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“Commercialisation and return on investment in research remain priorities. CNIO’s royalty income in 2016 is double that of 2012. A total of 44 inventors, about 10% of the researchers at CNIO, have contributed and benefited from this achievement.”

MARIA A. BLASCO
Director
This year, once again, the data on CNIO’s scientific performance illustrate that we are doing a superb job contributing towards scientific breakthroughs. During 2016, the CNIO authored a total of 239 papers, 28 of which were published in journals with impact factors (IF) ranging from 10 to 15, and 32 papers in journals with impact factors greater than 15. Comparison with previous years (2006-2016) indicates that CNIO has continued to increase the numbers of papers published in top journals with IF greater than 15.

With the aim of upholding the CNIO’s strategy to remain an international Research Centre of Excellence and an influential institution in cancer research worldwide, 2016 saw the establishment of a CNIO Department of International Affairs. This new Department is headed by Carolina Pola who was the Editor of Nature Medicine for over seven years, as well as the former Director of Communications at Pharmamar, the most successful Spanish pharma company devoted to anti-tumour therapies. Since the creation of the Department of International Affairs, Carolina has redefined our international approach to embed the European concept of ‘Responsible Research and Innovation’ (RRI) in our institutional and research core. This new perspective, alongside several other efforts made by the Department, have resulted in several international collaborations that will bear fruit next year in the form of joint scientific symposiums to strengthen our research groups, as well as a plan to boost our recruitment of international talent. Our capacity to attract and retain scientific talent, providing new investigators with an interdisciplinary and multicultural environment, has become an integral part of our international agenda, which we will continue to nurture in the upcoming years.

I am also happy to mention that we continue to attract new and talented young group leaders to the CNIO. After the incorporation, in 2015, of two Junior Groups working in metastasis (led by Hector Peinado and Manuel Valiente) in the Molecular Oncology Programme, we welcomed another new Junior Group to the Programme in 2016: the Metabolism and Cell Signalling Group, led by Alejo Efeyan who joined the CNIO at the beginning of 2016.
During 2016, we also created a new service unit at the CNIO: the Biological Text Mining Unit, which is focused on the application and development of biomedical text mining technologies that are becoming a key tool for the efficient exploitation of information contained in unstructured data repositories. This Unit is fully funded through the ‘Plan de Impulso de las Tecnologías del Lenguaje de la Agenda Digital (PITL)’, in the framework of an agreement (‘encomienda’) between the Secretary of State of Telecommunications of the Spanish Ministry of Energy, Tourism and Digital Agenda (MINETAD) and the CNIO. We also revised the organisation of the two Bioinformatics Units that were providing support and expertise to our investigators, namely, the Bioinformatics Unit of the Structural Biology and Biocomputing Programme, and the Translational Bioinformatics Unit of the Clinical Research Programme. Under a new single structure, the CNIO Bioinformatics Unit, headed by Fátima Al Shahrour, we have joined the efforts of both previous units in order to better serve the needs of the CNIO Research Groups and to better coordinate and manage bioinformatics projects.

The valorisation of the research results generated by CNIO’s scientists, with the aim of turning them into high-potential diagnostic or therapeutic products and services, is one of our ways of conducting high-impact science. During the year, the CNIO has contributed to biobanking benefit through improving cancer patient outcomes, in particular. Óscar Shahrour, we have joined the efforts of both previous units in order to better serve the needs of the CNIO Research Groups and to better coordinate and manage bioinformatics projects.

Industrial partnerships remain key to achieve valorisation of scientific knowledge. Valorisation is not just about ‘money’, but also about the impact that can be created through successful alliances with industry. In such collaborations, both the CNIO and its industrial counterpart are committed to working towards a common goal and to jointly undertaking all the steps needed to conduct the research, as well as to identifying the best possible protection and commercialisation of the results. In 2016, new contracts with industry secured future revenues for collaborative research that amount to nearly 4 million euros, which represents about 10% of CNIO’s annual income.

The CNIO External Scientific Advisory Board (SAB), currently chaired by Mariann Bienz, is of utmost importance for guiding the strategic plans of the CNIO as well as for the review of our research groups. We would like to wholeheartedly thank our current SAB members Jean Massagut (Memorial Sloan Kettering Cancer Center, New York, USA) for his committed dedication to the CNIO SAB that lasted for over a decade (2003-2016). We wholeheartedly thank our former SAB member Joan Massagut (Memorial Sloan Kettering Cancer Center, New York, USA) for his dedicated commitment to the CNIO SAB that lasted for over a decade (2003-2016). We also appreciate and thank the CNIO SAB from 2013 to 2014, and as member for the remaining 9 years. During 2016, we welcomed two new members to our SAB, namely Stephen Frye, Director of the Centre for Integrative Chemical Biology and Cancer Research at the University of North Carolina Eshelman School of Pharmacy in Chapel Hill (USA); and Ada E. Yonath, Nobel Prize winner in Chemistry (2009) and Director of the Helen and Milton A. Kimmel Center for Biomolecular Structure and Assembly at the Weizmann Institute of Science in Rehovot (Israel).

I would like to take this opportunity to thank all those who helped the CNIO by sponsoring our students, postdoctoral programmes or our research projects at the CNIO and the IR Business School course, the La Caixa Foundation for supporting the Visiting Scientists Programme and the Dean’s Office. During 2016, we hosted Patrick Sung, Head of YouTube Partnerships in Southern Europe and Russia, Margery Resnick, Professor at the Massachusetts Institute of Technology in Cambridge (USA), and President of the International Institute in Madrid, Pilar García, Oncologist at the Instituto Ramón y Cajal de Investigación Sanitaria (IRYCYC), Ramón y Cajal University Hospital, Madrid; Edurne Pasaban, Mountaineer, Tolosa, Spain; María Teresa Fernández de la Vega, President of Women for Africa Foundation (Fundación Mujeres por África), and former First Deputy Prime Minister of Spain; Tania Balló, documentalist and film director, Barcelona, Spain; and Francisco Juan Martínez Mojica, Professor at the University of Alicante, Spain; and Francisco J. Ayala, an evolutionary biologist and philosopher at the University of California, Irvine, USA. The Ramón y Cajal Foundation also sponsored one of the WISE seminars, given by Edurne Pasaban, Mountaineer, Tolosa, Spain. Furthermore, I would like to highlight the work that is being carried out at the CNIO Women and Science (WISE) Office. During 2016, we had the pleasure of listening to Maria Concepción Ferreras, Head of YouTube Partnerships in Southern Europe and Russia, Margery Resnick, Professor at the Massachusetts Institute of Technology in Cambridge (USA), and President of the International Institute in Madrid, Pilar García, Oncologist at the Instituto Ramón y Cajal de Investigación Sanitaria (IRYCYC), Ramón y Cajal University Hospital, Madrid; Edurne Pasaban, Mountaineer, Tolosa, Spain; Maria Teresa Fernández de la Vega, President of Women for Africa Foundation (Fundación Mujeres por África), and former First Deputy Prime Minister of Spain; Tania Balló, documentalist and film director, Barcelona, Spain; and Francisco Juan Martínez Mojica, Professor at the University of Alicante, Spain; and Francisco J. Ayala, an evolutionary biologist and philosopher at the University of California, Irvine, USA. The Ramón y Cajal Foundation also sponsored one of the WISE seminars, given by Edurne Pasaban, Mountaineer, Tolosa, Spain. Furthermore, I would like to highlight the work that is being carried out at the CNIO Women and Science (WISE) Office. During 2016, we had the pleasure of listening to Maria Concepción Ferreras, Head of YouTube Partnerships in Southern Europe and Russia, Margery Resnick, Professor at the Massachusetts Institute of Technology in Cambridge (USA), and President of the International Institute in Madrid, Pilar García, Oncologist at the Instituto Ramón y Cajal de Investigación Sanitaria (IRYCYC), Ramón y Cajal University Hospital, Madrid; Edurne Pasaban, Mountaineer, Tolosa, Spain; Maria Teresa Fernández de la Vega, President of Women for Africa Foundation (Fundación Mujeres por África), and former First Deputy Prime Minister of Spain; Tania Balló, documentalist and film director, Barcelona, Spain; Angeles Gonzalez-Sinde, scriptwriter, film director and former Spanish Minister of Culture; and Christina Rosenwaike, Spanish singer-songwriter, actress and producer. Together with Belén Yuste and Sonnina L. Rivás Caballero, from Rocavera Eventos, Madrid, we also organised and hosted the CNIO Exhibition ‘M creative’, a travelling exhibition, tour companies benefit through improving cancer patient outcomes, in particular. Óscar Fernández Capetillo, Vice-Director of Translational Research and Director of Innovation since 2015, has been leading these key strategic areas for the CNIO, enhancing translational research at CNIO in collaboration with the CNIO Experimental Therapeutics Programme. In 2016, we leveraged on public-private partnerships in order to bring our research results closer to the patient. A project based on Dr. Djouder’s findings that boosting research at CNIO in collaboration with the CNIO Experimental Therapeutics Programme, one focused on Pim kinase inhibitors and another on ATR inhibitors, both are very close to reaching the clinical testing stage.

Commercialisation and return on investment in research remain priorities. Royalty income and milestone payments collected in 2016 raised more than 600 thousand euros. This includes revenues from patent licences as well as from commercialisation of research tools such as monoclonal antibodies. Following CNIO’s policy of royalty revenue share, this income flows back to the CNIO’s research activities as well as to the inventors themselves. A total of 44 inventors, about 10% of the researchers at CNIO, have contributed and benefited from this achievement.

The valorisation of the research results generated by CNIO’s scientists, with the aim of turning them into high-potential diagnostic or therapeutic products and services, is one of our ways of conducting high-impact science. During the year, the CNIO has contributed to biobanking benefit through improving cancer patient outcomes, in particular. Óscar Fernández Capetillo, Vice-Director of Translational Research and Director of Innovation since 2015, has been leading these key strategic areas for the CNIO, enhancing translational research at CNIO in collaboration with the CNIO Experimental Therapeutics Programme. In 2016, we leveraged on public-private partnerships in order to bring our research results closer to the patient. A project based on Dr. Djouder’s findings that boosting research at CNIO in collaboration with the CNIO Experimental Therapeutics Programme, one focused on Pim kinase inhibitors and another on ATR inhibitors, both are very close to reaching the clinical testing stage.

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ORGANISATION OF RESEARCH

MÁRINA A. BLASCO DIRECTOR

ALFONSO VALENCIA VICE-DIRECTOR OF BASIC RESEARCH

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Manuel Serrano Programme Director

Manuel Serrano
Tumour Suppression Group

Mariano Barbacid
Experimental Oncology Group

Ari S. Blanco
Telomeres and Telomerase Group

Marcos Malumbrea
Cell Division and Cancer Group

Óscar Fernández-Capetillo
Genomic Instability Group

Ana Losada
Chromosome Dynamics Group

Juan Mendoza
DNA Replication Group

Maria S. Soria
Melanoma Group

Héctor Pinedo
Microenvironment and Metastasis Junior Group

Manuel Valiente
Brain Metastasis Junior Group

Alejo Eftehian
Metabolism and Cell Signalling Junior Group

CLINICAL RESEARCH PROGRAMME

Manuel Hidalgo
Programme Director

Manuel Hidalgo (until December)
Gastrointestinal Cancer Clinical Research Unit

Miguel Quintela-Fandino
Breast Cancer Junior Clinical Research Unit

David Olmos
Prostate Cancer Junior Clinical Research Unit

Luis Paz-Ares
H12O-CNIO Lung Cancer Clinical Research Unit

BIOBANK

Manuel M. Morente
Director

ÓSCAR FERNÁNDEZ-CAPETILLO VICE-DIRECTOR OF TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Javier Benitez Programme Director

Javier Benitez
Human Genetics Group

Mercedes Robledo
Breast Endocrine Cancer Group

Núria Malats
Genetic and Molecular Epidemiology Group

MIGUEL URIOSTE
Familial Cancer Clinic Unit

JUAN C. CIGUDA
Molecular Genomics and Genome Editing Unit

CLINICAL RESEARCH PROGRAMME

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Miguel Quintela-Fandino
Breast Cancer Junior Clinical Research Unit

David Olmos
Prostate Cancer Junior Clinical Research Unit

Luis Paz-Ares
H12O-CNIO Lung Cancer Clinical Research Unit

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Erwin F. Wagner
Growth, Development and Disease Group

Francisco X. Real
Epithelial Carcinogenesis Group

Mira Pérez-Moreno
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Alfonso Valencia Programme Director

Alfonso Valencia
Structural Computational Biology Group

Daniel Lärka
Cell Signalling and Adhesion Junior Group

Santiago Ramón-Maíques
Structural Bases of Genome Integrity Junior Group

Ramin Campos-Olivas
Spectroscopy and Nuclear Magnetic Resonance Unit

Nabil Djouder
Growth Factors, Nutrients and Cancer Junior Group

Massimo Squatrito
Soro Ballesteros Foundation-CNIO Brain Tumour Junior Group

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Joaquin Pastor Programme Director

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Biology Section

EXPERIMENTAL THERAPEUTICS PROGRAMME

Anabel Sanz Director

Diego Megías
Genomic Core Unit

Javier Muñoz
Proteomics Core Unit

Alba De Martino
Histopathology Core Unit

Íñigo Blanco
Animal Facility (Vivotecnia Management & Services)

TECHNOLOGY TRANSFER AND VALORISATION OFFICE
Vice-Direction of Basic Research

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“My main activity, as Vice-Director for Basic Research, is to work together with CNIO’s Basic Research Groups in order to enhance scientific excellence and foster collaboration.”

There have been several encouraging developments this year pertaining to the organisational aspects within the CNIO’s Basic Research domain. 1 Junior Group Leader, Alejo Efeyan, joined the CNIO to conduct a new line of research in the area of metabolism and cell signalling in cancer; the two previous Units that provided support in Bioinformatics and Translational Bioinformatics were reorganised and merged into one single Bioinformatics Unit headed by Fátima Al-shahrour (it has already started operations in collaboration with a number of CNIO Groups); the inclusion of CNIO’s protein production facility (previously integrated in the Proteomics Unit) in the Crystallography Unit, now called the Crystallography and Protein Engineering Unit; and the recruitment of Salvador J. Capella to lead the National Bioinformatics Institute (INB-ISCIII) Unit.

This constructive trend will be further strengthened in the coming year thanks to the creation of the Biological Text Mining Unit in the area of Computational Biology, headed by Martin Krallinger (this new Unit will become operational at the beginning of 2017); and the new recruitments in strategic areas of biochemistry-structural biology and electron microscopy - protein complexes. Furthermore, the increase of collaborations with internal and external groups, as well as the consolidation of the projects developed with the Experimental Therapeutics Programme, will also lead to a further bolstering of our overall scientific output.
MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO Programme Director

It is my pleasure to introduce the highlights of the Molecular Oncology Programme in 2016. First of all, my enthusiastic and warm welcome to Alejo Efeyan, who joined the CNIO early this year to lead the Metabolism & Cell Signalling Junior Group. Alejo is a brilliant young scientist who trained as a postdoctoral fellow with David Sabatini, at the Massachusetts Institute of Technology, Cambridge. Cancer cells are metabolically hyperactive and an exciting discovery in recent years has been the realisation that cancer cells have mutations in the pathways that detect nutrient availability. Understanding how the nutrient sensing mechanisms contribute to cancer is the main goal of the Metabolism & Cell Signalling Junior Group. Alejo’s outstanding career and his original project have been awarded with a prestigious and generous grant from the European Research Council. His Group is now settled and fully operative. We are all very proud of having him here with us and we wish him all the best!

It is also very gratifying that the two other Junior Groups that joined the CNIO during 2015 have continued to successfully consolidate their teams and their projects throughout 2016. The Brain Metastasis Junior Group, led by Manuel Valiente, now has promising candidate small compounds that inhibit metastasis initiation in brain slices. Likewise, the Microenvironment & Metastasis Junior Group, led by Héctor Peinado is making impressive progress towards detecting how the vesicles shed by tumours (known as exosomes) are distributed throughout the organism, modifying it, and thereby making it more receptive for metastatic seeding.

In the following pages, you will read about several surprising discoveries that expand our understanding of cancer and that might pinpoint new therapeutic strategies in the near future. For example, a protein that regulates mRNA stability, CEBP4, and contributes to cancer progression (Melanoma Group, Nat. Commun. 2016); or telomere-derived transcripts that play a key role in chromosomal integrity through the stabilisation of telomeres (Telomeres & Telomerase Group, Nat. Commun. 2016).

“Congratulations to Marcos Malumbres and Óscar Fernández-Capetillo for being elected as members of the European Molecular Biology Organization (EMBO).”

Other discoveries concern basic mechanisms that are altered in cancer. For example, it has been found that the infliction of damage to tissues triggers cell plasticity in the surrounding cells and that cytokine IL-6 is a key factor in this process (Tumour Suppression Group, Science 2016). You will also read about a rather unique DNA polymerase named PrimPol (DNA Replication Group), about how cohesins regulate transcription, an unsuspected role for these proteins traditionally involved in sister chromatid cohesion (Chromosome Dynamics Group), and about a kinase named MASTL that is upregulated in cancer and its inhibition blocks the proliferation of some cancer cells (Cell Division & Cancer Group).

The identification of preclinical anti-cancer treatments is the ultimate goal of the Molecular Oncology Programme. In this regard, we are proud of two relevant contributions: a novel therapeutic approach for lung adenocarcinoma based on two inhibitory molecules that are effective even against aggressive cancers lacking p53 (Experimental Oncology Group, Nat. Med. 2016); and the identification of compounds that block DNA repair with therapeutic efficacy against acute myeloid leukaemia (Genomic Instability Group, Sci. Signal. 2016).
OVERVIEW

Tumour suppressors are genes that can prevent the development of cancer. All our cells have a functional set of these genes, but they can become defective over time. The affected cells thus become partially unprotected and, combined with additional mutations in other genes, can give rise to cancer. Understanding how tumour suppressor genes work may help us to design drugs that block cancer. Tumour suppressor genes are now known to control many aspects of cell biology and organismal physiology, such as cellular pluripotency, cell senescence, and metabolism. We aim to achieve an integrated understanding of cancer protection.

Our goals are to:

→ Understand the mechanisms of tumour suppression and identify new tumour suppressor regulators.
→ Study the interplay between tumour suppression and ageing.
→ Analyse the involvement of tumour suppressors in the regulation of metabolism and protection from metabolic damage.
→ Characterise cellular senescence as a tumour suppression mechanism.
→ Investigate cellular pluripotency and the involvement of tumour suppressors in the process of reprogramming to induced pluripotent stem (iPS) cells.
→ Explore the role of cell plasticity in cancer, tissue regeneration, and ageing.
→ Search for new frontiers in cell plasticity.

“We have found that damaged cells secrete factors that promote reparative activities in the surrounding cells, including loss of differentiation and plasticity. This could be beneficial to repair the damaged tissues, but it could also favour the expansion of dormant cancer cells.”
New tumour markers for the prognosis of head and neck cancer

Head and neck cancers include a heterogeneous group of tumours located in the oral cavity, pharynx and larynx. The survival rate of patients with this pathology has hardly improved over the last decade. Stratification of patients has been limited, until now, to a clinical classification and not a molecular one. Analysis of patients’ biopsies showed that about half of them possess high levels of the p21 protein, while study of mTOR protein levels can be associated with head and neck cancer. The mechanisms by which mTOR and p21 are involved in the development of cancer are not fully understood.

Phosphorylation of mTOR by mTORC1 disrupts the 4E-BP/p21 complex ensuring stable p21 degradation and, this is associated to more aggressive squamous cancers. 4E-BP binds to p21 and promotes p21 accumulation, and this is associated to less aggressive squamous cancers.

Antioxidant defences delay ageing and age-related diseases

Accumulation of cell damage plays an important role in ageing. There is no clear answer about which types of cellular damage are more relevant for ageing. Although the accumulation of oxidative damage with ageing is undisputed, the large majority of attempts to prove that oxidative damage is relevant for ageing have failed. All these attempts, however, have manufactured only one component of the complex network of antioxidant defences. In contrast to these previous attempts, we have approached this issue by increasing the levels of NADPH, a simple co-factor required for almost all antioxidant reactions and whose levels are known to determine the global antioxidant capacity of cells. To achieve this, we generated transgenic mice with an increased expression throughout their bodies of glucose–6-phosphate dehydrogenase (G6PD), one of the most important enzymes for the production of NADPH. We found that G6PD transgenic mice have overall higher levels of NADPH and, consequently, a better protection against oxidative damages. Importantly, these mice are not predisposed to cancer and, indeed, have a modest increase in longevity. These observations point to novel strategies to delay age-related diseases, including cancer.

Senescence cells provide critical signals for cellular reprogramming

The mechanisms involved in the reprogramming of differentiated cells inside a living organism remain to be elucidated. Senescence is a cellular response to damage characterised by an abundant production of cytokines and other secreted factors that, together with the recruitment of inflammatory cells, results in tissue remodelling. We have shown that in vivo expression of the reprogramming factors OCT4, SOX2, KLF4 and cMYC (OSKM) triggers two divergent cellular outcomes: most cells undergo senescence, while other cells undergo reprogramming, both occurring in close physical association. OSKM-induced senescence requires the tumour suppressor locus Inksa/Arf, which, via the production of the cytokine IL6, creates an optimal tissue environment for in vivo reprogramming. We concluded that tissue injury or ageing, through cellular senescence and cytokine IL6, favour in vivo reprogramming through the accumulation of senescent cells.

RESEARCH HIGHLIGHTS

Figure 1. Model of mTOR–mediated regulation of p21. Non-phosphorylated 4E-BP binds to p21 and promotes p21 degradation, and this is associated to more aggressive cancers. 4E-BP1 phosphorylation by mTOR disrupts the 4E-BP/p21 complex ensuring stable p21 accumulation, and this is associated to less aggressive cancers.
OVERVIEW

KRAS oncogenes have been implicated in about one fifth of all human cancers including lung and pancreatic adenocarcinomas, 2 of the tumour types with the worst prognosis. Unfortunately, identification of suitable therapies to treat these tumours remains elusive and patients are still treated with cytotoxic compounds approved over 2 decades ago. The recent discovery that these tumours display intra-tumour heterogeneity adds another layer of complexity that needs to be addressed. Hence, our laboratory has decided to search for novel therapeutic targets that may contribute to the early stages of lung tumour development, hoping that these targets will be present in all tumour cells – including cancer initiating cells and cancer stem cells – and not only in limited populations of evolving clones. In addition, we have continued our quest to validate known targets (mainly those of the MAPK and PI3K pathways) using genetically engineered mouse tumour models with the ultimate goal of establishing rational combination therapies that may provide significant therapeutic benefits in the clinic.

Significance

→ We have shown that human lung tumours respond efficiently to combinations of DDR1 and NOTCH inhibitors in PDX models.
→ We have provided a mechanistic explanation for the exclusive presence of K-RAS or EGFR mutations in human lung adenocarcinomas.
→ We have demonstrated that the different incidence of H-RAS and K-RAS oncogenes in human tumours is due to the signalling intensity of their respective oncoproteins.
Identification of novel therapeutic targets for the treatment of K-Ras driven lung adenocarcinoma

The recent discovery that lung tumours display significant levels of clonal heterogeneity (Govindan, Science, 2014) implies that effective therapies must target early oncogenic events/alterations present in all tumour cells and not only in clonal variants that appear during tumour development. To provide potential solutions to this key issue we decided to search for novel therapeutic targets present in the earliest stages of lung tumour development, expecting that such targets will be present in the entire tumour population including the putative cancer initiating stem cells. Among the most highly expressed druggable genes we identified Drd1, a locus that encodes a tyrosine protein kinase receptor. As reported early this year (Ambrogio et al., Nat Med, 2016), genetic and pharmacological inhibition of Drd1 prevented progression of K-Ras driven p53 wild type, but not p53 mutant tumours. Yet concomitant inhibition of Drd1 and Notch, a downstream mediator of Drd1 activity, led to a significant anti-tumour effect even in aggressive K-RasG12V, p53 mutant adenocarcinomas. More importantly, this treatment induced regression of K-Ras, p53 mutant patient-derived lung ortho-xenografts (PDX) with a therapeutic efficacy superior to standard chemotherapy. Identification of additional targets present in these early K-Ras mutant driven lung cancer cells should expand the therapeutic opportunities to treat K-Ras mutant tumours in the clinic, thus by-passing the challenges derived from the development of intra-tumour heterogeneity.

Lack of selective advantage for lung cells expressing K-Ras and EGFR oncogenes

Activating mutations in KRAS and EGFR, the 2 most frequent oncogenic drivers in human lung adenocarcinoma, occur in a mutually exclusive manner suggesting functional redundancy and implying lack of positive selection. By means of a mouse model engineered to induce expression of mutant EGFR (Ambrogio et al., 2016), in advanced tumours driven by a resident KrasG12V oncogene, we show that, instead, their co-expression is detrimental for the progression of lung adenocarcinoma. In vivo expression of EGFR in KRAS-driven tumours triggers an immediate response with hallmarks of replicative stress resulting in apoptosis. Yet, a fraction of tumour cells survive, but enter a transient cystostatic state incompatible with tumour development that is fully reversible upon discontinuation of EGFR expression. Ultimately, continuous co-expression of both mutants results in the attenuation of the overall oncogenic signalling to levels compatible with cell proliferation and tumour growth. In sum, our results indicate that the mutual exclusivity of KRAS and EGFR activating mutations occurs as a combination of cellular toxicity and signal attenuation that results in the lack of selective advantage for those cells expressing both oncogenes.

Whereas the wild type H-Ras and K-Ras proteins are bioequivalent, their oncogenic isoforms H-RasL858R and K-RasG12V induce different tumour spectra

We have provided genetic evidence demonstrating that the H-Ras and K-Ras proteins are fully bioequivalent in mice. Previous studies have shown that replacement of the K-Ras alleles by H-Ras coding sequences resulted in viable mice (Potenza et al., EMBO Rep, 2005). Yet, these mice displayed cardiovascular defects. Now, we have shown that these defects were due to the presence of the 4 H-Ras expressing alleles in these mice. Ablation of the 2 endogenous H-Ras alleles, hence generating mice that only express the H-Ras protein from the 2 targeted K-Ras alleles, is absolutely normal.

These results appear to be at variance with the well-established observation that H-Ras and K-Ras oncogenes are involved in different human tumour types. To determine whether the oncogenic versions of the H-Ras and K-Ras proteins are also bioequivalent, we knocked-in H-RasG12V oncogene sequences into the K-Ras locus. Germline expression of H-RasG12V or K-RasG12V from the K-Ras locus resulted in equal embryonic lethality. However, their expression in adult mice led to different tumour phenotypes. Whereas H-RasG12V elicited papillomas and haematopoietic tumours, K-RasG12V induced lung tumours and gastric lesions. The reason why H-RasG12V expression failed to cause lung tumours is due to the induction of a senescence-like state due to excessive MAP kinase signalling. Likewise, H-RasG12V but not K-RasG12V induced oncogene-induced senescence in mouse embryonic fibroblasts (MEFs). Label-free quantitative assessment revealed that minor differences in H-RasG12V expression levels led to drastically different biological outputs, suggesting that subtle differences in MAP kinase signalling influence the differential tumour spectra induced by RAS oncogenes.
OVERVIEW

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

Our research aims are:

- Generating mouse models to validate telomeres and telomerase as therapeutic targets for cancer and age-related diseases.
- Deciphering the interplay between telomeres and DNA repair pathways.
- Studying the role and regulation of non-coding telomeric RNAs or TERRA.
- Testing telomerase gene therapy in ‘telomere syndromes’ and age-related diseases.
- Elucidating the role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

“‘We have demonstrated that TERRA long non-coding RNAs are essential for telomere protection.’”
Fighting aplastic anaemia using a therapy designed to delay ageing

Aplastic anaemia is a rare, potentially fatal disease of the blood, by which the bone marrow is unable to generate blood cells at the appropriate pace. The disease can be hereditary or acquired and develops at any stage of life. A subgroup of the inherited form is caused by replicative impairment of haematopoietic stem and progenitor cells owing to very short telomeres due to mutations in telomerase and other telomere components. An abnormal telomere shortening is also described in cases of acquired aplastic anaemia. We have tested the efficacy of our telomerase gene therapy, originally designed to delay ageing, in two independent mouse models of aplastic anaemia due to short telomeres. We found that a high dose targets the bone marrow compartment, including haematopoietic stem cells. Telomerase treatment following telomere attrition in bone marrow cells rescues aplastic anaemia and mouse survival. Improved survival is associated with a significant increase in telomere length in peripheral blood and bone marrow cells, as well as improved blood counts. Our telomerase gene therapy represents a novel therapeutic approach to the treatment of aplastic anaemia.

Telomere length is genetically determined, but in the past we were able to generate mouse embryonic stem (ES) cells with telomeres twice the size of normal ones. We now have used such ES cells with ‘hyper-long’ telomeres, traceable thanks to the co-expression of green fluorescent protein (GFP), to generate chimeric mouse containing cells with both hyper-long and normal telomeres. We showed that chimeric mice contain GFP-positive cells – bearing hyper-long telomeres – in all mouse tissues (FIGURE 1), display normal tissue histology, as well as normal survival. Both hyper-long and normal telomeres shorten with age, but GFP-positive cells manage to retain longer telomeres than the mice age. These chimeric mice also accumulate fewer cells with short telomeres and less DNA damage with age, and express lower levels of p53. Cells with long telomeres are longitudinally maintained or enriched with age in highly renewing compartments (i.e. blood). We demonstrated that mice with functional, longer and better preserved telomeres can be generated without the need for genetic manipulations, such as telomerase overexpression.

Telomere RNAs are essential to maintain telomeres

Despite their especially compact structure, which is difficult to access, telomeres transcribe information like the rest of the DNA generating long non-coding RNAs known as TERRA. Deciphering the role of TERRA was one of the unsolved issues of telomere biology in the past decade. This was, in part, due to a lack of knowledge on the TERRA loci, which had prevented functional genetic studies. We had already shown that mouse TERRA arise from the Xp locus, on the contrary, does not lead to decreased TERRA levels. The deletion of the 20q locus, which resulted in a dramatic decrease in TERRA levels. The deletion of the Xp locus, on the contrary, does not lead to decreased TERRA levels. These findings demonstrate that, although human TERRA arise from two loci, the 20q locus is the main origin of human TERRA. Thus, both in mice and in humans, TERRA arise from one or at most two loci (FIGURE 2). Ablation of 20q TERRA in human cells results in a dramatic loss of telomere sequences and in the induction of a massive DNA damage response. These latter findings represent the first demonstration, in any organism, of the essential role of TERRA in the maintenance of telomeres.

Telomeres are transcribed from the human 20q and Xp subtelomeres. We used the CRISPR-Cas9 technology to delete the 20q locus, which resulted in a dramatic decrease in TERRA levels. The deletion of the Xp locus, on the contrary, does not lead to decreased TERRA levels. These findings demonstrate that, although human TERRA arise from two loci, the 20q locus is the main origin of human TERRA. Thus, both in mice and in humans, TERRA arise from one or at most two loci (FIGURE 2). Ablation of 20q TERRA in human cells results in a dramatic loss of telomere sequences and in the induction of a massive DNA damage response. These latter findings represent the first demonstration, in any organism, of the essential role of TERRA in the maintenance of telomeres.

**RESEARCH HIGHLIGHTS**

- Fighting aplastic anaemia using a therapy designed to delay ageing
- Telomere RNAs are essential to maintain telomeres
- Ablation of 20q TERRA in human cells results in a dramatic loss of telomere sequences and in the induction of a massive DNA damage response.
- These latter findings represent the first demonstration, in any organism, of the essential role of TERRA in the maintenance of telomeres.

**REFERENCES**

1. Telomere length is genetically determined, but in the past we were able to generate mouse embryonic stem (ES) cells with telomeres twice the size of normal ones. We now have used such ES cells with ‘hyper-long’ telomeres, traceable thanks to the co-expression of green fluorescent protein (GFP), to generate chimeric mouse containing cells with both hyper-long and normal telomeres. We showed that chimeric mice contain GFP-positive cells – bearing hyper-long telomeres – in all mouse tissues (FIGURE 1), display normal tissue histology, as well as normal survival. Both hyper-long and normal telomeres shorten with age, but GFP-positive cells manage to retain longer telomeres than the mice age. These chimeric mice also accumulate fewer cells with short telomeres and less DNA damage with age, and express lower levels of p53. Cells with long telomeres are longitudinally maintained or enriched with age in highly renewing compartments (i.e. blood). We demonstrated that mice with functional, longer and better preserved telomeres can be generated without the need for genetic manipulations, such as telomerase overexpression.

2. Telomere RNAs are essential to maintain telomeres

- Despite their especially compact structure, which is difficult to access, telomeres transcribe information like the rest of the DNA generating long non-coding RNAs known as TERRA. Deciphering the role of TERRA was one of the unsolved issues of telomere biology in the past decade. This was, in part, due to a lack of knowledge on the TERRA loci, which had prevented functional genetic studies. We had already shown that mouse TERRA arise from the Xp locus, on the contrary, does not lead to decreased TERRA levels. The deletion of the 20q locus, which resulted in a dramatic decrease in TERRA levels. The deletion of the Xp locus, on the contrary, does not lead to decreased TERRA levels. These findings demonstrate that, although human TERRA arise from two loci, the 20q locus is the main origin of human TERRA. Thus, both in mice and in humans, TERRA arise from one or at most two loci (FIGURE 2). Ablation of 20q TERRA in human cells results in a dramatic loss of telomere sequences and in the induction of a massive DNA damage response. These latter findings represent the first demonstration, in any organism, of the essential role of TERRA in the maintenance of telomeres.

**PUBLICATIONS**


**AWARDS AND RECOGNITIONS**

- Miguel Catalan Cancer Achievement Award, Regional Government of Madrid. Scientific Committee Member, Innovative Medicines Initiative 2 (IMI2) Joint Under- taking, Brussels, Belgium.
- Member of the External Scientific Board of Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, Spain.
- Member, Alumni Advisory Board, University of Alicante.
- Founding Editor, Cell Stress.
- Editorial Board Member, Nutrition & Healthy Aging.
OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated. During the past few 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 the function of microRNAs in cell biology and tumour development, and iv) to understand how progenitor cells and cancer stem cells control their self-renewal and proliferative properties. As a final goal, we aim to generate information that may be useful for improving therapeutic strategies against cancer cell proliferation.

“In 2016, we investigated the relevance of several mitotic regulators during cancer progression and therapy, with special focus on kinases that are currently under preclinical and clinical evaluation.”
Controlling the proper number of cell divisions

The mammalian cell cycle is regulated by at least 2 families of inhibitors, the INK4 and Cip/Kip proteins. While elimination of individual members of these families is a frequent finding in human cancer, the consequences of eliminating this inhibitory mechanism in mammalian cells have not yet been explored. Using a combination of mutant alleles in the mouse, we have now observed that a major physiological function of cell cycle inhibitors is to prevent replicative stress. In a mouse model insensitive to INK4 proteins and deficient in p27<sup>INK4A</sup> and p27<sup>INK4B</sup>, we have observed that these inhibitors prevent the accumulation of DNA damage due to replicative stress in different tissues including the nervous system. Ablation of these inhibitors prevents mouse development. This effect is most likely due to hyperactivation of cyclin-dependent kinases as the replicative stress can be prevented by slightly inhibiting the enzymatic activity of these proteins (Quereda et al., 2016).

Cell cycle kinases as new targets for cancer therapy

Cell cycle progression is controlled by phosphorylation events and cell cycle kinases are currently the focus of multiple therapeutic strategies. Inhibitors of the Aurora and Polo-like kinases are evaluated in clinical trials with promising results, at least in haematopoietic malignancies. Over the last few years, we have generated mouse models with specific mutations in these kinases in order to understand their roles in different tissues and cell types. Our recent data have uncovered an unexpected function of Polo-like kinase 1 (Plk1) in the cardiovascular system, a role that we are studying in detail in order to understand possible toxicities derived from the use of Plk1 inhibitors in patients.

A relatively new serine/threonine kinase, known as MASTL (or Greatwall in flies and Xenopus), has been characterised as a critical node in cell division. We have previously shown that MASTL is essential for mouse embryonic development and cell cycle progression (Figure). This is due to mitotic collapse after nuclear envelope breakdown (NEB). MASTL is exported from the nucleus to the cytoplasm in a CRM1-dependent manner before NEB. Once at the cytoplasm, Greatwall inhibits the PP2A-B55 complexes to maintain the mitotic state. These data indicate that some breast cancer cells require MASTL kinase activity for proliferation, suggesting that a subset of breast tumours may benefit from strategies aimed at inhibiting this kinase. We are currently studying the consequences of inhibiting MASTL in the activity of the PP2A-phosphatase. Since MASTL specifically inhibits PP2A-B55 complexes, we are also characterising the relevance of the B55 family members present in the human genome.

Over the past few months, we have tested this hypothesis by studying the relevance of MASTL in tumour cell proliferation and its possible use as a cancer target. In collaboration with Miguel Quintela’s Group at the CNIO and Carlos Caldas at Cancer Research UK, we analysed MASTL expression in breast cancer. Our data suggest that this protein is overexpressed in a significant number of hormone-positive and -negative tumours and correlates with poor prognosis. In collaboration with researchers at Pfizer, we used different RNAi and CRISPR techniques to analyse the effect of MASTL knockdown or knockout in breast cancer cells both in vitro and in vivo. These data indicate that some breast cancer cells require MASTL kinase activity for proliferation, suggesting that a subset of breast tumours may benefit from strategies aimed at inhibiting this kinase. We are currently studying the consequences of inhibiting MASTL in the activity of the PP2A-phosphatase. Since MASTL specifically inhibits PP2A-B55 complexes, we are also characterising the relevance of the B55 family members present in the human genome.

![Figure](image)

The proper balance between cell cycle kinases and phosphatases is crucial, not only for cell cycle progression but also for the structure and function of different tissues.

- **Publications**

- **Awards and Recognition**
  - Elected EMBD Member.
Our laboratory has centred its research on trying to understand how cells respond to ‘replicative stress’ (RS), which is frequent in cancer and induced by several anticancer agents. In mammals, RS is suppressed by a signalling cascade initiated by ATR and CHK1 kinases. Throughout the years, our laboratory has developed a wide battery of cellular and animal tools for the study of RS. These tools include mice with enhanced or limited ATR-CHK1 function, cell lines in which the pathway can be activated at will, and chemical inhibitors of the ATR kinase. Our studies have revealed the impact of RS on cancer and ageing, have led to drugs that can be used to test our ideas on cancer therapy, and have also unveiled the mechanisms by which these drugs kill cancer cells. Altogether, our main aim is to understand how genome maintenance is safeguarded – particularly during replication – and to exploit this knowledge as a way to fight against cancer.

“During 2016, we have investigated which tumour types could best benefit from a treatment with ATR inhibitors, the potential mechanisms of resistance to these drugs, as well as new pathways that suppress RS.”
RESEARCH HIGHLIGHTS

Efficacy of ATR inhibition in two preclinical models of cancer

Repetitive stress (RS) is a widespread phenomenon in cancer cells that, when persistent, leads to DNA double-strand breaks and genomic instability. Besides from the basal level of RS that occurs in every cell division, the presence of oncogenes, or many of the agents used in chemotherapy, are potent inducers of RS. In mammals, RS is sensed and suppressed through a signalling cascade that is initiated with the activation of the ATR kinase. In mammals, RS is sensed and suppressed through a signalling cascade that is initiated with the activation of the ATR kinase. We previously hypothesised that due to the high levels of RS in certain cancers, they could be particularly dependent on a proficient RS response. In this regard, and in collaboration with the Experimental Therapeutics Programme, we have developed chemical inhibitors of ATR that presented some anti-tumour properties in vitro. During 2016, our work in this area was focused on the identification of tumours that are particularly sensitive to ATR inhibition, as well as on the discovery of mechanisms of resistance to these chemicals. For the first area of focus, we have shown efficacy of ATR inhibitors, as monotherapy, in 2 mouse models of Ewing Sarcoma and Acute Myeloid Leukaemia (FIGURE 1). Regarding the second area of focus, we discovered – via genomewide CRISPR-Cas9 screening – that the levels of CDC23A, a key phosphatase controlling mitotic entry, are a key determinant of the sensitivity to ATR inhibitors in mouse and human cells.

Two new players that suppress replication stress in mammalian cells

Besides ATR, several other factors participate in limiting the impact of RS in mammalian cells. Recent works have identified that POLD3, a subunit of the DNA polymerase Polδ, participates in the repair of the breaks generated by RS, and also suggest that limiting its activity could be specifically deleterious for cancer cells. By developing a novel conditional knockout mouse strain we found that POLD3 deletion is lethal during embryonic development and also when depleted in adult mice. These severe defects were explained by a complete destabilisation of the POLG complex in the absence of POLD3, which abrogates DNA replication, raising serious doubts regarding the potential of POLD3 as an anticancer target. In independent work, we have been investigating how SUMO and ubiquitin participate in the coordination of DNA replication. Here, we identified USP7 as the first chromatin-associated SUMO deubiquitinase (SUMDUB) and revealed its essential role during DNA replication.

• PUBLICATIONS


• AWARDS AND RECOGNITION

- Elected Member of the European Molecular Biology Organization (EMBO).
Our research focuses on a protein complex named cohesin that is essential for chromosome organisation. Cohesin mediates sister chromatid cohesion and, thereby, ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. It 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, organisation of DNA replication factories and locus rearrangement by recombination. Mutations in cohesin have recently been found in several tumour types, most prominently in bladder cancer and acute myeloid leukaemia. Mutations in cohesin and its regulatory factors are also at the origin of a group of human 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 in somatic cells. We use mouse models carrying knockout alleles of genes encoding cohesin subunits to investigate their functional specificity, both at the cellular level and in the context of an organism. We also take advantage of the Xenopus egg cell-free system to explore additional aspects of cohesin regulation.

“We aim to define the specific contributions of cohesin-SA1 and cohesin-SA2 to genome organisation. Our work may uncover vulnerabilities in cancer cells carrying mutations in the gene encoding SA2, which is one of the twelve genes most mutated in cancer.”
Cohesin-S2A regulates transcription independently of CTCF

Cohesin consists of four core subunits, SMC1, SMC3, RAD21 and SA. There are two versions of the SA subunit in vertebrate somatic cells, SA1 and SA2. We have previously reported that cohesin-SA1 is required for telomere cohesion, while cohesin-SA2 plays a major role in centromeric cohesion. In terms of transcriptional regulation, much less is known about the potential differences between these two complexes. A study in HeLa cells reported a similar genomic distribution for cohesin-SA1 and cohesin-SA2 that largely overlapped with the distribution of the architectural protein CTCF. Our initial comparison of distribution and transcription profiles of wild type and SA1-null mouse cells suggested that cohesin-SA1 could be more important for the regulation of transcription than cohesin-SA2. First, we detected an almost double number of cohesin-binding sites genome-wide in SA1 null cells, and the new sites displayed reduced overlap with CTCF and TSS (transcriptional start sites). Second, we found that in a number of genes whose expression was altered in SA1 null cells, cohesin-SA2 could not replace cohesin-SA1 efficiently since cohesin occupancy was clearly reduced in these cells (Remesero, Cuadrado et al., 2012). When we analysed chromatin states in adult mouse brain using histone marks, CTCF and cohesin distribution, we noticed again that cohesin-SA1 complexes co-occurred with CTCF at active enhancers and promoters (Cuadrado et al., 2015). More recently, using a new and better SA2 antibody, we examined the binding of cohesin-SA1 and cohesin-SA2 along the genome of human mammary epithelial cells. We found more than 20,000 positions common for both complexes in which CTCF is also present, but also found around 10,000 cohesin-SA2 specific positions, lacking SA1, in which the overlap with CTCF is significantly lower. Importantly, these cohesin-SA2 specific positions are highly enriched in enhancers and promoters and contain transcription factor binding motifs other than CTCF. Proteomic analysis of immunoprecipitates obtained with SA1 and SA2 antibodies detected several transcription factors among the SA2-specific interactors. Functional analyses, in which we compare changes in gene expression, cohesin distribution as well as chromatin architecture changes after downregulation of SA1, SA2 or CTCF, further support a prominent role of cohesin-SA2 in promoting local interactions between cis-regulatory elements independently of CTCF. While cohesin-SA1 would instead collaborate with CTCF in the demarcation of domain boundaries (FIGURE 1).

Pds5 proteins regulate cohesin dynamics

Two factors are associated with chromatin-bound cohesin, Pds5 and Wapl. Wapl promotes cohesin unloading and in its absence there is an excess of cohesin on chromatin, and chromosome organisation is altered both in interphase and mitosis. The role of Pds5 is less clear. Moreover, there are two versions of Pds5 present in vertebrate cells: Pds5A and Pds5B. In order to explore their specific functions we previously generated murine knock out (KO) alleles for these two genes. We showed that both Pds5A and Pds5B contributed to cohesion establishment during S phase by promoting cohesin acetylation and Sororin binding, with Pds5B being specifically required for cohesion at centromeres (Carretero et al. 2013). Now we have observed that cells lacking Pds5A, Pds5B, or both, have distinct alterations in their transcriptomes when compared to wild type cells. Genome wide distribution of cohesin is not obviously altered in the absence of either Pds5 protein, but does change in the absence of both. Under this condition, the dynamic association of cohesin to chromatin, measured in Fluorescence Recovery After Photobleaching (FRAP) experiments, is significantly decreased. Much milder effects are observed in cells lacking only Pds5A or Pds5B. Abrupt accumulation of cohesin in axial structures, known as vermicelli, previously described in Wapl depleted cells, can be observed only in the absence of the two Pds5 proteins (FIGURE 2). Thus, Pds5 proteins are required for proper cohesin dynamics and cohesin distribution, although no clear specificities can be found for Pds5A and Pds5B, at least globally. The gene expression differences described above might therefore be caused by preferential interactions with chromatin regulators at specific loci. It is also possible that Pds5 proteins have functions independent of cohesin. We are currently exploring both these possibilities.
Our laboratory studies the molecular mechanisms that underlie genomic duplication in mammalian cells. The ‘replisome’ complex in charge of DNA replication encounters natural obstacles (e.g. unusual DNA structures, collisions with transcription proteins), as well as exogenous challenges such as ionising radiation, UV light and chemicals that modify the DNA structure and block DNA polymerases. The situations in which replication forks are forced to slow down, stall or collapse are generically referred to as replicative stress (RS). Our Group investigates the ‘DNA damage tolerance’ pathways that facilitate DNA replication in the presence of RS or damaged DNA. In recent years, we have identified 2 mechanisms that counteract RS: (1) the conditional activation of dormant replication origins; (2) the participation of PrimPol, a primase-polymerase enzyme, in the restart of stalled forks. We continue to characterise the different cellular responses to RS (FIGURE 1).

“We have developed genetic tools to investigate the physiological impact of defective DNA replication, including mouse strains that suffer from a high incidence of haematological cancers due to their inefficient response to DNA damage during replication.”

DNA REPLICATION GROUP

Juan Méndez
Group Leader

Post-Doctoral Fellow

Sara Rodríguez

Graduate Students

Marcos Díaz, Daniel González, Karolina Jodkowska, Sergio Muñoz
**RESEARCH HIGHLIGHTS**

**Cellular functions of PrimPol protein**
We have continued to characterise PrimPol, a DNA primase-polymerase that participates in DNA damage tolerance during chromosomal replication. With this aim in mind, we have generated human and mouse cells in which PrimPol expression is either downregulated or completely ablated. PrimPol-deficient cells display a marked sensitivity to UV irradiation, including the accumulation of unrepaired Thy dimers (CPDs and 6-4pp) in the DNA. The skin of PrimPol KO mice also presents an inefficient healing response to UV irradiation and a higher frequency of benign papillomas. The importance of PrimPol as a tumour suppressor gene is currently being investigated.

**Effects of DNA re-replication in vivo**
Cdc6 and Cdt1 proteins are responsible for the loading of MCM2-7, the DNA helicase, at replication origins. After Cdc6 and Cdt1 execute their ‘origin licensing’ functions in the G1 phase, their activities are inhibited until mitosis is complete in order to prevent origin reactivation and DNA over-replication within the same cell cycle. However, these strict control mechanisms may be partially overridden in some cancer types, notably non-small cell lung carcinomas, by the overexpression of Cdc6 and/or Cdt1 genes.

We have recapitulated the deregulated expression of Cdc6 and Cdt1 using mouse strains that allow the inducible expression of both proteins, alone or in combination. While individual deregulation of Cdc6 or Cdt1 has only mild effects, their combination is lethal for developing embryos and also for adult individuals. Using single-molecule analysis of DNA replication, high-throughput confocal microscopy and histopathology, we have identified origin re-firing events that are sufficient to cause DNA over-replication and DNA damage in different tissues. These mouse models will allow a complete study of the physiological impact of DNA re-replication in vivo.

**Evidence for replicative stress in early embryonic cell cycles**
Replicative stress is normally studied in the context of cells undergoing external challenges. However, it also occurs in the unperturbed G1 phase when the replication machinery reaches special DNA structures (e.g. G-quadruplexes) that are difficult to replicate, or when it collides with a transcriptional fork. In 2016, we participated in a collaborative study led by Dr M. Lopes (University of Zurich, Switzerland), that identified unexpected levels of physiological RS. Mouse embryonic stem cells and early embryos by the blastocyst stage display a constitutive accumulation of RPA-covered ssDNA, fork slowing and fork remodelling events, all hallmarks of RS. These characteristics are related to the short duration of the G1 phase in embryonic stem cells and are lost upon the onset of cell differentiation. This result underscores the importance of the G1 phase to fully repair DNA that had been damaged in the previous cell cycle, before entering a new round of replication (Ahuja et al., 2016).

**Single-molecule analyses of DNA replication**
As replicative stress impinges on many cellular processes, the possibility of analysing DNA replication at the single-molecule level continues to attract the interest of many research groups at the CNIO and other institutions. In 2016, we collaborated in two projects led by Oscar Fernández-Capetillo (CNIO Genomic Instability Group) to demonstrate that USP7 ubiquitin protease targets SUMO and is essential for DNA replication (Lecona et al., 2016), and that PolD3 is haplosufficient for DNA replication in mice (Murga et al., 2016). In the latter project, the analyses of replication in stretched DNA fibres revealed a striking accumulation of asymmetric forks in the absence of PolD3, a regulatory subunit of DNA polymerase δ (Figure 2). Finally, a collaboration with R. Freire (Hospital Universitario de Canarias, Tenerife) revealed a novel function for USP7 ubiquitin protease in the control of DNA replication (Hernández-Pérez et al., 2016).

**Publications**
OVERVIEW

Melanomas are inherently aggressive cancers for which basic and translational research have significantly improved patient prognosis. Nevertheless, clinical responses are still incomplete. The long-term goals of our Group are to identify new progression biomarkers and therapeutic agents. Focusing on stress response programmes involving apoptosis, autophagy and endosome mobilisation, we have discovered lineage-specific oncogenes that define the melanoma ‘fingerprint’. Transcriptomic and proteomic analyses of the melanoma secretome have enabled us to define how tumour cells remodel the (lymph)angiogenic vasculature and avoid immune recognition. Moreover, we have generated a unique set of animal models for non-invasive imaging of melanoma progression in vivo. These systems have led to the validation of nanoparticle-based treatments that are currently being tested in clinical trials. Our ultimate objective is to improve the management of patients with otherwise refractory metastatic melanomas.

“We have identified oncogenic cascades that are uniquely deregulated in melanoma and as such, may represent novel targets for therapeutic intervention.”
CNSI Melanoma Group: objectives and model systems

Melanomas are aggressive solid tumours and provide a prime example of how integrated basic and clinical research have significantly improved patient prognosis. Yet, despite great success with targeted and immune-based therapies, sustained clinical responses are still limited. Moreover, the field lacks molecular markers of diagnosis, and the knowledge of how melanomas progress and metastasise is still largely incomplete. Therefore, these questions represent key unmet needs, as emphasised by a committee of experts in which M. Soengas participates (Merlino et al., Pigment Cell Melanoma Res 2016). In addition, one of the main hurdles slowing progress 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 areas of research (FIGURE 1):

→ **Aim 1. Oncogenic pathways, which are selectively deregulated in melanoma and may represent new diagnostic indicators**

→ **Aim 2. Risk factors and prognostic markers that underlie the unique ability of melanoma to metastasise from seemingly thin lesions**

→ **Aim 3. Animal models that allow for non-invasive monitoring of premetastatic niches, and as such, may serve as a platform for cost-effective genetic and pharmacological screens.**

LINEAGE-SPECIFIC ONCOGENIC DEPENDENCIES IN MELANOMA

One of the long-term objectives of the Melanoma Group is the discovery of new melanoma drivers. We previously identified a cluster of endosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Curbelo et al., Cancer Cell 2014 and Oncotarget 2015). In collaboration with the group of P. Aguinis (University of Leuven, Belgium), we further explored therapeutically-relevant regulatory mechanisms and functions of the endosomal machinery in different cell types (Marti et al., FEBS J 2016). More recently, we also discovered unique features of autophagy (another key lysosomal-associated process) in melanoma. Employing human melanoma biopsies, combined with newly-generated mouse models, we identified selective heterozygous losses of ATG5 as a new risk factor for melanoma progression and as a main mediator of the resistance to targeted therapy (García-Fernández et al., Autophagy 2016).

RNA-binding proteins and RNA-based anticancer agents in the control of melanoma cell proliferation and metastasis

Melanomas are long-known for being associated with a plethora of changes in mRNA gene expression profiles. Still, the specific contribution of RNA binding proteins (RBPs), particularly, splicing modulators, remains virtually unexplored in this disease. We have identified tumour-selective roles of RBPs in melanomas defined by selective allelic loss of ATG5 (Autophagy 12, 176-179). Pérez-Guzmán et al., PLoS One 11, e0150805. These unique features of autophagy (another key lysosomal-associated process) in melanoma. Employing human melanoma biopsies, combined with newly-generated mouse models, we identified selective heterozygous losses of ATG5 as a new risk factor for melanoma progression and as a main mediator of the resistance to targeted therapy (García-Fernández et al., Autophagy 2016).

We have also made great progress regarding one of the most pressing needs in the field of melanoma, namely, the mechanisms underlying immune suppression (reviewed in Cereno-Walls and Soengas, Curr Pharm Design 2016). This was achieved by combining the analysis of human melanoma biopsies with a new class of ‘Lymphoreporter’ mouse models that we generated in collaboration with Safragr Orteg’s Transgenic Mice Unit at the CNIO. Moreover, we have expanded the use of dsRNA nanoparticles as immunomodulatory agents. This information will be used to support clinical trials that are currently being performed by Bioncotech Therapeutics, a biotechnology company cofounded by M. Soengas.

RESEARCH HIGHLIGHTS

**PUBLICATIONS**


Our laboratory is focused on understanding metastatic progression. During this process, tumour cells communicate actively with the tumour microenvironment. Among all factors involved in metastasis, our laboratory is specifically interested in defining the role of secreted exosomes during pre-metastatic niche formation. Exosomes are actively involved in cell-cell communication during both physiological and pathological processes. Our data support that tumour-secreted exosomes modulate the molecular signature of exosomes secreted from highly metastatic tumours in lymphatic fluid as biomarkers to predict relapse and metastatic potential.

“Exosome secretion by metastatic cells is an adaptive strategy for tumour cells to corrupt the surrounding microenvironment, thereby favouring tumour progression.”

Role of tumour-derived exosomes in lymph node metastasis

Melanoma-secreted exosomes have been shown to home to specific niches in lymph nodes. We are studying how tumour-secreted exosomes promote cellular and molecular alterations in the lymph node microenvironment, fostering metastasis (FIGURE A). The goal of the current project is to determine the mechanisms through which tumour-secreted exosomes promote lymph node and distal metastasis. Our studies in melanoma patients will be the first ones evaluating the use of circulating vesicles in lymphatic fluid as biomarkers to predict relapse and metastatic potential.

Linking obesity with metastatic risk

Obesity has been associated with the increased risk of developing metastasis in certain cancers. Although the implication of obesity in cancer is clear, there is, to date, a lack of studies analysing the impact of obesity on metastasis. We are investigating the mechanisms involved in the crosstalk between the adipose tissue, platelets and tumour cells during the metastatic process (FIGURE B). We are dissecting the systemic effects of tumour-derived exosomes in adipose tissue as well as the involvement of platelets, determining their role in metastasis. Ultimately, we aim to determine specific signatures in circulating exosomes and platelets of cancer patients in order to define new prognostic and therapeutic markers that can be applied in the clinical setting.

Novel pathways involved in neurofibromatosis progression

Although neurofibromatosis is a genetic disorder, in this project we aim to develop a very innovative concept, which focuses on unveiling unknown pathways involved in exosome secretion during neurofibromatosis progression. We are investigating the molecular signature of exosomes secreted from highly metastatic neurofibromatosis models. Our data support that tumour-secreted exosomes carry a specific signature that can be detected in the circulation. This approach will result in the development of new diagnostic tests and therapies to block neurofibromatosis progression.

Figure (A) Analysis of exosome distribution in sentinel lymph nodes. Green-labelled exosomes from B16-F1R2 melanoma cells were injected in the footpad and followed for 16 hours. Analysis of lymph nodes demonstrated that exosomes reach popliteal (sentinel) lymph nodes with a specific distribution found mainly in subcuticular areas co-localising with lymphatic endothelial cells (in red).

Figure (B) Metastasis of breast cancer cell lines in lung metastatic niches. Tumour breast cancer cell lines (in red) were injected by tail vein in mice, in combination with platelets. Analysis of metastasis demonstrates that tumour cells reach metastatic lungs in areas surrounding terminal bronchiolies, formerly known as areas where pre-metastatic niches were formed.

Publications

The Brain Metastasis Group is seeking to identify novel ways to target both cancer cells and the associated microenvironment in order to reduce metastatic burden in the brain.”

The Brain Metastasis Group investigates the progression of cancer to the Central Nervous System (CNS). During 2016, we focused our efforts on various projects:

- Using a novel medium-throughput drug discovery platform, the laboratory identified two compounds with the potential to target established brain metastasis from experimental lung and breast cancer models.
- We identified two novel mediators of brain metastasis that are enabling us to explore the influence of epigenetics on brain colonisation as well as the ability of cancer cells to interact with neurotransmitters.
- We are evaluating the therapeutic potential of targeting specific components of the microenvironment that are only present surrounding metastatic lesions in the brain. Our research suggests that the viability of brain metastasis is highly dependent on altered components of the microenvironment, thus highlighting potential vulnerabilities.

Overview

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is poor given that available 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 extracranially but not in the brain. In this scenario, cancer cells present at this highly demanding secondary site have additional time to evolve and develop 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.
Mammals, including humans, have evolved in an environment where the ability to efficiently use limiting nutrient sources has been a key survival adaptation that has shaped all our responses to nutrients. Unprecedented nutrient overabundance is in conflict with our cellular and organismal responses, which are best tuned to operate under scarcity. These aberrant responses not only lie at the core of the pathogenesis of the metabolic alterations observed in diabetes, but are also key in cancer and the process of ageing. We use genetically engineered strains of mice as a physiological framework to understand the molecular bridges from elevated nutrient levels to human disease. In particular, we have genetically modified the RagA and RagC GTPases, key players in the sensing of nutrients that activate a master regulator of metabolism, a kinase called mTOR.

Mice with gain-of-function mutations in RagA—therefore unable to sense a drop in nutrient levels—have an increased glycaemia in spite of a normal food intake and decreased adiposity. Furthermore, these mice show intolerance to glucose, which means that when glucose is administered it remains in the circulation, and peripheral organs (such as liver and skeletal muscle) are unable to uptake it. These perturbations are tightly associated with the development of type 2 diabetes. Indeed, when we examined the ability of peripheral tissues to respond to insulin we observed an impaired response to insulin, also known as insulin resistance, which leads to increased levels of glucose in circulation. We are currently characterising other metabolic imbalances observed in these mice and are performing a genetic dissection of these alterations by deregulating nutrient sensing in an organ-specific manner.
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 5 Groups investigate how tumours grow as ‘extrinsic organs’; the spectrum of investigations ranges from epithelial cancers such as liver, skin and intestinal cancer, 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 cells (like macrophages and fibroblasts), to the role of inflammation, as well as cell adhesion, 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. At present, these aspects are successfully covered and integrated in an interactive and collaborative manner by the complementary research areas of 2 Senior and 3 Junior Groups.

The Senior Group, led by Francisco X. Real, studies epithelial tumours and focuses 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. Mirna Pérez-Moreno’s Group investigates the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer. Nabil Djouder’s Group aims to dissect the contribution of nutrient and growth factor signalling pathways to cancer development, and in particular to gastro-intestinal cancers. Massimo Squatrito’s Group, which is partly supported by the Seve Ballesteros Foundation, studies how brain tumours, mainly glioblastomas and medulloblastomas, 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, e.g. in the liver, lung, skin and bone. We are investigating the role of AP-1 in inflammatory skin diseases, such as psoriasis, but also aim to molecularly define the causes of lung fibrosis. We have continued our efforts to study how the whole organism responds to a locally growing tumour in the context of a complex metabolic impairment in cancer-associated cachexia.
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 that lead 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, 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 is for CNIO to remain an international and competitive institution. At present, 4 out of 5 Group Leaders in our department are foreigners, one of whom is partly funded by the Seve Ballesteros Foundation. Fourteen different nationalities from 4 continents are a testament to our international science culture and we all focus 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 pre-clinical studies based on these screenings.

Bone development, osteosarcomas and arthritis

We are studying the function of AP-1 proteins and their targets in bone development and disease using loss-of-LOF) and gain-of-function (GOF) mouse models. In mice, c-Fos expression leads to osteosarcomas (OSs) and chondrogenic hyperplasias. We found that loss of Wnt signalling delays OS development in c-Fos GOF mice, pointing to a novel mechanism linking c-Fos/ AP-1 and OS development.

Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA) and Osteoarthritis (OA) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the function of AP-1 factors and their target genes in the development of arthritis using GEMMs, experimental arthritis models and local gene manipulation approaches. Additionally, we are investigating how crossstalk from other organs, like skin and bone, may contribute to the development and progression of different types of arthritis, as well as whether inflammation generated from the joint, using GEMMs, experimental arthritis models and local gene manipulation approaches.

Lung fibrotic diseases and non-small cell lung cancer (NSCLC)

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) share the same target organ as well as similar characteristics such as higher incidence in smokers, high morbidity and lack of effective treatments leading to high mortality. Our studies using GEMMs provide experimental tools for studying the important contribution of Fra proteins to these diseases. While Fra-2 is required for the innate immune response associated with disease progression in experimental lung fibrosis, Fra-2 promotes tumour growth in NSCLC. We are currently testing the therapeutic value of Fra-2 inhibition in our pre-clinical models for fibrosis and NSCLC, and are validating our findings using patient’s tissue samples. These studies are conducted in collaboration with Daiichi Sankyo Company (Japan) and Mariano Barbacid’s Experimental Oncology Group at CNIO, respectively.

Skin cancer, inflammation and human disease

We have demonstrated that loss of epithelial Fra-2 protein results in skin barrier defects. Mechanistically, Fra-2 binds and transcriptionally regulates epidermal differentiation gene promoters. We are currently investigating the targets of Fra-2 in skin that play a role in barrier function and inflammation.

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone crosstalk. We have recently reported that IL-17A production in skin causes bone loss by inhibiting Wnt signalling in bone-forming osteoblasts. We have extended our studies and shown that psoriasis patients suffer from bone loss that correlates with IL-17A levels. We are currently evaluating the role of the microbiota in skin inflammation by antibiotic treatments, high-throughput microbiota sequencing and germ-free housing conditions.

High-throughput proteomics and transcriptomic analyses unravel novel pathways and molecules for targeted therapies, such as S100A8/A9 and complement C3. We have now generated new GEMMs to define the role of these novel target molecules in inflammatory skin disease with a focus on the systemic effects beyond the skin in arthritis and bone loss.

Another angle of research in psoriasis involves the analysis of the role of epithelial stem cells in the disease initiation and progression using state-of-the-art lineage-tracking models. Recent data suggest that a subtype of epithelial stem cells is important for disease progression; we are currently characterising these cells and expanding these studies to parallel tumourigenic skin models. The identification of novel target molecules in skin cancers, such as S100A8/A9 and complement C3, unravels novel pathways and molecules for targeted therapies.


del. Deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis, whereas additional inactivation in immune cells abrogates this protective effect. Ectopic c-Fos or expression of Fos-dimers leads to altered cholesterol and bile acid metabolism, inflammation, fibrosis, hepatocyte/hide duct proliferation and tumours with human HCC gene signatures. A robust connection between c-Fos expression and the activity of the LXR/Retinoid pathway, an important regulator of cholesterol homeostasis, was observed and it most likely contributes to the oncogenic function of c-Fos in hepatocytes.

Cancer-associated caebehia (CAC)

We previously demonstrated that ‘browning’, a switch from white to brown fat, is a contributor to the wasting process in CAC, and we also documented the importance of IL-6 and β-adrenergic signalling. Using GEMMs, as well as syngeneic mouse models, we are investigating the role of inflammation in CAC and are also studying the systemic events in CAC, such as the role of the neuro-endocrine system, e.g. the renin-angiotensin–aldosterone system. In collaboration with the Medical University of Vienna and Attenteous Diagnostics (Vienna), we are analysing human serum samples from cancer patients to validate the findings from the GEMMs. Our goal is to understand the molecular switch from a local inflammation-associated tumour to the systemic effects of CAC, and to potentially identify novel biomarkers (in collaboration with Drs R. Senaris, Santiago de Compostella, Spain and M. Petruzzelli, Cambridge, UK).

Defining a function for AP-1 in lung disease

Lung fibrotic diseases and non-small cell lung cancer (NSCLC) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the function of AP-1 factors and their target genes in the development of arthritis using GEMMs, experimental arthritis models and local gene manipulation approaches. Additionally, we are investigating how crossstalk from other organs, like skin and bone, may contribute to the development and progression of different types of arthritis, as well as whether inflammation generated from the joint, using GEMMs, experimental arthritis models and local gene manipulation approaches.

We found that loss of Wnt signalling delays OS development in c-Fos GOF mice, pointing to a novel mechanism linking c-Fos/ AP-1 and OS development.

Figure 1 - switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/ dimers in skin, bone, liver and lung, which are complemented by loss-of-function mouse models. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models of disease to human disease as well as to identify novel targets. Furthermore, we are investigating the systemic response of the mouse organism to a growing tumour in cancer cachexia. Preclinical studies are performed using different genetically engineered mouse models with compounds that target the identified molecules in order to determine the potential of translating our findings for the treatment of human disease.
We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial carcinoma (UC), adopting a disease-oriented approach. We use cultured cells, genetically modified mice, and patient samples, giving similar weight to the 3 model systems. Primary observations are made at either of these levels and are then extended through additional work. To translate the findings, we bring this knowledge to a ‘population’ level, harnessing information and samples from large patient cohorts.

In PDAC, we study cell differentiation as a potent tumour suppressor mechanism acting early during carcinogenesis. We use the excellent genetic mouse models available because these processes cannot be readily studied using human samples. PDAC can originate both in pancreatic progenitors and in acinar cells. Understanding the contribution of these cell types to PDAC 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 their mechanisms of action, and applying this knowledge for improved prediction of outcome and therapy.”
Pancreas cancer molecular pathophysiology

We are analysing the tumour suppressive role of several transcription factors involved in pancreatic differentiation. PI3C is characterised by highly prevalent alterations in KRAS, p16, TP53, and SMAD4, and by low-frequency alterations in a plethora of other genes converging in a few critical genetic pathways. The currently accepted progression model proposes that the sequential acquisition of these genetic changes drives the development of PanIN-1, -2, and -3 lesions. We have previously highlighted the weaknesses of this model. Using mutant Kras as the driving oncogene in a pancreatic Gata4-null background, we have shown that PDAC can be initiated from pancreatic progenitors or adult acinar cells without the development of acino-ductal metaplasia (ADM) or preneoplastic PanINs (FIGURE 1). These findings, together with recent evidence using whole genome sequencing of human PDAC, suggest the importance of a plethora of other genes converging in a few critical genetic patterns.

We are analysing the tumour suppressive role of several translation programmes, providing mechanistic evidence that these processes are linked at the transcriptional level in pancreatic cells. We are also exploring the potential of enhancing Nrs2a activity to suppress pancreatitis and tumour development.

This work benefits from a close collaboration with the CNIO Groups of E. Wagner and N. Malats.

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

Our goal is to refine current knowledge on the genomic landscape of UC and apply this in the clinical setting. Through exome sequencing we identified STAG2 and RBM10 as new UC genes that are more broadly involved in human cancer. STAG2 codes for a cohesin subunit; its inactivation in UC is not associated with aneuploidy, suggesting that regulation of chromatin architecture and gene expression mediate its tumour suppressor role. Transcriptomic analyses of human tumours and cultured urothelial cell lines provide mechanistic evidence that these processes are linked at the transcriptional level in pancreatic cells. We are also exploring the potential of enhancing Nrs2a activity to suppress pancreatitis and tumour development.

These studies will be complemented with the use of normal urothelial organoids, for which we have established robust culture methods and have shown their strict dependence on EGF and Wnt signalling. We have characterised an organoid cell-of-origin with stem cell properties in vitro and have identified conditions promoting urothelial differentiation (FIGURE 2). In addition, we are expanding these studies to human bladder cancers.

Within the context of a project funded by the Spanish Association Against Cancer (ARCC), we are analysing the clinical usefulness of the new UC taxonomy. The main aim is to identify predictors of outcome and response to cisplatin-based therapies in patients receiving percutaneous chemotherapy. These studies are linked to the design of clinical trials that include molecular stratification criteria. This work is carried out in collaboration with N. Malats at the CNIO and the SOGIG cooperative group.

RESEARCH HIGHLIGHTS

**Figure 1** Pancreatic KRAS G12D/G12D mice develop PanIN-less PDAC. Quantification of PanIN and PDAC in young (<40 weeks) and old (>1 year) mice (A): Acini from mice lacking Gata4 display reduced cyst formation upon culture in Matrigel. They also show a blunted APM response to EGF but are able to respond to IL7 (B).

**Figure 2** Normal urothelial organoids (NMU-o) express E-cadherin (A). Organoids have been passaged in vitro to the design of clinical trials that include molecular stratification criteria. This work is carried out in collaboration with N. Malats at the CNIO and the SOGIG cooperative group.
**OVERVIEW**

Tumour cells evolve into a progressively complex interplay between heterogeneous tumour cells and their tissue macroenvironment, which influences their proliferation and malignancy. Identifying the signalling mechanisms and cell types that sustain this complexity is one of the major goals in cancer biology. In adult skin, epithelial progenitor cells have been identified as the cell of origin of skin carcinomas. Several studies have been instrumental for defining regulatory pathways controlling their proliferation and/or differentiation. However, the identification of extrinsic factors modulating stem cell behaviour has not progressed very far to date. Using skin as a model system and employing mouse genetics and human samples, our research aims to understand how the interactions between epithelial progenitor cells, and also the interactions with their surrounding macroenvironment, sustain skin homeostasis, regeneration, and when perturbed lead to cancer. This information may provide insights for the future development of regenerative and anti-cancer therapies.

“During 2016, we continued our efforts to uncover novel events controlling the behaviour of skin stem cells in order to open up new insights into the mechanisms that control their regenerative characteristics, and how when disrupted they can lead to cancer.”

**RESEARCH HIGHLIGHTS**

**Regulation of epidermal progenitor cells self-renewal and differentiation**

During 2016, we continued exploring how tissues acquire an adequate control of cell division and differentiation. In particular, using mouse epidermal development as a model system, we investigated the contributions of mitotic and cytoskeletal proteins in the regulation of skin progenitors’ self-renewal through oriented cell divisions.

**Contributions of stromal cells to the skin stem cell niche in homeostasis**

We have recently identified a novel connection between macrophages and skin progenitor cells, which modulates their stem cell properties and regenerative potential. We are expanding these results to decipher how other signals, and cells from the stroma, are connected with the skin stem cell niche and regulate skin regeneration.

**Contributions of stromal cues in cancer stem cell maintenance and tumour progression**

The formation of tumours and their progression to malignancy undoubtedly involves the contributions of the tumour macroenvironment. Identifying the signalling mechanisms and cell types that contribute to tumour initiation and progression to malignancy is instrumental for detecting potential targets for clinical applications aimed at eradicating tumours.

The macroenvironment of many tumours is rich in cytokines, chemokines, and inflammatory enzymes. During 2016, we continued exploring the role of diverse cell-derived soluble mediators in modulating proliferation, migration and survival of skin cancer stem cells.

In addition, we focused our efforts on dissecting the contributions of immune cells to the cancer stem cell niche in tumour initiation and development. We are employing conditional loss- and gain-of-function studies in genetically modified mice in order to demonstrate the role of specific cell types and their derived soluble mediators in tumourigenesis; this may provide further insights for the potential development of immunotherapeutic approaches.

**Figure** Skin carcinoma showing the presence of a high density of inflammatory cells within the tumour. Inset shows a magnification of immune infiltrates within the tumour. ”Arrows point to some immune cells.”
ANNUAL REPORT 2016

VICE-DIRECTION OF BASIC RESEARCH

GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

Junior Group Leader
Nabil Djouder

Post-Doctoral Fellow
Hugo Bernard

RESEARCH HIGHLIGHTS

We have a particular interest in studying gastrointestinal tract disorders. Our work in this area focuses on metabolic organs such as the liver, intestine and pancreas, as these 3 organs are physiologically interconnected and influenced through their exocrine and/or endocrine functions and microbiota (Figure 1). Our task is thus to generate new mouse models mimicking human disease and to study mechanisms and events initiating disease development. We also use patient-derived xenograft models and organoids to translate our findings into clinical perspectives. Guided by experimental mouse models combined with the use of human data, we aim to provide a comprehensive study for a rational approach towards the development of novel mechanism-based therapeutics to prevent, ameliorate and treat diseases.

Identifying new components of growth factor and nutrient signalling cascades

We identified 2 components of the growth factor and nutrient signalling cascades regulating the mTORC1 pathway: Unconventional prefoldin RPBSD-interactor (URI) (Djouder et al., 2007) and Microsphereule protein 1 (MCRS1) (Fawal et al., 2015).

MCRS1, in an amino acid- dependent manner, maintains Rheb at lysosome surfaces, phosphorylated by S6K1 and has an oncogenic role in ovarian cancer and HCC development.

Research concepts from our laboratory

Metabolic alterations initiate tumorigenesis prior to genomic instability.

Inhibition of de novo NAD+ synthesis functions as a non-oncogene addiction pathway in liver and pancreas cancer.

Oncogene-induced NAD+ depletion in DNA damage.

“A our research focus is to generate mouse models recapitulating human disease associated to nutrient overload in order to guide research perspectives and applications.”

Milestones in growth factors and nutrients research in my laboratory. The scheme illustrates our present and future research. Time and effort are dedicated to better understand how manipulation of growth factor and nutrient signalling pathways can lead to gastrointestinal tract disease development.

Generation of genetically engineered mouse models

2 conditional knock-outs (URI and MCRS1 loss-of-function).

3 knock-ins (over-expression of URI (wt), URI (S371A) and MCRS1).

Research achievements

Inflammatory cues and nutrient overloads up-regulate hepatic URI.

URI is an oncogene initiating NASH and HCC.

Nicotinamide riboside to prevent liver and pancreas cancers.

MCRS1 activates mTORC1 in response to amino acids.

URI is the first identified OGT regulator in response to glucose fluctuations.

Glucose depletion can induce oncogenic signals through OGT/c-MYC regulation.

c-MYC is oncogenic and tumour suppressive depending on nutrient availability.

PUBLICATIONS


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GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

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c-MYC is oncogenic and tumour suppressive depending on nutrient availability.

PUBLICATIONS


OVERVIEW

Malignant gliomas (astrocytomas, oligodendrogliomas and oligoastrocytomas) are the most frequent form of brain tumour and Glioblastoma Multiforme (GBM), a grade IV astrocytoma, is the most lethal tumour of the central nervous system in the adult. Standard GBM therapy consists of tumour resection and postsurgical treatment with chemotherapy and ionising radiation (IR). Although there have been improvements in surgical and imaging techniques, available treatments for GBMs are still inefficient, most likely due to intrinsic resistance to the current therapeutic modalities and high cellular heterogeneity.

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

“The main focus of our Group is to uncover the genetic alterations present in GBM patients that are responsible for the aggressiveness and the poor treatment response of this tumour type.”

RESEARCH HIGHLIGHTS

Next generation glioma mouse models

A decade of studies has underlined the complexity of the glioma genome, however, the functional significance of the vast majority of the genetic alterations remains elusive. Understanding the genetic events that lead to glioma formation and the mechanisms of resistance to therapy will be instrumental for the development of new treatment modalities for gliomas. To accurately reproduce the high genetic heterogeneity observed in glioma patients, we would have to recreate not just a handful of genetic alterations, but possibly dozens. The advent of the CRISPR/Cas genome editing technology has now made it possible to target almost any candidate cancer gene in the in vivo setting. We are actively working to develop the ‘next-generation’ glioma mouse models that more faithfully recapitulate in vivo the complexity of the GBM genome, with a particular interest in tumour suppressor genes and complex gene rearrangements.

Overcoming therapy resistance in GBM

The standard therapies for GBM patients, IR and temozolomide (TMZ), generate double-stranded DNA breaks (DSBs), the most deleterious form of DNA damage. The DSBs are then responsible for the initiation of the DNA Damage Response (DDR) and, consequently, the activation of DNA repair pathways and cell-cycle checkpoints. 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. The most frequent resistance mechanism to TMZ treatment is the expression of the DNA-repair gene O6-methylguanine DNA methyltransferase (MGMT), however, other resistance mechanisms have still to be identified.

Through a variety of genetic approaches (Haploid cells transposone mutagenesis, gRNA and shRNA screenings) we have identified the main signalling pathways that mediate resistance to TMZ. We are currently performing a series of synthetic lethality screenings in order to bypass these mechanisms of resistance.

Figure Schematic representation of the RCAS-CRISPR-Cas9 system to generate gliomas with tailored genetic alterations.

PUBLICATIONS


AWARDS AND RECOGNITION

- Alberto J. Schuhmacher has been awarded a Research Contract from the Beca de Honor (Honour Grant) Programme (funded by the Ministerio de Economía, Industria y Competitividad, MEC), and the “Edwards Gallegos” Grant of the Francis Collins Foundation, Spain.
- Álvaro Curiel has received the Beca de Honor - Colegio Mayor Larraona given by the University of Navarra, Spain.
The objective of the Structural Biology and Biocomputing Programme is the mechanistic understanding of key cancer-related molecular systems. The Programme was designed to combine computational and structural approaches, and to collaborate with the CNIO Basic and Translational Research activities.

Our 3 main research goals are to:

- Reconstruct the structural details of protein complexes that are active in cancer and related processes.
- Predict the consequences of cancer-related alterations; we are focusing on alterations of compensatory nature (co-evolutionary related mutations) as well as those affecting alternative splicing patterns.
- Contribute to the analysis of cancer epigenomic and genomic information as part of international genome projects.

Currently the Programme includes 3 Research Groups and 6 Core Units that provide support to the CNIO’s research activities.

Following the recommendations of the CNIO’s External Scientific Advisory Board (SAB), resulting from the 2015 review of the Structural Biology and Biocomputing Programme, we started the process of recruiting additional Groups for the Programme. The selection of candidates for two Junior Group Leader positions was carried out with the help of an ad-hoc external and an internal selection committee. Six outstanding candidates were invited to visit the CNIO, defend their work and have a discussion with the corresponding committees. Finally, two candidates were selected covering the areas of biochemistry-structural biology and electron microscopy - protein complexes. These two new Groups will start their work at the CNIO in the first part of 2017.

Once these Groups are consolidated, the plan is to re-evaluate the possibility of hiring a senior crystallographer as recommended by the SAB.

On the computational side, the Programme has seen the departure of the Heads of the Bioinformatics and the National Bioinformatics Institute (INB-ISCIII) Units; they have since been replaced by Salvador Capella, as Head of the INB-ISCIII/ELIXIR Unit, and by Fátima Al-shahrour, who will coordinate the Bioinformatics Unit and absorb the activities of the previous Translational Bioinformatics Unit.

Of particular relevance for the CNIO’s activities in Computational Biology, was the finalisation of the negotiation with the Ministerio de Energía, Turismo y Agenda Digital for the implementation of a biological text mining platform in the framework of the ‘Plan de Impulso de las Tecnologías del Lenguaje’. Within the CNIO structure, this activity will fall under and be developed by a new ‘Text Mining’ Unit headed by Martin Krallinger. This Unit will start operations at the beginning of 2017.

Despite these positive developments, the CNIO still needs to reinforce the computational side of the Programme; particularly, research related to the Experimental Therapeutics Programme as well as Computational Cancer Genomics needs to be further strengthened.

“The Programme is about to undergo an important transition with the addition of two new Groups. Several very positive new activities in the area of data and text mining have also come about. The establishment of the new Groups and the reinforcement of the structural and computational activities at the CNIO remain challenges for the coming year.”
OVERVIEW

The main interest of our Group is the study of the molecular bases of cancer by bringing an evolutionary perspective to the study of the interplay between genomics and epigenomics in tumour progression.

Our research is largely carried out in the context of large-scale genome projects, in which we develop new computational methods for the study of genome-cancer relationships.

In this general scenario, the strategic goals of the Structural Computational Biology Group are to:

- Develop new ideas, methods and software platforms for the extraction, integration and representation of cancer data, including the analysis of molecular, genomic, epigenomic and phenotypic information in collaboration with large-scale genome projects.
- Include new technologies for data and text mining, together with Machine Learning methods, in our cancer genome analysis framework.
- Analyse the function, structure and specific interactions of cancer-related proteins.

“This year the initial phase of two large scale projects was completed; i.e. the International Human Epigenome Consortium (iHEC) and the Pancancer Analysis of Whole Genomes (PCAWG). In both cases, we have contributed to the computational analysis, including the implementation of the data analysis infrastructures and the development of new analysis methods, as well as collaborating towards the interpretation of the biological results.”

RESEARCH HIGHLIGHTS

The Group has contributed to several community efforts in different areas:

- Epigenomics with the BLUEPRINT EU flagship project, which is part of the iHEC consortium; the results from this work were published at the end of 2016.
- Pancancer Analysis of Whole Genomes (PCAWG), global analysis of 2500 complete cancer genomes; these results will be published in 2017.
- The BioCreative text mining challenge in chemical compounds resulted in a number of resources and publications that appeared throughout 2016.

We have introduced a new computational method for the prediction of pairs of residues in protein interfaces. This method can help in the analysis of cancer related mutations.

We have also introduced new methods for the analysis of epigenomes at the linear two dimensional level (chromatin states) and three dimensional level (chromatin structure in the nucleus).

The cancer genome analysis system

Our Group is deeply involved in the development of a computational framework for the analysis of human genomes with specific application to the analysis of cancer genomes. Over the years, this framework has been applied to a number of collaborative cancer projects, and it has been particularly instrumental in the CLL-ICGC project.

We have now moved on to a new phase in which the framework is used for the analysis of the large set of full cancer genomes.
of the Pancancer Analysis of Whole Genomes (PCAWG). It is one of the four frameworks for data organisation, analysis and exploration used by the consortium.

With regards to the future, given the characteristics of the framework in terms of its modular structure, capacity of integration of new methods in working pipelines, and ease of installation (e.g. adoption of docker and cloud technologies), we consider that it can be the seed of new developments in the overarching analysis of human disease genomes.

Protein structure prediction and cancer genomes

In the context of cancer genome analysis, and as part of the Pan Cancer global effort, we have developed a set of methods to analyse the consequences of mutations in the interface of proteins. The underlying logic is that cellular functions are governed by signals transmitted via protein interactions and protein complexes. In these interactions, the amino acids located in interacting surfaces determine the intensity of the interactions and, very importantly, the specificity of the interactions. The exquisite functioning of cellular systems between proteins depends critically on the pairing of the proteins with their correct partners, and the accuracy of the interactions depends on the correct formation of pairs of residues in the 2 proteins in the interface.

We have shown that cancer associated mutations tend to accumulate in the protein interfaces to the point that, with the information available, it is possible to say that cancer related mutations specifically target protein interfaces. Therefore, understanding the nature of protein-protein interactions is important for understanding the impact of cancer mutations.

We have developed a new methodology able to predict, with high accuracy, a small set of pairs of residues located in the interface of interacting human proteins. The new methodology, based on the study of the co-evolution of the corresponding protein families, does not require any information about the corresponding structures and it is applicable to many human protein complexes for which no other information is available. Furthermore, we have shown that the pairs of residues predicted to interact are very conserved in structural terms (they occupy the same position in space over the lengthy evolutionary time), to interact are very conserved in structural terms (they occupy the same position in space over the lengthy evolutionary time), and are very conserved in structural terms (they occupy the same position in space over the lengthy evolutionary time). The initial results show that the system is not only able to reproduce the structure of the lineage different uration during haematopoiesis, but also to detect what the main potential epigenetic driving factors of the differentiation are. The method, initially developed for the Blueprint datasets, is now being extended to other data types provided by the iHEC consortium.

Alternative splicing at the protein level

In the context of the BLUEPRINT iHEC project we have designed a system for the comparative analysis of epigenetic data (the Blueprint analysis portal http://blueprint-data.bsc.es/ release. 2016-08/, developed in collaboration with the RSC-CNS and EBI-EMBL). This portal is now the main point of access for the project’s results (e.g. chromatin states, ChiP-seq positions of histone modifications), enabling the direct comparison of the epigenetic structure of different cell types. Based on the information provided by the Blueprint Analysis Portal, we have developed the methodology to compare epigenomes at the level of their organisation in functional segments (chromatin states). The initial results show that the system is not only able to reproduce the structure of the lineage differentiation during haematopoiesis, but also to detect what the main potential epigenetic driving factors of the differentiation are. The method, initially developed for the Blueprint datasets, is now being extended to other data types provided by the iHEC consortium.

EPICGENOME analysis infrastructure and portal

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Biological Text Mining

Text mining, an important part of the Group’s activity, has broad implications in Biomedicine. In 2016, we completed this year an exhaustive review of the application of text mining to the area of chemistry (Kral et al., this work has been submitted to Chem Rev); this review was based on our experience in the analysis of text mining systems and the results in the context of the 2015 BioCreative Chemdner challenge (http://www. biocreative.org/tasks/biocreative-in/chemdner/).

During 2016, we reached an agreement with the Ministerio de Energía, Turismo and Agenda Digital for the implementation of a biological text mining platform in the framework of the ‘Plan de Impulso de las Tecnologías del Lenguaje’, this project is to develop tools and procedures in line with the recommendations of the European e-Infrastructure in text mining OpenMinted, in which we also participate.

between the results obtained at the level of gene and protein expression; a discrepancy that might have profound implications for our understanding of the role of mRNA in cells and the overall understanding of the biological function of processed RNAs.
We showed that Focal adhesion Kinase (FAK) interacts with PIP_2 lipids at cell adhesion sites and that this interaction induces FAK clustering and conformational changes, which trigger FAK autophosphorylation. Following this, Src is recruited to FAK and, in turn, phosphorylates the FAK kinase to induce full FAK activation. Currently, we are studying the architecture of FAK clusters bound to lipid membranes by electron microscopy, and we are investigating how force, induced at adhesion sites by actomyosin contraction, induces changes to these structures in order to activate focal adhesion signalling. We utilise these mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ experimental and virtual screening, using fragment based approaches, to identify allosteric ligands, and then use a structure based approach to develop these fragments into inhibitory lead compounds.

SH2-domain-containing inositol 5-phosphatases (SHIP) remove the 5-phosphate from PIP_3 and thereby, like PTEN, negatively regulate PIP_3 levels. Despite their importance, little is known about the mechanisms of SHIP regulation. We solved a crystal structure containing the catalytic and C2 domains of SHIP2, showing an extensive interface between the two domains. We have shown that the C2 domain of SHIP2 binds phosphatidylserine, and hence the rigid C2 interaction efficiently positions the active site towards its substrate. Although the C2 domain interacts with the phosphatase domain far from the active site, we show that the C2 interaction greatly enhances the catalytic activity of SHIP2. We employed molecular dynamics simulations to guide a mutagenesis study that has identified distinct allosteric signalling pathways emanating from hydrophobic or polar interdomain interactions, which differentially affect lipid chain or head group regions of the substrate. Furthermore, we confirmed via cell biology experiments that mutations at the domain interface affect downstream signalling to Akt.

We focus on mechanisms of growth and adhesion signalling that occur at the plasma membrane and involve specific phosphoinositides. In particular, we aim to answer two main questions: (i) what are the events occurring at the cell membrane at integrin adhesion sites that trigger Focal Adhesion Kinase signalling; and (ii) how are phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) levels regulated to affect signalling of the Akt pathway.

“*We obtain detailed structural and mechanistic insights in order to understand how growth and adhesion signals are triggered to cause tumour invasion, and we use this information for allosteric targeting.*”

**OVERVIEW**

Our Group studies regulatory mechanisms of key signalling switches controlling growth and adhesion signals. Such signals regulate important cellular processes such as proliferation, adhesion 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.

**RESEARCH HIGHLIGHTS**

We showed that Focal adhesion Kinase (FAK) interacts with PIP_2 lipids at cell adhesion sites and that this interaction induces FAK clustering and conformational changes, which trigger FAK autophosphorylation. Following this, Src is recruited to FAK and, in turn, phosphorylates the FAK kinase to induce full FAK activation. Currently, we are studying the architecture of FAK clusters bound to lipid membranes by electron microscopy, and we are investigating how force, induced at adhesion sites by actomyosin contraction, induces changes to these structures in order to activate focal adhesion signalling. We utilise these mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ experimental and virtual screening, using fragment based approaches, to identify allosteric ligands, and then use a structure based approach to develop these fragments into inhibitory lead compounds.

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**PUBLICATIONS**


\( \text{Lietha D (2016). Forcing FAK into Transcriptional Activity. Structure 24, 1223-1225.} \)

STRUCTURAL BASES OF GENOME INTEGRITY JUNIOR GROUP

Santiago Ramón-Maiques  
Junior Group Leader

Post-Doctoral Fellow

Maria Dolores Moreno

Graduate Students

Francisco Del Caño, Alba Ruiz (until February)

Technicians

Araceli Grande (TS)*, Igor Yefimenko (TS)*

*Titulado Superior (Advanced Degree)

RESEARCH HIGHLIGHTS

Unmasking CAD, a metabolic gatekeeper of cell proliferation

CAD is a 1.5 MDa multi-enzymatic complex formed by hexameric association of a ~240 kDa polypeptide with four functional domains: glutaminase (GLNase), carbamoyl phosphate synthetase (CPSase), aspartate transcarbamoylase (ATCase) and dihydroorotase (DHOase). Each domain catalyses one of the initiating steps in the de novo biosynthesis of pyrimidine nucleotides. CAD is tightly regulated by allosteric effectors and by phosphorylation through different signalling cascades, and its activity is key to fuel the high demand of pyrimidines during cell growth and proliferation. Despite its central role in metabolism and its potential as an anti-tumour target, there is no detailed information about the architecture of CAD or about the structure of any of its functional domains. We aim to decipher the structure of the complex and to understand its catalytic and regulatory mechanisms at the atomic level.

Structure and functioning of the ATC domain of human CAD

We resolved the crystal structure of the ATCase domain of human CAD – free or bound to carbamoyl phosphate, or to the anti-tumour drug PALA – confirming its overall similarity with bacterial homologues (Ruiz-Ramos et al., 2016). Unexpectedly, we found a decreasing affinity for PALA that could help to understand tumour resistance to this drug. Mutagenic and biochemical analysis linked the lowered PALA affinity to the communication of conformational changes between the ATCase subunits. The mutation of one key residue in this mechanism was recently found by others to cause the first CAD-related human disease (Ng B.C. et al., Hum Mol Genet, 2015).

Using CRISPR to understand the functioning of CAD in vivo

We generated fluorescent recombinant chimeras and used CRISPR to introduce green fluorescent protein (GFP) into the endogenous CAD gene and to knockout CAD in human cell lines. These tools enable us to interrogate important aspects of CAD functioning in vivo. By tracking the subcellular localisation of CAD in mammalian cells we demonstrated that, contrary to previous reports, CAD is located exclusively at the cytosol and does not translocate into the nucleus during the cell cycle. These engineered proteins and gene edited cells are also proving to be instrumental for the identification of interacting protein partners and for the testing of the disease-causing potential of newly identified clinical mutations in CAD.

OVERVIEW

Safeguarding genome integrity is essential for correct cell functioning and to prevent cancer. Our Group is interested in understanding central cellular processes that affect the integrity of the genome, such as the metabolism of nucleotides, DNA recombination or the maintenance and recognition of chromatin architecture. These processes depend on the assembly of large and dynamic macromolecular complexes. We combine protein engineering, X-ray crystallography, nuclear magnetic resonance (NMR) and single-particle electron microscopy (EM), together with biochemical and functional studies, in order to decipher the structure of these protein-protein and protein-DNA complexes, as well as to understand their catalysis and regulatory mechanisms at the atomic level. This knowledge should guide the design of compounds to modulate protein activity and provide novel opportunities for fighting tumours.

“We obtained an atomic view of the ATC domain of human CAD – a metabolic gatekeeper controlling cell proliferation – bound to the anti-tumour drug PALA, and localised CAD within the cell.”
OVERVIEW

The Unit unites the technical and scientific management of Nuclear Magnetic Resonance Spectroscopy (NMR) and other biophysical instrumentation available through the Structural Biology and Biocomputing Programme. It provides CNIO researchers with instrumentation and technical support for a variety of spectroscopic and biophysical techniques. 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 small molecule screening. With a broad range of instruments and expertise, the Unit can help structural biologists and functional geneticists answer their research questions. The Unit unifies the technical and scientific management of the Cancer Cell Biology Programme (from the Tumour Suppression Group (from the Molecular Oncology Centre), the Growth Factors, Nutrients and Cancer Groups (from the Cancer Cell Biology Programme). Collectively, with these and previous groups, we implemented sample preparation protocols and developed spectroscopic and analysis technology to characterise the metabolites present in different biological samples, as illustrated by two important publications.

“IN 2016, WE QUANTIFIED METABOLITES FROM CELL MEDIA, MOUSE BLOOD AND LIVER EXTRACTS, THEREBY CONTRIBUTING TO THE UNDERSTANDING OF THE CELLULAR AND PHYSIOLOGICAL METABOLIC RESPONSES TO FASTING AND TO ONCOGENE ACTIVATION, WHICH ARE IMPORTANT ASPECTS OF TUMOUR BIOLOGY.”

PUBLICATIONS


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, a multi-angle static light scattering apparatus, and a surface plasmon resonance (SPR) instrument. Research groups mostly from, but not limited to, the Structural Biology and Biocomputing Programme have extensively used these technologies throughout 2016. For example, we reported the results of a multidisciplinary intra-Programme collaboration this year (with the former Computational Biophysics Group), illustrated in the FIGURE, which combines various NMR and SPR experiments with enhanced sampling molecular dynamics simulations to shed light on the conformational dynamics associated with the binding of Imapitinib to the proto-oncogene c-Src. We found that both conformational selection and induced fit play a role in the binding mechanism, reconciling opposing views held in the literature.

The Unit hosts a 700 MHz NMR spectrometer, which is well equipped with probes, and a sample changer for running up to 120 samples automatically. This provided the required throughput for screening small molecule protein binders (together with the CNIO’s Structural Biology and Biocomputing and Experimental Therapeutics -ETP- Programmes), as well as for metabolomics measurements that were performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the ETP), the Tumour Suppression Group (from the Molecular Oncology Programme), as well as the Genes, Development and Disease and the Growth Factors, Nutrients and Cancer Groups (from the Cancer Cell Biology Programme). Collectively, with these and previous groups, we implemented sample preparation protocols and developed spectroscopic and analysis technology to characterise the metabolites present in different biological samples, as illustrated by two important publications.

Figure Free energies for local unfolding represented on the backbone structure of the kinase domain of Src in its free form (left) as well as their variation upon binding to Imatinib (right). The indicated values are coded both in the colour and in the thickness of the backbone coil. They were derived from the rates of exchange of backbone amide protons measured with traditional NMR measurements for all but grey-coloured residues. Red circles indicate α-helices (αD and αL), the regions with increased exposure to the solvent in the bound structure.

Figure Free energies for local unfolding represented on the backbone structure of the kinase domain of Src in its free form (left) as well as their variation upon binding to Imatinib (right). The indicated values are coded both in the colour and in the thickness of the backbone coil. They were derived from the rates of exchange of backbone amide protons measured with traditional NMR measurements for all but grey-coloured residues. Red circles indicate α-helices (αD and αL), the regions with increased exposure to the solvent in the bound structure.
OVERVIEW

Bioinformatics is a key discipline for understanding the cancer genome and, therefore, essential 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 between genotype and phenotype that are needed to identify cancer driver molecular alterations and new therapeutic targets.

Our Unit has several goals:

→ To provide bioinformatics support with data analysis and interpretation using computational and statistical methods.

→ To achieve genome analysis in cancer patients’ data in order to identify new biomarkers and mechanisms of drug response.

→ To develop new computational methodologies and bioinformatics tools for cancer research.

→ To maintain CNIO’s scientific computing facilities and to provide training in bioinformatics tools for cancer research.

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“We have developed SATIE, a tool that enables us to predict sequential treatments, second-line therapies or therapeutic interventions for acquired drug resistance.”

Researchers

Fátima Al-Shahrour
单元领导

Javier Perales
研究员

Hector Tejero
博士后研究

Technicians

Angel Carrero (TS)*, Coral Fustero (PEL A)*, Gonzalo Gómez (TS)*, Osvakko Graña (TS)*, Elena Piphero (TS)*, Miriam Ribis (TS)*, Kevin Troublé (PEL A)*

*Graduate Student

**Post-Doctoral Fellow

***Technicians

SELECTED PUBLICATIONS*


5. RubioSeq+ has been used in several projects, such as the analysis of the lynx genome (Abascal et al., 2016), and for the whole-exome sequencing analysis of patient-derived xenografts for lung cancer (Pereira et al., 2016).

Regarding our scientific collaborations, we helped to unveil mechanisms of cellular reprogramming and senescence (Mosteiro et al., 2016), and also to describe the role of p21 in fasting adaptation (Lopez-Guadamillas et al., 2016) in collaboration with Manuel Serrano’s Group (CNIO). Other bioinformatics analyses were performed together with Mariano Barbacid’s Group (CNIO) (Ambrogio et al., 2016), these identified DOR1/Notch inhibition as a novel therapy for KRAS-driven lung adenocarcinoma. Finally, within the context of our international collaborations with Harvard associated institutions, we have contributed to the study of leukaemia stem cells in AML (Pereira et al., 2016) and the mechanisms of CALR mutations in MPN cells (Elf et al., 2016).

RESEARCH HIGHLIGHTS

During 2016, the Bioinformatics Unit (former Head, David G. Pisano), and the Translational Bioinformatics Unit headed by Fátima Al-Shahrour from the Clinical Research Programme, were reorganised and merged into one single Bioinformatics Unit (BU). BU was established with the aim of providing resources to enable the integration of biological and clinical data, using computational biology approaches, as well as to contribute to research projects in need of bioinformatics support.

In 2016, BU published 22 peer-reviewed articles as a result of our ongoing research projects and scientific collaborations with CNIO Research Groups as well as other national and international research institutions. We developed several bioinformatics tools for the analysis of next-generation sequencing data in collaboration with the SING group from the University of Vigo – RubioSeq+ (Rubio-Camarillo et al., 2017), nextpresso (Graña et al., 2016) – and 2 web tools to guide the selection of therapies from genome-wide studies in cancer disease - PanDrugs (http://pandrugs.bioinfo.cnio.es) and SATIE (http://satie.bioinfo.cnio.es). RubioSeq+ has been used in several projects, such as the analysis of the lynx genome (Abascal et al., 2016), and for the whole-exome sequencing analysis of patient-derived xenografts for lung cancer (Pereira et al., 2016).

Welcome to SATIE

Figure: SATIE-Sequential Antitumour Treatment Inference and Enrichment tool. http://satie.bioinfo.cnio.es
The Spanish National Bioinformatics Institute (Instituto Nacional de Bioinformática, INB) is a component of the National Infrastructure of Biomolecular and Bioinformatics Resources Platform (Plataforma en Red de Recursos Biomoleculares y Bioinformáticos, PRBB) of the Spanish National Institute of Health Carlos III (Instituto de Salud Carlos III, ISCIII). The INB is the Spanish Node of ELIXIR, the permanent European Infrastructure of Biomolecular and Bioinformatics Resources (Nacional de Bioinformática). The INB Unit has actively participated in different Work Packages (WP) of the ELIXIR-EXCELERATE programme. It is also involved in other major projects such as RD-Connect, BLUEPRINT and eTOX. The Unit’s contribution can be divided into three main areas:

### Data resources and Bio-computing

The storage and processing of data have become fundamental tasks within almost all of the current research projects. Through a collaboration model, the Unit participates in several research projects studying the data requirements and developing solutions to store and process the data. An example of this is the BLUEPRINT data-portal (http://dcc.blueprint-epigenome.eu). BLUEPRINT is a high impact FP7 project aimed at producing epigenomes of haemopoetic cell lines from healthy and non-healthy human donors. In the current version, the data portal provides an epigenomic analysis, obtained from 1,019 samples, to the scientific community. Their associated epigenomes are characterised by: gene and transcript expression (from RNA-Seq experiments), hyper and hypomethylated regions (derived from WGBS experiments), chromatin accessibility (DNase-Seq), and 7 Histone marks binding activity (ChIP-Seq). Recently, a scientific article was published illustrating the possibilities offered by this portal (Fernandez JM et al., 2016).

### Infrastructure development

Within the infrastructure development aspect, there is a clear focus on developing a text-mining infrastructure for the processing of biomedical texts. The LiMTox system (http://limtox.bioinfo.cnio.es) has been built using the same system and focusing on the study of different aspects of melanomas.

### RESEARCH HIGHLIGHTS

Despite this Unit’s coordination role, the INB actively participates in different Work Packages (WP) of the ELIXIR-EXCELERATE programme. It is also involved in other major projects such as RD-Connect, BLUEPRINT and eTOX. The Unit’s contribution can be divided into three main areas:

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### End users applications and services

The Unit actively contributes to the creation of an integrated platform that connects databases, registries, biobanks and clinical bioinformatics for research on rare diseases. RD-Connect (http://rd-connect.eu) allows researchers and clinicians to explore the possible genetic causes of these diseases by combining genomic data with phenotypic information from patients across Europe, in a clear attempt to have enough statistical power to support findings. The INB unit has also developed APPRIS (http://appris.bioinfo.cnio.es) and keeps improving it in order to annotate genes and identify the principal isoform of every single gene. Currently, the GENCODE consortium uses APPRIS to annotate the principal human and mouse isoforms.

### PUBLICATIONS

The Electron Microscopy Unit (EMU) is a research facility that supports biological scientific projects ranging from the cellular to the macromolecular level. The EMU performs standard specimen preparation protocols, negative staining, cryo-EM, and data collection methods, as well as 2D and 3D data processing.

In collaboration with CNIO’s Telomeres and Telomerase Group (Molecular Oncology Programme) and the Cell Signalling and Adhesion Group (Structural Biology and Biocomputing Programme), we used the single-particle electron microscopy technique to obtain the first low resolution structures of full-length TRF1 dimer (shelterin component) and its structure in complex with telomeric DNA. We contributed to the understanding of the molecular mechanism that protects the ends of chromosomes: our results demonstrate that full-length TRF1 presents a molecular architecture that assists its interaction with telomeric DNA and at the same time makes TRFH domains accessible to other TRF1 binding partners. Furthermore, our studies suggest hypothetical models on how other proteins such as TIN2 and tankyrase contribute to regulate TRF1 function.

In collaboration with Iván Ventoso, from the Centro de Biología Molecular Severo Ochoa (CSIC-UAM) and the Departamento de Biología Molecular, Universidad Autónoma de Madrid (UAM), the EM Unit participated in the novel findings that illustrate how viral mRNA is threaded into the 40S subunit during the scanning process. Based on structural and functional data, we generated new insights into the scanning process, describing how a stem-loop in the proximal region of viral mRNA can promote a Eukaryotic Initiation Factor 2 (eIF2)-less translation initiation by trapping in RNA extensions of the ribosomal 40S subunit.

We continued our collaboration with the CNIO Cell Signalling and Adhesion Group (Structural Biology and Biocomputing Programme) on PI(4,5)P2-mediated induction of Focal Adhesion Kinase (FAK) clustering at the cell membrane, applying 2D electron crystallography.

"We have used single-particle electron microscopy to elucidate the molecular architecture of full-length TRF1 and to demonstrate how it assists its interaction with other proteins and telomeric DNA."

**PUBLICATIONS**

NOWAYS, knowledge of the three-dimensional (3D) structure of a protein is critical in order to gain a full understanding of its function. The structures of proteins, alone or in complex with other biological partners, reveal functional networks thereby providing a better understanding of the behaviour of the cell’s molecular machinery. This implies knowing how proteins move, providing a better understanding of the cell’s other biological partners, revealing functional networks thereby facilitating the discovery of new targets for drug design. The scaling up of the production of proteins, like full-length human MASTL, has permitted a wide range of biophysical experiments to take place. Other projects were directly focused on structural characterisation by x-ray crystallography in support of drug discovery, as in the case of the human proteins HASPIN and CDK4/CyclinC complex where we obtained several crystal structures of the protein-ligand complexes (FIGURE). Especially relevant was our continuous work on the production of proteins for the generation of antibodies by the CNIO Monoclonal Antibody Unit (Biotechnology Programme). Throughout 2016, we have worked closely with the Experimental Therapeutics Programme on several projects, some of them also in collaboration with other CNIO Groups. The scaling up of the production of proteins, like full-length human MASTL, has permitted a wide range of biophysical experiments to take place. Other projects were directly focused on structural characterisation by x-ray crystallography in support of drug discovery, as in the case of the human proteins HASPIN and CDK4/CyclinC complex where we obtained several crystal structures of the protein-ligand complexes (FIGURE). Especially relevant was our continuous work on the production of proteins for the generation of antibodies by the CNIO Monoclonal Antibody Unit (Biotechnology Programme). During 2016, this smooth collaboration has led to the production of several proteins involved in cancer such as CRC25A, IDO1, TDO2, ELI1, PDL1, PDL2 or NOM1.

The Unit also undertakes several collaborations with different CNIO groups. It is noteworthy to mention the collaborations established by CNIO’s Telomerases and Telomerase Group, the Gastrointestinal Cancer Clinical Research Unit, the Epithelial Carcinogenesis Group and the Structural Computational Biology Group. Additionally, the Unit maintains external collaborations with groups at the Physical Chemistry Department (University of Granada), the Environmental Biology Department (CB-CNIO), the Pharmacology and Therapeutics Department (Roswell Park Cancer Institute, USA), the Department of Biomedicine (University of Bergen, Norway), and the Department of Molecular Engineering (Aarhus University, Denmark).

Finally, the Unit has continued the study of the role of ephrinB2 in different pathologies. This was done by blocking its activity with specific recombinant antibodies generated by us, in collaboration with groups from the MRC Clinical Sciences Centre (UK) and the NCI Center for Cancer Research (USA).
Vice-Direction of Translational Research

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

Clinical Research Programme
- Gastrointestinal Cancer Clinical Research Unit
- Breast Cancer Junior Clinical Research Unit
- Prostate Cancer Junior Clinical Research Unit
- Molecular Diagnostics Unit
- H12O-CNIO Haematological Malignancies Clinical Research Unit
- H12O-CNIO Lung Cancer Clinical Research Unit

Biobank
“With our translational research efforts, at the CNIO we are trying to reduce the gap between research laboratories and cancer patients.”

The ultimate goal of the CNIO is to contribute to the global effort in the fight against cancer. This involves the work of many different laboratories and individuals, who collectively help to achieve this mission. During 2016, the CNIO maintained an important focus on translational research, placing significant importance on those research aspects that are in close proximity to the patients. These included the identification of novel mutations and altered pathways in samples from cancer patients that could perhaps be used to guide novel treatments. It is noteworthy to mention that a lot of this work was done under the framework of large international Consortia in which we participated, again underscoring CNIO’s active contribution towards the worldwide effort in cancer research. One example of our key role in collaborative efforts is the important function that the CNIO Biobank carries out in coordinating the Spanish National Biobank Network, which manages patient samples from 52 institutions across Spain. Finally, and in addition to our work in the discovery of molecular alterations that are present in cancer patients, the Clinical Research Programme tries to capitalise on these discoveries (as well as others from all around the world) by bringing them to the clinic. Besides from their efforts in trying to implement new clinical trials, the research from our clinical groups has revealed new insights that can be used to benefit cancer patients, such as novel strategies directed towards overcoming resistance to antiangiogenic agents. Finally, through our collaborative agreement with Hospital 12 de Octubre, two Clinical Research Units established by investigators from that hospital are also housed at the CNIO, in an effort to further strengthen our links with neighbouring hospitals.
The Human Cancer Genetics Programme is currently composed of three Research Groups: Human Genetics, Endocrine Cancer, and Genetic and Molecular Epidemiology Groups, and three Units: Human Genotyping CEGEN and Molecular Cytogenetics and Genome Editing Units, and the Familial Cancer Clinical Unit. In addition, there is a Familial Cancer Consultancy for the evaluation of families with cancer and the provision of genetic counselling. The Consultancy is located in the Hospital Universitario de Fuenlabrada and works in close collaboration with the Oncology Service at that Hospital. The number of consultancy days and amount of families attended have increased since we set it up five years ago. Currently, we are attending to around 350 families/year. This increase of families has resulted in a higher number of genetic and genomic diagnosis studies, which have been made possible thanks to the incorporation of a massive sequencing platform. This platform has been operational over the past year.

The Programme's core goals are centred on research, training and diagnosis. The genetic and cytogenetic study of tumours, genome editing, genetic interactions, data integration, the search for diagnostic and prognostic markers, the discovery of novel cancer-related genes and environmental factors that confer cancer susceptibility, are our main research priorities. A further complementary area of work is the application of Pharmacogenetics and Pharmacogenomics to identify genes that modify drug response. This research line focuses on a wide variety of tumours, taking advantage of the high-throughput genotyping technologies provided by the Genotyping Unit.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote in training and education. During this year, the Programme's groups have hosted 8 residents from different Spanish hospitals for 3-month periods. We also offer professionals from different international research centres the opportunity to join us, either as visitors or for longer training visits consisting of short-term stays of 1-3 months (a total of 3 international visitors from Latin America were hosted in 2016).

In terms of education, since the beginning of 2016, 1 foreign and 3 national Erasmus Master's students and 9 national and 2 international PhD students have worked on their research projects, 1 of whom has already successfully defended her thesis. We participate in many international and national consortia. This enables us to apply for international projects, hold international meetings and publish in the best journals. Likewise, a good collaboration with other CNIO Groups and Units is one of our main characteristics, allowing us to benefit from the internal exchange connecting people, techniques, technology and knowledge.

Milestones and major achievements of the Programme in 2016 include:

- The co-organisation of the 6th Familial Cancer Conference in collaboration with the European School of Oncology and Nature Reviews Clinical Oncology.
- Mercedes Robledo, Head of the Hereditary Endocrine Cancer Group, was awarded the International Medal bestowed by the Society for Endocrinology.
- Núria Malats, Head of the Genetic and Molecular Epidemiology Group, was elected Chair of the EUPancreas COST Action.
- The co-direction of the CNIO Canceromatics III - Tumor Heterogeneity Conference, November 2016.

“The programme continues with carrying out its translational work, connecting clinicians with scientists, studying human cancers and helping and advising professionals about the new genetic results generated by novel technologies.”
The Human Genetics Group is working on the study of human cancer from a genetic, cytogenetic and epidemiologic point of view. We want to understand why the inherited susceptibility to cancer doesn't follow a mathematical model in people, but rather ‘an apparently random model’, and why there are families with a large number of members suffering from cancer.

For these studies we work with individuals, families and the affected and normal population, trying to perform a correct diagnosis with known genes as well as looking for new genes that could explain cancer susceptibility in specific families. Our main objective is to work with every family by raising their awareness in regards to their own risk of developing cancer and how to prevent it. To this primary level, we have to add a secondary level of prevention, which will facilitate an important risk reduction in the population, through the development of non-invasive and non-genetic but yet extremely effective measures.

“During 2016, we showed how inhibitors, other than PARPi, could be used in patients with BRCA mutations. We started working on the identification of new treatments for cardiac tumours based on transcriptome analysis, and finally, we are also exploring a polygenic inheritance model in families with testicular cancer that is based on more than 25 identified genes associated with this disease.”

Breast cancer: PARPi and OGG1 inhibitors in BRCA1 mutation carriers

We have demonstrated that certain missense mutations in BRCA1 seem to make cells more sensitive to Poly (ADP-ribose) Polymerase (PARP) inhibitors than those mutations that give rise to the absence of the protein (frameshift mutations) (T. Valclová, Hum Mol Genet 2016). We are currently investigating the mechanisms underlying these differences with the aim of identifying new markers of sensitivity or resistance to these agents.

In parallel, we recently showed that the Single Nucleotide Polymorphism (SNP) rs2304277 located in the 3’ untranslated region (UTR) of the OGG1 DNA glycosylase gene of the Base Excision Repair pathway (BER), modified cancer risk in patients harbouring mutations in BRCA1 (Osorio A. et al., Plos Genetics, 2014). We have identified that the SNP is associated with a constitutive OGG1 transcriptional downregulation, which leads to a high genome and telomere instability in those patients harbouring BRCA1 and BRCA2 mutations, thereby explaining the contribution of this polymorphism to cancer risk. This association is most likely explained by a synthetic lethal/sick interaction between these 2 genetic events. (Benitez-Buelga C. et al., Oncotarget, 2016).

Familial cancer exome project

This project started several years ago with the objective of identifying new high susceptibility genes that explain families...
with rare tumours as well as deciphering the genetic heterogeneity present in some of them.

→ In 2015, we identified ATPα as being responsible for families with gastric neuroendocrine tumours. We are currently searching for new genes in families that cannot be explained by mutations in ATPα.

→ A second gene, POT1 ATP4a, was identified by mutations in two families that cannot be explained by mutations in POT1. We are currently investigating a large family with meningiomas across 3 generations. Analysis of the data generated using Blasco’s Telomeres and Telomerase Group, we are generating a knock-in mouse with the aim of recapitulating the disease, as well as enabling us to work with antimicrobial drugs.

→ We are currently investigating a large family with meningiomas across 3 generations. Analysis of the data generated using Blasco’s Telomeres and Telomerase Group, we are generating a knock-in mouse with the aim of recapitulating the disease, as well as enabling us to work with antimicrobial drugs.

**Publications**


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 major susceptibility gene for paraganglioma, a gene-net regulated by methylation in medullary thyroid carcinoma, and germline variants and tumour microRNAs that predict outcomes in cancer therapies.""
RESEARCH HIGHLIGHTS

A gain-of-function mutation in DNMT3A causes paraganglioma

The high percentage of patients carrying germline mutations makes phaeochromocytomas (PCC) and paragangliomas (PGL) the most heritable of all tumours. However, there are still cases that are not explained by mutations in the known susceptibility genes. We aimed to identify the genetic cause in patients strongly suspected of having hereditary tumours. Whole-exome sequencing was applied to the germline of a parent-in patients strongly suspected of having hereditary tumours. We aimed to identify the genetic cause

Identification of germline variant in tumor microRNAs to predict outcomes in cancer therapies

Personalised cancer treatment is of enormous clinical and social relevance since it can lead to safer and more efficient therapies. This year we focused our efforts on applying next generation sequencing to: i) understand how low frequency genetic variants impact paclitaxel-induced neutropathy; and ii) identify microRNAs predictive of the antiangiogenic drug response in renal cancer patients. Peripheral neutropathy diminishes the quality of life of many cancer patients, sometimes permanently, and limits the dose and efficacy of many cancer drugs. We found that low frequency variants in EIF4E, EPHAS and EPHA4 genes contribute to the susceptibility to paclitaxel-induced neutropathy. Furthermore, EPHA4 neural injury repair function suggests that these genes might constitute important neuropathy markers for many neurotoxic drugs. Regarding antiangiogenic therapies, these have drastically improved the survival of kidney cancer patients; however, a fraction of the patients are refractory to these drugs. The first miRNA deep sequencing study on an exceptional series of patients treated with sunitinib revealed microRNAs predictive of sunitinib response. Furthermore, a two microRNA-based classifier discriminated individuals with progressive disease upon sunitinib treatment (P=1.3x10^-4) with better predictive value than the commonly used clinicopathological risk factors. Thus, we provide new relevant markers that can help rationalise cancer treatment.

Multilayer OMIC data in Medullary Thyroid carcinoma identifies the STAT3 pathway as a potential therapeutic target in RETM918T tumours

Medullary thyroid carcinoma (MTC) is a rare disease with few genetic drivers that, when diagnosed at an advanced stage, remains incurable. Due to its rarity, its genomic dissection has not been comprehensively explored. Exploiting multilayer genomic data, considering the transcriptome, miRNome and methylome, it is possible to uncover genes negatively regulated by methylation, such as DNMT1, DNMT3B, DNMT3L, DNMT3A and miR-340; using ME-CHR1 and CT-cell lines. Moreover, hypomethylation may induce activation of key pathways related to the malignant behaviour of RETM918T-related MTCs. Functional annotation enrichment analysis identified the Jak/Stat pathway as a specific hallmark of RETM918T-harbouring MTCs. In vitro studies with MTC cell models pointed to a RETM918T genetic class-specific proliferative dependency on STAT3 activity. Remarkably, the inhibition of STAT3 increased the sensitivity of RETM918T-bearing MTC cells to the FDA-approved RET inhibitor Vandetanib. This combinational treatment could potentially overcome the adverse effects encountered in clinical practice when Vandetanib monotherapy is applied.
The scope of 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.

“We have undertaken in-depth analyses integrating omics and non-omics data to predict pancreatic and bladder cancer risk and outcome, and have assessed the challenges that epidemiology faces in this endeavour.”
RESEARCH HIGHLIGHTS

Research findings

During 2016, the Group mainly focused its research on pancreatic and bladder cancers. Regarding pancreatic cancer (PC), we further analysed the epidemiological and clinical data from the PanGenEU Study and have characterised the risk of PC associated with diabetes, multimorbidity patterns and family history of cancer, among others. We have completed the genome-wide association study (GWAS) and, in collaboration with the international consortia, we are now replicating the primary findings. We are exploring, in collaboration with experts in the field, genome-wide methylation data generated with the Illumina 850K array in cases and controls. We also participated in a study that identified 3 new pancreatic cancer susceptibility signals on chromosomes 1q21.2, 5p15.33 and 8q24.23. Regarding bladder cancer (BC), we showed that common SNPs have a limited role in predicting BC outcomes and reported, for the first time, a heritability estimate for disease outcome by assessing the predictive ability of the models, including up to 171,304 SNPs for tumour recurrence and progression. We have also provided proof of concept for the joint effect of genetic variants in improving the discriminative ability of clinical prognostic models by using innovative analytic approaches, and demonstrated that SNPs in inflammation-related genes were associated with BC prognosis (FIGURE1). Through international collaborations, the Group has participated in the exploration of common germline variants in the APOBEC3 region associated with BC and breast cancer risk, and observed a tissue-specific role of environmental oncogenic triggers. In line with this study, mutations in cancer driver genes were primarily found in high-risk BC, together with APOBEC-related mutational signatures. We also participated in the development of a urine-based peptide biomarker and a combined methylation/mutation panel for detecting both primary and recurrent BC.

Methodological contributions

We have proposed an epidemiological-based integration of omics and non-omics data by considering the ‘massive’ inclusion of variables in the risk assessment and predictive models (FIGURE 2). We also discussed the numerous challenges embedding this type of research and have proposed analytic strategies that allow considering both omics and non-omics data used in the models towards a personalised prevention. Furthermore, we have adapted Bayesian sequential threshold models in combination with LASSO and applied them to time-to-event and the censoring nature of data, in order to study 822 BC patients followed-up more than 10 years.

Translational activities

We coordinate the COST Action BM1204 EUPancreas (www.eupancreas.eu). This Action includes 250 multidisciplinary members from 22 EU countries, European and international nongovernmental organizations, and private companies. Several scientific, training, and dissemination activities have been conducted during 2016. By endorsing the Pancreatic Cancer Europe (PCE) multi-stakeholder platform, we have actively participated in several activities aimed at increasing the awareness of PC in the general population, the medical community, and among health policy makers. The Group has actively participated in setting up a European-based clinical registry of PC (PanCreD) jointly with the EPC; the Joint Research Centre from the European Community, and the European Network of Cancer Registries. The Group has also contributed to the development of recommendations for a state strategy for personalised/precision medicine, by the Roche Institute. Another area our Group contributed to was the identification of different sources of big data and the importance of unstructured data for potential future uses in drug discovery; the main practical and ethical challenges to unravel the full potential of big data in healthcare were discussed.

**PUBLICATIONS**

- **Zhao W et al.** (incl. Real FX, Malats N) (2016). Mosaic loss of chromosome 1 is associated with common variation near TCL1A. Nat Genet 48, 563-568.

**PATENT**


**AWARDS AND RECOGNITION**


**FOUNDERS**


**WEBSITES**

- **www.eupancreas.eu**
ANNUAL REPORT 2016

NGS, the utility of testing multiple genes with different modes is changing with the routine use of NGS. Despite the promise of comprehensiveness of clinical management of cancer, these syndromes can significantly enhance the quality and as to minimise toxicity and maximise efficacy. Vigilance of the refinement and optimisation of treatment strategies so for both the patient and his/her relatives, but also for facilitating for genetic counselling and for the design of a surveillance scheme heritable cancer predisposition syndrome is not only essential for cancer at an early age of onset deserve special attention because Individuals that present with an uncommon malignancy or with cancer at an early age of onset deserve special attention because they are more likely to harbour an inherited predisposition and may require unique treatment strategies. Identification of a heritable cancer predisposition syndrome is not only essential for genetic counselling and for the design of a surveillance scheme for both the patient and his/her relatives, but also for facilitating the refinement and optimisation of treatment strategies so as to minimise toxicity and maximise efficacy. Vigilance of these syndromes can significantly enhance the quality and comprehensiveness of clinical management of cancer. In addition, the evaluation of inherited cancer predisposition is changing with the routine use of NGS. Despite the promise of NGS, the utility of testing multiple genes with different modes of inheritance and with varying levels of penetrance has been questioned due to the increasing costs of surveillance and unnecessary treatments, and the uncertain consequences of the identification of variants of unknown significance. More than ever it is necessary to underline that NGS testing should only be offered in the context of expert genetic counselling. In the Cancer Genetics Consultation of the Familial Cancer Clinical Unit (FCCU) we work together with Fuenlabrada Hospital clinicians, as well as health-care providers from other Madrid hospitals and other Autonomous Communities, in order to heighten the vigilance of hereditary cancer syndromes and to better adapt the genetic counselling process in alignment with the introduction of new technologies. 

Individuals that present with an uncommon malignancy or with cancer at an early age of onset deserve special attention because they are more likely to harbour an inherited predisposition and may require unique treatment strategies. Identification of a heritable cancer predisposition syndrome is not only essential for genetic counselling and for the design of a surveillance scheme for both the patient and his/her relatives, but also for facilitating the refinement and optimisation of treatment strategies so as to minimise toxicity and maximise efficacy. Vigilance of these syndromes can significantly enhance the quality and comprehensiveness of clinical management of cancer. In addition, the evaluation of inherited cancer predisposition is changing with the routine use of NGS. Despite the promise of NGS, the utility of testing multiple genes with different modes of inheritance and with varying levels of penetrance has been questioned due to the increasing costs of surveillance and unnecessary treatments, and the uncertain consequences of the identification of variants of unknown significance. More than ever it is necessary to underline that NGS testing should only be offered in the context of expert genetic counselling. In the Cancer Genetics Consultation of the Familial Cancer Clinical Unit (FCCU) we work together with Fuenlabrada Hospital clinicians, as well as health-care providers from other Madrid hospitals and other Autonomous Communities, in order to heighten the vigilance of hereditary cancer syndromes and to better adapt the genetic counselling process in alignment with the introduction of new technologies.

> OVERVIEW

> PUBLICATIONS


Book chapter


PATENT

Chromosomal translocations are very common events involved in the development of several cancers, especially in sarcomas and haematological malignancies. The research activity of the Molecular Cytogenetics and Genome Editing Unit covers the main topics related to cancer cytogenetics and genome engineering: from classical cytogenetics techniques to new genome engineering tools, including the CRISPR-Cas9 system. We are focusing on the implementation and development of genome engineering tools, including the CRISPR-Cas9 system.

The Molecular Cytogenetics and Genome Editing Unit is the molecular cytogenetics component of the Human Cancer Genetics Programme. It also participates in collaborative projects with clinical and toxicological testing and biomarker identification. The Molecular Cytogenetics and Genome Editing Unit covers research on the molecular mechanisms underlying the initiation of human cancers, and can also be used for high-throughput drug screening, toxicological testing and biomarker identification.

From the patient’s chromosome translocations to their functional effects

We have worked on the oncogenic role of the translocation t(8;21)(q22;p22)/RUNX1-RUNX1T1, which occurs in 4% of acute myeloid leukemia patients. We deciphered a new function for the activation of MAP8, observed in t(8;21)+ cells, which is responsible for the stabilisation of SPI. Our data show the essential role of SP1 in t(8;21)+ cell maintenance through the regulation of key genes, such as CDKN1A. These results provide new evidence for the inclusion of pharmacological approaches leading to degradation of SPI in the treatment of these patients.

Optimising CRISPR-Cas9 to model cancer aberrations in primary cells

In vitro modelling of complex tumour-associated chromosome translocations at native loci is feasible with CRISPR. However, the generation of translocations must be optimised, especially for mimicking events in human primary cells. We have optimised our CRISPR protocol to efficiently obtain those cells, thereby enabling the rescue of translocation+ populations of human primary cells, including induced pluripotent stem (iPS) cells and mesenchymal stem cells (MSCs). These models can surely help us to understand the molecular mechanisms underlying the initiation of human cancers, and can also be used for high-throughput drug screening, toxicological testing and biomarker identification.

We provide state-of-the-art molecular cytogenetics and genome editing services. The Unit makes available various techniques to the CNIO Research Groups; these techniques provide more sensitive and accurate tools to analyse cancer cells, such as RNA-FISH, chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific FISH analysis to detect genomic integration sites of small constructs including LV particles. In 2016, we carried out over 1,000 assays for experimental and clinically-oriented projects.

**RESEARCH HIGHLIGHTS**

**By way of different molecular approaches, we generate human cancer cell models carrying tumour-associated chromosomal translocations in order to study their functional contribution to oncogenesis.”**

**PUBLICATIONS**

the promoter of ABC22 as the strongest association with tumour response in patients treated with docetaxel (P=0.009). We also identified a significant association for an intronic variant located in CYP1B1 associated with docetaxel tumour response (P=2.1×10⁻⁴). Our integrated pathway-based approach enables the revealing of promising genetic biomarkers of treatment outcome in breast cancer patients.

New low-frequency variant loci associated with anthracycline-induced cardiotoxicity (AIC) in cancer patients by Illumina HumanXome BeadChip

Anthracycline chemotherapeutic agents are widely used in the treatment of cancer; however, chronic anthracycline-induced cardiotoxicity (AIC) is a serious long-term complication leading to substantial morbidity. Our aim was to identify new genes and low-frequency variants influencing the susceptibility to AIC. We studied the association of variants on the Illumina HumanXome BeadChip array in a discovery cohort of breast cancer anthracycline-treated patients. Using gene-based tests (SKAT-O) that have greater statistical power to detect rare variant associations and that can evaluate the cumulative effect of multiple genetic variants, we identified novel variant associations in a gene with a major role in mitochondrial oxidative β-oxidation and the respiratory chain, involved in anthracycline-related toxicity via an oxidative stress mechanism. We replicated our association result in another cohort of anthracycline-treated paediatric cancer patients from Spain.

Functional characterisation at the 20q13.33 risk locus for capetitabine-induced hand-foot syndrome (CHFHS)

Capetitabine is a chemotherapy drug widely used in breast and colorectal cancer; the most frequent adverse drug reaction to this treatment (in 10% of the patients) is CHFHS, a cause of dose reductions and dose delays. By genome-wide association studies (GWAS), we identified four linked CHFHS regulatory variants (β2-haplopyotype) associated with the risk of CHFHS appearance (HR:2.48 p=1.43×10⁻⁶). The CHFHS gene encodes R-Cadherin, which is located in the granular layer of the epidermis and is involved in the cohesion of epidermal layers. We demonstrated that these regulatory variants are able to mediate chromatin structural changes in chromatin organisation, which results in the presence of the risk alleles and in decreased expression levels of CHFHS mRNA and R-Cadherin protein. Additional functional experiments are being performed. The study has been carried out in collaboration with CNIO’s Chromosome Dynamics Group and the Epithelial Cell Biology Group.

**OVERVIEW**

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

“Advances in understanding patients’ responses to therapy will help to individualise cancer patient care.”

**RESEARCH HIGHLIGHTS**

Identification of genetic variants associated with docetaxel and anthracycline efficacy

Taxanes and anthracyclines are widely used in the treatment of breast cancer, despite the benefit being limited to a small proportion of patients and that preoperative biomarkers, which are predictive of clinical outcome, still remain lacking. We carried out a pharmacogenetic study in 181 patients with locally advanced breast cancer, previously enrolled in a phase II randomised controlled trial (NCT00132929), in which patients were randomly assigned to receive docxorubicin (anthracycline) or docetaxel (taxane) neoadjuvant chemotherapy. We assessed whether genetic variants in 13 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 ABC22 as the strongest association with tumour response in patients treated with docxorubicin (P=0.009). We also identified a significant association for an intronic variant located in CYP1B1 associated with docetaxel tumour response (P=2.1×10⁻⁴). Our integrated pathway-based approach enables the revealing of promising genetic biomarkers of treatment outcome in breast cancer patients.

**PUBLICATIONS**


**TECHNIQUES**

- Chen Alzons, Núria Álvarez, Belén Herrera, Tak Moreno (until October) (TS), Guillelmo Pita (TS), Belco Núñez (since October) (TS)
- *Diploma Superior (Advanced Degree)
The Clinical Research Programme (CRP) aims to translate advances in cancer research into the prevention, diagnosis, and treatment of patients. The major goals of the CRP are the conduction of early clinical trials with novel drugs, the discovery of biomarkers of drug action and disease outcome, the implementation of a strategy for personalised medicine, and the launching of a training programme in drug development.

The CRP is composed of 5 Clinical Research Units (CRU) and 1 support Unit. The Gastrointestinal Cancer CRU, led by Manuel Hidalgo until December 2016, studies novel therapeutics and personalised medicine in pancreatic cancer. Miguel Quintela-Fandino leads the Breast Cancer CRU that works on the development of kinase and angiogenesis inhibitors in breast cancer, as well as on the understanding of the molecular taxonomy and metabolic vulnerabilities of this disease. The Prostate Cancer CRU, led by David Olmos, explores novel therapeutics and biomarkers of the disease, with a particular interest in understanding DNA damage repair deficiency mechanisms in prostate cancer. The Lung Cancer CRU, headed by Luis Paz-Ares, and the Haematological Malignancies CRU, led by Joaquín Martínez-López – both established as part of an agreement with the Hospital Universitario 12 de Octubre – focus on molecular and preclinical studies in non-small cell lung cancer and in multiple myeloma, respectively. The Molecular Diagnostics Unit, led by Luis Lombardía, provides support to medical professionals of the National Health system and the CRP through the provision of a wide variety of molecular tests that determine alterations in biomarkers involved in cancer. In 2016, the Programme continued the expansion of its clinical trials activities in collaboration with several hospitals in Spain.

“The Clinical Research Programme focuses on developing novel and more effective treatments against cancer.”
GASTROINTESTINAL CANCER CLINICAL RESEARCH UNIT

Clinical Investigator
M José De Miguel (until June), Laura Medina (TS)

Clinical Research Unit Head
Manuel Hidalgo (until December)

Post-Doctoral Fellows
Lucía Fernández (until June), Camino Menéndez, María Vela (until February)

Graduate Students
Spas Dimitrov, Beatriz Salvador

Technicians
Carolina Alonso (since December), Natalia Baños, Victoria Bonaña, Yolanda Durán, Manuel Muñoz

Clinical Investigators
Sofía Perea, Begoña Vázquez (since April)

Staff Scientists
Rodrigo De Almeida, Pedro P. López, Sofía Perea, Begoña Vázquez (since April)

Clinical Investigators

Clinical Investigators
Camino Menéndez, María Vela (until June), Lucía Fernández (until June), Camino Menéndez, María Vela (until February)

Clinical Investigators
Raul Calero (CNIO-HNJ Clinical Research Unit, Hospital Infantil Univesitario Niño Jesús, Madrid), Lucas Moreno (CNIO-HNJ Clinical Research Unit, Hospital Infantil Univesitario Niño Jesús, Madrid)

Clinical Investigators
Yolanda Durán, Manuel Muñoz, Natalia Baños, Victoria B.bonilla, Soraya Ardila (until March) (TS)*, Carolina Alonso (since December), Laura Gallardo, Beatriz Salvador, Gemma M. Sánchez (TS)*, Francesca Sarno (TS)*

Clinical Investigators
Raul Calero (CNIO-HNJ Clinical Research Unit, Hospital Infantil Univesitario Niño Jesús, Madrid)

Clinical Investigators

OVERVIEW

The Gastrointestinal (GI) Cancer Clinical Research Unit focuses on the clinical development of novel therapeutics for patients with cancers of the gastrointestinal tract as well as personalised medicine approaches for these patients. The work of the Group combines the preclinical assessment of novel anticancer agents in ‘Avatar’ mouse models with the design, conduction, and analysis of clinical trials with novel anticancer agents in patients with gastrointestinal tumours. Over the last few years the Group has implemented a growing portfolio of clinical trials with new agents spanning a broad range of mechanisms of action.

Key to the work is the development and characterisation of Avatar mouse models for drug screening, biomarker development, and personalised medicine. The Group has developed and has characterised the largest collection of these models in pancreatic cancer. Avatar models are used in 3 critical applications: (i) the screening of new anticancer agents; (ii) conduction of co-clinical trials, in which ongoing clinical trials are performed in parallel with studies using Avatar models of the same cancer type in order to elucidate mechanisms of action and biomarkers of drug response; resistance; and (iii) finally, the Avatar models for personalised cancer treatment integrated with next generation sequencing.

• PUBLICATIONS


Pharmacodynamic analysis of clinical trials with novel anticancer agents in patients with gastrointestinal tumours. Over the last few years the Group has implemented a growing portfolio of clinical trials with new agents spanning a broad range of mechanisms of action.

Key to the work is the development and characterisation of Avatar mouse models for drug screening, biomarker development, and personalised medicine. The Group has developed and has characterised the largest collection of these models in pancreatic cancer. Avatar models are used in 3 critical applications: (i) the screening of new anticancer agents; (ii) conduction of co-clinical trials, in which ongoing clinical trials are performed in parallel with studies using Avatar models of the same cancer type in order to elucidate mechanisms of action and biomarkers of drug response; resistance; and (iii) finally, the Avatar models for personalised cancer treatment integrated with next generation sequencing.

• PUBLICATIONS


ANNUAL REPORT 2016

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 therapeutic treatments. Our activities are directed towards personalized treatment, and range from preclinical models to the sponsoring of multicentric clinical trials. Specifically, our research areas are:

- Discovery of new targets for breast cancer prevention: role of fatty acid synthase (FASN).
- Breast cancer functional taxonomy: by using a systems biology approach, we are clustering the disease into subtypes defined by biologic features that constitute therapeutic targets.
- Study of the mechanisms of resistance against targeted therapies.
- Conduct investigator-initiated clinical trials.

Research Highlights

In the field of functional taxonomy, we have completed our study in triple-negative breast cancer. We have interrogated the disease from the bimodal relapse pattern point of view, and performed a phosphoproteomic screening that would reduce the countless patterns of genomic, epigenomic and transcriptomic aberrations into a discrete number of patterns of hardened signalling pathways. We found 6 kinases whose hyperactivity accounted for 94% of the relapsed cases. These kinases were grouped into a maximum number of 34 patterns, the largest of which (25%) was virtually associated with cure. This taxonomy was also useful because all the kinases in the final ‘relapse signature’ were also targetable nodes.

Regarding the study of targeted therapies, we have observed that the generally assumed hypothesis of vascular normalization upon exposure to antiangiogenics is not always true. In fact, resistance against antiangiogenics can originate after a vascular normalising or ‘abnormalising’ response. Whether a tumour experiences the former or the latter depends on the tumour type and the type of agent. What is quite important from the clinical point of view is that we can track, individually, whether a tumour experiences a normalising or an abnormalising response after less than 2 weeks of exposure to the agent, using a non-invasive imaging test with 18F-fluoromisonidazole. This has been demonstrated in animals and in patients. The applicability of this finding lies in the fact that we have also unravelled the mechanisms of resistance depending on whether the tumour reacts with normalisation or abnormalisation against antiangiogenics: in the first case, the tumour switches from glycolytic to mitochondrial metabolism, which is reversible by mitochondrial inhibitors. In the latter, the tumour experiences an immune-switch. Since both mechanisms are targetable, we can now individually track which pathway a tumour is undergoing upon exposure to antiangiogenics and tailor which synergistic agent that patient would need.

Publications

Prostate cancer (PrCa) is the most common cancer and the 2nd leading cause of cancer mortality among men in Western countries. Despite advances in diagnosis and early-disease treatment, up to 30% of PrCa patients will develop metastasis at some point and succumb after the acquisition of a castration-resistant status. Despite advances in diagnosis and early-disease treatment, up to 30% of PrCa patients will develop metastasis at some point and succumb after the acquisition of a castration-resistant status. In addition to AR aberrations following androgen-deprivation therapy leading to resistance to current treatment options, DNA repair defects have been identified in about 5% and 25% of early PrCa and mCRPC, respectively. Seminal work from our Group, and others, has established that some alterations, e.g. germline BRCA1/BRCA2 deleterious mutations, are linked to poor outcomes. Currently, we have established that some alterations, e.g. germline BRCA1/BRCA2 deleterious mutations, are linked to poor outcomes. Analyses of the clinical impact of germline and somatic mutations in outcomes are still undergoing. BRCRAD and BRCAP110S studies, although in a retrospective fashion, will address similar questions at an early prostate cancer stage.

**RESEARCH HIGHLIGHTS**

**PROCURE biomarkers platform**

This network was started by our Group in 2013; it currently has 5 ongoing prospective studies (PROREPAIR, PROFSTAC, PROSABI, PROSENA, PRORAD) in mCRPC in 63 participating centres with over 900 enrolled patients.

**PROREPAIR study**

This is a prospective multicentre cohort study involving 50 centres with over 900 enrolled patients.

**RESEARCH UNIT**

**PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT**

**OVERVIEW**

**RESEARCH HIGHLIGHTS**

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**Publications**


**AWARDS AND RECOGNITION**

- Scientific Committee Member: ESMO Congress, Copenhagen. Faculty Board Member, EDTIC-ECOG-ACRIN-ESMO Metastatic Prostate Cancer. Copenhagen.

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**Clinical Research Programme**

**PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT**

**Overview**

Prostate cancer (PrCa) is the most common cancer and the 2nd leading cause of cancer mortality among men in Western countries. Despite advances in diagnosis and early-disease treatment, up to 30% of PrCa patients will develop metastasis at some point and succumb after the acquisition of a castration-resistant status (mCRPCa). The early identification of PrCa patients with greater intratumour heterogeneity. Biological characterisation of BRCA2 and ATM mutated tumours

**SWITCH Phase II study**

In 2016, we also completed the enrolment and follow-up of our first clinical trial, ‘Phase II pilot study of the prednisone to dexamethasone switch in mCRPC patients with progression on abiraterone and prednisone’, aimed at analysing the role of certain steroids in the resistance and response to novel androgen-synthesis inhibitors in 28 patients. A simple change in prednisone to dexamethasone rescued the sensitivity to abiraterone and prolonged the time benefiting from this treatment in 40% of the patients; such responses could be linked to AR mutations detected in ctDNA.
**OVERVIEW**

The Molecular Diagnostics Unit (MDU) is mainly dedicated to developing, implementing, standardising and making available a wide variety of highly sensitive and specific molecular diagnostics assays that are scarcely available in the Hospitals of the Spanish National Health System. MDU’s portfolio of genetic tests enables the determination of alterations in the sequence or expression levels of key genes involved in cancer. In turn, these assays can be used for the early diagnosis of neoplasias, the detection of minimal residual disease in patients showing clinical remission, for monitoring the response to therapy in patients, as well as for facilitating decision-making amongst different treatment options. Furthermore, the Unit also provides support to the research needs of CNIO’s Clinical Research Units and Research Groups by checking their samples for alterations in the biomarkers included in our portfolio. Finally, MDU is very much committed to disseminating knowledge in the field of molecular diagnostics by hosting and mentoring biomedical students.

"In this transition phase of precision medicine, MDU is increasingly focused on the implementation of assays for the detection of biomarker alterations that could grant a more selective diagnosis for cancer patients."

**RESEARCH HIGHLIGHTS**

**Strengthening our support**

During 2016, our catalogue has grown with the addition of a new molecular diagnostics test based on the detection, by bi-directional Sanger sequencing, of mutations in exons 4 and 5 of the MYD88 gene. Waldenström’s macroglobulinemia (WM) is a rare form of blood cancer that is characterised by an excess of malignant white blood cells (lymphoplasmacytic cells) in the bone marrow. It has been shown that WM is the result of a multistep transformation process that accumulates sequential oncogenic alterations. The most prominent is the L265P somatic activating mutation in the MYD88 gene (present in 90% of WM). Hence, its detection would enable us to differentiate WM (but also diffuse large B-cell vitreoretinal lymphoma or marginal zone lymphomas) from indolent B-cell or other chronic lymphoproliferative disorders.

Additionally, because identification of several gene alterations involved in the onset of myeloproliferative neoplasms (MPNs) has revealed the huge complexity of these diseases and has challenged their accurate differential diagnosis, we started working on the implementation and validation of a new assay that will enable us to detect mutations in the TET2 gene; this will complement the diagnosis of MPNs patients. Mutations in this tumour suppressor gene (present in 13% of MPNs) lead to genomic instability via epigenetic modifications and foster cancer progression. Recent studies have revealed that the order in which these mutations are acquired is critical. Thus, patients with early mutations in TET2 were more likely to have better prognosis compared to patients who had previous mutations in other genes linked to MPNs (FIGURE).

Lastly, we have completed the initial experimental phase of a clinical trial sub project, FRAGANCE, led by the CNIO Gastrointestinal Cancer Clinical Research Unit, which is geared towards precision medicine for fragile patients with advanced pancreatic cancer.

**Tutoring**

MDU has also upheld its policy regarding training programmes in 2016 by welcoming one medical resident and one undergraduate student.
The most relevant achievements of our Group in 2016 were:

- We reported a phase I clinical trial based on an innovative cell therapy approach using activated and expanded NK cells for Multiple Myeloma (MM). The results of exploring this approach in phase II and III clinical trials are promising.
- We published the first report of exome sequencing in amyloidosis. Finally, we redefined the role of stringent complete response in Multiple Myeloma.
- We reported a phase I clinical trial based on an innovative approach in phase II and III clinical trials are promising.
- We published the first report of exome sequencing in amyloidosis. We have not found any recurrent mutation.
- Finally, we redefined the role of stringent complete response by next generation sequencing in Multiple Myeloma.

**OVERVIEW**

The Haematological Malignancies Clinical Research Unit focuses on 3 main objectives:

- Molecular research of haematological cancer: the study of cancer-induced changes at the proteomic and genomic levels. We aim to:
  - Find new genomic and proteomic biomarkers for a better diagnosis of these haematological diseases.
  - Identify new molecular alterations as predictors of response to treatment, e.g. to study minimal residual disease.
  - Study immune mechanisms of cancer control, with a special focus on NK cells.
- In vivo research: i) to establish the effects of new anticancer molecules in vitro models of the disease; ii) to determine the mechanisms of resistance to anticancer drugs.
- Clinical research: to translate preclinical findings to the patients through a phase I clinical trial unit.

“We contribute towards redefining the response criteria for Multiple Myeloma (MM) through the usage of new molecular techniques. In 2016, we published the first reports sequencing complete exomes of amyloidosis. Finally, we reported a new cell therapy approach based on infusion of NK cells in MM.”

**PUBLICATIONS**

- Selected publications at other institutions

**SELECTED PUBLICATIONS AT OTHER INSTITUTIONS**

- Selected publications at other institutions
Our Group combines basic preclinical studies with clinical and translational research, mainly in lung cancer and other solid tumours. In summary, the main research areas of our Group focus on 2 modalities: (1) the identification of new molecular biomarkers that can be used in the clinic for diagnostic, prognostic, predictive and pharmacogenomic purposes; and (2) developing novel treatment strategies. For example, we have comprehensively profiled bronchoalveolar lavage (BAL) fluids of developing novel treatment strategies. For example, we have comprehensively profiled bronchoalveolar lavage (BAL) fluids of non-small-cell lung cancers to test new drugs/targets. We were fully recruited in 2016, with a substantial contribution by investigators from our Group. These important data have been recently published in The Lancet Oncology. In addition, Luis Paz-Ares is the principal investigator of a phase I trial (JIVD) testing a novel combination of pembrolizumab plus ramucirumab in different types of solid tumours. Encouraging preliminary clinical data were presented at ASCO 2016 in the cohort of non-small-cell lung cancer, showing a response rate of 35% and 7-months of progression-free survival in pretreated patients. Finally, a first-in-human trial with a novel T-cell bispecific antibody targeting carcinoembryonic antigen (CEA) expressed on tumour cells and CDS on T-cells was initiated and is actively recruiting patients.

**New drug development and early clinical trials**

Our Group has been actively involved in pharmacogenomic, pharmacokinetic, translational and clinical studies with novel antituumour agents in several types of solid tumours, particularly lung cancer. Our principal clinical research area has been immunotherapy and immune-based early clinical trials. As a first relevant example we can mention the CheckMate CA 209-032 trial testing nivolumab +/- ipilimumab in recurrent or extensive-stage small-cell lung cancer, which was fully recruited in 2016, with a substantial contribution by investigators from our Group. These important data have been recently published in The Lancet Oncology. In addition, Luis Paz-Ares is the principal investigator of a phase I trial (JIVD) testing a novel combination of pembrolizumab plus ramucirumab in different types of solid tumours. Encouraging preliminary clinical data were presented at ASCO 2016 in the cohort of non-small-cell lung cancer, showing a response rate of 35% and 7-months of progression-free survival in pretreated patients. Finally, a first-in-human trial with a novel T-cell bispecific antibody targeting carcinoembryonic antigen (CEA) expressed on tumour cells and CDS on T-cells was initiated and is actively recruiting patients.

**Research highlights**

**New drug development and early clinical trials**

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**Conducting practice changing randomised controlled trials**

Our Group has also made a substantial contribution in conducting pivotal trials with immune checkpoint inhibitors. In particular, an important phase III trial, led by Dr Paz-Ares (the international principal investigator), with pembrolizumab in completely resected non-small-cell lung cancer patients is actively recruiting participants. Furthermore, the first randomised trial comparing second-generation (alatistinib versus first generation (gefitinib)) tyrosine-kinase inhibitors in patients with EGFR-mutated lung cancers, also internationally led by Dr Paz-Ares, was completed in 2016 and its results were recently published in The Lancet Oncology.

**Novel biomarker development and translation**

IL-11 and CCL-1 have been proposed as novel diagnostic biomarkers of lung adenocarcinoma in bronchoalveolar lavage fluid. This finding has potential implications in early lung cancer diagnosis. Moreover, different members of our Group contributed towards providing further insights into the role of PD-L1 expression and other potential immune biomarkers for the benefit of immune checkpoint inhibitors.
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. The CNIO Biobank facilitates 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.

The CNIO Biobank is a ‘biobank for biomedical research purposes’, as defined by the Spanish Law 14/2007 on Biomedical Research 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.

The biobank is organised as a technical unit with strict criteria for quality, order and purpose, regardless of whether or not it hosts other collections of biological samples for different purposes. Samples and their associated information are managed in compliance with Spanish legislation and international recommendations; all of this is consistent with quality criteria for sample collection and its subsequent management.

The Biobank has been authorised by the Health Authorities of the Autonomous Community of Madrid and the National Registry of Biobanks with reference B.000848. The Biobank has been accredited by the Health Authorities of the Autonomous Community of Madrid and the National Registry of Biobanks with reference B.000848.

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## Direction of Innovation

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“Innovation and research constitute both sides of the same coin. At the CNIO, we are making our effort so that the products of our research end up benefiting society.”

Scientific research often brings about the generation of knowledge and/or products that can be of use beyond the boundaries of academic research. These can include novel technologies, animal models, antibodies or chemical entities with interesting biomedical properties, among others. At the CNIO, we aim to bridge the gap between our researchers and potential outsources that could potentially be interested in further developing our inventions. These initiatives have materialised in the form of contracts with industry or in the licensing out of several of our products. Importantly, while the royalties deriving from these activities mostly benefit the CNIO as an Institution, they also circle back to the scientific Groups to fund their research as well as to the inventors themselves. To date, more than 40 investigators from 11 groups have benefited from this initiative.

A Programme that deserves to be singled out in terms of innovation is that of the Experimental Therapeutics Programme (ETP), a whole department that aims to develop chemical entities that could potentially lead to new anticancer therapies. With several of these molecules already licensed out to the Pharmaceutical industry, their current portfolio of projects includes several at an advanced stage. The Direction of Innovation promotes a pipeline based on collaborative drug development between ETP and the rest of the CNIO Groups, so that drug-development capitalises on the excellent research conducted by our basic scientists. Accordingly, all of our current early stage drug development projects have emerged from active collaborations with scientific research groups. In addition, we are trying to consolidate ETP as a strong node for anticancer drug development in Spain, so that our expertise can contribute towards the development of new therapies together with other cancer researchers in our country.

An important strength of our innovation activities derives from the excellent support provided by the Biotechnology Programme. Their work is not only vital for the progress of our scientific projects, but has also led to the realisation of important products and technologies that have contributed to our innovation portfolio. Some of the antibodies developed at the CNIO are contributing towards important aspects of current innovative treatments, such as those based on immunotherapies. The accreditation of our Animal Facility by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) also represents a key milestone in 2016 for the Biotechnology Programme.

Finally, several initiatives continue to be in place with the aim of fostering an innovation culture among our scientists, such as our continuous agreement with the prestigious IE Business School, through which many of our investigators have already obtained training in market-oriented innovation strategies.
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.

In 2016, the Programme was significantly reinforced with the recruitment of 9 young technicians who are funded for a 2-year period by the programme Ayudas para la Promoción de Empleo Joven e Implementación de la Garantía Juvenil en I+D+i del Ministerio de Economía y Competitividad.

This year, the CNIO Animal Facility obtained full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC); this recognition reflects the high standards achieved by the CNIO with regards to the use and care of animals for experimentation.

Faithful to its mission, a number of different technological innovations have been explored or implemented by the Core Units during this last year, often in collaboration with CNIO Groups. Noteworthy examples include the application of CRISPR technology for the generation of mouse models, the generation of monoclonal antibodies against small molecules, the application of proteomic approaches to the study of exosomes, the development of immuno-PET approaches for tumour imaging, the expansion of multicolour capabilities in flow cytometry studies, and the application of microfluidics-based setups to advanced microscopy, to name a few.

In 2016, the Programme and its Core Units have been particularly active in networking activities. This included the participation of several of our Programme members in the Core Technologies for Life Sciences (CTLS) meeting at EMBL in Heidelberg, Germany, where the first steps were taken towards the organisation of a new scientific association addressing core facilities issues (CTLS). In addition, several Unit Heads were very active in participating in networks and scientific societies from their corresponding fields. Also, the Programme Director was voted as President-Elect of the Spanish Society of Biotechnology (SEBiot), highlighting the prominent role of the CNIO in this area.

Also, as an indication of our high commitment to training and education, the Programme has been involved in the organisation of courses, workshops and specialised meetings. Moreover, an increasing number of our staff members undertook Masters and other training activities, at the CNIO and elsewhere.

This year, the Core Units were particularly successful in attracting funding from external sources through activities related to innovation; several contracts and agreements with private companies and public institutions, based on the technologies mastered by several of our Core Units, were formalised. Also, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit have grown by about 16% over the previous year, reaching a new historical maximum.

Last but not least, 2016 has once 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 nearly 30 publications, many of them appearing in top journals.
Genomics is the discipline that studies the structure and dynamics of the genome, its features, its regulation and expression. The genome is the core of life, the ensemble of the genetic material that conserves the assembly instructions of the species. Each cell in an individual keeps a copy of it deep in its nucleus. Chemically made of linear DNA macromolecules and distributed into chromosomes, it is packed with and interpreted by a myriad of protein cohorts acting in concert. It is expressed into RNA transcripts; some are functional in and of themselves, and others, constitute an intermediate step leading to the functional proteins that govern the cell. While less than a 2% fraction of a mammalian genome codes for protein, a vast majority of it (80%) has been found to participate in biochemical events. The genome is not immutable, it can suffer alterations. A chance for biological evolution or for damage. In fact, cancer derives from the accumulation of such alterations. Cells with a damaged genome can transform and develop into a tumour. The field of Genomics sheds light on this world of complexity.

RESEARCH HIGHLIGHTS

All tumours, even those of the same type and sharing a similar fate, are molecularly different and heterogeneous at the molecular level. By employing a distinct set of powerful methodologies, Genomics reveals the genetic diversity of cancer and helps to dissect molecular mechanisms. These methodologies have the capacity to interrogate a wide number of genetic loci, or even a whole genome in a single assay. Some tools detect modifications at a structural level: mutations, binding of protein factors, variations in chromatin folding. Others are suitable for observing functional choreographies, transcriptomic changes—for example, in response to treatments—that may uncover therapeutic targets and prognostic biomarkers.

The Genomics Unit provides services at two levels of complexity. The genomic wide level is addressed by both deep-sequencing and microarray technologies. Deep-sequencing permits a variety of applications, such as whole-genome or whole-exome tumour sequencing, transcriptome analyses by RNAseq, or location of interacting protein factors on chromosomal DNA by ChIPseq. As a novel sample type, the Unit successfully participated in the exome sequencing of cell-free DNA obtained from cancer patients’ blood. This year has seen a 40% increase in the overall demand and in the number of samples processed.

On the other hand, the DNA microarray platform can be efficiently used for transcriptome determinations or for the detection of chromosomal copy number abnormalities. At the single locus level other offers are available. A traditional DNA capillary sequencing service, based on a 3730xl DNA Analyzer from Applied Biosystems, is being used to find and confirm mutations in candidate genes as well as for 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. With a current, but continuously growing, catalogue of over 30 genetic modifications, the demand for genotyping services this year has almost doubled in comparison to former years.

Figure

An RNAseq experiment reveals the effects of a treatment at the RNA level. A blind analysis properly clusters samples of the same type together in columns. Genes whose transcription is significantly affected by treatment are shown in rows. Colour intensity reflects the transcriptional level. Some gene expression variability is apparent among replicates from the same condition.
Finally, the Unit also leads its own research projects focused on stem (iPS) cells, and embryo- and mouse model-based research. To research with embryonic stem (ES) cells, induced pluripotent genes in the mouse germ line. The Unit also provides support has generated more than 300 mutant alleles of cancer related stem cells and genome editing by targeted nucleases, the Unit art technology for the manipulation of the mouse genome. Using as for the assessment of experimental therapies at the preclinical mouse has become a crucial component of drug discovery as well the molecular mechanisms underlying tumour development and OVERVIEW

GENETICALLY ENGINEERED MICE

Core Unit Head

Sagrario Ortega

Student in Practice

Aleida Pujol

BIOTECHNOLOGY PROGRAMME

TRANSGENIC MOUSE CORE UNIT

Technicians

Edelfarne Ayala, M. Carmen Gómez, Jaime A. Muñoz (TS), Lucía M. Piret De Ayala (PEJ-L)**, Patricia Photo (TS), Pierfrancesco Vargiu (TS)**

*Titulado Superior (Advanced Degree)

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

RESEARCH HIGHLIGHTS

The CNIO Transgenic Mice Unit is dedicated to the generation, cryopreservation and derivation of genetically engineered mouse strains. We have created over 200 mutant strains, including knockout, knockin and conditional alleles, by gene targeting in embryonic stem (ES) cells, and over 100 mouse strains by conventional transgenesis. The Unit currently maintains a cryopreserved stock of over 1000 mouse strains, frozen at the Unit as sperm or embryos. This stock represents an invaluable resource of engineered strains for modelling and studying cancer in the mouse. Through our Unit, the CNIO shares part of this stock with EMMA (the European Mouse Mutant Archive) in order to make these models more accessible to the wider scientific community. We acknowledge the CNIO Animal Facility for their constant help and collaboration to make all these achievements possible.

The CRISPR/Cas9 system of Streptococcus pyogenes has expanded the currently available set of mammalian genome engineering tools, providing an easy, efficient, flexible and versatile method for creating targeted mutations in mammalian genes. We use the CRISPR/Cas9 system to generate knockout and knockin mice by introducing the components of the system, the guide CRISPR RNA and the Cas9 nuclease (either as messenger RNA or as protein) directly into mouse zygotes (FIGURE). In this experience, this system has proven to be extremely efficient for introducing new additional mutations in strains that are already carrying several engineered alleles, such as some mouse models of lung and pancreatic cancer that are used at the CNIO. We have also used the system to generate knockin alleles (point mutations) and tag insertions with efficiencies close to 20% directly in zygotes. The characteristics of the CRISPR system - efficient, fast and easy to implement - make it extremely useful for creating constitutive mutations in the mouse and to test certain biological questions before embarking on the creation of conditional or more sophisticated alleles. For these types of alleles, gene targeting in ES cells may still be the method of choice and we are currently optimising the use of CRISPR in ES cells to increase the efficiency of this technology.

Figure: Efficiency of GFP knockin via CRISPR in mouse embryos. Embryos are collected from B6.CBA females, crossed with DRD5KO/Rosa26Sortm1CAG-EGFP/Fyu (129Gt(ROSA)26Sortm1CAG-EGFP/Fyu).Luo (Ro/N) males, at E0.5. Embryos are injected with gRNA_GFP97 (50ng/µl) and commercial Cas9 protein (100ng/µl) in the cytoplasm at the zygote stage and cultured in vitro for 3 days up to the blastocyst stage. Confocal images (maximal projection) of GFP fluorescence.

PUBLICATIONS

The development of monoclonal antibody (mAb) technology has led to the generation of large panels of highly specific reagents that have had a tremendous impact on basic and applied research over the last four decades. MAbs have become indispensable tools for many of the laboratory techniques that are used to answer essential questions in biomedical research. Their outstanding specificity and sensitivity and reproducibility have allowed researchers to minimise the purchase of ineffective reagents in this new field. We also established collaborations with several big pharmaceutical companies (e.g. Merck, Lilly) for the production of mAbs against molecules of their interest, involved in cancer development.

**EuroMaBNet and its commitment with Ab validation**

In 2008, in collaboration with Oxford University, we founded EuroMaBNet (www.euromabnet.com), a non-profit organisation that includes internationally distinguished multidisciplinary academic laboratories specialised in antibody technologies. Their wealth of expertise ranges from the identification of new targets to the production of fully validated Abs and their use as research tools, clinically relevant diagnostic/prognostic reagents, and novel therapeutics.

The use of poorly characterised antibodies is of major concern to researchers. The Unit produces novel and high quality mAbs for use in basic research in order to gain new insights into the human cancer development process. We are also highly specialised in mAb characterisation, thereby providing CNIO researchers with reliable and well-validated reagents that give an added value to their research projects.”

EuroMaBNet has published a position paper (Roncador et al., 2016) and some easy to follow guidelines (http://www.euromabnet.com/guidelines) that provide a set of criteria and recommendations to help researchers select the most effective mAbs from those available in the market, and provide the strategic guidance needed to perform antibody validation.

EuroMaBNet also has a strong commitment to improving the education and training of junior scientists in Ab validation. With that in mind, we have started organising annual Antibody Validation Workshops (www.euromabnet.com) to provide practical guidelines about the principles underlying antibody validation, including the verification of Ab specificity, selectivity, sensitivity and reproducibility. These workshops outline the problems generated by the use of poorly validated reagents and educate researchers to minimise the purchase of ineffective Abs.
OVERVIEW

Molecular imaging involves specialised instrumentation, used alone or in combination with targeted imaging agents, to visualise tissue characteristics and/or biochemical markers. The data generated from molecular imaging studies can be used to help understand biological phenomena, identify regions of pathology, and provide insight regarding the mechanisms of disease. At the Molecular Imaging Unit, we offer state-of-the-art techniques such as Positron Emission Tomography (PET), Computed Tomography (CT), Ultrasounds (US) and Densitometry (DeXa).

“Molecular Imaging, especially PET, goes beyond the role of tumour detection and has also taken on the role of tumour characterisation.”

PUBLICATIONS


AWARDS AND RECOGNITION

- Scientific Advisory Board Chair and Faculty, the Madrid-MIT M+Vision Consortium, Spain.

RESEARCH HIGHLIGHTS

The main objectives of the Unit are to provide CNIO researches with state-of-the-art molecular imaging equipment and human resources in order to: guarantee the highest quality studies, develop and update protocols and techniques to optimise visualisation of tumours in both preclinical and clinical fields, as well as assess and advise researchers on the best-suited imaging modality for their research projects.

With the Immuno-PET strategy, the high specificity of the antibody is coupled with the high sensitivity of PET imaging to obtain a strong, non-invasive, tool for glioblastoma (GBM) and pancreatic carcinoma diagnosis and follow-up. In 2016, we published the results of our collaboration with the Sever-Ballesteros Foundation Brain Tumour Group and the Crystallography and Protein Engineering Unit at the CNIO. We reported the development of a new tracer (18Z-LEM2/15) for the efficient detection of MT1-MMP in preclinical GBM models.

We have also provided imaging support in clinical trials conducted under CNIO’s Clinical Research Programme. With the Breast Cancer Clinical Research Unit, we published the 18F-FMISO-PET imaging results from a clinical trial aimed at selecting patients who will benefit from treatment with angiomodulators knowing the degree of tumour hypoxia by using this PET biomarker (FIGURE). Furthermore, we continued our active participation in the international consortium focused on imaging, ‘M+Vision’ led by the Massachusetts Institute of Technology (MIT).
FLOW CYTOMETRY 
CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Ulan P. Cronin (TS)*, Elena Garrido (TS)*, Tania López (PEJ-L)**, Miguel Ángel Sánchez (TS)*

*Titulado Superior (Advanced Degree)
**Plan de Empleo Joven-Licenciado (Youth Employment Plan-Rad-Graduate)

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in setting up and optimising flow cytometry techniques of their interest. Some of the applications that have been developed and validated by our Unit include:

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

We have further developed our multicolour panels for the characterisation of the immune response by incorporating the new generation of Brilliant UV dyes from samples such as haematopoietic tissues, pancreas, skin, liver, and lung. Modifications in our analytical and cell sorters have also been applied to allow for this. Moreover, these panels could still be combined with the detection of proliferation and cell death. In terms of our cell sorting capabilities we included, at the end of the year, a MoFlo ASTRIOS in our portfolio of cell sorters. This cell sorter is equipped with 4 laser lines and 15 fluorescent detectors, which enable the isolation of up to 6 different populations simultaneously. The optical configuration in the ASTRIOS will allow for the use of the new generation of Brilliant UV dyes.

OVERVIEW

Flow cytometry is a very useful 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 the necessary technical and scientific advice regarding the use of flow cytometric technologies, collaborating with them for the design, acquisition, data analysis and interpretation.

With our 4 analysers and 3 high-speed cell sorters, with different configurations of lasers and detectors, we can cater to all our users’ needs. We also have an automated magnetic bead separation system (AutoMACS) and 2 automated cell counters. Analysers are available to users upon appropriate training and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6-defined populations at a time, as well as allow for single cell cloning. We can accept human samples to sort under Biosafety regulations.

“$\textit{In vivo}$ LacZ detection has always been a challenge. We have optimised a protocol for the identification and isolation of LacZ expressing cells from haematopoetic and lung tissues.”

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"In vivo LacZ detection has always been a challenge. We have optimised a protocol for the identification and isolation of LacZ expressing cells from haematopoetic and lung tissues."
The Confocal Microscopy Unit is equipped with 3 laser scanning confocal systems (Leica SP2 and SP5) that incorporate UV and multiphoton excitation, a white light laser and a Hybrid Detector, as well as 2 wide-field systems (a DeltaVision 4D deconvolution station and a Leica DMRI6000 system, equipped with microinjection). 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 multi-well plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorescence.

→ A Matrix Screening Application integrated into the SP5 confocal systems, allowing high-throughput feeding of the instrument, not only in multi-well plates, but also in tissue sections.

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

During 2016, the Confocal Microscopy Unit contributed to the microscopy field in several aspects. It improved the intelligent screening technique with new algorithms for image acquisition, thereby creating new applications in both confocal and conventional fluorescence microscopy. The use of microfluidics with live-cell assays in perfusion chambers has also experienced a significant increase in performance and demand. In addition, the Unit patented a new device for improving hardware autofocus that will be of great relevance in high-resolution automated image acquisition. Moreover, the Confocal Microscopy Unit continues to dedicate a significant effort towards the development and implantation of High-Content Screening technology at the CNIO; for example, in 2016, we provided support for the running of screening assays for compounds that could modify mitotic checkpoints, integrity of nucleoli, DNA Damage, BrdU, cell proliferation, etc.

Last but not least, in the field of intravital microscopy, we already have several ongoing projects that are focused on metastasis and skin alteration studies.
 Throughout 2016, the Unit continued its mission of implementing and optimising quantitative proteomic strategies. More specifically, we have introduced a new fractionation method using high pH reverse phase micro columns, which minimises sample loss and thus is highly suitable for low amounts of material. We used this approach to post-fractionate samples enriched in phosphopeptides, substantially increasing the number of identifications. This workflow was used to determine phosphorylation dynamics upon activation of WT and kinase-mutant platelets (in collaboration with the Cell Division and Cancer Group), as well as to identify potential substrates of CDK8 involved in the establishment of ground state pluripotency (in collaboration with the CNIO Tumour Suppression Group).

More recently, in collaboration with the Metabolism and Cell Signalling Group at the CNIO, we also used phosphoproteomics to better understand the molecular mechanism of the mTOR pathway. Together with the CNIO Genomic Instability Group, we are using a recent approach, named Thermal Proteome Profiling (Savitski et al., see FIGURE), to identify protein targets of certain inhibitors (e.g. target deconvolution). We have also performed several AP-MS/MS experiments for different proteins (STAG1, STAG2, PDS5A, PDS5B) belonging to the cohesion complex (with CNIO’s Chromosome Dynamics Group). Likewise, we have identified a large protein network (more than 300 proteins) that interacts with the RNA pol II complex (in collaboration with the Tumour Suppression Group). Over the last few years, the analysis of the protein content of exosomes has received great interest in the context of metastasis and the pre-metastatic niche. Along this line, we are conducting several proteomic analyses of exosomes from different origins in collaboration with CNIO’s Microenvironment and Metastasis Group, the Gastrointestinal Cancer Clinical Research Unit and the Melanoma Group.

OVERVIEW

Proteins catalyse and control almost all cellular processes in a living cell. 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. Recent developments in sample preparation, liquid chromatography, mass spectrometry and data analysis have enabled researchers to investigate diverse proteomic facets in a systematic high-throughput manner, currently comparable to next-generation sequencing platforms. As a result, proteomics is positioned as one of the most powerful technologies to study, at the protein level, complex cellular processes. This vast amount of data is providing new insights into the molecular mechanisms underlying diverse human pathologies such as cancer.

“Mass spectrometry-based technologies enable probing the composition, structure, function and regulation of the proteome, providing new insights into the underlying mechanisms of cancer.”

PUBLICATIONS


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 serves as the bridge between the basic sciences and clinical medicine.

The Histopathology Core Unit offers knowledge and expertise through a full range of services encompassing paraffin embedding and cutting, as well as the construction of tissue microarrays (TMAs). We also provide our users with histochemical stains upon request, research and diagnostic immunohistochemistry (IHC) testing, antibody validation, and in situ hybridisation (ISH) (ALU sequences for mouse xenograft characterisation). Furthermore, the Unit offers other services, such as laser-capture microdissection, slide digitalisation for brightfield, polarisation light and fluorescence, image analysis; and quantification. The Unit collaborates with researchers at any stage of their career in the histological characterisation of phenotypically relevant animal models of disease, thus providing them with the Pathology expertise required for the success of their research projects.

RESEARCH HIGHLIGHTS

In 2016, the Unit beat previous records in the Histopathology database with more than 15,000 new entries. This corresponded to about 40,000 requests processed with approximately 37,000 paraffin-embedded blocks; 40,000 histochemical techniques performed; 13,000 routine immunohistochemistry techniques performed (not counting optimisation tests); 7,000 scanning requests for histological slide scanning and image analysis; and 70 requests for laser microdissection.

All the developed techniques follow a standardised validation process. In 2016, the Unit added several new antibodies to its portfolio, which includes more than 3,000 tested and 1,000 currently available antibodies that have been optimised for both human and mouse tissue samples. The antibody validation process follows rigorous testing in order to achieve the best possible results and to demonstrate reproducibility between assay runs and between batches. This represents a highly valuable resource for CNIO researchers as well as for the external clinical and research community.

In respect of the importance that our researchers place on quality and reproducibility, our Unit participates in several External Quality Assessment Schemes, such as NordiQC and UKNEQAS, which evaluate the quality of the staining techniques performed at the Unit and in which more than 800 laboratories participate worldwide. In 2016, our Unit scored very high in the evaluated techniques, and several protocols developed by the Unit were incorporated into the ‘Best Methods section’ of the UKNEQAS Cellular Pathology Techniques website (PAS staining and Haematoxylin-Eosin, among others).

RESEARCH HIGHLIGHTS

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PUBLICATIONS

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), they 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. More than 950 companies, universities, hospitals, government agencies and other research institutions across 41 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 25 CNIO 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 rederivation. We also have an additional area with a capacity for 1,800 type II cages dedicated for the use of non-replicative strains of adenovirus, lentivirus and retrovirus, as well as for xenograft models. In this area, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are always manipulated in Type II biosafety cabinets.

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 maximize the productivity and ensure the quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility has harboured more than 2,500 genetically modified mouse lines; currently, there are more than 700 genetically modified lines and more than 45,000 live mice. The Facility also provides access to more than 80 tool strains, including constitutive and inducible Cre strains, Flp strains, reporter strains, Tet transactivator strains and others.

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

Additionally, the monitoring of the mouse models through non-invasive imaging technologies is provided by the Molecular Imaging Core Unit, which has integrated all its image acquisition instruments within the Animal Facility. Likewise, the work of the Transgenic Mice Unit is performed in a laboratory inside the SPF barrier. Finally, the necropsy laboratory is equipped with instruments for the haematological and biochemical analysis of blood and urine, which complement the pathology and clinical diagnostics.

All the work carried out by the Animal Facility complies with both national and EU legislation – Spanish Royal Decree RD53/2013 and EU Directive 2010/63/UE – for the protection of animals used for research experimentation and other scientific purposes. Experimental procedures and projects are reviewed and evaluated by the Research Ethics and Animal Welfare Committee of the Instituto de Salud Carlos III, as well as by the Institutional Animal Care and Use Committee (IACUC).

The Orden ECC/766/2015 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. Currently, the Animal Facility Service is performed by more than 35 qualified personnel between care workers, technicians, supervisors and veterinarians involved in the breeding and care of animals, ensuring the welfare of the animals. 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 line with our commitment to maintain the highest possible standards in regards to animal research issues, the CNIO has joined the Agreement on Openness on Animal Research promoted by the Federation of Scientific Societies in Spain (FCSOE), in collaboration with the European Animal Research Association (EARA), which was launched on September 2016. An institutional statement on the use of animals in research can be consulted in the CNIO website.
The current ETP-CNIO pipeline encompasses targeted and phenotypic projects. The following highlights summarise some of our achievement during 2016.

Our most advanced targeted project is dedicated to CDK8 inhibitors. We have selected our first lead, ETP-27, which has yielded positive results in PK-PD studies and has shown early signs of efficacy in a MOLM13 xenograft model after oral administration. Currently, we have embarked on the fine optimisation of the in vivo exposure within this series.

Additional targeted projects, focused on Mastl and Haspin inhibitors, are undertaken in collaboration with Marcos Malumbres (CNIO Cell Division and Cancer Group). The Mastl project started with a cell-based screening of several ETP-libraries, where a few ‘high-micromolar’ hits emerged as potential Mastl inhibitors. However, a direct target engagement experiment to unequivocally identify Mastl as their molecular target was not possible due to the unavailability of isolated active Mastl protein. ETP’s Biology Team has been able to set up highly efficient conditions for the purification, isolation and production of ‘full length active Mastl kinase’. This important achievement has enabled, for the first time, the biochemical profiling of the identified hits and an additional targeted biochemical screening of ETP-libraries. As a result of these activities, we have identified several families of compounds as biochemical Mastl inhibitors, including ETP-790 with an IC₅₀ of around 30 nM. These results shall pave the way for the discovery and development of advanced Mastl inhibitors in the near future. It is worth mentioning that the production of the protein was carried out by the CNIO Crystallography and Protein Engineering Unit.

Using the same cell-based assay, Malumbres’ Group identified compounds that efficiently produce ‘mitotic cell death’, a new avenue for cancer therapy. The knowledge of the biochemical profile of these hits obtained by ETP has contributed to the identification of Haspin kinase as the target responsible for the observed phenotype. Currently, ETP is working on the discovery of Haspin inhibitors where we have already identified highly potent compounds in the low-nanomolar range.

We are also collaborating with P. Carmeliet (VIB-KU Leuven, Belgium) for the discovery of novel inhibitors of a particular enzyme in the field of vascular normalisation. We have carried out a screening campaign and we are now working on hit generation activities to identify catalytic inhibitors of this enzyme and their associated intellectual property.

ETP collaborates with Manuel Serrano (CNIO Tumour Suppression Group) in a project dedicated to the discovery of novel targets and modulators against Cancer Stem Cells (CSCs). We have been focusing on target deconvolution activities around previously identified hits that have the ability to selectively kill CSCs and to inhibit the tumour-initiating capacity of pancreatic CSCs. Our Medicinal Chemistry Team has designed and synthesised chemical probes around those molecules by using ‘minimalist linkers’. These chemical fragments bear a photoactivatable group to achieve crosslinking of the modified hits with targeted proteins, and a special chemical group to perform ‘click chemistry’ that is useful to attach the ‘cross-linked complex’ to a reporter tag for imaging and/or pull-down experiments. The treatment of cells and cell lysates with these molecules has enabled the identification of a target candidate, which is currently under additional validation studies.

ETP is collaborating with Maria A. Blasco (CNIO Telomeres and Telomerase Group) in the discovery of TRF1 inhibitors, a project that also requires a ‘target deconvolution’ phase. During this year, we focused our attention on a series of TRF1 inhibitors with an unknown and perhaps innovative mechanism of TRF1 modulation. We have profiled the main hit, ETP-946, against large panels of enzymes and receptors representing more than 600 targets. Among them, we have retrieved 4 potential candidates, which are currently undergoing validation studies. The chemical modification of ETP-946 with ‘minimalist linkers’ has yielded potential affinity probes that are currently under evaluation. Last but not least, ETP has set up a ‘thermal stability assay’ to study the stabilisation of hTRF1 overexpressed in HER2+85 cells in the presence of ETP-946 and analogues. These experiments will inform us about direct interactions of those compounds with TRF1.

Phenotypic screenings have proven to be advantageous for the discovery of innovative molecular targets and modulators, as well as to establish their link with disease. Nevertheless, the molecular target responsible for a desired phenotype needs to be identified and this deconvolution phase implies an extra step of complexity in the process. As mentioned above, we are working on several phenotypic projects:

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Finally, I’d like to mention that other ‘phenotypic exploratory projects’ are also currently at the screening phase; these are undertaken in collaboration with the CNIO Researchers Manuel Valiente, Oscar Fernández-Capetillo and Massimo Squatrito.
The Medicinal Chemistry Section is part of the Experimental Therapeutics Interdisciplinary Programme that is dedicated to early Drug Discovery activities in the oncology field. Our mission is to discover and to develop new anticancer agents based on novel hypotheses and targets generated by CNIO’s Basic Research Groups, and to do so in close collaboration with these groups. Medicinal chemists are responsible for the design, the preparation and the optimisation of compounds for biological evaluation, as well as for the generation of Structure Activity Relationships (SARs) and the development of novel lead compounds with in vivo activity in appropriate animal models.

Recently, and due to the increasing number of projects related to the cell phenotype, our Programme is entering the chemical biology field in order to contribute to the deciphering of the precise protein target or mechanisms of action responsible for the observed phenotypes. In this regard, we chemically modify the active molecules into affinity probes—compounds with appropriate linkers that form either reversible or irreversible complexes with their targets inside living cells and/or cell lysates— that enable us to directly identify the target/s by extracting them via pull-down experiments, followed by mass spectrometry resolution and cellular localisation experiments using imaging techniques.

“We have successfully designed and synthesized affinity-based chemical probes used in imaging and pull-down/LC-MS/MS analysis experiments in order to discover novel targets/ inhibitors of CSC proliferation, in collaboration with the CNIO Tumour Suppression Group.”

RESEARCH HIGHLIGHTS

During 2016, our Section was involved in several projects at different phases of the drug discovery process, among them:

Cyclin-dependent protein kinase 8 inhibitors (CDK8) project

In this funded project (grant no. SAF2013-44267-R), we have identified lead compound ETP-27, which has served to demonstrate in vivo Proof of Concept (PoC) for CDK8 inhibition in cancer with positive preliminary results. This chemical series was protected by a patent application that reached PCT level in August 2016 and has been exemplified during 2016. Additionally, crystallographic studies with ETP-27 and some analogues from this chemical series have been performed by the CNIO Crystallography and Protein Engineering Unit confirming the expected binding mode of the molecules in the catalytic site.

Currently, we are dedicated to the fine optimisation of the lead compound, trying to increase the oral exposure levels vs time. Modifications in the molecule to reduce Clearance or to increase the Volume of Distribution by introducing basicity, for example, are being considered. The final objective of the project is to obtain an advanced product that is ready for preclinical regulatory development and further clinical studies.
Microtubule-associated serine/threonine protein kinase-like (MASTL) inhibitors

A chemical exploration around the hit identified in the biochemical High-Throughput Screening (HTS) with active full length human MASTL protein has been set up. The aim is to define the pharmacophore required for MASTL activity and to increase the activity of current hits, in order to obtain more potent inhibitors that can be used in biological assays as tool compounds. We will then use this information for the design of novel MASTL inhibitors, including Intellectual Property in their structures.

HASPIN inhibitors

Haspin inhibitors that produce a rapid and efficient mitotic cell death have been identified. We have started a chemical programme in order to explore the current hits and also to generate novel compounds. We are exploring 2 chemical series with haspin kinase activity. In a collaborative project with the CNIO Tumour Suppression Group, we identified several hits that are able to modulate CSC proliferation. Several reference compounds and analogues have been synthesised in order to help with the validation studies. After analysis of the hits from an HTS campaign, we have concluded that they were not good enough as starting points for further exploration. Therefore, a hit generation campaign, we have finalised the Hit-to-Lead phase in collaboration with VIB for the generation of novel compounds.

Inhibition of Cancer Stem Cell (CSC) proliferation

In a collaborative project with the CNIO Tumour Suppression Group, we have identified several hits that are able to modulate CSC proliferation, stemness and, at sublethal doses, inhibit the tumour initiating capacity of pancreatic CSCs. In order to decipher the target behind the observed phenotype, we have performed chemotype searches linking the structure with potential targets and profiling in broad panels of enzymes and receptors. As a result of the chemical exploration, we have found that the presence of basicity in these molecules is essential for activity. Based on this discovery, we have been able to successfully synthesise different affinity probes by adding a ‘minimalist linker’ to the basic centre, retaining the required cellular activity. These modified molecules, after treatment with cells or cell lysates and photo-irradiation, can covalently capture their binding proteins in a distance-dependent manner. The subsequent click chemistry reaction of the terminal alkyne group of the linker with different reporters (i.e. rhodamine-Ni or biotin-NJ3) enables, via pull-down experiments, the identification of potential cellular protein targets of the drug, as well as imaging-based determination of their cellular localisation. Because these experiments require the use of appropriate controls, we have also been able to synthesise inactive analogues by removing the basicity of the hit and have performed similar pull-down and imaging experiments. The already identified candidate targets are going through a validation process.

TeloMenic repeat binding factor 1 (TRF1) inhibitors

This project is undertaken in collaboration with the CNIO Telomeres and Telomerase Group. After a screening campaign, using a cell-based assay to measure the removal of TRFI from telomeres, we have identified several hits, among them ETP463. Our main objective during 2016 has been both the deconvolution of the molecular target behind the observed effect using this hit, as well as the chemical exploration to increase SAR knowledge within this chemical series. In the deconvolution studies, we carried out several chemotype searches and an extensive profiling of the compound against a broad range of enzyme and receptor panels. From these studies we have identified 4 candidate targets that are currently under study. Also, we have generated the first affinity probes by the introduction of appropriate linkers in the molecule that will allow for reversible or irreversible interactions with their molecular targets. Currently, we are testing these probes to address their TRF1 modulation in cells. If they show activity they will be very useful for further imaging localisation and pull-down experiments in order to identify the responsible molecular targets for TRFI modulation, including TRFI itself.

**Publications**

- **Patent**

In the Experimental Therapeutics Programme, we are working on both targeted and phenotypic-based drug discovery projects. In the targeted projects, the Biology Section is devoted to the biochemical, cellular, and in vitro/ in vivo pharmacological characterisation of the compounds synthesised within the Programme. Our aim is to obtain novel anticancer agents with optimised profiles that are able to demonstrate in vivo proof of concept in animal models of cancer.

In phenotypic-based projects, we perform target engagement and target deconvolution tasks using label-free techniques and chemical probes, respectively. The cellular thermal shift assay is a label-free technology that enables drug binding studies to target proteins in their relevant cellular contexts. Alternatively, we use engineered chemical probes to deconvolute the possible targets of our molecules. For that purpose, we first have to confirm that they behave similarly to the parent compound in the phenotypic screening.

These molecules bear a linker with functionalities that permit their crosslinking with targeted proteins and the attachment of reporter tags for imaging and pull down experiments in cells and cell lysates. Thus, through immunofluorescence assays, we can determine the cellular localisation of the complex of the chemical probe with its targets and, by pull down experiments, using active and inactive chemical probes also in competition with the parent compound followed by mass spectrometry analysis, we can identify candidate targets for the observed phenotype.

Furthermore, in exploratory screening projects carried out in collaboration with other CNIO Groups, we provide support by preparing customised compound assay plates from our ETP-libraries, adding the compounds using automated liquid-handling instruments that allow rapid, accurate, and reproducible compound dispensing and assay plate setup. All this instrumentation is integrated in a platform that allows sample tracking and recording.

“Upon production of active human full length MASTL protein, with the support of the CNIO Crystallography and Protein Engineering Unit, we have set up a biochemical assay that has allowed us to perform both target engagement validation with the hits obtained from a MASTL phenotypic screening, and a High-Throughput screening where we have identified a MASTL inhibitor with an IC_{50} of 300nM among other hits.”
DIRECTION OF INNOVATION

Compound is orally bioavailable as observed in pharmacokinetic as well as important toxicity related parameters. Moreover, the compound shows good solubility, permeability, metabolic stability a cellular selectivity of >30 fold for CDK8 vs. Haspin. This main off-target identified is Haspin; ETP-27 demonstrates inhibitor with picomolar cellular inhibition of P-STAT1-S727. We have identified ETP-27, a highly selective picomolar CDK8/CDK19 inhibitor with an IC50 of 300nM, which is a good starting point for SAR exploration and pharmacophore development.

Cyclin-dependent kinase 8 (CDK8)

During 2016, our Section was involved in several projects:

**Phenotypic drug discovery project**

- From phenotypic to target-based drug discovery, the MASTL case project. An HTS screening, together with a phenotypic screening designed to identify compounds that allow exit from mitosis of mitotically arrested cells, was used to identify potential MASTL inhibitors. Five hits were identified. To perform target engagement we produced and purified MASTL full length protein and set up a biochemical assay. We confirmed 3 hits as MASTL inhibitors with an IC50 between 12 and 50 µM. We performed a screening of 800 compounds with the biochemical targeted assay identifying a MASTL inhibitor with an IC50 of 300nM, which is a good starting point for SAR exploration and pharmacophore development.

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

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. For the CSC project, we collaborated in the deconvolution studies of the identified hits, evaluating their activity in kinase and receptor panels, as well as providing technical support for the assays using chemical probes. For the glioneogenesis project, we have performed pharmacokinetic profiles of the hits obtained in the screening, the most promising compound has been tested in vivo by our collaborator.

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

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. We have previously reported the production of active human full length MASTL protein to run biochemical assays. We used it to validate the hits coming from the phenotypic screening and to perform a biochemical screening of our ETP-640 library. In a single point screening assay, with the cut-off value set at 40% inhibition, we achieved a hit rate of 0.02. Hits were confirmed in a dose response assay and a number of analogues were tested. We have identified a 300nM hit, which is a good starting point for the exploration of Structure-Activity-Relationships (SAR) and for pharmacophore development. This information will be used later on for hit generation and subsequent hit-to-lead (HtL) exploration of novel inhibitors.

**Brain metastasis screening**

The CNIO Brain Metastasis Group has developed an ex vivo assay to search for drugs that kill human brain metastasis in mice. ETP-Biology has provided support in running the experiments and also to validate the in vitro screening results obtained with the approved or in clinical trial ETP-antitumour library. Two classes of drugs are under further characterisation.

**Cancer stem cells (CSC) and gluconeogenesis**

These projects are carried out in collaboration with the CNIO Tumour Suppression Group. For the CSC project, we collaborated in the deconvolution studies of the identified hits, evaluating their activity in kinase and receptor panels, as well as providing technical support for the assays using chemical probes. For the glioneogenesis project, we have performed pharmacokinetic profiles of the hits obtained in the screening, the most promising compound has been tested in vivo by our collaborator.

**Tumor Suppression Group**

We have given support to Manuel Serrano’s Group by analysing, with liquid chromatography-tandem mass spectrometry (LC-MS/MS), in tumour and host-mouse plasma samples, the levels of a standard-of-care-drug administered in nanoparticles in order to improve the delivery of chemotherapeutics to their site of action.

**Support to other CNIO Groups**

We have given support to Manuel Serrano’s Group by analysing, with liquid chromatography-tandem mass spectrometry (LC-MS/MS), in tumour and host-mouse plasma samples, the levels of a standard-of-care-drug administered in nanoparticles in order to improve the delivery of chemotherapeutics to their site of action.

**EXPERIMENTAL THERAPEUTICS PROGRAMME | BIOLOGY SECTION**

**PUBLICATIONS**


**PATENT**

**SCIENTIFIC CONTEXT**

The observation of an altered metabolic state in cancer cells dates back to the early 20th century when Otto Warburg observed that cancer cells preferentially utilise glycolysis over oxidative phosphorylation for growth, even in the presence of normal oxygen levels (Warburg 1956), a phenomenon known as the ‘Warburg effect’. Warburg argued that, ‘the altered metabolic state was the underlying cause for cancer’.

The molecular mechanisms driving an altered tumour metabolism have only recently begun to be understood as a result of large-scale genomic sequencing as well as advances in metabolic profiling technologies. Recent studies have shown that many oncogenes, including Myc and Ras, 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, and the one carbon pool.

Cellular metabolism is a fine tuned process. Tumours may rely heavily on specific metabolic pathways to obtain their energy while using other pathways to grow in order to give tumour cells a growth advantage. This situation may leave tumour cells in a frail position under certain treatments or circumstances, while normal cells may be able to compensate and survive (FIGURE, C).

Furthermore, the high requirements of nutrients and other soluble factors, and the release of metabolites with immunosuppressive properties, together with the hypoxic conditions found in tumours, creates a ‘non-friendly’ microenvironment for an anti-tumour immune surveillance, while facilitating the growth of other tumour-promoting cells such as the stroma and myeloid cells (FIGURE A,B). Thus, the mechanistic understanding of cancer metabolism has led to renewed interest in developing therapeutics that target key enzymes involved in this process. Checkpoint-blockade immunotherapy has been one of the most exciting advances made in cancer treatment in recent years. Metabolic interplay in the local microenvironment can mediate T cell differentiation and function. ‘Checkpoint-blockade’ antibodies can also influence cellular metabolism. Finally, recent clinical trials have shown that combination immunotherapy based on immune checkpoints blockade, provides even higher response rates than either approach alone. |  

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**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 the metabolic reprogramming regulated by oncogenes like RAS, as well as the characterisation of the metabolic status of tumours (Cerezo A. et al., February 2016; Keystone symposium meeting on ‘New Frontiers in Understanding Tumor Metabolism’ in Banff, Canada). 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 and metabolomics. Finally, each target goes through an *in vivo* validation process using xenografts, allografts and mouse models developed at the CNIO, this process includes the use of non-invasive *in vivo* imaging technologies, as well as the immunohistochemical characterisation of tumours for different metabolic, immune and tumour markers.
EXPERIMENTAL THERAPEUTICS PROGRAMME | CNIO - LILLY EPIGENETICS SECTION

SCOPE OF THE CNIO - ELI LILLY PARTNERSHIP

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

SCIENTIFIC CONTEXT

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 that are able to write, erase and read histone and DNA modifications (FIGURE). Thus, contrary to genetic mutations, epigenetic aberrations can be reversed by targeting 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, as well as the development of small molecular inhibitors of their activities, hold great promise for cancer therapy (FIGURE).

“Our goal is to identify epigenetic events that contribute to tumorigenesis and that might be susceptible to modulation by therapeutic agents.”

María José Barrero
Section Head
Staff Scientist
Sergio Ruiz
Technicians
Verónica García (TS)*, Jacinto Sarmentero (TS)*
*Titulado Superior (Advanced Degree)


Figure Strategies for targeting epigenetic regulators. The enzymatic activities of DNA methyltransferases (DNMTs), protein lysine methyltransferases (PKMTs), protein arginine methyltransferases (PRMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), or lysine demethylases (KDMs), are amenable to inhibition by small molecules. Additionally, molecular probes can be used to block the interactions of readers containing PHD, Bromo, Chromo or Tudor domains with modified histones, or to disrupt the interaction between critical core components of chromatin-related complexes.
The CNIO’s Technology Transfer and Valorisation Office at CNIO (TTVO) acts as a bridge that connects the research results generated by CNIO’s scientists to the Centre’s commercial partners, thereby helping to ensure that new products are developed for the public interest. Without this bridge the public, and in particular cancer patients, would not benefit from the full potential of the discoveries made by CNIO scientists. Close alignment of the technology transfer expertise with CNIO’s innovation strategy priorities serves to optimise the transfer of the research materials and novel scientific discoveries to the health sector. The TTVO performs an all-round management and follow-up of all aspects, including relationships with stakeholders, in order to ensure the appropriate intellectual property protection and commercial viability of the research results generated by CNIO’s scientist. Additionally, the Office proactively follows up on the progress of scientific activity at the CNIO in order to identify projects with high transfer potential; it plays a managerial and advisory role in the entire process in order to ensure the efficient use of the patent system, identify appropriate commercial partners for a timely development of technologies, negotiate licenses, monitor the activities of licensees regarding the achievement of milestones, and the payment of royalty fees.

In this context, the TTVO Office managed the execution of 182 agreements related to the CNIO’s intellectual property in 2016. About 70% of these were of international nature, which is a reflection of the international relevance of the scientific research at the CNIO. Many pertain to the exchange of research materials and data with the external scientific community and health-based companies. Other involved Research Collaboration Agreements (RCAs). RCAs are an important mechanism used by CNIO for successful cooperation with industry; they ensure that the risks and benefits related to research results and intellectual property are shared. In 2016, the industry investment secured via RCAs totalled 3.8 Mio euros, nearly 10% of CNIO’s yearly budget. This income reverts back to CNIO research activities as well as to the inventors themselves. A total of 44 inventors, about 10% of CNIO’s researchers, have contributed and benefited from this achievement.

Public-private partnerships are potenti tools for the valorisation of research results whereby scientific knowledge gets converted into diagnostic and therapeutic products and services. Valorisation of CNIO’s research results through alliances with industry is not just about ‘money’, but also about the impact that can be created for the public benefit and, in particular, for cancer patients. In 2016, a project based on the findings of CNIO scientists that boosting levels with nicotinamide riboside (NR) prevented and abolished aggressive tumour formation received nearly 1 Mio in funding from the MINECO Retos-Colaboración programme. The project was focussed on the development of a new NR-based therapy that could be used for Hepatocellular Carcinoma and other tumours.

The inventions of CNIO scientists that have the potential to be transferred to the market are protected through patents. The CNIO’s patent portfolio is composed of 26 families. Licensed patents are managed by our licensees and the rest is managed by the TTVO Office. Patents and unpatented research tools are licensed to increase their availability to the scientific community, as well as to create opportunities for our business partners and to provide a financial return on public investment. The royalty fees collected from licenses in 2016 exceeded 650,000 euros. Fostering an innovation culture among our scientists is one of our priorities. With the support of Fundación Banco Santander, we uphold our agreement with the prestigious IE Business School, through which many of our investigators − 3 new in 2016 − have already obtained training in market-oriented innovation strategies. Finally, these achievements stand testament to the excellence and hard work of our CNIO scientists and to CNIO’s unwavering encouragement of innovation and technology transfer activities.

“Valorisation of CNIO’s research results through alliances with industry is not just about ‘money’, but also about the impact that can be created for the public benefit and, in particular, for cancer patients.”

The CNIO’s Technology Transfer and Valorisation Office
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 2016. They play an inherent role in our present and future successes.”

One of the Fundación “la Caixa” main goals 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. This acclaimed ‘la Caixa’-Severo Ochoa International PhD Programme assures highly competitive standards by guiding exceptional students towards a career in oncology research; a basic principle is that the selection process is not to be limited to Spanish students only but also includes international students. During 2016, 2 pre-doctoral students received one of these internationally recognised fellowships. The Fundación “la Caixa” also helps finance our most prominent international conferences, the CNIO-“la Caixa” Foundation Frontiers Meetings.

The Fundación Seve Ballesteros is a private not-for-profit institution focused on securing, financing and promoting research projects centred on brain tumours. Fundación Seve Ballesteros supports the Seve Ballesteros Foundation – CNIO Brain Tumour Group, headed by Massimo Squatrito, since 2012. This Group focuses on the identification of markers for brain tumours as its principal activity.

The Fundación Banco Santander funds the Banco Santander Foundation-CNIO Fellowships for Young Researchers. These fellowships are aimed to support highly talented and motivated young scientists who trained in the UK/USA and wish to pursue their postdoctoral training at the CNIO. One young scientist, Ana Ortega, who came from the Sloan Kettering Institute for Cancer Research in New York, joined the CNIO thanks to a Banco Santander Foundation-CNIO Fellowship in 2016. Additionally, thanks to the support of the Fundación Banco Santander, a group of 3 young researchers received training on managerial and entrepreneurial skills, in collaboration with the IE Business School.

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 Manuel Serrano, Maria A. Blasco and Óscar Fernández-Capetillo.

The Fundación Jesús Serra-Catalana Occidente continues to fund the Visiting Scientists Programme that was established to support prestigious international professors for short stays at the CNIO. The beneficiary of the Jesús Serra Foundation’s Visiting Scientist Award in 2016 was Patrick Sung, Professor of Molecular Biophysics and Biochemistry and of Therapeutic Radiology, Yale University School of Medicine in New Haven (USA).

AXA Research Fund (ABF), a global initiative of scientific philanthropy run by the insurance group AXA, awarded an AXA-CNIO Endowed Permanent Chair in Molecular Oncology to Mariano Barbacid as part of its 2011 call.
Communication
This year, yet again, CNIO’s discoveries and activities were prominently featured in various domestic and international media outlets. In the printed and digital press the CNIO exceeded 2,600 media mentions; it is clear that the CNIO is being brought closer to the public eye, thereby increasing awareness of cancer research and slowly interweaving it into our national culture.

One of the most widely commented articles in 2016, published in the prestigious *Science* journal in November, concerned cell reprogramming; it was published under the title *Tissue Damage and Senescence Provide Critical Signals for Cellular Reprogramming in vivo*, and was authored by the researchers Manuel Serrano and Lluc Mosteiro, from the CNIO Tumour Suppression Group. The discovery, which places the focus on tissue damage as a tool for cellular reprogramming and for the potential regeneration of tissue, was featured in major media outlets, including the front page of the daily *El Mundo*, as well as receiving radio and TV coverage.

A major milestone in 2016 was the agreement that CNIO established with the Spanish Radio and Television Corporation (RTVE) on the occasion of the World Cancer Day, which took place on 4 February. Thanks to this collaboration, the Spanish public TV and radio broadcasting stations featured the voices of CNIO researchers and the latest developments in the oncology field.

As one of the top leading Cancer Research Centres in the world, the CNIO also hit the headlines beyond our borders. In 2016, the CNIO submitted 26 press releases to the global news service, EurekAlert! Throughout the year, these stories received nearly 83,000 hits from around the world and were taken up by prestigious international media such as the BBC, *The Guardian*, *The Scientist* or *Scientific American*.

“Our social networks are consolidating their communities. By December 2016, our Twitter channel had over 10,842 followers, with whom we keep an ongoing and valuable dialogue via the platform. The CNIO Friends’ social media has also become more consolidated; in December 2016, our Facebook page dedicated to this philanthropic initiative reached over 33,800 followers, highlighting, once again, the solidarity of our society and its growing interest in cancer research and the advances that can help fight the battle against cancer.

It’s another joy for us to mention that in May, the ‘CNIO Friends’ Facebook page received the Internet Day Award in its category. A distinction that, within the short period of the initiative’s existence, has served to boost its wider public dissemination.

“At the CNIO, we want to give science and research the strong voice they deserve as they are our true means of coping with cancer.”

Communications Officer (until April) Vanessa Pombo

Communications Officer Cristina de Marcos (since April)
Un hallazgo del CNIO puede ayudar a entender el inicio oncológico

Un hallazgo del CNIO puede ayudar a entender el inicio oncológico: los telómeros son estructuras proteínicas que se encuentran en el ácido desoxirribonucleico y determinan la gravedad del melanoma. Si un paciente con un melanoma se somete a cirugía, la elección de la revisión de las secuencias moleculares tumores es crucial. María Blasco, investigadora del CNIO, informa que ha decidido por lo que el sistema no siempre se decide a hacer un análisis de la existencia, definición y reperos de daño el organismo pierde avances que repercutirán en la calidad de vida que algunos padecen. La Rápida es una nueva era en el mundo de la salud, más orientada al paciente que al médico. La ciencia se ocupa de la evolución de la especie humana, pero cuando llega a la evolución de la especie humana, se verá que no se ha superado el 1,3%.

Después de ocho años de investigación, el Centro Nacional de Investigación (CNIO) ha contribuido esta semana a la medicina con la explicación de un nuevo hallazgo: el descubrimiento de que el envejecimiento para manipular las células de un organismo no es tan simple. Los telómeros, que al menos tres años y medio se caracterizan por su rápido envejecimiento, pueden ser manipulados en el futuro para generar ratones en los que todas sus células tienen el mismo envejecimiento.

Los telómeros son estrucuuras proteínicas que se encuentran en el ácido desoxirribonucleico y determinan la gravedad del melanoma. Si un paciente con un melanoma se somete a cirugía, la revisión de las secuencias moleculares tumores es crucial. María Blasco, investigadora del CNIO, informa que ha decidido por lo que el sistema no siempre se decide a hacer un análisis de la existencia, definición y reperos de daño el organismo pierde avances que repercutirán en la calidad de vida que algunos padecen. La Rápida es una nueva era en el mundo de la salud, más orientada al paciente que al médico. La ciencia se ocupa de la evolución de la especie humana, pero cuando llega a la evolución de la especie humana, se verá que no se ha superado el 1,3%.

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COMMUNICATION

INVITED GUEST SPEAKERS (Distinguished Seminar Series)

- Edurne Pasaban, March 8, 2016
- Diane Simeone, June 27, 2016
- Francisco J. Martínez Mojica, September 16, 2016
- Francisco J. Ayala, October 14, 2016
- Charles Brenner, October 28, 2016
- Celeste Simon, December 2, 2016

SOCIAL EVENTS

- The first distinction received by the ‘CNIO Friends’ initiative came from social media. Indeed, its Facebook page rose to first place in its category in the Internet Day Awards, sponsored by Google, Fénix Directo, Telefónica, Facebook, El Corte Inglés, and HP. May 12, 2016.

In 2016, the CNIO once again, participated in the European Researchers’ Night; an event funded by the EU Framework Programme. Over 200 participants visited the CNIO that opened its doors to the public as part of its commitment to scientific dissemination and education. The event provided guests with the opportunity to meet researchers in an interactive and entertaining way. September 30, 2016.

CNIO’s Director, Maria A. Blasco (left), and the President of the CLH Group, José Luis López de Silanes (right), signed an agreement to foster collaboration between both entities. Through this collaboration, the company joined ‘CNIO Friends’ in order to support research and training of research personnel, with the goal of advancing the development of innovative and specific therapies against cancer. March 18, 2016.

CNIO hosted an exhibition entitled ‘Marie Skłodowska-Curie: A Pole in Paris’, which revisited the personal and professional life of this key woman of the 20th century. This initiative of the CNIO Women in Science Office (WISE) stands testament to CNIO’s commitment to promoting and upholding the work of women scientists. November 2016.

Diane Simeone, June 27, 2016

CNIO’s Director, Maria A. Blasco (left), and the President of the CLH Group, José Luis López de Silanes (right), signed an agreement to foster collaboration between both entities. Through this collaboration, the company joined ‘CNIO Friends’ in order to support research and training of research personnel, with the goal of advancing the development of innovative and specific therapies against cancer. March 18, 2016.

Edurne Pasaban, March 8, 2016
International Affairs
The year 2016 marks the creation of the Department of International Affairs (IAs) at the CNIO, its setup underscores the Centre’s commitment with increasing its international impact to continue its growth in scientific and professional excellence. The overarching goal of the Department is to provide a strategic global vision for the CNIO to facilitate the coordination of efforts from the different departments with the aim of substantially strengthening our international reach with the research community, policy makers and society. The lines of work initiated this year will enable us to reinforce four of CNIO’s fundamental concepts: knowledge sharing, value creation, research and innovation advancement, and professional and academic development.

As an integral part of the CNIO, we strive to consolidate our Centre’s institutional leadership and reputation abroad, as well as to establish new robust partnerships and participate in European and International projects. This vision is now framed in the concept of ‘Responsible Research & Innovation (RRI)’ that will help us to further increase our impact on society by contributing to scientific advances and by endorsing innovative science policies.

The department focuses on the continuation of existing CNIO initiatives and on propelling novel projects abroad. One of our key activities for the upcoming year is the co-organisation of an Innovative Medicine Initiative (IMI) Oncology Workshop that will be hosted at the CNIO and spearheaded by the CDTI; its mission is to coordinate national efforts in upcoming cancer calls of this European public-private initiative. In this same vein, we have established a strategic workflow to coordinate CNIO’s efforts, keep investigators informed and facilitate their participation and leadership in H2020 projects—an effort to help CNIO researchers to reinforce their scientific potential and strengths in order to become even more competitive in the international scene.

We have already sown the seeds for exciting alliances with international research centres of excellence that will pan out throughout 2017, not only in Europe but also in other countries where we can complement and synergise in regards to science, innovation and training. The CNIO will be formulating new ways of collaboration to continue attracting international talent and to provide our investigators with the most exciting, diverse and global setting for their professional and scientific growth.

“Broadening our reach and scientific impact through international collaborations is crucial for the CNIO investigators. We will continue consolidating our presence abroad with new initiatives.”
CNIO Offices

Dean’s Office

CNIO Women In Science Office
knowledge dissemination. Members of CNIOSA and CNIOPDA have participated in various school visits and Open Doors activities such as the Semana de la Ciencia or the European Researchers’ Night. these events were highly attended, attracting over 250 participants of all ages.

A particularly inspirational event this year was our Annual CNIO Lab Day. We were fortunate to host Simon Gifford, co-founder of Mashauri Limited, Director of Genesis Management Consulting and Professor at the prestigious Instituto de Empresa. Gifford gave an inspiring talk on how MBAs and entrepreneurs think and behave differently. He also spoke about his personal experience in setting up various consulting companies, emphasising the value of risk-taking and independent thinking. We also had seven 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 discussed in over sixty posters, which together emphasised the breadth of research covered by 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 of 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 divulgence and outreach.

1. Awards for Excellence in Research by Predoctoral Fellows

Early in the year, the family Agüera-Nieto contacted the CNIO about giving a donation to support research conducted by young scientists. This led to the inauguration of the ‘Antonia Nieto Award’, in honour of their mother, to recognise the PhD student authoring the article with the highest impact. In 2016, the award went to Luc Mesteiro for her impressive work published in Science on the mechanisms of cellular reprogramming in vivo. We are still moved by the kindness and commitment of the Agüera-Nieto family, who came to the CNIO to hand over this award and to share their multiple ideas and suggestions for the Amigos del CNIO initiative. Their energy is yet an additional inspiration for us to further strive to make significant contributions towards the advancement of cancer research.

Additional awards in the PhD category were for Ana Teijeiro (Cancer Cell), Cristina Mayor-Ruiz (Mol Cell), Juan J. Montero (Nat Commun) and Eva Pérez-Guijarro (Nat Commun).

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

The awardee was Matilde Murga, for her outstanding contributions to the fields of DNA replication and genomic instability (Mol Cell and additional coauthored papers in Nat Struct and Mol Biol, Oncotarget and Sci Signaling).

3. Outstanding Contribution to Outreach and Awareness

This year’s recipient was Guillermo de Cárcer, for his tireless efforts in the organisation of the European Researcher’s Night. This is an event for which he coordinated over 50 volunteers, resulting in a flawless and exciting open-doors activity. The award was presented by Marcos Argumosa, who is himself an utterly impressive example of altruism, having run 10 consecutive marathons in 2015 to support the Amigos del CNIO initiative.

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

“At the CNIO we aim high: to carry out the most innovative and competitive basic and translational research, and to best prepare our trainees for the future, so that they can fulfill their potential as influential leaders.”
Although it is worrying to see the lack of female students in the latest data show that 55% of women pursue university studies. not seem to be that women are not present in academia; the scientific discipline, being awarded to a woman. The issue does another year has gone by without a single Nobel Prize, in any as recipients of prestigious scientific awards; as an example, and women. Furthermore, women are still underrepresented regarding the distribution of gender along the career ladder, the European Union still display the typical ‘scissors’ graphic career. Recent studies from different organisations in Spain and to be undertaken to ensure gender equality in the research Office is composed of CNIO volunteers from different areas of the Centre, people who believe there is still a real need for action among young girls to enter the scientific career. The CNIO Women in Science Office (WISE) was created at the end of 2012 with the aim to give visibility to women, to promote institutional awareness on gender equality, and to try to promote and support women in their professional careers. The WISE Office is composed of CNIO volunteers from different areas of so-called ‘STEM’ careers, they are still well represented at the pre- and post-doctoral stages. However, this representation drops to a meagre 25% of women at the Principal Investigator level, and it is even lower at the levels of Department Directors and beyond. When experts analyse the data and try to identify the causes, one factor that keeps coming up repeatedly is the existence of cultural stereotypes between men and women. Since those stereotypes need to be challenged at an early age, the Office has decided to, from 2016 onwards, open up its seminars to schools and high schools with 2 main purposes: to make teenagers aware of those gender issues, and to promote equality and hopefully vocations among young girls to enter the scientific career. We are certain that within the CNIO community, we all need to continue working together, with the other CNIO administrative offices as well as the Works Council, in order to reach our common goal, namely, to maintain the CNIO’s high level of scientific productivity and to ultimately make it an outstanding centre to work where gender barriers are completely eliminated and there is a sensible balance between work and life.

The Office consists of two working groups:

→ **Work/Life Balance** – aimed to promote and support initiatives to help improve the delicate balance between professional and personal life at the CNIO.

→ **Seminars and Events** – aimed to raise awareness of gender issues, and provide networking opportunities to all CNIO researchers.

In 2016, the WISE Office was again able to invite and welcome several top female leaders from different areas to tell us about their experience with gender issues, giving our young scientists ideas and advice on how to best overcome some of the hurdles that they may face during their careers, while also giving CNIO researchers the opportunity to expand their networks.

Some of the seminars organised by the WISE Office during 2016 are:

→ **EXPEDITION TO SUCCESS**: Achieving goals and overcoming difficulties. Edurne Pasaban. Mountaineer, Tolosa, Spain. March 8th.

→ **Recuerdos y Olvidos Feministas**. María Teresa Fernández de la Vega. President of the Women for Africa Foundation, former Vice President of Spain. May 10th.


→ **Marie Skłodowska-Curie**: Medical Physics pioneer and inspiration to female scientists. Dr Guadalupe Martín Martín. Medical Physicist. Puenslabrada University Hospital. Madrid, Spain. November 7th.

Also, in November, we hosted the itinerant exhibition on the life and work of two-time Nobel Laureate, Marie Skłodowska-Curie, who is still an inspiration to women scientists. We put together six organised visits to the exhibition combined with a tour to different CNIO laboratories for students and the general public; these were a great success. Since 1901, the year when Alfred Nobel established the awards bearing his name, only 17 women have been honoured with the recognition in the scientific field and unfortunately, many were left out despite their now widely recognised merits. We wish to contribute towards changing those numbers, and so we are working on developing different mentorship and leadership programmes for CNIO researchers.

Within the CNIO community, we continue to work together towards the elimination of gender barriers, the empowerment of women in Science and society and the promotion of scientific vocations among young girls. Scientific Excellence can only be fully achieved through gender equality.”
Facts & Figures

Scientific Management
Competitive Funding
Education and Training Programmes
Scientific Events

Administration
Board of Trustees
Scientific Advisory Board
Management
CNIO Personnel 2016
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 & 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 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 scientific divulgation events, and arranges the CNIO guided visits.

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

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.

“Every day, we work towards building a strong and flexible framework to support our scientists and to help them achieve excellence.”

Raquel Ares, Sonia Candés, Almudena del Codo (since March), M. Dolores Liébanes, Victoria López, Ana Merino, Juan Ramón Molina, Mercedes Moro, Leyre Vergés (since December)
COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies. In 2016, researchers at the CNIO were involved in 148 projects that received extramural funding.

CNIO actively participates in 64 collaborative projects in total: 30 were international collaborative projects (of which are coordinated by the CNIO) and 34 collaborative projects at the national level (12 of them coordinated by the CNIO). The international collaborative projects were funded by institutions such as the European Commission through the 7 Framework Programme and Horizon 2020, the Interreg SUDOE Programme, the US National Institutes of Health (NIH), the US Department of Defense (DoD), the Melanoma Research Alliance, the in addition to these collaborative projects, researchers at the CNIO attracted funding for projects carried out by individual groups. In 2016, 21 of these projects received international funds while 63 of them received national funding (mainly the Spanish Ministry of Economy, Industry and Competitiveness and the Institute of Health Carlos III). The international individual projects are funded by the European Commission (European Research Council (ERC) grants and the Marie Curie Actions), the Worldwide Cancer Research (WCR), the Howard Hughes Medical Institute (HHMI) and the European Foundation for the Study of Diabetes (EFSD).

INTERNATIONAL GRANTS

COST ACTION

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td>Malats, Núria (coordinator)</td>
<td>COST Action EU Pancreas: An integrated European platform for pancreas cancer research: from basic science to clinical and public health interventions for a rare disease (Ref.: COST BM1204)</td>
</tr>
</tbody>
</table>

EURATOM

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<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td>Serrano, Manuel</td>
<td>RISK-IR: Risk, Stem Cells and Tissue Kinetics-Ionising Radiation (Ref.: 321267)</td>
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</table>

INNOVATIVE MEDICINES INITIATIVE JOINT UNDERTAKING (IMI JU)

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<tr>
<th>Principal Investigator</th>
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<tbody>
<tr>
<td>Valencia, Alfonso</td>
<td>e-TOK: Integrating bioinformatics and chemoinformatics approaches for the development of expert systems allowing the in silico prediction of toxicities (Ref.: 15502)</td>
</tr>
<tr>
<td>Valencia, Alfonso</td>
<td>Open PHACTS: An open, integrated and sustainable chemistry, biology and pharmacology knowledge resource for drug discovery (Ref.: 15591-2)</td>
</tr>
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INTEGRATED PROJECT (IP)

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<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td>Valencia, Alfonso</td>
<td>BLUEPRINT: A BLUEPRINT of haematopoietic epigenomes (Ref.: 282510)</td>
</tr>
<tr>
<td>Valencia, Alfonso</td>
<td>ASSET: Analysing and striking the sensitivities of embryonal tumours (Ref.: 253948)</td>
</tr>
<tr>
<td>Valencia, Alfonso</td>
<td>RD-CONNECT: An integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research (Ref.: 305444)</td>
</tr>
</tbody>
</table>

MARIE CURIE ACTIONS (MCA)

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td>Fernández-Capetillo, Óscar</td>
<td>ITN 2dRed: Joint training and research network on chromatin dynamics and the DNA damage response (Ref.: 362390)</td>
</tr>
</tbody>
</table>

NETWORKS OF EXCELLENCE (NOE)

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<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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</thead>
<tbody>
<tr>
<td>Barbari, Mariam</td>
<td>EUROCANPLATFORM: A European platform for translational cancer research (Ref.: 260798)</td>
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### SMALL OR MEDIUM-SCALE FOCUSED RESEARCH PROJECTS

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<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tr>
<td><strong>Malats, Núria</strong></td>
<td>TransBiOBC: Translation of Novel Biomarkers for Bladder Cancer for clinical outcome prediction (Ref: 601913)</td>
</tr>
<tr>
<td><strong>Robledo, Mercedes</strong></td>
<td>ENSgT: CANCER: European network for the study of adenoma-structuring clinical research on adenocarcinomas in adults (Ref: 259713)</td>
</tr>
</tbody>
</table>

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

<table>
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<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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</thead>
<tbody>
<tr>
<td><strong>Malats, Núria</strong></td>
<td>Bio-Pac: Biomarkers of tumor recurrence in pancreatic cancer (financed by ISS) (Ref: AC1/00025)</td>
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</table>

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

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<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td><strong>Malumbres, Marcos</strong></td>
<td>MicroKin: Deciphering the multifaceted pathways underlying MCPH pathogenesis in the mouse and human (Financed by MEC) (Ref: PCIN-2015-007)</td>
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### HORIZON 2020 (2014-2020)

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<tr>
<th>Research Infrastructures, Including E-Infrastructures</th>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tr>
<td><strong>Valencia, Alfonso</strong></td>
<td>ELIXIR-EXCELERATE: Fast-track ELIXIR implementation and drive early user exploitation across the life-sciences (Ref: 676559)</td>
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<td></td>
<td>OpenMinTeD: Mining Infrastructure for TExt and Data (Ref: 614021)</td>
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### MARIE SKŁODOWSKA-CURIE ACTIONS (MSCA)

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td><strong>Soengas, María S.</strong></td>
<td>ITN IMMUTRAIN: Training network for the immunotherapy of cancer (Ref: 641549)</td>
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</table>

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

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<thead>
<tr>
<th>Principal Investigator</th>
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<tbody>
<tr>
<td><strong>Benítez, Javier</strong></td>
<td>BRIDGES: Breast cancer risk after diagnostic gene sequencing (Ref: 634935)</td>
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</table>

### INDUSTRIAL TECHNOLOGIES: ADVANCED MATERIALS AND NANOTECHNOLOGIES

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<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td><strong>Hidalgo, Manuel</strong></td>
<td>NeCanTec: Nanomedicine upscaling for early clinical phases of multimodal cancer therapy (Ref: 688795)</td>
</tr>
</tbody>
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1. This Programme is cofunded by the European Regional Development Fund (ERDF)
INTERNATIONAL GRANTS | INDIVIDUAL PROJECTS

EUROPEAN COMMISSION

7TH FRAMEWORK PROGRAMME (2007-2013)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Fernández-Capetillo, Óscar | ERC Consolidator Grant iHEALTH: Investigating the causes and consequences of replication stress in mammalian health (Ref.: 67840)

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Al-Shahrour, Fátima | PERSMEDomics: Bioinformatics and integrative genomics for a novel personalized cancer therapy (Ref.: 33430)
Peinado, Héctor | WHRI COFUND ADIPOMET: Analyzing the crosstalk of tumor and adipose tissue during metastasis (Ref.: 608765)
Rámón, Santiago; Moreno, María | WHRI COFUND CAD_FL: Revealing the functional mechanism of CAD and its potential as a therapeutic target (Ref.: 608765)
Squarrito, Massimo | GLDD: DNA Damage Response (DDR) signaling in tumor formation and therapeutic resistance of gliomas (Ref.: 63875)
Wagner, Erwin F.; Gago, Nuria | WHRI COFUND STEM-PSEO: Unraveling the contribution of Epidermal and Non-Epidermal Progenitor (Ref.: 608765)

HORIZON 2020 (2014-2020)

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Efeyan, Alejo | ERC Starting Grant NutrientSensingVivo: The Physiology of Nutrient Sensing by mTOR (Ref.: 638891)
Hidalgo, Manuel | ERC Advanced Grant AVATAR: Integrating Genomics and Avatar Mouse Models to Personalize Pancreatic Cancer Treatment (Ref.: 670582)
Serrano, Manuel | ERC Advanced Grant CELLPASTICITY: New Frontiers in Cellular Reprogramming: Exploiting Cellular Plasticity (Ref.: 669622)

EUROPEAN FOUNDATION FOR THE STUDY OF DIABETES (EFSO)

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Djouder, Nabil | Growth factors and nutrients in type 2 diabetes: role of URI in β cell plasticity and glucose homeostasis

HOWARD HUGHES MEDICAL INSTITUTE (HHMI)

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

MELANOMA RESEARCH ALLIANCE (MRA)

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Soengas, María S. | Prognostic and therapeutic impact of lymphovascular niches in melanoma (Ref.: 348673)

PROSTATE CANCER FOUNDATION

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Olmos, David | Integration of clinical, molecular and biological characteristics to define an aggressive subtype of prostate cancer based on deficient homologous recombination

WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Blasco, María | Targeting telomeres in cancer (Ref.: 16-1177)
Lietha, Daniel | Targeting regulatory mechanisms for allostERIC cancer drug discovery (Ref.: 15-1177)
Malumbres, Marcos | New therapeutic strategies by inhibiting Mastl in breast tumors (Ref.: 15-0278)
Peinado, Héctor | Evaluation of obesity as a novel risk factor in metastasis (Ref.: 16-1244)
Pérez Moreno, Mina A. | Defining the role of macrophage-derived Wnts in squamous cell carcinoma (Ref.: 15-1219)
Soengas, María S. | Harnessing ends/exocytosis for a coordinated targeting of melanoma cells, their vasculature and the immune system (Ref.: 15-1274)
Wagner, Erwin F. | Dissecting the roles of Fra proteins in lung adenocarcinoma progression and metastasis (Ref.: 15-026)

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

PRINCIPAL INVESTIGATOR | PROJECT TITLE
--- | ---
Peinado, Héctor | Role of exosomes and Endoglin in Neurofibromatosis Progression (Ref.: W81XWH-16-1-0131)

EUROPEAN RESEARCH COUNCIL (ERC)

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

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Serrano, Manuel | ERC Advanced Grant CELLPASTICITY: New Frontiers in Cellular Reprogramming: Exploiting Cellular Plasticity (Ref.: 669622)

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Soengas, María S. | Prognostic and therapeutic impact of lymphovascular niches in melanoma (Ref.: 348673)
NATIONAL GRANTS

COMMUNITY OF MADRID / COMUNIDAD AUTÓNOMA DE MADRID®

PRINCIPAL INVESTIGATOR
Barbacid, Mariano; Malumbres, Marcos (coordinator)

PROJECT TITLE
Programa DACODYCLE: El ciclo celular y las mismarNdo en la autoreparación y diferenciación de células progenitoras (Ref.: S201/BMD-2471)

PRINCIPAL INVESTIGATOR
Blanco, María A.; Semaro, Manuel (coordinator)

PROJECT TITLE
Programa REICalle: Reparación en cáncer y regeneración (Ref.: S201/BMD-2101)

PRINCIPAL INVESTIGATOR
Campos-Olivas, Ramón; Lleitha, Daniel

PROJECT TITLE
Programa BRIEPO 2: Plataforma integrada de bioinformática para el descubrimiento de nuevos fármacos basado en la estructura del receptor (Ref.: S201/BMD-2457)

PRINCIPAL INVESTIGATOR
González-Neira, Anna

PROJECT TITLE
Programa VISO4MARMAL: Modelos animales para el estudio de enfermedades de la visión (Ref.: S201/BMD-2439)

PRINCIPAL INVESTIGATOR
Martínez, Jorge L.

PROJECT TITLE
Programa ANGIOBODIES 2: Desarrollo de anticuerpos recombinantes para usos terapéuticos y diagnósticos en angiopatías patológicas y para la identificación de nuevos marcadores angiogénicos (Ref.: S201/BMD-2312)

PRINCIPAL INVESTIGATOR
Montoya, Guillermo

PROJECT TITLE
Programa INTERACTOMICS: Interactomics del oncotransfómer (Ref.: S201/BMD-2305)

PRINCIPAL INVESTIGATOR
Robledo, Mercedes

PROJECT TITLE
Programa TIRONET: Fisiopatología del tejido: Mecanismos implicados en cáncer, autoinmunidad y mecanismo de acción de HER-2 en oncológico (Ref.: S201/BMD-2328)

PRINCIPAL INVESTIGATOR
Soengas, María S.

PROJECT TITLE
Programa NANODEINMED: Nanosistemas dendríticos como agentes y vectores terapéuticos en distintas aplicaciones biomédicas (Ref.: S201/BMD-2351)

PRINCIPAL INVESTIGATOR
Real, Francisco X.

PROJECT TITLE
Programa CEI-EO: Líneas y competición celular en el desarrollo y la enfermedad (Ref.: P200/BMD-2275)

SUB-PROGRAMME OF COOPERATIVE HEALTH RESEARCH THEMATIC NETWORKS/SUBPROGRAMME DE REDES TEMÁTICAS DE INVESTIGACIÓN COOPERATIVA (METIC)*

PRINCIPAL INVESTIGATOR
Cigudosa, Juan C.

PROJECT TITLE
Red Temática de Investigación Cooperativa en Cáncer (RTICC) (Group RD12/0036/0034)

PRINCIPAL INVESTIGATOR
Malats, Núria

PROJECT TITLE
Red Temática de Investigación Cooperativa en Cáncer (RTICC) (Group RD12/0036/0050)

PRINCIPAL INVESTIGATOR
Real, Francisco X.

PROJECT TITLE
Red Temática de Investigación Cooperativa en Cáncer (RTICC) (Group RD12/0036/0050)

SUB-PROGRAMME OF GRANTS FOR RESEARCH SUPPORT PLATFORMS IN HEALTH SCIENCES AND TECHNOLOGY/SUBPROGRAMME DE AYUDAS PARA PLATAFORMAS DE DISEÑO EN CIENCIAS Y TECNOLOGÍAS DE LA SALUD*

PRINCIPAL INVESTIGADOR
Benitez, Javier

PROJECT TITLE
Plataforma de recursos biomoleculares y bioinformáticos, PRBB (Group PT13/0010/0005)

PRINCIPAL INVESTIGADOR
Moneite, Manuel M. (coordinator)

PROJECT TITLE
Plataforma de Bio Bancos (Coordination node and group) (PT13/0010/0006)

MINISTRY OF HEALTH, SOCIAL SERVICES AND EQUALITY / MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD (MSSSI)

RESEARCH PROJECTS IN HEALTH*

PRINCIPAL INVESTIGATOR
Blasco, María A.

PROJECT TITLE
Cellular aging in first episode early-onset psychosis. Collaboration with Gregorio Marañón Hospital (Ref.: EC10-278)

PRINCIPAL INVESTIGATOR
Blasco, María A.

PROJECT TITLE
Safety and efficacy of gene therapy with telomerase in acute myocardial infarction. Impact on ventricular remodeling in an experimental porcine model. Collaboration with Gregorio Marañón Hospital (Ref.: EC10-005)

MINISTRY OF ECONOMY, INDUSTRY AND COMPETITIVENESS / MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVENESS (MEIC)

EXCELLENCE NETWORKS / REDES DE EXCELENCIA

PRINCIPAL INVESTIGATOR
Barbacid, Mariano (coordinator); Blasco, María A.; Fernández-Capetillo, Óscar; Malumbres, Marcos; Real, Francisco X.; Semaro, Manuel

PROJECT TITLE

PRINCIPAL INVESTIGADOR
Malumbres, Marcos (coordinator)

PROJECT TITLE
NRCANCER- Desarrollo de nueva terapia antitumoral basada en nicotinamida-ribosido (Ref.: RTC-2016-5431-1)

PRINCIPAL INVESTIGADOR
Soengas, María S.

PROJECT TITLE
Ensayo Clínico Fase I de BO-110: un nuevo tratamiento para melanoma avanzado y otros tumores (Ref.: RTC-2016-5431-1)

CHALLENGES-COLLABORATION/RETOS-COLABORACIÓN*

PRINCIPAL INVESTIGADOR
Djouderra, Nadir

PROJECT TITLE
ARANCHES: Desarrollo de nueva terapia antimotoral basada en resistinol-ribosido (Ref.: RTC-2016-5431-1)

PRINCIPAL INVESTIGADOR
Soengas, María S.

PROJECT TITLE
Ensayo Clínico Fase I de BO-110: un nuevo tratamiento para melanoma avanzado y otros tumores (Ref.: RTC-2014-0441-1)
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</thead>
<tbody>
<tr>
<td>Barbacid, Mariano (coordinator)</td>
<td>Molecular analysis of Capicua, a novel tumor suppressor involved in RTK signaling and transcriptional repression (Ref.: 2013133031)</td>
</tr>
<tr>
<td>Fernández-Capetillo, Oscar</td>
<td>Exploring synthetic lethal interactions between PARP and the DNA damage response in cancer treatment (Ref.: 2013453031)</td>
</tr>
<tr>
<td>Soengas, Maria S.</td>
<td>Role of RNA binding proteins in melanoma progression: searching for new diagnostic markers and therapeutic targets (Ref.: GCB1512507)</td>
</tr>
<tr>
<td>Dean’s Office for Academic Affairs; Soengas, Maria S.</td>
<td>European Researchers’ Night 2014, organized by Madri+d Foundation and founded by European Commission on the framework of H2020 Programme</td>
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### National Grants - Individual Projects

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

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<th>Principal Investigator</th>
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<tbody>
<tr>
<td>Benítez, Javier</td>
<td>Biologic and genetic bases of telomere shortening in hereditary breast cancer: Searching for new high susceptibility genes in BRCAX families with short telomeres (Ref.: PI12/00370)</td>
</tr>
<tr>
<td>Casón, Alberto</td>
<td>Exome sequencing of trios, father-mother-proband, in pediatric patients with multiple phaeochromocytoma/paragangliomas (Ref.: PI12/00236)</td>
</tr>
<tr>
<td>Casón, Alberto</td>
<td>Next generation sequencing of genes directly and indirectly involved in the Krebs cycle, applied to phaeochromocytoma/paragangliomas with hypomethylated phenotype (Ref.: PI12/00783)</td>
</tr>
<tr>
<td>Cigudosa, Juan C.</td>
<td>Genetic diagnostics by next-generation sequencing in myeloid neoplasias: step towards its clinical use and characterization studies on the mutation genome and functional pathological effects (Ref.: PI12/00425)</td>
</tr>
<tr>
<td>García, María José</td>
<td>Definition of novel ovarian cancer susceptibility genes using next-generation sequencing technology and a LOH-candidate region approach in high-risk non-BRCA1/BRCA2 patients (Ref.: PI12/0039)</td>
</tr>
<tr>
<td>González-Nerja, Anna</td>
<td>Personalizing breast cancer treatment: prediction model construction for taxanes and anthracyclines efficacy thought the integration of different genomic approaches (Ref.: PI12/00226)</td>
</tr>
<tr>
<td>Hidalgo, Manuel</td>
<td>Targeted Pancreatic Cancer Stroma (Ref.: PI13/00230)</td>
</tr>
<tr>
<td>Malats, Núria</td>
<td>Aetiology of pancreas cancer: Application of “omics” technologies in the assessment of risk factors (Ref.: PI12/00815)</td>
</tr>
<tr>
<td>Malats, Núria</td>
<td>Building and validation of risk prediction models for pancreatic cancer. The application of a multi-omics approach (Ref.: PI13/00537)</td>
</tr>
<tr>
<td>Molina, María Esther</td>
<td>Dietary patterns, antioxidants and biomarkers of oxidant-antioxidant status in the EPIC-Granada and EPIC-Gipuzkoa (European Prospective Investigation into Cancer and Nutrition) cohort (Ref.: PI12/00002)</td>
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<tr>
<td>Pérez de Castro, Ignacio</td>
<td>An integrative study of Oromesoblastic Instability and Cancer: looking for prognostic markers and therapeutic opportunities (Ref.: PI14/00227)</td>
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<tr>
<td>Olmos, David</td>
<td>Homologous recombination DNA repair deficiency related chromosomal instability in aggressive prostate cancer (Ref.: PI13/00267)</td>
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<tr>
<td>Quintela, Miguel Angel</td>
<td>From systems biology to clinical trials: high-throughput studies and definition of predictive factors and resistance mechanisms against breast cancer drugs (Ref.: PI13/00430)</td>
</tr>
<tr>
<td>Robledo, Mercedes</td>
<td>Prognostic profiles in endocrine tumours identified by next generation sequencing, and definition of markers with clinical utility (Ref.: PI14/00240)</td>
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<tr>
<td>Rodríguez, Sandra</td>
<td>Exonic Sarcoma-Model: induction of the T(22:22) translocation in human mesenchymal stem and iP cells by the CRISPR-Cas9 system and study of the cellular context and other secondary events role (Ref.: PI14/00884)</td>
</tr>
<tr>
<td>Squarzini, Mattia</td>
<td>Investigating the role of Fasl and Fas2 in glioma tumor formation and treatment response (Ref.: PI15/00328)</td>
</tr>
<tr>
<td>Urioste, Miguel</td>
<td>PTEN-hamartoma tumour syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene (Ref.: PI14/00459)</td>
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</table>
This Programme is cofunded by the European Regional Development Fund (ERDF)

COMPETITIVIDAD (MEIC) ECONOMÍA, INDUSTRY AND MINISTRY OF ECONOMY, INDUSTRY AND

FACTS & FIGURES

Barbacid, Mariano PANTHER: A three prong strategy to fight pancreatic cancer (Ref.: SAF2015-70553-R)

Méndez, Juan REPLICON: Molecular mechanisms that control eukaryotic DNA replication (Ref.: BFU2013-49553-P)

Blasco, Maria A. TeloHealth: Telomeres, telomerase and disease (Ref.: SAF2015-40230-R)

Ruiz, Sergio RSHIPS: Replicative stress during somatic cell reprogramming (Ref.: SAF2013-49447-P)

Challenges-Research / Retos-Investigación

Barbacid, Mariano PANTHER: A three prong strategy to fight pancreatic cancer (Ref.: SAF2014-59864-R)

Blasco, Maria A. TelomHealth: Telomeres, telomerase and disease (Ref.: SAF2013-40230-R)

Ouyang, Özge PsSORTACEmiR21: Investigating the role of microRNA21 in ductal adenocarcinoma (Ref.: SAF2015-40869-R)

Fernández-Capotillo, Óscar BREAKINGRAD: Exploring the limits of radiosensitivity in mammals (Ref.: SAF2014-59449-R)

Loidiá, Ana COHESIN: Cohesin function and regulation: a multidisciplinary approach (Ref.: BFU2013-48481-R)

Ruiz, Sergio RSHIPS: Replicative stress during somatic cell reprogramming (Ref.: SAF2013-49447-P)

National plan for scientific and technological research and innovation (2013-2016)

Centres and units of excellence “severo ochoa” sub-programme / Subprograma de Apoyo a Centros y Unidades de Excelencia “severo ochoa”

Princípal Investigator Project Title

Blasco, Maria A. Center of Excellence “severo ochoa” (Ref.: SEV-2015-0510)

Blasco, Maria A. CNIO in Horizon 2020: support for proposal preparation (Ref.: EUC2014-51617)

Valencia, Alfonso Expression Patterns of Inverse Comorbidity (Ref.: BFU2015-71241-R)

Valiente, Manuel BrainMET: Deconstructing metastatic disease in the brain (Ref.: SAF2015-70553-R)

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

Serrano, Manuel CANCERGE: Cancer and agronomy-associated diseases: new frontiers and new strategies (Ref.: SAF2015-48256-R)

Soengas, María S. MEL-STOP: Vascular trafficking in melanoma progression and treatment response (Ref.: SAF2014-56688-R)

Vallejo, Manuel inVACtive BrainMET: Dissecting the role of reactive astrocytes in brain metastasis (Ref.: SAF2014-57043-R)

Valencia, Alfonso EPIC: Expression Patterns of Inverse Comorbidity (Ref.: BFU2015-71241-R)

Wagner, Erwin F. CANPSOR: Investigating Cancer Risk in Psoriasis (Ref.: SAF2015-70553-R)

National Research Plan 2008-2011

Sub-programme for non-targeted fundamental research projects / Subprograma de proyectos de investigación fundamental no orientada

Princípal Investigator Project Title

Uruñuela, Ósvaldo: Investigating the role of microRNA21 in ductal adenocarcinoma (Ref.: SAF2013-59470)

Valencia, Alfonso Development of biocomputing systems and subsequent mathematical methods for the analysis of oncologic personalised therapies (Ref.: BFI2013-40205)

Wagner, Erwin F. HaploP-i: From liver physiology to hepatic and hepatocellular carcinoma (HCC): role of AP-1 (Fos/Jun) proteins (Ref.: BFU2013-40205)

National plan for scientific and technological research and innovation (2013-2016)

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### SCIENTIFIC MANAGEMENT

#### COMPETITIVE FUNDING

<table>
<thead>
<tr>
<th>PRINCIPAL INVESTIGATOR</th>
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<tbody>
<tr>
<td>Olmos, David</td>
<td>Understanding the role of growth factors and nutrients in inflammatory bowel disease and colon cancer</td>
</tr>
<tr>
<td>Peinado, Héctor</td>
<td>Use of exosomes circulating as markers of progression in neurofibromatosis and for the determination of new therapeutic strategies</td>
</tr>
<tr>
<td>Valencia, Alfonso</td>
<td>PerMed: Precision Medicine from Big Data to Cognitive Computing CNIO (Ref.: 76/2016)</td>
</tr>
<tr>
<td>Peinado, Héctor</td>
<td>Tumour exosome integrins determine organotropic metastasis</td>
</tr>
<tr>
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<td>Cancer de próstata familiar y esporádico asociado a alteraciones genéticas, germinales y/o somáticas, en genes de la reparación del DNA</td>
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<tr>
<td>Quintela, Miguel Ángel</td>
<td>Reprogramación inmune en cáncer de mama presupuesto a antiangiépticos inductores de apoptosis</td>
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10. This Programme is cofunded by the European Regional Development Fund (ERDF) |
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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 2016, the CNIO signed new agreements with several Spanish Universities and other institutions, namely with the Universidad Autónoma de Barcelona, UNED and the International School of Protocol, University Claude Bernard Lyon, Universidad Francisco de Vitoria, Universidad de Navarra, Universidad de Córdoba, Universidad CEU San Pablo, Fundación Jesús Serra, Fundación la Caixa", and the ISFPS Claudio Galeno of Madrid.

TRAINING PROGRAMMES  PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES

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<tr>
<td>Training of PhD students</td>
<td>121</td>
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<td>108</td>
<td>105</td>
<td>110</td>
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<tr>
<td>Post-doctoral training</td>
<td>81</td>
<td>67</td>
<td>55</td>
<td>48</td>
<td>51</td>
</tr>
<tr>
<td>Training for MDs</td>
<td>16</td>
<td>21</td>
<td>14</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Laboratory training for MSc/BSc students</td>
<td>42</td>
<td>36</td>
<td>73</td>
<td>80</td>
<td>95</td>
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<tr>
<td>Laboratory training for technicians</td>
<td>26</td>
<td>19</td>
<td>21</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Master's Degree in Molecular Oncology (graduated)</td>
<td>37</td>
<td>37</td>
<td>34</td>
<td>29</td>
<td>25</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 (288 hours). During this time, the students actively participate in research projects in one of the CNIO groups. During 2016, 6 students from 3 different countries participated in this programme.

- Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO individual Research Groups or Units. This year, 95 students participated in these programmes, of which 6 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, in 2016, 10 students obtained their PhD degrees and 25 more joined the CNIO. More than 25% of the 130 students working at the CNIO in 2016 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 4 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 Ministry of Economy, Industry and Competitiveness has undertaken efforts to link the "la Caixa" CNIO International PhD Programme to distinguished research centres accredited as "Severo Ochoa Centres of Excellence". The third call of this new "la Caixa"-Severo Ochoa International PhD Programme was very successful, attracting around 130 eligible applications from undergraduates from 32 different countries. During 2016, 2 pre-doctoral students received one of these internationally recognised fellowships.

The distribution of students across the CNIO’s Research Programmes in 2016 was as follows: 54.9% of the students worked in the Molecular Oncology Programme, 11.8% in the Cancer Cell Biology Programme, 8.2% in the Structural Biology and Biocomputing Programme, 23.6% in the Human Cancer Genetics Programme, 9.1% in the Clinical Research Programme, 1.8% in the Biotechnology Programme and the remaining 0.9% in the Experimental Therapeutics Programme.

FUNDING OF PHD TRAINING

<table>
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<th>No. SPANISH ENTITIES</th>
<th>FUNDING OF PHD TRAINING</th>
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<td>86</td>
<td>Ministry of Economy, Industry and Competitiveness / Ministerio de Economía, Industria y Competitividad (MEIC) (Predoctoral fellowships)</td>
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<td>36</td>
<td>Ministry of Economy, Industry and Competitiveness / Ministerio de Economía, Industria y Competitividad (MEIC) (I+D Projects)</td>
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<tr>
<td>7</td>
<td>Ministry of Education, Culture and Sport / Ministerio de Educación, Cultura y Deporte (MECD) (Predoctoral fellowships)</td>
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<td>4</td>
<td>Institute of Health Carlos III / Instituto de Salud Carlos III (ISCIII) (I+D Projects)</td>
</tr>
<tr>
<td>6</td>
<td>&quot;la Caixa&quot; Banking Foundation / Fundación Bancaria &quot;la Caixa&quot; (Predoctoral fellowships)</td>
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<tr>
<td>23</td>
<td>Spanish Association Against Cancer (AECC) / Fundación Científica de la AECC (I+D Projects)</td>
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<tr>
<td>1</td>
<td>AlfrescoMedia Foundation / Fundación AlfrescoMedia</td>
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<tr>
<td>1</td>
<td>Cris Foundation / Fundación Cris</td>
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<td>24</td>
<td>INTERNATIONAL ENTITIES</td>
</tr>
<tr>
<td>2</td>
<td>European Commission Framework Programme / H2020</td>
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<td>2</td>
<td>Marie Skłodowska-Curie actions of the European Commission</td>
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<tr>
<td>4</td>
<td>European Research Council</td>
</tr>
<tr>
<td>5</td>
<td>Portuguese Foundation for Science and Technology (FCT)</td>
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<tr>
<td>1</td>
<td>China Scholarship Council</td>
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<tr>
<td>1</td>
<td>European Foundation for the Study of Diabetes</td>
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<tr>
<td>2</td>
<td>Boehringer Ingelheim Fonds</td>
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<tr>
<td>1</td>
<td>Boehringer Ingelheim International GMBH</td>
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<tr>
<td>1</td>
<td>Hoffman-La Roche</td>
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<tr>
<td>1</td>
<td>Pfizer</td>
</tr>
<tr>
<td>1</td>
<td>Prostate Cancer Foundation Young Investigator Award</td>
</tr>
<tr>
<td>1</td>
<td>Volkswagen Foundation</td>
</tr>
<tr>
<td>TOTAL 110</td>
<td></td>
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</tbody>
</table>
POST-DOCTORAL TRAINING

One of the CNIO’s prime objectives is to attract young researchers who have recently obtained their PhD or MD degrees and to offer them highly interesting research projects at the forefront of cancer research.

In 2016, 51 postdoctoral fellows worked at the CNIO. Notably, more than one third of these fellows were from outside of Spain, many coming from very prestigious international institutions.

For yet another year, the Fundación Banco Santander upheld its agreement with the CNIO in 2016 and continued the highly competitive fellowship programme aimed to support 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, who came from the Sloan Kettering Institute for Cancer Research (New York), joined the CNIO thanks to a Santander Foundation-CNIO Fellowship in 2016. Another scientist, coming from the City University of New York, was awarded one of these fellowships in late 2016.

Thanks to the donations received through the “CNIO Friends” platform we launched the inaugural Postdoctoral Contract “CNIO Friends” Programme in 2016, thereby enabling us to recruit 2 scientists for a period of 2 years each.

Furthermore, in 2016, as a result of an agreement signed with the Juegaterapia Foundation, we were able to create a third Postdoctoral Contract, “Juegaterapia-CNIO Friends”, which enabled us to contract a scientist to carry out a project on paediatric oncology.

POSTGRADUATE PROGRAMMES

In addition, the CNIO — in collaboration with several academic institutions in Spain — provides access to a variety of postgraduate programmes that cover the areas of Cellular & Molecular Biology, Molecular Biomedicine, Biotechnology, Biocomputing & Computational Biology, Clinical & Applied Cancer Research, Therapeutic Targets, and Molecular Oncology.

Official Postgraduate Programmes in 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) through 4 Official Postgraduate Programmes, namely the Doctorate in Biosciences, Masters in Molecular and Cell Biology, Masters in Molecular Biomedicine, and Masters in Biotechnology.

Master’s Degree in Biocomputing and Computational Biology

The Master in Bioinformática y Biología Computacional — directed by Alfonso Valencia, Director of CNIO’s Structural Biology and Biocomputing Programme — 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) and the Madrid Science Park (Parque Científico de Madrid, PCM).

Official Master’s Degree in Clinical and Applied Cancer Research

LABORATORY TRAINING FOR TECHNICIANS

This training programme has been developed for students in Anatomical Pathology and is organised through agreements with 18 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 20 weeks (707-712 hours) of laboratory training for students. Additionally, the CNIO offered real-life work experience to 1 student of Analytical Assays and Quality Control for 12 weeks (370 hours), and to 3 students of Clinical Diagnosis for 12 weeks (380 hours). Of the 26 students who participated in this programme in 2016, 5 were hired by the CNIO.

TRAINING FOR MDS

In line with CNIO’s commitment to bridge the gap between bench and bedside, the Centre offers 3 programmes providing excellent training opportunities to MDs and other health care professionals. Training usually consists of a 3-month period during residency. In 2016, 17 medical residents from 11 different hospitals enjoyed the benefits of rotations within the different Groups and Units of the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2016, the Ramón y Cajal Programme supported 8 scientists. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (now Spanish Ministry of Economy, Industry and Competitiveness) 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. Ten other scientists were funded by similar programmes, including the Juan de la Cierva programme (5 contracts); the ISCIII Miguel Servet (1 contract) and SEOM-Río Hortega (contract funded by the Spanish Society of Medical Oncology, 1 contract) programmes; and the Spanish Association Against Cancer (AECC, 3 contracts).

VISITING RESEARCHER PROGRAMME

The Jesús Serra Foundation, part of the Catalana Occidente Group, aims to help eminent international specialists work together with CNIO researchers for a few months in order to expand their knowledge in areas of common interest. During 2016, Patrick Sung from the Yale University in New Haven (USA) was beneficiary of the Jesús Serra Foundation’s Visiting Researcher Programme.

“SCIENCE BY WOMEN” PROGRAMME

Thanks to this Programme, launched by the Spanish “Fundación Mujeres por África”, Dorcas Osei Safa from the University of Ghana, Legon, stayed at the CNIO as a visiting scientist in the Experimental Therapeutics Programme, from January to July 2016.
The CNIO “La Caixa” Foundation Frontiers Meetings are the main international conferences that are jointly organised by the CNIO and the “La Caixa” Foundation. They focus on specific, cutting-edge aspects of cancer research, thus providing a unique platform for an intensive and dynamic exchange and debate of scientific ideas. The invited speakers – 20 internationally renowned leaders in oncology – present their latest findings during 2 and a half days. 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.

CANCEROMATICS III - TUMOR HETEROGENEITY
13-16 NOVEMBER 2016

ORGANISERS
- Fátima Al-Shahrour, Spanish National Cancer Research Centre, (CNIO), Madrid, Spain
- Núria Malats, Spanish National Cancer Research Centre, (CNIO), Madrid, Spain
- Chris Sander, Dana-Farber Cancer Institute, Boston, US
- Alfonso Valencia, Spanish National Cancer Research Centre, (CNIO), Madrid, Spain

SESSIONS
- Pan-Cancer analysis
- Analysis of mutations and functional consequences of mutations on pathway and network
- Tumor heterogeneity
- Tumor heterogeneity (part II)
- Drug prediction and repurposing
- Drug prediction and repurposing (part II)
- Translational Genomics
- Translational Genomics (part II)

SPEAKERS
- Bissan Al-Lazikani, The Institute of Cancer Research (IARC), London, UK
- Niko Beeremwinkel, ETH Zurich, Switzerland
- Diego Di Bernardo, Telethon Institute of Genetics and Medicine, Pozzuoli, Italy
- Paul Boutros, Ontario Institute for Cancer Research (OICR), Toronto, Canada
- Fabien Calvo, Cancer Core Europe, Paris, France
- Chris Evelo, Maastricht University, The Netherlands

OTHER MEETINGS & CONFERENCES
The CNIO annually hosts various international meetings and conferences. Within this category are the 5 international events held in 2016.

PANCREOS KICK-OFF MEETING
17 MARCH 2016
Participants by country and supporters of the PancreOS are presented:
- Pancreatic Cancer Europe - Alfredo Carrato
- EUPancreas COST - Nuria Malats

BLUEPRINT, ROADMAP, ENCODE AND 1000 GENOMES: BROWSE THROUGH THEM WITH ENSEMBL
4-5 APRIL 2016

ORGANISER
- CNIO Bioinformatics

SPEAKERS
- Denise Carvalho-Silva, Ensembl Team, European Bioinformatics Institute (EBI).
MAKING ACCESS TO PERSONALISED MEDICINE A REALITY FOR PATIENTS
15 SEPTEMBER 2016

SPEAKERS
- Nuria Malats, Spanish National Cancer Research Centre (CNIO)
- Denis Horgan, EAPM
- Antoni Andreu, Ministry of Health
- Ramon Gonzalez Carvajal, Regional Ministry of Health of Andalucia
- Maria Blasco, Spanish National Cancer Research Centre (CNIO)
- Natacha Bolaños, Spanish Group for Cancer Patients, GRPAC
- Emilia Sánchez Chamorro, Madrid Health Ministry
- Ruth Vera, Spanish Society of Medical Oncology (SEOM)
- Ivo Gut, Centro Nacional de Análisis Genómico CNAG-CRB
- Pablo del Pino, Celgene
- Federico Plaza, Roche Pharma / Roche Institute

VII CONGRESO NACIONAL DE BIOBANCOS Y EL I CONGRESO LATINOAMERICANO DE BIOBANCOS
16-18 NOVEMBER 2016

ORGANISERS
- Biobanco IDIS
- Complejo Hospitalario Universitario de Santiago-CHUS
- Fundación Ramón Domínguez
- Plataforma Red Nacional de Bio Bancos
- CNIO

SPEAKERS
- Maimuna Mendy, Agencia Internacional de Investigación en Cáncer (IARC). Biobanco IARC
- Gustavo Stefanoff, Biobanco del Instituto Nacional de Cáncer José Alencar Gomes da Silva (INCA)
- Balvir Matharoo-Ball, Nottingham University Hospital NHS Trust
- Gonzalo Héctor Arza, Hospital Central de las Fuerzas Armadas (HCFFAA)
- Hugo Campos, Biobanco del A. C. Camargo Cancer Center
- Luz María Ruiz Godoy, Biobanco del Instituto Nacional de Oncología (INCan)
- Liliana Virginia Siede, Ministerio de Ciencia, Técnica e Innovación Productiva de la Nación Argentina
- Pilar Nicolás, Universidad de Deusto, Bilbao
- Máximo Fragá, Biobanco CHUS/IDIS
- Jorge Pombo Otero, Servicio de Anatomía Patológica CHUAC
- Rocío Aguilar, Biobanco del Sistema Sanitario Público de Andalucía
- Ana Caroline Neuber, Biobank, Barretos Cancer Hospital, Barretos, SP
- Cristina Villena Portella, Centro Investigación Biomédica en Red, Enfermedades Respiratorias (CIBERES)
- Diego Santos, Proyecto Cepaphis
- Susana Tejeira, Banco de Cerebros. Biobanco Vigo. (BIOBANC-MUR)

SCIENTIFIC EVENTS

SENESCENCE & CANCER
2ND ANNUAL MEETING OF THE SPANISH NETWORK OF CELLULAR SENESCENCE
23 NOVEMBER 2016

GROUPS OF THE SPANISH NETWORK
- Joaquín Arribas (Vall d’Hebron Institute of Oncology, Barcelona, Spain) and members from his laboratory
- Manuel Collado (Instituto de Investigación Sanitaria – Complejo Hospitalario Universitario de Santiago de Compostela, Spain) and members from his laboratory
- Bill Keyes, (previously at the Centre of Genomic Regulation, Barcelona, and currently at the IGIBMC, Strasbourg, France) and members from his laboratory
- Ramón Martínez (Univ. Politécnica de Valencia, Valencia, Spain) and members from his laboratory
- Ignacio Palmero (Instituto de Investigaciones Biomédicas “Alberto Sols”, Madrid, Spain) and members from his laboratory

EXTERNAL INVITED SPEAKERS
- Juan Carlos Acosta, IGMM, Edinburgh, UK
- Marco Demaria, ERIBA, Groningen, The Netherlands
- Valery Kryzhanovsky, Weizmann Institute, Rehovot, Israel

- Silvia Sánchez, Instituto de Investigación Sanitaria Hospital La Fe
- Gema Huesa, Barcelona Albert Einstein Research Center, Fundación Puigvert Marquall
- Manuel Rodríguez Castro, AGABELA
- Carmen López Rodríguez, FROBREC
- Anna Bosch-Comas, Biobanc HCB-1DBAPS
- Ángela González Ferro, Hospital Universitario Lucus Augusti
- Nuria Ajenjo, Centro Nacional de Investigaciones Oncológicas, CNIO
- Natalia Cal Parriños, Fundación Profesor Nonoa Santos - Instituto Investigación Biomédica A Coruña (INEBIC)
- Roberto Bílbaí, Biobanco Vasco
- Pilar Nicolás, Universidad de Deusto, Bilbao
- Roberto Dienstmann, Instituto de Oncología del Hospital Vall d’Hebrons
- Juan Cruz Cigudosa, Centro Nacional de Investigaciones Oncológicas (CNIO)
- Abel González, Universitat Pompeu Fabra
- Manuel M. Morente, Plataforma Red Nacional de Bio Bancos (ISICHI)
- Inés Arco Siéndones, Biobanco Sistema Sanitario Público de Andalucía
- Verónica Valdivieso Gómez, Biobanco SSA
- Ana García Díaz, Centro de Diagnóstico de Enfermedades Moleculares
- Raquel Bermuda, Instituto d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)
- Rosa Maria Pinto Labajo, Banco Nacional de ADN Carlos III, Instituto de Investigación Biomédica de Salamanca
- Mara Ortega Gómez, Biobanco HUP Instituto de Investigación sanitaria Hospital Universitario de la Princesa
- Joan Ramón Gómez Cottij, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana
- Raquel Amigo, Hospital Universitari i Politècnic La Fe (HS-La Fe)
- Gustavo Stefanoff, Instituto Nacional de Cáncer (INCA)/ Ministerio de Salud
- Carol Aristimudo, Biobanco Vasco: Nido Hospital Universitario, Araba
- Joan Anoedo Cibeira, GoodGut SL
- Andrés García Montero, Banco Nacional de ADN Carlos III
- Máximo Fraga, Biobanco CHUS/IDIS
- Teresa Escames, Biobanco en Red de la Región de Murcia (BIOBANC-MUR)
The CNIO is committed to disseminating the results of state-of-the-art cancer research to the wider community, including medical professional and junior scientists, thereby enabling them to stay abreast of recent developments in specialised techniques. This is achieved through training courses and hands-on workshops organised by CNIO scientists and technologists.

TRAINING COURSES AND WORKSHOPS

FLOW CYTOMETRY SEMINAR SERIES
25-26 JANUARY 2016

• Rui Gardner, Head of the Flow Cytometry Lab, Instituto Gulbenkian de Ciência, Oeiras, Portugal
• Lola Martínez, Head of the Flow Cytometry Unit, CNIO, Madrid, Spain

ADVANCED CELL SORTING COURSE
26-29 JANUARY 2016

• Rui Gardner, Head of the Flow Cytometry Lab, Instituto Gulbenkian de Ciência, Oeiras, Portugal
• Lola Martínez, Head of the Flow Cytometry Unit, CNIO, Madrid, Spain

WORKSHOP REDIX EXO-IMAGING CNIO
26-28 JULY 2016

• Hernando del Portillo
• Héctor Peinado
• Isabel Guerrero
• Diego Megías
• Juan Monteaudo
• Susana García Silva
• Ana Amor
• Marta Hergueta
• María Yáñez-Mo
• Antonio Marcilla
• Francesc E. Borrás
• Juan Manuel Falchón-Pérez
• Antonio Osuna
• Lucía Robado

TALLER CEGEN- PRB2: ESTUDIOS DE ASOCIACION: DISEÑO Y ANALISIS DE DATOS
29 NOVEMBER 2016

• CEGEN

• Javier Benítez, Director del Nudo del CeGenISCIII en Madrid, Director del Programa de Genética Humana, Centro Nacional de Investigaciones Oncológicas, CNIO
• Anna Gonzalez-Neira, Jefe de Unidad de Genotipado Humano-CeGen-ISCIII, Centro Nacional Investigaciones Oncológicas, CNIO
• Guillermo Pita, Unidad de Genotipado Humano-CeGen-ISCIII, Centro Nacional Investigaciones Oncológicas, CNIO
• Rosario Alonso, Unidad de Genotipado Humano-CeGen-ISCIII, Centro Nacional Investigaciones Oncológicas, CNIO
• Pablo Fernández, Jefe de Área de Epidemiología Ambiental y Cáncer, Centro Nacional de Epidemiología ISCIII, Madrid. CIBER en Epidemiología y Salud Pública -CIBERESP
• Ana Osorio, Grupo de Genética Humana, Centro Nacional Investigaciones Oncológicas, CNIO
• Agustín Fernández Fernández, Unidad de epigenética del Cáncer, Instituto Universitario de Oncología del Principado de Asturias (IUOPA)
• Sara Ruiz –Pinto, Unidad de Genotipado Humano-CeGen, Centro Nacional Investigaciones Oncológicas, CNIO
• Lola Alonso, Grupo de Epidemiología Genética, Centro Nacional Investigaciones Oncológicas, CNIO

COURSE OF ANIMAL LABORATORY FROM C TO D
13-15 DECEMBER 2016

• CNIO
• Animalaria Formación y Gestión

• Manuel Berdoy, University of Oxford, UK
• Ignasi Sabul, PCR, Spain
• José M. Orellana, University of Alcala, Spain
• Sagrario Ortega, CNIO, Spain
• Ignacio Álvarez, UCM, Spain
• Pablo Fernández, Jefe de Área de Epidemiología Ambiental y Cáncer, Centro Nacional de Epidemiología ISCIII, Madrid. CIBER en Epidemiología y Salud Pública -CIBERESP
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• Lola Alonso, Grupo de Epidemiología Genética, Centro Nacional Investigaciones Oncológicas, CNIO
• Marlees Leenars, Radbound UMC, Netherlands
• Ángel Naranj, CNR, Spain
• Javier Guilén, AALAC International, Spain
• Pedro Pablo López, CNIO, Spain
• Violeta Solís, Glaxo Smithkline, Spain
• Alba de Martino, CNIO, Spain
• Belén Pintado, CBM, Spain
• Francisca Mulero, CNIO, Spain
• Isabel Blanco, CNIO, Spain
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 purpose of this international seminar series is not limited to bringing outstanding cancer researchers to the CNIO, but also serves to annually invite 3–4 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”. 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 23 distinguished speakers in 2016.

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<tbody>
<tr>
<td>15/01/2016</td>
<td>Giulio Desideri</td>
<td>Institute for Applied Cancer Science, The University of Texas MD Anderson Cancer Center, Houston, USA</td>
<td>Integrating functional genomics with drug discovery to overcome resistance to treatment in multiple cancer</td>
</tr>
<tr>
<td>05/02/2016</td>
<td>Sarah Teichmann</td>
<td>EMBL-European Bioinformatics Institute &amp; Wellcome Trust Sanger Institute, Cambridge, UK</td>
<td>Understanding Cellular Heterogeneity</td>
</tr>
<tr>
<td>12/02/2016</td>
<td>Romain Quillard</td>
<td>ICREA, ICF- The Institute of Photonic Sciences, Barcelona, Spain</td>
<td>Applications to oncology of light and nanotechnology</td>
</tr>
<tr>
<td>19/02/2016</td>
<td>Joseph Jonkers</td>
<td>Netherlands Cancer Institute, Amsterdam</td>
<td>Genetic dissection of breast cancer development, therapy response and resistance in mouse models</td>
</tr>
<tr>
<td>26/02/2016</td>
<td>Cory Brayton</td>
<td>Johns Hopkins University School of Medicine, Baltimore, USA</td>
<td>Research relevant immune variations in mice</td>
</tr>
<tr>
<td>04/03/2016</td>
<td>Michael Siwek</td>
<td>Center of Immunology, Marseille-Luminy, France</td>
<td>Beyond dam cells: Dissecting lineage identity and self-renewal</td>
</tr>
<tr>
<td>11/03/2016</td>
<td>Nicholas Dyson</td>
<td>James and Shirley Curvey MGH Research Scholar, Harvard Medical School, Boston, USA</td>
<td>The consequences of Rb inactivation</td>
</tr>
<tr>
<td>15/04/2016</td>
<td>András Nagy</td>
<td>Mount Sinai Hospital Lennard-Tanenbaum Research Institute, Toronto, Canada</td>
<td>Reprogramming Leads to Multiple States of Phosphorylation in the Artificial Cell Space</td>
</tr>
<tr>
<td>22/04/2016</td>
<td>Herbert Waldmann</td>
<td>Max Planck Institute of Molecular Physiology, Dortmund, Germany</td>
<td>Chemical Biological Modulation of Ras-Signaling</td>
</tr>
<tr>
<td>29/04/2016</td>
<td>Nandeep S. Chandel</td>
<td>Northwestern University, Feinberg Medical School, Chicago, USA</td>
<td>Mitochondria as signaling organs</td>
</tr>
<tr>
<td>06/05/2016</td>
<td>Andrés Moya</td>
<td>University of Valencia, Spanish Evolutionary Biology Society (SEBES), Valencia, Spain</td>
<td>Man: Nature and Future</td>
</tr>
<tr>
<td>13/05/2016</td>
<td>Anna M. Wu</td>
<td>Crump Institute for Molecular Imaging, David Geffen School of Medicine at UCLA, Los Angeles, US</td>
<td>ImmunoPET: Engineered antibodies for non-invasive imaging of tumors and immune cell subsets</td>
</tr>
<tr>
<td>20/05/2016</td>
<td>Mathias Heilemøller</td>
<td>DKFZ - German Cancer Research Center, Heidelberg, Germany</td>
<td>How cells of the immune system control development of fatty liver disease, non-alcoholic steatohepatitis and subsequent</td>
</tr>
<tr>
<td>03/06/2016</td>
<td>Stephan Herzig</td>
<td>Institute for Diabetes and Cancer IDC Helmholtz Center, Munich, Germany</td>
<td>Cancer and Metabolism: A bi-directional connection</td>
</tr>
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## Facts & Figures

**Michele de Palma**

### January

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<td>Jeremy Graff</td>
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<td>Imprime PGG, a Pathogen Associated Molecular Pattern (PAMP), triggers a coordinated, anti-cancer immune response</td>
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<tr>
<td>11/02/2016</td>
<td>Dana Simeone</td>
<td>University of Michigan Health System, Ann Arbor, USA</td>
<td>TRIM57: an Oncogenic Driver in Human Cancers</td>
</tr>
<tr>
<td>27/01/2016</td>
<td>Adriano Gentile</td>
<td>Columbia University Medical Center, New York, USA</td>
<td>Oncogenic circuits and mechanisms of resistance in acute lymphoblastic leukemia</td>
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<td>Metabolism of cancer cells: Lessons from astrocytic tumors</td>
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<td>20/02/2016</td>
<td>Michele de Palma</td>
<td>The Swiss Institute for Experimental Cancer Research (ISREC), Lausanne, Switzerland</td>
<td>microRNA regulation of tumor-associated macrophages and response to immunotherapy</td>
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<td>Hans-Christoph Gessner</td>
<td>University of Erlangen, Germany</td>
<td>The history behind «the CRISPR craze»</td>
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<tr>
<td>11/03/2016</td>
<td>Chris Thorne</td>
<td>Horizon Discovery Ltd., Cambridge, United Kingdom</td>
<td>Lessons learned from high throughput CRISPR targeting in human cell lines</td>
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<td>A new way of writing, discovering and sharing science</td>
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<td>13/04/2016</td>
<td>Robert L. Kamen</td>
<td>National Cancer Institute of Bangladesh, Kolkata, India</td>
<td>DNA and cancer in the general population</td>
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<td>Michael T. Heneka</td>
<td>University of Bonn, Clinic und Polysarc GmbH, Bonn, Germany</td>
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## Ad-Hoc Seminars

In addition to the CNIO Distinguished Seminar Series, the CNIO also hosts numerous ad-hoc seminars throughout the year. A total of 49 ad-hoc seminars were organised by CNIO researchers in 2016.

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**FACTS & FIGURES**

**Sophie Vasseur**  
29/11/2016

**Maria Sibilia**  
27/10/2016

**Marta Kovatcheva**  
22/11/2016

**Senthil**  
21/10/2016

**Arkaitz Carracedo**  
18/10/2016

**Masayuki**  
17/11/2016

**Yann Cormerais**  
16/11/2016

**Alejandro**  
13/10/2016

**Llucia Albertí**  
03/11/2016

**Ronald Koop**  
06/10/2016

**OCTOBER**

**Institute for Cancer Research. Medical Koff Laboratory, Memorial Sloan-Kettering Cancer Center, New York, US**

**Muthuswamy CIC bioGUNE, University of the Basque Country, Donostia, Spain**

**Sweet-Cordero Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, US**

**Arkaitz Carracedo CIC bioGUNE, University of the Basque Country, Donostia, Spain**

**Marta Karavchova Koff Laboratory, Memorial Sloan-Kettering Cancer Center, NY, US**

**El Giboa Miller School of Medicine, University of Miami, USA**

**Sophie Vasseur CBCH, Cancer Research Center of Marseille, France**

**Luis Amos Pérez Biomedical Informatics, Columbia University, New York, US**

**Mark A. Febrario Garvan Institute of Medical Research, Sydney, Australia**

**Functional analysis of long non-coding RNAs associated with Pancreatic Ductal Adenocarcinoma**

**Molecular mechanisms for the protective effects of physical exercise on cancer incidence**

**02/09/2016**

**James DeGregori University of Colorado, Aurora, US**

**Optogenetic control of Receptor Tyrosine Kinases with light-oxygen-voltage**

**09/08/2016**

**Guillem Panigada Soriano PhD student at Leiden University, Netherlands**

**Proteasome inhibitor acquired drug-resistance in Multiple Myeloma**

**14/09/2016**

**Yann Cormerais Centre Scientifique de Monaco**

**Amino Acids and Cancer: LAT1 a transporter essential for mTORC1 activity and tumor growth**

**26/09/2016**

**Ana Janic**

**The Walter and Eliza Hall Institute, Melbourne, Australia**

**Identification of the critical p53 tumour suppression mechanisms in vivo**

**26/09/2016**

**Iain Cheeseman**

**Whitehead Institute for Biomedical Research, Cambridge, US**

**Dissociating the Mechanisms of Cell Division using CRISPR/Cas9**

**06/10/2016**

**Ronald Koop**

**PerkinElmer, Waltham, US**

**Advanced In Vivo Optical Imaging: Tomography, Spectral Unmixing and Co-Registration**

**10/10/2016**

**Lloïc Alberti Serveira**

**Basel University, Switzerland**

**Single-cell analysis of early haematopoietic development: multipotentiality or heterogeneity?**

**18/10/2016**

**Masayuki Yamamoto**

**Tohoku University Graduate School of Medicine, Sendai, Japan**

**The Keap1-Nrf2 System and Cancer Development**

**21/10/2016**

**Senthil Mathivanan**

**Both Israel Deaness Medical Center, Harvard Medical School, Boston, US**

**Tumor and Normal Pancreas Organoids: A live biobank platform for discovery and translation**

**27/10/2016**

**Maria Sibilia**

**Institute for Cancer Research, Medical University of Vienna, Austria**

**Immune regulation in inflammation and cancer**

**DECEMBER**

**05/12/2016**

**Graham Robertson**

**Centenary Institute, Camperdown, Australia**

**Skin inflammation, type 2 immunity and the atopic march**

**05/12/2016**

**Amalia Telenti**

**Human Longevity Inc., San Diego, US**

**Genomes are just data**

**12/12/2016**

**Jorge Moscat**

**Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, US**

**Stroma-epithelium signaling crosstalk by p62 in cancer**

**14/12/2016**

**Alfredo Caro**

**CIC bioGUNE, Center for Cooperative Research in Biosciences, Derio, Bilbao**

**Anti-cholesterolemic treatment promotes prostate-cancer aggressiveness**

**15/12/2016**

**Federico Pietrocola**

**Cordeliers Research Center University of Paris Descartes, Paris, France**

**Caloric Restriction Mimetics: mechanisms and impact on anticancer therapy**

**16/12/2016**

**Albert Jordan**

**Molecular Biology Institute of Barcelona, Spain**

**Specificities and genomic distribution of somatic human histone H1 subtypes**

**21/12/2016**

**Aranzazu Villasante**

**Columbia University, New York City, US**

**Cancer Engineering: A Translation from Genomes are just data**

**05/12/2016**

**Maria Concepción Ferreras**

**You Tube, Head of Partnerships, Southern Europe and Russia**

**In Her Own Voice: MIT’s Earliest Women Scientists**

**23/02/2016**

**Pilar Garrido**

**IRYCS, Ramón y Cajal University Hospital, Madrid, Spain**

**Lung cancer in women: a different disease?**

**08/03/2016**

**Edurne Pasaban**

**Mountaineer, Tolosa, Spain**

**EXPERIENCY TO SUCCESS: Achieving goals and overcoming difficulties**

**10/05/2016**

**Maria Teresa Fernández de la Vega**

**President of Women for Africa Foundation (Fundación Mujeres por África), Former Vicepresident of Spain**

**Recuerdos y olvidos feministas**

**28/06/2016**

**Tania Baldí**

**Documentalista y Film Director, Barcelona, Spain**

**Las Simombres, sin ellas la historia no está completa**

**27/09/2016**

**Ángeles González-Sinde**

**Scriberi, Film Director, Former Spanish Minister of Culture**

**Out of focus: women and film (Fuera de foco: mujeres y cine)**

**07/10/2016**

**Guadalupe Martín Martín**

**Radiosfera Hospitalaria/Medical Physicist, Servicio de Radioscopia / Medical Physics Service, Fuenlabrada University Hospital, Madrid, Spain**

**María Skolodowska-Curie: Medical Physics pioneer and inspiration to female scientists**

**20/12/2016**

**Christina Rossminge**

**Spanish singer-songwriter, actress and producer**

**30 years of work: my view on the politics and procedures of the Spanish and global music scenes/30 años de trabajo: mi visión en la política y procedimientos en la escena musical Española y global**

**CNIO-WOMEN IN SCIENCE (WISE) SEMINARS**

These seminars are aimed to raise gender awareness via lectures given by gender experts and/or role models, and also to provide CNIO researchers with an opportunity to expand their networks.

**12/01/2016**

**María Concepción Ferreras**

**You Tube, Head of Partnerships, Southern Europe and Russia**

**Woman and Technology: a positive story**

**19/01/2016**

**Margery Baunick**

**Massachusetts Institute of Technology, Cambridge, USA and International Institute in Madrid, Spain**

**In Her Own Voice: MIT’s Earliest Women Scientists**

**23/02/2016**

**Pilar Garrido**

**IRYCS, Ramón y Cajal University Hospital, Madrid, Spain**

**Lung cancer in women: a different disease?**

**08/03/2016**

**Edurne Pasaban**

**Mountaineer, Tolosa, Spain**

**EXPERIENCY TO SUCCESS: Achieving goals and overcoming difficulties**

**10/05/2016**

**Maria Teresa Fernández de la Vega**

**President of Women for Africa Foundation (Fundación Mujeres por África), Former Vicepresident of Spain**

**Recuerdos y olvidos feministas**

**28/06/2016**

**Tania Baldí**

**Documentalista y Film Director, Barcelona, Spain**

**Las Simombres, sin ellas la historia no está completa**

**27/09/2016**

**Ángeles González-Sinde**

**Scriberi, Film Director, Former Spanish Minister of Culture**

**Out of focus: women and film (Fuera de foco: mujeres y cine)**

**07/10/2016**

**Guadalupe Martín Martín**

**Radiosfera Hospitalaria/Medical Physicist, Servicio de Radioscopia / Medical Physics Service, Fuenlabrada University Hospital, Madrid, Spain**

**María Skolodowska-Curie: Medical Physics pioneer and inspiration to female scientists**

**20/12/2016**

**Christina Rossminge**

**Spanish singer-songwriter, actress and producer**

**30 years of work: my view on the politics and procedures of the Spanish and global music scenes/30 años de trabajo: mi visión en la política y procedimientos en la escena musical Española y global**
SCIENTIFIC DIVULGATION EVENTS

RESEARCHERS’ NIGHT
30 SEPTEMBER 2016

This year, the CNIO participated in the 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 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 280 people came to the Spanish National Cancer Research Centre (CNIO) to attend Researchers’ Night (September 30, 2016) and to learn about cancer research. The activities, which were entirely organised by voluntary contributions from 30 young researchers, provided guests the opportunity to meet researchers in an interactive and entertaining way. These included hands-on experiments, view of a virtual tour through the facilities thanks to a video project recorded by scientists from CNIO “CNIO for Kids”, and a speed dating session with the researchers.

OPEN DOORS DAY: INVESTIGATING TO DISARM CANCER
7-20 NOVEMBER 2016

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 (XVI Semana de la Ciencia, 7-20 November, 2016). In 2016, 50 people participated in the guided visit to the Centre’s facilities.

MARIE CURIE EXHIBITION
NOVEMBER 2016

The CNIO hosted an exhibition entitled “Marie Sklodowska-Curie: A Pole in Paris”; the exhibition revisited the personal and professional life of one of the key women of the 20th century. The exhibition opened on 7 November — the birthday of this Polish-French scientist — and could be visited until the 30th of November. This initiative of the CNIO’s Women in Science Office (WISE) stands testament to our commitment to promoting and upholding the work of women scientists. The exhibition attracted 296 visitors.

ROCAVIVA EVENTOS
BCNMOMENTS
14 JULY 2016

During this year, the company bcnmoments organised the “Leading Program Madrid”; a programme sponsored by the “la Caixa” Foundation that awards the 20 highest selectividad test scores within the Community of Madrid. The selected students had the opportunity to get to know different success stories in a broad range of companies and institutions, including the CNIO. During their “Business Experience” at the CNIO in July 2016, the students had the chance to visit the labs guided by young scientists.

GUIDED VISITS

Throughout the year, the CNIO provides tailor-made opportunities to visit its installations and to learn about the essentials of cancer research. During 2016, more than 471 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**
- Luis de Guindos Jurado  
  Minister of Economy, Industry and Competitiveness  
  **Ministro de Economía, Industria y Competitividad**

**President**
- Carmen Vela Olmo  
  Secretary of State for Research, Development and Innovation of the Spanish Ministry of Economy, Industry and Competitiveness  
  **Secretaria de Estado de Investigación, Desarrollo e Innovación del Ministerio de Economía, Industria y Competitividad**

**Vice-President**
- Jesús Fernández Crespo  
  Director of the National Institute of Health Carlos III  
  **Director del Instituto de Salud Carlos III**

**Appointed Members**
- José Javier Castrodeza Sanz  
  Secretary General for Health and Consumer Affairs of the Spanish Ministry of Health, Social Services and Equality  
  **Secretario General de Sanidad y Consumo del Ministerio de Sanidad, Servicios Sociales e Igualdad**

- Marina Pilar Villegas Gracia  
  Director of the Spanish State Research Agency of the Ministry of Economy, Industry and Competitiveness  
  **Directora de la Agencia Estatal de Investigación del Ministerio de Economía, Industria y Competitividad**

- Cristina Ysasi-Ysasmendi Pemán  
  Director of the Department of National Affairs of the Cabinet of the Presidency of the Government  
  **Directora 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**

- Luis Gabilondo Pujol  
  Director General of Health of the Health Department of the Government of Navarre  
  **Director General de Salud de la Consejería de Salud del Gobierno de Navarra**

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

**Elected Members**
- Rafael Pardo Arvelanedo  
  Director, BBVA Foundation  
  **Director de la Fundación BBVA**

- Jaume Giró Ribas  
  CEO of “la Caixa” Banking Foundation  
  **Directora General de la Fundación Bancaria Caixa d’Estalvis i Pensions de Barcelona, “la Caixa”**

- Ignacio Polanco Moreno  
  Chairman, Grupo PRISA  
  **Presidente del Grupo PRISA**

- Caja Madrid Foundation (until September 2016)  
  Fundación Caja Madrid

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

**Legal Advisor**
- Fernando Arenas Escribano  
  Chief State’s Attorney of the Ministry of Health, Social Services and Equality  
  **Abogado del Estado Jefe en el Ministerio de Sanidad, Servicios Sociales e Igualdad**

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* In accordance with the Spanish Transparency Legislation (Spanish Royal Decree 451/2012, of March 5), the following information is hereby provided:
  - 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 203,845 Euros. This amount was received as base salary, seniority, and position supplement.
  - Members of the CNIO Board of Trustees are not remunerated.
• 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
  Director, 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, Translational Diagnostics; Director, Musculoskeletal Tumor Program
  Yale University School of Medicine
  New Haven, USA

• Sara Courtneidge, PhD, DSc (hc)
  Associate Director for Translational Sciences, Knight Cancer Institute
  Professor, Department of Cell, Developmental & 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
  Fred Hutchinson Cancer Research Center
  Research Professor of Microbiology at the University of Washington
  Seattle, USA

• Scott W. Lowe, PhD
  Chair, Geoffrey B. Greene Cancer Research Center
  Chair, Cancer Biology and Genetics Program
  Memorial Sloan-Kettering Cancer Center
  New York, USA

• Joan Massagué, PhD
  Director
  Sloan-Kettering Institute
  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
  P. Vall d’Hebron, Barcelona, Spain

• Janet M. Thornton, FRS, PhD
  Director Emeritus and Senior Scientist
  European Bioinformatics Institute (EMBL-EBI)
  Hinxton, United Kingdom

• Karen H. Vousden, PhD, CBE, FRS, FRSE, FMedSci
  Director
  The Beatson Institute for Cancer Research
  Cancer Research UK
  Glasgow, United Kingdom

• Alfred Wittinghofer, PhD
  Emeritus Group Leader
  Department of Structural Biology
  Max Planck Institute for Molecular Physiology
  Dortmund, Germany

• Ada E. Yonath, PhD
  Director
  The Helen and Milton A. Kimmel Center for Biomolecular Structure and Assembly
  Weizmann Institute of Science
  Rehovot, Israel
## MANAGEMENT

### DIRECTOR

<table>
<thead>
<tr>
<th>Role</th>
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<tbody>
<tr>
<td>Blasco</td>
<td>María A.</td>
</tr>
<tr>
<td>SECRETARIATE</td>
<td>Alcami</td>
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### MANAGEMENT OFFICE

<table>
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<tr>
<th>Role</th>
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<tbody>
<tr>
<td>Peláez</td>
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### COMMUNICATION

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<td>Nuria</td>
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<td>Fernández-Capetillo</td>
<td>Óscar</td>
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### INNOVATION

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<td>Anabel</td>
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### INTERNATIONAL AFFAIRS

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<tr>
<td>Pola</td>
<td>Carolina</td>
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### SCIENTIFIC MANAGEMENT

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### PROJECTS & CONSORTIA

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<td>Liébana</td>
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<td>Arencibia</td>
<td>Raquel</td>
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<td>Molina</td>
<td>Juan Ramón</td>
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<td>Muñoz</td>
<td>Ana</td>
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<td>Viegas</td>
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### EDUCATION & TRAINING PROGRAMMES

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<tr>
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### SCIENTIFIC EVENTS

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<td>López</td>
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<td>López</td>
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<td>Rodríguez</td>
<td>M. Carmen</td>
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### TECHNOLOGY TRANSFER & VALIDATION OFFICE

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<tr>
<td>Martín</td>
<td>M. Cruz (Technology Transfer Manager)</td>
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<td>Rocío</td>
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### DIRECTOR’S OFFICE

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<td>Ámez</td>
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### SECRETARIATE

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<td>María del Mar</td>
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<tr>
<td>Alcami</td>
<td>María Jesús</td>
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### SECRETARIATE (COMMUNICATION, INNOVATION, SCIENTIFIC MANAGEMENT)

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### SECRETARY (COMMUNICATION, INNOVATION, SCIENTIFIC MANAGEMENT)

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<tr>
<td>Pombo</td>
<td>Vanessa (Communications Officer) (until April)</td>
</tr>
<tr>
<td>De Martos</td>
<td>Cristina (Communications Officer) (since April)</td>
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### TECHNOLOGY TRANSFER & VALORIZATION OFFICE

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### SAP

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<td>Ferrer</td>
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### FINANCE & ADMINISTRATION

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<td>Alcaino</td>
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<td>Baviano</td>
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<td>García-Andrade</td>
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### HUMAN RESOURCES

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<tr>
<td>Pérez</td>
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<tr>
<td>Baraldi</td>
<td>Patricio</td>
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<td>Carbonell</td>
<td>David</td>
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<td>Francisco (until December)</td>
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### INFORMATION TECHNOLOGIES

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<tr>
<td>Molina</td>
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### AUDIT

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<td>García-Bisón</td>
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<tr>
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<td>Ana María</td>
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<td>Hernando</td>
<td>M. Elena</td>
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### INFRASTRUCTURE MANAGEMENT

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<tr>
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<td>Luis</td>
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<td>Luis</td>
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### MAINTENANCE

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<td>Garván</td>
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<td>Bertol</td>
<td>Narciso</td>
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### PREVENTION & BIOSECURITY

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<td>Crespo</td>
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### INFORMATION TECHNOLOGIES

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### EXTRAMURAL CLINICAL RESEARCH

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<td>López</td>
<td>Antonio</td>
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* Plan de Empleo Joven (Youth Employment Plan)
CNIO PERSONNEL 2016

464 TOTAL CNIO PERSONNEL

44 ADMINISTRATION 9%

420 RESEARCH 91%

GENDER DISTRIBUTION

312 FEMALE 67%

152 MALE 33%

AGE DISTRIBUTION

43 > 50 9%

152 50 33%

160 41-50 34%

Scientific Personnel 2016

DISTRIBUTION BY PROGRAMMES

<table>
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<tr>
<th>Programme</th>
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<td>Structural Biology and Bioinformatics</td>
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<tr>
<td>Biotechnology</td>
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<tr>
<td>Cancer Cell Biology</td>
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<td>Human Cancer Genetics</td>
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<tr>
<td>Clinical Research</td>
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<td>Molecular Oncology</td>
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<tr>
<td>Experimental Therapeutics</td>
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DISTRIBUTION BY PROFESSIONAL CATEGORY

<table>
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<th>Personnel</th>
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<td>Post-Doctoral fellows</td>
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<tr>
<td>Graduate students</td>
<td>104</td>
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<tr>
<td>Principal investigators</td>
<td>50</td>
</tr>
<tr>
<td>Technicians</td>
<td>151</td>
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GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

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<thead>
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<th>Category</th>
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<tr>
<td>Post-Doctoral fellows</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Graduate students</td>
<td>70%</td>
<td>30%</td>
</tr>
<tr>
<td>Principal investigators</td>
<td>50%</td>
<td>50%</td>
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<tr>
<td>Technicians</td>
<td>36%</td>
<td>64%</td>
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TOTAL SCIENTIFIC PERSONNEL 420

ADMINISTRATION | TOTAL SCIENTIFIC PERSONNEL

GENDER DISTRIBUTION IN SENIOR ACADEMIC AND MANAGEMENT POSITIONS

<table>
<thead>
<tr>
<th>Position</th>
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<th>Male</th>
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<td>Group Leaders, Heads of Clinical Research Unit/Section</td>
<td>32%</td>
<td>68%</td>
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<tr>
<td>Heads of Unit/Biobank</td>
<td>13%</td>
<td>87%</td>
</tr>
<tr>
<td>Scientific Directors, Heads of Area</td>
<td>50%</td>
<td>50%</td>
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<tr>
<td>Management Directors, Heads of Area</td>
<td>33%</td>
<td>67%</td>
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FACTS & FIGURES

TOTAL SCIENTIFIC PERSONNEL 420

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral fellows 16%</td>
<td>29</td>
</tr>
<tr>
<td>Graduate students 34%</td>
<td>71</td>
</tr>
<tr>
<td>Staff scientists 13%</td>
<td>32</td>
</tr>
<tr>
<td>Principal investigators 11%</td>
<td>23</td>
</tr>
<tr>
<td>Technicians 26%</td>
<td>56</td>
</tr>
<tr>
<td><strong>Total 100%</strong></td>
<td>211</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral fellows 10%</td>
<td>13</td>
</tr>
<tr>
<td>Graduate students 24%</td>
<td>30</td>
</tr>
<tr>
<td>Staff scientists 22%</td>
<td>27</td>
</tr>
<tr>
<td>Principal investigators 10%</td>
<td>13</td>
</tr>
<tr>
<td>Technicians 34%</td>
<td>43</td>
</tr>
<tr>
<td><strong>Total 100%</strong></td>
<td>126</td>
</tr>
</tbody>
</table>

**DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION**

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral fellows 16%</td>
<td>1</td>
</tr>
<tr>
<td>Graduate students 33%</td>
<td>3</td>
</tr>
<tr>
<td>Staff scientists 36%</td>
<td>13</td>
</tr>
<tr>
<td>Principal investigators 17%</td>
<td>14</td>
</tr>
<tr>
<td>Technicians 41%</td>
<td>52</td>
</tr>
<tr>
<td><strong>Total 100%</strong></td>
<td>83</td>
</tr>
</tbody>
</table>

SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spanish 83%</td>
<td>350</td>
</tr>
<tr>
<td>Non-Spanish 17%</td>
<td>70</td>
</tr>
<tr>
<td><strong>Total scientific personnel 100%</strong></td>
<td>420</td>
</tr>
</tbody>
</table>

FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral fellows 13%</td>
<td>14</td>
</tr>
<tr>
<td>Graduate students 24%</td>
<td>25</td>
</tr>
<tr>
<td>Staff scientists 22%</td>
<td>16</td>
</tr>
<tr>
<td>Principal investigators 10%</td>
<td>6</td>
</tr>
<tr>
<td>Technicians 6%</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total foreign scientific personnel</strong></td>
<td>70</td>
</tr>
</tbody>
</table>

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

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spanish 83.3%</td>
<td>350</td>
</tr>
<tr>
<td>America 3.6%</td>
<td>15</td>
</tr>
<tr>
<td>Asia 1.7%</td>
<td>7</td>
</tr>
<tr>
<td>Rest of Europe 11.4%</td>
<td>48</td>
</tr>
<tr>
<td>Other 0%</td>
<td>18</td>
</tr>
<tr>
<td>France 6%</td>
<td>4</td>
</tr>
<tr>
<td>Austria 6%</td>
<td>3</td>
</tr>
<tr>
<td>Portugal 13%</td>
<td>6</td>
</tr>
<tr>
<td>Italy 21%</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total scientific personnel 100%</strong></td>
<td>420</td>
</tr>
</tbody>
</table>

Percent values represent percentages of the total CNIO personnel in each category.
CNIO Friends

CNIO Friends

‘CNIO Friends’ Postdoctoral Contracts

Jugaterapia Foundation—‘CNIO Friends’

CNIO opens its doors to ‘CNIO Friends’

Benefactor Friends/Sponsor Friends

Donations to the CNIO
The ‘CNIO Friends’ initiative celebrated its first two years of existence at the end of 2016, and it did so by looking towards the future with all the enthusiasm, commitment, and effort it received from its donors. Since its inception, the community of Friends has continuously increased and more and more people decided to renew their commitment, and thereby their loyalty and trust, to the Centre’s scientific research. We closed 2016 with a community of about 800 Friends, in addition to inheritances and legacies from individuals who decided to support the CNIO’s cutting-edge research; our challenge now is to get our message across more effectively with every announcement we make.

In 2016, thanks to our Friends, we launched the first three cancer research postdoctoral contracts after a competitive assessment process based on scientific excellence. The first two were allocated to sponsor the researchers Paulina Gómez, from the Genetic and Molecular Epidemiology Group, and Vera Pancaldi, from the Structural Computational Biology Group. The former will be working on an international project aimed at analysing the relationship between multiple risk factors and pancreatic cancer, one of the cancers with the highest mortality rates today; the latter will be studying how DNA structures affect tumour aggressiveness, among other factors.

Both researchers stressed “the crucial importance of philanthropy as a driving force in scientific research and their surprise about the difference between the Anglo-Saxon system - where philanthropy is fully integrated - and our country’s system.” Indeed, philanthropy is a sign of commitment to a cause, as well as a tool for individuals and society in general to support cancer research regardless of the economic circumstances.

The third contract came from the Juegaterapia Foundation and their famous ‘baby pelones’ toys, by which they underlined their commitment to childhood cancer research. Last June, the CNIO welcomed one of the most acclaimed popular music stars in our country, Alejandro Sanz, to commemorate the collaboration between the Juegaterapia Foundation and our Centre. Thanks to this grant, the CNIO will host a researcher in 2017 in the Epithelial Carcinogenesis Group who, together with the CNIO-Hospital Niño Jesús Clinical Trials Unit, will study neuroblastomas - the most common tumour type in the first 2 years of life - and tumours of the central nervous system, the most common solid tumours in children.

Businesses also joined our community of Friends in 2016. Indeed, the CNIO signed a collaborative agreement with ASISA Vida in relation to a new product launched by the insurance company that offers specific coverage for gynaecological cancer. Thanks to the insurance company’s commitment to this cause, 5% of the premiums of this product will be donated to the ‘CNIO Friends’ initiative to fund cancer research. Another agreement was formalised with Grupo CLH, the leading company in Spain for the transportation and storage of oil products.

Our CNIO volunteers are another highly valuable asset. Dozens of them came out last November to meet new Friends at the La Vaguada Shopping Centre, where they installed a mini-laboratory to introduce the initiative to the residents and visitors of the popular Barrio del Pilar district in Madrid, where the centre is located. This initiative was one of many examples that helped raise funds for research: there was the event organised by the secondary school IES La Encantada de Rojales, Alicante, consisting of a charity run in May to raise funds for CNIO’s research; the Edinburgh Marathon for which participants put on their running shoes in name of cancer research; and the charity tuna music festival at the Madrid Polytechnic University. Thanks to all of you for supporting our work!

The latest cancer survival figures are encouraging – some types of cancer, such as breast cancer, now exceed 80% – however, there is still a long and difficult road ahead. Our Friends are key in the effort of finding new solutions to fight this disease. Our heartfelt appreciation goes out to each and every one of them.
At the beginning of the year, the CNIO organised the first recruitment programme funded by donations from the CNIO Friends' philanthropic initiative. After a thorough selection process of the candidates, the first two grants were allocated to sponsor the researchers Paulina Gómez, from the Genetic and Molecular Epidemiology Unit, and Vera Pancaldi, from the Structural Computational Biology Group.

Paulina Gómez is an expert in Genetic and Molecular Epidemiology. Her research mainly focuses on PanGenEU, a large European study involving six countries that delves into the relationship between multiple risk factors and pancreatic cancer, one of the carcinomas with the highest mortality rate today. These studies are aimed at defining the population with the greatest risk of suffering from pancreatic cancer, and thereby facilitate the early diagnosis of the disease.

Vera Pancaldi is an expert in Computational Biology. Specifically, she focuses on the study of how the DNA structure affects tumour aggressiveness and how it helps us to understand the context and impact of genetic modifications in patients. These studies could be used as a basis for improving tumour diagnosis and for developing personalised therapies in the clinic.

“This financial contribution has enabled me to ensure the continuity of this project without having to worry about looking for new external funding; it gives me the necessary independence to develop this research.”

In July, the Juegaterapia Foundation, which helps children suffering from cancer, signed a collaboration agreement with the CNIO Friends’ initiative to fund a 100,000 euro grant that will be dedicated to research projects related to childhood cancer. Alejandro Sanz came to the CNIO as Juegaterapia’s Goodwill Ambassador to commemorate this agreement. The singer visited the facilities and obtained first-hand information on the research being carried out. “The fight against cancer is a war and I am a soldier”, he said during his visit to the CNIO.
One of the most exciting events held in 2016 was the ‘Jornada Amigos del CNIO’, which took place in mid-May and welcomed our donors/CNIO Friends in order to bring CNIO science closer to them. Our donors spent an afternoon with us and once again demonstrated their great enthusiasm for being part of the important mission of conquering of cancer.

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 (around €361,000 this year); in doing so they have contributed to society for generations to come.
BENEFACTOR FRIENDS/SPONSOR FRIENDS

Benefactor Friends
- Alfonso Agüera Nieto
  Santa Ana-Cartagena, Murcia
- Álvaro Gil Conejo
  Mijas, Málaga
- Andrés Sánchez Arranz
  Madrid, Madrid
- Asociación Junta Local Casasimarro
  Cuenca, Cuenca
- Encarnación Fernández Pérez
  Madrid, Madrid
- Ferrán Nacher Carull
  Xativa, Valencia
- Francisco Javier Gálago Franco
  Barbastro, Huesca
- Gema Rubio González
  Madrid, Madrid
- IES La Encantá
  Rojales, Alicante
- Iluminada Hernández González
  Gijón, Asturias
- Instituto Preventivo de Galicia
  Arteixo, A Coruña
- Ismael Crespo Martín
  Cáceres, Cáceres
- Javier Com García
  Madrid, Madrid
- Jesús Miguel Iglesias Beturto
  Valladolid, Valladolid
- José Luís Catalá López
  Las Palmas de Gran Canaria, Las Palmas
- José Limiñana Valero
  Alicante, Alicante
- José Polo Criado
  Cáceres, Cáceres
- Juan Félix Ortigosa Córdoba
  Granollers, Barcelona
- Luis David Sanz Navarro
  Madrid, Madrid
- M. Begoña Rumbo Arcas
  Rutas-Vilaboa/Culleredo, A Coruña
- María Jesús Amores Moler
  Jáchaga, Cuenca
- María Rodríguez López
  Celada de los Calderones, Cantabria
- Miguel Muñoz Martín
  Alcalá de Henares, Madrid
- Nemesis Carro Carro
  León, León
- Paloma Fuentes González
  Madrid, Madrid
- Raúl Bueno Herrera
  Plasencia, Cáceres
- Santiago Crespo Martín
  Cáceres, Cáceres
- Sergio Recio España
  Madrid, Madrid
- Susana Sanz Fraile
  Mérida, Badajoz
- Asisa Vida Seguros S.A.U.
  Madrid, Madrid
- Compañía Logística de Hidrocarburos CLH, S.A.
- Fresia Group
  Salou, Tarragona
- Fundación Juegaterapia
  Madrid, Madrid
- María Josefa Azcona Peribañez
  Madrid, Madrid

DONATIONS TO THE CNIO

505,000€
TOTAL CNIO DONATIONS 2016

248,000€
CNIO FRIENDS
2016 100,000€
2015 100,000€
2014 100,000€

784,000€
LEGACIES
2016 381,000€
2015 400,000€
2014 23,000€

44,000€
DONATIONS BEFORE LAUNCH OF CNIO FRIENDS
2014 10,000€
2013 25,000€
2012 3,000€
2011 6,000€

100,000€
ATRESMEDIA
2015 100,000€
2014 50,000€
In order to pour the Annual Report into a more creative concept, the CNIO works closely with selected professionals in the artistic and creative sectors who ensure delivery of an end product that is attractive in more ways than one. We extend our thanks to the creative team, the visual artist Amparo Garrido, and the graphic design studio underbau whose invaluable work created the images and design that illustrate this Annual Report.

AMPARO GARRIDO  PHOTOGRAPHY

A Madrid-based visual artist working with photography and video, Amparo Garrido has been represented in individual and group shows both in Spain and abroad since 1998. Her work has been honoured in several prestigious competitions. She obtained the first place in the 2001 edition of the ABC Photography Prize, and second place in the 2007 Purificación García Prize. Other honourable mentions include the Pilar Citoler and Ciudad de Palma prizes. Her work can be found in major collections, including the Museo Nacional Centro de Arte Reina Sofía in Madrid, the photographic holdings of the Madrid regional government, the Coca-Cola Foundation, the Ex 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.

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 (Orquesta y Coro Nacionales de España, Instituto Cervantes, La Fábrica and Museo Thyssen-Bornemisza among others). 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.