“Thanks to the ‘CNIO Friends’ initiative, close to 600 donors have given us their philanthropic support, and this number is increasing every day.”

MARIA A. BLASCO
Director
First of all, I would like to thank all of those who have once again collaborated on the elaboration of this Annual Report, with especial thanks to Sonia Cerdá who is responsible for our CNIO publications, as well as to our photographer Amparo Garrido and the underbau graphic design team.

During 2015, the CNIO authored a total of 278 papers, 49 of which were published in journals with impact factors ranging from 10 to 15, and 21 papers in journals with impact factors greater than 15. Comparison with previous years (2006-2015) indicates that since 2010, the CNIO has further continued to increase its output of papers in top journals.

In line with our capabilities to produce top science, I am proud to mention that in 2015, the Spanish Ministry of Economy and Competitiveness renewed CNIO’s accreditation as one of the “Severo Ochoa” Centres of Excellence, distinguishing our Centre as the flagship of Spanish cancer research. The “Severo Ochoa” award brings with it important associated funding for the CNIO as well as human resources.

During 2015, 2 new Junior Groups joined the Molecular Oncology Programme at the CNIO: the Microenvironment and Metastasis Group, led by Héctor Peinado, a former Assistant Professor at Weill Cornell Medical College (New York, USA); and the Brain Metastasis Group, headed by Manuel Valiente, a former postdoctoral fellow in the laboratory of Joan Massagué at the Memorial Sloan Kettering Cancer Center (New York, USA). In addition, during 2015, we completed the recruitment of a third Junior Group that will be incorporated into the Molecular Oncology Programme in January 2016. In particular, Alejo Efeyan, a former postdoctoral fellow in David Sabatini’s Group at the Massachusetts Institute of Technology (MIT, Boston, USA), will lead the Metabolism and Cell Signalling Group at the CNIO. In 2015, Alejo received a European Research Council (ERC) starting grant.

In 2015, we created 2 new Core Units: a Protein Crystallography Unit, which is shared with the CNIO Experimental Therapeutics Unit, and a Laser Microscopy Unit.
Programme, and an Electron Microscopy Unit that works in collaboration with the Centre for Biological Research (Centro de Investigaciones Biológicas, CIB-CSIC) in Madrid. These new Units have already started their work in collaboration with several CNIO Groups.

I am very pleased to announce that in December 2015, Óscar Fernández Capetillo, Head of the Genomic Instability Group at the CNIO, was appointed Vice-Director of Translational Research and Director of Innovation. We are very excited about having Óscar leading these key strategic areas for the CNIO. Given his former achievements in translating his basic research discoveries into drug development, in collaboration with the CNIO Experimental Therapeutics Programme, we are confident that Óscar will help to greatly enhance translational research at the CNIO.

I take this opportunity to wholeheartedly thank our former Vice-Director of Translational Research, Manuel Hidalgo. Thanks to his efforts, clinical research at the CNIO is now a reality. In particular, during the last few years, we have increased the number of Clinical Research Units at the CNIO, as well as our agreements with Hospitals. We have now a total of six leading oncologists working at the CNIO in some of the cancer types with the highest social impact (breast, prostate, GI tumours, lung, oncohaematological tumours and paediatric cancer). They all run early phase clinical trials and have clinical practice in the Hospitals associated with the CNIO. In addition, the CNIO also conducts important clinical activity through the familiar Cancer Consultancy located at the Hospital Universitario de Fuenlabrada, as well as through the Molecular Diagnostics and Molecular Cytogenetics and Genome Editing Units at the CNIO.

Conducting world-class scientific research is paramount, but transferring the results to the life sciences value chain is of equal importance to the CNIO. In 2015, our partners have validated preclinical data in order to progress towards the clinical development of a number of previously licensed programmes. We expect that our collaborator Merck Serono will soon complete the preclinical development of ATR inhibitors, thereby accelerating the translation of CNIO’s research into new potential treatment options for cancer patients. Our monoclonal antibody commercial pipeline has also been strengthened. Altogether, the income for the CNIO’s research activities is expected to significantly increase over the next annual budget of the CNIO.

In recognition of these achievements, the CNIO’s ‘Innovation strategy’ was awarded with the Innovation Recognition Award handed out by the Spanish Association of Innovative Companies (Fondo de Empresas Innovadoras, FED); this award is an acknowledgement of the impact CNIO makes thanks to the innovative technologies that we foster and develop for the oncology sector.

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. In June 2015, 2 Research Programmes were evaluated by the SAB, namely the Molecular Oncology Programme (MOP) and the Structural Biology and Biocomputing Programme (SBBP), as well as the 6 Units associated with the SBBP that provide technical support to the entire Centre. Overall, the SAB was impressed by the scientific excellence of the Programmes, which continue to position the CNIO at the forefront of international biomedical science as well as the most promising institute in basic cancer research in Spain. Following the SAB’s recommendations, we will start recruiting several new groups for the SBP in 2016 with the aim of reinforcing structural biology at the CNIO.

During 2015, Julio Celis, from the Danish Cancer Society Research Center in Copenhagen, left the CNIO Scientific Advisory Board (SAB). Two new members have since been approved by our Board of Trustees to form part of the SAB, namely Stephen Frye, Director of the Center for Integrative Chemical Biology and Drug Discovery 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). We wholeheartedly thank Julio for his dedication to the CNIO during his 13 years with us. We would also like to welcome and extend our thanks to Drs. Frye and Yonath for their future commitment to the CNIO.

I would like to take this opportunity to thank all those who have helped the CNIO by supporting our students, postdoctoral programmes and the stays of several researchers. I hereby extend my gratitude to the Research Programmes that have provided collaboration for funding postdoctoral stays at the CNIO and the IE business school course, the Luis Vives Foundation for fostering international PhD fellowships, the Sève Ballerstein Foundation that supports the Sève-Ballerstein Foundation-CNIO Brain Tumour Group, and the Jesus Serra Foundation for supporting the Visiting Scientists Programme and the Dean’s Office. During 2015, the following scientists were the beneficiaries of the Jesus Serra Foundation’s Visiting Researchers Programme: Chaitanya R. Dregi, Professor of Radiology; Vice Chair, Research Department of Radiology at Columbia University in New York (USA); Marcin Nowotny, Head of the Laboratory of Protein Structure, the International Institute of Molecular and Cell Biology in Warsaw (Poland), Eva Nogales, investigator with the Howard Hughes Medical Institute and Professor at the University of California in Berkeley (USA); and, Patrick Song, Professor of Molecular Biophysics and Biochemistry and of Therapeutic Radiology, Yale University School of Medicine in New Haven (USA).

I also wish to thank the Foundation June Sabadell for sponsoring a series of Distinguished Seminars at the CNIO given by ‘outside-the-box speakers’, who provided novel perspectives that contribute to the CNIO’s transdisciplinary environment. During 2015, we had the privilege to listen to: María Jose García Borque, Head of the facility ISOLDE-CERN in Geneva (Switzerland); Ignacio Cirac, Director of the Theory Division, Max Planck Institute for Quantum Optics in Garching (Germany); Thijn Brunsmekamp, Group Leader in the Division of Biochemistry at the Netherlands Cancer Institute in Amsterdam (The Netherlands); and Elly Tanaka, Director of the DFG Research Centre for Regenerative Therapies Dresden – Cluster of Excellence of the TU Dresden CRTI (Germany).

Thanks to the sponsorship of the French Embassy in Spain we were also able to invite the following speaker to our Distinguished Seminar series: Hugues de Thé, Head of Molecular Pathology and Seminar series at the University Institute for Haematology CRTD (Germany).

Furthermore, I would like to give my special thanks to the CNIO Women and Science (WISE) Office for organising an outstanding series of seminars on gender issues. We had the pleasure of listening to Natalia González Valdés, Director of the Ovarian Cancer Programme at the University School of Medicine in New Haven (USA); Maria del Mar Martínez, Director at McKinsey’s Madrid Office; Margarita Salas Falgueras, Professor of Research at the Centre for Molecular Biology “Severo Ochoa” (CNB-CSIC-UAM), Institute for International Relations and Innovation of the Ministry of Economy and Competitiveness (MINRECO); and Blanca de Pablo, Professor of Research, Centre for Biological Investigation, Spanish National Research Council (CSIC).

In 2015, the presence of our Centre in the media continued its upward trend: over 2,000 mentions were tracked in the national and international press, representing a growth of 10% compared to the already remarkable figures of 2014. Likewise, our presence on radio and TV has also experienced a significant increase, almost tripling the highs of 2014. These include shots during the prime-time news bulletins of the main Spanish TV channels.

Two of our studies particularly caught society’s attention this year: in May, the research on TRF1 and cancer immortality – published in ‘EMBO Molecular Medicine’ by the CNIO Telomers and Telomerase Group – hit the front pages of the newspapers and was covered by numerous radio and TV news bulletins as well as international media outlets. In October, the international research published in ‘Nature’ – with the participation of Héctor Peinado, Head of the Microenvironment and Metastasis Group – on the first ‘molecular labels’ that can predict the organs where metastases will form, had a great resonance in Spain and abroad.

One of the most exciting adventures we faced in 2015 was the implementation of the ‘CNIO Friends’ initiative, a philanthropic platform launched in late 2014 to raise public awareness about the importance of supporting biomedical research in order to fight cancer. I am proud to say that this is revealing itself to be one of our most rewarding experiences in outreach activities conducted so far. After a year, the initiative resulted in us benefiting from several personal and unique interactions with our donors and friends. To the date, close to 600 donors have given us their philanthropic support, and this number is increasing every day.

In April, we received significant and remarkable support: the athlete Marcos Argumosa ran 10 consecutive marathons to familiarise society with ‘CNIO Friends’. His action caught the attention of the media and boosted the involvement of new donors. And in December and at Christmas time, a video homage to our Friends, produced by the visual artist Amparo Garrido, travelled the length and breadth of Spain in the national high-speed long-distance trains thanks to an agreement with RENFE, the Spanish rail transport operator.

In 2016, during its first year of existence, ‘CNIO Friends’ will make it possible for us to launch the Postdoctoral Contract ‘CNIO Friends’ Programme, through which we will recruit 2 scientists to reinforce our lines of research over the next 2 years. This setup serves as a great example of how combining the efforts of the research community with the input of society can lead to results that can truly make a difference for the future of medicine.
Vice-Direction of Basic Research

Molecular Oncology Programme
- Tumour Suppression Group
- Experimental Oncology Group
- Telomeres And Telomerase Group
- Cell Division And Cancer Group
- Genomic Instability Group
- Chromosome Dynamics Group
- DNA Replication Group
- Melanoma Group
- Microenvironment & Metastasis Junior Group
- Brain Metastasis Junior Group

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

Structural Biology And Biocomputing Programme
- Structural Computational Biology Group
- Macromolecular Crystallography Group
- Cell Signalling And Adhesion Junior Group
- Structural Bases Of Genome Integrity Junior Group
- Spectroscopy And Nuclear Magnetic Resonance Unit
- Bioinformatics Unit
- National Bioinformatics Institute Unit
- Electron Microscopy Unit
- Crystallography Unit
“My main goal, as Vice-Director, is to strengthen CNIO’s Basic Research domain by encouraging scientific excellence and fostering collaboration, so we can continue to make strides in cancer research”

There have been several encouraging developments this year pertaining to the organisational aspects of CNIO’s Basic Research domain: 2 Junior Group Leaders, Héctor Peinado and Manuel Valiente, joined the CNIO to conduct new lines of research in the area of metastasis; the establishment of 2 new Core Units − the Electron Microscopy and Protein Crystallography Units − that have already started operations in collaboration with a number of CNIO Groups; and, the very positive evaluations of both the Molecular Oncology Programme and the Structural Biology and Biocomputing Programme by the corresponding external Scientific Advisory Board.

CNIO’s Basic Research areas will be further strengthened in the coming year with the incorporation of another Junior Group leader, Alejandro Efeyan, who will conduct research focused on metabolism and cancer; new recruitments in strategic areas of structural biology; more collaborations with internal and external groups; and the consolidation of the projects developed with the Experimental Therapeutics Programme.
It is my pleasure to introduce the highlights of the Molecular Oncology Programme in 2015. First of all, my enthusiastic and warm welcome to the two new Junior Groups, namely, the Microenvironment & Metastasis Group, led by Héctor Peinado, and the Brain Metastasis Group, led by Manuel Valiente. The two Groups will work on different aspects of metastasis, thereby filling an important gap in the research carried out at CNIO.

Héctor Peinado joins the CNIO after an extraordinarily successful postdoctoral period in the laboratory of David Lyden, at Weill Cornell Medical College, New York, working on tumour-derived exosomes and on the pro-metastatic conditioning of distant tissues by primary tumours. During 2015, Héctor successfully secured funding from different public and funding agencies, both nationally and internationally, including a generous donation from La Sexta and the AXA Foundation. His Group is now a reality, composed of 5 members and several students in practice.

Manuel Valiente is a pioneer in the molecular analysis of the mechanisms underlying the metastatic colonisation of the brain, work that he undertook in the laboratory of Joan Massagué, at the Memorial Sloan Kettering Cancer Center, New York. At CNIO, Valiente is going to dissect the process of brain metastasis in further detail. For this, he already has in place a Group of 5 members and several students in practice.

My best wishes to the two new Groups!

In the following pages, you will read about the exciting scientific advances made by each of the groups of the Molecular Oncology Programme. They are all at the forefront of their respective areas of research. There are two scientists in particular that I want to put in the spotlight due to their impressive outputs this year, namely, Marcos Malumbres and Oscar Fernández-Capetillo.

This year, the Cell Division and Cancer Group, led by Malumbres, has published primary research in journals such as Nat. Cell Biol., Dev. Cell, Blood, and Mol. Cell Biol. On a similar level, the Genomic Instability Group, led by Fernández-Capetillo, has published work in journals such as Genes Dev., Nat. Commun., EMBO J. and Mol. Cell Biol.

My congratulations go out to Marisol Soengas, Head of the Melanoma Group, for her remarkable achievements in securing funding for melanoma research in 2015. Two consortia led by Soengas have been granted generous funds for the next few years; one is funded by the Asociación Española de Investigación sobre el Cáncer (€1.2M) and the other one is funded by the American Melanoma Research Alliance ($900,000). These grants put Soengas and the CNIO at the leading front of research against melanoma.

Oscar Fernández-Capetillo has been promoted to Vice-Director of Translational Research in order to capitalise on his experience in drug discovery. Also, Fernández-Capetillo has obtained a Chair as Professor in the prestigious Karolinska Institute in Stockholm. These are all major responsibilities that reflect Oscar’s extraordinary leadership, efficiency and dedication. I wish him the greatest possible successes in these new challenges.

The outstanding careers of our investigators have been further recognised by prestigious awards. In this regard, Mariano Barbacid, Head of the Experimental Oncology Group, has received the Cancer Research Award given by the Asociación Española de Investigación sobre el Cáncer. Also, Oscar Fernández-Capetillo has received the Carmen y Severo Ochoa Award, given by the Foundation of the same name, and the award Líder de Grupo Emergente en Investigación Biomédica, given out by the AXA Foundation.

“The Molecular Oncology Programme continues its tradition of scientific excellence and innovation, with the ultimate goal of moving forward basic and translational research in the cancer field.”
TUMOUR SUPPRESSION GROUP

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, in combination with additional mutations in other genes, can give rise to cancer. Understanding how these 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, including cellular pluripotency, cell senescence, and metabolism. Our Group aims to achieve an integrated understanding of cancer protection.

Our goals are:

→ To understand the mechanisms of tumour suppression and to identify new tumour suppressor regulators.
→ To study the interplay between tumour suppression and ageing.
→ To analyse the involvement of tumour suppressors in the regulation of metabolism and protection from metabolic damage.
→ To characterise cellular senescence as a tumour suppression mechanism.
→ To investigate cellular pluripotency and the involvement of tumour suppressors in the regulation of reprogramming to induced pluripotent stem (iPS) cells.
→ To explore the role(s) of cell plasticity in cancer, tissue regeneration, and ageing.
→ To search for new frontiers in cell plasticity.

“Pharmacological Notch inhibitors have anti-tumour effects in preclinical models of several types of cancers, including lung adenocarcinomas. However, caution must be taken in regards to their clinical application because Notch inhibition may increase the incidence of squamous-type tumours, like in the urinary bladder.”

Manuel Serrano

Group Leader

Staff Scientists
Susana Llanos, Daniel Muñoz, Cristina Pantoja

Post-Doctoral Fellows
Timothy Cash, Pablo J. Fernández-Marcos (until October), Cian J. Lynch, Gianluca Varetti

Graduate Students
Noelia Alcázar, Raquel Bernad, Selim Chaib (since August), Dafni Chondronasiou, Elena López-Guadamillas, Lluc Mosteiro, Miguel Rovira (since April)

Technician
Maribel Muñoz (TS)*

Student in practice
Isabel Calvo (since June)*

*Titulado Superior (Advanced Degree)
NOTCH pathway inactivation promotes bladder cancer progression and epithelial–mesenchymal transition

The NOTCH pathway is frequently altered in multiple cancers. Interestingly, NOTCH mutations fall into two distinct patterns depending on the tumor type. On one hand, gain-of-function mutations are present in acute T-cell lymphoblastic leukaemias, chronic lymphocytic leukaemias, and lung adenocarcinomas, thereby showing that the NOTCH pathway is oncogenic in these malignancies. On the other hand, loss-of-function mutations are detected in myeloid leukaemias and in squamous cell carcinomas (SCCs) of different origins, implying that the NOTCH pathway inactivation promotes bladder cancer in adult mice and shows multiple layers of keratin.

Pharmacological inhibition of PEEK reduces adiposity and metabolic syndrome

The NOTCH pathway inactivation promotes bladder cancer progression and epithelial–mesenchymal transition (EMT) in bladder cancer cells that is partly mediated by loss of its effector HES1. Our results indicate that NOTCH serves as a tumour suppressor in the bladder and that loss of this pathway promotes mesenchymal and invasive features.

Partial loss of Rpl11 in mice recapitulates Diamond-Blackfan anaemia and promotes lymphomagenesis

Mutations in ribosomal genes cause Diamond-Blackfan anaemia (DBA), a condition characterised by anaemia and cancer susceptibility. A subset of DBA patients carries loss-of-function haploinsufficiency mutations in the RPL11 ribosomal gene. The ribosomal protein RPL11 is particularly relevant because of its dual function: on one hand, it is an integral component of the ribosome; on the other hand, under conditions that perturb ribosome biogenesis, ribosome-free RPL11 activates p53 and inhibits CMV. We have generated mice with an inducible Rpl11 null allele and have shown that heterozygous loss of Rpl11 in adult mice results in anaemia associated to decreased erythropoietic progenitors and defective erythropoiesis.

Pharmacological inhibition of PEEK is an effective and safe anti-obesity intervention that could reverse the metabolic syndrome in humans.

NOTCH inactivation induces squamous bladder carcinoma. Representative example of a squamous cell carcinoma, stained with haematoxylin and eosin, showing multiple layers of keratin.

**Figure 1**

Partial loss of Rpl11 in mice recapitulates Diamond-Blackfan anaemia, including increased cancer susceptibility.

**Figure 2**

**PUBLICATIONS**

Our laboratory is interested in understanding the molecular events implicated in the development of K-Ras mutant lung and pancreatic cancers. 2 tumour types with some of the worst prognoses. We are also interested in identifying, and subsequently validating, targets of therapeutic value with the ultimate goal of establishing combination therapies that will have a profound effect on the progression of these tumours and could be ultimately translated to the clinic. We are addressing these ambitious goals by using a new generation of genetically engineered mouse tumour models that uses 2 independent recombinase systems to separate, both spatially and temporally, tumour development from target validation. Moreover, we are now conducting target validation by using conditional knocked-in mice that, upon Cre-mediated recombination, express a kinase dead isoform rather than ablative protein expression to better mimic pharmacological intervention. The outcome of these studies should pave the way for the development of more efficacious therapies in a clinical setting.

RESEARCH HIGHLIGHTS

Identification of novel targets for the treatment of K-Ras driven lung adenocarcinoma

Patients with advanced K-Ras mutant lung adenocarcinomas have poor clinical outcome and are currently treated with standard chemotherapy due to the lack of targeted therapies. We have reasoned that the identification of novel mediators of K-Ras signalling during the early stages of tumour development may unveil essential targets that would not be affected by the clonal heterogeneity observed in advanced and metastatic tumours. Indeed, transcriptional profiling of K-Ras-driven hyperplasias revealed inter-tumour heterogeneity with a subset exhibiting an aggressive transcriptional profile analogous to that of advanced human adenocarcinomas. This signature identified the tyrosine kinase receptor Ddr1 as having the top score. Genetic and pharmacological inhibition of Ddr1 blocked tumour development. Moreover, concomitant inhibition of Ddr1 and Notch signalling, a downstream mediator of Ddr1, thwarted progression of murine K-Ras12V; p53-null adenocarcinomas. Importantly, this combined treatment induced regression of K-Ras p53 mutant patient-derived lung xenografts (PDX) with therapeutic efficacy superior to standard chemotherapy and with significantly less toxic effects. Our data indicate that the combined inhibition of DDR1/NOTCH could be an effective therapy for K-Ras mutant lung adenocarcinoma patients.

DDR1 and NOTCH are involved in a regulatory loop that maintains MAPK activity, an essential pathway for K-Ras-driven lung adenocarcinoma. In this setting, NOTCH inhibition has been shown to impair ERK activity by de-repression of DUSP1. In addition, DDR1 itself is a direct ERK transcriptional target. We propose that DDR1, NOTCH and RAS are connected in a robust signalling network, which could explain the additive effect observed upon DDR1/NOTCH co-inhibition. Yet, we cannot exclude the existence of DDR1 and NOTCH independent roles, ensuing additional therapeutic effect upon combined inhibition. For instance, DLL4 is the major NOTCH ligand in remodelling vasculature and its blockade induces decreased vessel function, thereby affecting tumour growth. Therefore, targeting DLL4 may avoid the gastrointestinal toxicity associated to pan-NOTCH inhibition while providing synergistic therapeutic effects. The fraction of DDR1+ tumours exceeds the prevalence of K-Ras mutations, suggesting that DDR1 might be associated to tumours driven by alternative oncogenes. Indeed, analysis of TCGA expression data in lung adenocarcinoma revealed a tendency for co-occurrence of EGFR mutations and DDR1 expression. This is in agreement with our TMA where most lung adenocarcinomas with p53, LKB1 or EGFR mutations display DDR1+ immunostaining. Intriguingly, all tested EGFR mutant cell lines express high levels of both DDR1 and HEX1 and are sensitive to DDR1/NOTCH co-inhibition, suggesting that EGFR mutant patients could also benefit from this co-treatment.

In summary, our results suggest that proper stratification of lung adenocarcinoma patients may lead to the identification of cohorts that can benefit from concomitant DDR1/NOTCH inhibition.
**PUBLICATIONS**


KrasG12V-driven pancreatic tumorigenesis in mice is not detected in the chemotherapy-treated mice.

Combined inhibition of DDR1 and Notch signalling has comparable therapeutic activity to standard chemotherapy regimens (cisplatin/paclitaxel) but is significantly less toxic.

Figure 1

**AWARDS AND RECOGNITION**

1st Cancer Research Award, ASECa (Asociación Española de Investigación en Cáncer), Seville, Spain.

Keynote Lecturer, EACR-AMIC-SIC International Conference, Florence (Italy), June 2015.

Keynote Lectures, Oncocforum, Leuven (Belgium), June 2015.

Keynote Lectures, International Frontiers in Oncology, Pamplona (Spain), October 2015.

Keynote Lectures, 5th ASECa International Congress, Seville (Spain), October 2015.

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Season Chair, 40th FEBS Congress, Berlin (Germany), July 2015.

Season Chair, AACR-NCI-EORTC International Conference, Boston (USA), November 2015.

Member of the Scientific Committee and Session Chair, EPMI Signalizing Pathways Symposium, Barcelona, Spain.

Member, Lilly Americas Global Scientific Advisory Board.

Member, Novartis Lung Cancer Strategy Scientific Advisory Board.

**ANNUAL REPORT 2015**

**MOLECULAR ONCOLOGY PROGRAMME**

**EXPERIMENTAL ONCOLOGY GROUP**

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We study the mechanisms by which tumour cells are immortal and normal cells are mortal. The immortality of cancer cells is one of their most universal characteristics. 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. The 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.

Telomere uncapping as a potential new therapeutic target for lung cancer

Since unlimited cell division in cancer requires activation of mechanisms that ensure maintenance of telomere length, telomeres are considered anti-cancer targets. The targeting of telomeres in human cancer has been approached via targeting telomerase activity. However, therapeutic strategies based on telomerase inhibition to treat cancer will be effective only when telomeres shorten below a minimum length. We investigated whether the induction of telomere dysfunction, independently of telomere length, by targeting the TRF1 shelterin component could be used as a more universal way to block the growth of dividing cells. We found that the genetic ablation of Trf1 impairs the growth of p53-null K-RasG12V-induced lung carcinomas and increases mouse survival (FIGURE 1). This is accompanied by induction of telomeric DNA damage, apoptosis, decreased proliferation, and G2 arrest. This tumour-suppressive effect of Trf1 deficiency occurs already at the first mouse generation and is independent of telomere length. We also showed that chemical inhibition of TRF1 could be achieved in vivo by using small molecules, which effectively impair the growth of already established lung adenocarcinomas without affecting mouse and tissue viability. Our results constitute proof of concept that acute telomere uncapping by means of TRF1 abrogation is an effective therapeutic strategy to block the growth of aggressive lung...
Pulmonary fibrosis driven by telomere dysfunction

Idiopathic pulmonary fibrosis (IPF) is a degenerative disease of the lungs with an average survival, post-diagnosis, of 2–3 years. Mutations in components of telomerase or in proteins of the shelterin complex are found in both familial and sporadic IPF cases.

The lack of mouse models that faithfully recapitulate the human disease, however, has hampered new advances. We generated 2 independent mouse models that develop IPF owing to either short telomeres (Trf1Δ/-) or telomere dysfunction in the absence of telomere shortening (mice with a complex containing Ccl4 knock-in, and p53 pseudogene lncRNA). Maximum 18F-FDG-glucose uptake

A mutation in the shelterin component POT1 is responsible for cardiac angiosarcoma

Cardiac angiosarcoma (CAS) is a rare malignant tumour whose genetic basis is unknown. In collaboration with the CNIO Human Genetics Group and the Familial Cancer Clinical Unit, we have shown via whole–exome sequencing of a TP53-negative Li–Fraumeni-like (LFL) family including CAS cases, that a nonsense variant in the gene coding for the shelterin component POT1 is responsible for CAS. The same gene alteration is found in 2 other LFL families with CAS, supporting the causal effect of the identified mutation. We extended the analysis to TP53-negative LFL families with no CAS and found the same mutation in a breast AS family. Our functional and in vitro studies demonstrated that carriers of the mutation show reduced telomere–bound POT1 levels, abnormally long telomeres and increased telomerase fragility, highlighting a new role of POT1 as a high susceptibility gene in familial cancer and opening therapeutic opportunities for prognosis and treatment in families with CAS.
The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated. During the last few years, we have generated and characterized different mouse models in order to understand the relevance of several cell cycle regulators in the control of cell division and tissue physiology; these include cell cycle kinases and phosphatases, and proteins involved in ubiquitin-dependent degradation. Our interests are: i) to understand the basic control mechanisms that regulate the cell division cycles; ii) to characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) characterising the function of microRNAs in cell biology and tumour development, and iv), understanding 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 towards improving therapeutic strategies against cancer cell proliferation.

“In 2015, we investigated the relevance of several mitotic regulators during cancer progression and therapy. We have also described the metabolic changes imposed by microtubule poisons that are used to treat cancer and their therapeutic relevance.”
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 the prevention of replicative stress. In a mouse model insensitive to INK4 proteins and deficient in p21WAF1 and p27KIP1, we observed that these inhibitors prevent the accumulation of DNA damage due to replicative stress in different tissues including the nervous system. Moreover, ablation of these inhibitors prevents mouse development. This effect is most likely due to the hyperactivation of cyclin-dependent kinases, since the replicative stress can be prevented by slightly inhibiting the enzymatic activity of these proteins (Quereda et al., 2015).

Oncogenic effect of Aurora kinases in cancer

Aurora kinases are enzymes involved in the regulation of mitosis. These proteins are frequently overexpressed in human tumours and are currently considered as putative cancer targets. Yet, the effect of their overexpression in vivo is not well understood. We generated a new mouse model in which endogenous Aurora B can be overexpressed in a conditional manner (González-Loyola et al., 2015). Mice that overexpressed this kinase developed a wide variety of tumours. The molecular and cellular characterisation of these tumours suggested that Aurora B overexpression not only induces chromosomal instability, as previously expected, but also results in a dysfunctional p53 response, thus contributing to tumour development through multiple mechanisms.

Regulation of the megakaryocyte cell cycle

The cell cycle is widely considered as a universal mechanism for cell proliferation. However, some specialised cells display variants of the consensus cell cycle and understanding these differences may be crucial in the design of therapies against specific malignancies. Using mouse models with specific alterations in cell cycle regulators, we studied the relevance of endoreplication and endomitosis; two variants of the canonical cell cycle, in megakaryocytes. These cells undergo multiple rounds of genome amplification without generating daughter cells, thus increasing their ploidy. We have identified several mitotic kinases that, despite being essential for mitotic cell cycles (such as the ones used by cancer cells), are dispensable for the polypliodisation of megakaryocytes, thereby providing some new options for leukaemia treatment. Other kinases, such as Plk1, are still essential for megakaryocytes and their inhibition leads to thrombocytopenia (Trakala et al., 2015).

Control of cellular metabolism in mitosis

Microtubule poisons, such as taxanes, block mitosis and eventually lead to cell death in a process frequently known as mitotic catastrophe. However, some cells are able to bypass this mitotic arrest and survive, thus contributing to chemo-resistance to those therapies. We have recently observed that mitotic arrest induces an early autophagic flux response, which results in autophagy-dependent mitochondrial degradation and a dramatic energetic deficit (Domeńich et al., 2015). The subsequent increase in the AMP:ATP ratio results in the activation of the metabolic sensor AMPK followed by phosphorylation and activation of PFKFB3, an enzyme required for glycolysis. Thus, mitophagy can be considered as a critical effector of the therapeutic effect of mitotic therapies, while both AMPK and PFKFB3 are critical for survival. The manipulation of these molecular routes may have therapeutic benefits in the presence of microtubule poisons (Esteban-Martínez et al., 2015).

Figure 1. Different variants of the mammalian cell cycle are found in mammals. Megakaryocytes normally undergo endomitosis by skipping late mitotic events. In the absence of Cdk1, they undergo repeated S-G phases (endoreplication), whereas the DNA is replicated more than once (re-replication) in the absence of both Cdk1 and Cdk2.
Overview

DNA damage is the source of pro-cancerous mutations. In addition, recent evidence has suggested that the reverse connection might also exist, namely that oncogenes can promote the generation of DNA damage. However, the nature of the damage that is caused by oncogenes is still poorly understood. Our laboratory has centred its research on trying to understand how cells respond to ‘replicative stress’ (RS), a type of DNA damage that unavoidably occurs every time a cell replicates its DNA, and which is mainly prevented by ATR and Chk1 kinases. Unfortunately, the essential nature of these kinases poses important limitations on their study, particularly at the organism level. In order to overcome these limitations, a significant part of our work over the last few years has been focused on the development of cellular and animal tools for the study of ATR and Chk1. These tools include mice with enhanced or limited ATR-Chk1 function, cell systems in which the pathway can be activated at will, and chemical inhibitors of the ATR kinase. Our studies have revealed the impact of replication stress on cancer and ageing, and have provided drugs that can be used to test our ideas on how to approach cancer therapy. 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 2015, we worked on the development of a new CRISPR-based pipeline for screening genes that are implicated in chemotherapy resistance, and described a new protein complex (SMC5/6) that is essential to suppress cancer and ageing in mammals.”

Oscar Fernandez-Capetillo

Group Leader

Staff Scientists
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(Titulo Superior (Advanced Degree))
## RESEARCH HIGHLIGHTS

### NSMCE2 suppresses cancer and ageing in mice

The structural-maintenance-of-chromosome (SMC) complexes play key roles in chromosome architecture and dynamics. Three heterodimeric SMC complexes have been identified in eukaryotes; these are named SMC1/3 (cohesin), SMC2/4 (condensin) and SMC5/6. The latter one has been shown to prevent DNA damage, but how or where it does this is still a matter of debate. During this past year, we focused our research on NSMCE2, a SUMO ligase that is part of the so-called SMC5/6 complex. By developing a conditional knockout and a sumo dead knock-in, we have found that NSMCE2 suppresses cancer and ageing in mice.

### Searching for mechanisms of resistance to anticancer therapies

Cells are protected from the accumulation of replication stress through a phosphorylation-based signalling cascade that is coordinated by the ATR and Chk1 kinases. It is well known that cancer cells have high levels of replicative stress, and that massive accumulation of this type of DNA damage can rapidly lead to cell death. For this reason, targeting the RS-checkpoint kinase ATR has been extensively studied as an anti-cancer strategy in our laboratory. As a result, in collaboration with the Experimental Therapeutics Programme, we developed ATR inhibitors, some of which were licensed to the pharmaceutical company Merck Serono for clinical development. At present, we are trying to understand the mechanisms of resistance to this drug by using a novel CRISPR-based screening pipeline. Survival of therapy-resistant tumour cells is one of the main reasons for tumour relapse and, therefore, a capital problem in clinical oncology. During the last few years, the implementation of the prokaryotic innate immune system CRISPR-Cas9 into eukaryotic cells and animal organisms has become a very potent, worldwide-used tool for genome editing. Making use of this knowledge, we generated inducible-Cas9 mouse ES cells, in which we subsequently transduced a library of sgRNA’s targeting 20,000 genes of the mouse genome. We subsequently used the library for further screens and, for instance, identified mutations that render cells insensitive to ATR inhibitors (FIGURE 1). We are currently validating our findings, which we hope can help us to predict the mechanisms of resistance that might emerge in the clinic, as well as offer us a rational way to predict which patients will particularly benefit from a therapy with ATR inhibitors.

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**Figure 1** Isolation of mES cells resistant to ATR inhibition: (A) Growth of 2 different clones of mES cells at doses of ATR inhibitor (ATRi) that are toxic for wild type (WT) cells. (B) Quantification of the DNA damage generated by ATRi (measured by High-Throughout Microscopy as the nuclear intensity of gH2AX, per nucleus) in WT and ATR-resistant clones. (C) Confocal images illustrating the lower levels of DNA damage (gH2AX staining) upon ATRi treatment in resistant clones compared to WT cells.

**Figure 2** Nsmce2 is a haplosufficient tumour suppressor. (A) Kaplan-Meier survival curves of Nmce2+/− and Nmce2+/− mice at time of death.
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 knock out 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.

"Chromosome instability is a hallmark of many tumours and one key mechanism to prevent it is sister chromatid cohesion mediated by cohesin. During cell division, cohesion is particularly strong at centromeres. We have discovered an unanticipated role of CENP-C in promoting cohesion at this chromosomal region."

Ana Losada
Group Leader

Ana Cuadrado
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Visiting Scientist
A novel role of Centromere Protein C (CENP-C) in centromeric cohesion

Centromeric chromatin containing the Centromere Protein A (CENP-A) directs kinetochore assembly through a hierarchical binding of CENPs, starting with CENP-A and CENP-T. Centromeres are the chromosomal regions where cohesion, mediated by cohesins, is most prominently maintained in mitosis. Cohesion is essential for faithful chromosome segregation and therefore for preventing chromosome instability, a common feature of many solid tumours. While most cohesins dissociate from chromosome arms in prophase, Shugoshin 1 (Sgo1) prevents this process at centromeres. Centromeric localisation of Sgo1 requires histone H2A phosphorylation by the kinase Bub1. Using the Xenopus egg cell-free system, we have now found that both CENP-C and CENP-T can independently drive centromeric accumulation of Sgo1 through recruitment of Bub1 to the kinetochore KNL1, MIS12, NDC80 (KMN) network. Moreover, we have also shown that targeting of Bub1 is the only requirement for Sgo1 accumulation, since forced targeting of Bub1 kinase domain rescues Sgo1 recruitment in the absence of any kinetochore component other than CENP-A. The kinase Mps1 regulates this pathway. Even though Sgo1 targeting is similarly impaired in chromosomes assembled in extracts lacking Mps1, CENP-T or CENP-C, centromeric cohesion defects are most prominent in the absence of CENP-C. These findings reveal that CENP-C plays a second role in cohesion in addition to Sgo1 recruitment. We are currently investigating the nature of this role, which could be related to cohesion deposition at centromeres, cohesion establishment, or recruitment of some other cohesin-independent regulator of cohesion.

The contribution of cohesin to gene expression and chromatin architecture

To determine how cohesin contributes to the establishment of tissue-specific transcriptional programmes, we have compared the genome-wide distribution of cohesin, gene expression and chromatin architecture in the cerebral cortex and pancreas of adult mice. For this purpose, in close collaboration with the CNIO Bioinformatics Core Unit, we have used Next Generation Sequencing (NGS) technologies. Chromatin Immunoprecipitation (ChIP) sequencing, RNA sequencing and a Chromosome Conformation Capture technique known as 4C. We have found that more than one third of cohesin binding sites differ between the two tissues. The tissue-specific sites show reduced overlap with CCCTC-binding factor (CTCF) and are enriched at the transcription start sites (TSS) of tissue-specific genes. Analyses of chromatin contacts at the Protocadherin (Pcdh) and Regenerating islet-derived (Reg) gene clusters, mostly expressed in the brain and pancreas respectively, revealed remarkable differences in locus architecture that correlate with the differential distribution of cohesin. Since there are two versions of cohesin in somatic tissues, cohesin-SA1 and cohesin-SA2, we also investigated their specific contributions. At the Pcdh locus, chromatin organisation is not significantly altered in brains of SA1 null embryos, suggesting that cohesin-SA2 can also perform the architectural function when SA1 is not present. In contrast, reduced dosage of SA1 altered the architecture of the Roy locus and decreased the expression of Roy genes in the pancreas of SA1 heterozygous mice (FIGURE2). Given the role of Roy proteins in inflammation, such reduction may explain the increased incidence of pancreatic cancer observed in these animals. This is the first reported example of how heterozygous mutations in cohesin may contribute to tumourigenesis.

**Publications**


Our laboratory studies the process of DNA replication. All cells that proliferate synthesize a replica of their genome before undergoing mitotic division. DNA replication makes the genome vulnerable to mutations and chromosomal reorganizations, and constitutes one of the unavoidable risk factors in developing cancer. On the other hand, as cancer cells are highly proliferative, the proteins that synthesize DNA are useful targets for chemotherapeutic drugs. Our current research interests are: (1) the control of DNA replication through the regulation of replication origins (the genomic positions where ‘replisomes’ are assembled to start the synthesis of new DNA); (2) the study of specific proteins that mediate replication of damaged DNA, such as the primase-polymerase PrimPol; (3) the consequences of deregulated replication in cancer and ageing. In our different projects we use a combination of technical approaches including biochemistry, molecular biology and mouse genetics.

“We have found that certain blood cell types are highly sensitive to replication stress. A partial loss of the replicative DNA helicase affects hematopoietic stem cells and erythroblasts, causing anaemia and increasing the frequency of haematological cancers.”

Juan Méndez
Group Leader

Post-Doctoral Fellow
Sara Rodríguez

Graduate Students
Marcos Díaz, Daniel González, Karolina Jodkowska, Sergio Muñoz
Lethal anemia caused by hypomorphic expression of MCM helicase

We have shown, using a mouse strain with hypomorphic expression of the Mcm3 gene, that limiting the number of potential replisomes in vivo affects the functionality of haematopoietic stem cells and the differentiation of rapidly-dividing erythrocyte precursors (Figure 1, A and B). In addition, the lifespan of Mcm3-hemizygous mice is reduced due to early-onset lymphomas and mesenchymal tumours. When the concentration of MCM3 protein becomes <1/3 of its normal levels, embryos die in utero because the foetal liver fails to make enough red blood cells to sustain oxygen delivery to all tissues. During the last year, we demonstrated the link between Mcm3-deficient foetal liver (Figure 1) and the formation of benign papillomas. Furthermore, older K5-Cdc6 mice displayed better fur preservation than their wild-type littermates (Figure 2). This unanticipated ‘anti-aging’ effect was analyzed in collaboration with the laboratory of C. Blanpain (Université Libre de Bruxelles, Belgium), that CDC6 overexpression extended the resting stage of the hair follicle growth cycle (Bua et al., 2015).

Figure 1 Impaired red blood cell production in Mcm3-deficient mice. (A) Competitive transplantation (1:1) between Mcm3-deficient foetal liver cells and Mcm3-competent bone marrow cells expressing fluorescent Tomato protein. (B) Red blood cell chimerism in recipient mice after transplantation. (C) Accumulation of immature erythroblasts in Mcm3-deficient peripheral blood. Adapted from Álvarez et al. (2015).

Cdc6 overexpression affects papillomagenesis and influences hair growth

In 2015, we also completed a study to monitor the effect of Cdc6 deregulation in vivo. Cdc6 encodes a protein responsible for the recruitment of MCM helicase to replication origins and is overexpressed in several cancer types, including subsets of brain tumours, mantle cell lymphomas and non-small cell lung carcinomas. To date, no model has been described to test the proto-oncogenic effects of Cdc6 deregulation in mammalian tissues. The K5-Cdc6 mice strain generated at the CNIO displayed higher levels of CDC6 protein in the skin and other tissues with stratified epithelia. Cdc6 “gain of function” was revealed by the enhanced loading of MCM complexes in keratinocytes. Deregulated Cdc6 by itself did not promote skin tumours, but in combination with chemical carcinogens it favoured the formation of benign papillomas. Furthermore, older K5-CDC6 mice displayed better fur preservation than their wild-type littermates (Figure 2). This unanticipated ‘anti-aging’ effect was analyzed in collaboration with the laboratory of C. Blanpain (Université Libre de Bruxelles, Belgium), that we found that CDC6 overexpression extended the resting stage of the hair follicle growth cycle (Bua et al., 2015).

Applications of single-molecule analysis of DNA replication

One of our approaches for the study of DNA replication consists of the analysis of replisome progression and origin activity in individual DNA molecules. To this end, we extensively use a ‘stretched DNA fibre’ technique that has attracted the interest of other CNIO Research Groups. In 2015, single-molecule analysis of DNA replication was applied to several projects, including the study of megakaryocyte polyploidisation mechanisms led by Marcos Malumbres (Trakala et al., 2015) and the study of NSMCE2 in cancer and ageing, led by Oscar Fernandez-Capetillo (Jacome et al., 2015). We are currently working on the development of novel applications for this powerful technique.

Figure 2 Cdc6 deregulation affects the hair growth cycle. Distribution of wild-type and K5-Cdc6 mice (105 weeks old) in 3 phenotypic categories according to their fur preservation.

CDC6 overexpression extended the resting stage of hair follicles, increasing hair preservation. Adapted from Búa et al. (2015).
OVERVIEW

Cutaneous melanomas are a prime example of aggressive cancers, for which basic and translational research have significantly improved patient prognosis. Nevertheless, sustained clinical responses are still incomplete in this disease. The long-term goal of our Group is to identify new tumour drivers and progression biomarkers as a platform for a more rational drug design. Specifically, we are interested in stress response programmes (involving apoptosis, autophagy, senescence and endosome mobilisation), with a particular focus on lineage-specific oncogenes. RNA-binding proteins are also central themes in our research. Our experimental settings include human biopsies isolated from early, intermediate and late stages of melanoma development, combined with a unique set of animal models engineered for non-invasive imaging of metastatic processes. These studies are performed in the context of large multidisciplinary consortia and biotechnology companies in order to facilitate the translation of our discoveries to the bedside.

“We have identified oncogenic signals that are uniquely deregulated in melanoma. These lineage-specific drivers increase the risk for metastasis and inhibit the response to current targeted therapies, and as such, may have direct translational implications.”

Maria S. Soengas
Group Leader

Staff Scientists
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Technicians
Tonantzin Calvo, Estela Cañón (TS)*

*Titulado Superior (Advanced Degree)
RESEARCH HIGHLIGHTS

New lineage-specific roles of the endolysosomal machinery in melanin progression

One of the long-term objectives of the Melanoma Group is the discovery of new melanoma drivers. In particular, we pursue lineage-specific oncogenes, which, due to their unique functions in melanoma, may represent alternative targets for therapeutic intervention. We have identified a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies. Within this gene cluster, melanomas were found to depend on the vesicular trafficking modulator RAB7 as a dose-dependent rheostat of progression and invasion (Alonso-Curbelo et al., Cancer Cell 2014 and Oncotarget 2015). In collaboration with the CNIO Experimental Therapeutics Programme, we have now traced this ‘RAB7-based addiction’ right back to the level of transcriptional control. Newly generated mouse models confirmed the functional relevance of the heterogeneous deletion of Atg5 in melanoma metastasis and importantly, in the resistance to targeted therapy (FIGURE 2B). These data have relevant translational implications for drug design, as an inadvertent partial blockade of autophagy may worsen (instead of counteracting) the malignant phenotype of metastatic melanoma cells (García-Pascual et al., Autophagy, in press).

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

Melanomas are long-known for their highly deregulated mRNA expression profiles. Still, the specific contribution of RNA binding proteins and spliceosome modulators remains virtually unexplored in this disease. In collaboration with the groups of J. Valcárcel, F. Gebauer (the Centre for Genomic Regulation, CRG) and R. Méndez (the Institute for Research in Biomedicine, IRB), we have identified tumour-selective roles of modulators of mRNA stability, alternative splicing, transcription and translation, with targets involving master specifiers of melanocyte lineage. We are very excited about the prospect of RNA regulators as diagnostic markers and therapeutic targets in melanoma.

We have also made great progress in dsRNA nanoparticles as anticancer treatments. Comprehensive functional analyses in vivo now demonstrate a three-pronged activity of these compounds: (i) killing melanoma cells by further deregulating their endolysosomal machinery, (ii) abrogating the function of cytokine-activated endothelial cells and (iii) engaging potent immunomodulatory activity (Olmeda et al., submitted). This information will support clinical trials that are under consideration by the CNIO spin-off company Biostech Therapeutics.

Metastatic risk and resistance to BRAF inhibitors in melanoma defined by selective allelic loss of ATG5

We then questioned whether other lysosomal-dependent degradative processes, such as autophagy, could also be regulated and act in a melanoma lineage-specific manner. Curiously, a meta-analysis of RNA expression and copy number variation, together with the assessment of prognostic values of all the genes constituting the core autophagy machinery, identified a unique feature in melanoma that involved selective heterozygous losses of ATG5 (FIGURE 2A). This ATG5 heterozygosity predicted poor overall patient survival, in melanoma, in a manner not shared by other autophagy factors and not recapitulated in other cancer types. We have also made great progress in dsRNA nanoparticles as anticancer treatments. Comprehensive functional analyses in vivo now demonstrate a three-pronged activity of these compounds: (i) killing melanoma cells by further deregulating their endolysosomal machinery, (ii) abrogating the function of cytokine-activated endothelial cells and (iii) engaging potent immunomodulatory activity (Olmeda et al., submitted). This information will support clinical trials that are under consideration by the CNIO spin-off company Biostech Therapeutics.

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Role of tumour-derived exosomes in pre-metastatic niche formation

We have demonstrated that exosomes are biomarkers and functional contributors to pre-metastatic niche formation in metastatic organs. Exosomes can serve as vehicles for horizontal transfer of oncoproteins, thus promoting additional modifications in the tumour and metastatic microenvironments. We showed that melanoma-derived exosomes expressing c-MET influence bone marrow-derived cell mobilisation and recruitment to pre-metastatic and metastatic niches, thus promoting metastasis in a process that we have termed ‘education’ (Peinado et al., *Nature Med.*, 2012).

More recently, we have observed that pancreatic cancer-derived exosomes expressing macrophage migration inhibitory factor (MIF), preferentially acted upon Kupffer cells (Costa-Silva et al., *Nat Cell Biol.*, 2015). Strikingly, MIF and c-MET levels in plasma exosomes demonstrate the potential of using exosomal protein levels as an early biomarker for liver pre-metastatic niche formation and for predicting patient outcomes, respectively.

Tumour-derived exosomes define metastatic organotropism

Our studies demonstrated that tumour exosomes are a major tumour-derived factor that acts systemically to promote bone marrow-derived cells (BMDCs) recruitment to the tumour and metastatic microenvironments (Peinado et al., *Nature Med.*, 2012). Our recent results demonstrate that tumour-derived exosomes are uptaken by organ-specific cells preparing the pre-metastatic niche. Exosome proteomics revealed distinct intrametapatic expression depending on their specific organ of metastasis. Therefore, we postulate that exosome integrins could serve as a “ZIP” code for exosomes to home in metastatic organs triggering local effects in different metastatic microenvironments (Peinado et al., *Nature Med.*, 2012).

We focus on understanding the interactions of the tumour with its microenvironment, thereby defining new targets to block metastasis.

**“Our work has highlighted that tumour-secreted exosomes promote the ‘education’ of the tumour microenvironment, thus reinforcing metastasis.”**

**REFERENCES**


**AWARDS AND RECOGNITION**

- 2015. Consellera y Xifiles Prize for Metastasis Research, La Jolla and the AXA Foundation, Spain.
OVERVIEW

Brain metastasis is the most common neurological complication of cancer. When metastatic cells reach the brain, prognosis is always poor given that current therapy (i.e. surgery and radiation) has limited benefits for patients and the disease inevitably relapses. The rise in the number of patients with brain metastasis is partially due to the increasing number of systemic therapies that work extracranially. Paradoxically, given the inefficiency of these therapies to work in the brain, they give cancer cells extra-time to colonise this highly demanding secondary site. In the laboratory we study why and how cells from different cancer types (breast and lung cancer among others) 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.

RESEARCH HIGHLIGHTS

The Brain Metastasis Group has established a new line of research at the CNIO focused on the progression of cancer to the Central Nervous System (CNS). During 2015, we have initiated various projects:
→ By exploring the role of commonly de-regulated genes in brain metastasis experimental models, we are now investigating unknown aspects of brain colonisation by cancer cells.
→ We have identified 2 distinct sources of resistance to irradiation, which we are currently testing using novel brain metastasis models that incorporate this therapy.
→ We are dissecting the heterogeneity present in the brain metastasis associated microenvironment by targeting transcription factors that we identified in subpopulations of glial cells associated with brain metastasis.
→ The lab has validated a new experimental platform to test brain metastasis sensitivity to drugs, which maintains cancer cells within their microenvironment and that is compatible with human specimens and medium-throughput optimisation.

“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 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 to liver, bone and brain tumours. The research covers various 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.

Mirna Pérez-Moreno’s Group investigates the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer development. Nabil Djouder’s Group aims to dissect the contribution of nutrient and growth factor signalling pathways to cancer development, and in particular to gastrointestinal cancers. Massimo Squatrito’s Group, which is partly supported by the Seve Ballesteros Foundation (F-SB), studies how brain tumours, mainly glioblastomas and medulloblastomas, develop and how they respond to therapy. The Senior Group, led by Francisco X. Real, studies epithelial tumours focusing mainly on pancreatic and bladder cancer. The Group employs an integrative approach to understand the molecular pathophysiology of these tumours and applies this knowledge in the clinical setting. 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, skin and bone. We are investigating the role of AP-1 in skin cancer and in inflammatory skin diseases, such as psoriasis, but we also aim to molecularly define the causes of lung fibrosis and cancer. We have also embarked on a new project in order to study how the whole organism responds to a growing tumour in the context of a complex metabolic disorder, termed cancer cachexia.

“Our main goal is to render CNIO globally more competitive and to ensure that CNIO remains an international institution. Fifteen different nationalities are represented in our Programme and we aim to perform first-class cancer cell biology as well as to train students and postdocs to become the next-generation of promising scientists.”
OVERVIEW

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

- Elucidating a causal link between inflammation, cancer and AP-1 (Fos/Jun) expression, using cell type-specific, switchable genetically engineered mouse models (GEMMs).
- Developing and characterising new GEMMs for cancer and human diseases, such as bone loss, 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.

“We aim to ensure that CNIO remains an international 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. Fifteen different nationalities from the 5 continents ensure an international science culture and all focus on unraveling the mysteries of 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 and skin. 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 screens.

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-function/mutation mouse models. c-Fos expression leads to osteosarcoma (OS) and chondrogenic hyperplasias. We found that c-Fos modulates Wnt signalling in OS cells, which constitutes a potential link between c-Fos/AP-1 and OS development. Furthermore, we generated adenovalvar constructs expressing Cre recombinase under the control of cell-specific promoters, which allow restricting AP-1 expression or inactivation to specific cells of the bone lineage. Bone/cartilage dysplasias are observed when c-Fos is locally expressed in the knee joint using this approach.

Rheumatoid (RA), Psoriatic (PsA) and Osteo-Arthritis (OA) are destructive joint pathologies linked to chronic inflammatory diseases. We are studying the functions of AP-1 factors and their target genes in the development of arthritis using GEMMs, experimental arthritis models and local gene manipulation approaches.

Liver disease – inflammation, metabolism, fibrosis and cancer

Fra-1/2 proteins appear to be dispensable for liver fibrosis, while they are important modulators of hepatic lipid metabolism by controlling PPARα expression. Strikingly, specific AP-1 dimers can either induce or repress PPARα expression. Therefore, fatty liver disease and obesity most likely depend on AP-1 dimmer composition. In addition, while both Fra proteins protect against steatosis, ectopic expression of Fra-2, but not Fra-1-containing AP-1 dimers in hepatocytes, leads to hepatoxemia and liver dysplasia in aged mice. Mechanistically, molecular analyses point to the involvement of the Wnt/γ-catenin pathway, often connected to human hepatocellular carcinoma (HCC).

Ectopic c-Fos expression and its dimers lead to spontaneous liver inflammation, fibrosis, hepatic/bile duct proliferation and tumours with human HCC gene signatures. Deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis, whereas deletion in immune cells abrogates this protective effect. Moreover, a robust connection between c-Fos expression and the activity of the liver X receptor/retinoid X receptor (LXR/RXR) pathway, an important regulator of cholesterol homeostasis, was observed and most likely contributes to the oncogenic function of c-Fos in hepatocytes.

Cancer-associated cachexia (CAC)

We are studying the role of white adipose tissue (WAT) ‘browning’ as a contributing factor to the wasting process, thus providing a promising new target to prevent/delay cachexia in cancer patients. Other aspects of the syndrome are being investigated using GEMMs, as the role of inflammation, the metabolic impairment and the systemic changes occurring in the neuro-endocrine system, in order to delineate the course of events during CAC and to potentially identify biomarkers for the onset of CAC.

A function for AP-1 in lung disease

We recently documented the connection between the Fos protein Fra-1 and major transcription factors controlling epithelial to mesenchymal transition, a process important in epithelial cancers. The contribution of Fra-1/2 proteins to lung fibrosis and non-small cell lung cancer (NSCLC) is currently being studied using GEMMs, as well as lung cancer samples from patients, in order to unravel the relevant cellular and molecular mechanisms. Our preliminary data from the mouse models indicate that Fra-2 might be crucial for the development of fibrosis and protection from cancer in the lung. These findings may represent new diagnostic and therapeutic opportunities for these diseases and lay grounds for further pre-clinical testing. This study is conducted in collaboration with Mariano Barbacid’s Experimental Oncology Group at CNIO and Daiichi Sankyo Company (Japan).

Skin cancer, inflammation and human disease

Since Squamous Cell Carcinomas (SCCs) have increased c-Fos expression, we modelled SCC development in mice with inducible c-Fos expression. We identified an essential role of c-Fos in modulating immune cell recruitment to the skin, which contributes to skin cancer development. We are currently evaluating the role of p53 deletion in epidermal cells lacking c-Fos. These mice develop SCCs upon ageing. The goal is to understand the initiation of SCCs in relation to ageing. We also demonstrated that loss of epidermal Fra-2 protein results in skin barrier defects. Mechanically, Fra-2 binds and transcriptionally regulates epidermal differentiation gene promoters, which are co-occupied by the transcriptional repressor EzH2 through post-translational mechanisms involving the BRK pathway.

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone crosstalk. JunB represses the expression of pro-inflammatory cytokines that affect the differentiation of bone-forming osteoblasts. We extended our studies to psoriasis patients and have shown that they have bone loss. We are currently planning to evaluate the role of the microbiota in skin inflammation by antibiotic treatments, high-throughput microbiota sequencing and germ-free housing conditions.

New approaches including genetic and biochemical analyses by proteomics of mouse and human skin samples were performed; these unravelled novel pathways and molecules for targeted therapies, such as Sirtuin4/Ap-1 and complement C1. In addition, a potential role of specific miRNAs, e.g. miR21 involved in the pathogenesis of psoriasis, was established showing that miR21 inhibition can be a novel therapeutic intervention in psoriasis. Human skin samples are provided by our collaborator Esteban Daudén from the Hospital Universitario de La Princesa (Madrid, Spain). Another angle of research in psoriasis involves the understanding of the role of epidermal stem cells in the initiation and progression of psoriasis using state-of-the-art lineage-tracking models.
OVERVIEW

Our Group focuses on pancreatic ductal adenocarcinoma (PDAC) and urothelial carcinoma (UC) by adopting a disease-oriented approach. Our strategy accords equal importance to the 3 models that we use: cultured cells, genetically modified mice, and patient samples. Our primary observations can be made at either of these levels and they are then expanded through additional work. We translate this knowledge to the “population-wide” level by harnessing information and samples from large patient cohorts.

Our research on PDAC focuses on the role of cell differentiation as a potent tumour suppressor mechanism that acts early on during carcinogenesis. We use the excellent genetic mouse models that are available because these processes cannot be readily studied using human samples. PDAC can originate both in pancreatic progenitors as well as in acinar cells. The elucidation of 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 the context of UC, we focus our efforts on identifying new genes, using them for improved tumour taxonomy, as well as for characterising the mechanisms through which they participate in cancer. This knowledge is then applied for improved prediction of outcome and therapy.

“In 2015, we exploited the PDAC mouse models to highlight the role of GATA6 as a tumour suppressor and the requirement of BPTF for c-Myc- and KRas-driven carcinogenesis. We continued our genomic studies of UC and generated new information to improve the molecular classification of tumours and cell lines.”
Pancreatitis molecular pathophysiology

Cell differentiation as a tumour suppressor mechanism in the pancreas. PDAC is characterised by highly prevalent alterations in KRAS, p16, TP53, and SMAD4, as well as by low-frequency alterations in a plethora of other genes converging in a few critical genetic pathways. Our main interest is to identify new players involved in this tumour. We have continued our work on NRAS2 and have shown that the pancreas of heterozygous mice is histologically normal but that it displays a basal phenotype and the relationship with response to neoadjuvant and adverse therapy is being analysed.

Through exome sequencing we have identified new genes and pathways involved in UC and we are focusing on the STAG2/COH2/cohesin. We are analysing the mechanisms through which this gene contributes to UC, as well as the clinical significance of STAG2 inactivation as it relates to patient outcome and response to therapy, and we are generating conditional Stag2-null mice. A relationship between Stag2 inactivation and the Luminal phenotype has been established using several strategies.

UC cell lines have provided useful tools to study bladder cancer biology and to perform preclinical studies; we have generated the largest UC line genomic resource, which has evidenced the limitations of these strategies. Therefore, during the last year we have started to apply ‘organoids’ technology to study urothelial cells. We have established reproducible methods to produce normal mouse urothelial organoids and are characterising their requirements for growth and differentiation. Normal organoids have been cultured uninterruptedly for over 1 year. We have also initiated the culture of mouse tumour organoids and plan to move to using human samples in the near future.

This work is being conducted in close collaboration with the Group of Núria Malats at the CNIO, as well as with SUGOG and a European Consortium of collaborators.
EPITHELIAL CELL BIOLOGY JUNIOR GROUP

During 2015, we continued our efforts to uncover novel events controlling the behaviour of skin stem cells, with the aim of opening up new insights into the mechanisms that control their regenerative characteristics and how, when disrupted, these may result in cancer.

OverVIEW

Tumour cells evolve into a progressively complex interplay of heterogeneous tumour cells with their tissue microenvironment, 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 in defining regulatory pathways controlling their proliferation and/or differentiation. However, the identification of extrinsic factors modulating stem cell behaviour is not yet fully established. Using the skin as a model system, based on mouse genetics and human samples, our research aims to understand how the interactions between epithelial progenitor cells, and also the interactions with their surrounding microenvironment, sustain skin homeostasis and regeneration, and how, when perturbed, this may lead to cancer. This information may provide insights for the future development of regenerative and anti-cancer therapies.

RESEARCH HIGHLIGHTS

Regulation of epithelial progenitor cells’ self-renewal and differentiation

We continue to explore how tissues acquire an adequate control of cell division and differentiation. In particular, we study the contributions of mitotic and cytoskeletal proteins in the regulation of skin progenitor’s self-renewal through oriented cell divisions, using mouse epidermal development as a model system.

Contributions of immune cells to the skin stem cell niche in homeostasis

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

Contributions of inflammatory responses in cancer stem cell maintenance and tumour progression

The formation of tumours and their progression to malignancy undoubtedly involves the contributions of the tumour microenvironment. Identifying the signalling mechanisms and cell types that contribute to tumour initiation and progression to malignancy is instrumental for the identification of potential targets for clinical applications to eradicate tumours.

The microenvironment of many tumours is rich in cytokines, chemokines, and inflammatory enzymes. During 2015, 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 have directed our efforts towards 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.

Publications

The incidence of metabolic diseases and cancer has increased to epidemic proportions possibly due to a high-calorie diet and nutrient overload along with a more sedentary lifestyle. In this regard, we are interested in deciphering the growth factor and nutrient signalling pathways to find new effectors as well as in generating mouse models recapitulating human diseases. This will allow us to better understand how growth factors and nutrients impact on the patho-physiological states of metabolic disorders and cancer. Using cell biological and biochemical techniques, combined with in vivo mouse models and human data, our lab devotes significant effort to develop innovative mechanism-based therapeutics, thereby providing the development of novel avenues to treat metabolic dysfunctions and cancer.

“Our research focus is to apply complex cancer models to guide clinical research perspectives and applications.”

### RESEARCH HIGHLIGHTS

Our lab studies molecular mechanisms of diseases associated to the dysregulation of the growth factor and nutrient signalling cascades. We have a particular interest in 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. Our task is to find new components of growth factor and nutrient circuits and elucidate their role and functions in vivo by generating genetically engineered mouse models (GEMMs). Overall, Mouse model-based preclinical platforms (GEMMs and patient-derived xenograft (PDX) models) guide our research directives for future clinical applications.

#### Identifying new components of growth factor and nutrient circuits

Unconventional prefoldin RPB5 interactor (URI). We reported URI as a direct downstream effector of the mammalian/mechanistic target of rapamycin (mTOR)/ribosomal protein S6 kinase -1 (S6K1), the growth factor- and nutrient-sensing node. We demonstrated that URI is an oncogene inducing nicotinamide adenine dinucleotide (NAD) depletion to induce hepatocellular carcinoma. Further, we developed URI-based fluorescence resonance energy transfer (FRET) probes to screen for new components of the growth factor and nutrient signalling cascade using live-cell imaging.

Microspherele protein 1 (MCRS1). We recently showed that MCRS1, in an amino acid-dependent manner, connects Rheb to mTORC1 activation. MCRS1 depletion inactivates mTORC1.

**Genetically engineered mouse models**

In order to better understand the role and functions of URI and MCRS1 in the liver, pancreas and intestinal disorders, the following GEMMs were generated in our laboratory:

- 2 conditional knock-out mouse models (URI and MCRS1 loss-of-function).
- 3 knock-in mouse models (over-expression of URI (wt), URI (S371A) and MCRS1).

**Biological concepts from our laboratory**

We have proposed the following concepts:

- Oncogene-induced NAD+ depletion results in DNA damage; thus suggesting that metabolic reprogramming initiates tumourigenesis prior to genomic instability.
- Nicotinamide riboside, a vitamin B3 derivative and an NAD+ booster may be used for cancer prevention and treatment.

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### OVERVIEW

The CNIO devotes significant effort to develop innovative mechanism-based therapeutics, thereby providing the development of novel avenues to treat metabolic dysfunctions and cancer.

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**PUBLICATIONS**

- MCRS1 blocks and couples Rheb to amino acid-dependent mTORC1 activation. Dev Cell 33, 1-18.
- Tumor-modified PDXs. Cancer Res 73, 5992-5997.
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**Figure** Strategic development of growth factors and nutrients research at the CNIO: finding new effectors of the growth factor- and nutrient-sensing pathway, validating genomic predictors and oncogenic drivers in early stages of disease development, and generating new mouse models mimicking different steps of human sicknesses and offering new therapeutic strategies to prevent and cure metabolic dysfunctions and cancer.
The standard therapies for GBM patients — ionising radiation (IR) and Temozolomide (TMZ) — generate double-strand DNA breaks (DSBs), which are the most deleterious form of DNA damage. 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 loss of components of a specific DNA repair pathway might be compensated by the increased activity of other components or pathways. Upregulated DNA repair pathways could lead to resistance to radiotherapy and DNA-damaging chemotherapy; therefore, inhibitors of these pathways could potentially increase the sensitivity of the cells to these therapies.

We have performed a series of shRNA-based genetic screenings to identify DDR genes that modulate the response to TMZ in glioma cells. In collaboration with Fátima Al-Shahrour from the CNIO Translational Bioinformatics Unit, we have been able to uncover novel biomarkers of TMZ response in GBM and, more importantly, novel targets to be inhibited in combination with TMZ treatment.

GlioVis, data visualisation tools for glioma datasets

We are currently living in the ‘genomic era’. Scientific literature is flooded with an impressive amount of cancer genetic data: somatic mutations, copy number, gene expression, miRNA expression, DNA methylation, clinical information, etc. Most of the data are available in raw and processed forms; however, the analysis and interpretation of such information requires specialised software and training.

To render the data more accessible for the glioma research community we have developed GlioVis (http://gliovis.bioinfo.cnio.es): a user-friendly web application for data visualisation and analysis to explore brain tumour datasets.

“Most effective treatment for GBM patients at present is a combination of radiotherapy and alkylating agents. Increasing the sensitivity of the tumour cells to these therapies will possibly extend the survival of the patients.”

The most effective treatment for GBM patients at present is a combination of radiotherapy and alkylating agents. Increasing the sensitivity of the tumour cells to these therapies will possibly extend the survival of the patients.
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 as well as 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 cell adhesion and metabolism.
→ Predict the consequences of cancer related alterations; we are focusing on alterations with a compensatory nature (co-evolutionary related mutations) as well as those affecting alternative splicing patterns.
→ Contribute to the analysis of cancer epigenomic and genomic information within the framework of international genome projects.

The Programme is currently composed of 3 Research Groups and 6 Core Units that provide support to the CNIO’s research activities.

Recently, 2 key groups left the CNIO in order to follow their scientific development elsewhere. Francesco Gervasio, an expert in biophysical simulations, went to the University College of London, and Guillermo Montoya, a senior crystallographer, moved to the Novo Nordisk Foundation Center for Protein Research in Copenhagen, while maintaining during 2015 a reduced research activity.

In order to maintain a reasonable level of activity and to provide the necessary support to internal projects, we established 2 new Core Units this year: a Protein Crystallography Unit, which is shared with the CNIO Experimental Therapeutics Programme; and an Electron Microscopy Unit that works in collaboration with the laboratory of O. Llorca (Centro de Investigaciones Biológicas, CIB-CSIC, Madrid), where cell biology samples are prepared for further observation and imaging using our microscopes. These new Units have started their independent work by re-organising the equipment and operational aspects; they have already commenced work with a number of CNIO groups. Moreover, we also share the Translational Bioinformatics Unit, led by Fátima Al-Sharour, with the Clinical Research Programme.

"After a very positive initial period, the Programme is now in a phase of reorganisation, consisting of the renewal of the equipment, the replenishment of its Core Units, and most importantly, the recruitment of new group leaders in Structural and Computational Biology."

Of particular relevance to the CNIO’s activities in Computational Biology, was the finalisation of the negotiation for membership in the European Bioinformatics Infrastructure ELIXIR. ELIXIR is a large international consortium that is now associated to a European grant (EXCELERATE), in which the Spanish participation is led by the CNIO. CNIO’s National Bioinformatics Institute Unit (INB-ISCIII) has played a key role in developing the technical aspects of the Spanish participation in ELIXIR and EXCELERATE.

During 2015, the 3-year extension of the 5+3 contracts of the 2 junior Group Leaders was evaluated by an ad-hoc expert committee and approved by the CNIO. Later in the year, the Programme was reviewed in depth by the CNIO’s External Scientific Advisory Board (SAB). The SAB evaluated the activity of the Programme very positively and endorsed the reorganisation of its Units, for which it made very specific recommendations in regards to the need to focus on the proper equipping of these Units. Very importantly, the SAB strongly recommended the appointment of at least one senior structural biologist. The CNIO has taken these recommendations very seriously, allocating additional funds for the renewal of the equipment of the Units, and has started the search for a senior and a junior structural biologist.
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, where 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 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.

→ Analyse the function, structure and specific interactions of cancer-related proteins.

→ Develop methods, tools and ideas to understand and model processes relating to genome structure, organisation and evolution, with a special focus on tumour progression.

“...This year we carried out a new research effort regarding the analysis of genome/epigenome data, including chromatin 3D structure data, by combining methods for functional genome segmentation with network biology strategies. The results reveal interesting properties of chromatin organisation, including fundamental components for its organisation and dynamics...”

RESEARCH HIGHLIGHTS

The work of our Group can be described in terms of our contributions to 3 large-scale community efforts.

Our Group contributes to the organisation of the data flow and analysis of the Spanish Chronic Lymphocytic Leukaemia (CLL) project, which is part of the International Cancer Genome Consortium (ICGC) that ended in 2015; and to the BLUEPRINT epigenome EU flagship project, which is now entering its final phase of data production. We have participated in the main published studies of the CLL consortium, as well as in the white papers on analysis technology of the ICGC consortium. In 2016, our main efforts will be dedicated to the analysis of the massive amounts of data produced by the largest cancer genome sequencing consortia, ICGC and TCGA, in what is known as PanCancer, as well as to the completion of the integrated analysis of the BLUEPRINT data.

Organisation of chromatin and the interaction between its components

Inspired by the work of the CLL-ICGC and BLUEPRINT projects, we have developed a new framework for the analysis of large scale epigenetic and chromatin capture data. Based on concepts developed in the area of network biology, we have used the available information to reconstruct the network of interactions between a large set of more than 70 chromatin features, including DNA and histone modifications, as well as a large number of chromatin binding proteins. The analysis of the network reveals interesting properties of the components related to their specific functional activity and evolutionary history, including detection of the importance of the 5hmC modification of DNA as a network organiser.
Alternative splicing at the protein level

This year we continued the work on alternative splicing in the context of the NIH-funded GENCODE project. We have produced a new release of the APPRIS system for the systematic annotation of protein isoforms. APPRIS annotates binding sites, evolutionary rates, the existence of protein structures or models, as well as the presence of membrane proteins, and determines the most likely complete isoform for each gene (“Principal Isoform”). APPRIS annotations are part of the information used for the annotation of the human genome.

The Principal Isoform is the only one that has protein-like features, including presence of binding sites, coverage of known protein structures, evolutionary conservation and normal rates of evolution, and it is the genuine representative of the function of the corresponding genes. The systematic analysis of the Principal Isoforms has several interesting consequences.

Firstly, it allows a better definition of the mutations that might be relevant in cancer studies, rejecting others that map outside the boundaries of the Principal Isoforms, and therefore are expected to not have functional consequences at the protein level.

Secondly, we have been able to compare the evolutionary consequences of splicing versus gene duplication by reconstructing the history of a selected set of genes that after duplication have retained 2 very similar paralogs, or have produced 2 splice isoforms differing solely by the presence of membrane proteins. This finding is in clear contradiction with studies that have analysed expression at the level of transcription but, perhaps not surprisingly, the finding seems fit well with the large scale analysis of gene expression performed in multiple tissues, carried out by the ENCODE/GTEX (www.gtexportal.org) consortium.

Thirdly, by combining APPRIS with the systematic exploration of all available large scale MS data sets – carried out in collaboration with the group of Jesus Vazquez (CNIC) – we have demonstrated that the immense majority of genes only express 1 isoform at the protein level, at least for those genes with medium-high levels of expression. This finding is in clear contradiction with studies that have analysed expression at the level of transcription but, perhaps not surprisingly, the finding seems fit well with the large scale analysis of gene expression performed in multiple tissues, carried out by the ENCODE/GTEX (www.gtexportal.org) consortium.

We have produced a new release of the APPRIS system for the systematic annotation of protein isoforms. APPRIS annotates binding sites, evolutionary rates, the existence of protein structures or models, as well as the presence of membrane proteins, and determines the most likely complete isoform for each gene (“Principal Isoform”). APPRIS annotations are part of the information used for the annotation of the human genome.

Text mining is an increasingly important component of computational biology with numerous applications in biology and biomedicine.

As part of the BioCreative international effort for the evaluation of text mining systems, we organised the 2015 competition (Seville, Sept 2015), with a special focus on the extraction of mentions of chemicals (drugs and other compounds), diseases and gene names from patient records. For the competition, we created a large, systematically annotated corpus, as well as the corresponding annotation guidelines and tools to score the results of the teams participating in the competition.

Beyond the BioCreative benchmarking effort, in the context of the IMI eTOX project, we completed the development of a system specialised in the extraction of information related to the toxicology of biological compounds and drugs from scientific publications and toxicity reports. The relationships between administered drugs, genes, proteins and cytotoxic variants are systematically linked to the related databases and underlying text.

We have also completed the development of a text mining system to extract information related to melanoma from the literature. The system systematically explores melanoma related papers to detect mentions of genes, mutations, and drugs, as well as a number of medical/pathology related terms, and exposes the information to the end-users together with facilities for navigating the information in the context of the bulk of information available from the large cancer genome projects.
Macromolecules and their interactions underlie all biological processes and play either, dynamic roles in catalysis or signalling, or static roles in scaffolding or information storage. Our Group focuses on the molecular understanding of the role played by macromolecules involved in oncogenic processes. There is an information gap between our current knowledge and our understanding of the molecular mechanisms that govern the function of different cellular machines. Structural determination reveals an unparalleled view of the design principles of living systems at levels that span from basic mechanistic questions regarding protein function, to the evolutionary relationships between cellular components. To achieve this, our work focuses on the structural and dynamic interactions of these biomolecules and their complexes.

“We have visualised, for the first time, the dynamics of DNA phosphodiester hydrolysis by an endonuclease.”

Guillermo Montoya
Group Leader

Jesús Prieto
Staff Scientist

Rafael A. Molina (until September)
Post-Doctoral Fellow

Igor Yefimenko (until September)
Technician
Structural design of protein-DNA interactions for gene targeting

We have observed, for the first time, the hydrolitic reaction performed by a specific endonuclease on its target DNA; we watched how an endonuclease generates a double-strand break in a DNA molecule following a two-metal ion mechanism. To investigate this process we developed a procedure to slow down the enzymatic reaction. This method allowed us to monitor the kinetics of enzyme catalysis using a time-resolved crystallography approach and the structures of successive reaction intermediates. Thus, we have provided a uniquely detailed view of the dynamic processes of this key biological reaction. Our work interlaced structural and molecular dynamic analyses to dissect the hydrolysis of a phosphohistidine bond, thereby precisely defining the catalytic mechanism of an endonuclease. We have solved more than 150 crystal structures to obtain key snapshots of different catalytic stages, showing the orchestrated conformational changes in the amino acids, nucleotides and metals during catalysis. This work provides the first ‘live’ and visual proof of this key biological mechanism (FIGURE 1).

The telomeric nucleosome

The telomere is a specialised region of the chromatid that contains repetitive DNA sequences. This ‘buffer’ DNA is truncated during chromosome replication and needs to be further expanded by the telomerase. Different proteins and protein-DNA complexes are implicated in the assembly of the supramolecular structure of the telomeric chromatin that caps the telomere end avoiding the activation of the DNA Damage Response and harmful chromosome fusion. One of the essential protein components involved in this assembly is the telomere complex, consisting of different proteins: TRF1, TRF2, TIN2, POT1, TPP1 and RAPI. The current literature contains scarce knowledge of these mechanisms. In this proposal we aim to decipher the molecular basis of telomere capping and telomere organisation (FIGURE 2). These data will help us understand how the loss of telomere protection contributes to genome instability.

Mitotic complexes

Cellular growth and division are regulated by an integrated protein network that ensures the genomic integrity of all eukaryotic cells during mitosis. Microtubules play an important role in several cellular processes, particularly in the formation of the mitotic spindle. The regulation of microtubule dynamics during mitosis is key for spindle formation. Spindle defects, arising from failures in setting up the microtubules, lead to chromosomal instability and aneuploidy, a common cause of tumour development. One of the most effective strategies for cancer treatment so far has been to interfere with the highly dynamic mitotic spindle microtubules; tubulin remains the most successful spindle targeted molecule in cancer. To date, novel anti-mitotic agents have demonstrated limited efficacy in clinical trials and classical anti microtubule drugs are still considered as being the best approach for cancer therapy. We are attempting to dissect the molecular working mechanism of CCT/TRIC, the molecule responsible for the folding of tubulin and actin, which are the essential building blocks of the cytoskeleton. This molecular machine is essential for sister chromatid separation through the folding of key anaphase promoting factor subunits, such as Cdc20. Using a hybrid approach, we are aiming to dissect the molecular recognition of these key substrates by the chaperone.

- **PUBLICATIONS**

- **AWARDS AND RECOGNITION**
  - Co-organiser, EMBO Workshop “Cell division: molecular machineries and cancer targeted therapies” with co-sponsorship by UNIA.
CELL SIGNALLING AND ADHESION JUNIOR GROUP

OVERVIEW

Our Group studies regulatory mechanisms of key signalling switches that control 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 deregulate these events. The structural understanding allows us to design potential anti-cancer therapeutics that interfere with oncogenic deregulation.

RESEARCH HIGHLIGHTS

We focus on mechanisms of growth and adhesion signalling that occur at the plasma membrane and involve specific phosphoinositides. Specifically, we aim to answer the following questions: (i) how does the phosphoinositide phosphatidylinositol 4,5-bisphosphate (PIP₂) activate Focal Adhesion Kinase (FAK), (ii) how does phosphoinositide 3-kinase (PI3K)-generated phosphoinositide 3,4,5-trisphosphate (PIP₃) lead to activation of serine/threonine protein kinase B/Akt (PKB/Akt), and (iii) how are the SH2-domain-containing inositol 5-phosphatases (SHIP) regulated to reduce PIP₃ levels in the plasma membrane.

Regarding Protein Kinase B (PKB), we performed biochemical and cellular studies to understand which regulatory mechanisms control intrinsic activity and which ones regulate selective phosphorylation of specific PKB substrates. We confirmed that PKB phosphorylation is essential for activity, however, surprisingly, we found that membrane targeting, which has been regarded as essential for PKB activity, mainly affects substrate specificity. Furthermore, we showed that in addition to the canonical activation mechanism via PI3K, initial C-terminal phosphorylation and association of PKB with PDK1 in the cytosol can also activate PKB resulting in phosphorylation of different PKB substrates.

SHIP phosphatases remove the 5-phosphate from PIP₂ and thereby, like PTEN, negatively regulate PKB levels. Despite their importance, little is known about mechanisms of SHIP regulation. We recently solved a crystal structure of the catalytic and C2 domains of SHIP2, showing an extensive interface between the 2 domains (FIGURE). Although the C2 domain interacts with the phosphatase domain far from the active site, biochemical studies showed that the C2 interaction greatly enhances the catalytic activity of SHIP2 and, interestingly, affects substrate recognition. We employed molecular dynamics (MD) simulations to guide a mutagenesis study that revealed how the C2 domain, via an allosteric mechanism, affects the dynamics of loops close to the substrate binding site, affecting SHIP2 catalysis.

• PUBLICATIONS

“We elucidated mechanisms by which the SHIP2 inositol phosphatase is regulated to reduce PIP₃ levels. This information can aid in the design of novel small molecules, targeting SHIP2, to reduce oncogenic signals.”
Safeguarding genome integrity is essential for correct cell functioning and for preventing 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 provide further insight into the design of compounds to modulate protein activity, as well as 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. We proposed novel mechanisms of DNA targeting during transposition.”

Revealing the structure and functioning of CAD, a metabolic gatekeeper of cell proliferation

The de novo synthesis of pyrimidines is essential to fuel the high demand of nucleotides during cell proliferation. This pathway is initiated by CAD, a 243 kDa multifunctional polypeptide with 4 enzymatic activities: glutaminase, carbamoyl phosphate synthetase (CPS), aspartate transcarbamoylase (ATC) and dihydroorotase (DHO). CAD also limits the flux through the pathway and its activity is allosterically controlled and it is also modulated by phosphorylation. Despite its central metabolic role and its potential as an anti-tumour target, there is no detailed information about CAD other than that it self-assembles forming a 1.5 MDa complex. We are interested in characterising the architecture of CAD to understand its catalytic and regulatory mechanisms. Giving its large size and modular organisation, we tackle this challenge by combining single-particle EM and X-ray crystallography. Recently, we reported the crystal structure and biochemical characterisation of the DHO domain of human CAD. Now, we have determined the crystal structure of the ATC domain and we are producing larger CAD complexes for EM studies. In addition, we have labelled full-length human CAD with GFP or Cherry to investigate the localisation of CAD in the cell during the cell cycle.

Basic mechanisms of DNA recognition

MuB is an ATP-dependent nonspecific DNA-binding protein that selects the target DNA for transposition. A detailed mechanistic understanding of how MuB juggles the DNA is unknown. We demonstrated that MuB is an AAA+-ATPase that assembles into helical filaments around the DNA, and identified critical residues for the ATPase activity, DNA binding and filament assembly, as well as for the interaction with the transposase. However, the function of a 7 kDa N-terminal domain (NTD) has remained uncharacterised. In collaboration with the CNIO NMR Unit, we have determined the structure of the NTD, which reveals a striking similarity to DNA-binding proteins. We have also demonstrated that the NTD directly mediates the ability of MuB to establish filament–filament interactions. We propose a ‘zippering’ mechanism by which the NTD favours filament clustering and the bridging of distant DNA regions during transposition.

• PUBLICATIONS


• Book Chapter


• AWARDS AND RECOGNITION

- 1st Prize Poster Award, 33rd Congress of the Sociedad Española de Biología Molecular (SEBBM), Francisco del Caño, Araceli Grande-García, Santiago Ramón-Maiques. Subcellular localisation of the multifunctional protein CAD and production of knock-out cell lines by the CRISPR/Cas9 system.
RESEARCH HIGHLIGHTS

Our Unit incorporates a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, afimeter, isothermal titration and differential scanning calorimeters, a circular dichrograph, a multi-angle static light scattering apparatus, analytical ultracentrifugation, and a surface plasmon resonance (SPR) machine. Research Groups mostly from, but not limited to, the Structural Biology and Biocomputing Programme have extensively used these technologies throughout 2015. Two important intra-Programme collaborations carried out this year are: the NMR solution structure determination of the N-terminal domain (NTD) of MuB, an essential protein for DNA transposition specificity (with the Structural Bases of Genome Integrity Group), as illustrated in the FIGURE; and lipid binding studies of different PKB/Akt constructs using SPR (with the Cell Adhesion and Signalling Group).

The Unit also hosts a 700 MHz NMR spectrometer that is well equipped with probes and a sample changer for running up to 120 samples automatically. This provides the required throughput for the screening of small molecule protein binders (with the CNIO's Structural Biology and Biocomputing and Experimental Therapeutics -ETP- Programmes), as well as for metabolomics studies. Furthermore, we use NMR to characterise the metabolic profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples.

“...we quantified abundant cell metabolites, thereby contributing to the understanding of the metabolic response to chemotherapeutic drugs causing mitotic arrest and the metabolic plasticity of pancreatic cancer stem cells, which are crucial aspects of tumour treatment and biology.”
In collaboration with the laboratory of C. Heeschen (Barts Cancer Institute, London), we helped to unveil specific metabolic features of pancreatic cancer stem cells (CSCs) (Sancho et al. 2015), and also to describe the pancreatic ductal adenocarcinoma (PDAC) microenvironment in order to better understand its biology (Sainz et al. 2015). Our long-standing collaboration with CNIO’s Chromosome Dynamics Group (A. Losada) yielded interesting insights into cohesin’s contribution to the establishment of tissue-specific transcriptional programmes, by jointly interpreting genome-wide cohesin distribution, gene expression and chromatin architecture in the cerebral cortex and pancreas of adult mice (Cuadrado et al. 2015).

Other bioinformatics analyses were performed together with M. Serrano’s laboratory (CNIO) (Morgado-Palacin et al. 2015, Pallà et al. 2015). J. Benitez (CNIO) (Matamala et al. 2015, Vlačlová et al. 2015), and A. Muñoz (IIB) ( Aguilera et al. 2015).

We helped the Confocal Microscopy Core Unit (CNIO) to design and implement iMSRC, a new software tool that converts a conventional automated microscope into an intelligent screening platform (Carr et al. 2015). In collaboration with D. Glez-Peña (University of Vigo) we published miGate (Andres Leon et al. 2015), a curated database of miRNA-mRNA targets with more than 125 million predictions on a consistent sequence space.

Another genomic resource for the UBC-40 urothelial bladder cancer cell line (Earl et al. 2015) was released in collaboration with FX. Real’s laboratory (CNIO). Previously published works have allowed us to deliver additional data and protocols as genomic data resources (Tanic et al. 2015, Foronda et al. 2015).
The Unit has contributed to the creation of a text-mining infrastructure that identifies, for a large number of compounds, the associated toxicological effects based on the evidences extracted from several literature corpus.

- Coordinate the activities of the Institute.
- Design (with the support of all the nodes) the INB scientific/technical programme and to ensure its execution.
- Design (with the support of all the nodes) the INB training programme.
- Coordinate the participation of Spain in ELIXIR.
- Mediate the collaboration between the INB and third parties including National and International research consortia, other infrastructures, SMEs and the Industry.

The INB Unit differs from the other Units in the Structural Biology and Bioinformatics Programme in the sense that its offering is not restricted to the CNIO Groups, and that its budget is funded entirely by an external agency, the ISCIII.

RESEARCH HIGHLIGHTS

The INB service offering is distributed amongst three horizontal Work Packages (WP) as well as the Training WP. These services are integrated into six major research areas: Health, Genomics, Functional Genomics, Structural Biology, Biological Networks and Data mining. Besides from its coordination role, the Unit also participates in the delivery of the services.

Data resources and Bio-computing

The storage and processing of data have become fundamental tasks for 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://blueprint-dev.bioinfo.cnio.es/). BLUEPRINT is a high impact FP7 project aimed at producing a blueprint of haemopoetic epigenomes. In the current version, the data portal provides an epigenomic analysis obtained from 439 samples to the scientific community. Their associated epigenomes are characterised by: gene and transcript expression (from RNA-Seq experiments), hyper and hypo methylated regions (derived from WGBS experiments), chromatin accessibility (DNase-Seq), and 7 Histone marks binding activity (ChIP-Seq).

Infrastructure development

Within the infrastructure development, special attention is paid to the text-mining infrastructure for the processing of biomedical texts. The LiMTox system (http://limtox.bioinfo.cnio.es/) is the first text mining approach that extracts associations between compounds and a particular toxicological end point at various levels of granularity and evidence types, all inspired by the content of toxicology reports.

End users applications and services

The Unit actively contributes to the creation of an integrated platform that connects databases, registries, bioinformatics and clinical bioinformatics for research on rare diseases.

The application allows clinicians and researchers to explore the possible causes of the disease by combining patient's genomic data with phenotypic annotations. The Unit has also developed APPRIS (http://appris.bioinfo.cnio.es/), a service that automatically annotates genes and transcripts. The GENCODE consortium uses APPRIS to annotate the principal isoforms of several species.

OVERVIEW

The Electron Microscopy (EM) Unit is a research laboratory and a central core facility that provides CNIO researchers, and the wider research community, with access to Transmission Electron Microscopy, as well as supplying expertise in EM image analysis. As a core facility, we offer standard specimen preparation techniques for proteins and protein complexes, data collection and data processing tailored to the specific needs of the users.

We also provide training for regular users on the use of equipment, as well as guidance regarding specimen preparation. For cell biology samples, we have established collaboration with the Centre de Investigaciones Biológicas (CIB-CSIC, Madrid), where the samples are prepared for further observation and imaging with our microscope.

RESEARCH HIGHLIGHTS

The Electron Microscopy Unit is a research facility that provides support for biological science projects at scales ranging from the cellular level to the macromolecular complex level. The Electron Microscopy Unit implements sample preparation protocols and data collection methods, as well as performing 2D and 3D data processing.

In collaboration with the CNIO Cell Signalling and Adhesion Group (Structural Biology and Biocomputing Programme) we demonstrated that PI(4,5)P₂ induces Focal Adhesion Kinase (FAK) clustering at the cell membrane. We are currently employing 2D electron crystallography to elucidate the arrangement of the FAK protein and its conformation within the clusters. We have obtained preliminary 2D crystals of the 4.1-ezrin-radixin-moesin (FERM)+kinase domain of FAK on a lipid monolayer.

In collaboration with the CNIO Telomeres and Telomerase Group (Molecular Oncology Programme) we are pursuing the structural characterisation of TRF1, a central component of the shelterin complex, which is known to protect mammalian telomeres and regulate telomerase activity. We have obtained negative-staining images of the purified TRF1 oligomer. 2D averages and the preliminary 3D structure indicate that TRF1 forms dimers, as previously suggested. Furthermore, in collaboration with the CNIO Melanoma Group (Molecular Oncology Programme), we have developed a protocol that enables, via electron microscopy, the visualisation of different extracellular vesicles produced by melanoma cell lines.

Additionally, under the framework of a recently established agreement with the CIB-CSIC and in line with the needs of CNIO’s researchers, we also provide ultrastructural analysis and immunodetection by EM at the cellular level. Thus, cell and tissue samples that are prepared by the Electron Microscopy Facility at the CIB-CSIC can be visualised and analysed at the CNIO.

“PIKKs are attractive targets for anti-cancer therapies as they regulate their kinase activity in response to diverse stimuli. In collaboration with Óscar Llorca from CIB-CSIC, we have summarised the findings on the structure of PIKKs in a review article.”

Figure 2D electron crystallography of Focal Adhesion Kinase (FAK) clusters. (A) Schematic representation of the FAK cluster. (B) Preliminary 2D crystals of FERM+kinase domains.

PUBLICATIONS

The aim of the Crystallography Unit is to provide the CNIO Research Groups with a three-dimensional characterisation of the structure of biological macromolecules at high-resolution (X-ray crystallography) and low-resolution (SAXS). The knowledge of the 3D structure of proteins and protein complexes is essential for understanding their function in cellular processes. The crystal structures give us a picture – at atomic resolution – of the protein. With this knowledge, we know where to introduce mutations that can alter (or improve) the specificity of the protein and its affinity to other molecules. In turn, this can lead to the design of drugs to block or control the activity of proteins involved in disease. Small-angle X-ray scattering (SAXS) is a complementary technique to X-ray crystallography. It permits delineation of the dynamic changes in shape and size undergone by the proteins in solution, giving a structural picture of the thermodynamic behaviour of these biological molecules, including changes induced upon ligand binding.

This Unit is shared between the Structural Biology and Biocomputing Programme and the Experimental Therapeutics Programme.

RESEARCH HIGHLIGHTS

The Crystallography Unit began its journey in February 2015, with the aim of providing state-of-the-art, high-throughput protein crystallization, X-ray crystallography and SAXS services to meet the demands of the Research Groups at the CNIO and special collaborative efforts outside our institute.

The full-service Unit provides access to sophisticated equipment and technologies, including the European synchrotron light sources. We also offer consultancies, guidance, and technical assistance at every stage of the 3D structure determination process. Non-crystallography groups benefit from expert aid in the use of existing 3D structures for the design and interpretation of experiments, including the possibility to crystallize their target proteins in the presence of inhibitors, for structure-based drug design.

Since its formation, the Unit works in close collaboration on the drug discovery projects led by the Experimental Therapeutics Programme. We also run a number of collaborations with different Groups at the CNIO involved in the following Programmes: Molecular Oncology (Telomeres and Telomerase and Brain Metastasis Groups), Clinical Research (Gastrointestinal Cancer Clinical Research Unit), and Cancer Cell Biology (Epithelial Carcinogenesis Group). Additionally, the Unit has initiated external collaborations with the Physical Chemistry Department (University of Granada), the Environmental Biology Department (CB-CSIC), and the Pharmacology and Therapeutics Department (Roswell Park Cancer Institute, USA).

“"Our goal is to provide CNIO investigators with 3D structural information of their target macromolecules to understand the mechanism(s) that regulate their biological functions, including their modulation by novel therapeutic ligands.""
Vice-Direction of Translational Research

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The activity and impact of CNIO’s scientists in the field of translational cancer research continues to be outstanding. While the contribution of the Centre towards making discoveries in basic science is undoubtedly critical, the resolution to making these discoveries more clinically applicable is fundamental. In 2015, several important advances in translational studies have taken place at the CNIO, ranging from studies of cancer risk factors, biomarker discovery, to the advanced preclinical testing of new treatments and clinical trials. A key aspect is the expanding portfolio of ongoing clinical trials conducted in collaboration with hospitals in the Community of Madrid, other Regional Communities and abroad.
The Human Cancer Genetics Programme is currently composed of 3 Research Groups: Human Genetics, Endocrine Cancer, and Genetic and Molecular Epidemiology Groups, and 3 Units: Human Genotyping-CEGEN, Molecular Cytogenetics and Genome Editing, and the Familial Cancer Clinical Unit. In addition, the Programme includes a Familial Cancer Consultancy for the evaluation of cancer families and the selection of appropriate candidates for genetic studies in order to perform a correct diagnosis and to provide genetic counselling. The Consultancy is located at the Hospital Universitario de Fuenlabrada and works in close collaboration with the Hospital’s Oncology Service. This year, we have doubled the number of consultancy days undertaken due to the increase in the number of families who attended for genetic counselling; 320 families versus 180 in 2014. This increase in families has led to a higher number of genetic and genomic diagnosis studies being possible with the incorporation of a massive sequencing platform in the Programme.

The core goals of the Programme are geared towards research, training and diagnosis. Our main interest in clinical diagnosis is based on the genetic characterisation of families with cancer. The Programme’s research priorities are the genetic and cytogenetic study of tumours, the search for diagnostic and prognostic markers, as well as the discovery of novel cancer-related genes. These research activities are complemented by another area of work that studies the genetic and environmental factors that confer cancer susceptibility and modulate drug response (pharmacogenetics). 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 training and education. During this year, the groups in the Programme hosted 6 resident physicians from different hospitals in Spain for 3-month periods. Furthermore, we offer professionals from different international research centres the opportunity to join us either as visitors, or for a short training of 1-3 months (a total of 6 international visitors from Europe (3) and Latin America (3) were hosted in 2015).

In terms of education, since the beginning of 2015, 1 foreign Erasmus Master’s student and 8 national and 5 international PhD students worked on their research projects; 4 of them have already successfully defended their thesis.

We participate in many international and national Consortia, which enables us to apply for international projects, hold international meetings and publish in the best journals. This year we were awarded a COST Action and a European project. Likewise, a good collaboration with other CNIO Groups and Units is one of our main characteristics, enabling us to benefit from the valuable internal feedback generated by people, techniques, technology and knowledge.

Milestones and major achievements of the Programme:

- The co-organisation of the European Pancreatic Club.
- The organisation of the VII Congress of the Spanish Society of Pharmacogenetics and Pharmacogenomics.
- The identification of 3 new genes responsible for families with rare cancers.

“Genetics, lifestyle, exogenous factors... one cause alone is unlikely to lead to cancer; it takes all of them to cause cancer. It is a difficult task to decipher the weight that each factor carries, living in our ‘contaminated first world’. However, that’s exactly what our work aims to uncover.”

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- The organisation of the VII Congress of the Spanish Society of Pharmacogenetics and Pharmacogenomics.
- The identification of 3 new genes responsible for families with rare cancers.
OVERVIEW

The Human Genetics Group has been working on research aiming to better understand the genetic bases of familial cancer for several years. Our main objective is to translate our discoveries into clinical practice. We continue working on our Familial Cancer Exome Project, which aims to identify new high-susceptibility genes, via whole exome sequencing, in order to explain some families with infrequent tumours. Similarly, we are interested in the identification of modifier genes that modulate factors such as age of onset, disease evolution and cancer risk. Finally, we are gaining further insight into the role of miRNAs as early diagnostic biomarkers of breast cancer.

Our strategic goals are:

→ To better define the genetic landscape of familial breast and ovarian cancer.
→ To discover new genetic markers associated with diagnosis and prognosis.
→ To improve our knowledge of families with rare or infrequent cancers by using massive sequencing.

“We have discovered two high-susceptibility genes that explain two different types of rare familial tumours, and we have advanced in the identification of genetic risk factors for breast and high-grade ovarian tumours.”
Breast cancer

We previously identified an SNP in the OGG1 gene that conferred a higher susceptibility to ovarian cancer in carriers of the BRCA1 gene (HR 1.12, p= 4.8x10^{-7}). This gene is related to oxidative stress. We have now demonstrated that carriers of this SNP and the BRCA1/2 mutation have higher expression levels and shorter telomeres than non SNP carriers, suggesting a higher genomic instability in the carriers, which would explain their higher risk of cancer.

We have found that normal cells derived from heterozygous BRCA1 mutation carriers are halpo-insufficient for DNA repair. Moreover, we have demonstrated that certain missense mutations in BRCA1 seem to make the cells more sensitive to Poly (ADP-ribose) Polymerase (PARP) inhibitors than those mutations that give rise to the absence of the protein (frameshift mutations). We are currently investigating the mechanisms underlying these differences with the aim of identifying new markers of sensitivity or resistance to these agents.

Ovarian cancer

By using array-comparative genomic hybridisation, we have defined and validated a region of genomic loss at 6q24-26 that is associated with an improved outcome in patients with high-grade serous ovarian cancer (HGSOC). We have selected several candidate genes in the region and have performed functional analyses in cell models as well as survival association analyses. We have found that low expression of the NEU-related gene GTF2H5 is associated with a better prognosis in HGSOC patients and may also be predictive of the response to platinum-based chemotherapy.

Familial cancer exome project

There are a number of families that have rare or infrequent cancers with an unknown genetic base. We have started a massive sequencing project with the objective of identifying some of these high-susceptibility genes. In 2014, we discovered that the ATP4a gene was responsible for type I gastric carcinoid. In collaboration with S. Orgaz’s Transgenic Mice Core Unit, we generated a knock-in mouse model to further the pathophysiological evolution of gastric carcinoid. We generated heterozygous and homozygous (H1e) mice for the ATP4a mutation and demonstrated that H1e mice mimic the human biochemical and pathological alterations, however, they only get the preneoplastic step (dysplasia). We have also demonstrated that by adding hydrochloric (HCl) at low concentrations (3%) to the water, it is possible to prevent and/or recover the ‘normal clinical, morphological and analytic conditions’ of the stomach (FIGURE).
Our Group is interested in identifying high and low genetic risk factors involved in endocrine tumour susceptibility. To this end, we analyse tumour samples and look for differences between genomic features according to the different individual genetic backgrounds. Such comprehensive characterisation allows us, not only to define diagnostic and prognostic markers associated with primary mutations, but also to pinpoint specific altered pathways that can lead to the identification of future therapeutic targets.

We are also interested in defining genetic markers associated with differences in anticancer drug response and toxicity. We are applying a candidate gene approach, as well as whole genome association studies, to a large series of biological material with associated data, in order to identify therapeutic interventions and other clinically relevant outcome variables. These efforts will collectively improve the diagnosis, prognosis and treatment of patients.

“We proposed a methylome profile to stratify phaeocromocytoma patients according to their metastasis risk, identified miRs that act as ‘master regulators’ of thyroid transformation, and proved that EPHAs play a key role in chemotherapy-induced neuropathy.”
DNA methylation profiling in phaeochromocytoma and paraganglioma reveals diagnostic and prognostic markers

Phaeochromocytoma and paraganglioma (PPGLs) are rare tumours that often present highly variable, post-operative management challenges. The current diagnostic and therapeutic strategies are limited, and PPGL patients may benefit from more effective management and targeted therapies. DNA methylation profiling data from 2 large, well-characterised discovery and primary validation series of tumours (FIGURE). By integrating these miRNA data with gene expression data, we were able to identify target genes for these key miRNAs, which is a novel finding for thyroid pathologies. Furthermore, based on an analysis of clinical follow-up information, we propose a prediction model for disease relapse based on the expression of 2 miRNAs (miR-192 and let-7a) and clinicopathological features. On the whole, our Group has contributed with a comprehensive and clinically relevant characterisation of oncogenic and parathyroid factors for the neuropathy (rs7349683: HR=2.3, P=0.007; rs301927: HR=1.9, P=0.012, respectively). From a biological perspective, Eph receptors represent a family of receptor kinases, involved in axon guidance and other neural-related functions. Furthermore, because Eph proteins mediate neural injury repair, these polymorphisms could act as broad-spectrum neuropathic drug targets relevant for many neuropathic disorders.

Polymorphisms in ephrin type A receptor (EphA) genes are potential markers for taxane-resistant neuroendocrine neoplasms

Improving the quality of life for cancer patients is of enormous clinical and social relevance. In this regard, the peripheral neuropathy induced by anti-cancer drugs, can result in symptoms and disabilities in up to 40% of cancer patients as a consequence of the chemotherapeutic drugs and also the taxane-induced neuropathy. Therefore, the identification of efficacious therapies for these drugs, and diminishes the quality of life of the patients, sometimes permanently. In a previous GWAS analysis, we proposed that genetic variants in the EphA genes were important factors influencing taxane-induced neuroendocytotoxicity. To follow-up on our initial results, we analysed data from patients treated with first-line paclitaxel and with exceptional neuroendocrine neoplasms, data recorded by cycle. Polymorphisms in EPHA5, EPHA6 and EPHA8 were confirmed as risk factors for the neuropathy (rs7349683: HR=2.3, P=0.007; rs301927: HR=1.9, P=0.012).

**Figure DNA methylation patterns associated with metastatic PPGLs.** (A) Ouletwood test provides a genome-wide scan for chromosomal hotspots. Heatmaps show methylation levels for 52 confirmed CpGs associated with metastatic PGL. (B) miRNA expression in discovery series (VS) tumours without metastases in red or blue, respectively. Hyper- and hypomethylated CpGs are indicated in red or blue, respectively.
The scope of the research carried out by our Group ranges from the identification of aetiological agents and mechanisms, to the translation of the findings into the clinical and public health domains, focusing on bladder, pancreatic, and breast cancers. We employ a wide variety of biomarkers to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. Omics data provide a unique opportunity in this regard and the Group explores its integration in epidemiologic studies.

The strategic goals of the Group are to:

→ Identify non-genetic and genetic factors, as well as their interactions, associated with cancer development and progression and with their 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 developed and applied statistical approaches to integrate omics data, predict cancer risk and outcome and to identify the role of both genetic and non-genetic factors in cancer development and progression.”
During 2015, the Group has mainly focused its research on pancreatic and bladder cancers.

In pancreatic cancer (PC), we have substantially contributed to the field by characterising the reverse association between atopic disease and risk of PC, analysing data from the PanGenEU study. A meta-analysis, including our own study, concluded on the protective effect of asthma in PC development (FIGURE 2). Aims of the study included the identification of relationships at the whole-genome level, providing some new biological insights and highlighting the importance of integrating omics data (FIGURE 2). Furthermore, we developed a permutation-based method to concomitantly assess significance and to correct by multiple testing with the MacT algorithm, and applied it with penalised regression methods (LASSO and ENET) when exploring relationships between different genetic variants, DNA methylation and gene expression measured in bladder tumour samples. In addition, Bayesian-based approaches are being explored for predicting BC outcomes and using whole exome and genome sequencing data.

We coordinate and lead certain projects of the COST Action BM1204 EU. Pancreatic Cancer (www.eupancreas.com). This Action includes 200 multidisciplinary members from 22 EU countries, EU governmental and nongovernmental institutions, as well as private companies. Several scientific, training, and dissemination activities have been conducted during 2015. By endorsing the European Multi-stakeholder Platform on Pancreatic Cancer, we have actively participated in several activities aimed at increasing PC awareness in the general population, the medical community and among health policy makers, as well as in setting a European-based clinical registry of PC (PanCreOS). A White Paper on PC has been jointly written with the European Alliance of Personalised Medicine and has been distributed among European Parliamentarians.

**Methodological contributions**

By adopting an integrative research approach, we participate in large, international multidisciplinary studies requiring the development of methodological innovations in all aspects of epidemiology. In this regard, we have proposed an integrative framework that allows the identification of relationships at the whole-genome level, providing some new biological insights and highlighting the importance of integrating omics data (FIGURE 2). Furthermore, we developed a permutation-based method to concomitantly assess significance and to correct by multiple testing with the MacT algorithm, and applied it with penalised regression methods (LASSO and ENET) when exploring relationships between different genetic variants, DNA methylation and gene expression measured in bladder tumour samples. In addition, Bayesian-based approaches are being explored for predicting BC outcomes and using whole exome and genome sequencing data.

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Genetic susceptibility plays an important role in several types of cancer. The identification of mutations in genes that predispose to cancer has clinical utility for patient management. Furthermore, a better knowledge of these genes will improve our understanding of the molecular pathways involved in cancer initiation.

The Familial Cancer Clinical Unit (FCCU) aims to identify genetic alterations that confer cancer susceptibility. Patients with hereditary cancer syndromes carry constitutionally rare alterations in high-penetration genes. The most widely known examples are mutations in mismatch repair genes (in high-penetrance genes. The most widely known examples are mutations in mismatch repair genes (in high-penetrance genes.

The FCCU visits patients with suspected genetic susceptibility to cancer at our consultation at HUF. The referral system for the HUF area was clearly established during 2015. Discussions of protocols and clinical guidelines are held in monthly sessions conducted by the hospital’s Hereditary Cancer Clinical Committee, which was created in March 2015.

At this location the Cpg Island Methylator Phenotype (CIMP) is predominant and Chromosomal Instability (CI) is rare. Left-sided EOCC appears as a transitional or intermediate location, except for CI tumours that seem to predominate at this location. Finally, rectal EOCC shows microsatellite stability, low CIMP, and low CI, possibly in relation with MACS (Microsatellite and Chromosomal Stable) tumours (FIGURE).

Establishing relationships with cancer patient associations is one of FCCU’s main goals. During 2015, the FCCU strengthened its relationships with ASACO (Asociación de Afectados por Cáncer de Ovario) and AEAS (Asociación de Afectados por Sarcomas). Members of ASACO are regularly evaluated in the FCCU consultancy for genetic counselling. We also participated in the ASACO annual meeting last May. In addition, we have started a specific collaboration with ASACO members focused on the evaluation of familial antecedents of cancer. A survey was distributed among the members during the first semester of 2015 and the global results were presented in the ASACO annual meeting – hosted by the CNIO – that took place last September.

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MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

Juan C. Cigudosa
Unit Head

Sandra Rodríguez
Staff Scientist

Technicians
Angelo Bertini (since May) (TS)*, M. Carmen Carrión, Luis Espinosa (until April) (TS)*, M. Carmen Martín, Francisco J. Moya (since June) (TS)*

*Titulado Superior (Advanced Degree)

OVERVIEW

Improvements in whole-genome sequencing have generated abundant data from cancer cells, revealing the complexity of the cancer genome, which undergoes small changes in the DNA sequence as well as structural rearrangements (translocations, inversions, deletions and amplifications). We study the role of acquired chromosomal abnormalities in cancer through: (1) the molecular characterisation of genetic and cytogenetic markers, and (2) the design of human stem cell models carrying chromosome rearrangements. The Molecular Cytogenetics and Genome Editing Unit is a state-of-the-art facility that performs customised conventional and molecular cytogenetic analyses of cells from human and animal sources. Our experience with the versatile CRISPR-Cas9 genome editing technology enables us to engineer human cells to recapitulate cancer genome alterations. The Unit also participates in collaborative projects with clinical and basic science investigators across the CNIO and other institutions.

“We identify aberrant genomic profiles in leukaemias and sarcomas, opening up new avenues for targeted therapies. By genome engineering, we create human stem cell models with unique genetic events, thereby providing invaluable tools for future basic and clinical research.”

RESEARCH HIGHLIGHTS

Optimising CRISPR technology to model human cancer aberrations in primary cells

Methodologies to reproduce tumour-associated chromosome aberrations are necessary to perform the functional identification and detailed characterisation of cancer genes. We have developed an efficient strategy, based on the CRISPR genome editing system, to generate human cell models bearing tumour-specific chromosomal alterations (Nat Commun 2014), demonstrating that the in vitro modelling of tumour-initiating cells is feasible with CRISPR. We have optimised our CRISPR protocol to increase the efficiency of engineered chromosome aberrations in poorly transfectable human primary cells, reducing the labour and costs associated with single cell cloning and genotyping after cell modelling.

From the patient chromosome translocations to the human haematopoietic progenitor (hHSC) cell models

We have genetically engineered 2 human hHSC models to carry novel fusion genes: i) a RUNX1 truncated protein as a result of the t(1;21)(p13;q22) translocation identified in a leukaemia patient – this model demonstrates that C-terminally truncated RUNX1 proteins can contribute to leukaemogenesis in a similar way to the RUNX1-ETO fusion gene; ii) an hHSC model bearing the NUP98/HOXA9 fusion gene that has allowed deciphering its leukaemogenic molecular mechanisms, opening up new therapeutic possibilities.

Technological and translational activities

Our Unit provides state-of-the-art molecular cytogenetic and genome editing services. We are developing various techniques that may provide more sensitive and accurate tools to analyse cancer cells, such as in situ analysis of RNA expression by FISH analysis, and implementing the use of CRISPR libraries to perform high-throughput functional analysis for the identification of cooperative driver genes essential for cancer development. In 2015, we carried out over 1,500 assays for experimental and clinically-oriented projects.

* Awards and Recognition
- Nominated Member of the Scientific Committee of the Fundación Leucemia y Linfoma: a charitable organisation that provides funds for research and educational projects within the field of oncological disorders.
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 us to identify genes underlying complex diseases, such as cancer, and drug response. In this Unit we implement different high-throughput and cost-effective methods to measure from one, to millions of SNV and CNVs. In addition, epigenetic studies using whole-genome methylation arrays are performed in the Unit. Complementarily, research focused on the identification of predictive biomarkers for personalised cancer therapy is also undertaken.

Identification of rare variants associated with capcetibina-induced hand-foot syndrome (HFS)

Capcetibina (Xeloda) is an oral prodrug of 5-fluorouracil (5-FU) that is used in the standard treatment of breast cancer and colorectal cancer. One of the most relevant dose-limiting adverse effects of capcetibina is HFS, characterised by redness, tenderness, and peeling of the palms and soles. With this study we aimed to identify genetic variants associated with HFS. Using the Illumina HumanExome Beadchip, we investigated 239,800 variants across the genome in 106 Spanish and UK patients suffering from extreme HFS toxicity grades (grade 0 vs. grades 3 & 4) and diagnosed with breast and colorectal cancer. We also found a new intronic variant in ENOSF1 not previously associated with HFS (OR=0.61, p=6.681x10-05). Recently, polymorphisms in ENOSF1 have been reported to be associated with capcetibina-related severe toxicity, mainly HFS. Remarkably, ENOSF1 is one of the main targets of 5-FU expression through degradation of TYMS mRNA via an antisense mechanism.

Replication analysis in Ewing’s sarcoma (ES) survival genes

Ewing sarcoma (ES) is relatively uncommon, despite being the second most frequent primary malignant bone tumour in children and adolescents after osteosarcoma. Despite considerable progress made during the past decades, many individuals still relapse or suffer from adverse drug reactions; this has motivated the search for predictive factors. We selected 24 genes reported to be involved in the biotransformation of the mediators used as the standard chemotherapy regimen for ES, and a total of 384 SNPs were selected across these candidate genes. We identified 3 SNPs in the Spanish population –rs7919447, rs4487373 and rs11881477, located in the ARCC6, ARCB1 and CTPS1 genes respectively - that are significantly associated with overall survival. These associations were confirmed in a large, independent replication cohort of 495 patients from 5 European countries.

Further explored the joint effects of common and low-frequency variants (MAF <5%) on AIC, taking into account genes with at least 3 variants that were genotyped using a gene-based test (SKAT). We identified a protein-coupled receptor (p=1.70x10-4) as the most strongly associated gene. This study demonstrates that both single-variant and gene-based tests – taking into account the joint effects of common and low-frequency variants - can elucidate genetic markers predictive of AIC in children.

Identification of rare variants associated with capcetibina-induced hand-foot syndrome (HFS)
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 3 support Units. The Gastrointestinal Cancer CRU, led by Manuel Hidalgo, 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, and 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 Par-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, focuses on the implementation of molecular markers in clinical trials, and the Clinical Trials Management Unit coordinates our clinical trials activities.

Finally, the Translational Bioinformatics Unit, led by Fátima Al-Shahrour, works on applying knowledge of cancer genetics to patient care.

In 2015, we continued the expansion of our clinical trials activities in collaboration with several hospitals in Spain. Operational units at the Hospital Universitario de Fuenlabrada, Hospital de Madrid, Fundación Jiménez Díaz, Hospital Ramón y Cajal, Hospital Niño Jesús, Hospital “12 de Octubre” and the Hospital Virgen de la Victoria in Malaga, have treated over 350 patients in early clinical trials this year. We have also established multicentre clinical trials in breast, prostate and pancreatic cancers that involve the participation of several Spanish hospitals. Finally, we launched the ‘Avatar’ Clinical Trial aimed at personalising the treatment of patients with pancreatic cancer.

“We focus on developing novel and more effective treatments against cancer.”
The Gastrointestinal (GI) Cancer Clinical Research Unit focuses on the clinical development of novel therapeutic strategies for patients with cancers of the gastrointestinal tract as well as personalized medicine approaches for these patients. Our work combines the preclinical assessment of novel anticancer agents in 'Avatar' mouse models with the design, conduct, and analysis of clinical trials with novel anticancer agents in patients with gastrointestinal tumours. Over the last few years we have implemented a growing portfolio of clinical trials with new agents spanning a broad range of mechanisms of action. An important development in this area has been the recent report that nab-paclitaxel – an agent that we helped to develop – has demonstrated improved survival in patients with pancreatic cancer; this has led to the approval of the drug to treat this disease. Our Group has demonstrated that SPARC expression is not a predictor of nab-paclitaxel activity.

Key to our work is the development and characterisation of Avatar mouse models for drug screening, biomarker development, and personalised medicine. We have developed and have characterized the largest collection of these models in pancreatic cancer. We use the Avatar models in 3 critical applications: (i) the screening for immunotherapy studies. Biological studies. Examples of some of the most recent agents that we have tested include the PanHer inhibitor SYM013 – which has been shown to have activity in KRAS mutant p53 WT tumours – and Palbociclib, an inhibitor of CDK4/6 that targets p16 defective tumours, and phase 1 studies of palbociclib, a CDK4/6 inhibitor that has substantial preclinical activity in mouse models. (ii) The avatar models with the design, conduction, and analysis of clinical trials with novel anticancer agents in patients with gastrointestinal ORIGIN<vector-1000x0.png>

In 2015, we initiated phase 2 clinical trials with demcizumab, a new drug that targets cancer stem cells in KRAS mutant tumours, and phase 1 studies of palbociclib, a CDK4/6 inhibitor that has substantial preclinical activity in mouse models. (iii) Finally, we are using the Avatar models for personalized cancer treatment integrated with next generation sequencing. We have initiated several projects, the generation of 2D and 3D models, using Zebrafish to generate ZvATAR models, and the generation of Avatar models suitable for immunotherapy studies.

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novel anticancer agents, spanning a wide range of mechanisms of action, such as signalling inhibitors. Notch inhibitors, stromadirected agents and conventional chemotherapy. More recently we have been involved in studies with immune targeting agents and oncolytic adenoviruses. These studies include first-in-class/first-in-human clinical trials and analyses of clinically important biomarkers, as well as co-clinical studies in mouse models. We are particularly interested in building upon the national pan-cancer clinical registries that we have developed. To build on positive results obtained in both preclinical and phase 1 clinical studies, we are now leading a multicentre phase 2 study of demcizumab in patients with advanced PDA. Furthermore, we have initiated the phase I development of the CDRX 4/6 inhibitor palboicitab in combination with nab-paclitaxel in patients with advanced PDA. In addition, we continue to test multiple new agents, alone and in combination, in animal models of PDA in preclinical studies. The overarching goal is to identify agents that warrant pan-cancer clinical studies. In order to increase the number of agents that can be tested as well as to explore combinations and perform more studies in a rapid and efficient manner, we have developed 2D and 3D screening platforms, as well as a new strategy to use Zebrafish (ZaVATARS) to this end.

Personalized treatment of pancreatic cancer

Our goal in this area is to implement an integrated approach that combines next generation sequencing with Avatar mouse model development. In pilot study, we performed whole-exome sequencing analyses in 25 patients with advanced solid tumours in order to identify putatively actionable tumour-specific genomic alterations. Avatar models were used as an in vivo platform to test proposed treatment strategies. A total of 15 patients received a personalized treatment, of which 6 achieved durable remissions. Based on these results we launched the Avatar clinical trial, in which we developed with advanced PDA are randomised to either a standard of care approach or a personalised approach in which we perform a tumour biopsy followed by exome analysis and generate an Avatar model to experimentally test treatment options resulting from the genetic analysis. With funding from a European Research Council (ERC) Advanced Grant, we are now launching a multicentre randomised study in collaboration with hospitals in Madrid. This unique clinical trial will provide an important collection of biomaterials and a prospective clinical follow-up of patients with pancreatic cancer. Furthermore, in regards to colorectal cancer (CRC), we have conducted a clinical trial in the first-line setting to show that the monitoring of RAS mutation status in circulating cell-free DNA (cfDNA) predicts patient outcome (Figure 2B).

**PUBLICATIONS**

- Mire, Loitz, A, Saria A, Karmo-Stiles S, Lozzi M, Antonio B, Azevedo MM, Cioffi M, Tatari M, Miram- da-Lorenzo I, Hidalgo M, Gomez-Lopez G, Zebrafish. (2015). A Cancer Research Platform to Test Proposed Treatment Strategies. A total of 15 patients received a personalized treatment, of which 6 achieved durable remissions. Based on these results we launched the Avatar clinical trial, in which we developed with advanced PDA are randomised to either a standard of care approach or a personalised approach in which we perform a tumour biopsy followed by exome analysis and generate an Avatar model to experimentally test treatment options resulting from the genetic analysis. With funding from a European Research Council (ERC) Advanced Grant, we are now launching a multicentre randomised study in collaboration with hospitals in Madrid. This unique clinical trial will provide an important collection of biomaterials and a prospective clinical follow-up of patients with pancreatic cancer. Furthermore, in regards to colorectal cancer (CRC), we have conducted a clinical trial in the first-line setting to show that the monitoring of RAS mutation status in circulating cell-free DNA (cfDNA) predicts patient outcome (Figure 2B).
The Breast Cancer Clinical Research Unit (BCCRU) focuses on the translational interface of therapeutic development. Breast cancer is a heterogeneous disease, and thus, there are large inter-patient variations in terms of disease course, prognosis, relapse and resistance to conventional or targeted therapeutics. Our activities are directed towards personalised treatment, and range from preclinical models to the sponsoring of multicentric clinical trials. Specifically, our research areas are:

- Discovery of new targets for breast cancer prevention: 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.

**OVERVIEW**

“This year, we completed a taxonomic project that enables the detection of virtually 100% of the patients with early hormone-receptor positive breast cancer who will experience distant relapse.”

**RESEARCH HIGHLIGHTS**

**FASN in breast cancer**

Carbon tracing experiments have demonstrated that, in the absence of FASN, the carbon skeletons incorporated through glucose in the glycolysis process are not redirected to other anaerobic pathways such as the pentose-phosphate shunt or the Krebs cycle. We are currently pinpointing the mechanism that can explain why glycolysis comes to a stop after the synthesis of pyruvate in FASN+ cells.

**Breast cancer taxonomy**

We have identified a set of 3 genes that, when altered (mutated, amplified, or showing deregulated levels of RNA expression), are almost invariably linked to relapse of early hormone-receptor positive breast cancer. Alterations in these genes explain almost half of the relapses in this subset. They are also implicated in hormone resistance.

**Resistance to targeted therapies**

We have continued our research in antiangiogenic agents. Antiangiogenic agents can exert a hypoxic or normalising response. Last year, we pinpointed the mechanism that explains resistance after induction of a normalising response. This year, we discovered that when these drugs induce hypoxia in the microenvironment, they also induce a change in the immune infiltrate that promotes tumour tolerance. This mechanism of resistance is reversible.

**Clinical trials**

We have launched the trials CNIO BR-007, 008 and 009 along with the BIG-NABCG collaboration (2015). These trials are based on information generated in our preclinical discoveries. More information about the trials can be found in the research group website.
OVERVIEW

Prostate cancer (PrCa) is the most common cancer and the second leading cause of cancer mortality among men in Western countries. Despite the advances in PrCa diagnosis and early-disease treatment achieved over the last 25 years, up to 20% of PrCa patients will still develop metastatic disease at some point. The majority of these metastatic PrCa patients will succumb after the acquisition of a castration-resistant status (Castration Resistant Prostate Cancer; CRPC), even when treated with novel therapies that have shown to improve survival and quality of life (QoL) in this advanced-resistant setting. The early identification of PrCa patients who have a more aggressive biology and a greater predisposition to develop aggressive metastatic disease could lead to improved outcomes. Currently, we lack the adequate biological knowledge and reliable biomarkers to select the right treatment for the right patient at the right time.

RESEARCH HIGHLIGHTS

**DNA repair defects in early prostate cancer**

The driving androgen receptor (AR) signalling in PrCa has been implicated in the acquisition of DNA damage, such as single- (SSBs) and double-strand breaks (DSBs). Interestingly, AR activity also regulates a network of DNA repair genes. Genes directly regulated by the AR are involved in homologous recombination (HR), non-homologous end-joining repair, DNA mismatch repair, Fanconi anemia and base-excision repair pathways. To date, a small number of familial cancer syndromes have been associated with an increased risk of prostate cancer. The majority of these genes are associated with inherited mutations in HR DNA repair genes (e.g. *BRCA1*). We have previously shown that *BRCA1* carriers have worse outcomes than non-carriers, when conventionally treated with radiotherapy or prostatectomy, as they relapsed and progressed earlier to lethal metastatic disease. In 2015, we have shown that, despite of their aggressive behaviour, *BRCA1* mutated tumours are androgen-dependent, which is of relevance for tailored treatment. We are currently working on the molecular characterisation of *BRCA1* mutated PrCa, in collaboration with the Institute of Cancer Research (UK), IKoonFab and the Peter McCallum Cancer Centre (Australia), as well as several Spanish centres. In 2015, we have also identified several features, previously associated with poor PrCa outcome, to be significantly more common in *BRCA2* mutated PrCa than in sporadic tumours, which may help to explain their adverse prognosis and be of relevance for targeted therapies.

Circulating biomarkers in CRPC

In the current metastatic CRPC scenario, there are several drugs with diverse mechanisms showing activity in a subset of patients, while others remain primarily resistant. The efficient ‘a priori’ discrimination between both populations is still required. The development of novel biomarkers, which are truly indicative of the tumour biology and/or the tumour-host interaction, should facilitate individual patient risk stratification and improve treatment benefit prediction. We have launched a network of 55 centres across Spain (the PROCuRE Program) in order to conduct several prospective, multicentre, and parallel biomarker studies in patients receiving docetaxel, cabazitaxel, radium-223 or abiraterone acetate; these studies will involve over 900 CRPC patients during the next 3 years. With these studies, we aim to analytically qualify and clinically validate a series of blood-borne biomarkers including: ctDNA, ctRNA, exosomes and CTCs. Its characteristics make this study unique in the CRPC field.

**Publications**


**Awards and Recognition**

- 1st Prize for Young Researchers in Oncology, Adolfo Calvo Foundation, Spain.
- Expert Panel Member, 1st Advanced Prostate Cancer Consilium Conference (APCCC), Switzerland.

**Post-Doctoral Fellow**

Carolina Navas (since March)

**Clinical Research Fellow**

Nuria Romero

**Clinical Investigator**

Elena Castro

**Junior Clinical Research Unit Head**

David Olmos

**Technician**

Patricia Cozar

**Visiting Scientists**

Teresa García (January-September) (Fundación de Investigación Hospital Madrid)
OVERVIEW

The main duty of the Molecular Diagnostics Unit (MDU) is to provide support to medical professionals of the National Health System (NHS) and the CNIO Clinical Research Units, through the provision of a wide variety of sensitive and specific molecular tests, with the aim of determining alterations in DNA sequences or changes in expression levels of key genes that are involved in cancer. These assays enable the improvement of early diagnosis, the detection of minimal residual disease in patients showing clinical remission, the monitoring of the response to therapy, as well as facilitate decision making amongst different treatment options. MDU is also entrusted with the development, implementation, standardisation and supply of the very latest technologies and methodologies in the field of molecular diagnostics, in order to improve cost, reliability and flexibility. Finally, we are very much committed to disseminating knowledge pertaining to the field of molecular oncology diagnostics by hosting and mentoring biomedical students.

RESEARCH HIGHLIGHTS

Expanding our support

In 2015, we developed a new assay based on the detection of mutations by Sanger sequencing in exons 5-8 of the TP53 tumour suppressor gene. This sequence encodes the DNA binding domain of the protein and contains approximately 90% of the detected mutations (FIGURE). TP53 is the most commonly mutated gene in human cancer, especially in pancreatic, skin, oesophagus, head/neck, and colorectal cancers where more than a third of the patients carry mutations in TP53. Recently, mutations in TP53 have become of particular interest as there is evidence that the mutated gene can elicit gain-of-function effects by acquiring oncogenic properties that alter the expression of several other genes and thus, favour the progression and dissemination of cancer.!sf

Additionally, in August 2015, MDU started a collaboration with the CNIO Gastrointestinal Cancer Clinical Research Unit in a sub project of a national clinical trial − FRAGANCE (Phase I/II Study to Assess the Efficacy and Safety of Nab-paclitaxel in Combination With Gemcitabine for the Treatment of Fragile Patients With Advanced or Metastatic Pancreatic Cancer) − geared towards personalised medicine. This study aims to investigate alternative drug therapy regimes in patients with advanced pancreatic cancer by using the chemosensitivity expression profiles of total RNA extracted from their Circulating Tumour Invasive Cells (CTICs). Data from whole gene expression experiments will be analysed using a proprietary algorithm that is able to generate a list of potentially sensitive and resistant drugs for each patient, which can then be used to guide anti-cancer therapies in these patients.

Tutoring

MDU has remained committed towards its policy regarding training and educational programmes; in 2015, our Unit welcomed a medical resident and 3 undergraduate students.

“Since its creation a decade ago, in 2005, MDU has helped 271 haematologists, pathologists and oncologists, active in 98 NHS hospitals. During this period of time, MDU has carried out more than 4,273 tests on samples from 2,178 patients with cancer.”
The paradigm of personalised medicine is the identification of the appropriate drug for the right patient, using molecular profiles. The success of personalised treatment depends on each individual molecular profile, which can a priori be considered as being very heterogeneous. High-throughput technologies are being used to dissect the genetic heterogeneity of tumours, and in parallel, bioinformatics has emerged as a critical discipline to transform the huge amount of genomic data into comprehensive models. However, these analyses have resulted in the identification of hundreds (or thousands) of mutations and other alterations in the same tumour; therefore, we need new approaches to establish the relevance of these changes, and more importantly, to prioritise those that could be of clinical use for cancer therapy.

Our main research objective is to gain a better understanding of the impact of cancer genomics on making clinical decisions by developing a new computational pipeline. This pipeline is to relate and prioritise drug therapies, based on the wide range of actionable genomic alterations for each individual patient instead of the population average data.

This novel computational approach (PanDrugs, http://pandrugs.bioinfo.cnio.es) is based on the analysis and integration of genomic data (mutations, copy number variations or gene expression levels), with functional data (protein essentiality) and pharmacological data (sensitivity or resistance to antitumour drugs). PanDrugs integrates several public pharmacological resources with a curated target-drug resource that includes single gene-drug and pathway-drug associations and an extensive classification of these drugs based on their status (approved, clinical candidates or experimental probes and drug/target family). We have validated the approach by applying it to publicly available data (ICGC and TCGA cancer genome projects).

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

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

**RESEARCH HIGHLIGHTS**

The most relevant achievements of our group in 2015 are:

- We characterised, in collaboration with the MD Anderson Clinical Research Unit, a haiplosufficient tumour suppressor that regulates proliferation and differentiation programmes in haematological malignancies.
- We published, in collaboration with the Spanish Myeloma Group, that both the sequential and alternating administration of VMP (Bortezomib plus melphalan and prednisone) and Rd (lenalidomide plus low-dose dexamethasone) in elderly patients with newly diagnosed multiple myeloma yields impressive results, even in high-risk patients.
- Finally, we have redefined the role of stringent complete response in multiple myeloma.

**PUBLICATIONS AT OTHER INSTITUTIONS**


**AWARDS AND RECOGNITION**

- “Theresio Dr J. Font 2015” Award for the best medical publication by a Spanish Physician. Mutual Midrce, Spain.
H12O-CNIO LUNG CANCER
CLINICAL RESEARCH UNIT

Our Group has been actively involved in pharmacogenomic, pharmacokinetic, translational and clinical studies with novel antimitour agents in several types of solid tumours. In the case of lung cancer, we have actively participated in successful genomically-driven phase I trials in the setting of BIGFRP acquired resistance, in particular in the development of new TP53M selective third-generation EGFR inhibitors. Moreover, our Centre has made a notable contribution in the development of an immune checkpoint blockade therapy (PD-L1) for small-cell lung cancer: this treatment regimen has shown encouraging clinical results against this deadly disease. As far as colorectal cancer is concerned, we have conducted a first-in-human phase I trial with a novel anti-EFGR monoclonal antibody showing treatment responses in cetuximab/panitumumab refractory patients. Finally, new drugs targeting TGF, PI3KCA, MEK1/2 have been tested in histology-agnostic early phase I trials conducted by our group leaders.

Conducting practice changing randomised controlled trials

Probably one of our Group’s major clinical research achievements has been the co-leadership of 3 of the most important NSCLC and renal-cell carcinoma phase III trials conducted during the last decade. Programmed death-1 ligand 1 (PD-1/L1) blockers have been demonstrated to improve overall survival – compared to standard second-line therapy – in pulmonary adenocarcinomas, squamous cell carcinomas and renal-cell carcinomas, changing treatment paradigms for these diseases. Drugs targeting these immune checkpoints are currently being approved for use in lung and renal-cell cancers and these research findings are rapidly being translated to the first-line and adjuvant settings. Our Group is leading some of these important pivotal trials. In the case of colorectal cancer, a significant contribution was made with an oral agent (TX-102) that has been shown to increase overall survival in standard treatment refractory patients. Finally, important contributions have also been made in establishing new standards for prostate cancer.

Novel biomarker development and translation

PD-L1 expression, assessed by immunohistochemistry, was investigated as a potential predictive marker for evaluating the benefit of anti-PD-1 therapies in 2 pivotal phase III lung cancer trials. Our Group has presented the first data suggesting a potential predictive effect of PD-L1 expression in non-squamous non-small-cell lung carcinoma. This is the first-time ever that PD-L1 expression appears to be useful for selecting candidates for treatment with these therapies in solid tumours. However, the issue of predictive markers for evaluating the benefit of immune checkpoint inhibitors remains an unresolved area of debate, and intensive research, including that of our Group, is currently ongoing in this field. Research from our laboratory has also validated PD-L1 expression assessed by immunohistochemistry as a potential prognostic factor in malignant pleural mesothelioma. This may provide a good rationale to test anti-PD-L1 drugs for this disease. In addition, our Group has validated, for the first time, a transcriptomic gene signature that is predictive for chemotherapy benefit in metastatic colorectal cancer patients. If further confirmed in independent cohorts, this could have potential clinical implications, as it may help to optimise chemotherapy delivery and selection in these subsets.

New drug development and early clinical trials

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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, as defined by the Spanish Law 14/2007 on Biomedical Research and the Royal Decree RD 1716/2011, a “Biobank for biomedical research purposes”. 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 it hosts other collections of biological samples for different purposes. Samples and their associated information are collected 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 Community of Madrid – in accordance with the regulation established by RD1716/2011 – and is registered in the National Registry of Biobanks with reference B000848.

**RESEARCH HIGHLIGHTS**

**Biology**

- Collection, management, manipulation and custody of human biological samples and associated documentation, in accordance with the legal framework for biobanking.
- Transfer of samples and clinical biomedical information to research projects, under the approval of the corresponding scientific and ethical committees.

**Management of other collections**

- Custody service of collections of biological samples and/or information related to biomedical research as promoted by the CNIO or other external research groups.
- Coordination of sample collections in multicentre studies.
- Processing of products derived from human samples for research (tissue arrays, DNA, RNA, etc.).
- Researchers who want to deposit their collections at the CNIO-Biobank facilities, or who wish to request samples, must sign an MTA (Material Transfer Agreement) that specifies the terms and conditions under which the Biobank will custody the samples and data.

**Ethico-legal advice for CNIO researchers regarding the use of human samples in biomedical research**

- Technical, scientific and ethical advice regarding the collection, storage and management of human samples used for biomedical research, as well as in regards to the creation and management of new collections that are beyond the Biobank’s scope.
- Institutional registry of all human samples residing in the Centre for research purposes.

**Other services**

- Collaboration with CNIO researchers in human pathology.
- Collaboration in diagnostic activities as specialists in human pathology.

The CNIO Biobank participates in and coordinates the Spanish National Biobank Network. This nationwide platform of services integrates 52 institutions (www.redbiobancos.es) and is an initiative of the National Institute of Health Carlos III (ISCIII).

During 2015, the CNIO-Biobank has processed 19 tissue requests from 11 scientific research projects. Additionally, as the Spanish National Biobank Network Coordination Office, we have managed 31 scientific research projects of high complexity.

The mean impact factor of the 14 publications published in 2015, for which our Unit provided support was 6.381. We also provided sample and/or documental support for the familial cancer activities of the CNIO Human Cancer Genetics Programme.

Finally, the Unit has spearheaded many activities in the national and international biobanking scene through its participation and leadership in numerous forums, working groups and national and international scientific societies. These include the European, Middle Eastern and African Society for Biopreservation and Biobanking (ESBB), the International Society for Biological and Environmental Repositories (ISBER), international think tanks such as the Marble Arch International Working Group on Clinical Biobanking, BC-Net LARC-WHO/NCI initiative, EurocanPlatform (7th FP), Plan de Cáncer Familiar de la Comunidad de Madrid, and the Sociedad Española de Anatomía Patológica (SEAP).

**OVERVIEW**

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Direction of Innovation

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

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

Technology Transfer and Valorisation Office

Private Sponsors
Research results should eventually lead to innovations and practical applications that can be implemented within the life sciences sector, and, in our case, particularly, within the Spanish National Health System.

The CNIO establishes strategic industrial collaborations in order to gain access to broader expertise, greater resources and to contribute to a faster application of research results in society. The CNIO maintains a successful network of industrial collaborators ranging from big pharmaceutical to smaller biotechnology companies, including Eli Lilly, Roche, Merck Serono, Boehringer Ingelheim, Daiichi Sankyo, Celgene and MEI Pharma. Significantly, in 2015, CNIO renewed its collaboration with the Roche ‘Extending the Innovation Network (EIN) Programme’ for 2 more years to support innovative research approaches. Furthermore, the CNIO entered into a new collaboration with Pfizer. As a result of this 3 year collaboration, we expect to generate knowledge and tools to improve the efficacy and safety of the therapeutic interventions that could also be applied to personalised medicine.

Additionally, the CNIO collaborates with patient-focused stakeholders such as the Paradifference Foundation that supports research on rare tumours at the CNIO, with the aim of developing more efficient treatments and essentially finding a cure for the disease.

It’s now been 4 years since the Innovation strategy was launched at the CNIO with an emphasis on collaborative drug discovery. Harnessing the innovative power of CNIO’s scientists through the fostering of intra collaborations has resulted in a significant increase in the number and diversity of projects in the early drug discovery pipeline developed at ETP-CNIO (Experimental Therapeutics Programme). Currently, ETP-CNIO is working on several projects in various stages of development, ranging from HTS Assay Definition to Lead Optimisation. External collaborations with leading institutes such as VIB (Flemish Institute for Biotechnology) further contribute to enrich the portfolio.

The CNIO maintains a leading position within the academic sector when it comes to the commercialisation of Monoclonal Antibodies. CNIO’s 2015 antibody catalogue contained antibodies against 20 mouse and 69 human protein antigens.

Fostering an innovation culture is key for maximising the innovation potential of a Centre like ours. A number of intra and extramural initiatives have been organised. A debate about ‘Innovation as a Bridge Between Science and Society’ was held at the CNIO; it highlighted how researchers are working towards pursuing their dreams to unravel discoveries that can become useful for patients.

In 2015, with the support of the Banco Santander Foundation, 3 students from the CNIO were granted the opportunity to attend the 4th edition of the ‘Management Fundamentals for Scientists and Researchers’ course hosted at the reputed IE Business School. This course will enable them to transfer their scientific knowledge and research to the marketplace in the form of a spin-off company or a transfer accord.

In 2015, in recognition of its innovation strategy, the CNIO was awarded with the Innovation Recognition Award by the Spanish Association of Innovative Companies (Foro de Empresas Innovadoras, FEI), for its accomplishments in fostering and developing innovative technologies for the oncology sector.

“Harnessing the innovative power of CNIO’s scientists and collaborators as a driver for drug discovery; time to set up our efforts.”
The main mission of the Biotechnology Programme is to provide expert technical support and advice to CNIO Research Groups in a number of disciplines and technologies that are 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 from the CNIO Research Groups, they also provide support and collaborate with groups from other institutions, both public and private.

2015 has been a year of consolidation for the Programme’s activities. Our resources have been kept essentially stable; however, towards the end of the year, the Programme was significantly reinforced by 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. We are convinced that this inflow of young people undergoing this training step in their professional careers will be refreshing for the Core Units, and will also help enhance their training activities and capabilities, in addition to providing additional resources to face future challenges and respond to the demands from the users.

As an indication of our high level of commitment towards training and education, the Programme has been involved in the organisation of courses, workshops and specialised meetings, such as the Course on Flow Cytometry, the Workshop on New Applications and Technologies in Confocal Microscopy, and the Course on Animal Experimentation. Moreover, members of our staff have participated in an increasing number of Masters and other training activities hosted at the CNIO and elsewhere. Finally, the number of PhD and Master’s students in the Programme has reached a peak this year.

This year, the Core Units were particularly active in attracting funding from external sources through activities related to innovation. As many as six contracts and agreements have been signed with private companies and public institutions, based on the technologies mastered by several of our Core Units. Also, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit have grown by about 40% compared to the previous year, thus reaching a historical maximum.

“It is not possible to stay at the forefront of cancer research without the support provided by first-class Core Units mastering their technologies and disciplines. Thus, CNIO’s excellence is mirrored by the outstanding achievements of the Biotechnology Programme’s Core Units.”

Last but not least, 2015 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 about 30 publications, including several papers in top journals.
Genomics is the discipline that uncovers the life of the genome, its structural features, regulation and expression. The genome is the blueprint of life, the ensemble of the genetic material that keeps the detailed assembly instructions of the species. Any given cell of an individual keeps a copy of the genome deep in its nucleus. Chemically made of linear DNA macromolecules and distributed into chromosomes, the genome is packed with and interpreted by a myriad of protein cohorts. It is expressed into RNA transcripts that constitute the intermediate step between the genetic material and the functional proteins that run the cell. While less than a 2% fraction of a mammal’s genome codes for protein, a vast majority of the genome (80%) has been found to participate in some biochemical event or another. The genome is not immutable; it suffers damage and alterations. Cancer derives from the accumulation of such alterations. Cells with a damaged genome can transform and escape control, and develop into a tumour. The field of Genomics sheds light on this complexity.

Each cancer genome is different. Even an individual tumour harbours a number of subclonal genomes, each bearing some unique alteration. By employing a distinct set of powerful methodologies, Genomics reveals the genetic diversity of cancer genomes and helps to dissect transformation 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 can detect modifications at a structural level: mutations, binding of protein factors, variations in chromatin folding. Others are capable of examining functional choreographies: changes in the transcriptome in response to treatments, which may uncover therapeutic targets and prognostic biomarkers.

The Genomics Unit provides services at two levels of coverage. 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 and small RNAseq, or genome-wide location of interacting protein factors on chromosomal DNA by ChIPseq. These applications are based on the use of the sequencing-by-synthesis technology from Illumina. On the other hand, gene expression or transcriptome and detection of chromosomal copy number anomalies can be addressed with Agilent DNA microarrays. 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 mutations in candidate genes as well as for the verification of cloned genes or inserts. A cDNA clone repository from the IMAGE-MGC consortium provides scientists with reagents to transfect genes or to express a given protein of interest. The Unit also provides a transgenic mouse genotyping service, based on allele-specific quantitative PCR for a quick and efficient turnaround time.

“The Genomics Unit helps CNIO scientists to understand the molecular processes underlying cancer by providing them with a toolbox for DNA and RNA analyses that is dedicated to an array of applications, either at the single locus or at more global genomic levels.”

**PUBLICATIONS**


In 2015, the Unit has contributed to 6 peer review articles, in collaboration with CNIO and external groups, including projects related to the characterisation of cell cycle regulator functions in vivo and in tumour development, as well as of new mechanisms of lymphatic system development."

"Finally, the Unit leads its own research projects focused on the generation of mouse models to study tumour biology, as well as on the screening of cancer-related genes.

During 2015, the Transgenic Mice Unit has focused on the establishment of 2 new technologies: the use of mouse haploid embryonic stem cells (hESCs) for cancer-related genetic screenings, and the incorporation of the CRISPR/Cas9 system for genome editing in mice.

The first mouse hESC lines were established in 2011 by Anton Wutz and Joseph Penninger, from wild type parthenogenetic embryos. Since then, they have been shown to be a powerful tool for genomewide forward- and reverse- genetic screenings in mice using transposons or gene-trap lentiviruses for genomewide mutagenesis. The haploid karyotype enables direct phenotypic selection of recessive mutations that would be silent in a diploid context. We are interested in exploiting the potential of mouse hESCs for cancer related screenings by generating parthenogenetic hESCs from cancer mouse models and mutant mice available at the CNIO. For this purpose we are creating a collection of mutant hESCs, called HaploESCancer collection, derived from CNIO mice. This year we have established haploid mouse embryonic stem cell lines from p53 KO, Bcra1.lox and ATR.lox mice by SgrI activation of oocytes. These cells will be mutagenised using PiggyBac transposition − ENU or CRISPR/Cas9 − in order to obtain mutant clones covering nearly the whole set of protein/RNA coding loci in the mouse genome.

The CRISPR/Cas9 system, imported from bacterial and archaeal genomes, has expanded the currently available set of mammalian genome engineering tools, providing an easy, efficient, flexible and versatile method of introducing targeted mutations in mammalian genes. The CRISPR/Cas9 system has also been used to generate knockout and knockin mice by introducing the guide CRISPR RNA and the Cas9 RNA directly into mouse zygotes. We have successfully used this system directly in mouse zygotes for generating deletions of regulatory regions and knockout alleles by open reading frame alteration. We are now interested in exploring the advantages of this system for precise genome editing with respect to gene targeting in mouse embryonic stem cells (Figure)."

Figure Use of the CRISPR/Cas9 system for genome editing in the mouse. The figure illustrates the different strategies and delivery mechanisms used to target any given sequence of genomic DNA in the germ line of the mouse using zygotes or mouse embryonic stem cells (ES cells).

**PUBLICATIONS**

The Unit produces novel and high-quality mAbs that are used in basic research in order to gain new insights into the human cancer development process. We are also highly specialised in mAbs characterisation and provide CNIO researchers with reliable and well-validated reagents that add value to their research projects.

During the last 15 years, the Monoclonal Antibodies Unit has generated a large number of mAbs (against more than 120 different antigens), mostly targeting molecules for which mAbs are not commercially available. Many of these mAbs have been licensed to external companies, in turn generating revenues from royalties that represent an important source of funding for the CNIO.

Each year we prepare and update a detailed catalogue of CNIO mAbs that contains the datasheets of more than 70 thoroughly validated, high-quality mAbs (accessible at http://www.cnio.es/ing/servicios/anticuerpos/default.aspx).

In 2008, in collaboration with Oxford University, we founded EuroMAbNet (www.euromabnet.com), the first European non-profit organisation of academic laboratories with an internationally recognised reputation for generating and using validated mAbs. This international network is essential as an arena in which mAb laboratories can exchange knowledge, share cutting-edge methodology and create common strategies in order to standardise and improve the production of these important resources.

The number of mAbs generated as research tools by the research community has exponentially increased. Although this wealth of reagents has exciting scientific potential, a substantial number of mAbs fail even the most fundamental tests of activity or specificity. Furthermore, many research laboratories are unaware of this problem or lack the skills to test these reagents themselves. A lack of technical standards for mAbs often makes their selection a hopeless endeavour, wasting both time and valuable research funds. The use of poorly characterised reagents is of major concern to the scientific community, as perpetuation of serious scientific misconceptions inevitably compromises the advancement of science.

To help address mAb unreliability, EuroMAbNet has published a position paper (Roncador et al., 2015) and we have posted some easy to follow guidelines on our website (http://www.euromabnet.com/guidelines); these guidelines provide a set of criteria and recommendations that will help researchers to select the most effective mAbs from amongst those available in the market, as well as to provide the strategic guidance needed to perform antibody validation.

The development of hybridoma technology in 1975 has led to the generation of large panels of highly specific reagents that have had a tremendous impact in basic and applied research. The availability of monoclonal antibodies (mAbs) has enabled investigators to ask new questions and to develop new insights and applications that will benefit the diagnosis and treatment of cancer.

The Monoclonal Antibodies Unit provides CNIO Research Groups with an à la carte generation of mAbs, which can then be used as tools to characterise new pathways involved in cancer development. We are highly specialised in the production of mouse and rat monoclonal antibodies. The Unit also offers mAb production in gene-inactivated mice, mAb characterisation and validation, medium-scale mAb production and a Mycoplasma testing service for the cell culture facility.

RESEARCH HIGHLIGHTS

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Molecular imaging is revolutionising the way we study the inner workings of the human body, diagnose diseases, approach drug design, and assess therapies, clinically as well as preclinically. The field as a whole is helping to enable the real-time visualisation of complex biochemical processes involved in normal physiology and disease states in living cells, tissues, and intact subjects. Generally speaking, 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 pathology regions, and provide insight regarding the mechanisms of disease. Molecular imaging shows enormous promise in the areas of diagnostics, therapy monitoring, drug discovery and development, and can contribute towards our understanding of nanoscale reactions such as protein-protein interactions and enzymatic conversion.

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

In 2015, we continued our grant project with the Massachusetts Institute of Technology (MIT), titled ‘Improved Molecular Imaging by Multi-tracer PET’, which focuses on the use of dual isotopes to simultaneously assess different biological changes. We also provided imaging support in clinical trials conducted under CNIO’s Clinical Research Programme.

Furthermore, we continued our active participation in the international consortium focused on imaging, M+Vísisán, led by the MIT.
FLOW CYTOMETRY CORE UNIT

Lola Martínez
Core Unit Head

Technicians
Ultan P. Cronin (TS)*, Elena Garrido (TS)*, Tania López (since December)

(PESA)*, Miguel Ángel Sánchez (TS)

*Titulado Superior (Advanced Degree)

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

OVERVIEW

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

Our aim is to provide the CNIO Groups with the necessary technical and scientific advice regarding the use of flow cytometric technologies, and to collaborate with them in the design, acquisition, data analysis and interpretation.

We count with 4 analysers and 2 high-speed cell sorters with different configurations of lasers and detectors, which enable us to cater for 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 the appropriate training, whereas cell sorters are operated by the Unit’s staff. Our sorters can separate up to 4 defined populations at a time, as well as perform single cell cloning. We can accept human samples to sort according to Biosafety regulations.

RESEARCH HIGHLIGHTS

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

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

We have developed several new multicolour panels for the detection of different cellular subtypes (progenitors, T, B and inflammatory cells) from different sample types, such as haematopoietic tissues, pancreas, skin, liver, lung, etc., and have combined these panels with the detection of proliferation and cell death.

In collaboration with the CNIO Tamour Suppression Group, we used some of these multicolour panels for the detection of erythroid progenitors, and combined them with cell death and cell proliferation assays in order to characterise a Diamond–Blackfan anaemia mouse model based on the heterozygous deficiency of the ribosomal gene Rpl11.

Furthermore, the Unit participated in the development of a method to assess the instrument performance of cell sorters, based on the centre stream catch, and calculation of the maximum recovery for a particular sorting condition, Rmax. This method will be very useful for assessing instrument performance during the sorting of rare populations.

The Unit also continues its commitment to developing comprehensive flow cytometry training for different applications at the CNIO. This year we delivered a basic flow cytometry course, as well as a specific course focused on multicolour immunophenotyping and another specific course geared towards cell proliferation and cell death modules. We also launched a 2-day hands-on cell sorting course that was especially designed for operators. All courses were well attended and received excellent feedback.

• PUBLICATIONS.

• CHAPBOOK

• AWARD AND RECOGNITION
  • Elected Member of the Directiva Board “Sociedad Ibérica de Citometría” (SIC), Spain and Portugal.

100% (which is the usual). The right bottom panel is a representative plot of the purity and Rmax values, while screening the break-off point (BOP) of the sorter. As shown, the purity is not much affected by being slightly off the BOP but the recovery is indeed affected.
**CONFOCAL MICROSCOPY CORE UNIT**

Diego Magias
Core Unit Head
Technicians
Jesús Gómez (since December)
(PE-L), Manuel Pérez (TS)**

Joaquim Soriano (TS)**
*Industrial Engineer, Junior Researcher (Jr. Researcher)*
**Industrial Engineer, Junior Researcher (Jr. Researcher)*

**RESEARCH HIGHLIGHTS**

The Confocal Microscopy Unit is equipped with a 3D laser scanning confocal system (Leica SP2 and SP5) that incorporate UV and multiphoton excitation, as well as a white light laser and a Hybrid Detector, and 2 wide-field systems (a Deltavision 4D deconvolution station and a Leica DM16000 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 multiwell plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) through the use of fluorochromes.

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

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

During 2015, the Confocal Microscopy Unit contributed to the microscope field in several aspects: it published a new tool, named iMSRC, that implements the latest advances in intelligent screening; in its portfolio of techniques, it included new microfluidics devices for live-cell assays in perfusion chambers; and it also continued to establish scientific collaborations with CNIO researchers, covering several aspects of cancer studies such as multiphoton intravital imaging in mice. Moreover, the Confocal Microscopy Unit is dedicating a significant effort towards the development and implementation of High-Content Screening technology at the CNIO, for example, during this last year, we helped to run screenings aimed at testing compounds that could modify histopathological features, integrity of nuclei, DNA Damage, BrdU, cell proliferation, etc.

**PUBLICATIONS**


OVERVIEW

The proteome is a system in which highly interconnected proteins are dynamically modified, through physical interactions and/or post-translational modifications, in order to carry out specific cellular functions. Recent developments in sample preparation, liquid chromatography, mass spectrometry (MS) and data analysis have enabled researchers to investigate diverse facets of proteomics in a systematic, high-throughput manner, and data analysis have enabled researchers to investigate diverse facets of proteomics in a systematic, high-throughput manner, offering access to state-of-the-art technologies in the field. “Our main goal is to implement and provide MS-based proteomic technologies to research groups in order to better understand the molecular causes of cancer. The Unit also provides support for recombinant protein expression, offering access to state-of-the-art technologies in the field.”

RESEARCH HIGHLIGHTS

Throughout 2015, the Unit has implemented and optimised several aspects of the quantitative proteomics workflow. We have introduced an orthogonal separation technique (high-pH reversed-phase chromatography) to pre-fractionate samples in order to increase proteome coverage (FIGURE). Likewise, precursor isolation efficiency was optimised in the 2 MS platforms to improve accuracy and precision. Finally, we introduced a statistical model for data obtained from relative protein expression, which allows us to identify truly significant changes with higher sensitivity. All these approaches are being implemented in different projects that are currently ongoing at the Unit, such as the profiling of exosomes (in collaboration with the CNIO Microenvironment and Metastasis Group) and secretomes (in collaboration with the CNIO Melanoma Group) as molecular markers of metastasis. We are also investigating the role of transcriptional regulators in the development of pancreatic diseases (in collaboration with the CNIO Epithelial Carcinogenesis Group). Together with the CNIO Genomic Instability Group, we are using proteomic expression profiling to understand haploidy. Also, in close collaboration with the CNIO Tumour Suppression Group, we are conducting several proteomic analyses to study the foundations of ground state pluripotency.

Pertaining to the other section activities, the Unit has mainly focused on the production of cancer-related proteins for structural studies using X-ray crystallography and Small-angle X-ray scattering, in collaboration with the CNIO Crystallography Core Unit and the CNIO Experimental Therapeutics Programme. The obtained data results will be used to rationally guide the development of small molecule inhibitors and novel bioactive compounds as potential therapeutic agents. Finally, the Unit has continued collaborating with the CIEMAT and the CNIO Molecular Imaging Unit, in setting-up a platform to radiolabel engineered antibodies against either validated or novel tumour targets for positron emission tomography imaging (immunoPET). The aim is to develop noninvasive methods to obtain information about the in vivo status of the specific target for diagnostic and prognostic purposes.
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, in situ hybridisation (ISH) (microRNAs and ALU sequences), and TUNEL in FFPE tissues. Furthermore, the Unit offers laser-capture microdissection, slide digitalisation, image analysis and quantification services. 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

Throughout 2015, the Unit incorporated new equipment such as the Axio Scan.Z1 Slide Scanner for brightfield, fluorescence and polarised light; and the Ventana Discovery Ultra IHC research instrument, as the solution for non-conventional IHC and ISH research protocols including multi-labelling and immunofluorescence. These instruments, along with the new Definiens Tissue Studio software, will enable us to obtain comprehensive and consistent data from tissue-based assays for biomarker and morphological assessment in research and drug discovery.

All the developed techniques follow a standardised validation process. In 2015, the Unit has added several new antibodies to its portfolio, which includes more than 1,000 currently available antibodies that have been optimised for both human and mouse tissue samples. The antibody validation process follows rigorous testing 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.

Aware 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 independently evaluate the quality of the stains performed at the Unit. In 2015, several protocols developed by the Unit were incorporated into the ‘Best Methods section’ of the UKNEQAS Cellular Pathology Technique website.

PUBLICATION

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 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 Research Groups, Sections and Units from all our Research Programmes, except the Structural Biology and Biocomputing Programme.

Our Animal Facility has the capacity to house 18,000 type III cages (each with an average capacity for 3.5 mice). 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. Bedding, water, and cages are sterilised by autoclaving, and the feed is irradiated. 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 housing xenograft models. To maintain clean air in the premises, mice are housed in ventilated racks with integration of Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are manipulated in Type II biosafety cabinets.

Daily operations and husbandry procedures are highly automated in order to safely 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 generate the highest productivity possible and ensure the quality standards in our washing and sterilising areas. All records concerning breeding protocols and animal inventory are computerised and stored in a web-based application accessible via the CNIO intranet.

The Animal Facility currently harbours more than 1,500 genetically modified mouse lines, either as live animals or as cryopreserved embryos or sperm, carrying more than 300 gene targeted alleles and close to 200 transgenic integrations. More than 100 gene targeted alleles and 50 transgenic mouse strains of cancer-related genes have been generated by the Research Groups at the CNIO, and approximately 200 genetically modified lines have been imported from other research centres. The Facility also provides access to more than 70 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, 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/566/2015 stipulates that all animal procedures are to be carried out by qualified people in the possession of the corresponding accreditation as issued by the competent authority. The Animal Facility offers CNIO’s new staff a short course focused on the training of personnel performing work with laboratory animals; this is complementary to the online courses that are a requisite to gain access to the facility.
The main focus of the Programme is the development of Early-Drug Discovery projects. Over time, we have progressed to reach in vivo proof-of-concept (PoC) and/or have entered into licensing agreements with our advanced lead compounds. As already known, the ataxia telangiectasia and Rad3-related protein (ATR) inhibitors discovered at the CNIO have been licensed to Merck Serono for further clinical development, where one of the ETP-CNIO inhibitors continues its progression in the company pipeline towards its characterisation as a potential candidate for clinical trials.

We have also made progress with our CDK8 project. Our Medicinal Chemistry Group has successfully generated and optimised novel CDK8 inhibitors, yielding highly potent, selective and orally bioavailable compounds with intellectual property rights. The use of these compounds has allowed us to identify cell lines that are sensitive to CDK8 inhibition. Selected cell lines will be used to establish in vivo models for PoC studies.

The telomeric repeat binding factor 1 (TRF1) inhibition project, carried out in collaboration with the Telomeres and Telomerase Group (Maria Blasco), has advanced during 2015. A first chemical series of TRF1 inhibitors identified during a preliminary screening campaign, in particular compound ETP-007, has been used by CNIO’s researchers to demonstrate that TRF1 inhibition leads to significant antitumour effects in a mouse model of one of the most aggressive types of lung cancer (KrasG12V p53-/- NSCLC). The deconvolution studies using series 1 compounds and related inhibitors have allowed us to identify PI3K inhibition as a novel mechanism for TRF1 modulation. Interestingly, we have identified a second and unrelated chemical series of TRF1 inhibitors; this series is currently being explored with the aim of increasing the TRF1 inhibition potency, as well as to design and prepare chemical affinity probes for the investigation of the molecular target responsible for their TRF1 inhibition profile, including TRF1 itself. Other screening activities and combinations of TRF1 modulating agents are planned for the next stage.

We have continued the development of Kinase X inhibitors. The ETP has generated highly potent compounds that have demonstrated good general selectivity. Only 3 main off-targets, among more than 400, have been identified and characterised at the cellular level. Some representative inhibitors have displayed excellent Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties, as well as oral bioavailability. These candidates will be used in target validation studies, including in vivo PoC.

The Experimental Therapeutics Programme (ETP) is a well-established early-Drug Discovery Group (e-DD) at the CNIO. The integration of e-DD activities, alongside the Centre’s excellence in basic research, is contributing towards CNIO’s transformation into a more Comprehensive Cancer Research Centre that endeavours to help bring new therapies to the patients.**

In 2015, ETP participated in several Exploratory Projects. The collaboration with Manuel Serrano’s Group (CNIO Tumour Suppression Group) in the field of Gluconeogenesis and Cancer Stem Cell (CSC) therapeutics deserves special attention. Under this framework, we have identified interesting hit compounds for both indications. In particular, Cpd-1 and Cpd-2 have demonstrated their ability to selectively kill CSCs at submicromolar concentrations. Importantly, these hits inhibit the tumour-initiating capacity of pancreatic CSCs after the inoculation of pretreated cells in mice, in comparison with control untreated cells. Currently, we are investigating the target profile of these hits in order to identify the mechanism of action behind this effect. We expect to decipher the mechanism of action of the identified hits for both projects during the next stage of the project. The identification of the molecular targets involved will pave the way for the future development of targeted therapeutic agents. Other Exploratory Projects include collaborations with CNIO researchers Marcos Malumbres (Mastl and Haspin kinases) and Daniel Letha (PAK inhibitors).

Finally, we have made progress in our second collaborative project with VIB (target X**). The ETP-Biology Group has completed the assay development activities and has carried out a screening campaign with more than 5K compounds. Several hits have been identified and they are currently under characterisation.

"Target X (confidential), P. Carmeliet, VIB Department of Transgene Technology and Gene Therapy, KU Leuven (Belgium)."
The process of generating advanced compounds that demonstrate their in vivo proof-of-concept in different early drug discovery projects begins with the discovery or generation of ‘hits’, initial molecules that exhibit a desirable effect at the biochemical or cellular assay level on a particular oncological target. In the following phases - Hit-to-Lead and Lead Optimisation - each hit series will be modified systematically and in a multifactorial manner in order to establish, on the one hand, Structure-Activity-Relationships (SAR), the key modifications to improve potency and selectivity; and on the other hand, to confer drug-like properties to the molecules that will improve how molecules behave in the organism. Structural information about the target, obtained through structure-based drug design techniques using molecular modelling and methodologies such as X-ray crystallography, can sometimes be applied to develop the SAR in a faster and more focused manner. At the end of the process, we will obtain more potent and selective compounds with acceptable pharmacokinetic (PK) properties that can be tested in several in vivo models.

“We were involved in the generation of a proprietary chemical series of CDK8 inhibitors with in vivo properties that can be used in mechanistic studies (PoC), as well as in the identification and exploration of tool compounds in several target-validation oncology projects, e.g. TRF1 inhibition.”
During 2015, our Section was involved in several projects:

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

During this year, we focused our chemical efforts on the multifactorial optimisation of compounds in our main chemical series. We have synthesised more than 50 compounds and have obtained very potent compounds with a low nanomolar biochemical activity range and cellular β-catenin modulation at low micromolar concentrations. This chemical series consists of CDK8 and CDK9 inhibitors that have been profiled in terms of other cyclin kinases showing high selectivity versus CDK2, CDK1, CDK4, CDK5, CKD7 and CDK9. During the chemical exploration, we identified ETP-27 as an advanced compound (CDK8 IC50 = 0.5 nM; β-catenin IC50 = 310 nM), which was profiled against a panel of 468 kinases (KinomeScan profile/DiscoverX) showing Selectivity Scores of S(35) = 0.069 and S(10) = 0.017. The Selectivity Score or S-score is a quantitative measure of compound selectivity and it is calculated by dividing the number of kinases that compounds bind to by the total number of distinct kinases tested, excluding mutants. This compound showed acceptable Clearance (< 30 % hepatic blood flow) and good oral levels in pharmacokinetic studies. Additional in vitro and in vivo characterisation of ETP-27 is planned.

Kinase Y inhibitors

The project Kinase Y inhibitors is another collaborative undertaking with VIB. Complementary to the High-Throughput Screening (HTS) campaign carried out in the Biology Section of the Programme, we have also been involved in the synthesis of several reference compounds, as well as in the generation of potential hits by applying rational design strategies. The biological activity of the synthesised compound will be followed using biochemical and cellular assays.

Telomeric repeat binding factor 1 (TRF1) inhibitors

This is a collaborative project with the CNIO Telomeres and Telomerase Group, in which several hits have been identified in a screening assay that measures the removal of TRF1 from telomeres. During this period, we have assisted in the validation of the hits, providing a more advanced compound, ETP-037, with an optimised profile for in vivo use. The compound effectively impaired the progression of already formed lung tumorigenesis and decreased the association of TRF1 to telomeric DNA in mice. We have conducted deconvolution studies, using the identified hits to determine the molecular target behind this effect. The results point to PI3K inhibition as the mechanism of action for TRF1 modulation. Additionally, we are exploring a second chemical series and 30 compounds have been synthesised to date. We have identified compounds that displayed a significant TRF1 downregulation in KrasG12V- and p53-/- NSCLC cells when treated at concentrations of 10 micromolar for 48h. Affinity probes will be designed and prepared to determine the molecular mechanism of action of this class of TRF1 inhibitors.

Collaboration in Exploratory Projects

The Experimental Therapeutics Programme is collaborating with several CNIO researchers in the initial phases of drug discovery, for example, with the Groups of Manuel Serrano in the fields of Glucogenesiosis and Cancer Stem Cells, Daniel Liebba in the synthesis of atalosterone FAK inhibitors, and Marcos Mallumbers in the Mast and Haplin kinase field.

**PUBLICATIONS**

The Biology Section is devoted to the biochemical, cellular, and in vitro/in vivo pharmacological characterisation of the compounds synthesised within the Experimental Therapeutics Programme (ETP), with the aim of obtaining novel anticancer agents with optimised profiles that demonstrate in vivo proof-of-concept in animal models of disease.

The molecular pharmacological characterisation of novel agents is very important for establishing the tumour types and/or molecular backgrounds in which these new therapies could show efficacy as a single agent or in combination settings. Our experimental approach is based on the evaluation of the inhibition of proliferation in a minimum panel of 40 tumour cells from different origins. Subsequently, taking into account the growth inhibition data (\( GI_{50} \)), we evaluate 3 different doses of the new molecule in combination with a library of 100 approved, or still in clinical trials, therapeutic agents, covering both chemotherapy and targeted therapies. The final aim is to identify sensitive tumours and potential therapeutic combinations.

“We purified human MASTL full length protein, which allowed us to set up a novel biochemical assay in order to run a screening, and also carried out the biochemical, cellular and pharmacological characterisation of proprietary chemical compounds such as CDK8 inhibitors.”
The CDK8 and its paralog CDK19 kinases are components of a multi-protein Mediator complex involved in transcription control. Several studies have indicated that high overexpression of CDK8 could be drivers of malignant progression in colorectal cancer. The CDK8 and its paralog CDK19 kinases are components of a multi-protein Mediator complex involved in transcription control. Several studies have indicated that high overexpression of CDK8 could be drivers of malignant progression in colorectal cancer.

During 2015, our Section was involved in several projects:

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

The CDK8 and its paralog CDK19 kinases are components of a multi-protein Mediator complex involved in transcription control. Several studies have indicated that high overexpression and activity of CDK8 could be drivers of malignant progression in colorectal cancer.

We seek to obtain selective CDK8 inhibitors against other CDKs, either transcriptional or cell cycle regulator CDKs. For this purpose, we have tested 54 newly prepared compounds in a panel of CDKs described in the graph. Dose-dependent inhibition of kinase activity profile. Dose-dependent inhibition of CDK8/CDK19 inhibitor with good cellular inhibition of the β-catenin reporter assay in the HCT116 colon cell line. (B) Cellular inhibition of CDK8 activity measured by β-catenin reporter assay in the HCT116 colon cell line. (B) Cellular inhibition of CDK8 activity measured by β-catenin reporter assay in the HCT116 colon cell line. (B) Cellular inhibition of CDK8 activity measured by β-catenin reporter assay in the HCT116 colon cell line.

**Kinase X**

This project is undertaken in collaboration with VIB (Belgium). Newly synthesised compounds from 2 different chemical series have been characterised at the biochemical, cellular and ADMET level. We have tested 410 compounds in the primary biochemical assay, 64 of them were screened in selectivity panels looking for off-target activities and profiled in ADMET assays. The target has 3 different isoforms and the 22 more promising compounds were evaluated in all the isoforms, our compounds behave as pan–isoform inhibitors. The determination of the solubility and permeability of the compounds has enabled a better interpretation of our cellular data. Pharmacokinetic studies of the 6 more promising compounds were performed, showing that the compounds reached sufficient levels to be tested in mouse tumour models.

**Kinase Y**

In order to evaluate the feasibility of initiating an early drug discovery process for this undisclosed target, we performed a biochemical screening of our ETP-5K library in collaboration with VIB. We set up and validated the High/Throughput Screening (HTS) assay. In a single point screening assay with cut-off values set at 50% inhibition, we achieved a hit rate of 0.77. Hits were confirmed in a dose-response assay and we obtained inhibitors in the high nanomolar or low micromolar activity range; we will evaluate a number of analogues of the identified hits in order to obtain better starting points for the Hit to Lead (HtL) exploration.

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

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. We have purified active human MASTL full protein from insect cells. We have set up a biochemical assay with the obtained protein for the validation of hits previously identified in a phenotypic cell-based screening. Some of the hits are inhibitors of hMASTL at the micromolar level. We plan to run a biochemical screening to identify more potent hMASTL inhibitors as starting points for the HTL phase.

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

This project is undertaken in collaboration with the CNIO Telomeres and Telomerase Group. A phenotypic assay to measure the association of TRF1 to telomeres, developed by Maria Blasco’s Group, has been transferred to ETP. During a previous screening, we identified PEIκ inhibitors as modulators of TRF1 binding to telomeres that might have therapeutic implications. We have contributed towards the validation of this initial finding by testing other known structurally unrelated PEIκ inhibitors, and correlating TRF1 association with the inhibition of AKT phosphorylation. We have also tested around 110 analogues from a second hit that were identified in the initial screening, some of these have shown an improved activity as TRF1 inhibitors.

**Systematic identification of new therapies for the Avatar mouse models**

In collaboration with the CNIO Gastrointestinal Cancer (GIC) Clinical Research Unit, we have started a project to accelerate the identification of potential new therapies that could be translated from Avatar mouse models to patients. For this purpose, we treat freshly disaggregated cells from already established Avatar mouse models with a single point library of 80 approved or in clinical trials drugs. We have determined the growth inhibition data (GI₅₀) for the more promising drugs and the results have been transferred to the GIC Group. The active drugs will be tested in the Avatar mouse model.

**Cancer Stem Cells (CSCs) and Gluconeogenesis**

The CNIO Tumour Suppression Group, led by Manuel Serrano, has developed a 2 assay to search for specific killers of CSCs and for gluconeogenesis inhibitors. ETP-Biology has provided support in running the experiments and to interpret the screening results obtained with the diverse ETP library 640. The identified hits are under further characterisation.

**Support to other CNIO Groups**

We have given support to the CNIO Breast Cancer Clinical Research Group by analysing, with liquid chromatography-tandem mass spectrometry (LC-MS/MS), the levels of several standards of care drugs in tumour and host-mouse plasma samples from different mouse models of cancer.

**EXPERIMENTAL THERAPEUTICS PROGRAMME | BIOLOGY SECTION**

**PUBLICATION**

SCOPE OF THE ELI LILLY - CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer metabolism. 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, or by acting synergistically with other anti-tumour agents. A combination of in vitro and in vivo approaches are being used 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. 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 and mouse models developed at the CNIO; the process includes the use of non-invasive in vivo imaging technologies, and immunohistochemical characterisation of tumours based on different metabolic and tumour markers (FIGURE).

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 use glycolysis over oxidative phosphorylation for growth, even in the presence of normal oxygen levels; a phenomenon known as the ‘Warburg effect’ (Warburg 1956). Warburg argued that this altered metabolic state was the underlying cause for cancer.

The molecular mechanisms that drive an altered tumour metabolism have only recently begun to be understood as a result of large-scale genomic sequencing and advances in metabolomic profiling technologies. Recent studies have shown that many oncogenes, including Myc and Ras, impart an altered metabolic phenotype in cancer cells by regulating 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 (FIGURE, A); 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 (FIGURE, B). This situation may leave tumour cells in a frail position when exposed to certain treatments or under certain circumstances, while normal cells may be able to compensate. Furthermore, the high requirements of nutrients and other soluble factors, together with the hypoxic conditions found in tumours, creates a ‘non-friendly’ microenvironment for the anti-tumour immune surveillance, while facilitating the growth of other tumour-promoting cells such as stroma and myeloid cells (FIGURE C). Thus, the mechanistic understanding of cancer metabolism has led to renewed interest in developing therapeutics that target key enzymes in this process.

“...We are using a combination of in vitro and in vivo approaches to obtain a complete understanding of the metabolic status of tumours.”
**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 are being validated *in vitro* and *in vivo* using animal models developed at the CNIO (FIGURE). 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 do not only occur in the DNA sequence, but also occur 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 last 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. Thus, contrary to genetic mutations, epigenetic aberrations can be reversed through the targeting of 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 therapeutics.

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

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**CNIO - LILLY EPIGENETICS SECTION**

María José Barrero  
**Section Head**  
Staff Scientist  
Sergio Ruiz  

Technicians  
Verónica García (TS)*, Jacinto Sarmentero (TS)*  
*Titulado Superior (Advanced Degree)

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**Biinformatics search for potential epigenetic targets**  
**Knock down of targets using shRNAs or gene editing techniques**

**In vitro effects:**  
- Cell cycle  
- Growth curves  
- Colony forming assays  
- Soft agar

**In vivo effects:**  
- Xenograft models

**Molecular mechanism:**  
- RNA-seq  
- ChIP-seq  
- Mass spectrometry

**Figure**  
*In vitro and in vivo strategies for target validation.*
The CNIO continues to consolidate its leading position among cancer research centres with the production of scientific knowledge through the publication of papers in peer-reviewed journals. The Technology Transfer and Valorisation Office is dedicated to creating world-class impact through world class-research.

The Technology Transfer Team supports the activities of the CNIO Innovation Department to help bridge the gap between research and innovation. In order to channel knowledge towards applications, the Office advocates and boosts collaborative research, contract research, consultancy, spin off companies, licensing, and patenting.

The CNIO implements a rational intellectual property protection strategy based on technical and valorisation dossiers that address major issues regarding the patentability of the results and their commercial viability. The patent portfolio is composed of 23 live patent families that cover numerous territories. In 2015, new patent applications have been filed for 4 new inventions derived from the work of CNIO scientists and collaborators. The CNIO portfolio of patents is particularly strong in small molecules.

In 2015, the CNIO entered into a number of important partnerships. To name just a few, a new collaboration with Pfizer was established whereby 4 research groups at the CNIO will coordinate their efforts to better understand the biological processes underlying major tumours, such as those in breast, lung or pancreatic cancer, with the aim to design new diagnostic and therapeutic interventions. Other important collaborations include those with Boehringer Ingelheim, Eli Lilly, Daiichi Sankyo, Roche and Merck Serono. Additionally, a number of agreements were also signed with smaller biotech companies. Through these collaborations, CNIO scientists will explore new therapeutic indications of commercial or experimental drugs, and take part in the discovery and validation of novel anti-cancer targets, the use of ‘avatar’ mouse models in personalised medicine, as well as the generation of ground-breaking knowledge and technologies with the potential to revolutionise disease therapy. In 2015, 20 such contracts were negotiated, thereby securing future revenues for collaborative research that amount to 4.5 million euros, this amount represents more than 10% of the total annual budget of the CNIO.

In addition, 144 non-disclosure, material transfer and other IPR agreements were signed with both public and private entities. Of these agreements, 75% (108 out of 144) were with foreign organisations; this number provides a good indication of the internationalisation of the CNIO’s scientific activity.

The successful licensing of these technologies provides significant financial support for CNIO’s activities as well as for the inventors themselves; the revenues are distributed according to the CNIO royalty distribution policy and regulations. Altogether, the income generated from Intellectual Property Rights (IPR) in 2015 amounts to about 800,000 €, thanks to the efforts of more than 40 CNIO Inventors who have contributed to this IPR. This income will contribute to the economic sustainability of the CNIO, as well as support its research activities.

Once again this year, the technology transfer potential of the CNIO was successfully recognised by renowned stakeholders such as the Botín Foundation who nominated one of our scientists, Maria Blasco, as a 2015 ‘Botín Researcher’; a distinction that only a handful of prestigious researchers hold. Through this award the Botín Foundation will extend its support to the technology transfer activities related to the research of the Telomeres and Telomerase Group at the CNIO for 5 more years.

A world-class research institute such as the CNIO cannot thrive without driven individuals who are passionate about pushing the frontiers of present-day knowledge and who have the ambition to share new-found knowledge with others. CNIO is proactive when it comes to fostering the entrepreneurial activities of its researches and, therefore, in collaboration with the IE Business School, it provides business training for scientists who are interested in translating their findings into solutions that will benefit cancer patients and society in general.
PRIVATE SPONSORS

“We would like to thank all our sponsors and donors for the generous support that we received from them in 2015. They play an inherent role in our present and future successes.”

One of the Fundación Seve Ballesteros’ 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 the CNIO. This acclaimed 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.

The 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. One young scientist, Ana Ortega, who came from the Sloan Kettering Institute for Cancer Research in New York, was the recipient of a Santander Foundation-CNIO Fellowship in 2015. 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 scientific programmes. These 2 well-recognised organisations collaborate with the CNIO in this regard by supporting 3 research groups: Manuel Serrano, Head of the Tumour Suppression Group; María A. Blasco, Head of the Telomerases and Telomeres Group; and Oscar Fernández-Capetillo, Head of the Genomic Instability Group.

The Centre also benefits from generous donations from other companies and foundations, as well as from local associations that are equally dedicated to the battle against cancer. During 2015, our activities and seminars were also supported by the French Embassy, Fressia Group, the Fundación Antoni Serra, and the Fundación Banco Sabadell.

We would also like to express our gratitude to all the ‘CNIO Friends’ donors, sponsors and benefactors who – with their generous donations to support cancer research at the CNIO – have ensured the continuation of our research endeavours throughout 2015.
Communication
In 2015, the presence of our Centre in the media kept its rising trend: over 2,600 mentions were tracked in the national and international press, representing an increase of 15% compared to the already remarkable figures of 2014. Our presence on radio/TV programmes and in news bulletins has also experienced a significant increase. Figures for radio and TV hits almost tripled those of 2014; these include shots during prime-time newscasts and magazine programmes broadcasted by public as well as private media channels.

In 2015, the CNIO submitted 24 press releases to the global news service EurekAlert! Throughout the year, these stories received over 83,000 hits from around the world, surpassing the grand total of 300,000 visits.

Two CNIO discoveries in particular caught the attention of the media and society: in May, research on TRF1 and cancer immortality published under the title ‘Therapeutic inhibition of TRF1 impairs the growth of p53-deficient K-RasG12V-induced lung cancer by induction of telomeric DNA damage’ hit the front pages of the newspapers, among other media outlets; in October, the international research on metastasis titled ‘Tumour exosome integrins determine organotropic metastasis’, in which the CNIO participated, had an outstanding media impact and was also covered by the main TV and radio stations in Spain.

An exceptional achievement in 2015 was the agreement CNIO established with the TV & Media Group Atresmedia, one of the leading media groups in Spain. Through this agreement, ‘Constantes y Vitales’ – the social responsibility initiative of the TV channel laSexta and the AXA Foundation – launched the engaging action #CadaPasoEsVital, to support the research conducted by the Microenvironment and Metastasis Group led by Héctor Peinado at the CNIO.

Our social networks are also consolidating their communities. By December 2015, our Twitter channel had over 8,700 followers, with whom we keep up an ongoing and valuable dialogue via the platform. Furthermore, in February, we announced the launch of the ‘CNIO Friends’ social networks. In only 10 months, our Facebook account reached over 26,000 followers – who constantly send us words of encouragement, affection and congratulations – and reached more than 44.6 million people as a whole.

The ‘CNIO Friends’ initiative was greatly successful during its first year of existence. Both Marcos Argumosa’s 10 consecutive marathons in April, and the video homage in honour of our donors, produced by the visual artist Amparo Garrido in June, resulted in almost 120 media hits, including digital and print media outlets, radio and TV broadcast. The video received almost 23,500 views on Youtube as well.

To strengthen our bonds with our donors, we launched the ‘CNIO Friends’ newsletter, via which we keep them informed about our Centre’s latest news every other month. And thanks to an agreement with the Spanish railway operator RENFE, our video homage in their honour was aired in the Spanish high-speed long-distance trains in December and at Christmas time, helping us to spread the word about our cancer research during one of the most public-spirited times of the year.

“A year of growth together with media and society.”

Communications Officer Vanessa Pombo
INVITED GUEST SPEAKERS (Distinguished Seminar Series)

On World Cancer Day, the CNIO organised an open public debate on cancer research and clinical practice with CNIO researchers, the Spanish Association Against Cancer (AECC), and the San Carlos Clinical University Hospital. The event was sponsored by Bristol-Myers Squibb. February 4, 2015.

Carmen Vela, Secretariat of State for Research, Development and Innovation, talked about her professional career from a gender perspective, for the seminar series of the CNIO Women in Science Office (WISE). March 10, 2015.

Over 200 participants enjoyed the European Researchers’ Night at the CNIO; this unique event aims to encourage scientific careers and foster an entrepreneurial spirit in young people. September 25, 2015.

The event ‘Innovation: Bridge between Science and Society’ hosted a dialogue between the former Minister of Education, Ángel Gabilondo, and CNIO’s Director, Maria A. Blasco. The event was organised by the CNIO, the Banco Santander Foundation and the Instituto de Empresa Business School. October 19, 2015.

Throughout the year, Benefactors and Sponsors of ‘CNIO Friends’ could visit the Centre in order to meet its Director Maria A. Blasco, as well as to enjoy a guided tour of its facilities.

COMMUNICATION

SOCIAL EVENTS
CNIO Offices

Dean’s Office
CNIO Women In Science Office
The CNIO is recognised for the relevance and international projection of its scientific groups. Key to this success is a solid core of undergraduate students, predoctoral and postdoctoral fellows, medical residents and a broad spectrum of visiting scientists. In fact, personnel in training constitute over 60% of the workforce in our institute. As such, the CNIO dedicates particular emphasis to career development, supported in part by highly competitive PhD and Postdoctoral programmes. Agreements are also in place with active medical centres, to ultimately bridge the gap between academic and clinical environments.

Importantly, the CNIO Student Association (CNIOSA) and Postdoc Association (CNIOPDA) are active organisers of talks and seminars coordinated by the Dean’s Office. Examples of topics that we covered last year include Effective Job-Hunting and Interviewing, as well as Negotiation, Leadership and Confidence Workshops conducted by the expert scientific coach Rob Thompson. These events are performed in concert with CNIO’s Training Programmes and the Innovation and Communication Offices, which are deeply committed to providing the best environment for our personnel. We are most grateful to the Fundación Jesús Serra for their generous donation that helps us to strengthen career development programmes at the CNIO.

We believe that an informed society is better prepared to understand (and if needed, face) the diseases that constitute human cancer. Therefore, we are actively involved in knowledge dissemination. 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; all these events were highly attended, attracting numerous participants of all ages.

A particularly inspirational event this year was our Annual CNIO Lab Day. We were fortunate to host John Diffley (The Francis Crick Institute, UK) who spoke about his personal experience in setting up his laboratory, emphasising the value of perseverance, risk-taking and independent thinking. It was also exciting (and encouraging) to learn about the success stories of alumni from CNIO laboratories who now have productive careers in academia (Iva González-Suárez, IDIBELL, Barcelona), non-profit organisations (Marta Poyol, AECC, Madrid) and industry (Sara Álvarez, NIMGenetics, Madrid). We also had six outstanding talks given by CNIO trainees that covered exciting discoveries in the fields of stem cell biology, epidemiology, proteomics, tumour metastasis and drug development. Progress made in other basic and translational aspects of cancer were discussed in over fifty posters, which together emphasised the breadth of research covered by our different Scientific Programmes.

Finally, we also had the pleasure of announcing the establishment of the ‘Director’s List’, an initiative promoted and endorsed by CNIO’s leadership as a formal platform to recognise and give specific visibility to outstanding contributions made by our personnel in 3 categories: (1) predoctoral fellows with publications of the highest scientific impact; (2) excellence in research by postdoctoral and staff investigators; and (3) altruistic volunteering to further the mission of the Centre in regards to training, scientific divulgation and outreach. After a motivating review by our Director María Blasco on how the CNIO has consolidated and expanded our basic and clinical Scientific Programmes, solidified innovation and drug development activities and in general, reinforced our international recognition, it was with great satisfaction that we could present the first edition of our Director’s List:

1. Awards to Excellence in Research by Predoctoral Fellows
   - Elena Doménech (for an outstanding publication in Nat Cell Biol), Elena López-Guardamillas (Cell Metab), Julia Specks (Genes & Dev), Silvia Álvarez (Nat Commun) and Laia Richart (Nat Commun).

2. Award to Excellence in Research by Postdoctoral/Staff Investigators
   - Sergio Ruiz, for outstanding contributions in the fields of genomic instability and stem cell pluripotency.

3. Outstanding Contribution to Outreach and Awareness
   - Lisa Osterloh, for her tireless and altruistic efforts in the organisation and supervision of the European Researcher’s Night, visits by high- and middle-schools, as well as at the multiple talks and seminars given on career development.

In summary, we are as proud as ever for all that this vibrant community of young investigators at the CNIO has achieved, while mentored by a committed faculty at the forefront of cancer research.

“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.”
“No struggle can ever succeed without women participating side by side with men.”
Muhammad A. Jinnah, 1940.

The recent data published by the main scientific organisation in Spain (CSIC) in their report “Informe Mujeres y Ciencia 2015” speak for itself, and continue to show during 2014, the consistent classical ‘scissors’ pattern displayed in the comparative career paths of male and female professionals. Although about 50% of women are represented in the pre-doctoral and post-doctoral stages of the scientific career, those percentages go down significantly as we move up the scientific career ladder: a meagre 25% of women are represented at the Principal Investigator level versus 75% of men, and this representation continues to shrink as we move up to the levels of department directors and beyond.

Similar scenarios can be found all over Europe, and this global picture is also true even in countries such as the United States of America where policies to address those imbalances were implemented many years ago. Therefore, there is still a lot of work to be done in order to achieve gender equality in science.

At the CNIO Women in Science Office (WISE), we believe there is still a real need for actions to be undertaken to ensure gender equality in the research career. At the end of 2012, our office was created with the aim to promote awareness about these important aspects and to help correct the observed imbalances in the career ladder at the CNIO community. We are convinced that everyone within the CNIO community needs to work together towards a common goal: to help outstanding female researchers to reach the top as they have lots of fresh ideas to offer to science and they can most certainly contribute towards the CNIO’s scientific productivity.

The Office has two main working groups:

- Work/Life Balance: their aim is to promote and support initiatives to help improve the delicate balance between the professional and personal life, one of the main challenges faced by researchers at the CNIO.

- Seminars and Events: their aim is to raise awareness about gender issues in the scientific field (and others) and point out the difficulties that female researchers may experience in their quest to reach the top, as well as to provide networking opportunities to all CNIO researchers.

In 2015, the WISE office managed to invite several top female leaders from different areas to come and tell us about their experience with gender issues, thereby giving our young scientists ideas and advice on how to best overcome some of the hurdles that they may face during their careers. These seminars are aimed to stimulate institutional 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. Some of the talks given during 2015 include:

- Sex, Science and Society: a triangle that matters? Flora de Pablo, Professor, Centro de Investigaciones Biológicas, CSIC. Madrid, Spain. February 17.


In addition, the Office proposed several initiatives to the CNIO Direction in relation to work/life balance issues. As an example, a proposal was elaborated and put forward to the General Secretary of the ISCHI for the organisation of an urban camp during non-school days for children of CNIO employees; this was done in collaboration with members of the ISCHI.
Facts & Figures

Competitive Funding
Education & Training Programmes
Scientific Events
Administration
  Board of Trustees
  Scientific Advisory Board
  Management
  CNIO Personnel 2015
The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international funding bodies. In 2015, researchers at the CNIO were involved in 143 projects that received extramural funding.

The CNIO actively participates in a total of 66 collaborative projects: 31 are international collaborative projects (for 4 of them the CNIO acts as project coordinator) and 35 are collaborative projects at the national level (8 of which are coordinated by the CNIO). The international collaborative projects were funded by institutions such as the European Commission through the 7th Framework Programme and Horizon 2020, the US National Institutes of Health (NIH), the United States Department of Defense (DoD), the Melanoma Research Alliance, the Paradifference Foundation and the AXA Research Fund.

In addition to these collaborative projects, researchers at the CNIO attracted funding for projects carried out by individual groups. In 2015, 19 of these projects received extramural funding and 58 received national funding. The international individual projects are also funded by the European Commission (through the European Research Council (ERC) Advanced, Consolidator and Starting grants and the Marie Curie Actions), the Worldwide Cancer Research (WCR, formerly AICR), the Howard Hughes Medical Institute (HHMI) and the European Foundation for the Study of Diabetes (EFSD).
### Networks of Excellence (NGE)

**Principal Investigator** | **Project Title**
--- | ---
Barbacid, Mariano | EUROCANPLATFORM: A European platform for translational cancer research

### Small or Medium-Scale Focused Research Projects

**Principal Investigator** | **Project Title**
--- | ---
Barbacid, Mariano | LUNGTARGET: New approaches for the targeted therapy of non-small cell lung cancer
Malats, Núria | TransBlBC: Translation of novel Biomarkers for Bladder Cancer for clinical outcome prediction
Robledo, Mercedes | ENSGTCancer: European network for the study of adrenal tumours-structuring clinical research on adrenal cancers in adults

### ERA-NET on Translational Cancer Research (TRANSCAN)

**Principal Investigator** | **Project Title**
--- | ---
Malats, Núria | Bio-Fac: Biomarkers of tumor recurrence in pancreatic cancer ERA-NET on European Funding for Neuroscience Research (NEURON II)

### ERA-NET on European Funding for Neuroscience Research (NEURON II)

**Principal Investigator** | **Project Title**
--- | ---
Malumbres, Marcus | MicroKin: Deciphering the multifaceted pathways underlying MCPH pathogenesis in the mouse and human

### Horizon 2020 (2014-2020)

**Research Infrastructures, including E-Infrastructures**

**Principal Investigator** | **Project Title**
--- | ---
Valencia, Alfonso | ELIXIR-EXCELERATE: Fast-track ELIXIR implementation and drive early user exploitation across the life-sciences
Valencia, Alfonso | OpenMinToD: Mining Infrastructure for Text and Data

### Marie Skłodowska-Curie Actions (MSCA)

**Principal Investigator** | **Project Title**
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Soengas, María S. | ITN IMMUTRAIN: Training network for the immunotherapy of cancer

### Societal Challenge 1: Health, Demographic Change and Wellbeing

**Principal Investigator** | **Project Title**
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Benítez, Javier | BRIDGES: Breast cancer risk after diagnostic gene sequencing

### Massachusetts Institute of Technology (MIT)

**Principal Investigator** | **Project Title**
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Pérez Moreno, Mima Alicia | Team Leuko: Home-based neutrophil blood testing to tailor chemotherapy regimens to personal toxicity limits

### World Wide Cancer Research (WCR, FORMERLY AICR)

**Principal Investigator** | **Project Title**
--- | ---
Malats, Núria (Coordinator) | Oral microbiotic profiles and its association with risk of pancreatic ductal adenocarcinoma

### Volkswagen Foundation / Fundación Volkswagen

**Principal Investigator** | **Project Title**
--- | ---
Lietha, Daniel | Nanoapertures loaded with individual molecules

### Competitive Funding

**Principal Investigator** | **Project Title**
--- | ---
Peinado, Héctor | Imaging and therapeutic targeting of lymphangiogenesis in melanoma (Soengas, María S. (Coordinator))
Robledo, Mercedes | SDHA-related metastatic paraganglioma: search for the cure
Peinado, Héctor | Radiolabeled exosomes for the early detection of metastases and to predict breast cancer premetastatic niche
Peinado, Héctor | Exosomes in development and therapy of malignant mesothelioma
Peinado, Héctor | Characterization and functional analysis of breast cancer secreted exosomes in malignant progression
Peinado, Héctor | Exosome-mediated transfer of c-MET to bone marrow progenitors promotes metastasis
Malats, Núria | GENCODE 2: integrated human genome annotation: generation of a reference gene set

### US National Institutes of Health (NIH)

**Principal Investigator** | **Project Title**
--- | ---
Peinado, Héctor | Organ-tropic metastatic secretomes and exosomes in breast cancer
Peinado, Héctor | Exosomes in development and therapy of malignant mesothelioma

### Congressionally Directed Medical Research Programs (CDMRP)/United States Department of Defense

**Principal Investigator** | **Project Title**
--- | ---
Peinado, Héctor | PARADIFFERENCE FOUNDATION
Peinado, Héctor | CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS (CDMRP)/UNITED STATES DEPARTMENT OF DEFENSE

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### Horizon 2020 (2014-2020)

**Research Infrastructures, including E-Infrastructures**

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<tr>
<td>Barbaric, Mariano</td>
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<td>RAS AHEAD: Ras genes in health and disease</td>
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<tr>
<td>Fernández-Capetillo, Oscar</td>
<td>ERC Consolidator Grant</td>
<td>RSHARED: Investigating the causes and consequences of replication stress in mammalian health</td>
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<td>Heeschen, Christopher</td>
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<td>Serrano, Manuel</td>
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<td>CANCERIA2GEN: Common mechanisms underlying cancer and ageing</td>
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<td>Wagner, Erwin F.</td>
<td>ERC Advanced Grant</td>
<td>AP-1-FUN: AP-1 (Fos/Jun) functions in physiology and disease</td>
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**MARIE CURIE ACTIONS (MCA)**

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<tr>
<td>Al-Shahrour, Fátima</td>
<td>PERCHOMICS: Bioinformatics and integrative genomics for a novel personalized cancer therapy</td>
</tr>
<tr>
<td>Squatrito, Massimo</td>
<td>GLDIN: DNA Damage Response (DDR) signaling in tumor formation and therapeutic resistance of gliomas</td>
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<tr>
<td>Ramón-Maiques, Santiago Moreno, María</td>
<td>COFUND CAD_FL: Revealing the functional mechanism of CAD and its potential as a therapeutic target</td>
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**HORIZON 2020 (2014-2020)**

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<td>Djouder, Nabil</td>
<td>Growth factors and nutrients in type 2 diabetes: role of URI in β-cell plasticity and glucose homeostasis</td>
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**COMMUNITY OF MADRID / COMUNIDAD AUTÓNOMA DE MADRID (CAM)**

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<td>Barbaric, Mariano</td>
<td>Programa ONCOCYCLE: El ciclo celular y los microRNAs en la autorenovación y diferenciación de células progenitoras</td>
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<td>Malumbres, Marcos (coordinator)</td>
<td>Programa ReCaRe: Reprogramación en cáncer y regeneración</td>
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<tr>
<td>Campos-Olivas, Ramón</td>
<td>Programa VISIONANIMAL: Modelos animales para el estudio de enfermedades de la visión</td>
</tr>
<tr>
<td>Martínez, Jorge L.</td>
<td>Programa ANGIOBODIES 2: Desarrollo de anticuerpos recombinantes para uso terapéutico y diagnóstico en angiogénesis patológica y para la identificación de nuevos marcadores angiogénicos</td>
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<tr>
<td>Soengas, María S.</td>
<td>Prognostic and therapeutic impact of lymphovascular niches in melanoma</td>
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### PROSTATE CANCER FOUNDATION

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<tr>
<td>Olmos, David</td>
<td>Integration of clinical, molecular and biological characteristics to define an aggressive subtype of prostate cancer based on deficient homologous recombination</td>
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### WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)

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<td>Lietha, Daniel</td>
<td>Targeting regulatory mechanisms for allosteric cancer drug discovery</td>
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<td>Malumbres, Marcos</td>
<td>New therapeutic strategies by inhibiting Mastl in breast tumors</td>
</tr>
<tr>
<td>Pérez Moreno, Mina Alicia</td>
<td>Defining the role of macrophage-derived Wnts in squamous cell carcinoma</td>
</tr>
<tr>
<td>Soengas, María S.</td>
<td>Harnessing endo/exocytosis for a coordinated targeting of melanoma cells, their vasculature and the immune system</td>
</tr>
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<td>Wagner, Erwin F.</td>
<td>Dissecting the roles of Fra proteins in lung adenocarcinoma progression and metastasis</td>
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## Competitive Funding

**Spanish National Cancer Research Centre, CNIO**

### Annual Report 2015

#### Competitive Funding

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### Facts & Figures


**Excellence Networks/Redes de Excelencia**

- **ONCObio**: Biología del Cáncer
  - Malumbres, Marcos (Coordinator)
- **CellSYS**: Functional and Systems Biology of Cell Proliferation
  - Peinado, Héctor
- **REDiEX**: Red de Excelencia en Investigación e Innovación en Exosomas
  - Serrano, Manuel (Coordinator)

**Challenges/Collaboration/Retos-Collaboración**

- **SENESTHERAPY**: Cell senescence in cancer therapy
  - Soengas, María S.

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

### Research Projects in Health

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<tr>
<td>Blasco, Maria A.</td>
<td>Cellular aging in first episode early-onset psychosis</td>
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**Spanish Association Against Cancer / Asociación Española contra el Cáncer (AECC)**

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<td>Benítez, Javier</td>
<td>Ensayo Clínico Fase I de BO-110: un nuevo tratamiento para melanoma avanzado y otros tumores</td>
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<td>Malats, Núria</td>
<td>Ex vivo study of melanoma metastatic dissemination in different tumor microenvironments. Implications in the validation of new tumor biomarkers and therapeutic targets</td>
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**Ministry of Economy and Competitiveness / Ministerio de Economía y Competitividad (MINECO)**

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**La Marató TV3 Foundation / Fundación La Marató TV3**

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<tr>
<td>Soengas, María S.</td>
<td>Ven a conocer a los científicos, convéntrate en un científico! European Researchers’ Night 2014, organized by Madrid+D Foundation and founded by European Commission on the framework of H2020 Programme</td>
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**MADRID+D Foundation / Fundación Madrid+D**

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

### Sub-programme of competitive health research thematic networks/subprograma de redes temáticas de investigación cooperativa en salud (retics)

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<td>Gómez, Carlos Jesús</td>
<td>Cytosensitivity profiles for the personalized therapy of advanced colorectal cancer</td>
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<td>Hidalgo, Manuel (starting in June)</td>
<td>Personalized treatment for pancreatic cancer patients</td>
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**Spanish Association Against Cancer / Asociación Española contra el Cáncer (AECC)**

### Excellence Networks/Redes de Excelencia

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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</thead>
<tbody>
<tr>
<td>Barbacid, Mariano</td>
<td>ONCObio: Biología del Cáncer</td>
</tr>
<tr>
<td>Blasco, María A.</td>
<td>Exploring synthetic lethal interactions between PARP and the DNA damage response in cancer treatment</td>
</tr>
<tr>
<td>Fernández-Capetillo, Óscar</td>
<td>Role of RNA binding proteins in melanoma progression: searching for new diagnostic makers and therapeutic targets</td>
</tr>
<tr>
<td>Malumbres, Marcos</td>
<td>Cancer and immunodeficiency in children</td>
</tr>
<tr>
<td>Real, Francisco X.</td>
<td>Invasive bladder cancer: towards precision medicine</td>
</tr>
<tr>
<td>Serrano, Manuel (coordinator)</td>
<td>KeyYS: Functional and Systems Biology of Cell Proliferation</td>
</tr>
</tbody>
</table>

**Madrid+D Foundation / Fundación Madrid+D**

### Research Projects

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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</thead>
<tbody>
<tr>
<td>Benítez, Javier</td>
<td>Cancer and immunodeficiency in children</td>
</tr>
<tr>
<td>Malats, Núria</td>
<td>Invasive bladder cancer: towards precision medicine</td>
</tr>
<tr>
<td>González Pisano, David</td>
<td>Distinct routes of metastatic dissemination in different melanoma subtypes. Implications in the validation of new tumor biomarkers and therapeutic targets</td>
</tr>
<tr>
<td>Soengas, María S. (coordinator)</td>
<td>KeyYS: Functional and Systems Biology of Cell Proliferation</td>
</tr>
</tbody>
</table>
NATIONAL GRANTS  INDIVIDUAL PROJECTS

INSTITUTE OF HEALTH CARLOS III / INSTITUTO DE SALUD CARLOS III (ISCIII)

RESEARCH PROJECTS IN HEALTH

PRINCIPAL INVESTIGATOR  PROJECT TITLE

Benítez, Javier  Biologic and genetic bases of telomere shortening in hereditary breast cancer. Searching for new high susceptibility genes in BRCA1 familiies with short telomeres

Cascón, Alberto  Exomic sequencing of tios, mother-father-sonbad, in pediatric patients with multiple pheochromocytomas/paragangliomas

Cigudosa, Juan C  Genetic diagnostics by next-generation-sequencing in myeloid neoplasias: step towards its clinical use and characterization studies on the mutation genomic and functional pathological effects

García, María José  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

González-Neira, Aina  Personalizing breast cancer treatment: prediction model construction for taxanes and anthracyclines efficacy thought the integration of different genomic approaches

Hidalgo, Manuel  Targeting Pancreatic Cancer Stroma

Malats, Núria  Aetiology of pancreas cancer: Application of "omics" technologies in the assessment of risk factors

Molina, María Esther  Dietary patterns, antioxidants and biomarkers of oxidant-antioxidant relationships in cancer and breast cancer risk

Olmos, David  Homologous recombination DNA repair deficiency related chromosomal instability in aggressive prostate cancer

Pérez de Castro, Ignacio  An integrative Study of Chromosomal Instability and Cancer: looking for prognostic markers and therapeutic opportunities

Quintela, Miguel Ángel  From systems biology to clinical trials: high-throughput studies and definition of predictive factors and resistance mechanisms against breast cancer drugs

Robledo, Mercedes  Prognostic profiles in endocrine tumours identified by next generation sequencing, and definition of markers with clinical utility

Rodríguez Sandra  Ewing Sarcoma Model: induction of the t(11;22) translocation in human mesenchymal stem and iPS cells by the CRISPR-Cas9 system and study of the cellular context and other secondary events role

Squarizo, Massimo  Investigating the role of Frla and Frl2 in glioma tumor formation and treatment response

Urriola, Miguel  PTEN-hamartoma tumour syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene

MINISTRY OF ECONOMY AND COMPETITIVENESS / MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD (MINECO)

RESEARCH PROJECTS IN HEALTH

PRINCIPAL INVESTIGATOR  PROJECT TITLE

Montoya, Guillermo  Macromachines: Structural biology of macromolecular machines involved in chromosome dynamics

Malumbres, Marcos  MitoSYS: Physiological and therapeutic relevance of mitochondrial kinases and phosphatases

Pérez Moreno, Mina Alicia  CrosSkin: Intercellular crosstalk in skin physiology and disease

Real, Francisco X.  Transcriptional control of acinar cells differentiation and pancreatic cancer

Rodríguez, Cristina  Identification of markers predictive of paclitaxel severe neurotoxicity using genome-wide platforms

Usák, Özge  PosfACEmiR5: Investigating the role of microRNAs in TACE inhibitor in breast cancer - evaluating the potential therapeutic implications

Valencia, Affonso  Development of biocomputing systems and subsequent computational methods for the analysis of oncologic personalised therapies

Wagner, Erwin F  HepAP-1: From liver physiology to hepatitis and hepatocellular carcinoma (HCC): role of AP-1 (Fos/Jun) proteins

SPANISH NATIONAL CANCER RESEARCH CENTRE, CNIO

PRINCIPAL INVESTIGATOR  PROJECT TITLE

Brisco, María A  Acreditación del CNIO como Centro de Excelencia

Méndez, Juan  IERLICON: Molecular mechanisms that control eukaryotic DNA replication

Ramón-Maques, Santiago  CADOSTRUCTU: Structural determination of the architecture of CAD, an antitumoral target that controls the biosynthesis of pyrimidines

Ruíz, Sergio  RSHPS: Replicative stress during somatic cell reprogramming

Serrano, Manuel  HaploEScancer: Haploid ES cells for cancer research

Blasco, María A.  Genetic diagnostics by next-generation sequencing technology and a LOH-candidate region approach in high-risk non-BRCA1/BRCA2 patients

Hidalgo, Manuel  Targeting Pancreatic Cancer Stroma

Molins, María Esther  Dietary patterns, antioxidants and biomarkers of oxidant-antioxidant relationships in cancer and breast cancer risk

Peinado, Héctor  METASTAXOMES: Role of tumor-secreted exosomes in lymph node-microenvironment reprogramming during metastasis

Losada, Ana  COHESIN: Cohesin function and regulation: a multidisciplinary approach

Muñoz, Daniel  REMODEL: Cellular senescence as an active player in tissue remodeling

Muñoz, Javier  SARGUS: Understanding ground state pluripotency of embryonic stem cells through mass spectrometry-based proteomics

Bartabás, Mariano  PANTHER: A three prong strategy to fight pancreatic ductal adenocarcinoma

Blasco, María A.  TelomeHealth: Telomeres, telomerase and disease

Díaz, Nabila  MBL: Metabolic inflammation in liver cancer

Soengas, María S.  BRCAX: New frontiers and new strategies for the integration of different genomic approaches

Losada, Ana  COHESIN: Cohesin function and regulation: a multidisciplinary approach

Ortega, Sagrario  HaploEScancer: Haploid ES cells for cancer research

Pérez, Joaquín  CDK8eDD: CDK8 a novel target in cancer therapy. Relevance of CDK8 kinase activity, discovery and optimization of selective orally bioavailable CDK8 inhibitor

Olmos, David  Homologous recombination DNA repair deficiency related chromosomal instability in aggressive prostate cancer

Pérez de Castro, Ignacio  An integrative Study of Chromosomal Instability and Cancer: looking for prognostic markers and therapeutic opportunities

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Squarizo, Massimo  Investigating the role of Frla and Frl2 in glioma tumor formation and treatment response

Urriola, Miguel  PTEN-hamartoma tumour syndrome research: Phenotypic spectrum, associated cancers, molecular basis and search of new gene

Vallejo, Manuel  IVACTIVE BrainMET: Dissecting the role of reactive astrocytes in brain metastasis
## Competitive Funding

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spanish National Cancer Research Centre, CNIO</strong></td>
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<tr>
<td><strong>ANNUAL REPORT 2015</strong></td>
<td></td>
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<tr>
<td><strong>Spanish Oncology Genitourinary Group/Grupo Español de Tumores Genitourinarios (SOGUG)</strong></td>
<td></td>
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<tr>
<td>García, David</td>
<td>Validación multi-institucional de perfiles de expresión génica en sangre periférica en pacientes con cáncer de próstata resistente a la castración</td>
</tr>
<tr>
<td><strong>Spanish Group of Neuroendocrine Tumours/Grupo Español de Tumores Neuroendocrinos (GETNE)</strong></td>
<td></td>
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<tr>
<td>Robledo, Mercedes</td>
<td>ParafeOMICS: Identificación de marcadores diagnósticos y pronósticos en feocromocitomas y paragangliomas a través de la integración de cuatro plataformas de análisis masivo</td>
</tr>
<tr>
<td><strong>Mutua Madrileña Foundation / Fundación Mutua Madrileña</strong></td>
<td></td>
</tr>
<tr>
<td>Jiménez, María</td>
<td>JunBEAP-1 supresor tumoral en la piel. Mecanismos moleculares e interacción funcional con p53</td>
</tr>
<tr>
<td><strong>Ramon Areces Foundation / Fundación Ramón Areces</strong></td>
<td></td>
</tr>
<tr>
<td>Montoya, Guillermo</td>
<td>Desarrollo de bisturíes moleculares para la reparación de genes implicados en enfermedades monogénicas</td>
</tr>
<tr>
<td>Serrano, Manuel</td>
<td>Reprogramación nuclear in vivo e interrelación funcional entre p27 y Sox2</td>
</tr>
<tr>
<td><strong>Spanish Society of Medical Oncology / Sociedad Española de Oncología Médica (SEOM)</strong></td>
<td></td>
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<tr>
<td>Olmos, David</td>
<td>Validación de una firma de expresión con utilidad pronóstica en cáncer de próstata resistente a la castración en una cohorte multi-institucional de pacientes tratados con docetaxel</td>
</tr>
<tr>
<td><strong>AstraZeneca Foundation / Fundación AstraZeneca</strong></td>
<td></td>
</tr>
<tr>
<td>Olmos, David</td>
<td>Cáncer de próstata familiar y esporádico asociado a alteraciones genéticas, germinales y/o somáticas, en genes de la reparación del DNA</td>
</tr>
<tr>
<td><strong>BBVA Foundation / Fundación BBVA</strong></td>
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<tr>
<td>Sánchez, Alberto</td>
<td>Desarrollo de nuevas herramientas diagnósticas no invasivas por imagen para el diagnóstico del glioblastoma multiforme, el tumor cerebral más maligno</td>
</tr>
<tr>
<td><strong>Fero Foundation / Fundación Fero</strong></td>
<td></td>
</tr>
<tr>
<td>Valiente, Manuel</td>
<td>Predictive biomarkers for brain metastasis in small cell lung cancer</td>
</tr>
</tbody>
</table>

### Acquisition of Scientific and Technical Equipment

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>González, David</td>
<td>Scientific data storage infrastructure</td>
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</tbody>
</table>

### Action to Promote the Communication of Scientific and Technical Results or Innovation in High-Level International Conferences

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peñuelas, Héctor</td>
<td>Metastasis Initiation: Mechanistic and Therapeutic Opportunities</td>
</tr>
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</table>

### Excellence-Europe / Europa Excelencia

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodríguez, Cristina</td>
<td>ANGIOMARKER: Predicting antiangiogenic drug response in cancer: markers and mechanisms</td>
</tr>
<tr>
<td>Valente, Manuel</td>
<td>BrainMET: Deconstructing metastatic disease in the brain Research-Europe / Europa Investigación</td>
</tr>
</tbody>
</table>

### Research-Europe / Europa Investigación

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valencia, Alfonso</td>
<td>CancerCanAdvisor: An open bioinformatics platform for personalized treatment of cancer</td>
</tr>
</tbody>
</table>

### Networks and Scientific Managers-Europe / Europa Redes y Gestores

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasco, María A.</td>
<td>CNIO in Horizon 2020: support for proposal preparation and project management Young Researchers Program/Programa Jóvenes Investigadores</td>
</tr>
</tbody>
</table>

### Young Researchers Program / Programa Jóvenes Investigadores

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Project Title</th>
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<tbody>
<tr>
<td>Álvarez, Mónica</td>
<td>GPGenCan: Functional relevance of Greatwall/PP2A pathway in the maintenance of genomic stability: therapeutic implications in cancer</td>
</tr>
<tr>
<td>Lecona, Emilio</td>
<td>UBQREP: Modulation of DNA Replication by ubiquitination of chromatin proteins</td>
</tr>
</tbody>
</table>
Additionally, students can apply for laboratory training offers international opportunities for bright and dynamic university graduates, of all nationalities, to pursue an ambitious PhD project. To attest this, 15 students obtained their PhD degrees in 2015 and 26 more joined the CNIO in that same year. One third of the 105 students working at the CNIO in 2015 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

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’s 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 year of study in the biomedical field. The Programme encompasses 8 weeks of full-time laboratory training (300.5 hours). During this time the students actively participate in research projects in one of the CNIO’s groups. During 2015, 9 students from 5 countries participated in this programme.

→ Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO’s individual Research Groups or Units. This year, 73 students participated in these programmes, of which 3 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, 15 students obtained their PhD degrees in 2015 and 26 more joined the CNIO in that same year. One third of the 105 students working at the CNIO in 2015 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 the 4 such centres, to launch a programme for outstanding pre-doctoral students from all over the world who have an interest in pursuing an ambitious PhD project. Since 2013, the Ministry of Economy 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 2015, 2 pre-doctoral students received one of these recognised fellowships.

The distribution of students across the CNIO’s Research Programmes in 2015 was as follows: 54.3% of students worked in the Molecular Oncology Programme, 13.3% in the BBVA Foundation-CNIO Cancer Cell Biology Programme, 9.5% in the Structural Biology and Biocomputing Programme, 12.4% in the Human Cancer Genetics Programme, 6.7% in the Clinical Research Programme, 2.9% in the Biotechnology Programme and the remaining 1.0% in the Experimental Therapeutics.

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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 successfully advance their careers in the healthcare sector. During 2015, the CNIO signed several new agreements with Spanish Universities and other institutions such as Ministries and secondary schools; namely, with the Universidad Politécnica de Madrid, Universidad Católica de Murcia, Universidad de Castilla La Mancha, Universidad Autónoma de Barcelona, Universidad Complutense de Madrid, Universidad de la Coruña, Universidad de Sevilla, Ministerio de Educación, Cultura y Deporte, IES Moratalaz (Madrid), IES Benjamin Bust (Móstoles, Madrid), IES Jaime Fernández Claro (San Fernando de Henares, Madrid), IES Ramón y Cajal (Valladolid), Centro de Formación Baxona (Avilés, Asturias) and Centro Educativo OFESA (Madrid). These agreements formalise the procedures that are to be followed in order to enable students from the aforementioned institutions to perform laboratory practices.

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POST-DOCTORAL TRAINING

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

In 2015, 48 postdoctoral fellows worked at the CNIO. Notably, 42% of these fellows were from outside of Spain, many coming from very prestigious international institutions.

Once again in 2015, the Fundación Banco Santander agreement with the CNIO resumed 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 Memorial Sloan Kettering Institute of Cancer Research (New York), was awarded a Santander Foundation-CNIO Fellowship in 2015.

FUNDING SOURCES OF POST-DOCTORAL RESEARCHERS

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<tr>
<td>Ministry of Economy and Competitiveness / Ministerio de Economía y Competitividad (I+D Projects)</td>
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<tr>
<td>Institute of Health Carlos III / Instituto de Salud Carlos III (ISCIII) (I+D Projects)</td>
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<td>Community of Madrid / Comunidad Autónoma de Madrid (I+D Projects)</td>
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<td>Madrid-MIT M+Vision (Post-doctoral Fellowships)</td>
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<td>European Research Council</td>
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<td>Association for International Cancer Research</td>
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<td>AYA</td>
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<td>Daiichi Sankyo Agreement</td>
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<tr>
<td>Federation of the Societies of Biochemistry and Molecular Biology (Post-doctoral Fellowship)</td>
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<tr>
<td>Hoffmann-La Roche</td>
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<tr>
<td>Leukemia and Lymphoma Society (Post-doctoral Fellowship)</td>
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<td>PRzer</td>
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TOTAL 48

POSTGRADUATE PROGRAMMES

In addition, the CNIO – in collaboration with 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, Master’s in Molecular and Cell Biology, Master’s in Molecular Biomedicine, and Master’s 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


Programas de Postgrado Universidad CEU San Pablo

Máster Universitario en Investigación Clínica y Aplicada en Oncología.
LABORATORY TRAINING FOR TECHNICIANS

This training programme has been developed for students in Anatomical Pathology and is organised through agreements with 16 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 (704-712 hours) of laboratory training for students. Additionally, the CNIO offered real-life work experience to 1 student of Analytical Assays and Quality Control for 11 weeks (370 hours); to 2 students of Clinical Diagnosis for 14 weeks (380 hours); and to 3 students of Medical Archiving and Recording for 14 weeks (440 hours). Of the 27 students who participated in this programme in 2015, 5 were hired by the CNIO.

TRAINING FOR MDS

In line with CNIO’s commitment to bridge the ‘bench to bedside’ gap, the Centre offers excellent training opportunities in molecular diagnostics and familial cancer genetics to MDs and other health care professionals; this initiative is a collaborative effort with the Spanish Ministry of Health (current Ministerio de Sanidad, Servicios Sociales e Igualdad). Training usually consists of a 3-month period during residency. In 2015, 25 medical residents from 14 different hospitals enjoyed the benefits of rotations within the different Groups and Units of the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2015, 11 scientists were supported by the Ramón y Cajal Programme. This special initiative, established in 2001 by the former Spanish Ministry of Science and Technology (now Spanish Ministry of Economy 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. Twenty other scientists were funded by similar programmes, including the Miguel Servet (3 contracts), Sara Borrell (1 contract) and Río Hortega (2 contracts) programmes, Juan de la Cierva programme (Spanish Ministry of Economy and Competitiveness, 3 contracts) and the Spanish Association Against Cancer (AECC, 8 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 2015, Eva Nogales from the University of California in Berkeley (USA), Chaitanya Divgi from the Columbia University in New York (USA), Marcin Nowotny from the Institute of Molecular and Cell Biology in Warsaw (Poland) and Patrik Sung from the Yale University in New Haven (USA) were beneficiaries of the Jesús Serra Foundation’s Visiting Researcher Programme.

‘SCIENCE BY WOMEN’ PROGRAMME

The Women for Africa Foundation launched the ‘Science by Women’ programme aimed at promoting access for African women to science and technology by inviting them to “Severo Ochoa” Centres of Excellence to benefit from sabbatical stays in Spain. CNIO will host 3 of them.
CNIO FRONTIERS MEETINGS

CNIO Frontiers Meetings are the main international conferences that are organised by the CNIO. 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.

NEW TRENDS IN ANTICANCER DRUG DEVELOPMENT

25-25 MARCH, 2015

ORGANISERS
- Manuel Hidalgo, CNIO, Madrid, Spain
- Alberto Bardelli, IRCC, Torino, Italy
- Lillian Siu, Princess Margaret Cancer Centre, Toronto, Canada
- Josep Tabernero, VHI0, Barcelona, Spain

SESSIONS
- New Targets-Pathways in Clinical Development (1)
- Innovative Approaches in Drug Development
- New Targets-Pathways in Clinical Development (2)
- Immunotherapy Approaches for Cancer Treatment
- Personalizing Cancer Treatment

SPEAKERS
- Gerhard Attard, ICR, London, UK
- Mariano Barbacid, CNIO, Madrid, Spain
- Alberto Bardelli, University of Turin - Candido Cancer Institute IRCCS, Italy
- Maria Blasco, CNIO, Madrid, Spain
- Hilary Calvert, UCL, London, UK
- Luis Diaz, Johns Hopkins University, Baltimore, US
- Jeffrey Engelman, Massachusetts General Hospital Cancer Center, US
- Manel Esteller, IDIBELL, Barcelona, Spain
- Oscar Fernández Capetillo, CNIO, Madrid, Spain
- Manuel Hidalgo, CNIO, Madrid, Spain
- Sunil R Hingorani, Fred Hutchinson Cancer Research Center, Seattle, US
- Timothy Hoey, OncoMed Pharmaceuticals, Redwood City, US
- Tak W. Mak, Princess Margaret Cancer Centre, University of Toronto, Canada
- Ignacio Molero, CIMA & Clinica Universidad de Navarra, Pamplona, Spain
- Drew Purdie, Johns Hopkins University, Baltimore, US
- William Pao, Roche Pharma Research & Early Development, Basel, Switzerland
- Ingrid Sasson, Sanofi Oncology, Paris, France
- David J. Shields, Pfizer Inc., NY, US
- Lillian Siu, Princess Margaret Cancer Centre, Toronto, Canada
- Mario Sznol, Yale University, New Haven, US
- Josep Tabernero, VHI0, Barcelona, Spain
- Jaap Verweij, Erasmus University Medical Center, Rotterdam, The Netherlands
- Elisabeth G. de Vries, University Medical Center, Groningen, The Netherlands
- Ann L. White, University of Southampton, UK

In addition, 5 short talks were selected among participants’ contributions and 9 posters were presented.

MECHANISTIC INSIGHTS AND THERAPEUTIC OPPORTUNITIES

28-30 SEPTEMBER, 2015

ORGANISERS
- David Lyden, Weill Cornell Medical College, New York, US
- Yihin Kang, Princeton University, New Jersey, US
- Gemma Alderton, Nature Reviews Cancer, London, UK
- Victoria Aranda, Nature Medicine, New York, US
- Li-Kuo Su, Cancer Cell, Cambridge, US
- Héctor Peinado, CNIO, Madrid, Spain

SESSIONS
- Cell Fate Regulation, Stem Cells and Metastasis
- Epithelial-to-Mesenchymal Transition
- Circulating Factors: Microvesicles and Exosomes
- Circulating Factors: Circulating Tumor Cells/Platelets/Circulating DNA and RNA
- Pre-Metastatic Niche
- Disseminated, Dormant and Metastasis-Initiating Tumor Cells
- Organ-Specific Metastasis and Micrometastatic Disease
- Imaging Early Metastatic Events
- Targeting Metastasis
- Modeling Metastasis

SPEAKERS
- Julio Aguirre-Ghiso, Mount Sinai Medical Center, New York, US
- Salvador Aznar Benitah, Institute for Research in Biomedicine, Barcelona, Spain
- Thomas Brabletz, University of Erlangen- Nuremberg, Germany
- Janine Erler, Biotech Research & Innovation Centre (BRIC), University of Copenhagen, Denmark
- Brunhilde H. Felding, The Scripps Research Institute, La Jolla, US
- Cyrus Ghajari, Fred Hutchinson Cancer Research Center, Seattle, US
- Amato Giaccia, Stanford School of Medicine, US
- Kent W. Hunter, Center for Cancer Research National Cancer Institute, Bethesda, US
- Joan Massagué, Memorial Sloan Kettering Cancer Center, New York, US
In addition, 12 short talks were selected among participants’ contributions and 42 posters were presented.
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, 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 COURSE**
  - 9-10-11 FEBRUARY, 2015
  - Speakers/Organisers
    - Rui Gardner, Institute Gulbenkian of Science, Portugal
    - Lola Martínez, CNIO, Spain

- **CELL SORTING COURSE**
  - 12-13 FEBRUARY, 2015
  - Speakers/Organisers
    - Rui Gardner, Institute Gulbenkian of Science, Portugal
    - Lola Martínez, CNIO, Spain

- **HANDS-ON INTRODUCTION TO R 2015**
  - 1 JULY, 2015
  - Organiser
    - CNIO Bioinformatics
  - Speaker
    - Ramón Díaz Uriarte, Institute of Biomedical Research Alberto Sols, Spain

- **ACCESS TO ENCODE DATA THROUGH THE UCSC GENOME BROWSER**
  - 4 NOVEMBER, 2015
  - Organiser
    - CNIO Bioinformatics
  - Speakers
    - Osvaldo Graña and David G. Pisano, CNIO, Spain

- **INTRODUCTION TO FUNCTIONAL ANALYSIS OF GENE EXPRESSION EXPERIMENTS**
  - 25 NOVEMBER, 2015
  - Organiser
    - CNIO Bioinformatics
  - Speakers
    - Gonzalo Gómez and Daniel Rico, CNIO, Spain
**CNIO Distinguished Seminars**

The purpose of the Distinguished Seminars Series is to invite outstanding and internationally renowned scientists to give a seminar and to meet with researchers at the CNIO. Distinguished Seminars are recurrent events that are open to the 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. This year, the French Embassy sponsored one of these seminars. The purpose of the Distinguished Seminars Series is to invite a speaking program of high quality, with about 30 extended talks, of which about 15 will be distinguished speakers. In total, the CNIO hosted 21 distinguished speakers in 2015.

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<tr>
<th>DATE</th>
<th>SPEAKER</th>
<th>ORGANISATION</th>
<th>TITLE</th>
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</thead>
<tbody>
<tr>
<td>16/01/2015</td>
<td>Maria José García Borge</td>
<td>ISODIE, CERN, Geneva, Switzerland</td>
<td>The Nucleus: a trip to the heart of the matter, and its societal applications</td>
</tr>
<tr>
<td>23/01/2015</td>
<td>Ignacio Cirac</td>
<td>The Max Planck Institute, Munich, Germany</td>
<td>Quantum Physics: From the Schrödinger cat to the most powerful computers</td>
</tr>
<tr>
<td>06/02/2015</td>
<td>Margaret C Frame</td>
<td>Edinburgh Cancer Research Centre, College of Medicine and Veterinary Medicine, University of Edinburgh, UK</td>
<td>Imaging and targeting cancer processes</td>
</tr>
<tr>
<td>20/02/2015</td>
<td>Maria Elena Torres Padilla</td>
<td>Institute of Genetics and Molecular and Cellular Biology, (IGBMC), Illkirch France</td>
<td>Epigenetic mechanisms in early mammalian development</td>
</tr>
<tr>
<td>29/05/2015</td>
<td>James Berger</td>
<td>Johns Hopkins University School of Medicine, Baltimore, USA</td>
<td>Running rings (and spirals) around DNA—molecular mechanisms for initiating replication</td>
</tr>
<tr>
<td>19/06/2015</td>
<td>Elya Tanaka</td>
<td>Center for Regenerative Therapies, Dresden - CRTD, Germany</td>
<td>Proliferation to patterning during vertebrate limb regeneration</td>
</tr>
<tr>
<td>04/09/2015</td>
<td>James Hurley</td>
<td>University of California, Berkeley, USA</td>
<td>From HIV pathogenesis to coated vesicles, and back again</td>
</tr>
<tr>
<td>11/09/2015</td>
<td>Roger Williams</td>
<td>MRC Laboratory of Molecular Biology, Cambridge, UK</td>
<td>Structures and dynamics of phosphoinositide 3-kinase complexes in cellular signaling and sorting</td>
</tr>
<tr>
<td>18/09/2015</td>
<td>Sianon Gordon</td>
<td>University of Oxford, UK</td>
<td>Macrophage receptors and immune interactions</td>
</tr>
<tr>
<td>25/09/2015</td>
<td>Megan C. King</td>
<td>Yale University, New Haven, USA</td>
<td>The cell biology of DNA repair</td>
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**SCIENTIFIC EVENTS**

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<th>DATE</th>
<th>SPEAKER</th>
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<tr>
<td>12/09/2015</td>
<td>Jean-Pierre Cluzet</td>
<td>Pasteur Institute, Collège de France, Institut Pasteur, Paris, France</td>
<td>“Out of the box” seminars in contemporary and future technological, societal and cultural challenges that goes far beyond the frontiers of cancer research.</td>
</tr>
<tr>
<td>14/10/2015</td>
<td>David Brar</td>
<td>Biotech Research and Innovation Centre, Amsterdam, The Netherlands</td>
<td>Epigenetic mechanisms in early mammalian development</td>
</tr>
<tr>
<td>16/11/2015</td>
<td>Marita Howard</td>
<td>The National Center for Scientific Research, Cambridge, USA</td>
<td>“Emerging topics in cancer research” and “The role of inflammation in cancer”</td>
</tr>
<tr>
<td>01/12/2015</td>
<td>Valeria Niculescu</td>
<td>IE Business School, Madrid, Spain</td>
<td>“The impact of lifestyle on cancer” and “The role of inflammation in cancer”</td>
</tr>
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**ANNUAL REPORT 2015**

The ANNUAL REPORT 2015 provides an overview of the activities and achievements of the CNIO during the year 2015. It highlights the contributions of the CNIO faculty and staff to the advancement of cancer research and medicine. The report includes information on the CNIO’s research programs, publications, grants, and collaborations. It also features an extensive list of the CNIO’s distinguished speakers and their contributions to the field of cancer research.

**FACTS & FIGURES**

- **18/09/2015**: “Out of the box” seminars in contemporary and future technological, societal and cultural challenges that goes far beyond the frontiers of cancer research.
- **14/10/2015**: Epigenetic mechanisms in early mammalian development.
- **16/11/2015**: “Emerging topics in cancer research” and “The role of inflammation in cancer”.
- **01/12/2015**: “The impact of lifestyle on cancer” and “The role of inflammation in cancer”.

The report also includes a comprehensive list of the CNIO’s distinguished speakers and their contributions to the field of cancer research.
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<tr>
<th>DATE</th>
<th>SPEAKER</th>
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<tbody>
<tr>
<td>JANUARY</td>
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<tr>
<td>01/02/2015</td>
<td>Fabio Rinaldi</td>
<td>Institute of Computational Linguistics, University of Zurich, Switzerland</td>
<td>Large-scale biomedical text mining for knowledge discovery</td>
</tr>
<tr>
<td>02/02/2015</td>
<td>Tyler Alioto</td>
<td>Genome Assembly and Annotation Team Leader CNAG, Barcelona, Spain</td>
<td>A comprehensive assessment of somatic mutation calling in cancer genomes</td>
</tr>
<tr>
<td>27/01/2015</td>
<td>Monika Hegi</td>
<td>University of Lausanne, Switzerland</td>
<td>Epigenetic deregulation in glioma, biomarkers and new opportunities</td>
</tr>
<tr>
<td>30/01/2015</td>
<td>Tony Mok</td>
<td>The Chinese University of Hong Kong, China</td>
<td>Blood-based genomic biomarkers for lung cancer</td>
</tr>
<tr>
<td>30/01/2015</td>
<td>Joan Via Domenach</td>
<td>REGICOR, Barcelona, Spain</td>
<td>Generación de informes reproducibles utilizable para la salud</td>
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<tr>
<td>FEBRUARY</td>
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<tr>
<td>16/02/2015</td>
<td>Ignacio I. Wistuba</td>
<td>Anderson Clinical Faculty Chair for Cancer Treatment and Research - Department of Translational Molecular Pathology - UT MD Anderson Cancer Center, Houston, USA</td>
<td>Molecular pathogenesis of lung cancer</td>
</tr>
<tr>
<td>17/02/2015</td>
<td>Flora de Pablo</td>
<td>CSIC, Madrid, Spain</td>
<td>Sex, Science and Society: a triangle that matters?</td>
</tr>
<tr>
<td>24/02/2015</td>
<td>Guillaume Filion</td>
<td>CRG, Barcelona, Spain</td>
<td>Promoters interpret the chromatin context in different ways</td>
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<tr>
<td>MARCH</td>
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<tr>
<td>10/03/2015</td>
<td>Maria Carmen</td>
<td>Secretariat of State of Research, Development and Innovation, Ministry of Economy and Competitiveness, Madrid, Spain</td>
<td>A professional career with a gender perspective</td>
</tr>
<tr>
<td>26/03/2015</td>
<td>Manuel Fernando Ganvito</td>
<td>University of the Andes, Bogotá, Colombia</td>
<td>Targeting the pyrimidine metabolism in a devastating plant pathogen</td>
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<tr>
<td>27/03/2015</td>
<td>Nuria Flames</td>
<td>Biomedical Institute of Valencia, (IBV-CSIC), Valencia, Spain</td>
<td>Regulatory logic of serotonergic neuron terminal differentiation in CateGlias</td>
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<tr>
<td>APRIL</td>
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<tr>
<td>10/04/2015</td>
<td>Roel Verhaak</td>
<td>Department of Genomic Medicine, and the Department of Bioinformatics and Computational Biology at the UT MD Anderson Cancer Center in Houston, USA</td>
<td>Genomic characterization of disease evolution in glioma</td>
</tr>
<tr>
<td>10/04/2015</td>
<td>Alan Clarke</td>
<td>Cardiff University, UK</td>
<td>PS1K and Wnt pathway-driven neoplasia modelled in the mouse</td>
</tr>
<tr>
<td>13/04/2015</td>
<td>Curtis Harris</td>
<td>National Cancer Institute, Bethesda, USA</td>
<td>Interweaving the threads of JASp, microRNA, DNA methylation and information networks into the tapestry of cancer and aging</td>
</tr>
<tr>
<td>14/04/2015</td>
<td>Margarita Salas Felipueas</td>
<td>CIBER, Madrid, Spain</td>
<td>My life with phage-29</td>
</tr>
<tr>
<td>16/04/2015</td>
<td>Liset Menendez de la Prada</td>
<td>Instituto Cajal-CSIC, Madrid, Spain</td>
<td>Electrophysiological biomarkers of epileptogenesis</td>
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<tr>
<td>16/04/2015</td>
<td>Alejo Eleyan</td>
<td>MIT, Cambridge, USA</td>
<td>Physiology of nutrient sensing by mTOR</td>
</tr>
<tr>
<td>23/04/2015</td>
<td>Jean Pierre David</td>
<td>Universitätsklinikum Hamburg, Germany</td>
<td>From twelfth to loss, from arthritis to tumors, in the skeleton, Ral2 makes it all</td>
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<tr>
<td>MAY</td>
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<tr>
<td>05/05/2015</td>
<td>Robert Lowe</td>
<td>Medical University of Vienna, Department of Dermatology, Austria</td>
<td>Identification of a chemokine profile associated with melanoma progression</td>
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<tr>
<td>06/05/2015</td>
<td>Katherine Hoadley</td>
<td>Lineberger Comprehensive Cancer Centre, UNC Chapel Hill, USA</td>
<td>TCGA pan-cancer subtype analysis</td>
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<tr>
<td>14/05/2015</td>
<td>Jesús Rojo</td>
<td>The Marie Sklodowska-Curie Action (MSCA) Spanish National Contact Point. Fundación para el Conocimiento madríd</td>
<td>2015 call from the EU-funded Horizon 2020 programme, Marie Sklodowska Curie Actions (MSCA) -- Individual Fellow (IF)</td>
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<td>14/05/2015</td>
<td>Caroline Beckett &amp; Joanne Wodarcz</td>
<td>Sigma-Aldrich Corporation, St. Louis, USA</td>
<td>Targeted genome editing using CRISPR technology</td>
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<td>21/05/2015</td>
<td>Carme Castells</td>
<td>Biochemistry &amp; Molecular Biology, School of Pharmacy, University of Barcelona, Spain</td>
<td>Regulation ofelin signaling by JNK: consequences on systemic insulin resistance</td>
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<tr>
<td>22/05/2015</td>
<td>Erica Sloan</td>
<td>Monash Institute of Pharmaceutical Sciences, Monash University, Faculty of Pharmacy, Victoria, Australia</td>
<td>Beta-blockade of cancer: repurposing old drugs to block metastasis</td>
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<tr>
<td>Name</td>
<td>Affiliation</td>
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<tr>
<td>Sandra Rodríguez</td>
<td>Bioinformatics approaches for chromatin, The University of Melbourne, Australia</td>
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<td>Laura Soucek</td>
<td>The University of Melbourne, Australia</td>
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<td>Daniel Gianola</td>
<td>North Carolina State, Dublin City University, Ireland</td>
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<td>Theresa Guise</td>
<td>BioMoDeL; University of California, US</td>
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<td>ACRISP Research Associate, PhD student, Accredited Exercise Physiologist (ESSA) Federation University of Science, Victoria, Australia</td>
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<tr>
<td>Women at the top: making it happen</td>
<td>WISE SEMINAR</td>
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<td>Myc-driven transcriptional programs in tumor development: toward new therapeutic opportunities</td>
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<td>Microbial and cytokine drivers of tumor elicited inflammation in colorectal cancer</td>
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<td>Deconstructing molecular machines using single molecule methods</td>
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<td>John Harrington</td>
<td>IBM-Watson. IBM Watson Oncology: “A rapid way to accelerate human expertise in oncology”</td>
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<td>Rudolf Zechar</td>
<td>Institute of Molecular Biosciences, Karl Franzens Universität Graz, Austria</td>
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<tr>
<td>Pau Riu</td>
<td>Cell fate decision-making by the numbers: development and differentiation</td>
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<td>Laura Sonneck</td>
<td>Vall d’Hebron Institute of Oncology, Barcelona, Spain</td>
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<tr>
<td>Chaltanya R. Dirgi</td>
<td>Molecular imaging of the cancer phenotype</td>
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<tr>
<td>Mª Dolores Alonso Guirado</td>
<td>Bioinformatics and Biostatistics Service, Biological Research Center, CSIC, Madrid, Spain</td>
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<tr>
<td>Genome assembly of a filamentous fungus from Illumina sequencing reads using different approaches</td>
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<tr>
<td>Ana Barat</td>
<td>Dublin City University, Ireland</td>
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<tr>
<td>Bioinformatics approaches for chromatin, development and cancer research</td>
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<tr>
<td>Department of Animal Sciences, Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, USA</td>
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<tr>
<td>Prediction of complex traits</td>
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<td>Babraham Institute, Nuclear Dynamics Programme, Cambridge, UK</td>
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<td>Genomic regulatory architecture in human hematopoiesis links disease-associated variants with their target genes</td>
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<td>Uppsala University, Sweden</td>
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<td>A cell of origin-based strategy to decipher glioblastoma biology</td>
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<td>The University of Melbourne, Australia</td>
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<tr>
<td>Protein tyrosine phosphatases: molecular switches in pathology</td>
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<tr>
<td>Graham Robertson</td>
<td>School of Molecular Biosciences, University of Sydney, Australia</td>
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<td>Prem Premrunit</td>
<td>Mirimu, Inc., a spin-off company of Cold Spring Harbor Laboratory and Stony Brook University School of Medicine, NY, US</td>
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<td>Dimirrio Morakis</td>
<td>National Institutes of Health (NIH), Rockville, USA</td>
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<td>Natalia González-Valdés</td>
<td>Corporate Communications and CSR (Corporate Social Responsibility) Director of L’Oréal Spain</td>
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<tr>
<td>Roberto Buccione</td>
<td>EMBO Molecular Medicine, Heidelberg, Germany</td>
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<td>Data reproducibility, research integrity and the EMBO Press transparent editorial process</td>
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<td>Data reproducibility, research integrity and the EMBO Press transparent editorial process</td>
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This year, the CNIO participated in Researchers’ Night, an activity aimed at bringing researchers closer to the general public and concerned families in order to give them the opportunity to learn more about what researchers do for society. Each year, more than 300 European cities participate, in parallel, in 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 200 people came to the Spanish National Cancer Research Centre (CNIO) to attend Researchers’ Night (September 25, 2015) and learn about cancer research. The activities were entirely organised by voluntary contributions from 30 young researchers, and 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.

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 (XV Semana de la Ciencia, November 2–15, 2015). In 2015, 50 people participated in guided visits to the Centre’s research facilities.

This year, the CNIO participated for a third time in the annual meeting of the Spanish Group of Patients with Cancer (GEPAC), whose membership includes foundations such as the CRIS Foundation against cancer, AEAI, AECAT and the Sandra Ibarra Foundation – all supporters of the CNIO. This large congress, held in Madrid, is open to the members of the general public who are affected by or interested in cancer. Various societies, interest groups and pharmaceutical companies affiliated with oncology also participate in this event. It was a privilege for us to participate with our stand for the third year running. The idea was to be present so that we could answer people’s questions about cancer research and the latest developments.

Throughout the year, the CNIO provides tailor-made opportunities to visit its installations and to learn about the essentials of cancer research. During 2015, more than 498 people participated in such guided visits; most of them were ESO and Bachillerato student groups, but also professionals in the health sector.

On the 4th of February, the CNIO celebrated World Cancer Day by hosting an open-doors day. The event, sponsored by Bristol-Myers Squibb, welcomed patients, associations, relatives and anyone with an interest in learning more about recent advances in cancer research.

The CNIO Director, María Blasco, opened the event by introducing the Centre and its lines of research. Afterwards, a first debate entitled ‘The future of cancer research and clinical oncology’, was led by Manuel Hidalgo, the Director of CNIO’s Clinical Research Programme, and Eduardo Díaz-Rubio, the Director of the Medical Oncology Service at the San Carlos Clinical University Hospital. After the talks, the registered attendees had the opportunity to visit the Centre’s facilities.

During this year, the company bcnmoments organised the ‘Leading Program Madrid’, a programme sponsored by the ‘la Caixa’ Foundation that awards the 20 highest selected 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, the students had the chance to visit the labs guided by young scientists.

On the 19th of October, we hosted the “Innovation: Bridge between Science and Society” event at the CNIO, together with the Banco Santander Foundation and the Instituto de Empresa Business School. The event consisted of a dialogue between the Autonomous University of Madrid professor and former Minister of Education, Ángel Gabilondo, and the CNIO Director, María Blasco, they highlighted the CNIO’s commitment to innovation and the translation of scientific knowledge for the benefit of society.
ADMINISTRATION

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  Ministro de Economía y Competitividad

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  Secretariat of State for Research, Development and Innovation
  Secretaría de Estado de Investigación, Desarrollo e Innovación

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

Appointed Members
- José Javier Castrédexa
  Secretary General for Health and Consumer Affairs
  Secretario General de Sanidad y Consumo

- Marina Villegas Gracia
  Director General for Scientific and Technical Research, Ministry of Economy and Competitiveness
  Directora General de Investigación Científica y Técnica del Ministerio de Economía y Competitividad (MINECO)

- Cristina Ysasi-Ysaseñendi Pemán
  Director of National Affairs of the Cabinet of the Presidency of the Government
  Directora de Asuntos Nacionales del Gabinete de la Presidencia del Gobierno

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

- Sandra García Armesto
  Managing Director, Aragon Institute of Health Sciences
  Directora Gerente del Instituto Aragonés de Ciencias de la Salud

- 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

- Isabel Ansa Erice
  Director General of Health, Health Department of the Government of Navarra
  Directora General de Salud de la Consejería de Salud del Gobierno de Navarra

- Luis Ángel León Mateos
  Director of Health Research Planning, Galician Agency for Health Knowledge Management
  Director del Área de Planificación de Investigación Sanitaria de la Agencia Gallega para la Gestión del Conocimiento en Salud

Elected Members
- Jaume Giró Ribas
  Director General of “la Caixa” Banking Foundation Caixa d’Estalvis i Pensions de Barcelona
  Director General de la Fundación Bancaria Caixa d’Estalvis i Pensions de Barcelona, “la Caixa”

- Rafael Pardo Avellaneda
  Director, BBVA Foundation
  Director de la Fundación BBVA

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

- Pío Díaz de Tuesta Vázquez
  Legal Advisor, Cuja Madrid Foundation
  Aesor Jurídico de la Fundación Cuja Madrid

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

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

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 213,353 Euros. This amount was received as base salary, seniority, small bonuses.
- Members of the CNIO Board of Trustees are not remunerated.
SCIENTIFIC ADVISORY BOARD

- 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 and Professor
  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

- 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

- Genevieve 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

- Julio E. Celis, PhD
  Professor and Associate Scientific Director
  Danish Cancer Society Research Center
  Copenhagen, Denmark

- 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
  Director
  Clare Hall Laboratories
  Cancer Research UK London Research Institute
  Potters Bar, Hertfordshire, United Kingdom

- Denise Galloway, PhD
  Associate Director, Human Biology Division
  Fred Hutchinson Cancer Research Center
  Research Professor of Microbiology at the University of Washington
  Seattle, USA

- Scott W. Lowe, PhD
  Chair, Geoffrey Beene 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
MANAGEMENT

DIRECTOR
Blasco, Maria A.

SECRETARIATE
Alcamí, María Jesús

DIRECTOR’S OFFICE
Peláez, Fernando

COMMUNICATION
Noriega, Nuria
Head

SECRETARIATE (COMMUNICATION, INNOVATION, SCIENTIFIC MANAGEMENT)
Rodríguez, M. Carmen

INNOVATION
TECHNOLOGY TRANSFER & VALORIZATION OFFICE
Sanz, Anabel
Director
M. Cruz Marín (Technology Transfer Manager)

Rocío Manzano (since December)

ROBOTS & AUTOMATION

SCIENTIFIC MANAGEMENT
Barthelemmy, Isabel
Director

PROJECTS & CONSORTIA
Lílñanes, M. Dolores
Head
Almendros, Amaia

Araiza, Raquel (since February)
Merino, Ana

EDUCATION & TRAINING PROGRAMMES
Molina, Juan Ramón
Head

SCIENTIFIC EVENTS
More, Mercedes
Head

SCIENTIFIC PUBLISHING
Cerdá, Sonia
Head

LIBRARY & ARCHIVES
López, Victoria
Head

SECRETARIATE (COMMUNICATION, INNOVATION, SCIENTIFIC MANAGEMENT)
Vanessa Pombo
(Communications Officer)

DIRECTOR’S OFFICE
Peláez, Fernando

COMMUNICATION
Noriega, Nuria
Head

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Head

SCIENTIFIC PUBLISHING
Cerdá, Sonia
Head

LIBRARY & ARCHIVES
López, Victoria
Head

SECRETARIATE (COMMUNICATION, INNOVATION, SCIENTIFIC MANAGEMENT)
Vanessa Pombo
(Communications Officer)

MANAGING DIRECTOR
Arroyo, Juan

SECRETARIATE
Ámez, María del Mar

FINANCE & ADMINISTRATION
Fontaneda, Manuel
Director

PURCHASING
Álamo, Pedro
Head
Baviano, Marta

García-Andrade, Javier
Novillo, Angélica

HUMAN RESOURCES
Pérez, José Lorenzo
Head
Bardaji, Paz
Carbó, David
Martin, Francisco

ECONOMIC MANAGEMENT
Salido, M. Isabel
Head
Galindo, José Antonio
García, Juan A.

Rodríguez, M. José
Fernández, Bat
(since December)

AUDIT
García-Risco, Silvia
Head
Hernando, M. Elena

Doyagüez, Laura
(since December)

INFRASTRUCTURE MANAGEMENT
de Dios, Luis Javier
Director

MAINTENANCE
Vicente, Miguel
(Head, until May)
Garrido, Fernando
(Head, since December)

PREVENTION & BIOSECURITY
Cospín, Constantino
Head
Bertol, Narciso

INFORMATION TECHNOLOGIES
Fernández, José Luis
Head
de Miguel, Marcos

Escurriola, Rebeca
(since December)

EXTRAMURAL CLINICAL RESEARCH
López, Antonio
Director

* Plan de Empleo Joven (Youth Employment Plan)
CNIO PERSONNEL 2015

446 TOTAL CNIO PERSONNEL
401 RESEARCH 90%
45 ADMINISTRATION 10%

GENDER DISTRIBUTION
157 MALE 35%
289 FEMALE 65%

AGE DISTRIBUTION
102 31-40 23%
167 31-40 37%
36 > 50 8%
141 ≤30 32%

DISTRIBUTION BY PROGRAMMES
EXPERIMENTAL THERAPEUTICS 9%
CANCER CELL BIOLOGY 10%
BIOTECHNOLOGY 12%
HUMAN CANCER GENETICS 12%
CLINICAL RESEARCH 17%
MOLECULAR ONCOLOGY 29%
EXPERIMENTAL THERAPEUTICS 9%

DISTRIBUTION BY PROFESSIONAL CATEGORY
POST-DOCTORAL FELLOWS 9%
GRADUATE STUDENTS 23%
STAFF SCIENTISTS 17%
PRINCIPAL INVESTIGATORS 13%
TECHNICIANS 26%

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY
POST-DOCTORAL FELLOWS
FEMALE 61%
MALE 39%

GRADUATE STUDENTS
FEMALE 70%
MALE 30%

STAFF SCIENTISTS
FEMALE 65%
MALE 35%

PRINCIPAL INVESTIGATORS
FEMALE 37%
MALE 63%

TECHNICIANS
FEMALE 73%
MALE 27%

TOTAL SCIENTIFIC PERSONNEL
FEMALE 59%
MALE 41%

TOTAL SCIENTIFIC PERSONNEL 100%
401
### Distribution by Professional Category in: Basic Research

<table>
<thead>
<tr>
<th>Category</th>
<th>Scientific Personnel</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Doctoral Fellows</td>
<td>27</td>
<td>13%</td>
</tr>
<tr>
<td>Graduate Students</td>
<td>67</td>
<td>33%</td>
</tr>
<tr>
<td>Staff Scientists</td>
<td>37</td>
<td>18%</td>
</tr>
<tr>
<td>Principal Investigators</td>
<td>23</td>
<td>11%</td>
</tr>
<tr>
<td>Technicians</td>
<td>48</td>
<td>24%</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Distribution by Professional Category in: Translational Research

<table>
<thead>
<tr>
<th>Category</th>
<th>Scientific Personnel</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Doctoral Fellows</td>
<td>16</td>
<td>14%</td>
</tr>
<tr>
<td>Graduate Students</td>
<td>23</td>
<td>20%</td>
</tr>
<tr>
<td>Staff Scientists</td>
<td>20</td>
<td>17%</td>
</tr>
<tr>
<td>Principal Investigators</td>
<td>14</td>
<td>12%</td>
</tr>
<tr>
<td>Technicians</td>
<td>42</td>
<td>37%</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Distribution by Professional Category in: Innovation

<table>
<thead>
<tr>
<th>Category</th>
<th>Scientific Personnel</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Doctoral Fellows</td>
<td>1</td>
<td>7%</td>
</tr>
<tr>
<td>Graduate Students</td>
<td>4</td>
<td>5%</td>
</tr>
<tr>
<td>Staff Scientists</td>
<td>12</td>
<td>14%</td>
</tr>
<tr>
<td>Principal Investigators</td>
<td>14</td>
<td>5%</td>
</tr>
<tr>
<td>Technicians</td>
<td>53</td>
<td>41%</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Scientific Personnel: National Origin

- **Spanish**: 333 (83%)
- **Foreign**: 68 (17%)

### Distribution of Scientific Personnel by National Origin

- **Spain**: 333 (83%)
- **Rest of Europe**: 53 (13%)
- **Other**:
  - **America**: 10 (3%)
  - **Asia & Australia**: 5 (1%)
  - **Other**: 20 (5%)
  - **France**: 4 (1%)
  - **Austria**: 3 (1%)
  - **Portugal**: 6 (1%)
  - **Germany**: 5 (1%)
  - **Italy**: 15 (4%)

**Total scientific personnel**: 401
CNIO Friends

CNIO Friends
Super friend Marcos
‘Constantes y Vitales’ Campaign
Benefactor Friends/Sponsor Friends
Donations to the CNIO
One of the most exciting adventures we faced in 2015 was the implementation of the ‘CNIO Friends’ initiative, a philanthropic platform launched in late 2014 aimed at involving all social stakeholders in cancer research.

Under the slogan ‘More research, less cancer’, the CNIO is seeking a way to better fight the disease: through research and with everyone’s commitment, we shall combat cancer. The initiative not only responds to the need to appeal for economic support, but also aims at sharing, with the general public, the knowledge generated at a top-level centre like the CNIO, knowledge that can be used in medicine in the future.

This year, more than 560 friends, benefactors and sponsors have placed their trust in and directed their generosity towards the Centre’s research projects. The figures are extremely good and we hope to be able to continue progressing over the course of the next few years just as we have done to date. All our supporters can follow the Centre’s latest discoveries and developments through our ‘CNIO Friends’ newsletter, which was launched at the beginning of the year every other month, as well as enjoy other benefits. These benefits come in the shape of guided visits to the centre or the inscription of their names on the seats of the Auditorium, one of the most emblematic and special places in the Centre where researchers discuss and share new ideas and discoveries that revolutionise biomedicine day after day; this gesture enables our friends to symbolically partake in this important activity.

To promote our message, the Centre announced the launch of the ‘CNIO Friends’ social networks in February, which is aimed at creating a community and to connect with those who are fully acquainted with cancer research. Many messages of encouragement, support and congratulations are received each day through these channels; especially via our Facebook page, which, in only 10 months, has exceeded 26,000 followers and reached more than 44,600,000 people.

In June, after 6 months of the initiative’s launch, and to celebrate the occasion, the Centre paid tribute to its donors with a video that enabled them to explain the reasons that moved them to contribute so generously to the Centre’s research projects. The video, produced by the visual artist Amparo Garrido, has been watched almost 23,500 times on YouTube channels. In October, CNIO reached an agreement with RENFE (the Spanish rail transport operator), which resulted in the video production travelling the length and breadth of Spain in December by being broadcasted in its high speed - long distance trains.

Thanks to all the contributions received last year, the CNIO will launch ‘CNIO Friends’ contracts in 2016; a programme aimed at postdoctoral researchers developing new projects against cancer. Almost half of all tumours can be cured today, but there is still a long way to go. ‘CNIO Friends’ will serve to unite everyone’s efforts in this important task.

“Thanks to all the contributions received last year, the CNIO will launch ‘CNIO Friends’ contracts in 2016; a programme aimed at postdoctoral researchers developing new projects against cancer. Almost half of all tumours can be cured today, but there is still a long way to go. ‘CNIO Friends’ will serve to unite everyone’s efforts in this important task.”
“We must support research studies and the work of researchers. In Spain, these Centres of Excellence are essential; they should not only exist, but also have steady funding to carry out their work.”

JAVIER GÁLLEG

I’m investing in something that is being constructed and will endure over time. I have confidence in the team of researchers, whom I trust fully. They are a team of highly qualified professionals, and on a humane level it is number one.

CRUZ DÍAZ
“I felt I also had to do my bit to help. It gives you a sense of self-esteem and personal satisfaction to say: I am contributing a little to our society. A way of giving back to society in return for all it has provided me in other ways.”

NEMESIO CARRO

“I donate with my family. We consider research is a fundamental pillar for the advancement of society as a whole. You feel a certain satisfaction in saying: “They are doing something, partly ‘in memory of’, but that will also benefit future generations to come.”

MARINA LIMIÑANA
“Every Euro invested on this is a Euro invested in happiness. The other day I read that the CNIO is one of Europe’s leading Cancer Research Centres. And it is in Madrid, so you don’t have to go too far to collaborate here.”

MARCOS ARGUMOSA

In April, the sports world allied its efforts with the CNIO when the athlete Marcos Argumosa, our Super Friend Marcos, began a difficult but not impossible challenge: to run 10 consecutive marathons from Santander (Cantabria) to Madrid — 420 km in 10 days — to raise funds in support of ‘CNIO Friends’. All the companies and individuals interested in supporting Marcos could do so by purchasing kilometres through our online platform.

During the challenge, Marcos was not alone and received the support of city councils and provincial governments. On the 9th marathon, the finish line was set up at the CNIO headquarters with the support of the Banco Sabadell Foundation. That same day, CNIO scientists, including the Director, also grabbed their shoes and joined him for the last kilometres of the challenge.

As his endeavour received attention in the media, both in local radio stations and the news bulletins of Spain’s main TV channels, the engagement of new donors was boosted during those days, and in the weeks and months to come. We thank Marcos and our friends for his generosity and inspiration to help us spread the word of the initiative.
Another one of our achievements in 2015 was the agreement established with the TV & Media Group ‘Atresmedia’, one of the leading media groups in Spain. Through this agreement, ‘Constantes y Vitales’ – the social responsibility initiative of the TV channel laSexta and the AXA Foundation – launched the engaging action #CadaPasoEsVital (#EachStepMatters) with the aim of raising €100,000 in support of metastasis research at the CNIO led by Héctor Peinado, Group Leader of the Microenvironment and Metastasis Group. This campaign received enormous support from the community: more than 6,000 people donated 650,000 exercise kilometres through this platform, raising €100,000 destined to fund metastasis research led by Héctor Peinado at the CNIO.

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 €400,000 this year), in doing so they have contributed to society for generations to come.
**Benefactor Friends**

- Alberto Otero Hermida
  Bilbao, Bizkaia

- Alfonso Agüera Nieto
  Santa Ana-Cartagena, Murcia

- Amando Palomino López
  Cáceres, Cáceres

- Andrés Sánchez Arranz
  Madrid, Madrid

- Andrés Viedma Medina
  Jaén, Jaén

- Concepción Garófano Obregón
  San Fernando, Cádiz

- Diana Limiñana Gregori
  Mutxamel, Alicante

- Francisco Javier Gálibo Franco
  Barbastro, Huesca

- Gema Rubio González
  Madrid, Madrid

- José Carlos Fernández Martínez
  Madrid, Madrid

- José Limiñana Gregori
  Mutxamel, Alicante

- José Limiñana Valero
  Alicante, Alicante

- Jesús Miguel Iglesias Betuerto
  Valladolid, Valladolid

- Lesley Jackman
  Club femenino social, Castalla, Alicante

- Laisa Vázquez Bejarano
  Leganés, Madrid

- Marcelino Cordero Hernández
  Madrid, Madrid

- María Alonso Vaquero
  Madrid, Madrid

- María Begoña Toca
  Irún, Guipúzcoa

- María del Carmen Pérez Laborda
  Murcia, Murcia

- María Dolores Díaz Almagro
  Sevilla, Sevilla

- María Rodríguez López
  Colada de los Calderones, Cantabria

- Marina Limiñana Gregori
  Alicante, Alicante

- Miguel Ángel Fernández de Betsoño Villanueva
  Madrid, Madrid

- Nemessio Carro Carro
  León, León

- Patricia Limiñana Gregori
  Alcoi, Alicante

- Santiago Rodríguez Uriel
  Bruselas, Bélgica

**Sponsor Friends**

- Ana Cristina Jardón Ruiz
  Madrid, Madrid

- María Rosa Fernández García
  Selaya, Cantabria

**Benefactor Friends/Sponsor Friends**

**Donations to the CNIO**

- **Benefactor Friends**
  100,000€

- **Sponsor Friends**
  44,000€

- **Total CNIO Donations 2015**
  600,000€

- **CNIO Friends 2015**
  100,000€

- **CNIO Friends 2014**
  4,000€

- **CNIO Friends 2013**
  25,000€

- **CNIO Friends 2012**
  3,000€

- **CNIO Friends 2011**
  6,000€

- **423,000€ Legacies**
  2015 423,000€
  2014 44,000€

- **600,000€ Total CNIO Donations 2015**
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.

A Madrid-based visual artist working with photography and video, Amparo Garrido has been represented in individual and group shows both in Spain and abroad since 1998. Her work has been honoured in several prestigious competitions. She obtained the first place in the 2001 edition of the ABC Photography Prize, and second place in the 2007 Purificación García Prize. Other honourable mentions include the Pilar Citoler and Ciudad de Palma prizes. Her work can be found in major collections, including the Museo Nacional Centro de Arte Reina Sofía in Madrid, the photographic holdings of the Madrid regional authority, the Coca-Cola Foundation, and the Unicaja Foundation, among many others. Most recently, her latest exhibition at the Romantic Museum in Madrid, “Tiergarten” – a romantic German garden – a project that shows the relationship between contemporary art and romanticism, has received numerous praises and recognition.

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 and Museo Thyssen-Bornemiza 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.