in melanoma (Ref.: 348673)

- **Principal Investigator**: Valiente Cortes, Manuel
  - **Project Title**: Blocking melanoma brain metastasis by targeting the environment (Ref.: 498053)

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

- **Principal Investigator**: Peinado, Héctor
  - **Project Title**: Role of exosomes and Endoglin in Neurofibromatosis Progression (Ref.: W81XWH-16-1-0131)

### CANCER RESEARCH INSTITUTE

- **Principal Investigator**: Valiente, Manuel
  - **Project Title**: Brain-specific strategies to improve responses to immunotherapy (Ref.: 54545)

### BEUG FOUNDATION FOR METASTASIS RESEARCH

- **Principal Investigator**: Valiente, Manuel
  - **Project Title**: Altered brain vessels as a novel target in brain metastasis

---

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

#### Excellence Networks/Redes de Excelencia

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efeyan, Alex (Coordinator)</td>
<td>METABOCANCER: Crosstalk between systemic and cellular metabolism in cancer (Ref.: SAF2016-8975-REDT)</td>
</tr>
<tr>
<td>Fernández-Capetillo, Oscar (Coordinator)</td>
<td>UBIRef: Ubiquitin like proteins in signaling, proliferation and cancer (Ref.: SAF2017-89000-REDT)</td>
</tr>
</tbody>
</table>

#### Challenges-Collaboration Projects/Proyectos Retos-Colaboración

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbacid, Mariano</td>
<td>New approaches for treatment of lung cancer (Ref.: RTC-2017-6576-1)</td>
</tr>
<tr>
<td>Djouder, Nabil</td>
<td>iNCANCER: Desarrollo de nueva terapia antimetastásica en trastornos degenerativos (Ref.: RTC-2019-5435-1)</td>
</tr>
<tr>
<td>Muñoz, Inés</td>
<td>ATTACK: Cancer immunotherapy with bispecific antibodies that engage T-lymphocytes (Ref.: RTC-2017-5344-1)</td>
</tr>
<tr>
<td>Real, Francisco X.</td>
<td>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-2017-6523-1)</td>
</tr>
</tbody>
</table>

### State Research Agency, Ministry of Science, Innovation and Universities


<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbacid, Mariano</td>
<td>A multifaceted approach to target pancreatic cancer (Ref.: GC16173694BARB)</td>
</tr>
<tr>
<td>Malats, Núria</td>
<td>Invasive bladder cancer: towards precision medicine (Ref.: GCB14142293REAL)</td>
</tr>
<tr>
<td>Gómez, Gonzalo</td>
<td>Distinct routes of metastatic dissemination in different melanoma subtypes. Implications in the validation of new tumor biomarkers and therapeutic targets (Ref.: GCB15152978SOEN)</td>
</tr>
<tr>
<td>Valiente, Manuel</td>
<td>Study of the molecular mechanisms involved in primary (glioblastoma) and secondary (metastasis) brain tumors to identify novel therapeutic targets and anti-cancer agents, biomarkers to select treatments and novel non-invasive methods for molecular diagnosis (Ref.: GCTRA16015SEOA)</td>
</tr>
</tbody>
</table>

#### Community of Madrid / Comunidad Autónoma de Madrid

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Shahrour Núñez, Fátima Roncador, Giovanna</td>
<td>Programa LINFOMAS-CM: Linfomas agressivos, análisis clínico y genómico integrado para una medicina de precisión (Ref.: B2017/BMD-3776)</td>
</tr>
<tr>
<td>Blasco, Maria</td>
<td>Programa RyPSE-CM: RNA y proteínas de unión al RNA. Implicaciones en salud y enfermedad (Ref.: B2017/BMD-3730)</td>
</tr>
<tr>
<td>Djouder, Nabil</td>
<td>Programa TomoXliver-CM: Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar (Ref.: B2017/BMD-35817)</td>
</tr>
<tr>
<td>Malumbres Marcos (Coordinator) Barbacid M., Mariano</td>
<td>Programa PRINCIPAL INVESTIGATOR</td>
</tr>
<tr>
<td></td>
<td>Programa LINFOMAS-CM: Linfomas agressivos, análisis clínico y genómico integrado para una medicina de precisión (Ref.: B2017/BMD-3776)</td>
</tr>
<tr>
<td></td>
<td>Programa RyPSE-CM: RNA y proteínas de unión al RNA. Implicaciones en salud y enfermedad (Ref.: B2017/BMD-3730)</td>
</tr>
<tr>
<td></td>
<td>Programa TomoXliver-CM: Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar (Ref.: B2017/BMD-35817)</td>
</tr>
<tr>
<td></td>
<td>Programa PRINCIPAL INVESTIGATOR</td>
</tr>
<tr>
<td></td>
<td>Programa LINFOMAS-CM: Linfomas agressivos, análisis clínico y genómico integrado para una medicina de precisión (Ref.: B2017/BMD-3776)</td>
</tr>
<tr>
<td></td>
<td>Programa RyPSE-CM: RNA y proteínas de unión al RNA. Implicaciones en salud y enfermedad (Ref.: B2017/BMD-3730)</td>
</tr>
<tr>
<td></td>
<td>Programa TomoXliver-CM: Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar (Ref.: B2017/BMD-35817)</td>
</tr>
</tbody>
</table>

#### R&D Activities Programme in Biomedicine

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Shahrour Núñez, Fátima Roncador, Giovanna</td>
<td>Programa LINFOMAS-CM: Linfomas agressivos, análisis clínico y genómico integrado para una medicina de precisión (Ref.: B2017/BMD-3776)</td>
</tr>
<tr>
<td>Blasco, Maria</td>
<td>Programa RyPSE-CM: RNA y proteínas de unión al RNA. Implicaciones en salud y enfermedad (Ref.: B2017/BMD-3730)</td>
</tr>
<tr>
<td>Djouder, Nabil</td>
<td>Programa TomoXliver-CM: Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar (Ref.: B2017/BMD-35817)</td>
</tr>
<tr>
<td>Malumbres Marcos (Coordinator) Barbacid M., Mariano</td>
<td>Programa PRINCIPAL INVESTIGATOR</td>
</tr>
<tr>
<td></td>
<td>Programa LINFOMAS-CM: Linfomas agressivos, análisis clínico y genómico integrado para una medicina de precisión (Ref.: B2017/BMD-3776)</td>
</tr>
<tr>
<td></td>
<td>Programa RyPSE-CM: RNA y proteínas de unión al RNA. Implicaciones en salud y enfermedad (Ref.: B2017/BMD-3730)</td>
</tr>
<tr>
<td></td>
<td>Programa TomoXliver-CM: Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar (Ref.: B2017/BMD-35817)</td>
</tr>
</tbody>
</table>

### Scientific Management | Competitive Funding

#### Madrid+D Foundation / Fundación para el Conocimiento Madrid+D

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dean’s Office for Academic Affairs Soengas, María S.</td>
<td>European Researchers’ Night 2018-2019, organized by Madrid+D Foundation and founded by EU-H2020 Programme. Marie Skłodowska-Curie actions GA 818528</td>
</tr>
<tr>
<td>Malats, Núria</td>
<td>Invasive bladder cancer: towards precision medicine (Ref.: GCB14142293REAL)</td>
</tr>
<tr>
<td>Gómez, Gonzalo Pérez, Héctor Soengas, María S. (Coordinator)</td>
<td>Distinct routes of metastatic dissemination in different melanoma subtypes. Implications in the validation of new tumor biomarkers and therapeutic targets (Ref.: GCB15152978SOEN)</td>
</tr>
<tr>
<td>Valiente, Manuel</td>
<td>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.: GCTRA170013SEOA)</td>
</tr>
</tbody>
</table>

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)
Facts & Figures scientific Management

This Programme is cofunded by the European Regional Development Fund (ERDF) and the State Research Agency.

NATIONAL GRANTS / INDIVIDUAL PROJECTS

RESEARCH PROJECTS IN HEALTH

PRINCIPAL INVESTIGATOR PROJECT TITLE

Benitez, 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 phaeochromocytomas/pheochromocytomas with hypermethylation of their promoters (Ref.: PI15/00785)

Qintilán, Miguel Ángel
Tumor-tolerant immune reprogramming secondary to hypoxia-inducing angiogenesis in breast cancer: physiopathogenic mechanisms and potential therapeutic utility (Ref.: PI16/0035A)

Robledo, Mercedes
Progression-related mechanisms in endocrine and neuroendocrine tumors (Ref.: PI17/01796)

Robledo, Sandra
Ewing Sarcoma Model: induction of the (11;22) translocation in human mesenchymal stem and iPSC 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) + Ewing cells (Ref.: PI15/02303)

Urioste, Miguel
PTEN-hamartoma tumor syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene (Ref.: PI14/00459)

Institute of Health Carlos III / Instituto de Salud Carlos III (ISCIII)

UNIVERSITY / INNOVATION AND MINISTRY OF SCIENCE, STATE RESEARCH AGENCY.

SALUD CARLOS III / CARLOS III / INSTITUTE OF HEALTH NATIONAL GRANTS

The programme is funded by the European Regional Development Fund (ERDF) and the State Research Agency.

NATIONAL GRANTS / INDIVIDUAL PROJECTS

RESEARCH PROJECTS IN HEALTH

PRINCIPAL INVESTIGATOR PROJECT TITLE

Blasco, María
TELOHEALTH: Telomeres and Disease (Ref.: SAF2017-82623-R)

Barbacid, Mariano
PANTHER: A three-prong strategy to fight pancreatic ductal adenocarcinoma (Ref.: SAF2014-85864-R)

Blasco, María
TELOHEALTH: Telomeres and Disease (Ref.: SAF2017-82623-R)

Este documento es financiado por el Fondo Europeo de Desarrollo Regional (FEDER) y la Agencia Estatal de Investigación.

Institute of Health Carlos III / Instituto de Salud Carlos III (ISCIII)

UNIVERSITY / INNOVATION AND MINISTRY OF SCIENCE, STATE RESEARCH AGENCY.

SALUD CARLOS III / CARLOS III / INSTITUTE OF HEALTH NATIONAL GRANTS

The programme is funded by the European Regional Development Fund (ERDF) and the State Research Agency.

NATIONAL GRANTS / INDIVIDUAL PROJECTS

RESEARCH PROJECTS IN HEALTH

PRINCIPAL INVESTIGATOR PROJECT TITLE

Blasco, María
Centre of Excellence "Severo Ochoa" (Ref.: SEV-2015-0510)

Méndez, Juan
REPLICON: Control of eukaryotic DNA replication (Ref.: BFU2016-70402-R)

Muñoz, Javier
EPI-MASS: Epigenetic modifiers in pluripotency: a proteomic analysis of non-histone protein methylation (Ref.: SAF2016-74962-R)

Ortega, Sagrario
EPI-MASS: Epigenetic modifiers in pluripotency: a proteomic analysis of non-histone protein methylation (Ref.: SAF2016-74962-R)

Soengas, María
MEL-STOP: Whole-body imaging of melanoma metastasis as a platform for gene discovery and pharmacological testing (Ref.: SAF2014-86554-R)

Valente, Manuel
Sta3Ca2+ACTIVE: Biology of Sta3+ reactive astrocytes in brain metastasis (Ref.: SAF2014-75243-R)

Wagner, Elin F.
CAPPS2R: Investigating Cancer Risk In Pernicious Anaemia (Ref.: SAF2016-70867-R)

Institute of Health Carlos III / Instituto de Salud Carlos III (ISCIII)

UNIVERSITY / INNOVATION AND MINISTRY OF SCIENCE, STATE RESEARCH AGENCY.

SALUD CARLOS III / CARLOS III / INSTITUTE OF HEALTH NATIONAL GRANTS

The programme is funded by the European Regional Development Fund (ERDF) and the State Research Agency.

NATIONAL GRANTS / INDIVIDUAL PROJECTS

RESEARCH PROJECTS IN HEALTH

PRINCIPAL INVESTIGATOR PROJECT TITLE

Blasco, María
Non-canonical treatment for neurodegenerative diseases: telomerase gene therapy (Ref.: SAF2015-72455-R)

Díaz, Nabil
Thermo-regulation of NAD+ to protect from age-related diseases and cancer (Ref.: SAF2017-92729-R)

Efeyan, Alejo
NUTRIENTOR: Physiology of nutrient sensing and signaling by the mTOR complex I (Ref.: SAF2015-67358-R)

Fernández-Capetillo, Óscar
BREAKINGRAD: Exploring the limits of radiosensitivity in mammals (Ref.: SAF2014-84869-R)

Lleida, Daniel
FUNCTION: Structural studies elucidating the activation mechanism of Focal Adhesion Kinase (Ref.: BFU2016-77665-R)

Lozada, Ana
COHESIN2: Molecular mechanisms of variant cohesion function (Ref.: BFU2016-79645-R)

Malumbres, Marcos
Cycletor: Physiological and therapeutic relevance of mitotic kinases and cell cycle proteins (Ref.: SAF2015-69920-R)

Méndez, Juan
REPLICON: Control of eukaryotic DNA replication (Ref.: BFU2016-70402-R)

Muñoz, Javier
EPI-MASS: Epigenetic modifiers in pluripotency: a proteomic analysis of non-histone protein methylation (Ref.: SAF2016-74962-R)

Ortega, Sagrario
EPI-MASS: Epigenetic modifiers in pluripotency: a proteomic analysis of non-histone protein methylation (Ref.: SAF2016-74962-R)

Orozco, Ana

Peinado, Héctor
EXO-NGFR: Analyzing the role of exosome-derived NGFR during pre-metastatic niche formation (Ref.: SAF2017-82924-R)

Plaza, Iñaki
ESFORET: Structure-function studies of oncogenic RAS and RAF kinases in breast and ovarian cancers: from mechanism of action to targeted therapy (Ref.: BFU2017-8670-R)

Real, Francisco X.
TRANS-PPAC: Transcriptional control of pancreatic cancer development (Ref.: SAF2015-70553-R)

Rodriguez, Cristina
PREDICT: Identification of genetic markers and physiopathological factors predictive of the peripheral neuropathy of paclitaxel and of other oncologic drugs: massive sequencing of candidate genes (Ref.: SAF2015-64850-R)

Soengas, María
MEL-STOP: Vascular trafficking in melanoma progression and treatment response (Ref.: SAF2014-56666-R)

Soengas, María
MEL-STOP: Whole-body imaging of melanoma metastasis as a platform for gene discovery and pharmacological testing (Ref.: SAF2014-86554-R)

Valente, Manuel
RxACTIVE BrainMET: Dissecting the role of reactive astrocytes in brain metastasis (Ref.: SAF2014-75243-R)

Valente, Manuel
Sta3Ca2+ACTIVE: Biology of Sta3+ reactive astrocytes in brain metastasis (Ref.: SAF2014-75243-R)

Wagner, Elin F.
CAPPS2R: Investigating Cancer Risk In Pernicious Anaemia (Ref.: SAF2016-70867-R)
ANNUAL REPORT 2018

Facts & Figures scientiFic ManageM ent

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

CONTRA EL CÁNCER (AECC)
LA ASOCIACIÓN ESPAÑOLA FUNDACIÓN CIENTÍFICA DE "LA CAIXA" BANKING FOUNDATION AND CAIXA CAPITAL RISC

SPANISH NATIONAL CANCER RESEARCH CENTRE, CNIO
FUNDACIÓN BBVA
BBVA FOUNDATION / FUNDACIÓN BBVA
FUNDACIÓN INOCENTE
FUNDACIÓN FERO
ATRESMEDIA CORPORATION
INFRAESTRUCTURAS CIENTÍFICO-TECNOLÓGICAS/
SCIENTIFIC INFRASTRUCTURES/INFRASTRUTTURES CIENTÍFICO-TECNOLÓGICAS/

PRINCIPAL INVESTIGADOR PROJECT TITLE

Squatrito, Massimo "Idea Semilla" Grant: Identification of biomarkers of tumor treating fields (Ref.: BIO2017-9272-EXP)

Lecca, Emilio (until September 2018) UBQREP: Modulation of DNA replication by ubiquitination of chromatin proteins (Ref.: BFU2014-53168-JIN)

Peinado, Héctor Liquid biopsy by nanoplasmonic detection of exosomes: predicting response to (immuno- and radio)-therapy (Ref.: IN18_BBM_TRA_0293)

Rodríguez, Sandra Delivery of functional CRISPR component by pseudotyped virus-like particles (Ref.: BIO2017-9272-EXP)

Rodríguez, Sandra Gene therapy for human cancers driven by fusion genes (Ref.: CI18-00017)

Álvarez, Mónica GPGeoCan: Functional relevance of Greatwall/PP2A pathway in the maintenance of genomic stability: therapeutic implications in cancer (Ref.: SAF2016-60442-JIN)

Valiente, Manuel Predictive biomarkers for brain metastasis in small cell lung cancer (Ref.: EUC2014-51617)

Molina, Mª Esther Evaluación del valor pronóstico de diabetes mellitus tipo II (DM2) en pacientes con cáncer de páncreas (Ref.: PRE18-BBM_TRA_0313)

Malats, Núria Maniobras microbianos para el diagnóstico del adenocarcinoma ductal de páncreas (Ref.: SAF2014-60364-JIN)

Ortega, Ana "Marcos Fernández" Grant: Functional characterization of RagC mutations in Follicular Lymphoma pathogenesis (Ref.: BIO2017-91272-EXP)

Blasco, María Tumour exosome integrins determine organotropic metastasis (Ref.: CICPF18004BLAS)

Fernández-Capetillo, Óscar Premio Constantes y Vitales en la categoría "Joven talento en investigación biomédica" 2015

Squatrito, Massimo Leonardo Grant: Precision glioma mouse models by somatic genome editing (Ref.: CICPF18004BLAS)

Peinado, Héctor Análisis de Hsp90 como una nueva diana en neurofibromatosis (Ref.: CICPF18004BLAS)

Al-Shahrour, Fátima Gene therapy for human cancers driven by fusion genes (Ref.: CI18-00017)

Rodríguez, Sandra Delivery of functional CRISPR component by pseudotyped virus-like particles (Ref.: BIO2017-9272-EXP)

Ortega, Ana "Marcos Fernández" Grant: Functional characterization of RagC mutations in Follicular Lymphoma pathogenesis (Ref.: BIO2017-91272-EXP)

Molina, Mª Esther Evaluación del valor pronóstico de diabetes mellitus tipo II (DM2) en pacientes con cáncer de páncreas (Ref.: PRE18-BBM_TRA_0313)

Malats, Núria Maniobras microbianos para el diagnóstico del adenocarcinoma ductal de páncreas (Ref.: SAF2016-60442-JIN)

Ortega, Ana "Marcos Fernández" Grant: Functional characterization of RagC mutations in Follicular Lymphoma pathogenesis (Ref.: BIO2017-91272-EXP)

Blasco, María Tumour exosome integrins determine organotropic metastasis (Ref.: CICPF18004BLAS)

Fernández-Capetillo, Óscar Premio Constantes y Vitales en la categoría "Joven talento en investigación biomédica" 2015

Squatrito, Massimo Leonardo Grant: Precision glioma mouse models by somatic genome editing (Ref.: CICPF18004BLAS)

Peinado, Héctor Análisis de Hsp90 como una nueva diana en neurofibromatosis (Ref.: CICPF18004BLAS)

Al-Shahrour, Fátima Análisis de Hsp90 como una nueva diana en neurofibromatosis (Ref.: CICPF18004BLAS)

Squatrito, Massimo Leonardo Grant: Precision glioma mouse models by somatic genome editing (Ref.: CICPF18004BLAS)

Peinado, Héctor Tumour exosome integrins determine organotropic metastasis (Ref.: CICPF18004BLAS)

Djouder, Nabil Metabolic Inflammation-Associated IL-17A Causes Non-alcoholic Steatohepatitis and Hepatocellular Carcinoma (Ref.: IN18_BBM_TRA_0313)
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 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

<table>
<thead>
<tr>
<th>Training of PhD students</th>
<th>108</th>
<th>105</th>
<th>110</th>
<th>112</th>
<th>109</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral training</td>
<td>55</td>
<td>48</td>
<td>51</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>Training for MDs</td>
<td>14</td>
<td>25</td>
<td>17</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Laboratory training for MSc/BSc students</td>
<td>73</td>
<td>80</td>
<td>95</td>
<td>99</td>
<td>128</td>
</tr>
<tr>
<td>Laboratory training for technicians</td>
<td>21</td>
<td>27</td>
<td>26</td>
<td>20</td>
<td>15</td>
</tr>
</tbody>
</table>

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, 10% in the Human Cancer Genetics Programme, 3% 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 Fellowship Programme that offered one position to carry out a PhD degree. Thanks to an individual donation received through the ‘CNIO Friends’ platform launched in 2016, the third call of the ‘CNIO Friends’ Predoctoral Fellowship Programme, in 2018, resulted in the recruitment of 4 scientists for a 2-year period each. Also, 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 PhD degree, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract, in 2018, one scientist was able to continue with her PhD degree, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract, in 2018, one scientist was able to continue with her PhD degree, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract, in 2018, one scientist was able to continue with her PhD degree, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract, in 2018, one scientist was able to continue with her PhD degree, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract, in 2018, one scientist was able to continue with her PhD degree, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract, in 2018, one scientist was able to continue with her PhD degree, thanks to a ‘Juegaterapia-CNIO Friends’ Postdoctoral Contract, in 2018, one scientist was able to continue with her PhD degree.
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 (UAM) for the Máster Oficial en Dianas Terapéuticas en Sinalización 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 ORGANISMS 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
- Alejandro Efeyan, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- Thomas Rando, Stanford University, US

SESSIONS

- STEM CELLS AND REGENERATION
- GENOMÈNE INSTABILITY
- NUTRIENTS
- SENERECE
- 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
- Alejandro 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
- 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
- Graham Pawelec, Ecole Polytechnique Fédérale de Lausanne, Switzerland
- Joao Monteiro, The Perelman School of Medicine at the University of Pennsylvania, US
- Mark Sabatini, Institute for Research in Biomedicine, Barcelona, Spain
- Carola García de Vinuesa, The Bellvitge Biomedical Research Centre, Barcelona, Spain
- Guido Kroemer, Erasmus MC Rotterdam, Netherlands
- Cynthia Kenyon, University of California at San Francisco, US
- Jose Antonio Enríquez, Spanish National Cancer Research Centre, Madrid, Spain
- Celeste Rey, Stanford University, US
- Jessica de Vries, Erasmus MC Rotterdam, Netherlands
- Guido Kroemer, Erasmus MC Rotterdam, Netherlands
- Kathleen Collins, University of California at Berkeley, US
- Alejandro 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
- 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

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
- Mercedes Rincon, University of Vermont Medical Center, Burlington, US
- Andrea Schietinger, Memorial Sloan Kettering Cancer Center, NY, US
- Ton Schumacher, The Netherlands Cancer Institute (NKI), 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

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
- Laura Barbfwaj, Icahn School of Medicine at Mount Sinai, New York, US
- Marcus W. Rosenberg, Yale Cancer Center, New Haven, US
- Peter Carmeliet, VIB–EU Leuven Center for Cancer Biology, Belgium
- Thomas Gajewski, The University of Chicago, US
- Carola Garcia 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 University of California at San Francisco, US
- Ton Schumacher, The Netherlands Cancer Institute (NKI), Amsterdam, Netherlands
- Jennifer Wargo, The University of Texas MD Anderson Cancer Center, Houston, US
- Laurence Zitvogel, Gustave Roussy Institute, Villejuif, France
- Mercedes Rincon, University of Vermont Medical Center, Burlington, US
- Andrea Schietinger, Memorial Sloan Kettering Cancer Center, NY, US
- Ton Schumacher, The Netherlands Cancer Institute (NKI), Amsterdam, Netherlands
- 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 & CONFERENCES

The CNIO annually hosts various international meetings and conferences.

RETOS DE LA MEDICINA PERSONALIZADA DE PRECISIÓN EN ESPAÑA
4 JUNE 2018

ORGANISERS
- Fátima Al-Shahrouq, 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-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

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 & Molecular Epidemiology Group, CNIO
- Francisco X. Real, Epithelial Carcinogenesis Group, CNIO

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

CNIOS 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 Martinez, 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

TALLER CEGEN-PB2: 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 Martinez, 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

WORKSHOP DE VESÍCULAS EXTRACELULARES -EXOMAS: BIOLOGÍA Y APLICACIONES EN EL CAMPO DE LA BIOMEDICINA  
7 - 9 NOVEMBER 2018  
ORGANISERS  
· BIOCENBOS - CIBERES - GEIVEX - CIBERONC

SESSIONS  
· Taller A1: "Recolección de Muestras Sólidas"  
· Taller A2: "Análisis de Riesgos y Oportunidades"  
· Taller A3: "Recolección de Muestras Prospective"  
· Taller A4: "¿Qué es un Biobanco?"  
· Taller B1: "Cuidado de Muestras y/o Colaboración Científica"  
· Taller B2: "Poblaciones de Referencia"  
· MISIÓN DE DEBATE: "Intercarcelar con stakeholders externos: Plataformas ISCIII/Biotecs/Pacientes/Instituciones/Salud Pública"  
· Conferencia de Clausura "QUALITY MATTERS: INTERNATIONAL STANDARDS FOR BIOPREPARING"
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.

<table>
<thead>
<tr>
<th>DATE</th>
<th>SPEAKER</th>
<th>ORGANISATION</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>JANUARY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19/01/2018</td>
<td>Antoni Castells</td>
<td>Hospital Clinic of Barcelona, Spain</td>
<td>Clinical management of polyposis and non-polyposis colorectal cancers</td>
</tr>
<tr>
<td>26/01/2018</td>
<td>Andrés Aguilara</td>
<td>Andalusian Center for Molecular Biology and Regenerative Medicine, CABIMER, CSIC, Sevilla, Spain</td>
<td>Interplay between RNA and chromatin in the maintenance of genome integrity</td>
</tr>
<tr>
<td>FEBRUARY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02/02/2018</td>
<td>Raúl Méndez</td>
<td>Institute for Research in Biomedicine (IRB Barcelona), Spain</td>
<td>The CPEB family of RNA-binding proteins, mechanisms of action and new functions in cell-cycle and cancer</td>
</tr>
<tr>
<td>16/02/2018</td>
<td>Jörg Hoheisel</td>
<td>DKFZ German Cancer Research Center, Heidelberg, Germany</td>
<td>Pancreatic cancer: mechanistic insights, personalised diagnostics, and novel therapy options</td>
</tr>
<tr>
<td>23/02/2018</td>
<td>John Rubinstein</td>
<td>The Hospital for Sick Children Research Institute, Toronto, Canada</td>
<td>Electron cryoemicroscopy of rotary ATPases</td>
</tr>
<tr>
<td>26/02/2018</td>
<td>Shirley Kutter</td>
<td>Hebrew University of Jerusalem, Israel</td>
<td>The women of the Start-up Nation</td>
</tr>
<tr>
<td>MARCH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25/03/2018</td>
<td>Kiyoshi Nagai</td>
<td>MRC Laboratory of Molecular Biology, Cambridge, UK</td>
<td>CryoEM snapshots of the spliceosome provide insights into the molecular mechanism of pre-mRNA splicing</td>
</tr>
<tr>
<td>APRIL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06/04/2018</td>
<td>Stefan Kubicek</td>
<td>CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria</td>
<td>Targeting chromatin for cancer cell synthetic lethality</td>
</tr>
<tr>
<td>13/04/2018</td>
<td>Arlene Sharpe</td>
<td>Harvard Medical School, Boston, US</td>
<td>Biology of PD-1 Checkpoint Blockade</td>
</tr>
<tr>
<td>MAY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25/05/2018</td>
<td>Edith Heard</td>
<td>Professor at Collège de France / Institut Curie, Paris, France</td>
<td>Exploring epigenetic dynamics in development and disease using the paradigm of X inactivation</td>
</tr>
<tr>
<td>JUNE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01/06/2018</td>
<td>Kun-Liang Guan</td>
<td>Sanford Consortium for Regenerative Medicine (SCRM) The University of California, San Diego, US</td>
<td>The Hippo pathway in cell growth, organ size, and cancer</td>
</tr>
<tr>
<td>15/06/2018</td>
<td>Roger Lo</td>
<td>Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, US</td>
<td>Strategies to overcome therapeutic resistance in melanoma</td>
</tr>
</tbody>
</table>
### 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-à-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.

<table>
<thead>
<tr>
<th>DATE</th>
<th>SPEAKER</th>
<th>ORGANISATION</th>
<th>TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEPTEMBER</td>
<td>Karim Labib</td>
<td>Sir James Black Centre, School of Life Sciences, University of Dundee, Scotland</td>
<td>Destroying the eukaryotic replisome</td>
</tr>
<tr>
<td>OCTOBER</td>
<td>Rafael Yuste</td>
<td>The Neural Technology Center at Columbia University Biological Sciences, US</td>
<td>Reading the neural code: emergent properties of neural circuits</td>
</tr>
<tr>
<td>NOVEMBER</td>
<td>Nicolas Winsinger</td>
<td>University of Geneva, Switzerland</td>
<td>DNA-templated assemblies and reaction in chemical biology</td>
</tr>
<tr>
<td></td>
<td>Caetano Reis e Sousa</td>
<td>The Francis Crick Institute, London, UK</td>
<td>Dendritic cells in immunity to infection and cancer</td>
</tr>
<tr>
<td>DECEMBER</td>
<td>Jonathan Kipnis</td>
<td>Center for Brain Immunology and GLa (BIG), University of Virginia, Charlottesville, US</td>
<td>Meniscal lymphatics in brain function (and dysfunction)</td>
</tr>
</tbody>
</table>

### SCIENTIFIC EVENTS

<table>
<thead>
<tr>
<th>MARCH</th>
<th>Paul Janne</th>
<th>Dana Farber Cancer Institute and Harvard University, Boston, US</th>
<th>Strategies to prevent and overcome resistance to EGFR inhibitors in lung cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRIL</td>
<td>Doryn Babcock</td>
<td>Imperial College, London, UK</td>
<td>Cryo-EM of the membrane attack complex reveals a flexible nanopore</td>
</tr>
<tr>
<td></td>
<td>Giuseppe Bosso</td>
<td>SAPIENZA University of Rome, Italy</td>
<td>Insights into the epigenetic maintenance of Descemet’s telomeres as revealed by the Hpla - Effete/O6D1 relationship</td>
</tr>
<tr>
<td></td>
<td>Mario Rossi</td>
<td>National Scientific and Technological Research Council (CONCET); Max Planck Partner Institute of Biomedicine of Buenos Aires (BioBA-MSPS), Argentina</td>
<td>Role of protein Ubiquitylation in tumor-cell migration and invasion</td>
</tr>
<tr>
<td></td>
<td>Catharina von-Nicolai</td>
<td>Division of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden</td>
<td>A second DNA binding site in BRCA2 promotes homologous recombination</td>
</tr>
<tr>
<td></td>
<td>Sander van den Heuvel</td>
<td>Institute of Biodynamics and Biocomplexity (IBB); Faculty of Science, Utrecht University, The Netherlands</td>
<td>Cell division, differentiation and tumor formation; insights from C. elegans</td>
</tr>
<tr>
<td></td>
<td>Manuel Saldivia Concepcion</td>
<td>Centre for Immunology &amp; Infection, The University of York, UK</td>
<td>Lighting up parasite development: How I met your kinase</td>
</tr>
<tr>
<td>MAY</td>
<td>Guillaume Bellotier</td>
<td>Centre National de la Recherche Scientifique (CNRS), Montpellier, France</td>
<td>Innovative strategies to isolate CTCs with stemness properties</td>
</tr>
<tr>
<td></td>
<td>Rubén Fernández Buenoálogo</td>
<td>Max-Planck-Institute of Biochemistry, Dept. of Molecular Structural Biology, Martinsried, Germany</td>
<td>Cryo-electron tomography: The cell biology that came in from the cold</td>
</tr>
<tr>
<td></td>
<td>Eri Sakata</td>
<td>Max-Planck-Institute of Biochemistry, Dept. of Molecular Structural Biology, Martinsried, Germany</td>
<td>Conformational landscape of the 26S proteasome gives insights into the gate-opening</td>
</tr>
<tr>
<td></td>
<td>Heinz Neumann</td>
<td>Max-Planck-Institute of Molecular Physiology Dortmund, Germany</td>
<td>Studying chromatin with neo-functionalized proteins</td>
</tr>
<tr>
<td></td>
<td>Miguel Jimenez Alcázar</td>
<td>University Medical Center Hamburg-Eppendorf, Germany</td>
<td>The Role of Neutrophil Extracellular Traps in Thrombosis</td>
</tr>
<tr>
<td></td>
<td>Florian Karthoff</td>
<td>R. Leo Moffitt Cancer Center, Florida, USA</td>
<td>Maximizing melanoma modeling in the mouse</td>
</tr>
<tr>
<td></td>
<td>Ike Rooman</td>
<td>Vrije Universiteit Brussel, Belgium</td>
<td>Pancreatic cancer - where did it go wrong?</td>
</tr>
<tr>
<td>JUNE</td>
<td>Daniel Herranz</td>
<td>Rutgers Cancer Institute of New Jersey, USA</td>
<td>Dissociating NOTCH1-controlled transcriptional and metabolic oncogenic programs in T-ALL</td>
</tr>
<tr>
<td></td>
<td>Mélania Sauri</td>
<td>Karolinska Institutet, Stockholm, Sweden</td>
<td>Finding cancer Achilles’ heels in metabolic pathways</td>
</tr>
<tr>
<td></td>
<td>Luis Álvarez-Vallina</td>
<td>Aarhus University, Denmark</td>
<td>Engineering the Immune System for Enhanced Cancer Immunotherapy</td>
</tr>
</tbody>
</table>
Paweł Kordowiczki  
Institute of Animal Reproduction and Food Research PAS (IARRF) of the Polish Academy of Sciences, Olsztyn, Poland

Maria Ibarra Dauden  
EMBL, Heidelberg - The European Molecular Biology Laboratory, Heidelberg, Germany

ANNUAL REPORT 2018
Facts & Figures scientific Management
Sonia Laín
17/10/2018
Carlos Fernandez
18/09/2018
Fernando Martín
17/07/2018
Israel Sanchez
23/08/2018
Joana Nunes
20/06/2018
Sedeño
Escritora y traductora literaria
Molero
Philosopher, Professor of Ethics
Marte Macho
UPV (University of the Basque Country), Spain
Entre el suelo pegajoso y el techo de cristal: la realidad de las mujeres en el ámbito laboral

In vivo versus? A large animal model for human reproductive aging
Structure basis of the RNA binding on the Elongator sub-complex
Cell fate decisions after DNA damage: location, location, location?
Characterizing the human exposome: from phenotype to genotype at the clinic to the lab and back in 7 days
The Metabolic Needs of Epithelial to Mesenchymal Transition
What do crickets and ribosomes have to do with cancer?
Epithelial tube organization and patterning in development and disease
Mechanisms of Aging and Age-Associated Diseases: Neuroimmunology of Parkinson’s Disease
Dissecting the genetic complexity of embryonic stem cell differentiation
Personalized Oncology for primary tumors and brain metastasis: from the clinic to the lab and back in 7 days
Defining Genetic Drivers of Melanoma and a key step for Precision Medicine
Regulation of inflammation by the TPL-2 complex
The structural basis for regulation of acetyl-CoA carboxylase I
Genetic evolution of cerebral cortex size determinants
IL-17A is pathogenic in CNS autoimmunity by promoting IL-17 production that drives encephalotrigeminal T-cells
Role of the Notch pathway in lung adenocarcinoma: beyond the KrasG12V mouse model
Decisiones raciales e imacionales: una breve historia de cómo llegué a ser escritora

SCIENTIFIC MANAGEMENT

NOVEMBER
06/11/2018
Oriol Galligo  
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
Steve C. Lay  
Imperial College London, UK
Regulation of inflammation by the TPL-2 complex

27/11/2018
Timm Maier  
University of Basel, Switzerland
The structural basis for regulation of acetyl-CoA carboxylase I

29/11/2018
Víctor Borrell  
The Institute of Neurosciences, Alicante, Spain
Gene evolution of cerebral cortex size determinants

29/11/2018
Aoiya McGinley  
School of Biochemistry and Immunology at Trinity College Dublin, Ireland
IL-17A is pathogenic in CNS autoimmunity by promoting IL-17 production that drives encephalotrigeminal T-cells

DECEMBER
03/12/2018
Aline Bozec  
Cytek Biosciences Inc. Fremont, CA, US
Full-spectrum flow cytometry: How new technologies may drive changes in multicolor flow cytometry

12/12/2018
Yacine Kharraz  
University of Basel, Switzerland
HIF’s expression in immune cells regulates Autimmune and Infection Diseases

20/12/2018
Antonio Maraver  
Oncogenic Pathways in Lung Cancer; Institut de Recherche en Cancrologie de Montpellier (IRCM), France
Role of the Notch pathway in lung adenocarcinoma: beyond the KrasG12V mouse model

WOMEN IN SCIENCE SEMINARS

30/01/2018
Laura Ferrero Carballo  
Writer, freelance editor and literary advisor Instituto Vacllo Estepans, Barcelona
Decisions raciales e imacionales: una breve historia de cómo llegué a ser escritora

20/02/2018
Elisa Martín Garrido  
Chief Technology Officer for IBM, Madrid, Spain
Yes, we-can

06/03/2018
Fatima Bosch  
Autonomous University of Barcelona, Spain
Translational Gene Therapy Approaches to Treat Metabolic and Neurodegenerative Diseases

24/04/2018
Victoria Camps  
Philosopher, Professor of Ethics Universidad Autónoma de Barcelona, Spain
La filosofía como instrumento de emancipación

05/06/2018
Laura González Molero  
Consejera Independiente de Atenoex y Eventos
En el lado oscuro del género y la biografía

25/09/2018
Elvira Sadre  
Escrivana y traductora literaria; Writer and literary translator
Escrivanas, más allá del género y la biografía

23/10/2018
Eulàlia Pérez Sedó  
Department of Science, Technology and Society, IE-CCCS CSIC, Madrid, Spain
Conocimiento y reinos de género

11/12/2018
Marta Macho  
UPV (University of the Basque Country), Spain
Entre el sueño pegasiano y el hecho de cristal: la realidad de las mujeres en el ámbito laboral

WASHINGTON, D.C.
CANCER RESEARCH CENTRE: ONCO

ANNUAL REPORT 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.

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.

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.

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

Honorary President

Pedro Francisco Duque
Minister of Science, Innovation and Universities
Ministro de Ciencia, Innovación y Universidades

President

Rafael Rodrigo Montero
General Secretary for Scientific Policy Coordination of the Spanish Ministry of Science, Innovation and Universities
Secretario General de Coordinación de Política Científica del Ministerio de Ciencia, Innovación y Universidades

Vice-President

Raquel Yotti Álvarez
Director of the National Institute of Health Carlos III
Directora del Instituto de Salud Carlos III

Appointed Members

Faustino Blanco González
General Secretary for Health and Consumer Affairs of the Spanish Ministry of Health, Consumer Affairs and Social Welfare
Secretario General de Sanidad y Consumo del Ministerio de Sanidad, Consumo y Bienestar Social

Rosa Menéndez López
President of the Spanish National Research Council (CSIC), as representative of the Spanish Ministry of Science, Innovation and Universities
Presidenta de la Agencia Estatal del Consejo Superior de Investigaciones Científicas, como representante del Ministerio de Ciencia, Innovación y Universidades

Borja Luis Cabezón Royo
Director of the Department of National Affairs of the Cabinet of the Presidency of the Government
Director del Departamento de Asuntos Nacionales del Gabinete de la Presidencia del Gobierno

Margarita Blázquez Herranz
Deputy Director General for Networks and Cooperative Research Centres of the National Institute of Health Carlos III
Subdirectora General de Redes y Centros de Investigación Cooperativa del Instituto de Salud Carlos III

Carlos Pesquera González
Head of Cabinet of the Healthcare Counsellor of the Government of Cantabria
Jefe de Gabinete de la Consejera de Sanidad del Gobierno de Cantabria

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 Avelanedo, General Director / Francisco González Rodriguez, 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 Escrivano
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 Biology  
  Cambridge, United Kingdom

- **Lauri A. Aaltonen, MD, PhD**  
  Academy Professor  
  Director, Genome Scale Biology Research Programme  
  Biomedicum, University of Helsinki  
  Helsinki, Finland

- **Geneviève Almouzni, PhD**  
  Director, Institut Curie Research Centre  
  Head of Nuclear Dynamics & Genome Plasticity Unit  
  Institut Curie, Paris, France

- **J. Michael Bishop, MD**  
  Chancellor Emeritus and Professor in the Dept. of Microbiology and Immunology  
  Director of the G.W. Hooper Research Foundation  
  University of California at San Francisco  
  San Francisco, USA

- **José Costa, MD, FACP**  
  Professor of Pathology and of Orthopaedics and Rehabilitation  
  Director of the Translational Diagnostics and the Musculoskeletal Tumor Programs  
  Yale University School of Medicine  
  New Haven, USA

- **Sara Courtneidge, PhD, DSc (hc)**  
  Associate Director for Translational Sciences, Knight Cancer Institute  
  Professor, Departments of Cell, Developmental and Cancer Biology and Biomedical Engineering  
  Oregon Health & Science University  
  Portland, USA

- **John F.X. Diffley, PhD**  
  Associate Research Director  
  The Francis Crick Institute  
  London, United Kingdom

- **Stephen Frye, PhD**  
  Director, Center for Integrative Chemical Biology and Drug Discovery  
  Fred Eshelman Distinguished Professor  
  The University of North Carolina at Chapel Hill  
  Chapel Hill, USA

- **Denise Galloway, PhD**  
  Associate Division Director, Human Biology Division at Fred Hutchinson Cancer Research Center  
  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

- **Ángela Nieto, PhD**  
  Full Professor and Head of the Developmental Neurobiology Unit  
  Neuroscience Institute of Alicante (CSIC-UMH)  
  Alicante, Spain

- **Josep Tabernero, MD PhD**  
  Director, Vall d’Hebron Institute of Oncology (VHIO)  
  Head, Medical Oncology Department of Vall d’Hebron University Hospital  
  Barcelona, Spain

- **Alfred Wittinghofer, PhD**  
  Emeritus Group Leader  
  Department of Structural Biology  
  Max Planck Institute for Molecular Physiology (MPI)  
  Dortmund, Germany

- **Ada E. Yonath, PhD**  
  Director, the Helen and Milton A. Kimmel Center for Biomolecular Structure and Assembly  
  Martin S. and Helen Kimmel Professor of Structural Biology  
  Weizmann Institute of Science  
  Rehovot, Israel
# CNIO Personnel 2018

## Distribution by Programmes

<table>
<thead>
<tr>
<th>Programme</th>
<th>Personnel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural Biology</td>
<td>47</td>
</tr>
<tr>
<td>Biotechnology</td>
<td>88</td>
</tr>
<tr>
<td>Cancer Cell Biology</td>
<td>35</td>
</tr>
<tr>
<td>Human Cancer Genetics</td>
<td>45</td>
</tr>
<tr>
<td>Clinical Research</td>
<td>74</td>
</tr>
<tr>
<td>Molecular Oncology</td>
<td>117</td>
</tr>
<tr>
<td>Experimental Therapeutics</td>
<td>29</td>
</tr>
</tbody>
</table>

## Distribution by Professional Category

<table>
<thead>
<tr>
<th>Category</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral fellows</td>
<td>37</td>
<td>21</td>
</tr>
<tr>
<td>Graduate students</td>
<td>303</td>
<td>103</td>
</tr>
<tr>
<td>Staff scientists</td>
<td>69</td>
<td>46</td>
</tr>
<tr>
<td>Principal investigators</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>Technicians</td>
<td>16</td>
<td>21</td>
</tr>
</tbody>
</table>

## Gender Distribution

- **Total CNIO Personnel: 435**
- **Research: 87%**
- **Administration: 15.9%**
- **Technicians: 40%**
- **Principal Investigators: 11%**
- **Staff Scientists: 16%**
- **Graduate Students: 24%**
- **Post-doctoral fellows: 9%**
- **Gender Distribution**
  - **Female: 339 (66%)**
  - **Male: 178 (34%)**

## Age Distribution

- **18-30: 93**
- **31-40: 147**
- **41-50: 137**
- **50+: 63**

## Gender Distribution in Senior Academic and Management Positions

<table>
<thead>
<tr>
<th>Position</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Leaders, Heads of Clinical Research Unit/Section</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Heads of Unit/Biobank</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Scientific Directors, Heads of Area</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Management Directors, Heads of Area</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

## Gender Distribution by Professional Category

<table>
<thead>
<tr>
<th>Category</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral fellows</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Graduate students</td>
<td>70</td>
<td>68</td>
</tr>
<tr>
<td>Staff scientists</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Principal investigators</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>Technicians</td>
<td>41</td>
<td>23</td>
</tr>
<tr>
<td>Total Scientific Personnel</td>
<td>180</td>
<td>155</td>
</tr>
</tbody>
</table>
**DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH**

- **POST-DOCTORAL FELLOWS**: 27 (6%)
- **GRADUATE STUDENTS**: 67 (16%)
- **STAFF SCIENTISTS**: 31 (7%)
- **PRINCIPAL INVESTIGATORS**: 22 (5%)
- **TECHNICIANS**: 52 (12%)
- **TOTAL 100%**: 199 (46%)

**TOTAL SCIENTIFIC PERSONNEL**: 435

---

**DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH**

- **POST-DOCTORAL FELLOWS**: 8 (2%)
- **GRADUATE STUDENTS**: 32 (7%)
- **STAFF SCIENTISTS**: 27 (6%)
- **PRINCIPAL INVESTIGATORS**: 11 (2%)
- **TECHNICIANS**: 41 (9%)
- **TOTAL 100%**: 119 (27%)

**TOTAL SCIENTIFIC PERSONNEL**: 435

---

**DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION**

- **POST-DOCTORAL FELLOWS**: 2 (1%)
- **GRADUATE STUDENTS**: 4 (1%)
- **STAFF SCIENTISTS**: 11 (2%)
- **PRINCIPAL INVESTIGATORS**: 13 (3%)
- **TECHNICIANS**: 87 (20%)
- **TOTAL 100%**: 117 (27%)

**TOTAL SCIENTIFIC PERSONNEL**: 435

---

**SCIENTIFIC PERSONNEL: NATIONAL ORIGIN**

- **SPANISH**: 386 (88.74%)
- **REST OF EUROPE**: 32 (7.36%)
- **AFRICA**: 9 (2.07%)
- **ASIA**: 7 (1.61%)
- **OTHER**: 1 (0.23%)
- **TOTAL SCIENTIFIC PERSONNEL**: 435

**FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY**

- **POST-DOCTORAL FELLOWS**: 10 (21%)
- **GRADUATE STUDENTS**: 21 (43%)
- **STAFF SCIENTISTS**: 17 (35%)
- **TECHNICIANS**: 8 (17%)

**DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN**

- **SPANISH**: 386 (88.74%)
- **FRANCE**: 4 (0.91%)
- **AMERICA**: 2 (0.46%)
- **AUSTRIA**: 2 (0.46%)
- **PORTUGAL**: 5 (1.16%)
- **TOTAL SCIENTIFIC PERSONNEL**: 435

Percent values represent percentages of foreign employees of the total CNIO personnel in each category.
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 important 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.

The Fundación CRIS is dedicated to the promotion and development of research with the aim of eliminating the serious health threat of cancer. Fundación CRIS generously supports 3 research groups at the CNIO: the Prostate Cancer Clinical Research Unit (CRU), headed by David Olmos; the Breast Cancer CRU, headed by Miguel Quintela; and the H12O-CNIO Haematological Malignancies CRU, led by Joaquín Ballesteros. 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 María A. Blasco and Óscar Fernández-Capetillo.

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.

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.

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: Fundación Juegaterapia, Fundación Inocente Inocente, Fundación Española de Hematología y Hemoterapia, 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.
## CNIO Friends

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNIO Friends</td>
<td>249</td>
</tr>
<tr>
<td>CNIO Arte</td>
<td>250</td>
</tr>
<tr>
<td>‘Juegaterapia-CNIO Friends’ postdoctoral contract</td>
<td>251</td>
</tr>
<tr>
<td>A meeting starred by our ‘Friends’</td>
<td>252</td>
</tr>
<tr>
<td>Benefactor Friends/Sponsor Friends</td>
<td>253</td>
</tr>
<tr>
<td>Donations to the CNIO</td>
<td>255</td>
</tr>
</tbody>
</table>
"‘CNIO Friends’ places us on society’s visible spectrum.”

CNIO Friends postdoctoral contract was funded, leading to the incorporation of a young researcher, Miquel 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.

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 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.

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.
→ Sponsor Friends
- Asociación Bandera Rosa
  Algeciras, Cádiz
- Asociación de Mujeres Afectadas de cáncer de mama ROSAE
  Valdepeñas, Ciudad Real
- Colectivo de afectados “El árbol de la Vida”
  Las Pedroñeras, Cuenca
- Compañía Logística de Hidrocarburos CLH, S.A.
- Freesia Group
  Salou, Tarragona
- Fundación Inocente Inocente
  Madrid, Madrid
- Fundación Juegaterapia
  Madrid, Madrid
- Javier Campos
  Granada, Granada
- María Josefa Azcona Peribañez
  Madrid, Madrid
- Petroplast
  Logroño, La Rioja
- Santa Lucía Seguros
  Madrid, Madrid

Last but not least, we would also like to extend our heartfelt thanks to all the anonymous benefactors who have donated their legacies to support cancer research at the CNIO, in doing so they have contributed to society for generations to come.

Donations to the CNIO

- **1,533,000€** total CNIO donations
- **879,000€**
  - 2018: 371,000€
  - 2017: 261,000€
  - 2016: 156,000€
  - 2015: 91,000€
- **371,000€**
  - Total CNIO friends donations in 2018
  - (100,000€ from the Juegaterapia Foundation)
- **654,000€**
  - Legacies
  - 2018: 296,000€
  - 2017: 28,000€
  - 2016: 330,000€

* 453,000€ 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 Sofia 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 22nd 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.
cnoiō stop cancer