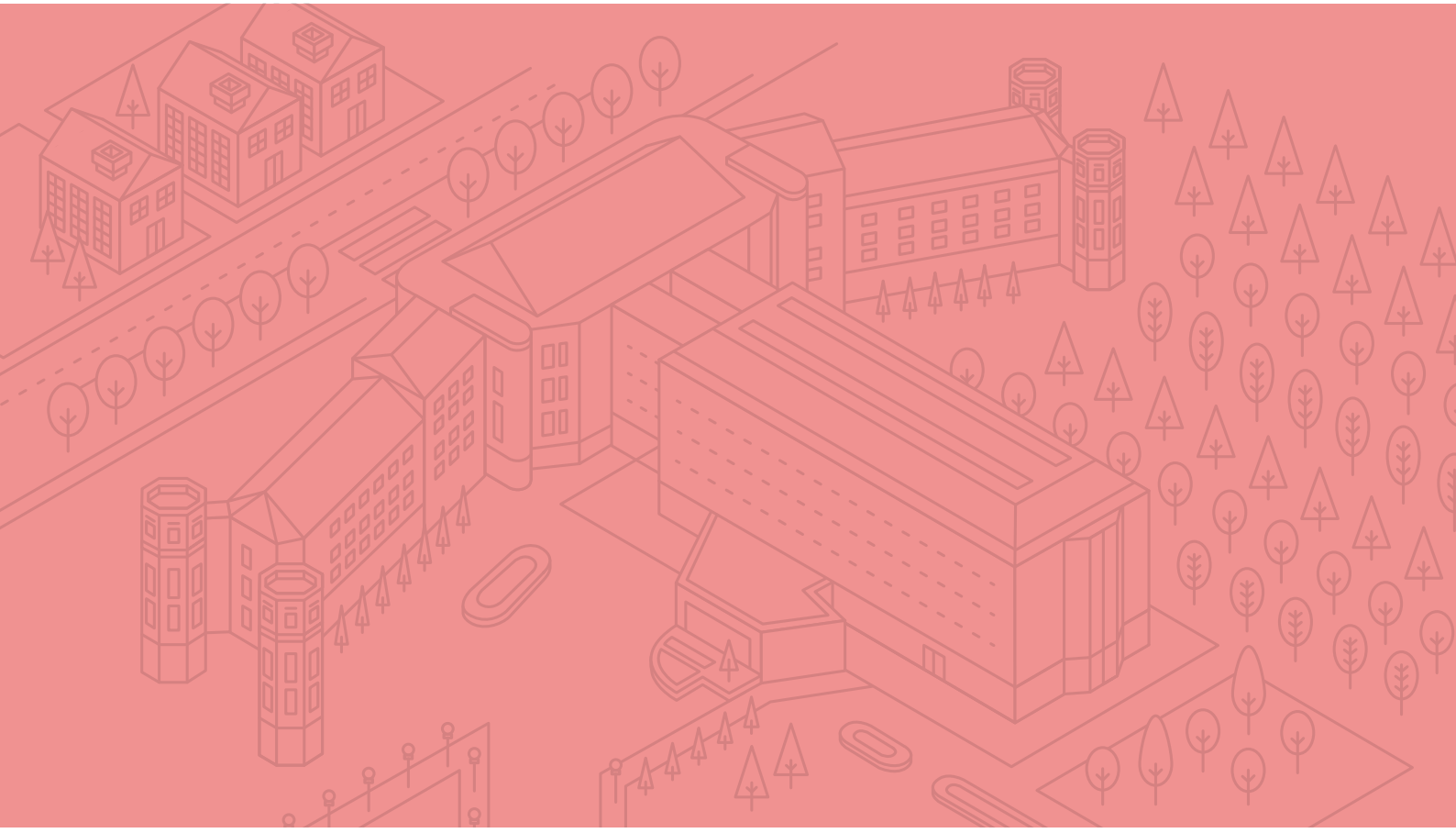
SPANISH NATIONAL
CANCER RESEARCH CENTRE, 2014

SCIENTIFIC REPORT 2014



Centro Nacional
de Investigaciones
Oncológicas

SCIENTIFIC REPORT 2014

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“During 2014, the income generated through research collaborations with companies nearly doubled that of 2013, reaching 5 million EUR.”

MARIA A. BLASCO
Director

FOREWORD

Maria A. Blasco Director

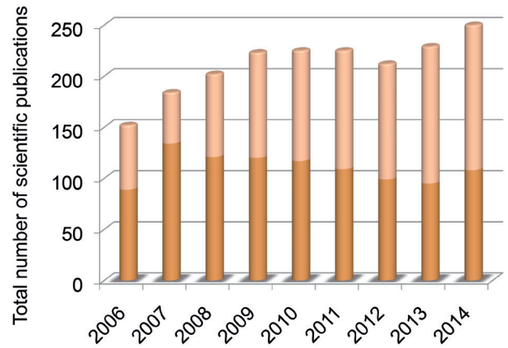
First of all, I would like to thank all of those who have once again collaborated in the elaboration of this Annual Report. A special thanks goes out to Sonia Cerdá who has coordinated all the efforts.

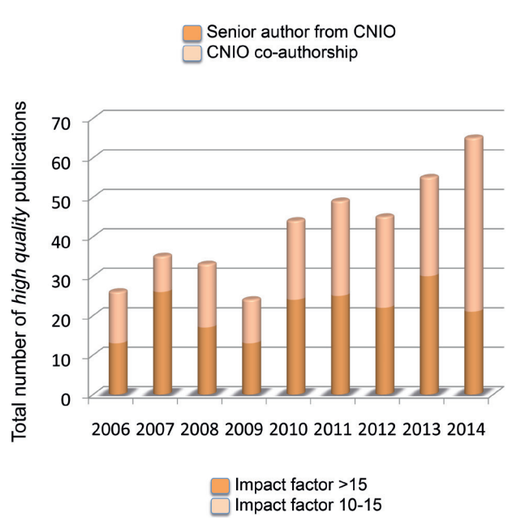
During 2014, the CNIO authored a total of 249 papers, 44 of which were published in journals with impact factors ranging from 10 to 15, and 21 in journals with impact factors greater than 15. Comparison with previous years (2006-2014) indicates that since 2010, the CNIO has further continued to increase its output of papers in top journals.

During 2014, we have also witnessed the growth of our Clinical Research Programme through the signing of a landmark agreement with one of the major public hospitals in Madrid, the *Hospital 12 de Octubre*. This has allowed the creation of two new Clinical Research Units at the CNIO. These new Units are led by oncologists from the *Hospital 12 de Octubre;* Luis Paz-Ares heads the H12O-CNIO Lung Cancer Clinical Research Unit and Joaquín Martínez heads the H12O-CNIO Haematological Malignancies Clinical Research Unit. With these two new Units, the CNIO covers the majority of the most prevalent cancers, namely GI tumours, lung cancer, haematological tumours, as well as breast and prostate cancers.

In addition to the Clinical Research Programme – whose oncologists are coordinating early phase clinical trials – the CNIO also conducts important clinical activity through the Familiar Cancer Clinical Unit located at the *Hospital Universitario de Fuenlabrada*. During 2014, we provided genetic counselling to 163 patients and performed 871 genetics tests, including the determination of 40 genes.

The year 2014 will be remembered in our annals as the year of the first approval and implementation of a royalty distribution policy at the CNIO. This scheme has the objective of recognising and rewarding the creative efforts of CNIO's researchers by promoting the commercial development of industrial and tangible property derived from their inventions.





During 2014, the income generated through research collaborations with companies nearly doubled that of 2013, reaching 5 million EUR. In addition, our network of collaborators has been extended, laying the groundwork to position the CNIO as a preferred research partner for the oncology industry.

It is noteworthy to mention that both the *Botín Foundation* and the *Banco Santander* – two well-recognised organisations committed to fostering science and technology transfer – have renewed their funding arrangement with two CNIO researchers, Manuel Serrano and myself, and have also further extended their support to a third CNIO researcher, Óscar Fernández-Capetillo.

Several important changes took place in our External Scientific Advisory Board (SAB) in 2014. In June, Mariann Bienz, Group Leader and Joint Divisional Head, MRC Laboratory of Molecular Biology, Cambridge, UK, was appointed as the new Chair of the CNIO SAB by the CNIO Board of Trustees. Mariann Bienz will take over the position that was excellently fulfilled by our former CNIO SAB Chair, Joan Massagué, who will continue with us as a SAB member. Additionally, in December 2014, the CNIO Board of Trustees approved the renewal of the SAB members who had completed their 3-year commitment to the CNIO SAB. In particular, we would like to thank Carlos López-Otín, José Baselga, Jesús San Miguel and Elías Campo for their invaluable dedication to our centre.

During 2014, we carried out the 5-Year Evaluation of the Junior Group Leaders of the Clinical Research, and Structural Biology and Biocomputing Programmes. The three Junior Group Leaders – namely, Miguel Quintela-Fandino from the Breast Cancer Clinical Research Unit, Daniel Lietha from the Cell Signalling and Adhesion Group, and Santiago Ramón-Maiques from the Structural Bases of Genome Integrity Group – were very positively evaluated and were granted a 3 year extension of their contracts at the CNIO. Congratulations Daniel, Santiago and Miguel!

I would like to take this opportunity to thank all those who have helped the CNIO by sponsoring our students, postdoctoral programmes and the stays of several researchers. I hereby extend my gratitude to the *Banco Santander* Foundation for funding postdoctoral stays at the CNIO and the *IE business school* course, the *La Caixa* Foundation for fostering international PhD fellowships, the *Seve Ballesteros* Foundation for supporting the Seve-Ballesteros Foundation-CNIO Brain Tumour Group, and the *Jesus Serra* Foundation for supporting the Visiting Scientists Programme and the Dean's Office. During 2014, the following scientists were the beneficiaries of the Jesús Serra Foundation's Visiting Researchers Programme: Andre Nussenzweig, Chief of the Laboratory of Genome Integrity at the National Cancer Institute, NIH, USA; Peter Petzelbauer, Head of the Skin & Endothelium Research Division of the Medical University of Vienna, Austria; and Eva Nogales, Head of the Biophysical Graduate Program at the University of California, Berkeley, USA.

I also wish to thank the Foundation *Banc Sabadell* for sponsoring a series of Distinguished Seminars at the CNIO given by out-of–the box speakers, who provided novel perspectives that contribute to the CNIO's transdisciplinary environment. During 2014, we were privileged to listen to: Ada Yonath, winner of the Nobel Prize in Chemistry and current Director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly at the Weizmann Institute of Science, Israel; Felix Goñi, Professor at the University of the Basque Country and Director of the Biophysics Unit; Enrique Dans, Professor of Information Systems and Technology at the IE Business School in Madrid (one of the leading business schools in the world); Francois Burgat, a political scientist and Arabist, as well as a Senior Research Fellow at the French National Centre for Scientific Research; and Joan Margarit, architect and world-renowned poet in both the Spanish and Catalan languages.

Thanks to the sponsorship of the *French Embassy* in Spain we were also able to invite the following speakers to our Distinguished Seminar series: Emmanuel Barillot, Director of Bioinformatics and Computational Systems Biology of Cancer (Curie Institute-INSERM/Mines Paris Tech), whose research focuses on the statistical analysis of large-scale biological data and biological network modelling in the context of cancer; Shahragim Tajbakhsh, Head of the Department of Developmental and Stem Cell Biology at the Pasteur Institute; and Jean Pierre Changeux, also from the Pasteur Institute, a French neuroscientist known to the general public for his ideas regarding the connection between the mind and the physical brain.

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 Dr. Margarita Alonso, Director of the *Fundación Instituto de Empresa*, Spain; Dr. Martha Gray (Harvard-MIT HST, MIT EECS, Research Laboratory of Electronics, IMES), Cambridge, USA; and, Dr. Capitolina Díaz from the University of Valencia and President of the Spanish Association of Women in Science and Technology. The WISE office also organised a coaching workshop entitled "The enzyme of the change" by Beatriz Ajenjo and Carlos Ferrari, from the International Coach Federation of Spain.

Last but not least, the number of times that our Centre's news was featured in the media in 2014 far exceeded the figures from previous years: 2,300 mentions in the national and international media, representing an increase of 41% over 2013. The productivity of our researchers is a key component of this success, and so I am proud to see the growing interest that the media and society in general are taking in our work. A good example are the findings that were published in July by the BBVA Foundation-CNIO Cancer Cell Biology Programme, led by Erwin Wagner, establishing that a third of all cancer deaths can be attributed to cachexia, a 'fat burning' process' that could

be used as a new target in oncology. This study, published by the prestigious journal 'Cell Metabolism', was featured on the front page of *El País*, one of the most popular newspapers in Spain, among other national and international media channels.

The global recognition given to the CNIO's work is also extended to the researchers themselves. I am proud of the fact that, in May, Óscar Fernández-Capetillo, leader of the CNIO Genomic Instability Group, was included in *Cell*'s '40 under 40'; a group of the most remarkable young scientists in the world. In the following days, many national and local Spanish media outlets told his heartening story. He is a truly inspiring role model for the young, teaching them the values of hard work and encouraging creative approaches to solving scientific problems.

Throughout 2014, CNIO's press releases in the global news service Eurekalert! received over 100,000 hits, representing an average of 8,500 page views per month. The CNIO has also increased its visibility via social media channels. More than 6,500 followers are part of our Twitter community, and even more importantly, the degree of their engagement and esteem is constantly growing. Direct feedback from this platform is very valuable for a qualitative and quantitative measurement of the human relevance of our research. Twitter has also revealed itself to be an essential channel of direct communication with journalists and more particularly, with patients, families and associations: all the people who give meaning to our work.

Finally, one of the most exciting experiences in 2014 was the launch of the 'CNIO Friends' initiative; a new philanthropic modality that seeks to involve citizens in biomedical research and to raise public awareness about the need of R + D + i to fight the disease. After only a few months, the initiative provided us with several personal and unique interactions with our donors and friends; something that motivates us even more to continue working on cancer research. ■

ORGANISATION OF RESEARCH

MARIA A. BLASCO DIRECTOR

ALFONSO VALENCIA VICE-DIRECTOR OF BASIC RESEARCH

MOLECULAR ONCOLOGY PROGRAMME

Manuel Serrano Programme Director

Manuel Serrano
Tumour Suppression Group

Mariano Barbacid
Experimental Oncology Group

Maria A. Blasco
Telomeres and Telomerase Group

Marcos Malumbres
Cell Division and Cancer Group

Óscar Fernández-Capetillo
Genomic Instability Group

Ana Losada
Chromosome Dynamics Group

Juan Méndez
DNA Replication Group

María S. Soengas
Melanoma Group

BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY PROGRAMME

Erwin F. Wagner Programme Director

Erwin F. Wagner
Genes, Development and Disease Group

Francisco X. Real
Epithelial Carcinogenesis Group

Mirna Pérez-Moreno
Epithelial Cell Biology Junior Group

Nabil Djouder
Growth Factors, Nutrients and Cancer Junior Group

Massimo Squatrito
Seve Ballesteros Foundation-CNIO Brain Tumour Junior Group

STRUCTURAL BIOLOGY AND BIOCOMPUTING PROGRAMME

Alfonso Valencia Programme Director

Alfonso Valencia
Structural Computational Biology Group

Guillermo Montoya
Macromolecular Crystallography Group

Daniel Lietha
Cell Signalling and Adhesion Junior Group

Santiago Ramón-Maiques
Structural Bases of Genome Integrity Junior Group

Ramón Campos-Olivas
Spectroscopy and Nuclear Magnetic Resonance Core Unit

David G. Pisano
Bioinformatics Core Unit

Alfonso Valencia
National Bioinformatics Institute Core Unit

Jasminka Boskovic
Electron Microscopy Core Unit

MANUEL HIDALGO VICE-DIRECTOR OF TRANSLATIONAL RESEARCH

HUMAN CANCER GENETICS PROGRAMME

Javier Benítez Programme Director

Javier Benítez
Human Genetics Group

Juan C. Cigudosa
Molecular Cytogenetics Group

Mercedes Robledo
Hereditary Endocrine Cancer Group

Núria Malats
Genetic and Molecular Epidemiology Group

Miguel Urioste
Familial Cancer Clinical Unit

Anna González-Neira
Human Genotyping-*CEGEN* Core Unit

CLINICAL RESEARCH PROGRAMME

Manuel Hidalgo Programme Director

Manuel Hidalgo
Gastrointestinal Cancer Clinical Research Unit

Miguel Quintela-Fandino
Breast Cancer Junior Clinical Research Unit

David Olmos
CRIS Foundation-CNIO Prostate Cancer Junior Clinical Research Unit

Luis J. Lombardía
Molecular Diagnostics Unit

Fátima Al-Shahrour
Translational Bioinformatics Unit

Joaquín Martínez-López (since November)
H12O-CNIO Haematological Malignancies Clinical Research Unit

Luis Paz-Ares (since November)
H12O-CNIO Lung Cancer Clinical Research Unit

BIOBANK

Manuel M. Morente Director

Christopher Heeschen (in Transition)
Stem Cells and Cancer Group

DIRECTION OF INNOVATION

BIOTECHNOLOGY PROGRAMME

Fernando Peláez Programme Director

Orlando Domínguez
Genomics Core Unit

Sagrario Ortega
Transgenic Mice Core Unit

Giovanna Roncador
Monoclonal Antibodies Core Unit

Francisca Mulero
Molecular Imaging Core Unit

Lola Martínez
Flow Cytometry Core Unit

Diego Megías
Confocal Microscopy Core Unit

Javier Muñoz
Proteomics Core Unit

Alba De Martino (since April)
Histopathology Core Unit

Isabel Blanco (Vivotecnia Management & Services)
Animal Facility

EXPERIMENTAL THERAPEUTICS PROGRAMME

Joaquín Pastor Programme Director

Sonia Martínez
Medicinal Chemistry Section

Carmen Blanco
Biology Section

Susana Velasco
CNIO-Lilly Cell Signalling Therapies Section

María José Barrero
CNIO-Lilly Epigenetics Section

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

Anabel Sanz Director

Vice-Direction of Basic Research

ALFONSO VALENCIA
Vice-Director of Basic Research

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

There have been several encouraging developments this year pertaining to the organisational aspects within the CNIO’s Basic Research domain; the recruitment of Hector Peinado as a Junior Group Leader, the creation of the Electron Microscopy and Protein Crystallography Units, and the positive evaluation of our Junior Group Leaders by the corresponding external Scientific Advisory Board.

This constructive trend will be further strengthened in the coming year with the new recruitments in the strategic areas of metastasis and structural biology, the increase in collaborations with internal and external groups, and the consolidation of the projects developed with the Experimental Therapeutics Programme.

MOLECULAR ONCOLOGY PROGRAMME

MANUEL SERRANO Programme Director

It is my pleasure to introduce the highlights of the Molecular Oncology Programme. I will start with the Melanoma Group that has expanded the repertoire of therapeutic targets in melanoma. Cancer cells have a very active metabolism that involves endo and exocytosis. Surprisingly, in the case of melanoma, this endosomal activity relies on a set of factors that are uniquely elevated in melanoma but not in all other cancer types tested (*Cancer Cell* 2014). Among them, the small GTPase RAB7 is critically needed for the tumourigenic potential of malignant melanomas. This GTPase or its regulators constitute attractive new targets for therapeutic interventions.

The Cell Division and Cancer Group has reported that the activity of topoisomerase poisons, often used as chemotherapeutic agents, can be highly increased when the ubiquitin ligase APC/C is simultaneously inhibited (*Cell Reports* 2014). This new connection provides the basis for an innovative and efficient combination of chemotherapeutic compounds that is currently in development.

The Ras oncogene and the tumour suppressor p53 are arguably the most intensively studied cancer-related proteins. The two proteins counteract each other in the context of cancer, however, little was known about their interplay in normal, non-tumourigenic, contexts. This year, the Experimental Oncology Group reported (*Proc Natl Acad Sci USA* 2014) that one of the basal functions of Ras signalling is to attenuate p53 activity through deacetylation. This is an unprecedented finding that provides new insight into the normal function of these central proteins.

The Chromosome Dynamics Group has continued challenging previous ideas about a chromosomal protein complex known as cohesin, which is frequently mutated in cancer. Cohesins were discovered as mediators of sister chromatid cohesion and are essential for faithful chromosome segregation. However, partly through the work of Ana Losada and her group, the function of cohesins has been extended beyond mitotic chromosomes to the interphase chromatin where cohesins play a critical role in the regulation of transcription. Recent evidence indicates that this last function could be responsible for the oncogenic effects of mutated cohesins (reviewed in *Nat Rev Cancer* 2014).

Telomeres are the end of chromosomes and are characterised by a complex nucleoprotein structure. A few years ago, the Telomeres and Telomerase Group discovered telomere-derived RNA transcripts known as TERRA. These atypical transcripts are induced upon cellular damage, binding and protecting all the telomeres. This year, Maria Blasco and her team reported that the telomere of chromosome 18 is the main source of TERRA transcripts (*Nat Commun* 2014). TERRAs could be another therapeutic target to destabilise telomeres in cancer cells.

“The mentioned studies are some of the exciting findings that took place within the Molecular Oncology Programme. They stand testament to the fact that the Programme is upholding its tradition of scientific excellence and innovation.”

The DNA Replication Group, in collaboration with researchers in the University of California San Francisco, have discovered that the “quality” of the replication machinery deteriorates with ageing in haematopoietic stem cells (*Nature* 2014). This underlies, at least to some extent, the weak haematopoietic potential characteristic of old individuals. Defective replication could also promote genomic alterations in haematopoietic cells, thereby explaining why many types of leukaemia and lymphomas are more frequent at an older age.

The links between metabolism and disease, including cancer and ageing, are an emerging theme. Maintenance of sufficiently high levels of nucleotide precursors is critical for normal physiology. The Genomic Instability Group has generated the first mouse model with supraphysiological amounts of nucleotides. So far, their work has revealed that increased nucleotide levels can reduce spontaneous chromosomal breakage and extend the lifespan of a mouse model of accelerated ageing.

In 2014, the Tumour Suppression Group reported that the pluripotency transcription factor NANOG is expressed in selective adult tissues, specifically in the basal layer of stratified epithelia (*Nat Commun* 2014). This contradicts the deeply entrenched idea that NANOG is only expressed during the very early stages of embryonic development. Interestingly, human cancers derived from stratified epithelia, including oesophageal and head-and-neck carcinomas, all express high levels of NANOG. The possibility that cancers could possess an aberrant pluripotency circuitry opens up new and exciting possibilities to understand and treat cancer.

TUMOUR SUPPRESSION GROUP

Manuel Serrano
Group Leader

Staff Scientists
Han Li (until May), Susana Llanos, Cristina Pantoja

Post-Doctoral Fellows
María Abad, Timothy Cash, Pablo J. Fernández-Marcos, Cian J. Lynch, Daniel Muñoz, Gianluca Varetti (since August)

Graduate Students
Noelia Alcázar, Elena López-Guadamillas, Lucía Morgado-Palacín, Lluc Mosteiro, Adelaida R. Palla (until June), Dafni Chondronasiou (since October), Raquel Bernad

Technician
Maribel Muñoz (since October)

Visiting Scientist
Liming Gui (since March)

OVERVIEW

Tumour suppressors are genes that can prevent the development of cancer. All our cells have a functional set of these genes. However, despite their efficient protection against cancer, these genes can become defective over time. The affected cells thus become partially unprotected from cancer and, upon additional mutations in other genes, can give rise to cancer.

Understanding how tumour suppressor genes work may help us to design drugs that block cancer. Our Group also manipulates the mouse genome to create novel alterations that increase or decrease tumour suppression potency.

The goals of our Group 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 the 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, in tissue regeneration, and in ageing.

"This year, we have obtained further evidence linking pluripotency genes to cancer. We have identified compounds that activate p53 without inflicting DNA damage. And, finally, we have reported the first DNA sequence analysis of families with unusual longevity."

RESEARCH HIGHLIGHTS

Cancer and pluripotency

NANOG is a key pluripotency transcription factor in embryonic stem cells (ESC), whose role in adult tissues and cancer is still largely unexplored.

NANOG is linked to tumours derived from stratified epithelia. We have found that NANOG is selectively expressed in stratified epithelia, including the skin, the oesophagus, and external mucosas such as the tongue (FIGURE 1). Interestingly, genetic overexpression of NANOG in mice selectively affected stratified epithelia, where it produced an increase in cellular proliferation and hyperplasia. NANOG promotes cell proliferation in these tissues by binding and activating the promoter of the *AURKA* gene, which encodes the mitotic factor Aurora kinase A. In collaboration with the CNIO Cell Division and Cancer Group, we have shown that genetic overexpression of AURKA in mice recapitulates the same effects as NANOG in the oesophagus. Importantly, NANOG and AURKA levels are positively correlated in oesophageal and head-and-neck squamous cell carcinomas (SCCs), and inactivation of NANOG in SCC cells reduces their proliferation.

NANOG promotes squamous cell carcinoma. A direct link between NANOG and SCCs had yet to be established. Interestingly, inducible overexpression of NANOG in mouse skin epithelia favours the malignant conversion of skin tumours induced by chemical carcinogenesis, leading to increased SCC formation. Gene expression analyses in pre-malignant skin indicated that NANOG induces genes associated to epithelial-mesenchymal transition (EMT). Endogenous NANOG binds to the promoters of these genes and induces EMT features in primary keratinocytes. These results provide direct *in vivo* evidence for the oncogenic role of NANOG in stratified epithelia.

Reprogramming activity of NANOGP8: a NANOG family member widely expressed in cancer. The human genome contains 11 *NANOG* paralogs, of which only *NANOG1*, *NANOG2* and *NANOGP8* encode full-length proteins. In collaboration with the CNIO Genes, Development and Disease Group, we have found that NANOGP8 is expressed in many human cancer cells and that it is as active as NANOG1 in promoting reprogramming to pluripotency. Therefore, NANOGP8 can contribute to cancer, possibly by promoting cell de-differentiation and/or plasticity.

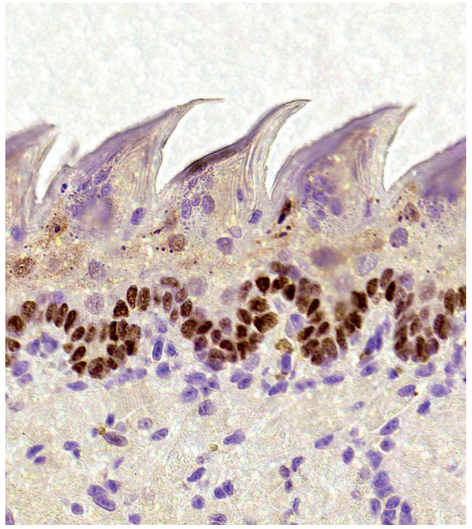


Figure 1 Histological section of the mouse tongue (dorsal surface) stained with an anti-NANOG antibody (brown nuclei). The wavy layer of cells with brown nuclei (expressing NANOG) corresponds to the basal layer that contains the epithelial stem cells.

Ribosomal stress and cancer

Genotoxic chemotherapeutic agents produce lasting mutagenic damage to the organism. To circumvent this serious drawback, there is great interest in identifying chemotherapeutic agents that activate p53 in a non-genotoxic manner. Nucleolar disruption is a non-genotoxic mechanism that results in free ribosomal protein RPL11, which binds and inhibits HDM2, thereby activating p53. In collaboration with the CNIO Experimental Therapeutics Programme, we have identified a group of acridines that efficiently produce nucleolar disruption and activate p53, without inflicting DNA damage (FIGURE 2). These acridines inhibit the transcription of the ribosomal RNA genes in a process that includes the selective degradation of the RPA194 subunit of RNA polymerase I. Our findings provide the basis for non-genotoxic chemotherapeutic approaches that selectively target the nucleolus.

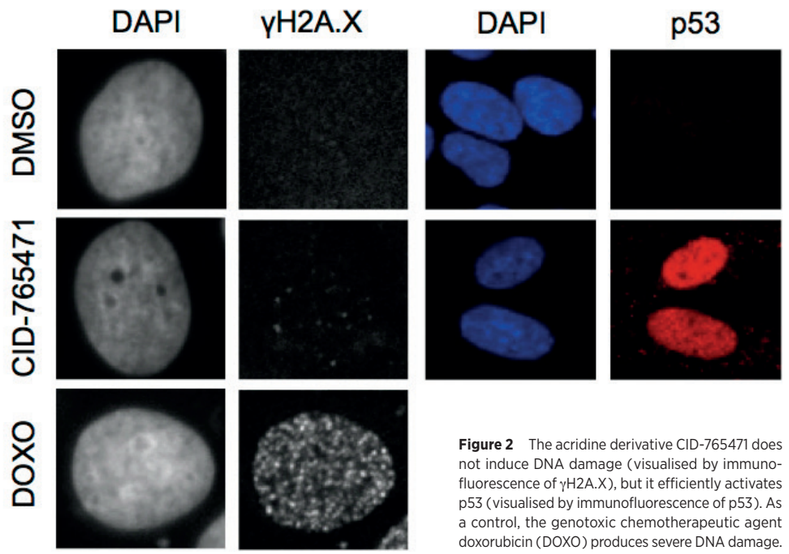


Figure 2 The acridine derivative CID-765471 does not induce DNA damage (visualised by immunofluorescence of γH2A.X), but it efficiently activates p53 (visualised by immunofluorescence of p53). As a control, the genotoxic chemotherapeutic agent doxorubicin (DOXO) produces severe DNA damage.

Genetics of human longevity

Exceptional longevity (EL) is a rare phenotype that can cluster in families. In collaboration with the CNIO Human Genotyping-CEGEN Unit, the CNIO Genomics Core Unit and the Spanish Network of Ageing and Frailty (RETICEF), we have sequenced a total of 7 exomes from exceptionally long-lived individuals (>100 yrs old) that come from 3 separate families with at least 2 centenarian siblings. We focused on rare functional variants (RFVs) and only 1 gene, *APOB*, carried RFVs in all members of the three families. Proteins APOB and APOE are components of lipoproteins specialised in the transport of cholesterol and triglycerides. Interestingly, variants in these 2 genes, *APOB* and *APOE*, had been previously associated with human longevity. We have also identified candidate longevity genes shared between 2 families or within individual families. Our work provides a catalogue of candidate genes that could contribute to exceptional familial longevity. ■

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EXPERIMENTAL ONCOLOGY GROUP

Mariano Barbacid
Group Leader

Staff Scientists
Matthias Drosten, Carmen Guerra, Monica A. Musteanu, David Santamaría

OVERVIEW

Our laboratory is interested in deciphering the molecular mechanisms involved in the initial stages of neoplastic transformation induced by K-Ras oncogenes in lung and pancreatic adenocarcinomas. We are particularly interested in understanding how specific cellular contexts dictate tumour development and also in identifying the key players involved in this process. We believe that those events that contribute to the early phases of tumour development are likely to represent key elements for the maintenance of the disease, and hence should provide a source of relevant therapeutic targets.

We are also interested in addressing questions that will have a direct impact on the management of K-ras driven tumours in the clinic. We plan to identify and functionally validate molecular targets that – if inhibited in a combined and systematic way – will reveal therapeutic strategies capable of eradicating tumour cells in such a way that they will not have a chance to develop resistance, as currently observed in the clinic.

We are addressing these ambitious goals using sophisticated genetically engineered mouse (GEM) models of cancer. The advent of the CRISPR/Cas9 editing techniques is opening up new opportunities for the interrogation of therapeutic strategies that could effectively eradicate aggressive tumours. The outcome of these studies should pave the way for the development of more efficacious therapies in a clinical setting in a not too distant future.

Main contributions towards improving the understanding, prevention, diagnostics or treatment of cancer:

- → We have identified the initiating cancer cells in K-Ras driven lung adenocarcinoma.
- → We have established that Ras proteins are essential for epidermal proliferation.
- → We have uncovered an unsuspected link between Ras signalling and the p53/p21 tumour suppressor axis.
- → We have developed a genetically engineered mouse model for Noonan syndrome.

Post-Doctoral Fellows
Chiara Ambrogio, Raquel García, Harrys K.C. Jacob

Graduate Students
Maria Teresa Blasco, Magdolna Djurec, Isabel Hernández, Patricia Nieto, Lucía Simón, Catherine E. Symonds

Technicians
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Research Associate
Jose Javier Berenguer, Juan Velasco

RESEARCH HIGHLIGHTS

Ras and p53: an unsuspected liaison in mitogenic signalling

The Ras family of small GTPases constitutes a central node in the transmission of mitogenic stimuli to the cell cycle machinery. The ultimate receptor of these mitogenic signals is the Rb family of pocket proteins, whose inactivation is a required step to license cell proliferation. However, little is known regarding the molecular events that connect Ras signalling with the cell cycle. We have used an unbiased small hairpin RNA (shRNA) library screen in Rasless cells to identify genes whose inactivation will allow proliferation of quiescent cells lacking Ras proteins. This screen unambiguously identified the p53/p21Cip1 axis as an essential mediator of Ras mitogenic signalling. That is, efficient knock-down of either p53 or p21Cip1 expression fully restored the proliferative properties of Rasless cells (FIGURE A). Similar results were obtained upon inactivation of the Rb tumour suppressor. Further studies revealed that loss of Ras proteins caused widespread transcriptional activation of p53 through a mechanism involving acetylation of 2 specific residues in its DNA binding domain. Surprisingly, phosphorylation and/or protein stabilisation of p53 was not required. These experiments suggest that Ras signalling regulates the acetylation state of p53 to control S phase entry via induction of p21Cip1 transcription (FIGURE A). Taken together, these results unveil a novel role for p53 in preventing cell proliferation under unfavourable mitogenic conditions.

In contrast, knock down of the p53/p21Cip1/Rb axis did not restore proliferation in cells lacking Raf, Mek or Erk kinases; the immediate downstream effectors of Ras mitogenic signalling (FIGURE B). A solution to this apparent conundrum came when we observed that knock-down of any of the members of the p53/p21Cip1/Rb tumour suppressor axis in Rasless cells resulted in the activation of the Raf/Mek/Erk signalling pathway in a Ras-independent manner. Thus, we can conclude from these studies that, at least in certain cells such as MEFs and keratinocytes, there is a retro-activation circuitry that, in the absence of these tumour suppressors, maintains an active MAP Kinase cascade. Moreover, these kinases, unlike the Ras proteins, are essential for cell proliferation even in the absence of the p53/p21Cip1 axis (FIGURE B). In other words, inactivation of the p53/p21Cip1/Rb tumour suppressor pathway per se is not sufficient to license cells to enter the cell cycle. They require active Raf/Mek/Erk signalling.

These results open up a series of important questions regarding the cellular circuitries responsible for handling mitogenic signals and their connection with the cell cycle machinery. These results may also have implications for the role of the MAPK pathway in those tumours that carry p53 mutations.

Target validation: therapeutic GEM tumour models

One of the main areas of interest in our laboratory has been the identification and validation of targets with therapeutic value in K-Ras driven lung and pancreatic tumours. Previous studies have shown that ablation of c-Raf, but not A-Raf or B-Raf, prevents tumour development. Thus, indicating that the Raf kinases do not have compensatory roles in K-Ras driven tumour development. Similar results were obtained with the interphase Cdks, where Cdk4, but not Cdk2 and Cdk6, prevented tumour formation. Other K-Ras downstream kinases, including Mek1/2 and Erk1/2, displayed compensatory activities and it was necessary to ablate both kinase isoforms to prevent the appearance of tumours. Unfortunately, systemic ablation of either Mek1/2 or Erk1/2 in adult mice resulted in multi-organ failure leading to the rapid death of the animals.

These studies, however, failed to address the potential therapeutic value of these targets in pre-existing tumours. To this end, we have generated GEM tumour models in which we can temporally separate tumour development from target ablation by using 2 independent recombinases. Consequently, we generated strains of mice in which we can activate a resident K-Ras oncogene and eliminate the p53 tumour suppressor with the FLp(o) recombinase; targets could be ablated by activating an inducible CreERT2 recombinase once the tumours had reached a desired size. We are currently using these GEM tumour models to validate those targets previously shown to prevent tumour development when ablated at the time of oncogene induction. ■

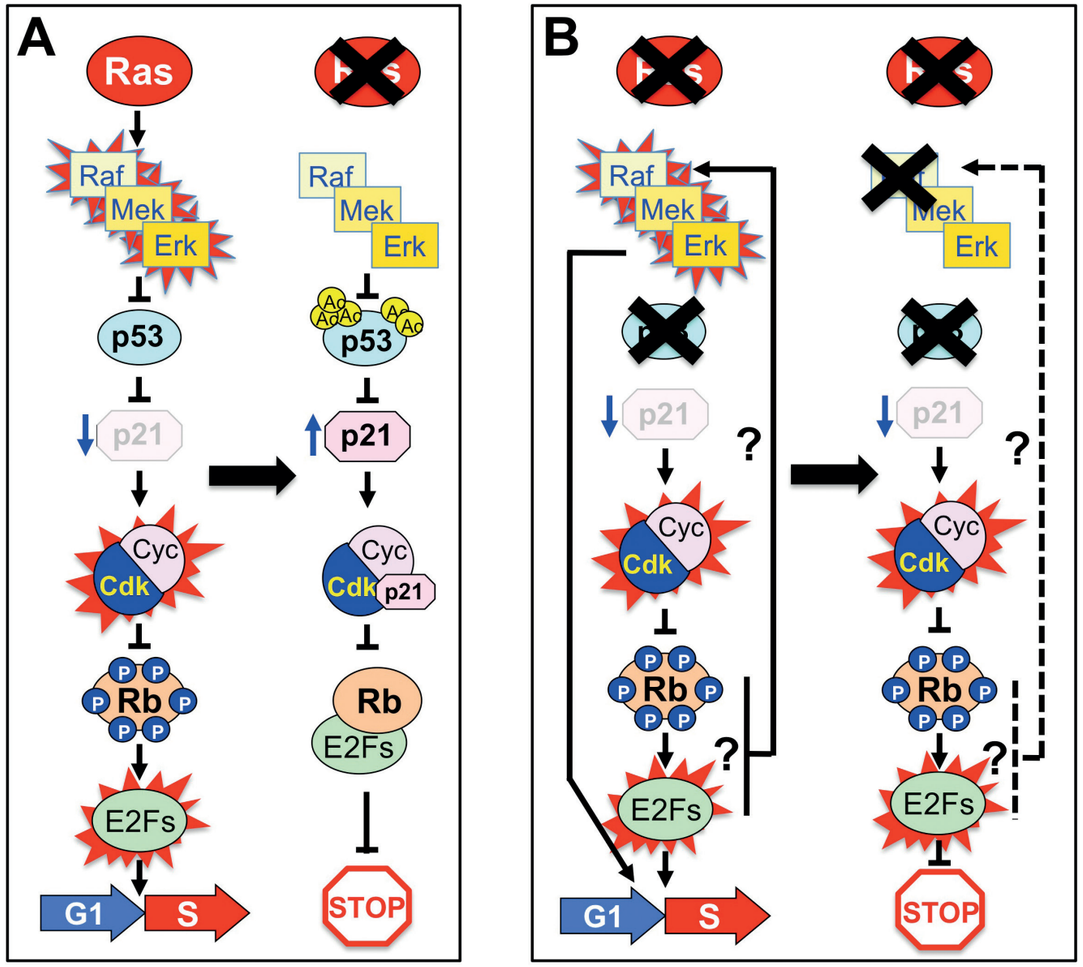

Figure Ras mitogenic signalling. (**A**) Schematic representation of the role of the p53/p21Cip1 axis in (left) wild type and (right) Rasless cells. (**B**) (Left) Schematic representation of the Ras signalling pathway, illustrating that Rasless cells can proliferate in the absence of p53 due to retroactivation of the Raf/Mek/Erk signalling cascade in a Ras-independent manner. Similar results were observed in Rasless cells lacking p21 and Rb tumour suppressors (see text). (Right) Unlike Rasless cells, cells lacking the Raf kinases (Rafless cells), the Mek kinases (Mekless cells) or the Erk kinases (Erkless cells) cannot proliferate in the absence of any of the main components of the p53/p21Cip1/Rb axis. Blue arrows indicate variation in expression levels. Red spikes indicate activated proteins. X-shaped crosses indicate that the corresponding protein has been genetically ablated. Ac, acetylated lysine residues. P, phosphorylated Ser/Thr residues. The dotted line indicates that in Rafless, Mekless or Erkless cells we do not have experimental evidence for the existence of a retroactivating circuitry observed in Rasless cells. The represented active state of the E2F transcription factors has not been determined experimentally; it is deduced from the inactive state of the Rb protein.

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AWARDS AND RECOGNITION

- Honorary Doctoral Degree (*Doctor Honoris Causa*), *Universidad de Barcelona*, Barcelona.
- Elected Fellow of the Academy of the American Association for Cancer Research.
- Eladio Viñuela Award in Life Sciences.
- President, Search Committee, Koerber European Science Award, Hamburg, Germany.
- Member of the Jury, BBVA Foundation Frontiers of Knowledge Award.
- Member of the Scientific Advisory Board, Cancer Science Institute, Singapore.
- Member of the Scientific Advisory Board, the Helmholtz Alliance PCCC, Heidelberg, Germany.
- Member of the Program Committee, 2015 Annual Meeting of the American Association for Cancer Research.
- Keynote Speaker: EMBO conference on Cell Signalling and Cancer Therapy, Dubrovnik, Croatia.
- Keynote Speaker: Symposium on Translational Oncology, Lausanne, Switzerland.
- Chairperson: 26th EORTC-NCI-AACR Symposium on "Molecular Targets and Cancer Therapeutics", Barcelona, Spain.

TELOMERES AND TELOMERASE GROUP

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OVERVIEW

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 but is not present in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes that are essential for chromosome protection and genomic stability. One of the many factors that lead to ageing is the progressive shortening of telomeres associated with organism ageing. When telomeres are altered (in their length or their integrity) adult stem cells have a maimed regenerative capacity.

"Our discovery of the genomic origin of telomeric RNAs will facilitate the design of molecular and genetics tools in order to better understand their role in normal development and in disease."

Telomere length defects are associated with cancer and ageing processes, and have a profound effect on stem cell behaviour. We aim to determine the role of genetic and epigenetic telomere regulators in cancer and ageing by generating new mouse models and studying the role of these factors in stem cell biology.

Our research aims are:

- → Understanding the biology of the telomeres and telomerase by generating mouse models to probe the role of telomeres and telomerase in cancer and ageing.
- → Deciphering the interplay between telomeres and DNA repair pathways.
- → Studying the role and regulation of non-coding telomeric RNAs or TERRAs.
- → Developing strategies for telomerase activation.
- → Elucidating the role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to induced Pluripotent Stem (iPS) cells.

RESEARCH HIGHLIGHTS

Identification of the genomic origin of telomeric RNAs

Telomeric RNAs (TERRAs) are UUAGGG repeat-containing RNAs that rise from the transcription of the telomeric C-strand by RNA polymerase II. TERRAs are thought to be transcribed from the subtelomere towards the telomere. The precise genomic origin of TERRA has remained elusive. Using a whole-genome RNA-sequencing approach, we identified novel mouse transcripts arising mainly from the subtelomere of chromosome 18, and to a lesser extent chromosome 9, which resemble TERRA in several key aspects. Chromosome 18 transcripts contain UUAGGG-repeats, are found at telomeres with the same frequency as TERRA transcripts, are heterogeneous in size, fluctuate in abundance in a TERRA-like manner during the cell cycle, are bound by the same TERRA RNA-binding proteins that bind TERRA, are regulated by stresses in a manner similar to TERRA, and are induced upon induction of pluripotency like TERRA. These transcripts can bind to chromosome 18 telomeres as well as to the other chromosome ends (FIGURE 1), although not all at once. We showed that downregulation of the transcripts that originate from chromosome 18 causes a reduction in TERRA abundance. Interestingly, downregulation of either chromosome 18 transcripts or TERRA resulted in similar induction of telomere dysfunction-induced foci, suggesting a protective role at the telomeres.

Integrity of induced pluripotent stem cells

The SIRT1 protein has been suggested to play a role in pluripotency. In mice, SIRT1 attenuates telomere attrition *in vivo* and it is recruited at telomeres in induced pluripotent stem cells (iPSCs). Being telomere elongation an iPSC hallmark, we studied the role of SIRT1 in pluripotency in the setting of mouse embryonic fibroblasts reprogramming into iPSCs. We found that SIRT1 is required for efficient post-reprogramming telomere elongation, and that SIRT1-deficient iPSCs accumulate chromosomal aberrations and form larger teratomas that are poorly differentiated. Our work demonstrated a role for SIRT1 in regulating genomic integrity and telomere expansion.

A gene that links stem cells, ageing and cancer

Sox4 is a transcription factor whose expression in mammals is restricted to embryonic structures and some adult tissues, such as the lymphoid organs, pancreas, intestines, and skin. During embryogenesis, Sox4 regulates mesenchymal and neural progenitor survival, as well as lymphocyte and myeloid differentiation, and contributes to pancreas, bone, and heart development. Aberrant Sox4 expression in adult tissues is linked to malignant transformation and metastasis in several types of cancer in mice and humans. We have addressed the roles of Sox4 in adult tissue homeostasis and cancer. We first generated a mouse model with reduced whole-body Sox4 expression. These mice display accelerated ageing and are cancer resistant. To specifically address a role for Sox4 in adult stem cells (FIGURE 2), we conditionally deleted *Sox4* (*Sox4*^{cKO}) in stratified epithelia. *Sox4*^{cKO} mice showed increased skin stem cell quiescence and strong resistance to tumour development when subjected to a chemically induced carcinogenesis skin protocol; this concomitantly with downregulation of cell cycle, DNA repair, and activated hair follicle stem cell pathways. Our findings shed light on some crucial functions of the Sox4 protein in cancer and ageing and provide new tools for elucidating *Sox4* function in adult tissue homeostasis.

Telomerase expression confers cardioprotection

Short telomeres are risk factors for age-associated diseases, including heart disease. We addressed the potential of telomerase (*Tert*) activation in the prevention of heart failure after myocardial infarction (MI) in adult mice. We used adeno-associated viruses for cardiac-specific *Tert* expression. We found that upon MI, hearts expressing Tert showed an improvement in cardiac functional and morphological parameters, concomitant with reduced mortality by heart failure after MI. Tert re-activation also resulted in elongated telomeres and a gene expression switch towards a regeneration signature of neonatal mice. Our work suggests that telomerase activation could be a therapeutic strategy to prevent heart failure after MI. ■

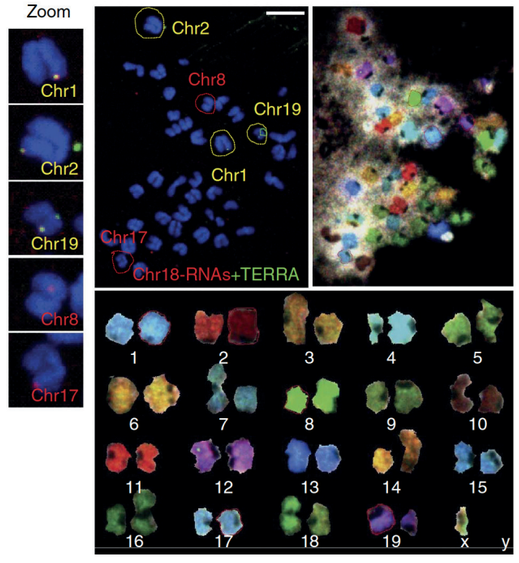

Figure 1 Identification of the chromosomes bound by chromosome 18-TERRAs. RNA-FISH targeting both chromosome 18-RNAs and TERRA's telomeric track: (top-left) RNA-FISH staining in metaphases, (top-right) SKY hybridisation and (bottom) chromosome identification by SKY. Scale bar, 10 μm.

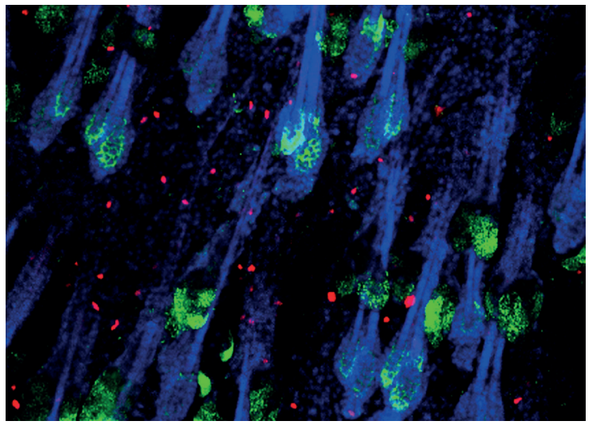

Figure 2 Sox4 protein maintains tissue homeostasis in stem cells. Stem cells from adult mouse epidermis (green, cytokeratin 6; blue, cell nucleus).

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AWARDS AND RECOGNITION

- Doctorate Honoris Causa, *Universidad Carlos III*, Madrid, Spain.

CELL DIVISION AND CANCER GROUP

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Technician
David Partida

OVERVIEW

Deregulation of the cell cycle is a common feature in human cancer. Our group focuses on deciphering the mechanisms by which cell division and cell proliferation are regulated. Over the last few years, we have used mouse models in order to understand the relevance of several cell cycle regulators in the control of cell division and in normal physiology of different cell types or tissues; these include cell cycle kinases and phosphatases, as well as regulatory complexes involved in ubiquitin-dependent degradation of proteins.

Our current interests are:

- → To understand the basic control mechanisms that regulate the various cell division cycles that are present in mammalian cells.
- → To characterise the physiological and therapeutic consequences of cell cycle deregulation *in vivo*.
- → To characterise the function of microRNAs in cell biology and tumour development, as well as their potential use in cancer therapy.
- → To understand how progenitor cells and cancer stem cells control their self-renewal and proliferative properties.

As a final goal, we aim to generate information that may be useful to improve therapeutic strategies against cancer cell proliferation.

"In 2014, our Group proposed new therapeutic combinations based on genetic and proteomic studies of the cell cycle. In addition, we have reported on how oncogenic kinases – currently under evaluation in clinical trials – are able to limit the effect of cell cycle inhibitors during tumour development."

RESEARCH HIGHLIGHTS

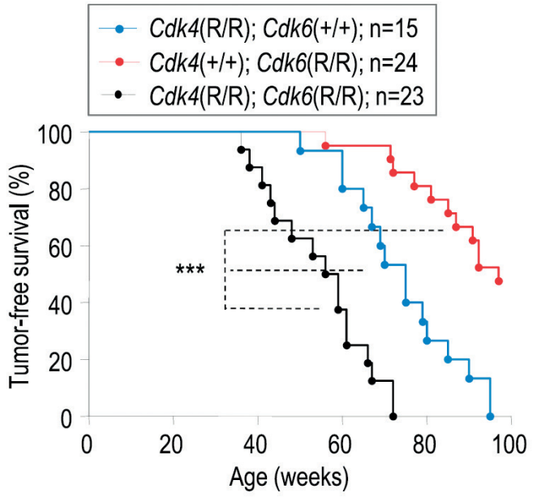

Figure 1 Tumour-free survival of *Cdk4*(R/R); *Cdk6*(R/R) or double *Cdk4*(R/R); *Cdk6*(R/R) mutant mice (taken from Rodríguez-Díez et al., *Blood* 2014).

Cdk4 and Cdk6 cooperate in counteracting the INK4 family of inhibitors during murine leukaemogenesis

Cdk4 and Cdk6 are 2 related protein kinases that bind D-type cyclins and regulate cell-cycle progression. Cdk4/6 inhibitors are currently being used in advanced clinical trials and show great promise against many types of tumours. Cdk4 and Cdk6 are inhibited by INK4 proteins, which exert tumour-suppressing functions. To test the significance of this inhibitory mechanism, we have generated knock-in mice that express a Cdk6 mutant (Cdk6 R31C) insensitive to INK4-mediated inhibition. *Cdk6*(R/R) mice display altered development of the haematopoietic system without enhanced tumour susceptibility, either in the presence or absence of p53. Unexpectedly, Cdk6 R31C impairs the potential of haematopoietic progenitors to repopulate upon adoptive transfer or after 5-fluorouracil-induced damage. These defects are overcome by eliminating the sensitivity of the cells to INK4 inhibitors through introduction of the INK4-insensitive Cdk4 R24C allele; INK4-resistant mice are more susceptible to haematopoietic and endocrine tumours. In BCR-ABL-transformed haematopoietic cells, Cdk6 R31C causes an increased binding of $p16^{INK4a}$ to wild-type Cdk4, whereas cells harbouring Cdk4 R24C and Cdk6 R31C are fully insensitive to INK4 inhibitors, resulting in an accelerated disease onset (FIGURE 1). Our observations reveal that Cdk4 and Cdk6 cooperate in haematopoietic tumour development and suggest a role for Cdk6 in sequestering INK4 proteins away from Cdk4. This study has been carried out in collaboration with Pierre Dubus from the University of Bordeaux, Marta Cañamero and Dolores Martínez from CNIO's Histopathology and Flow Cytometry Units, and Veronika Sexl from the Institute of Pharmacology and Toxicology of the Veterinary University of Vienna. Our results are of significant importance for cancer treatment; in 2013, Cdk4 and Cdk6 inhibitors were designated as a "Breakthrough Therapy" by the U.S. Food and Drug Administration (FDA) for their potential to double the life expectancy of breast cancer patients. Thus, these findings could help us to understand the molecular basis underpinning the success of these inhibitors and, therefore, contribute to the development of novel and more effective drugs and therapies.

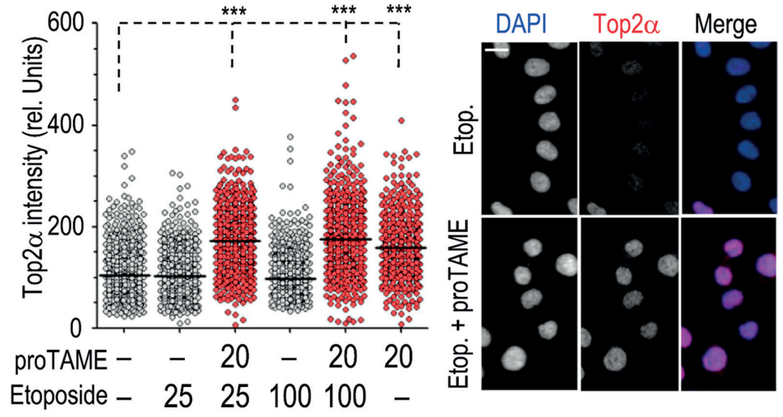

Figure 2 Cdh1 inhibition sensitises human cancer cells to topoisomerase poisons. Quantification of Top2α levels in HeLa cells treated with the indicated dose of proTAME or etoposide (μM). Horizontal bars indicate the mean. Representative images of cells treated with 25 μM etoposide or 25 μM etoposide + 20 μM proTAME. Top2α, red; DAPI, blue. Scale bars, 10 μm. (taken from Eguren et al., *Cell Rep* 2014).

A new synthetic lethal interaction between the Anaphase-Promoting Complex/Cyclosome (APC/C) and topoisomerase poisons

The APC/C cofactor Cdh1 modulates cell proliferation by targeting multiple cell-cycle regulators for ubiquitin-dependent degradation. We had previously shown that lack of Cdh1 results in structural and numerical chromosome aberrations; a hallmark of genomic instability. By using a proteomics approach in Cdh1-null cells and mouse tissues, we have identified kinesin Eg5 and topoisomerase 2α as Cdh1 targets involved in the maintenance of genomic stability. These proteins are ubiquitinated and degraded through specific KEN and D boxes in a Cdh1-dependent manner. Whereas Cdh1-null cells displayed partial resistance to Eg5 inhibitors such as monastrol, lack of Cdh1 resulted in a dramatic sensitivity to Top2α poisons as a consequence of increased levels of trapped Top2α-DNA complexes (FIGURE 2). Interestingly, we demonstrated that chemical inhibition of the APC/C in cancer cells using proTAME, which is currently undergoing preclinical trials, results in an increased sensitivity to Top2α poisons. Moreover, our data suggests that patient stratification based on their tumour's Cdh1 status could improve the effect of etoposide in that patient's treatment. This work identifies *in vivo* targets of the mammalian APC/C-Cdh1 complex and reveals the relevance of synthetic lethal interactions for anticancer treatments. This work has been conducted jointly with the CNIO Groups of Oscar Fernández-Capetillo and Javier Muñoz, and with Hiroyuki Yamano's team at the University College London (UCL) Cancer Institute. ■

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AWARDS AND RECOGNITION

- Scientific Advisory Board Member, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague.
- Editor-in-Chief, *Inside the Cell* (Wiley).

GENOMIC INSTABILITY GROUP

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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 that a cell replicates its DNA, and which is mainly prevented by ATR and CHK1 kinases. Unfortunately, the essential nature of these kinases poses significant limitations on their study, particularly at the organism level. In order to overcome these limitations, a major part of our work over these past years has 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 putative 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 cancer.

"During 2014, we focused on the identification of tumours with high levels of RS that would best benefit from a therapy with ATR inhibitors. In addition, we worked on various projects related to RS, such as its influence during somatic cell reprogramming or the role of nucleotide biosynthesis on ATR signalling."

RESEARCH HIGHLIGHTS

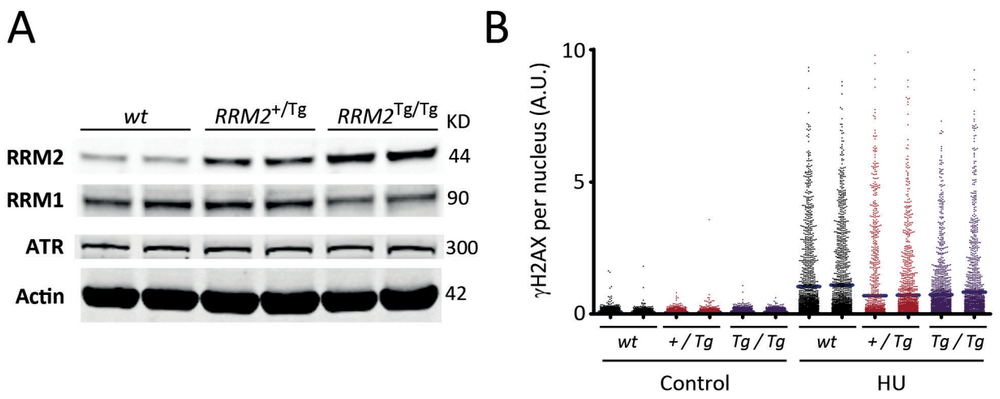

Figure 1 Increased RNR activity on *Rrm2*^{TG} MEF. (**A**) Western blot illustrating the increase in expression of the RNR regulatory subunit RRM2 in BAC transgenic MEF. (**B**) Resistance of RRM2 transgenic MEF to the RNR inhibitor hydroxyurea (HU). The figure illustrates the lower levels of HU-induced genotoxicity (measured by high-throughput microscopy as the nuclear intensity of H2AX phosphorylation, gH2AX, per nucleus) that are observed on *Rrm2*^{TG} MEF.

A conserved role of nucleotide biosynthesis on ATR biology in mammals

ATR is an essential kinase that coordinates the response to replication stress (RS); a type of DNA damage that drives genomic rearrangements at stalled replication forks, and which is abundant in certain tumours. How ATR suppresses RS is still poorly understood. Checkpoint activation, p53 induction or the stabilisation of stalled forks, are some of the widely studied functions of mammalian ATR. However, yeast studies have revealed another role that has so far remained elusive in mammals. In *S cerevisiae*, deletion of the ATR ortologue Mec1 is viable only upon concomitant deletion of Sml1; an inhibitor of the ribonucleotide reductase (RNR) complex. Whereas ATR is also essential in mammals, the extent to which its function is related to nucleotide metabolism remains unclear. We have now explored how increased RNR activity affects the ATR-response in mammals. To this end, we have generated BAC transgenic mice carrying extra alleles of the RNR regulatory subunit RMM2 (*Rrm2*^{TG}). These super-RNR mice present increased RNR activity, which is evidenced by a supra-physiological protection against RNR inhibitors (FIGURE 1). *In vitro*, *Rrm2*^{TG} cells present reduced chromosomal breakage in response to ATR inhibitors. Most importantly, we have been able to show that *Rrm2*^{TG} can significantly extend longevity in a mouse model of the Seckel Syndrome; which presents reduced levels of ATR. This line of work suggests that regulating a proper supply of nucleotides is most likely the mechanism behind the essential role of ATR in mammals. In addition, *Rrm2*^{TG} mice constitute a new tool that can be used to explore the involvement of nucleotide metabolism in several physiological contexts including cancer.

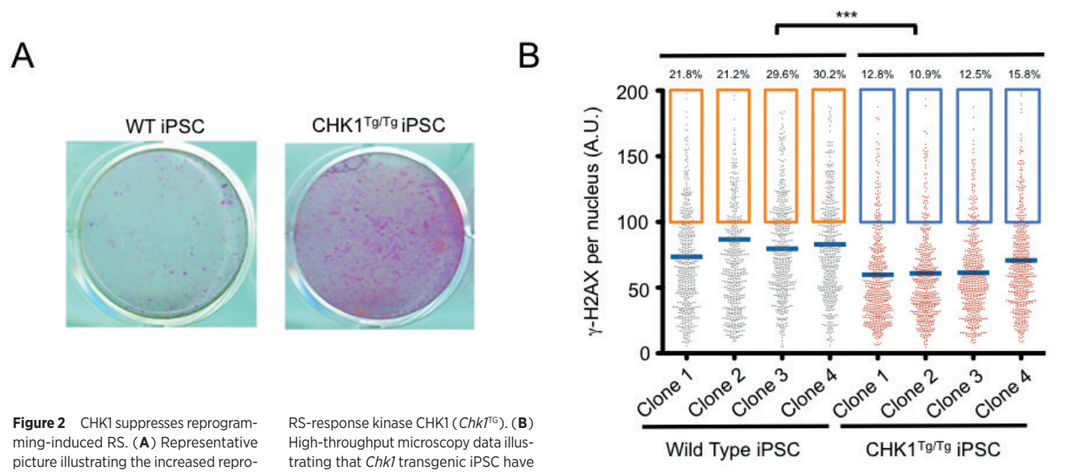

Figure 2 CHK1 suppresses reprogramming-induced RS. (**A**) Representative picture illustrating the increased reprogramming efficiency that is present on MEF carrying extra alleles of the RS-response kinase CHK1 (*Chk1*^{TG}). (**B**) High-throughput microscopy data illustrating that *Chk1* transgenic iPSC have constitutively lower levels of DNA damage than wild type controls.

Replication stress limits reprogramming to pluripotency

Reprogramming of somatic cells into induced pluripotent stem cells (iPSC) can be achieved by the expression of defined sets of transcription factors (e.g. OCT4, SOX2, KLF4 and cMYC). However, recent reports have shown evidence of DNA damage and genomic instability in iPSC, raising concerns about their potential biomedical use. The source of genomic instability on iPSC remains unresolved, although there is sufficient evidence to suggest that it could be linked to replicative stress (RS); a type of DNA damage occurring at stalled replication forks, which is limited by ATR and CHK1 kinases. According to the oncogene-induced DNA damage model of cancer development, the expression of oncogenes leads to genomic instability in cancer cells through the generation of RS. Interestingly, and besides from the well-established role of cMYC, the 3 remaining factors of the reprogramming set have also been shown to play oncogenic roles. Hence, we hypothesised that, similarly to oncogene-induced RS, an analogous reprogramming-induced RS could drive genomic instability in iPSC. In support of this view, we have seen that mouse embryonic fibroblasts (MEF) with reduced levels of ATR, which are highly sensitive to RS and resistant to transformation by oncogenes, are also refractory to reprogramming. Contrarily, MEF from a Super-CHK1 strain – that are resistant to RS – previously developed in our lab, reprogram at higher efficiencies than wild types and with lower amounts of RS (FIGURE 2). Our work reveals that RS is an important contributor to genomic instability on iPSC, and that strategies directed at lowering RS during reprogramming might serve to obtain more and safer iPSC. ■

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AWARDS AND RECOGNITION

- Selected by the scientific journal *Cell* as one of the 40 most accomplished young scientists under the age of 40; "40-under-40" (http://www.cell.com/40/under40).
- "*Fundación Botín*" Investigator, Spain.

CHROMOSOME DYNAMICS GROUP

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OVERVIEW

Proper development of a multicellular organism entails 2 major processes. One is proliferation, i.e. the cell duplicates its genetic material and divides into 2 identical daughter cells. The other is differentiation, i.e. the specialisation of naive precursors into specific cell types. This is accomplished through the activation of tissue-specific transcriptional programmes that establish cell identity. Higher order genome structure is a major determinant of such regulation of gene expression. Our research focuses on a protein complex named cohesin that occupies a central position in both of these processes. On the one hand, it mediates sister chromatid cohesion and, thereby, ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. On the other hand, it contributes to the spatial organisation of the genome by promoting or stabilising the formation of chromatin loops. 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 addition to the study of cohesin, we are interested in the centromere, a key element for chromosome segregation. Centromeres, which are defined epigenetically by the presence of a histone H3 variant known as CENP-A, direct the formation of the kinetochore. This macromolecular assembly of more than 100 proteins provides the interface through which spindle microtubules interact with the sister chromatids to power their separation in mitosis and meiosis.

“We have found that the partial deficiency of cohesin-SA1 results in altered chromatin contacts at the *Reg* gene cluster as well as in the transcriptional downregulation of 3 *Reg* genes that are involved in pancreatitis, suggesting a mechanism for the increased incidence of pancreatic cancer observed in SA1 heterozygous animals.”

RESEARCH HIGHLIGHTS

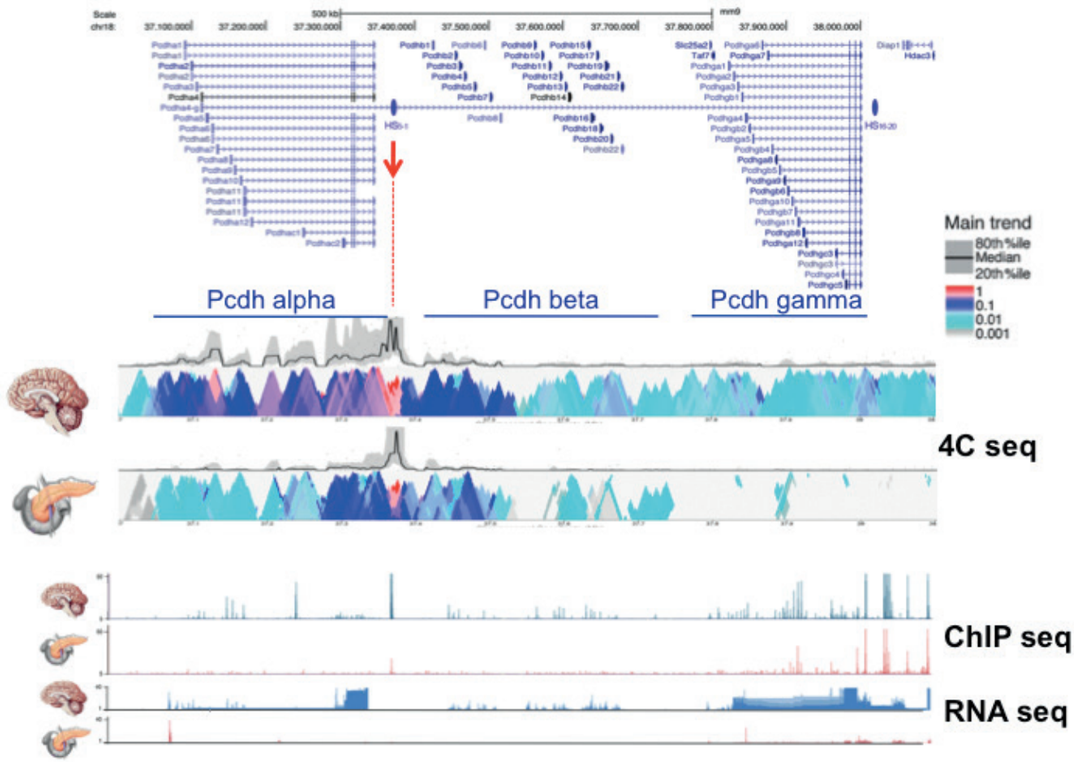

Figure 1 Distinct chromatin architecture of the *Pcdh* locus in murine cortex and pancreas viewed from the $HS_{5\text{-}1}$ enhancer (red arrow), as measured by 4C seq, correlates with differential cohesin distribution and gene expression, analysed by ChIP seq and RNA seq, respectively.

The contribution of cohesin to gene expression and chromatin architecture in 2 murine tissues

Cohesin, which in somatic vertebrate cells consists of SMC1, SMC3, RAD21 and either SA1 or SA2 subunits, mediates higher-order chromatin organisation by promoting or stabilising chromatin loops. To determine how cohesin contributes to the establishment of tissue-specific transcriptional programmes, we have compared the genome-wide cohesin distribution, gene expression and chromatin architecture in the cerebral cortex and pancreas from adult mice. For this purpose, in close collaboration with the Bioinformatics Unit, we have used 3 Next Generation Sequencing (NGS) technologies: 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 2 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. Cohesin/CTCF sites located at active enhancers and promoters need to contain cohesin-SA1, whereas either cohesin-SA1 or cohesin-SA2 can be present at active promoters independently of CTCF. Analyses of chromatin contacts at the Protocadherin (*Pcdh*) and Regenerating islet-derived (*Reg*) gene clusters, mostly expressed in brain and pancreas respectively, revealed remarkable differences in locus architecture that correlate with the differential distribution of cohesin (FIGURE 1). 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, we found reduced transcription and cohesin binding at the Regenerating islet-derived (*Reg*) gene cluster in the pancreas of SA1 heterozygous mice, as well as altered chromatin contacts. Given the role of Reg proteins in inflammation, we speculate that such reduction may contribute to the increased incidence of pancreatic cancer observed in these animals.

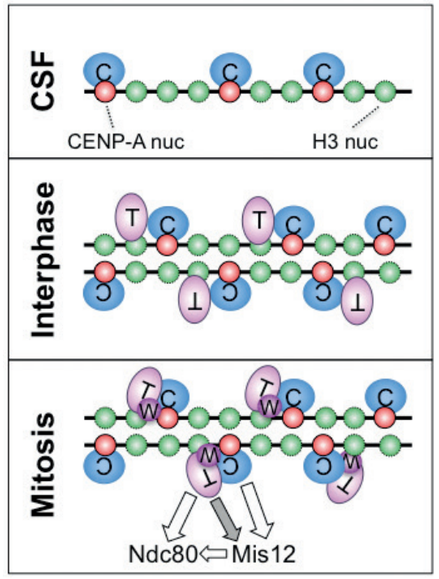

Figure 2 A model for the sequential recruitment of CCAN components CENP-C, CENP-T and CENP-W to centromeres assembled in the *Xenopus* cell-free system. Sperm chromatin is added to CSF extracts (arrested in meiosis II), which are then driven into interphase and subsequently into mitosis. Kinetochore components Mis12 and Ndc80 assemble only in mitosis.

The distinct functions of CENP-C and CENP-T/W in centromere propagation and function in *Xenopus* egg extracts

Centromeres are defined by the presence of CENP-A, and propagation of centromere identity entails the deposition of new CENP-A upon exit from mitosis in vertebrate cells. A group of 16 proteins that coimmunoprecipitate with CENP-A, the Constitutive Centromere Associated Network or CCAN, contribute to kinetochore assembly and function. For the majority of these proteins, it is still unclear how and when they are recruited to centromeres and whether they play a role in CENP-A deposition. Taking advantage of the *Xenopus* egg cell-free system, we have addressed these issues for CCAN proteins CENP-C, CENP-T and CENP-W. One advantage of this experimental system is the use of a naïve template, sperm chromatin, in which centromeres are marked by the sole presence of CENP-A nucleosomes, whereas all the rest of the CCAN components must be recruited from the soluble egg extract, to which the template DNA is added. Another advantage is that we can analyse the defect caused by removing a protein in a single cell cycle, without the accumulation of errors from previous cycles in down-regulation conditions (e.g., by siRNA). We have found that CENP-C recruitment occurs as soon as sperm chromatin is added to the egg extract, and continues after *de novo* incorporation of CENP-A in early interphase (FIGURE 2). In contrast, centromeric recruitment of CENP-T occurs in late interphase and precedes that of CENP-W, which occurs in mitosis. While loading of CENP-T in interphase does not require CENP-C, its maintenance at centromeres in mitosis together with CENP-W does. We have further shown that, unlike CENP-C, CENP-T and CENP-W do not participate in CENP-A deposition. However, like CENP-C, they are essential for the recruitment of outer kinetochore components, i.e. the KMN network. Our results support the existence of 2 pathways for kinetochore assembly directed by CENP-C and CENP-T/W that are partially redundant, and which can be reconstituted in *Xenopus* egg extracts. ■

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DNA REPLICATION GROUP

Juan Méndez
Group Leader

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OVERVIEW

Our laboratory studies the molecular mechanisms underlying DNA replication. As our cells proliferate, e.g., during the natural renewal of tissues, they undergo mitotic division cycles that require the precise duplication of the genome. The process of genomic duplication starts at thousands of different genomic locations ("origins") that serve as assembly sites for the protein machinery responsible for DNA synthesis. This machinery includes essential enzymatic activities, such as a DNA helicase known as MCM that separates the parental double helix and 2 DNA polymerases, which synthesise each of the new DNA strands. The biochemical activities of helicase and polymerase are coordinated to avoid the exposure of stretches of fragile ssDNA; a phenomenon referred to as "replication stress", which is characteristic of some tumour types.

In recent years, we have focused on two aspects of the genomic duplication process: (1) the identification of proteins and/or molecular mechanisms that counteract replication stress, such as the recently discovered PrimPol protein that mediates fork progression through damaged DNA; and (2), the impact of deregulated DNA replication *in vivo*, particularly on ageing and cancer predisposition. To this end, we have generated genetically modified mouse strains that recapitulate conditions of "gain-of-function" and "loss-of-function" of genes involved in DNA replication. Our results indicate that both scenarios constitute a challenge to genomic stability.

"We have shown that reduced expression of the *MCM3* gene in mice impairs erythropoiesis and favours the development of haematological cancers. In a collaborative study with E. Passegué's laboratory at the University of California, San Francisco (UCSF, USA), we have found that DNA replication stress underlies the functional decline in ageing haematopoietic stem cells."

RESEARCH HIGHLIGHTS

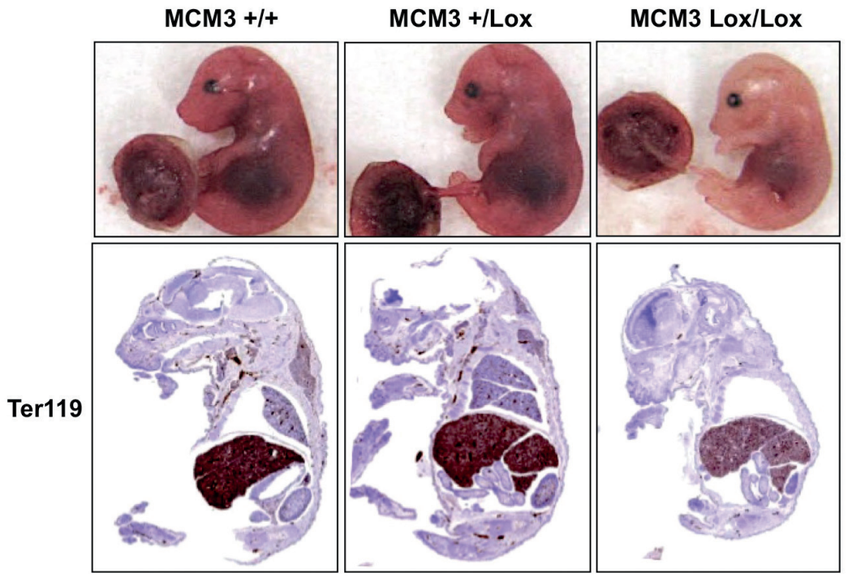

Figure 1 Impaired foetal erythropoiesis in MCM3-deficient embryos. Top: mid-gestation embryos; The *MCM3*$^{GFPLuc\text{-}LoxP}$ allele is abbreviated as *MCM3* Lox. Bottom: Immunohistochemical (IHC) staining with Ter119, which marks mature erythrocytes in the foetal liver.

Structure-function analyses in PrimPol; a DNA primase/polymerase that appears mutated in several cancer types

In 2013, we were involved in the discovery of a new human enzyme with DNA primase and polymerase activities, named PrimPol (García-Gómez et al., *Mol Cell*; Mourón et al., *Nat Struct Mol Biol*). PrimPol facilitates DNA replication under conditions of stress, promoting the restart of replication forks that have been temporarily arrested by UV light-induced DNA lesions, such as CPD and (6-4)pp thymine adducts. Our bioinformatics searches indicate that PrimPol appears deleted or mutated in several types of human cancers, including stomach adenocarcinoma, as well as diffuse large B-cell lymphoma and lung squamous cell carcinoma. In addition, mutations in the gene encoding PrimPol have been linked to familial cases of high-grade myopia. In 2014, we generated mutant versions of PrimPol that recapitulate some of the most frequent mutations detected in human cancers and in familial high myopia. In collaboration with the laboratory of L. Blanco at the *Centro de Biología Molecular "Severo Ochoa"* in Madrid, we have characterised the *in vitro* biochemical activities of these mutant versions of PrimPol, and their functionality *in vivo* using complementation assays. In addition, a viable PrimPol knock-out (KO) mouse strain has been generated in order to monitor the relevance of this protein in ageing and in cancer susceptibility.

Haematopoietic defects associated to hypomorphic expression of *MCM3*

The *MCM3* gene encodes one of the components of the DNA helicase acting at replication forks. We have generated a mouse strain carrying a genetically modified *MCM3* allele, MCM3$^{GFPLuc\text{-}loxP}$, which is hypomorphically expressed (approximately 1/3 of wild type *MCM3* levels). Over the last year, we have monitored the impact of *MCM3* downregulation on the haematopoietic system. Heterozygous MCM3$^{+/GFPLuc\text{-}LoxP}$ and MCM3$^{+/-}$ mice are viable, but their lifespan is compromised by a high frequency of early-onset lymphomas and mesenchymal tumours. The phenotype is more severe in homozygous MCM3$^{GFPLuc\text{-}LoxP/GFPLuc\text{-}LoxP}$ embryos, which die *in utero* at late gestational stages when the foetal liver fails to build a functional erythropoietic system. Foetal erythrocyte maturation requires 3-5 rounds of cell division, and we have uncovered a dynamic DNA replication programme coupled to this process. In wild-type erythroblasts (EBs), the frequency of origin firing increases as cells mature from primitive pro-EBs to basophilic and chromatophilic EBs. *MCM3*-deficient EBs fail to follow this pattern and the production of mature erythrocytes is limited (FIGURE 1). We have addressed whether this aberrant erythrocyte maturation could be traced to defects in haematopoietic stem cells (HSCs), the most upstream haematopoietic pluripotent precursors. In collaboration with the laboratory of E. Passegué (UCSF, USA), we have found that *MCM3*-deficient foetal HSCs have limited reconstitution potential upon transplantation into lethally-irradiated recipient mice. These results indicate that *MCM3* downregulation has a major impact on haematopoietic lineages.

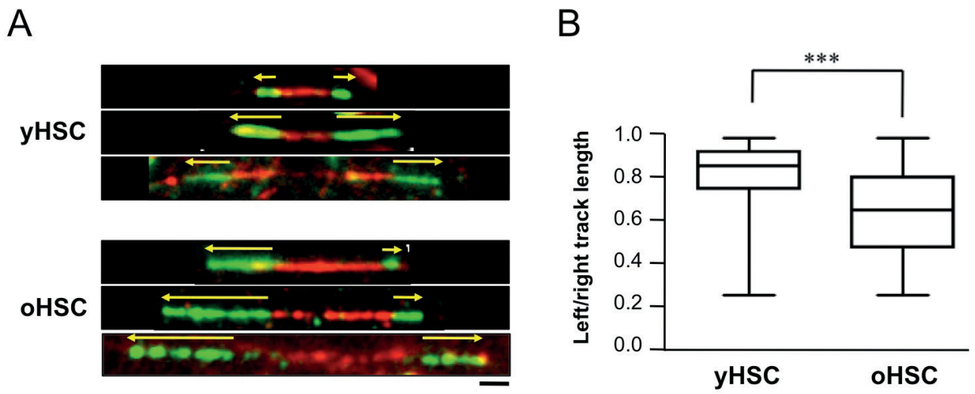

Figure 2 Replication stress in young (yHSC) versus old (oHSC) HSCs. (**A**) Origin structures in stretched DNA fibres derived from young and old HSCs. The ratio between the lengths of the 2 forks moving away from each origin indicates fork symmetry. (**B**) Box plot quantification of fork symmetry in young HSC vs. old HSC (n=100 origins in each case; *** denote p<0.001 in Mann-Whitney test).

In parallel, we have collaborated in a study, led by E. Passegué, to determine the causes of the functional decline of adult HSCs associated with natural ageing. Gene expression profiles of HSCs isolated from the bone marrow of young versus old mice revealed that *MCM2-7* genes were downregulated with age. We have performed single-molecule analysis of DNA replication in young and old HSCs, and have found a higher degree of fork asymmetry in the latter (FIGURE 2). As fork asymmetry is an indicator of fork stalling events, our results underscore the emerging notion that DNA replication stress is a driving force behind HSC functional decline with age. ■

PUBLICATION

Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, Reynaud D, Alvarez S, Diolaiti ME, Ugarte F, Forsberg EC, Le Beau MM, Stohr BA, Méndez J, Morrison CG, Passegué E (2014). Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature* 512, 198-202.

MELANOMA GROUP

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Tonantzin Calvo, Estela Cañón, Ángel Colmenar (until March)

OVERVIEW

Cutaneous melanoma is a prime example of a traditionally deadly disease, for which basic and translational research have significantly improved patient prognosis. Frequent mutations in oncogenic pathways and the identification of inhibitory immune checkpoints have paved the way for unprecedented clinical responses. Nevertheless, the portion of melanoma patients that benefit from treatment in a sustained way is still unsatisfactory. 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 emphasis on genes that are deregulated in a melanoma-specific manner. Modulators of RNA stability, transcription and translation, are also central themes in our research. Our experimental settings combine human biopsies isolated from early, intermediate and late stages of melanoma development, with a unique set of animal models engineered for non-invasive imaging of metastatic processes. These studies are performed within the context of multidisciplinary consortia composed of specialists in biology, chemistry, pharmacy, nanotechnology, molecular imaging, dermatopathology and clinical oncology. We also work in partnership with biotechnology companies in order to facilitate the translation of our discoveries to the bedside.

"We have identified a cluster of genes that distinguish melanoma from over 35 other tumour types, we have identified new functional vulnerabilities that can be exploited for drug delivery, and have generated animal models for the visualisation and targeting of early metastasis (a main ongoing challenge in this disease)."

RESEARCH HIGHLIGHTS

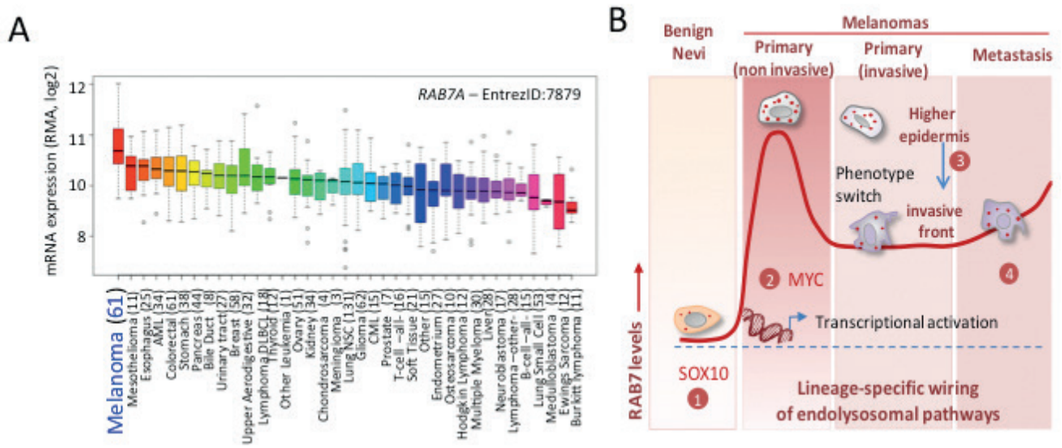

Figure 1 Identification of RAB7 as a new lineage-specific melanoma driver. (**A**) Relative RAB7 mRNA expression across a broad spectrum of cancers included in the Cancer Cell Line Encyclopedia. (**B**) Model summarising RAB7 regulators (SOX10 and MYC) and dose-dependent functions in melanoma progression and invasion.

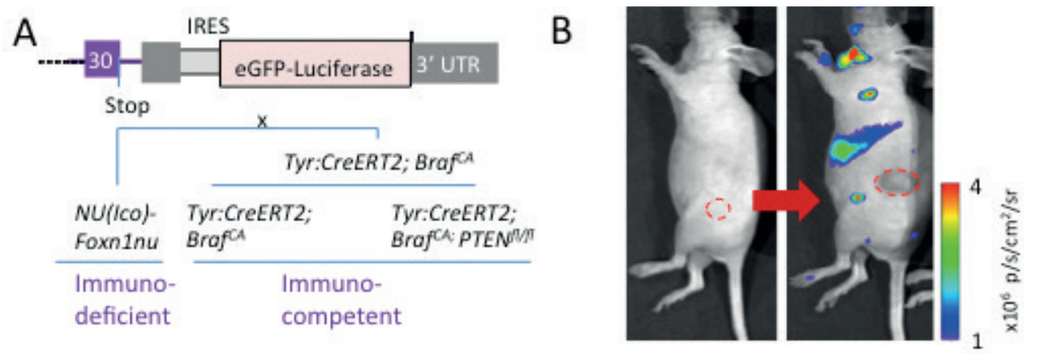

Figure 2 Melanoma "Lymphoreporter" mouse models. (**A**) Schematic illustration of the immunocompetent and immunodeficient strains generated from the *Vegfr3*^{GFPLuc} knock in mice. (**B**) Example of early activation of Vegfr3 driven by patient-derived melanoma xenografts as visualised by luciferase imaging.

New lineage-specific drivers of melanoma progression

The search for tumour drivers and druggable vulnerabilities in melanoma has traditionally involved "within lineage" comparisons of benign and malignant melanocytic cells or lesions. This approach has revealed over 80,000 mutations in melanoma tumours, representing a daunting challenge in the separation of byproducts from functionally relevant genetic alterations. A less explored strategy for the discovery of pro-oncogenic factors is to perform "across lineage" analyses (i.e. assessing different cancer types). To this end, we teamed up with researchers from the Bioinformatics Core Unit at the CNIO, the *Hospital 12 de Octubre* in Madrid and the Memorial Sloan-Kettering Cancer Center in New York. These studies identified a cluster of endolysosomal-associated genes that are selectively enriched in melanoma in a manner that is not shared by over 35 other additional malignancies. One of the most melanoma-upregulated factors of this gene set was the GTPase RAB7 (FIGURE 1A). Analyses in human cells, clinical specimens and mouse models demonstrated that RAB7 is an early-induced melanoma driver, with unanticipated dose-depending roles in melanoma cell proliferation and invasion (FIGURE 1B). Importantly, we have described the neuroectodermal master modulator SOX10 and the oncogene MYC as novel RAB7 regulators. Further live-time imaging of vesicular trafficking demonstrated that melanoma cells are highly efficient at incorporating large extracellular substrates in macropinosomes. Interestingly, this macropinocytic activity was largely silent in normal skin cells, potentially allowing for a tumour-cell selective uptake of nanosize-based anticancer agents. Collectively, these data support the endolysosomal machinery as a driver and therapeutic target in melanoma.

Further comparative analyses of melanoma cells versus normal melanocytes – performed 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) – have led to the identification of tumour-selective roles of modulators of mRNA stability, alternative splicing, transcription and translation. We are very excited about these results seeing as they open up new avenues of research on RNA regulators as diagnostic markers and therapeutic targets in melanoma.

Genetically engineered mouse melanoma models for gene discovery and target validation by non-invasive imaging techniques

A key limitation for rational drug design in cancer, particularly in melanoma, is the lack of physiologically-relevant models and tracers to monitor metastatic cells *in vivo*. In particular, a long-established clinical observation in the melanoma field, which remains unaddressed from a mechanistic perspective, is the contribution of the lymphatic system to distal metastasis, and ultimately to overall patient survival. Thus, one of the most accepted indicators of poor prognosis in this disease is the presence of melanoma cells in neighbouring (sentinel) lymph nodes. Still, removal of these lymph nodes does not significantly improve patient prognosis. In collaboration with the CNIO Transgenic Mice Core Unit, led by Sagrario Ortega, we have generated the first in-class melanoma "lymphoreporter" mice. These animals are based on a knock-in of a GFP-Luciferase fusion cassette at the 3' UTR region of *Flt4* (*Vegfr3*), a main driver of lymphangiogenesis. These animals were crossed into inducible models that mimic benign nevi (*Vegfr3*^{GFPLUC}*; Tyr::CreERT2; Braf*^{CA}) or malignant melanoma (*Vegfr3*^{GFPLUC}*;* Tyr::*CreERT2*; *Braf*^{CA}; *Pten*$^{fl/fl}$). In addition to these immunocompetent backgrounds, immunodeficient *Lymphoreporter* mice were used for mechanistic analyses of melanomas driven by human biopsies (patient derived xenografts or "avatars") or by genetically engineered melanoma cells (see examples in FIGURE 2). These mice are being used as a cost-effective platform for gene discovery and pharmacological testing of anticancer agents. ■

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AWARDS AND RECOGNITION

- "*Premio Gallego del Año 2014*" Award, *Correo Gallego* Group, Spain.

BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY PROGRAMME

ERWIN F. WAGNER Programme Director

The overall strategic goals of the BBVA Foundation-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. In March 2014, a Senior Research CNIO Group, which was previously part of the Molecular Pathology Programme and headed by Francisco X. Real, joined our Programme. Our Groups investigate how a tumour can grow as an 'extrinsic organ'. 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, angiogenesis, 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.

My own Research Group focuses on understanding the role of the transcription factor complex AP-1 (Fos/Jun) in physiological and pathological processes. We work on liver fibrosis and fatty liver disease, inflammation and cancer, on bone homeostasis and osteosarcomas, and also aim to molecularly define the causes of skin cancer and inflammatory skin diseases, such as psoriasis. Mirna Pérez-Moreno's Group concentrates on the role of cell adhesion, inflammation and cellular signalling in normal skin physiology and cancer development, whereas Nabil Djouder's Group aims to dissect the contribution of nutrient and growth factor signalling pathways to cancer development. Massimo Squatrito's Group – in part 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 focussing on pancreatic and bladder cancer. The Group employs an integrative approach to understand the molecular patho-physiology of these tumours and to apply this knowledge to the clinical setting.

"Our main goal is to make CNIO internationally more competitive and that it remains an international institution; 15 different nationalities are represented in our Programme. We aim to perform first-class cancer cell biology research and to train students and postdocs to become the next-generation of promising scientists."

GENES, DEVELOPMENT AND DISEASE GROUP

Erwin F. Wagner
Group Leader

Staff Scientists
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Albanderi Alfraidi (Since September), Rainer W. Hamacher, Michele Petruzzelli (Until July), Álvaro Ucero

Graduate Students
Lucía T. Díez (Since September), Stefanie Wurm

Technicians
Vanessa Bermeo, Ana Guio, Jakob Schnabl (March-September), Stephania Tocci (Since October)

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 development and to identify novel therapeutic targets. 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 apply these to preclinical studies.
- Using large-scale genomic or proteomic approaches to compare mouse models of disease to human disease and identify therapeutically relevant targets.

"We aim to make CNIO a more international institution. At present 4 out of 5 Group Leaders in our department are foreigners; 1 is partly funded by the Seve Ballesteros Foundation. Fifteen different nationalities ensure an international science culture and all focus on unravelling the mysteries of cancer."

RESEARCH HIGHLIGHTS

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

Bone development and sarcomas

We are studying the function of Fos proteins and their targets such as TGFBI using loss- and gain-of-function mouse models. We found that Fos protects osteoblasts from replicative stress and DNA damage through Chk1 upregulation; a mechanism that is most likely relevant to osteosarcoma development.

Liver disease – inflammation, metabolism, fibrosis and cancer

In hepatitis, c-Jun is a mediator of cell survival specifically in hepatocytes, while the absence of JunB in immune cells is beneficial. Mechanistically, JunB promotes cell death during acute hepatitis by regulating interferon-γ production, thus functionally antagonising the hepato-protective function of c-Jun.

Fra-1/2 proteins appear to be dispensable for liver fibrosis, while they are important novel modulators of hepatic lipid metabolism. AP-1 modulates hepatic lipid storage and steatosis formation by controlling PPARγ transcription. Strikingly, AP-1 dimers can either induce or repress PPARγ expression. Therefore, fatty liver disease and obesity most likely depend on the composition of AP-1 dimers.

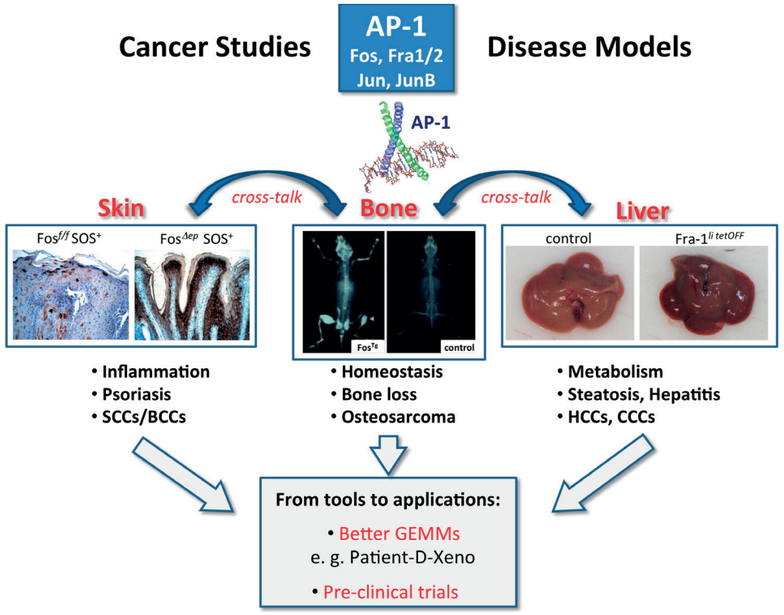

Figure 1 Tet-switchable AP-1 transgenic mice were generated for ectopic expression of specific AP-1 monomers/dimers in the liver, skin and bone. Proteomics, expression profiling, RNA-sequencing and ChIP-sequencing are employed to compare mouse models of disease to human disease and to identify novel targets. Preclinical studies are performed in our AP-1-dependent mouse models with compounds that target the identified molecules to determine the potential of translating our findings for treating human disease.

Ectopic c-Fos expression and its dimers lead to spontaneous liver inflammation, fibrosis, hepatocyte/bile duct hyperproliferation and cancer. Deletion of c-Fos in hepatocytes protects from chemically-induced liver carcinogenesis, whereas deletion in immune cells abrogates this protective effect.

Role of white adipose tissue in cancer-associated cachexia

Various cancer mouse models were employed to discover a consistent metabolic and phenotypic switch from white to brown fat (browning) in cachectic mice. The role of browning as a contributor to the wasting process was further characterised, providing a promising new target to prevent/delay cachexia in cancer patients.

A function for AP-1 in the lung

We have recently documented the connection between the Fos protein Fra-1 and major transcription factors controlling epithelial to mesenchymal transition; a process crucial to epithelial cancers. The contribution of Fra1/2 proteins to lung fibrosis and cancer is currently being studied using GEMMs, as well as lung cancer samples from patients. This study is conducted in collaboration with Mariano Barbacid's Experimental Oncology Group at the CNIO and the Daiichi Sankyo Company in Japan.

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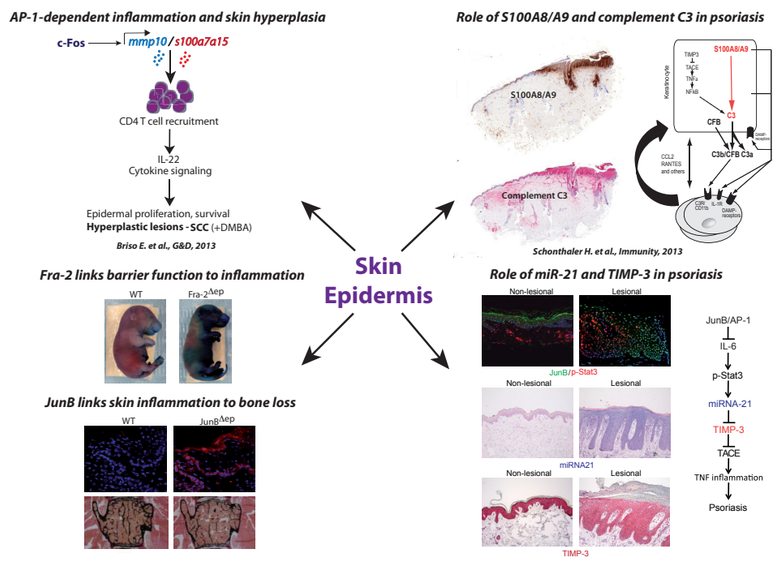

Figure 2 Defining AP-1(Fos/Jun) functions in skin/epidermis. On the left side, the functions of the AP-1 proteins c-Fos, Fra-2 and JunB in skin inflammation, barrier function and the link to bone loss are depicted. On the right side, a newly discovered pathway with novel targets for psoriasis is described.

Skin cancer, inflammation and human disease

Since we found increased c-Fos expression in Squamous Cell Carcinomas (SCCs), 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 also demonstrated that loss of epidermal Fra-2 protein results in skin barrier defects. Mechanistically, Fra-2 binds and transcriptionally regulates epidermal differentiation gene promoters, which are co-occupied by the transcriptional repressor Ezh2.

Characterisation of the epidermal inflammatory disease in mice lacking JunB suggests a skin to bone cross-talk. 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.

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 S100A8/A9 and complement C3 (FIGURE 2). In addition, a potential role of specific miRNAs, e.g. miR21 involved in the pathogenesis of psoriasis was established. Human skin samples are provided by our collaborator Esteban Daudén from *Hospital Universitario de La Princesa* (Madrid, Spain). ■

EPITHELIAL CARCINOGENESIS GROUP

Francisco X. Real
Group Leader

Staff Scientists
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OVERVIEW

We focus on the molecular/cellular mechanisms involved in pancreatic and bladder cancer with a disease-oriented approach. Our strategy resembles a pyramid having as base an equilateral triangle. The 3 vertexes correspond to the used models: patient samples, cell cultures, and genetically modified mice. The third dimension comes from the projection of this knowledge to the "population" level: we bring the biology to large-scale studies with patients. We are interested in the genetic susceptibility to cancer and in developing better molecular tools to predict patient outcomes or response to therapy. Our primary observations can be made at either of these levels and can then be extended through additional work.

In regards to pancreatic ductal adenocarcinoma (PDAC), we are interested in the early events involved in tumour development with a particular focus on cell differentiation as a critical tumour suppressor mechanism. These processes cannot be readily studied using human samples. Therefore, we use the excellent genetic mouse models that are available to us. PDAC can originate both in pancreatic progenitors and in acinar cells. The elucidation of the

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Technicians
Inmaculada Almenara (February-October), Natalia Del Pozo, Carme Társila Guerrero (April-September), María Tania Lobato, Ana Sagrera

contribution of these cell types to PDAC is crucial to design better strategies for early tumour detection and prevention in subjects at risk. Regarding urothelial cell carcinoma (UCC), we are interested in identifying new genes that can then be used for improved tumour taxonomy, characterising the mechanisms through which they participate in cancer, and apply this knowledge for improved prediction of outcome and therapy.

"Our work has highlighted novel aspects of the role of transcription factors involved in acinar differentiation (i.e. GATA6, HNF1A) as important players in PDAC; we have shown that the MNK1 kinase is key to the stress response in acinar cells. Furthermore, we have characterised new aspects of the molecular genetics of bladder cancer."

RESEARCH HIGHLIGHTS

Pancreas cancer 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* and 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 the early steps of tumour development. We have acquired evidence that transcription factors involved in the fine-tuning of acinar cell differentiation (i.e. *Gata6, Hnf1a,* and *Nr5a2*) also play an important role. *Gata6* inactivation in pancreatic progenitors accelerates *KRas*-driven PDAC progression in mice and GATA6 is deleted in a subset of human PDAC, supporting its role as a tumour suppressor. GATA6 controls the epithelial-mesenchymal transition and also regulates a "basal signature" in PDAC that is shared with breast and bladder tumours. Other genes that participate in development, differentiation, pancreatitis, and PDAC include *Nr5a2*, *Hnf1a*, *Foxa1/2*, and *Myc*. RNA-Seq and ChIP-Seq experiments have enabled us to identify intricate relationships between them through regulatory networks controlling tumour suppressors, epigenetic regulation, metabolic processes, and inflammatory cytokine cascades. Factors supporting acinar cell differentiation also repress – directly or indirectly – inflammatory genes. Among the signalling components, we have identified the stress kinase Mnk1 that is selectively expressed at high levels in acinar cells, is regulated during pancreatitis and cancer, and is required for the secretory response (FIGURE 1). We are currently evaluating its role in mutant *KRas*-driven PDAC using mouse models. This work benefits from a close collaboration with the other groups working on PDAC at the CNIO (Marinano Barbacid from the Experimental Oncology Group; Christopher Heeschen from the Stem Cells and Cancer Group; Manuel Hidalgo from the Gastrointestinal Cancer Clinical Research Unit; and Núria Malats from the Genetic and Molecular Epidemiology Group).

Urothelial cell carcinoma (UCC) genetics and biology

Our goal is to refine current knowledge on the genomic landscape of UCC and to apply this in the clinical setting. Hotspot *TERT* promoter mutations are the most common genetic change in UCC and occur early on during tumour progression. *TERT* mutant tumours do not display higher levels of TERT mRNA than wild type counterparts, pointing to the notion that they may act through mechanisms other than increased transcriptional activity. Intriguingly, *TERT* mutations are not associated with genetic or environmental risk factors for UCC and they are promising candidates for non-invasive tumour detection in exfoliated cells in urine.

Through exome sequencing we have identified new genes and pathways involved in UCC and we are focusing on STAG2, a cohesin component. *STAG2* inactivation is more frequent in non

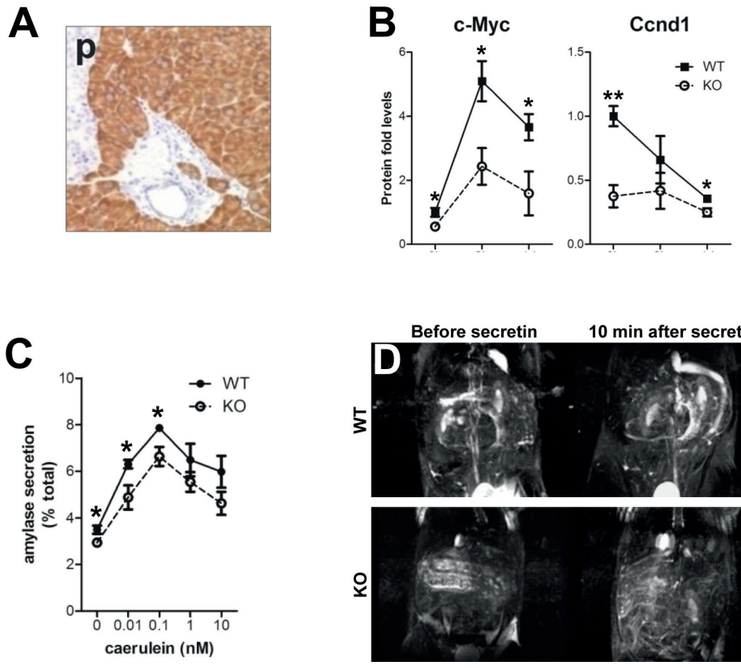

Figure 1 Mnk1 is an acinar-specific stress kinase required for cell proliferation upon pancreatic damage and for optimal exocrine secretion. (**A**) Mnk1 expression in mouse pancreas. (**B**) c-Myc and Ccnd1 levels in WT and KO pancreata upon acute pancreatitis (n=4). (**C**) Caerulein-induced amylase release by isolated WT and KO acini. (**D**) MRCP images showing secretin-induced fluid secretion into the duodenum of WT and KO mice.

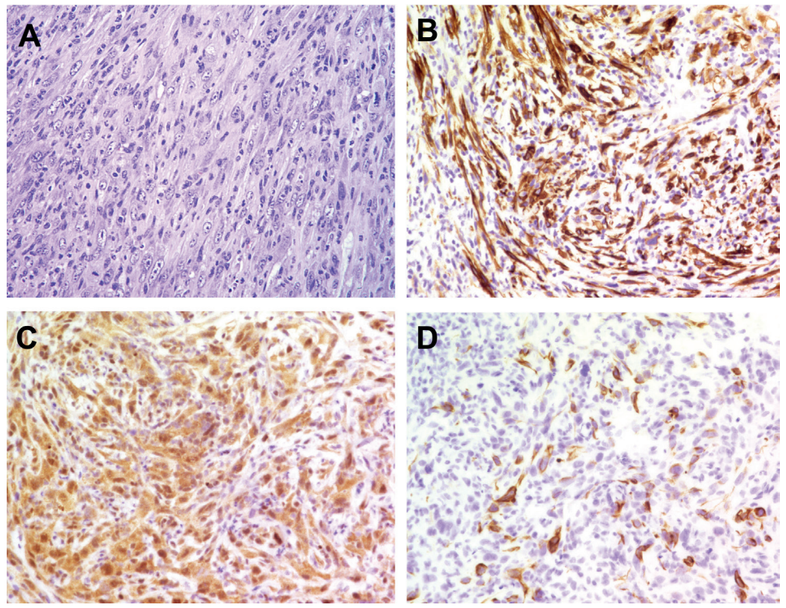

Figure 2 Adeno-Cre-mediated deletion of Pten and p53 in the bladder epithelium leads to the development of highly aggressive carcino-sarcomatoid tumours (**A**), expressing smooth muscle actin (**B**), KRT5 (**C**) and KRT14 (**D**). According to the recent taxonomical classifications, these features are typical of "genomically unstable" and "p53-like"-type human tumours.

muscle-invasive tumours and is not associated with aneuploidy, suggesting that STAG2 participates in UCC through mechanisms different from those involved in chromosome segregation. We are generating conditional Stag2-null mice to study how Stag2 inactivation contributes to urothelial tumour development/progression.

In the last few years, a new molecular taxonomy of UCC with broad clinical implications is emerging. Using genetic mouse models we have shown that the concurrent inactivation of *Pten* and *Tp53* in the urothelium leads to carcino-sarcomatoid tumours, which is in line with this taxonomy, and demonstrates specific therapeutic sensitivities (FIGURE 2). We are exploring whether "luminal", "basal", and "inflammatory" signatures are associated with clinical/pathological features and with patient outcomes. This information will be integrated with germline and somatic genomic analyses and will be used for the design of clinical trials in collaboration with the SOGUG (Spanish Oncology Genitourinary Group) cooperative group.

This work is being conducted in close collaboration with Núria Malats' Group at the CNIO, with SOGUG, and with a European Consortium of collaborators. ■

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AWARDS AND RECOGNITION

- Associate Editor, *Bladder*.
- Associate Editor, *Bladder Cancer*.
- European Pancreatic Club Council President (2015).

EPITHELIAL CELL BIOLOGY JUNIOR GROUP

Mirna Pérez-Moreno
Junior Group Leader

Post-Doctoral Fellow
Donatello Castellana

Graduate Students
Ljiljana Dukanovic,
Marta N. Shahbazi (until June)

Technician
Francesca Antonucci

OVERVIEW

Our research interests are directed at advancing our understanding of the events that regulate the physiology of epithelial tissues and how, when perturbed, these result in disease, including cancer. The primary epithelial tissue we study is the skin. In adult skin, epithelial progenitor cells have been identified as the cells of origin of skin carcinomas, the most common cancers in the world. These cells reside in the basal proliferative layer of the epidermis, whereas in the hair follicle they localise in a restricted area known as the bulge. In particular, we focus on dissecting how the interactions between epithelial progenitor cells, as well as the interactions with their surrounding microenvironment, maintain tissue architecture and modulate cell adhesion, proliferation, migration, and gene expression. In order to investigate how alterations in the interactions between epithelial progenitor cells with their neighbours and/or with the surrounding tissue microenvironment correlate with hair and epidermal diseases – including cancer – we employ mouse genetics, *in vitro* culture systems, and human skin sample analyses.

RESEARCH HIGHLIGHTS

Mechanisms regulating epidermal progenitor cells' self-renewal and differentiation

One of the fundamental questions in the biology of tissues is how they acquire an adequate control of cell division and differentiation. We continue exploring the role of novel players, including mitotic and cytoskeletal proteins, in the regulation of epidermal progenitor's self-renewal through oriented cell divisions, using mouse epidermal development as a model system. In addition, we are investigating how these novel players may be involved in regulating proper epidermal differentiation, tissue architecture and barrier function. We believe these results may spawn new concepts about how these molecules regulate tissue homeostasis and how, when disrupted, they lead to disease.

Interactions between epithelial progenitor cells, as well as their interactions with the surrounding microenvironment, in skin homeostasis

The contributions of progenitor cells and their lineages to skin regeneration, tissue repair and tumourigenesis, along with the intrinsic genetic alterations that regulate their behaviour, have been the subject of intense investigation. However, the environmental cues arising from cells in the tissue microenvironment that affect several aspects of progenitor cell behaviour, such as proliferation, survival and migration, are less understood.

We have uncovered a mechanism by which epidermal progenitor cells sense injury and promote repair of epithelial layers. This involves the adherens junctions protein p120-catenin, whose role extends beyond intercellular adhesion to the regulation of inflammatory responses and epithelial remodelling upon tissue injury, as well as being potentially implicated in chronic inflammation and cancer.

Moreover, we have identified a novel connection between skin progenitor cells and macrophages that modulates their regenerative potential. Under physiological conditions, in non-inflamed, non-transformed skin, a subset of skin macrophages surround hair follicle stem cells (<30μm) and contribute to their activation via Wnt ligands. No polarisation into the M1/M2 phenotypes was observed under these physiological conditions. The pharmacological inhibition of Wnt activity derived from the macrophages – via an IWP2 inhibitor delivered in liposomes – delayed activation of hair follicle stem cells and hair growth. Thus, perifollicular macrophages contribute to the activation of skin epithelial stem cells as a novel and additional cue that regulates their regenerative activity. These findings may have translational implications for skin repair, inflammatory skin diseases and cancer. ■

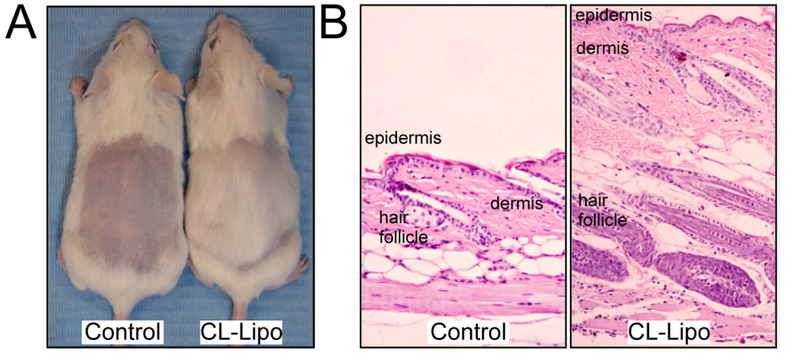

Figure Macrophages regulate the activation of hair progenitor cells and hair follicle growth. (**A)** Mice were shaved and injected subcutaneously with empty liposomes (control) or Clodronate liposomes (CL-Lipo). Observe that the treatment with CL-Lipo induced hair growth. (**B**) Histological analysis of skin sections showing that CL-Lipo induces hair growth compared to controls.

PUBLICATIONS

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AWARDS AND RECOGNITION

- Scientific Advisor, French Evaluation Agency for Research and Higher Education (AERES).
- Evaluation Committee Member, Le Département "*Oncogenèse et Biotechnologie*, *Institut Albert Bonniot* in Grenoble, France.

GROWTH FACTORS, NUTRIENTS AND CANCER JUNIOR GROUP

Nabil Djouder
Junior Group Leader

Post-Doctoral Fellows
Hugo Bernard, Stefan Burén (until September), Mohamad-Ali Fawal, Ana Gomes

OVERVIEW

As Western society has shifted to a higher caloric diet with nutrients overload and a more sedentary lifestyle, the incidence of metabolic syndrome and cancer has increased to epidemic proportions. Using mouse models combined with biochemical techniques, our laboratory is interested in delineating the growth factor and nutrient signalling cascades that impact on the patho-physiolological states of metabolic diseases and cancer. Successful outcomes in new mechanistic insights of circuits associated to growth factors and nutrients may improve the predictive clinical potential and should facilitate development of innovative mechanism-based therapeutics to treat metabolic dysfunctions and cancer.

"Developing new mouse models that mimic different stages of human diseases, and the identification and validation of gatekeeper pathways in early disease stages may offer new therapeutic strategies to prevent and cure metabolic dysfunctions and cancer."

Graduate Students
Marta Brandt, Almudena Chaves, Ana Teijeiro, Krishna Seshu Tummala

RESEARCH HIGHLIGHTS

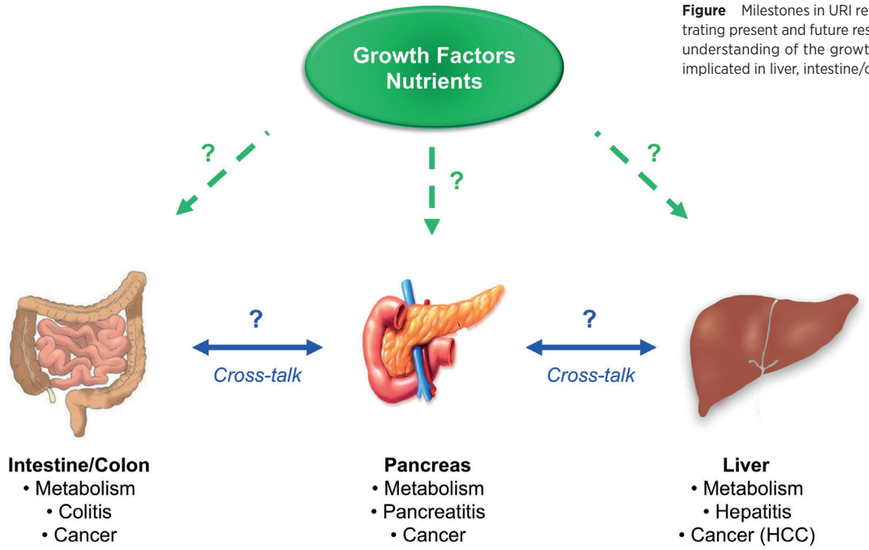

Figure Milestones in URI research at the CNIO: scheme illustrating present and future research at CNIO, involving a better understanding of the growth factor and nutrient circuitries implicated in liver, intestine/colon and pancreas disorders.

Our laboratory studies the molecular mechanisms of diseases associated to dysregulations in 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. Identifying new components of growth factor and nutrient circuitries, as well as elucidating their role and functions *in vivo* by generating new mouse models, will help us to better understand how growth factors and nutrients impact on the patho-physiological state of metabolic disorders and cancer.

Identifying new components of growth factor and nutrient circuits

To decipher the growth factor and nutrient signalling cascades, in addition to biochemical approaches, we screened for new components of the growth factors and nutrients cascade using live-cell imaging based on fluorescence resonance energy transfer (FRET).

Genetically engineered mouse models

We identified new components of the growth factors and nutrients signalling cascades. Mouse models were generated in our lab in order to study their impact on liver, pancreas and intestinal diseases. Elucidating molecular mechanisms will offer strategic therapeutic interventions to prevent and cure human diseases. ■

PUBLICATION

- Tummala KS, Gomes AL, Yilmaz M, Graña O, Bakiri L, Ruppen I, Ximénez Embún P, Sheshappanavar V, Rodriguez-Justo M, Pisano D, Wagner EF, Djouder N (2014). Inhibition of *De Novo* NAD$^+$ Synthesis by Oncogenic URI Causes Liver Tumorigenesis through DNA Damage. *Cancer Cell* 26, 826-839.

PATENT

- Djouder N, Tummala KS (2104). Methods for Treating Cancer. *EP14382298.9.*

SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito
Junior Group Leader

Staff Scientists
Bárbara Oldrini, Alberto J. Schuhmacher

Graduate Students
Carolina Almeida,
Alvaro Curiel (since February)

Technician
Claudia S. Troncone (since August)

"The central focus of our Group is to uncover the genetic defects, present in GBM patients, responsible for the aggressiveness of this tumour type. In particular, we are interested in the identification of the genetic alterations that lead to the modulation of the activity of the DNA damage response (DDR)."

OVERVIEW

Malignant gliomas (astrocytomas, oligodendrogliomas and oligoastrocytomas) are the most frequent forms of brain tumour, of which Glioblastoma Multiforme (GBM), classified as grade IV astrocytoma, is the most lethal tumour of the central nervous system in adults. Standard treatment for GBM consists of surgical resection of the tumour and postoperative treatment with chemotherapy and ionising radiation. Despite advances in surgical and imaging techniques, the treatments that are available for GBM are still ineffective; this is largely due to its intrinsic resistance to the current therapeutic modalities and its high cellular heterogeneity.

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

RESEARCH HIGHLIGHTS

New imaging tools for GBM tumours

Positron Emission Tomography (PET) is an imaging modality that is widely used in oncology for staging, monitoring the efficacy of a given treatment, and to follow-up tumour recurrence; it offers an *in vivo* quantitative and functional evaluation at the molecular level. ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) is the most frequently used radiopharmaceutical in clinical imaging. ^{18}F-FDG, however, has limited usefulness in brain tumours, such as GBM, due to the high uptake of glucose by the brain, which causes the image to have a low signal/background ratio thereby hindering the identification of the signal from the tumour. Immuno-Positron Emission Tomography (immunoPET) is a novel and attractive option to improve diagnostic imaging as it combines the high resolution and quantitative capabilities of PET, with the specificity and selectivity of monoclonal antibodies (mAb) against a given tumour cell surface marker.

In collaboration with Francisca Mulero (the Molecular Imaging Unit) and Jorge Luis Martínez (the Proteomics Unit) from the CNIO, and other collaborators at the CNIC and CIEMAT, we have identified a potential novel target for immunoPET imaging GBM. MT1-MMP is a membrane-anchored matrix metalloproteinase whose expression has been shown to be increased with tumour grade in gliomas; MT1-MMP expression levels in GBM patients are associated with worse prognosis. Preliminary studies, using an antibody vs. MT1-MMP labelled with the positron-emitting radioisotope ^{89}Zr, have shown promising results regarding the high specificity of such a probe, and suggest multiple potential clinical applications for this novel imaging tool.

Therapy resistance mechanisms in GBM

Standard therapy for GBM includes resection of the tumour mass, followed by concurrent radiotherapy and chemotherapy with an alkylating agent known as Temozolomide (TMZ). The most frequent resistance mechanism to TMZ treatment is the expression of the DNA-repair gene, O^6-methylguanine-DNA-methyltransferase (MGMT), although other resistance mechanisms still have to be identified. In collaboration with the CNIO Genomic Instability Group, we are conducting genetic screenings in haploid human cells in order to identify novel modulators of TMZ sensitivity. ■

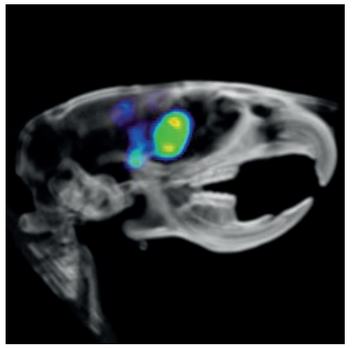

Figure PET-CT image of a GBM mouse xenograft performed after injection of a ^{89}Zr-labelled MT1-MMP antibody.

PUBLICATIONS

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- Vivanco I, Chen ZC, Tanos B, Oldrini B, Hsieh WY, Yannuzzi N, Campos C, Mellinghoff IK. A kinase-independent function of AKT promotes cancer cell survival. *Elife*. PMID: 25551293.

STRUCTURAL BIOLOGY AND BIOCOMPUTING PROGRAMME

ALFONSO VALENCIA Programme Director

The main objective of the Structural Biology and Biocomputing Programme is the mechanistic understanding of key cancer-related molecular systems. The strength of the Programme resides in its capacity to combine computational and structural approaches. The Programme collaborates with CNIO's Basic and Translational Research Groups, and participates in a number of international consortia.

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.
- Integrate the analysis of epi- and genomic information related to cancer progression, and use this information to propose potential therapeutic targets.

Research highlights of the year include: 1) the development of a first model of the complex interactions in the epigenetic network of proteins, histone and DNA modifications; 2) the initial investigation regarding the possibilities to repurpose drugs that are currently being used for the treatment of central nervous system diseases to treat cancer, based on the use of molecular information on inverse co-morbidities; 3) the proposal of a mechanistic model of the activation of the membrane clustering of Focal Adhesion Kinase (FAK) by phosphatidylinositol 4,5-biphosphate (PIP_2); 4) the investigation of the structure of the components of the complex carrying out *de novo* pyrimidine biosynthesis (carbamoyl-phosphate synthetase II, aspartate transcarbamylase and dihydroorotase); 5) the participation of the Bioinformatics Unit in top CNIO publications; and 6) the creation of a new Electron Microscopy Unit. ■

"Our Programme's work – on the building of mechanistic models that capture the details of the underlying molecular interactions – provides the essential link between basic biological knowledge and actionable biomedical applications."

STRUCTURAL COMPUTATIONAL BIOLOGY GROUP

Alfonso Valencia
Group Leader

Staff Scientists
Federico Abascal, Nicole Dölker, Milana Morgenstern, Tirso Pons, Daniel Rico, Michael Tress

Post-Doctoral Fellows
Simone Marsili, Vera Pancaldi, Miguel Vázquez

Graduate Students
Simone Ecker, Paolo Maietta (until October), Maria Rigau (since September), Juan Rodríguez

Technicians
David Juan, Martin Krallinger, Florian Leitner (until February), Miriam Rubio

OVERVIEW

The main interest of our group is the study of the molecular bases of cancer progression. We bring 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 computational methods in order to reveal the general properties of the genome-cancer relationships, via analysis of high-throughput datasets.

In this framework, 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 statistical 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 started a new line of research by analysing, at the population and molecular levels, the relationship between cancer and diseases of the central nervous system (CNS). Our results have contributed to corroborating the existence of an inverse comorbidity between the CNS and some specific cancer types; i.e patients with Alzheimer’s disease or schizophrenia have a statistically reduced probability of having cancer. In 2014, we published the first approach to the molecular basis underlying the observed inverse comorbidity. These results open up a new opportunity for repositioning drugs, indicated for CNS disorders, to treat cancer.”

RESEARCH HIGHLIGHTS

Our Group's endeavours can be summed up by our contributions to three large-scale genome efforts.

Gene expression variability in cancer progression

In the context of the Chronic Lymphocytic Leukaemia project, which is part of the International Cancer Genome Consortium (ICGC), we have contributed to the analysis of deep sequencing-based RNA-seq results. The classical clustering of chronic lymphocytic leukaemia (CLL) samples, according to gene expression levels, has provided consistent groups of cases that did not correspond to the CLL groups known to have different clinical outcomes.

We decided to reanalyse this surprising result with new eyes. Instead of focusing on a classification based on overexpressed and repressed genes, we classified the cases according to the differences in the level of expression between cases, under the assumption that the variability in expression levels will favour the adaptation to new environments, and in the case of disease, the more aggressive form of the disease. The results obtained with different CLL datasets indicate that, indeed, expression variability is a better predictor of disease outcome than the levels of gene expression.

Challenging the established paradigm in alternative splicing

Computational biology offers the possibility of revisiting fundamental concepts in biology by carefully analysing available data sources. We have demonstrated, in a succession of papers, that the majority of genes produce a single protein isoform and that this is the same isoform that is produced in most tissues. We have combined peptide identification from proteomics data, information from the best annotated databases, and detailed evolutionary analysis to determine potential splice isoforms in a wide range of tissues and cell types. For the vast majority of genes we have found that the peptides map to a single protein isoform, suggesting that there is a single main protein isoform present in most tissues. Indeed, we have found that it is only possible to find convincing evidence of the existence of only 276 genes with 2 or more splice isoforms expressed at the protein level. More than one third of the alternative protein isoforms generated by these splicing events are only subtly different from the main isoform, and over 20% of the splice events identified in the analysis were homologous exon substitutions. The functional importance of the homologous exon splicing events is evident when considering that they are evolutionarily highly conserved; expressed at high levels and expressed in specific tissues, i.e. the heart and brain.

Therefore, the evidence at the protein level is very strong for most of the genome, for which there is enough data in the proteomic databases. In almost all these cases, only one of the isoforms has protein-like features, including presence of binding sites, coverage of known protein structures, evolutionary conservation, and normal rates of evolution among others. The single isoform with protein-like features is therefore the genuine representative of the function of the corresponding genes. In this case, our results are clearly challenging a well-established paradigm. Unfortunately, this makes it difficult to publish our results, since most journals prefer to publish confirmatory research.

We still do not know what the biological explanation is behind the discrepancy between the numerous isoforms expressed at the RNA and protein level, but surely, just knowing that there is a discrepancy is the first step in being able to solve this biological dilemma.

This research is part of the ongoing effort to annotate the human genome and has been carried out in the context of the NIH-funded GENCODE project.

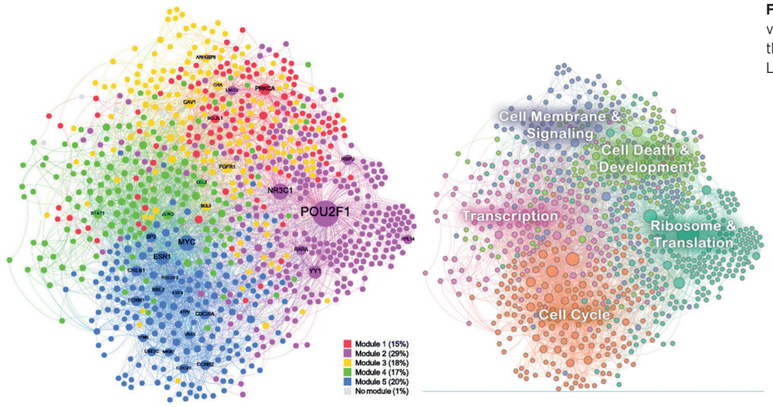

Figure Network of genes with highly variable expression levels associated to the more aggressive subtype of Chronic Lymphocytic Leukaemia.

Evolutionary analysis of large-scale epigenome datasets

We have extended our Group's experience in the study of co-evolution to the analysis of the relation between proteins and complexes that are involved in epigenomic regulation. In this context, we have developed a new methodology to study the co-evolution of the core components of the epigenetic network (chromatin-related proteins, CrPs). This new method was applied to a large collection of more than 12,000 phylogenetic trees, which include over a million protein sequences from 88 metazoan species. For the co-evolutionary analysis, we built a maximum-entropy model of pairwise interacting proteins based on the similarities between the evolutionary trees of orthologs for 58 mouse CrPs, while the whole set of 13,579 trees was used to evaluate the statistical significance of the results. To analyse the functional context of this co-evolutionary network, we have reconstructed their molecular environment, i.e. their preferential co-localisation with DNA methylation and Histone modification marks in the genome of mouse embryonic stems cells. This co-localisation map was obtained by analysing a large collection of 77 epigenomic features (ChIP-Seq experiments for histone marks and chromatin-related proteins) and NGS data for 3 DNA modifications (5mC, 5hmC and 5fC).

A detailed analysis of the general epigenetic map will allows us to detect interesting cases, such as the balance between co-evolving proteins with opposing roles in DNA modification; for example, proteins involved in DNA methylation (MBD2 and CBX3) and hydroxymethylation (TET1 and KDM2A).

This work was carried out in collaboration with the laboratory of M. Vingron at the Max Planck Institute of Molecular Genetics in Berlin (Germany), in the context of the Blueprint EU Consortium, which is part of the International Human Epigenome Consortium (IHEC). ■

PUBLICATIONS

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MACROMOLECULAR CRYSTALLOGRAPHY GROUP

Guillermo Montoya
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Post-Doctoral Fellow
Rafael A. Molina

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Technicians
Pablo Mesa (until April), M. Pilar Redondo (until September), Igor Yefimenko

OVERVIEW

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.

"The human genome is a sophisticated and complex coding system that is capable of producing thousands of different proteins in a tightly controlled way, regulated in time and location. Proteins interact with other macromolecules, forming assemblies that perform particular cellular tasks. The structural determination of these complexes will help us to decipher the mechanisms that regulate these processes."

RESEARCH HIGHLIGHTS

S-phase and replication

DNA replication is indispensable for the reliable inheritance of the genome at each cell division. To which extent this process is determined by similar mechanisms in bacteria and complex organisms is still under debate. Higher eukaryotic organisms require supplementary factors to cope with larger genomes, diverse cell fates, and to increase DNA replication fidelity; thus adding extra complexity to the process. In order to replicate, a DNA double helix must open up to allow the DNA synthesis machinery to copy each DNA strand. In mammals, thousands of origins of replication are activated at each cell cycle. However, not all origins are activated at the same time; their activation follows the specific timing of DNA replication during the cell cycle. To initiate replication, a number of protein complexes assemble at a given replication origin in a tightly regulated and temporally controlled manner. Among these complexes, we studied a module of proteins that contains the hexameric minichromosome maintenance (MCM) 2-7 complex. This complex is responsible for the unwinding of DNA after origin firing during S phase in association with 2 additional partners: the initiation factor Cdc45 and a 4-subunit complex called GINS. Together, they form the CMG complex that has ATP dependent helicase activity. Our Group attempts to decipher the molecular mechanisms of this essential cellular machinery for eukaryotic DNA replication. With this aim in mind, we have been able to obtain the structural information of an MCM homologue that contains a domain bearing primase and polymerase activities. This study has helped us to propose a working mechanism for our understanding of the helicase, which may have important implications for the eukaryotic complex. Thus, besides from the eukaryotic CMG, we are also attempting to gain mechanistic information about the MCM complex using X-ray crystallography and electron microscopy studies in order to decipher its structure.

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 the best approach for cancer therapy. This study could aid towards the development of new anti-microtubule agents that possess different structure and binding sites for tubulin; by exploring new agents and strategies, more effective therapeutic options to treat cancer may be provided. To fully understand the functional implications of TACC3-chTOG interaction and the regulation of microtubule dynamics during mitosis, a hybrid approach was used to dissect key interactions. Two conserved residues were identified in XTACC3, which, when mutated, impair XMAP215 binding and the efficient recruitment to the spindle (FIGURE 1).

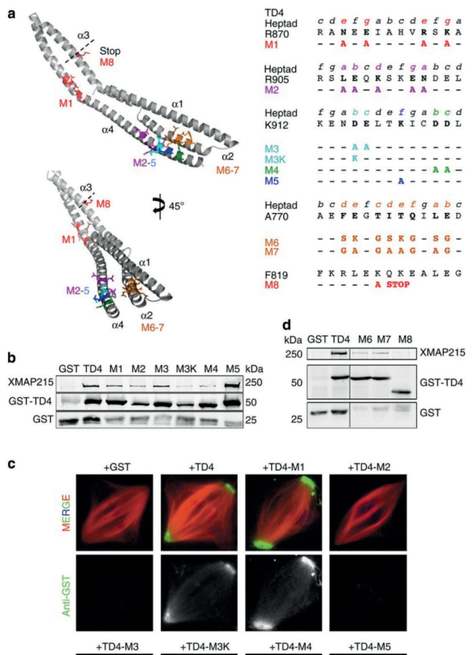

Figure 1 Mapping the XMAP-Ct binding site on TD4 structure. (**A**) SAXS structure of a TD4 monomer, highlighting regions where mutations were performed in stick representation. Two regions on α4 were detected by NMR to change upon XMAP-Ct binding. (**B**) Western blot analysis of anti-GST pull-downs from egg extracts containing various GST fusion proteins corresponding to the different mutations on α4 of TD4. The blots were examined with anti-XMAP215 (upper lane) and anti-GST (middle and bottom lanes). GST-TD4, TD4-M1, TD4-M3 and TD4-M5 can pull down endogenous XMAP215. GST-TD4-M2 can also pull down XMAP215, but less efficiently. However, the mutants affecting the polarity of this heptad region (TD4-M3K and M4) strongly abrogated XMAP215–TD4 interaction. (**C**) Representative images of spindles assembled in egg extracts containing GST or the different GST-TD4 mutants as indicated. The samples were processed for immunofluorescence using the anti-GST antibody. Images were taken with identical camera settings. GST-TD4, TD4-M1, TD4-M3 and TD4-M5 localise at the spindle poles, whereas GST, -M2, -M3K and -M4 do not. DNA is in blue, tubulin in red and GST in green. Scale bar, 10 μm. (**D**) Western blot analysis of anti-GST pull-downs from egg extracts containing various GST fusion proteins corresponding to the different mutations on α2 and α3 of TD4, all of which affected the XMAP215–TD4 interaction. The blots were examined with anti-XMAP215 (upper lane) and anti-GST (middle and bottom lanes).

Structural design of protein-DNA interactions for gene targeting

We have observed, for the first time, the hydrolytic 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 capturing 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 computational analyses to dissect the hydrolysis of a phosphodiester 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 2). This information may be used to engineer even more precise cutters. These scaffolds can present new perspectives for a wide range of applications, such as the correction of mutations linked to monogenic inherited diseases. Our Group has solved the crystallographic structures of different variants, revealing the molecular basis of new target DNA recognition domains. In addition, we have shown that the repair of the damaged gene can occur at its locus in human cells, opening up avenues to identifying possible therapeutic applications. ■

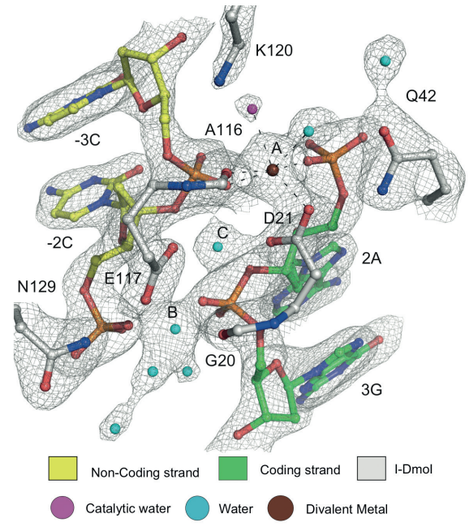

Figure 2 Detailed view of the I-DmoI active site, superimposed with the *2Fo – Fc* electron density contoured at 1.2σ. Metal-ion coordination is shown as dashed lines. The different metal-binding sites are alphabetically labelled (site A, B and C) according to the order of cation entrance.

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CELL SIGNALLING AND ADHESION JUNIOR GROUP

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Technicians
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OVERVIEW

Our Group studies the regulation of molecular switches that control growth and adhesion signals. Such signals control key cellular processes such as proliferation, adhesion and survival. We use X-ray crystallography and biochemical techniques to study how some of the important signalling molecules are regulated and how oncogenic events switch the signals on. We study these mechanisms at atomic resolution, which allows us to use structural information for the rational design of potential anti-cancer therapeutics.

Several growth and adhesion signalling molecules are regulated by specific phosphoinositides in the plasma membrane. We focus on three related systems: (i) how does the phosphoinositide phosphatidylinositol 4,5-bisphosphate (PIP_2) activate focal adhesion kinase (FAK); (ii) how does phosphoinositide 3-kinase (PI3K)-generated phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) 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 PIP_3 levels in the plasma membrane.

RESEARCH HIGHLIGHTS

Focal Adhesion Kinase

FAK is an important integrator of growth and adhesion signals. We recently reported a detailed, multi-step activation mechanism for FAK: the phospholipid PIP_2 induces FAK clustering on the cell membrane. In these clusters, FAK adopts a relaxed conformation allowing efficient autophosphorylation. FAK autophosphorylation recruits the tyrosine-protein kinase Src, which in turn phosphorylates the FAK kinase to induce full opening and FAK activation.

Our Group utilises such detailed mechanistic insights to discover highly specific allosteric FAK inhibitors. We employ experimental and computational approaches to identify fragment compounds (smaller than drug-like) interacting with allosteric sites, with the aim of extending or combining fragments to obtain inhibitory lead compounds. We have already discovered several novel compounds interacting with FAK (FIGURE).

Protein kinase B

In canonical PKB activation, PIP_3 co-localises PKB with PDK1 at the cell membrane, resulting in PKB activation. We performed a detailed biochemical study on the regulatory mechanisms of PKB. Our data indicate that, in addition to the canonical activation mechanism, initial C-terminal phosphorylation and association with PDK1 in the cytosol can also activate PKB. We show that this could result in phosphorylation of a different set of PKB substrates.

SHIP

The SHIP phosphatase removes the 5-phosphate from PIP_3 and thereby, like PTEN, negatively regulates PIP_3 levels. Despite its importance in physiology and disease, little is known about the mechanisms regulating SHIP activity or membrane targeting. We recently solved a crystal structure of the catalytic and C2 domains of SHIP, and we are now performing extensive biochemical studies to define the role of the C2 domain. We have found that the catalytic efficiency is greatly enhanced by the C2 domain and that the C2 domain is important for substrate recognition. We are currently employing molecular dynamics simulations and mutagenesis to understand the allosteric communication between the C2 and catalytic domains. ■

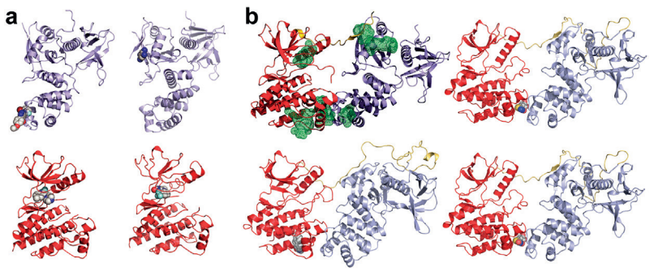

Figure (**A**) Using an experimental nuclear magnetic resonance (NMR) based screening method we have identified several fragment compounds interacting with the FERM (blue) or kinase (red) domain of FAK. Shown are crystal structures for 4 bound compounds. (**B**) We performed virtual screening against potential transient pockets (green mesh) and confirmed binding for 3 compounds (shown bound according to virtual screening results).

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STRUCTURAL BASES OF GENOME INTEGRITY JUNIOR GROUP

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Alba Ruiz

Technician
Araceli Grande

"We have obtained the first atomic views of human CAD – the protein complex responsible for *de novo* synthesis of pyrimidines – and we got important insight into the mechanism underlying its resistance to the anti-tumour drug PALA."

OVERVIEW

Safeguarding genome integrity is essential for correct cell functioning and to prevent cancer. Our group is interested in gaining a better understanding of the 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, 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. The knowledge gained from this work should guide the design of compounds that modulate protein activity and provide novel opportunities for fighting tumours.

RESEARCH HIGHLIGHTS

Revealing the structure and function of CAD and its potential use as a therapeutic target

Pyrimidine nucleotides are essential building blocks for nucleic acid synthesis and DNA repair. In animals, the *de novo* biosynthesis of pyrimidines is initiated and controlled by CAD, a 243 kDa multifunctional polypeptide that harbours the first 3 enzymatic activities of the pathway: glutamine-dependent carbamoyl phosphate synthetase (GLN-CPS), aspartate transcarbamoylase (ATC) and dihydroorotase (DHO). CAD is under strict allosteric control and its activity is modulated by phosphorylation through the ERK, PKA and mTORC1 signalling cascades. Up-regulation of CAD is essential for the proliferation of normal and tumour cells. Thus, targeting this central pathway opens up new possibilities for the development of novel therapeutic strategies. There is, however, no structural information about CAD, other than that it forms a ~1.5 MegaDa complex, which for unclear reasons, shuttles between the nucleus and the cytoplasm during the cell cycle. In order to shed some light on the architecture and functioning of human CAD, we resolved the crystal structures of the DHO and ATC (FIGURE, A) domains and proceeded to biochemically characterise both activities.

Basic mechanisms of DNA recognition

MuB is an ATP-dependent DNA-binding protein that selects the target DNA during the transposition of phage Mu. The mechanism by which MuB binds and selects the DNA was unknown. We previously showed that MuB contains an AAA+ ATPase module that forms helical filaments around the DNA. Now, we have discovered that the N-terminal region appended to the AAA+ module is an independent protein domain (NTD). In collaboration with the CNIO Spectroscopy and Nuclear Magnetic Resonance Unit, we have determined the structure of the NTD domain (FIGURE, B), revealing a striking similarity to a large family of DNA-binding proteins. We have also found that NTD favours the assembly of MuB filaments into bundles. We propose a molecular "zippering" mechanism by which NTD mediates MuB-DNA filament interactions and, possibly, the bridging of distant DNA regions. ■

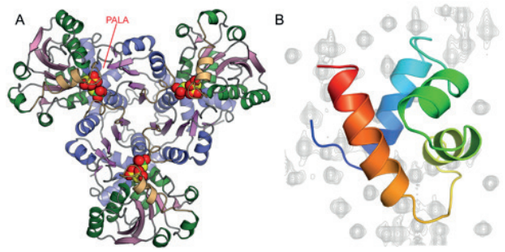

Figure (**A**) Crystal structure of the ATC domain of human CAD. Each subunit within the trimer is bound to the anti-tumour compound PALA. (**B**) Structure of the N-terminal domain of the MuB protein determined by NMR spectroscopy.

PUBLICATION

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SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE CORE UNIT

Ramón Campos-Olivas
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Technician
Blanca López-Méndez

"In 2014, we quantified metabolites in a variety of cell and mouse models of human cancer. Thus, we provided essential information to help understand crucial aspects of tumour biology such as the cellular response to glucose deprivation or to chemotherapeutic drugs inducing mitotic arrest, the metabolic plasticity of pancreatic cancer stem cells, and tumour-induced cachexia."

OVERVIEW

The Unit unifies the technical and scientific management of Nuclear Magnetic Resonance Spectroscopy (NMR) as well as other biophysical instrumentation available at the Structural Biology and Biocomputing Programme. It provides CNIO researchers with instrumentation and technical support for a variety of spectroscopic and other biophysical techniques. This includes the application of NMR to the *in vitro* characterisation of the structure and dynamics of biomolecules (proteins in particular) and their interactions with other biopolymers, as well as with small molecules that could represent initial hits in the drug discovery process or research compounds for biophysical and functional studies. Furthermore, we use NMR to characterise the metabolic profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples.

RESEARCH HIGHLIGHTS

Our Core Unit incorporates a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions. This includes spectrophotometers, a fluorimeter, 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 2014. Two important intra-Programme collaborations that were established this year are: 1) NMR solution structure determination of the N-terminal domain of the MuB protein; an essential protein for DNA transposition specificity (with the Structural Bases of Genome Integrity Group); and 2) DNA-binding studies of the BurrH protein (with the Macromolecular Crystallography Group), which are illustrated in the FIGURE. The BUDs (BurrH Domains) are highly specific DNA binding proteins with a simple amino acid to base code, and may thus become very valuable tools for genome engineering applications.

The Unit also hosts a 700 MHz NMR spectrometer that is well-equipped with probes (HR-MAS, dual fluorine/proton, and triple and quadruple resonance) and a sample changer for running up to 120 samples automatically. This provides the required throughput for the screening of small molecule protein binders (in collaboration with CNIO's Structural Biology and Biocomputing and Experimental Therapeutics -ETP- Programmes), as well as for metabolite analytics that are performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the ETP), the Cell Division and Cancer Group (from the Molecular Oncology Programme), the Genes, Development and Disease Group and the Growth Factors, Nutrients and Cancer Group (BBVA Foundation-CNIO Cancer Cell Biology Programme), as well as the former Stem Cells and Cancer Group (from the Clinical Research Programme). Collectively with these groups, we have implemented sample preparation protocols and have developed spectroscopic and analysis technologies to characterise and quantify metabolites present in different biological samples. ■

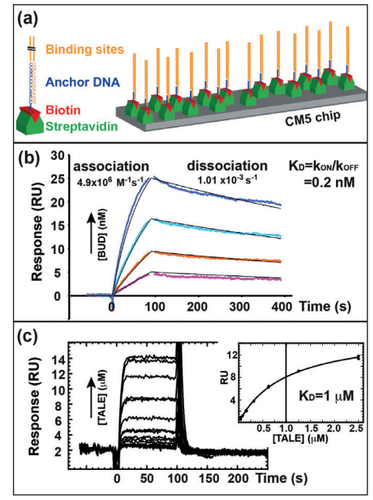

Figure Binding analysis using SPR. (**A**) Experimental setup used to measure protein-DNA binding. (**B**) SPR sensorgrams of BurrH injected at increasing concentrations (0.4, 0.8, 1.6 and 3.2 nM) over its immobilised target DNA; K_D=0.2 nM. (**C**) TALE AvrBs3 binds BurrH DNA target with a 5000-fold lower affinity (K_D=1 M).

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BIOINFORMATICS CORE UNIT

David G. Pisano
Core Unit Head

Technicians
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OVERVIEW

The Bioinformatics Unit helps CNIO scientists to analyse and interpret their data when it requires complex numerical or computational analyses. We design and maintain the Centre's scientific computing facilities, including the scientific storage, and train students and scientists in the use of bioinformatics tools and methods. In collaboration with Alfonso Valencia from the Structural Computational Biology Group (CNIO), Fátima Al-Shahrour from the Translational Bioinformatics Unit (CNIO), and the National Bioinformatics Institute (INB), we contribute to several cancer genomics initiatives ranging from the personalised to the population levels.

"Molecular and genomic cancer research is already a full-grown quantitative science, as hard as physics or mathematics, and new analytical methods and computational approaches are constantly needed to explore and understand novel experimental datasets, and to answer new questions."

RESEARCH HIGHLIGHTS

During 2014, we helped Marisol Soengas (CNIO) to demonstrate the role of RAB7 as a lineage-specific driver, and to elucidate the unique wiring of the lysosomal pathway for tumour progression in melanoma (FIGURE). We also collaborated with Nabil Djouder (CNIO) to show that restoration of NAD+ pools through a nicotinamide riboside (NR) rich diet prevents DNA damage and tumour formation in mice with liver damage and hepatocellular carcinoma (HCC), due to high expression levels of URI. With R. Guigó (CRG, Barcelona), in the context of the International Cancer Genome Consortium (ICGC), we contributed to the characterisation of new molecular and clinical subdivisions based on the expression profiles of a large cohort of chronic lymphocytic leukaemia (CLL) patients.

Using a whole-genome RNA-seq approach, we explored the genomic origin of telomeric RNAs (TERRAs) with Maria Blasco (CNIO), also identifying novel transcripts similar to TERRA in their regulation. Another study, with Manuel Serrano (CNIO), explored the function of NANOG as a lineage-restricted mitogen in stratified epithelia. With M. Sánchez-Beato (IDIPHIM, Madrid) and M.A. Piris (IFIMAV, Santander), we carried out massive sequencing of a panel of T-cell receptor signalling related genes, and found activating mutations in PLCG1 as a frequent occurrence in cutaneous T-cell lymphomas (CTCL).

With Alfonso Valencia and Michael Tress (CNIO), we contributed to the deployment of a new version of FireDB; a compendium of biologically relevant small ligand-binding residues. With Maria Blasco (CNIO), we also helped to address the role of Sox4 in adult stem cells, and its importance for the regulation of adult tissue homeostasis and cancer. Novel miRNA expression signatures, predictive of BRCA1/2 mutation status in breast tumour samples, were developed in collaboration with Javier Benítez (CNIO). ■

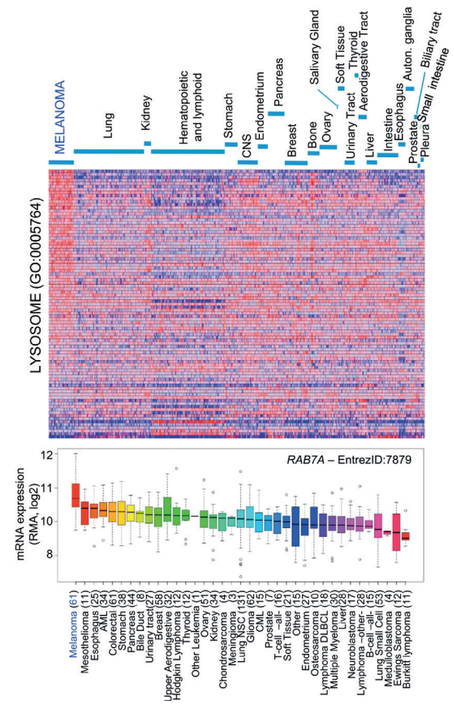

Figure (**Up**) GSEA heat map showing a selective enrichment of the Lysosome Gene Ontology gene set (GO:0005764) in melanoma cell lines, compared to non-melanoma tumour cell lines in the Cancer Cell Line Encyclopaedia (CCLE). (**Down**) Box plots showing the relative RAB7A mRNA levels across different tumour types.

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NATIONAL BIOINFORMATICS INSTITUTE CORE UNIT

Alfonso Valencia
Core Unit Head

Technicians
Andrés Cañada, Victor De La Torre, José M. Fernández, José M. Rodríguez

OVERVIEW

The Spanish National Bioinformatics Institute (*Instituto Nacional de Bioinformática*, INB) is a platform of the *Instituto de Salud Carlos III*. The INB integrates 10 geographically dispersed nodes that cover the main areas of bioinformatics. The CNIO hosts the Central Coordination Node as well as the Node specialised in genome scale annotation.

The INB is the Spanish Node of the European Bioinformatics Infrastructure (ELIXIR).

"During this year, the National Bioinformatics Institute Core Unit has focused its efforts on developing the technical and organisational strategy for its integration within the European Bioinformatics Infrastructure ELIXIR."

The main objectives of the INB Core Unit are to:

- → Generate and supply bioinformatics solutions to genomics projects with a particular emphasis on providing solutions related to human health.
- → Collaborate with national and international bioinformatics activities and consortia.
- → Support the development of bioinformatics and computational biology in Spain.
- → Provide training and support training activities in bioinformatics.
- → Integrate the Unit's activities within the framework of the European Infrastructure for Bioinformatics (ELIXIR).

RESEARCH HIGHLIGHTS

In 2014, the Core Unit has focused its efforts on developing the technical and organisational strategy for its integration within ELIXIR.

At the organisational level, the Unit coordinated the participation of the institutions that support the INB for the signing of the ELIXIR international consortium agreement. The INB is involved in the proposal for the implantation of ELIXIR as part of the INFRADEV EU call.

At the technical level, the Unit coordinated the INB's efforts to develop the areas that are central to the proposed contributions of the INB/ELIXIR-Spain Node to the global ELIXIR infrastructure.

These areas are:

- → Implementation of text mining and other information extraction resources that are applicable to biology.
- → Provision of computational and cloud resources in biology.
- → Genome analysis strategies in the context of common and rare diseases.
- → Organisation of web services and web servers through the implementation of methods, which are useful for biologists, under the proper registry and classification systems.
- → Development of the infrastructure for the benchmarking of bioinformatics methods. ■

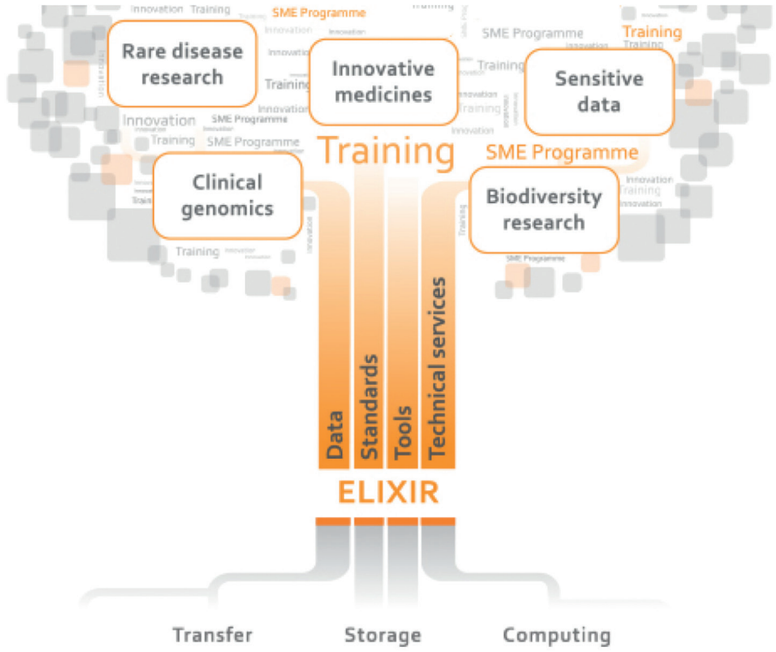

Figure The scheme illustrates key activities of the INB as node of the European Bioinformatics Infrastructure ELIXIR.

PUBLICATION

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ELECTRON MICROSCOPY CORE UNIT

Jasminka Boskovic
Core Unit Head

OVERVIEW

The Electron Microscopy (EM) Unit functions as a central research infrastructure that grants CNIO researchers, and the wider research community, access to Transmission Electron Microscopy, as well as providing expert analysis of EM images. The EM Unit offers specialised services, ranging from sample preparation and data collection, to data processing according to the specific requirements of the users.

"The Focal Adhesion Kinase (FAK) is a validated cancer target. In collaboration with the CNIO Cell Signalling and Adhesion Group, we discovered that PI(4,5)P2 promotes FAK autophosphorylation by inducing the formation of FAK clusters on PI(4,5)P2 rich membranes or soluble forms of PI(4,5)P2."

RESEARCH HIGHLIGHTS

The Electron Microscopy Unit was created in May 2014. The Unit hosts a Tecnai G2Transmission Electron Microscope (FEI). This instrument operates at accelerating voltages of up to 120 kV using a lanthanum hexaboride (LaB6) cathode. The microscope is equipped with a 4k TVIPS TemCam-F416 camera for the direct recording of digital images. There is also an EM-TOOLS software package that controls the camera for the acquisition of images under low-dose conditions. The Unit also possesses a cryo-transfer holder (Gatan model 626.DH) with a workstation for loading the grid into the cryo-holder and a control unit for adjusting and monitoring specimen temperature; this allows experiments to be performed under liquid nitrogen conditions (cryoEM) for the preservation of high-resolution structural features.

The Electron Microscopy Unit implements several sample preparation protocols and data collection methods, as well as undertaking data processing. In collaboration with the CNIO Cell Signalling and Adhesion Group (Structural Biology and Biocomputing Programme) we have developed a sample preparation protocol for visualising the formation of protein clusters on lipid vesicles or on soluble lipids. By measuring the volume occupied by these clusters, we managed to estimate the number of protein molecules per cluster. Together with the CNIO Structural Bases of Genome Integrity Group (Structural Biology and Biocomputing Programme), we have also optimised the protocols for monitoring the formation of protein-DNA filaments and filament bundles. Furthermore, in collaboration with the CNIO Melanoma Group (Molecular Oncology Programme), we have developed a protocol that allows the visualisation of exosomes by electron microscopy in order to validate the methods for the purification of exosomes produced by melanoma cell lines.

Additionally, in collaboration with the Electron Microscopy and Three Dimensional Reconstruction of Macromolecules Group at the Centre of Biological Research (CIB-CSIC, Madrid), we implemented a cryo-electron microscopy technique for several biological samples. ■

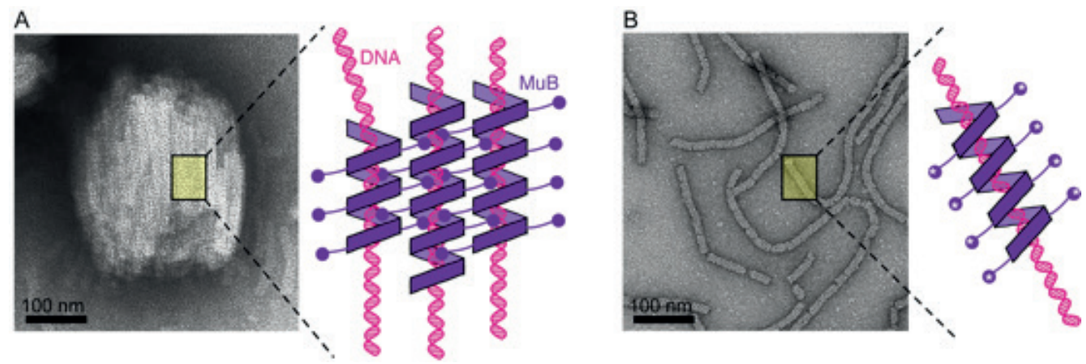

Figure EM images of negatively stained MuB filaments and bundles. Wild type filaments assemble into bundles (**A**) while a point mutation (*) in the protein hinders filament interaction (**B**). This work was performed in collaboration with the CNIO Structural Basis of Genome Integrity Group.

PUBLICATION

Goñi GM, Epifano C, Boskovic J, Camacho-Artacho M, Zhou J, Bronowska A, Martín MT, Eck MJ, Kremer L, Gräter F, Gervasio FL, Perez-Moreno M, Lietha D (2014). Phosphatidylinositol 4,5-bisphosphate triggers activation of focal adhesion kinase by inducing clustering and conformational changes. *Proc Natl Acad Sci USA* 111, E3177-E3186.

Vice-Direction of Translational Research

MANUEL HIDALGO
Vice-Director of Translational Research

“Our collective work that is carried out in collaboration with clinical centres is making an impact on the prevention, diagnosis and treatment of cancer.”

One of the key missions of the CNIO is to conduct research that benefits cancer patients and thereby favourably impacts society overall. During 2014, we have continued to work on the identification of cancer-predisposing genes, pharmacogenomic studies of drug efficacy and toxicity, genetic counselling for individuals and families at risk for cancer, as well as to collaborate with Hospitals to perform molecular diagnostics and bioinformatics studies. A key area of focus has been the expansion of our portfolio of ongoing clinical trials conducted in collaboration with Hospitals. To this end, we have initiated steps to create a consortium of Hospitals in Madrid in order to conduct collaborative, molecularly-driven, clinical trials.

HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ Director

The Human Cancer Genetics Programme currently incorporates 4 Research Groups, a Genotyping Core Unit and a Familial Cancer Clinical Unit. The Programme also includes a Familial Cancer Consultancy that undertakes the assessment of cancer families and the selection of appropriate candidates for genetic studies in order to perform a correct diagnosis and provide genetic counselling. The Consultancy is located at the *Hospital Universitario de Fuenlabrada* and works in close collaboration with the Oncology Service. The common goals of the Programme are geared towards research, training and diagnosis.

The Programme's research priorities are the genetic characterisation and diagnosis of families with cancer, genetic and cytogenetic studies of tumours, the search for diagnostic and prognostic markers, as well as the discovery of novel cancer-related genes. Another area of work that complements our research activities is the study of genetic and environmental factors that confer cancer susceptibility and 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.

Our Programme works in close collaboration with the clinical community to foster cooperation in genetic diagnosis and to promote training and education. During 2014, the Programme's Research Groups hosted 4 resident physicians from different hospitals in Spain for a 3 month training. We also offer short-stay opportunities of 2-6 weeks for professionals from different international research centres (3 national visitors in 2014). In terms of education, a total of 4 Master's students worked on their research projects this year and, since the beginning of 2014, 11 national and 5 international students worked on their PhD research projects, 4 of whom have successfully defended their doctoral theses in the meantime.

We participate in various international and national consortia; this permits us to apply for international project funding, hold international meetings and publish in the best scientific journals.

"Cancer is a dreadful disease that affects not only the afflicted person but also his/her entire family. We fight against the disease and its predisposing factors and we suffer when we are unable to be victorious and we lose the battle. We are not just scientists, we are human beings first and foremost."

Major milestones of the Programme in 2014 include:

- The agreement signed with the *Instituto Roche* to fund and improve training programmes in familial cancer for primary care medicine.
- The agreement signed with the *Instituto de Salud Carlos III* (*ISCIII*) to fund the Human Genotyping Unit-*CEGEN* over the next 4 years.
- The organisation of the 6th Familial Cancer Conference in collaboration with the European School of Oncology.
- The organisation of the 11th International Von Hippel-Lindau Symposium in close collaboration with the International Von Hippel-Lindau Alliance of patients.
- The organisation of the ISCO2014 Meeting "Cancer Genome: From Structure to Function"; a meeting of the International Society of Cellular Oncology.

HUMAN GENETICS GROUP

Javier Benítez
Group Leader

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Post-Doctoral Fellows
Oriol Calvete, Javier Gayarre

Graduate Students
Nerea Matamala, Beatriz Paumard, Alejandra Tavera, Tereza Vaclová

Technicians
Alicia Barroso, Carlos Benítez-Buelga, M. Victoria Fernández

OVERVIEW

For several years, the Human Genetics Group has been working on research aiming to better understand the genetic bases of familial cancer and more particularly breast and ovarian cancer. Our main objective is to translate our discoveries into clinical practice in order to improve knowledge of these cancers.

This year, the majority of our activities were focused on the Familial Cancer Exome Project that we have been working on over the past three years. This project aims to identify new high-susceptibility genes by using whole exome sequencing in order to gain insight into families with rare and infrequent tumours. We are also interested in identifying modifier genes that modulate the age of onset, disease evolution and cancer risk. Finally, we are working on the discovery of diagnostic and prognostic biomarkers using novel high-throughput technologies.

The Group's strategic goals are to:

- → Better define the genetic profile of familial and sporadic breast and ovarian cancer by identifying new cancer susceptibility genes.
- → Discover new genetic markers associated with diagnosis and prognosis.
- → Improve our knowledge of families with rare or infrequent cancers by using massive sequencing.
- → Translate our discoveries into clinical practice.

"Our Group has identified 2 low susceptibility alleles in genes within the Base Excision Repair pathway that modify the risk of developing breast and ovarian cancer in *BRCA1/2* mutation carriers. We have also established the impact of chemotherapy on telomere length; defined a chromosomal region in ovarian cancer that has prognostic value; established a miRNA signature that allows us to identify women at high risk of developing breast cancer at an early stage; and, discovered via Next Generation Sequencing 1) a gene responsible for type I gastric carcinoid, 2) a gene that contributes to the development of Barrett's oesophagus, and 3) a gene responsible for familial angiosarcoma."

RESEARCH HIGHLIGHTS

Breast cancer

By analysing a set of familial and sporadic cancer cases involved in a clinical trial with taxanes, we have demonstrated that chemotherapy causes telomere shortening. The impact of treatment on telomere shortening lasts around 2 years and the telomeres recover 1 year later (FIGURE 1). In addition, we have identified a correlation between oxidative stress and telomere shortening; this is currently being studied in depth.

At present, we are conducting a miRNA study in order to identify differentially expressed plasma miRNAs in the breast cancer population. A large group of tumours were initially analysed and the miRNAs that were found to be significant were subsequently validated in a second set of breast tumours. Finally, we selected 16 miRNAs that were further studied in plasma samples from 100 cases and 100 controls. Five of them were significant and represent good candidate markers for early detection of breast cancer in healthy women. We are currently validating these results in a second set of 100 cases and 100 controls.

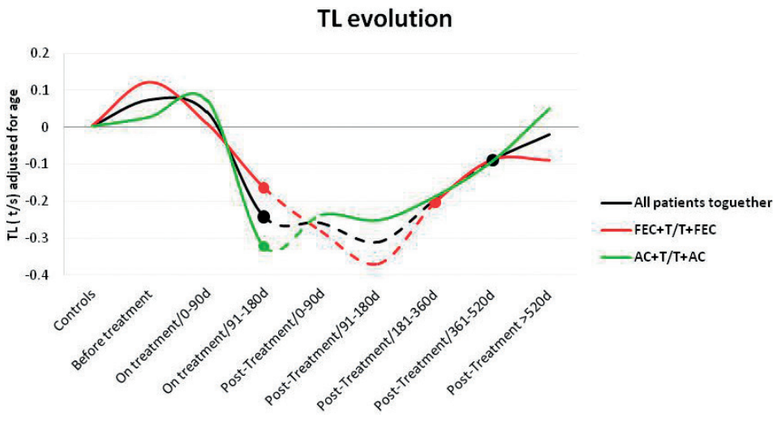

Figure 1 From the treatment's onset, we observed a shortening effect on Telomere Length (TL), which became statistically significant in patients treated for more than 90 days. TL of patients after treatment remained significantly shortened, compared to controls, up to 360 days post- treatment. TL of patients whose samples were extracted after 360 days of treatment discontinuation tended to recover to normal TL values, and we were not able to find significant differences when compared to controls. Red and green lines represent different treatment combinations.

We have demonstrated that *BRCA1* heterozygote mutation carriers are haplo-insufficient for DNA double-strand break repair by homologous recombination. Moreover, we have found that cells from patients harbouring *BRCA1* missense mutations are more sensitive to treatment with Poly (ADP-ribose) Polymerase (PARP) inhibitors than those harbouring mutations that cause total loss of the BRCA1 protein (frameshift mutations). We are currently investigating the mechanisms underlying these differences in drug response with the aim of identifying novel markers of sensitivity or resistance to these agents.

Ovarian cancer

By using array-Comparative Genomic Hybridization (CGH), 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, independently of known prognostic factors. Since the deletion can be analysed by Fluorescence *In Situ* Hybridisation (FISH) in paraffin sections, it may represent a marker that is suitable for routine clinical applications. We have selected some candidate genes whose loss could be associated with an enhanced chemotherapeutic response. Functional experiments are currently ongoing in order to test whether the deletion has prognostic and/or predictive significance.

Familial cancer exome project

There are a number of families that have rare or infrequent cancers with an unknown genetic basis. We have started a massive sequencing project with the aim of identifying some of these high-susceptibility genes. During 2014, we discovered that the ATP4a gene is responsible for type I gastric carcinoids.

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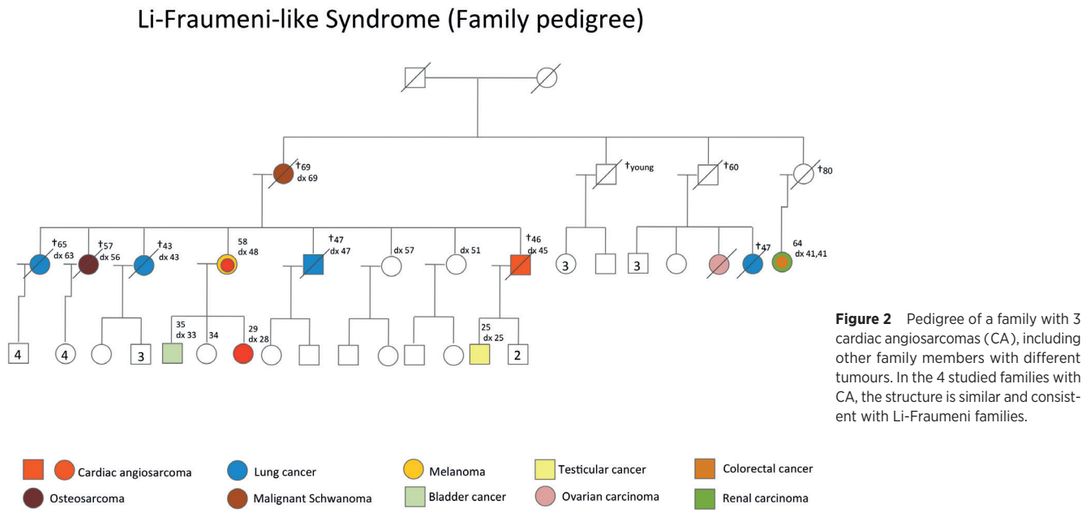

Figure 2 Pedigree of a family with 3 cardiac angiosarcomas (CA), including other family members with different tumours. In the 4 studied families with CA, the structure is similar and consistent with Li-Fraumeni families.

ATP4a is a proton pump in charge of gastric acidification and its maintenance. In collaboration with Sagrario Ortega's Transgenic Mice Core Unit at the CNIO, we have generated a knock-in mouse line containing an ATP4a alteration in order to gain insight into the pathologic evolution of gastric cancer. Furthermore, we have already generated 3 different mouse lines – wild type, heterozygous and homozygous for the mutation – and have started the clinical and pathological follow-up studies.

We found a second gene that is linked to a family with Barrett's oesophagus, a condition that predisposes to oesophageal carcinoma. A missense mutation alters the expression of the gene in this family. Finally, a third gene responsible for cardiac angiosarcoma in families – of which different family members develop different types of cancer – has also been discovered (FIGURE 2). This gene is related to telomeres and several functional and structural studies have been carried out. In collaboration with Maria Blasco's Telomeres and Telomerase Group at the CNIO, we have started to work on the generation of a knock-in mouse model.

In addition, several sequenced families with breast cancer following a recessive model, including some families with ovarian cancer, are currently being analysed using bioinformatics tools. ■

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MOLECULAR CYTOGENETICS GROUP

Juan C. Cigudosa
Group Leader

Staff Scientists
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Graduate Student
Ana Del Rio (until October)

Technicians
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OVERVIEW

State-of-the-art genetics and genomics have demonstrated that the chromosomes of tumour cells show small changes in the DNA sequence as well as structural rearrangements, in the form of translocations, deletions, amplifications, and/or aneuploidy. We are especially interested in those rearrangements that generate chimaeric genes that display new or altered biological activities, as well as in the molecular mechanisms by which these newly created fusion genes play a major role in oncogenesis.

Our work is mainly based on myeloid leukaemia and childhood sarcomas, paradigms of genetic neoplasia where fusion genes and point mutations collaborate to explain the abnormal cell proliferation and the disruption of the differentiation programme of haematopoietic and mesenchymal stem cells. Our research activity is currently focused on two major areas: (1) the molecular characterisation of genetics, cytogenetic and epigenetic markers; and (2) the design of human stem cell models carrying chromosome rearrangements that will allow us to study their effects on the biology of the tumour and the molecular pathways involved in causing these effects. In line with the other Groups and Units of the Human Cancer Genetics Programme, our Group's foremost goal is the translation of our findings to the clinical setting through the provision of molecular tools such as spectral human and mouse karyotypes, FISH probes, and NGS sequencing panels that can be used for both research purposes and as clinical reagents.

"We identify the genetic profiles present in patients with leukaemias and sarcomas; these mutations open up new avenues for targeted therapies. Via genetic engineering, we create human stem cell models with unique genetic events that provide invaluable tools for future basic and clinical research."

RESEARCH HIGHLIGHTS

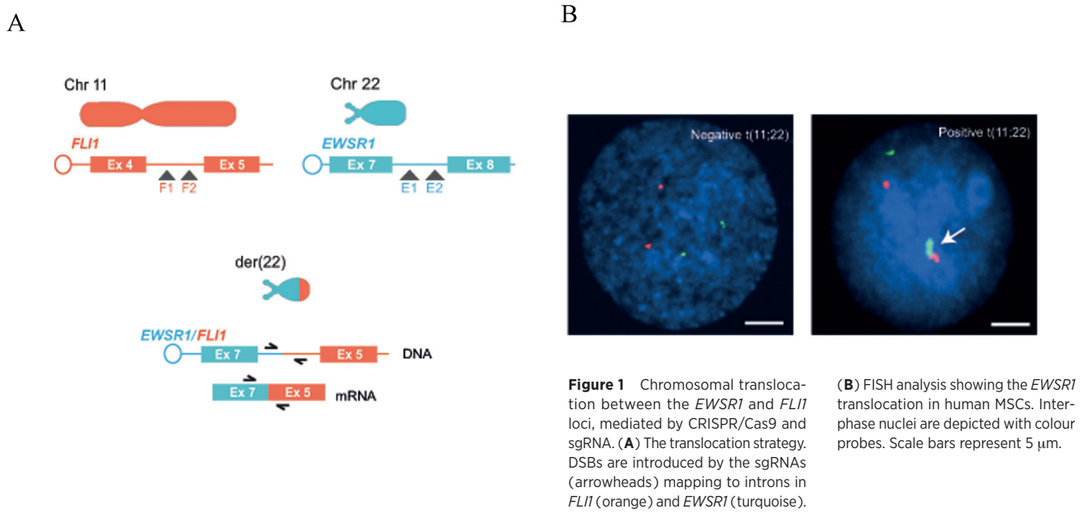

Figure 1 Chromosomal translocation between the *EWSR1* and *FLI1* loci, mediated by CRISPR/Cas9 and sgRNA. (**A**) The translocation strategy. DSBs are introduced by the sgRNAs (arrowheads) mapping to introns in *FLI1* (orange) and *EWSR1* (turquoise). (**B**) FISH analysis showing the *EWSR1* translocation in human MSCs. Interphase nuclei are depicted with colour probes. Scale bars represent 5 μm.

Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system

The recent expansion of the CRISPR/Cas9 system, which can cut the genome at any desired location, gave us the opportunity to generate "*à la carte*" chromosomal translocations. We have been able to recreate the Ewing's sarcoma t(11;22) translocation, which generates the *EWSR1-FLI1* fusion gene (FIGURE 1), and the t(8;21), which joins the *RUNX1* gene with the *ETO* gene in acute myeloid leukaemia. Firstly, we tested the system in HEK293A cells; FISH, PCR, RT-PCR and Western assays demonstrated the occurrence of the translocation. We also confirmed the functionality of the fusion protein by studying the transcription of known target genes. We then used the same strategy to generate the t(11;22) in mesenchymal stem cells (MSCs) and the t(8;21) in haematopoietic stem cells (hHPSCs), showing the effectiveness of this technique in human primary cells. With this method of recreating chromosome translocations that are common in human cancers, we offer a new, easy and cheap tool for studying both the mechanisms and the effects of these translocations on the initiation and progression of neoplastic diseases.

From the patient's chromosome translocations to the human haematopoietic progenitor cell models

Since we are committed to transferring our research activities into putative clinical applications, we are generating biological models and tools to study the role of chromosome translocations in cancer. During this last year, we developed 2 human haematopoietic stem cell models that have been genetically engineered to carry the novel chimaeras. Firstly, we cloned a fusion gene that was identified in a patient with a myelodysplastic syndrome, in which a t(1;21)(p32;q22) translocation resulted in the expression of a truncated RUNX1 protein lacking several regulatory domains. Since similar truncated RUNX1 proteins are generated by point mutations, we used the t(1;21) translocation as a model to demonstrate that C-terminally truncated RUNX1 proteins can contribute to leukaemogenesis, in a similar way as the *RUNX1-ETO* fusion gene.

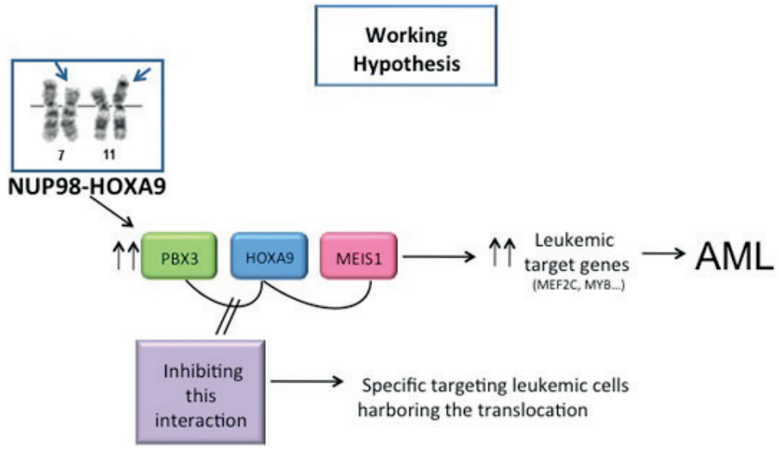

Figure 2 Working model for the action of the *NUP98-HOXA9* fusion gene. Deducted from our human haematopoietic progenitor that over-expresses the fusion gene, we have identified one of its molecular mechanisms of action. The fusion gene directly induces high expression of 3 proteins (PBX1, MEIS1 AND HOXA9) that interact to form a complex with known leukaemogenic potential. This complex is able to induce the over-expression of a number of genes directly involved in transforming normal haematopoietic progenitors into aberrantly differentiated cells with an increased proliferative activity. We are trying to interrupt this pathway by inhibiting the formation of the complex.

Secondly, we have been working on the chromosomal translocation t(7;11)(p15, p15), which results in the oncogenic fusion protein Nup98-Hoxa9. This is a rare but recurrent oncogenic event in acute myeloid leukaemia (AML) that is associated with very poor prognosis and short overall survival. We have been successful in the generation of a human haematopoietic progenitor model that overexpresses the fusion gene and we have described, for the first time, its DNA binding sites, most of which are regulatory regions of genes involved in the development of AML. The fusion protein is able to activate and repress the expression of the target genes. Thus, we have been able to provide a new biological rationale for widening the therapeutic repertoire for these patients (FIGURE 2).

Advances in the full genomic and epigenomic characterisation of myelodysplastic syndromes (MDS)

Moreover, we have also been working on 2 types of MDS: on the one hand, the subgroup characterised by the presence of the cytogenetic marker known as deletion 5q; and on the other hand, a cohort of samples from patients suffering from the less aggressive form of myelodysplasia (low grade). These patients are currently not treated as a general approach, however, and some of them progress to acute leukaemia within a very short period of time. Our aim is to find genetic biomarkers with prognostic value that can be translated into routine clinical laboratory practice.

Our Group also provides state-of-the-art molecular cytogenetic services. In 2014, we carried out over 1,500 assays including karyotyping of leukaemia and other tumours, design of FISH probes, spectral karyotyping, aneuploidy analysis for mouse models, and aCGH for experimental and clinically oriented projects. As a reference laboratory in Molecular Cytogenetics, we participate in several clinical assays, collaborative networks, and quantity performance studies, both at national and at European level. ■

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AWARDS AND RECOGNITION

- Editorial Board Member, *Genética Médica* News, a Spanish reference journal in the field of translational Clinical Genetics and Genomics.

HEREDITARY ENDOCRINE CANCER GROUP

Mercedes Robledo
Group Leader

Staff Scientists
Alberto Cascón, Cristina Rodríguez

PostDoctoral Student
Cristina Montero (since December)

Graduate Students
María V. Apellániz, Iñaki Comino, María Curras, Aguirre Andrés De Cubas, Lucía Inglada, Veronika Mancikova

Technicians
Alvaro Gómez, Rocío Letón, Lara Sánchez

OVERVIEW

Our Group is interested in identifying high and low genetic risk factors involved in endocrine tumour susceptibility. To this end, we classify patients according to primary gene mutations associated with their tumour development. We are interested in revealing differences between tumour transcriptomes, mirnomes, methylomes and chromosomal gains and losses according to the different individual genetic backgrounds. We have therefore obtained a large collection of endocrine tumours from patients, either with germline mutations in any of the known major susceptibility genes related to these diseases or without mutations (sporadic cases), as well as clinical follow-up data that is regularly updated. 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. In order to do so, we are applying a candidate gene approach as well as whole genome association studies to a large series of biological material and associated data regarding therapeutic interventions and other clinically relevant outcome variables. These efforts will collectively increase our genetic and molecular knowledge about these tumours and improve the diagnosis, prognosis and treatment of patients.

"We have discovered a new susceptibility phaeochromocytoma gene, again linking the disruption of the Krebs cycle to cancer aetiology, we have proposed defective *CYP3A4* alleles as a cause of paclitaxel-induced neuropathy, and we have identified high levels of promoter methylation as predictors of a shorter progression-free survival in thyroid carcinomas."

RESEARCH HIGHLIGHTS

MDH2 is a new phaeochromocytoma/paraganglioma susceptibility gene

Disruption of the Krebs cycle is a hallmark of cancer. *IDH1* and *IDH2* mutations are found in many neoplasms, and germline alterations in *SDH* and *FH* genes predispose to phaeochromocytoma/paraganglioma (PCC/PGL) and other cancers. Whole-exome sequencing applied to tumour DNA – obtained from a patient diagnosed with multiple malignant PGLs – has allowed us to reveal a mutation in *MDH2*, which encodes a Krebs cycle enzyme. The presence of the mutation in germline DNA, segregation of the variant with disease and the absence of MDH2 in mutated tumours, demonstrate that *MDH2* is a novel PCC/PGL tumour susceptibility gene. In addition, we showed that *MDH2*-mutated tumours do not only have a global transcriptional profile similar to that of *SDH* gene-mutated tumours, but they also have a CpG island methylator phenotype (CIMP)-like signature that is consistent with Krebs cycle disruption (FIGURE 1). Finally, we found that stable silencing of *MDH2* expression in HeLa cells led to the accumulation of malate as well as of the oncometabolite fumarate. This finding further links the disruption of the Krebs cycle to cancer aetiology,

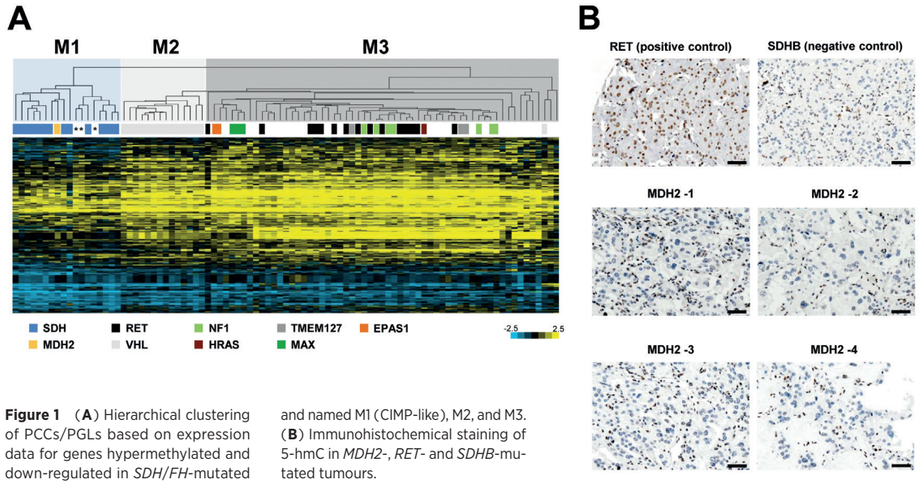

Figure 1 (**A**) Hierarchical clustering of PCCs/PGLs based on expression data for genes hypermethylated and down-regulated in *SDH/FH*-mutated tumours. Three clusters were observed and named M1 (CIMP-like), M2, and M3. (**B**) Immunohistochemical staining of 5-hmC in *MDH2*-, *RET*- and *SDHB*-mutated tumours.

and highlights that alterations in this major metabolic pathway may explain additional PCC/PGL cases.

Defective *CYP3A4* variants: severe drug toxicity and Spanish founder effect

Paclitaxel is a widely used cytotoxic agent, which frequently causes a peripheral neuropathy that can limit treatment success and can seriously impact the quality of life of the patients. Whole-exome sequencing performed on patients with extreme paclitaxel-induced neuropathy revealed rare defective variants in *CYP3A4*: a premature stop codon (*CYP3A4 *20*) and a novel missense variant (*CYP3A4 *25*). Further studies in independent series, confirmed an over-representation of defective *CYP3A4* variants in patients with high-grade paclitaxel-induced neuropathy (P=0.045) and with paclitaxel treatment modifications ($P=6x10^{-5}$), providing a basis for paclitaxel treatment individualisation. Because of the elevated number of *CYP3A4 *20* carriers found among the Spanish cancer patients, and because CYP3A4 metabolises more than 50% of all clinical drugs, we followed up this finding in 4,000 individuals from 12 world populations. The *CYP3A4 *20* allele was only detected in the Spanish peninsula, where 1.2% of the population (3.8% in specific regions) carried it in a haplotype that suggests a Spanish founder effect for this mutation, which is closely related to adverse drug reactions.

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AWARDS AND RECOGNITION

- Veronika Mancikova has been awarded the Young Investigators' Award at the 38th Annual Meeting of the European Thyroid Association.
- Iñaki Comino-Méndez has been awarded with the Young Investigator prize during the Fourth International Symposium on Pheochromocytoma.

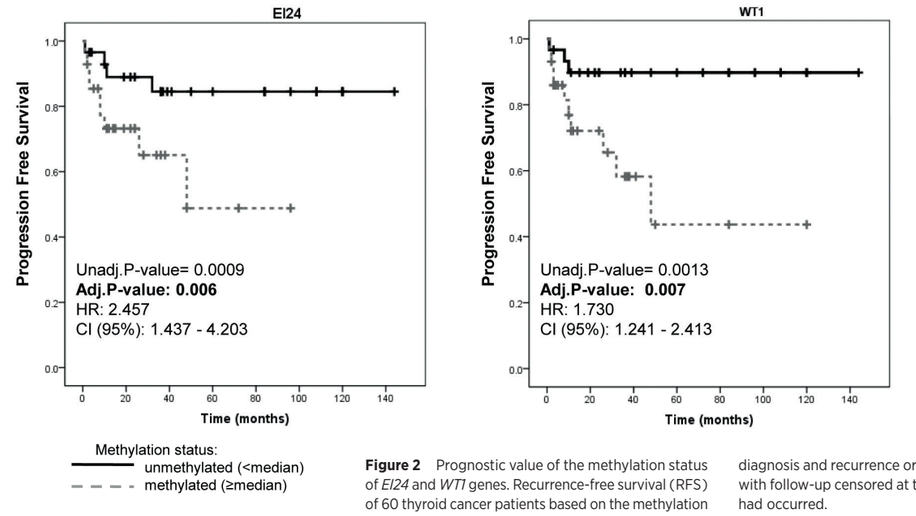

Figure 2 Prognostic value of the methylation status of *EI24* and *WT1* genes. Recurrence-free survival (RFS) of 60 thyroid cancer patients based on the methylation levels. RFS was defined as the time between initial diagnosis and recurrence or death due to the disease, with follow-up censored at the last contact if no event had occurred.

DNA methylation profiles reflect histology and RAS/BRAF mutational status in well-differentiated thyroid cancer

Follicular cell-derived carcinoma is the most common endocrine malignancy. By characterising, at the genome-wide level, the DNA methylation patterns of the largest series of well-differentiated thyroid tumours described to date – including follicular adenomas and carcinomas, papillary carcinomas and normal thyroid tissue – we provided novel insights into the biology underlying, on the one hand, the histological heterogeneity, and, on the other hand, differential patient outcomes of this disease. We describe distinct subtype- and mutational-specific methylation profiles, as well as propose novel markers associated with recurrence-free survival that could provide an improved classification of patients. According to the results, methylation levels increase in a progressive manner along the tumourigenic process from adenomas to carcinomas. Moreover, we describe distinct subtype- and mutational-specific methylation profiles, suggesting that methylation profiles may identify a subset of patients that could benefit from treatment with demethylating agents. In this regards, elevated levels of promoter methylation of etoposide-induced 2.4 and Wilms tumour 1 were independent predictors of shorter progression-free survival in the studied cohort (FIGURE 2). Altogether, this is the result of an exhaustive work addressing the involvement of DNA methylation in the aetiology of thyroid cancer. ■

GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

Núria Malats
Group Leader

Staff Scientist
José C. Martínez (since April)

OVERVIEW

The scope of research carried out by the Cancer Epidemiology Group ranges from the identification of aetiological agents and mechanisms, to the translation of the research findings into the clinical and public health domains, with a particular focus on bladder, pancreatic, and breast cancer.

Epidemiology now demands the alignment and synergy of scope, objectives, data, and tools across all disciplines. By adopting an integrative research approach, we are able to participate in large, international multidisciplinary studies requiring the development of methodological innovations in all aspects of epidemiology.

We employ a wide variety of biomarkers to better characterise exposures and cancer outcomes, as well as the genetic patterns predisposing or protecting against the disease, including variability in its clinical course. *Omics* data provide a unique opportunity to further dissect complex exposures, genetic susceptibility, and phenotypes. The Group explores the integration of this data in epidemiologic studies.

The strategic goals of the Group are to:

- Identify environmental exposures and genetic susceptibility factors, as well as gene-environmental & gene-gene interactions involved in cancer development and progression.
- Study the differential association of germline genetic variants and environmental exposures with cancer subphenotypes characterised at the molecular/omics level.
- Develop and apply statistical/informatics tools to model the risk, prediction, and clinical course of patients with cancer and to integrate epidemiologic with *Omics* information (genomics, transcriptomics and metagenomics).
- Assess clinical and public health strategies for cancer control, using current genomic tests and data.

Post-Doctoral Fellows
Paulina Gómez, M. Evangelina López De Maturana

Graduate Students
Alexandra Masson-Lecomte (until November), Silvia Pineda

Technicians
Ana Alfaro, Marina Arranz (March-September), Marien Castillo, Mirari Márquez, Marta Rava (since February), Ana Villajero (June-September)

Visiting Student
Laura Leroi (May-August)

"Integrative statistical approaches have allowed us to identify the role of both environmental and genetic inflammatory-related factors in cancer development and progression, and have enabled us to establish that as of now, common genetic variants have a limited predictive value in cancer risk prediction."

RESEARCH HIGHLIGHTS

Urothelial bladder cancer (UBC)

We built a predictive model for UBC risk by combining both genomic and nongenomic data, and by applying 3 Bayesian threshold models. The study confirmed the difficulty of predicting complex diseases using only genetic data, and suggested a limited potential for translating the results into public health interventions (FIGURE 1). We are currently conducting a whole-exome sequencing study to improve the performance of the genetic predictive models. In a simulation study, we demonstrated the advantage of applying multi-marker models rather than single-marker regression, due to their larger power and smaller type I error. Furthermore, the multi-marker models provided novel associations with UBC when applied to real data. We also found no strong evidence that common variants in the *TP53* pathway are associated with UBC susceptibility in an in-depth candidate-pathway assessment. Additional evidence of gene-environment interactions for tobacco and UBC was provided by an international study that tested for both multiplicative and additive interactions. We continued exploring the association between LINE1 and UBC and found a positive association between the %5mC and trihalomethane (THM) levels among controls, as well as that the %5mC status may modify the association between UBC risk and THM exposure. In 2 large studies, conducted with the CNIO Epithelial Carcinogenesis Group, analysing voided urine from healthy individuals (TERT-School/TERT-Adult), we found no mutations in *TERT*. Transcriptomics, methylomics, and genomics data from a small set of individuals are being used to identify the limitations of *OMICS* data integration as well as to develop novel appropriate statistical approaches.

Pancreatic ductal adenocarcinoma (PDAC)

We continued the exploration of PDAC risk factors, both genetic and non-genetic, following an integrated conceptual model for PDAC aetiology (FIGURE 2) that suggests the importance of chronic inflammatory processes. To this end, we analysed data from the PanGenEU study that includes 2,333 cases and 1,016 controls from 6 European countries. Regarding the genetic susceptibility of PDAC, we conducted an in-depth exploration of the phospholipid metabolism pathway that identified single-nucleotidepolymorphisms (SNPs) pointing to *DGKK*; a gene involved in the conversion of diacylglycerol to phosphatidic acid,

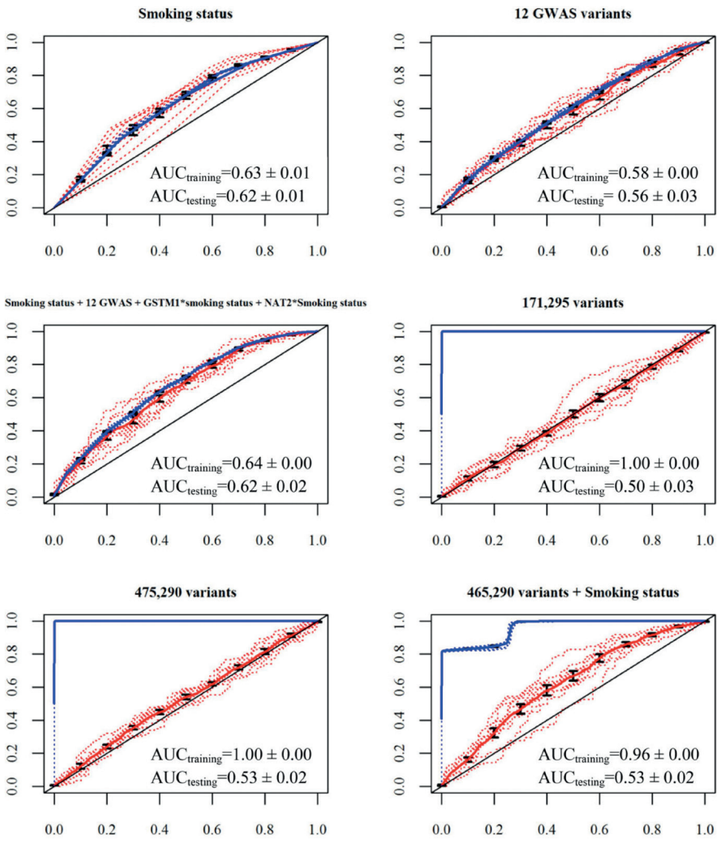

Figure 1 Assessment of the performance of the predictive models for UBC risk, combining both genomic and nongenomic data, and applying 3 Bayesian threshold models using the receiver operating characteristic curve (ROC) graphs for each model. Each displays the training (blue dots) and testing (red dots) cross-validation results, as well as the averaged curves (continuous lines) and estimated standard error bands (vertical bars).

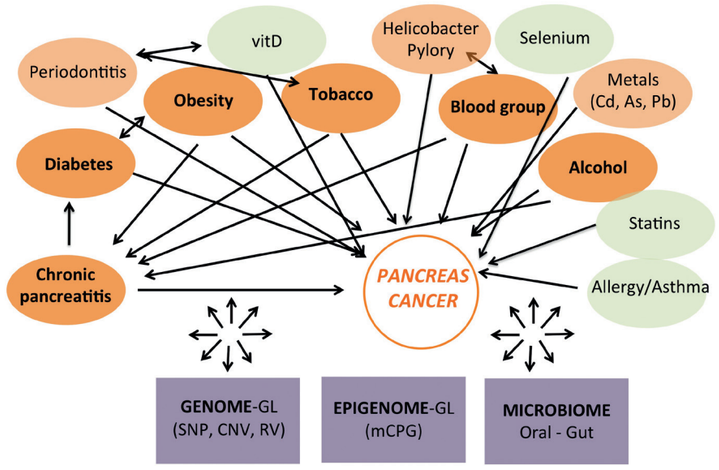

Figure 2 An integrated conceptual model for pancreatic cancer aetiology: a chronic inflammatory-related disease.

which is an important signalling molecule for growth regulation. We participated in a multistage genome-wide association study (PANSCAN3) that identified multiple new susceptibility alleles for PDAC. The collaboration with the International Cancer Genome Consortium (ICGC-pancreas) enables dissecting the genetic susceptibility of PDAC by applying whole genome and exome sequencing strategies. We analysed inflammatory polymorphisms associated with PDAC risk and showed, in collaboration with an international group, that some of these polymorphisms are also associated with chronic pancreatitis (CP), which is a risk factor for PDAC. We also contributed to an international study that identified SNPs at *PRSS1-PRSS2* and *CLDN2-MORC4* loci as being associated with CP. We continue to support, together with colleagues at the *Hospital Ramón y Cajal* in Madrid, the Spanish Registry of Familial Pancreatic Cancer. This project includes a screening programme for high-risk relatives. We coordinate the COST Action BM1204 *EU_Pancreas (www.eupancreas.com)*, which includes >160 multidisciplinary members from 21 European Union (EU) countries, EU governmental and non-governmental institutions, as well as private companies. Several scientific, training, and dissemination activities have also been conducted.

Breast cancer (BC)

As a result of a large gene-environment interaction assessment, in which 71,527 candidate variants in 34,475 BC cases and 34,786 controls from the European multi-consortia project (COGS) were analysed, we observed statistically significant associations between 3 SNPs in 2 independent loci and their interactions with parity, age at menarche, and adult body mass index. An ongoing study aims to estimate the heritability for breast and prostate cancer in families through the application of a new individual measure for assigning the genetic additive value of cancer. ■

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FAMILIAL CANCER CLINICAL UNIT

Miguel Urioste
Clinical Unit Head

Technicians
Samuel Domingo (since September), Maika González (until February), Fátima Mercadillo, Gema Arribas (April-August)

OVERVIEW

Genetic risk assessment and testing for cancer predisposition genes is generally a multistep process that involves: the identification of individuals who may be at an increased risk for potentially harmful mutations, followed by genetic counselling by suitably trained health care professionals, and genetic testing of selected high-risk individuals when indicated. Tests for high-penetrance mutations have clinical utility, meaning that they can be informative for clinical decision-making thereby facilitating the prevention or amelioration of adverse health outcomes.

The aim of the Familial Cancer Clinical Unit (FCCU) is the assessment, diagnosis, prevention and researching of familial and hereditary forms of cancer. The FCCU is involved in a network of clinical units and diagnostic laboratories of the Madrid Regional Government, with the aim of offering full patient evaluations. Furthermore, FCCU provides support to other Regional hospitals that lack this type of diagnostic service. Our Unit also collaborates with the Groups in the Human Cancer Genetics Programme, by supplying biological samples and clinical and familial data of patients with a genetic predisposition to cancer.

RESEARCH HIGHLIGHTS

This year the FCCU has continued to work towards consolidating the consultancy in the Medical Oncology Service of the *Hospital Universitario de Fuenlabrada*. A closer relationship with other medical specialists has been established in order to define a clear process that favours the best care and follow-up of the patients. Also, the first steps for the creation of a Hereditary Cancer Clinical Committee within the hospital have been taken.

During 2014, the FCCU evaluated 163 patients with suspected predisposition to cancer via the consultancy. Furthermore, 314 genetic diagnostic studies were carried out in the FCCU laboratory. We are currently working with 15 different genes whose constitutional harmful mutations confer a high susceptibility to develop cancer at an early age. Our portfolio of services is in permanent review and this year we have introduced new genes, like *POLE* and *POLD1*, to extend the diagnostic possibilities for patients with hereditary forms of colorectal cancer, or with other predisposition syndromes, e.g. *CDK4* in familial malignant melanoma.

An important part of the inherited predisposition to colorectal cancer has yet to be explained. We have continued our research of familial forms of colorectal cancer. In collaboration with the *Instituto Catalán de Oncología*, we have identified a Fanconi anaemia pathway gene that is probably implicated in the inherited susceptibility to mismatch repair-proficient colorectal tumours. The identification of new genes associated with hereditary colorectal cancer will facilitate the development of appropriate surveillance guidelines and the clinical management of these patients.

Also in collaboration with the Surgery Department of the *Hospital 12 de Octubre* in Madrid, we are working on the characterisation of early-onset colorectal cancer. We are researching the role of chromosomal instability in carcinogenesis in 2 groups of colorectal tumours categorised according to the age of cancer onset. Our initial data indicate that DNA copy number profiling reveals different patterns of chromosomal instability within colorectal cancer, according to the age of onset (FIGURE).

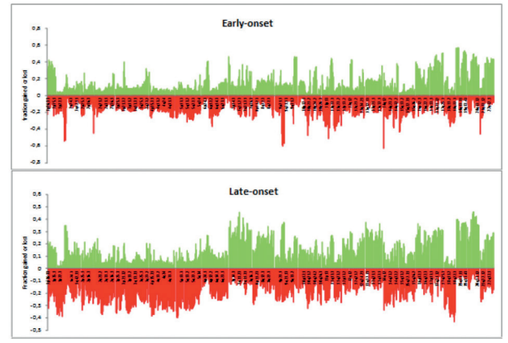

Figure Frequency plots of copy number gains (in green) and losses (in red) defined for early-onset (<45 years) and late-onset (>70 years) colorectal cancer. The fraction gained or lost is plotted on the y axis versus genomic location on the x axis.

Educational activities are other main goals of the FCCU. We have just edited a guideline that contributes to the improved identification and management of patients and families with Bannayan-Riley-Ruvalcaba syndrome; a rare disease that predisposes to cancer. The *Centro de Investigaciones Biomédicas en Red de Enfermedades Raras* (CIBERER) is involved in disseminating this guideline. ■

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HUMAN GENOTYPING-*CEGEN* CORE UNIT

Anna González Neira
Core Unit Head

Graduate Student
Sara Ruiz

Technicians
Charo Alonso, Núria Álvarez, Belén Herráez, Tais Moreno, Guillermo Pita

OVERVIEW

The most abundant types of genetic variation are single nucleotide variants (SNVs) and copy number variants (CNVs). Association studies involving large-scale analysis of both SNVs and CNVs in thousands of patients can help to identify genes that underlie complex diseases such as cancer, as well as help to predict drug response. Our Unit has implemented different high-throughput and cost-effective methods to measure as little as one up to millions of SNVs and CNVs. Epigenetic studies using whole-genome methylation arrays are also performed in the Unit. Complementarily, pharmacogenomic studies have been undertaken to identify predictive biomarkers for personalised cancer therapy.

"Advances in our understanding of why patients have different responses to cancer treatment will help to improve cancer patient care."

RESEARCH HIGHLIGHTS

Replication analysis in Ewing's sarcoma survival genes

Ewing's sarcoma (ES) is relatively uncommon, despite being the second most frequent primary malignant bone tumour in children and adolescents, after osteosarcoma. Although considerable progress has been made during the past decades, many individuals still relapse or suffer from adverse drug reactions; this has motivated the search for predictive factors. We examined 24 genes that have been reported to be involved in the biotransformation of the 6 agents 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 -rs7190447, rs4148737 and rs11188147, located in the *ABCC6*, *ABCB1* and *CYP2C8* genes, respectively- that are significantly associated with overall survival (FIGURE). These associations were confirmed in a large, independent, replication cohort of 495 patients from 5 European countries.

Meta-analysis for genetic markers of toxicity from capecitabine and other fluorouracil-based regimens

Meta-analysis was performed of 927 patients with colorectal cancer and 16 published studies (n = 4,855 patients), to examine the candidate polymorphisms of toxicity from capecitabine and other fluorouracil-based regimens. Global capecitabine toxicity was associated with the rare, functional *DPYD* alleles 2846T>A and *2A (combined odds ratio, 5.51; P = .0013) and with the common *TYMS* polymorphisms 5`VNTR2R/3R and 3`UTR 6bp ins-del (combined odds ratio, 1.31; P = 9.4 × 10−6).

Cis-acting regulatory genetic variants in the *CDH4* gene and capecitabine-induced hand-foot syndrome (CiHFS)

A two-stage genome-wide association study was carried out to identify genetic variation associated with the risk of suffering CiHFS. We identified genetic variants in the locus 20q13.33 that were associated with this adverse event and replicated the results in an independent cohort of patients. To determine the target gene(s) at the 20q13.33 risk locus, we conducted a circular chromosome conformation capture assay followed by high- throughput sequencing (4C-seq) in keratinotytes. We have demonstrated that the region containing the risk SNPs physically interacts with an enhancer element located downstream, which binds with the *CDH4* promoter. Evidence of direct looping suggests that our SNPs could modulate expression of *CDH4* through a competing mechanism by blocking the enhancer from looping with the promoter. ■

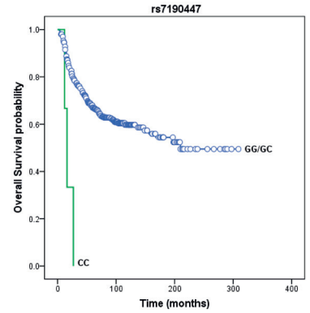

Figure Kaplan-Meier survival curve for Ewing's sarcoma patients according to genotypes for rs7190447 in *ABCC6* (X^2=14.8, P=1.19X10^{-4}) (N=408).

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CLINICAL RESEARCH PROGRAMME

MANUEL HIDALGO Programme Director

The Clinical Research Programme (CRP) aims to translate advances in cancer research into the prevention, diagnosis, and treatment of cancer 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.

"Our Research Groups strive to fight cancer by developing novel and more effective medicines against this dreadful disease."

The CRP is composed of five Clinical Research Units (CRU) and three 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, as well as on the understanding of the molecular taxonomy 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. In 2014, through a collaborative agreement with the *Hospital Universitario 12 de Octubre*, we have incorporated two new CRUs: the Lung Cancer CRU, headed by Luis Paz-Ares, and the Haematological Malignancies CRU, headed by Joaquín Martínez-López. 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 the knowledge gained from cancer genome studies to patient care.

In 2014, we have continued the expansion of our clinical trials activities in collaboration with several Hospitals in Spain. Operational Units at the *Hospital de Madrid, Fundación Jiménez Diaz, Hospital Ramon y Cajal* and the *Hospital Niño Jesus* have treated over 250 patients in early clinical trials this year. We have also formalised collaborative agreements with other Hospitals in Madrid in order to establish an early clinical trials consortium that aims to create a platform for molecular testing, as well as to conduct coordinated clinical trials in patients with defined molecular abnormalities. Furthermore, we have also established multicentre clinical trials in breast, prostate and pancreatic cancer 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.

GASTROINTESTINAL CANCER CLINICAL RESEARCH UNIT

Manuel Hidalgo
Clinical Research Unit Head

Staff Scientist
Pedro P. López

Post-Doctoral Fellows
Lucía Fernández, Lucas Moreno

Graduate Students
Spas Dimitrov, Raquel Martínez (until August), Beatriz Salvador (since September)

Technicians
Natalia Baños, Alejandro Márquez (July-October), Marina Mendiburu-Eliçabe (since November), Camino Menéndez, Manuel Muñoz

Clinical Investigator
Victor Moreno

OVERVIEW

The Gastrointestinal (GI) Cancer Clinical Research Unit focuses on the clinical development and personalised application of novel therapeutics for patients with cancers of the pancreas and colon. Our work combines the preclinical assessment of novel anticancer agents in 'Avatar' mouse models with the design, conduction, and analysis of clinical trials using these agents. 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 – improves survival in patients with pancreatic cancer; the results from this trial have led to the approval of the drug to treat this disease.

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 characterised the largest collection of these models for pancreatic cancer. We use the Avatar models in 3 critical applications, including 1) the screening of new anticancer agents; 2) conduction of co-clinical trials, in which ongoing clinical trials are performed in parallel with studies using Avatar models of the same cancer type in order to elucidate mechanisms of action and biomarkers of drug response/resistance; and 3) finally, we are using the Avatar models for personalised cancer treatment integrated with next generation sequencing.

"In 2014, nab-paclitaxel, a drug that we helped to develop, was approved to treat patients with pancreatic ductal adenocarcinoma (PDA). We have also shown promising results with demcizumab; a new drug that targets cancer stem cells in KRAS mutant tumours, and whose development is advancing rapidly in clinical trials conducted by our Group."

RESEARCH HIGHLIGHTS

In 2014, we continued the lines of work summarised below that were initiated in the previous year:

Avatar mouse model development and characterisation

Our Group has continued its efforts to develop and characterise Avatar models from patients with GI malignancies, as well as other tumour types, for drug screening, development of drug combinations, biomarker discovery, and personalised medicine. This collection of patient-derived xenografts (PDX) from PDA is the largest and best characterised collection available so far, and represents an important resource for academic and industry investigators within the framework of the European PDX Consortia. We have also co-authored several papers, listed below, based on collaborative work performed using our collection for drug development and 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 cancers.

Development of novel anticancer agents

We have significantly expanded our portfolio of early clinical trials in patients with GI cancer and other malignancies. At present, the GI Cancer Unit is conducting more than 20 clinical studies with novel anticancer agents, spanning a wide range of mechanisms of action, such as signalling inhibitors (FGFR, RAF, MEK, HER), Notch inhibitors, conventional chemotherapy and angiogenesis inhibitors. 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 analysis of clinically important biomarkers, as well as co-clinical studies in mouse models. Our most relevant contribution to this field has been the work that led to the approval of *nab*-paclitaxel for PDA. Over the past year, we have intensively investigated biomarkers that predict response to this drug. Our initial efforts focused on the expression of the SPARC protein but, unexpectedly, these studies did not reveal a predictive role for SPARC. Interestingly, these clinical results were predicted by mouse model studies (FIGURE 1). Based on these data, we are now exploring other markers such as Cav1 and FAP.

Importantly, we are conducting a clinical trial in which Avatar models will be generated from metastatic tumours derived from 100 patients treated with *nab*-paclitaxel and gemcitabine; these models will serve as an important platform to investigate and validate new biomarkers. Likewise, our clinical efforts in PDA are now geared towards improving the outcome of patients treated with this new combination. As mentioned above, preclinical work has suggested that drugs targeting the Notch pathway such as demcizumab, an antagonist of DLL4, have shown dramatic activity. In a phase I-II study, we have shown promising clinical activity with this agent in patients with PDA (FIGURE 2). Based on this data, our group is now leading the clinical development of this agent in an international phase II trial.

Personalised 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 a pilot study, we performed whole-exome sequencing analysis 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 13 patients received a personalised treatment, of which 6 achieved durable remissions. Based on these results we launched the Avatar clinical trial, in which patients with advanced PDA are randomised to either a standard of care approach or to a personalised approach; for the latter we perform a tumour biopsy followed by exome analysis and the generation of an Avatar model to experimentally test treatment options resulting from the genetic analysis. So far, we have enrolled 20 patients in this trial. ■

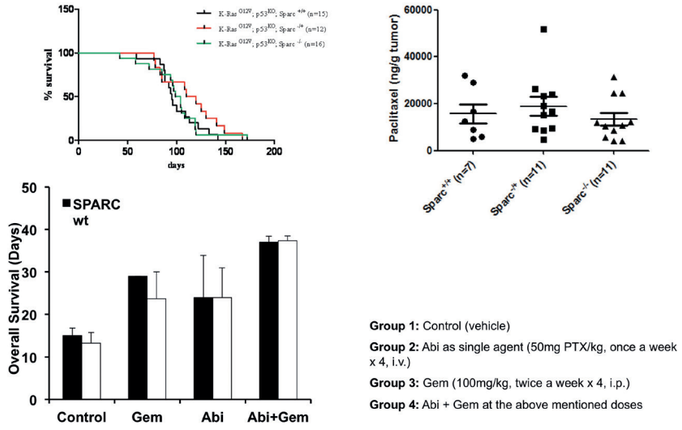

Figure 1 Effects of SPARC deletion in the PDA *KRAS*-driven mouse model. The impact of SPARC knock-down in a KRAS-p53 mouse model of PDA is shown. PDA developed in these models independently of SPARC levels. Likewise, the intratumour distribution of *nab*-paclitaxel is not affected by the levels of SPARC. *Nab*-PTX efficacy was similar in models with varying degree of SPARC expression.

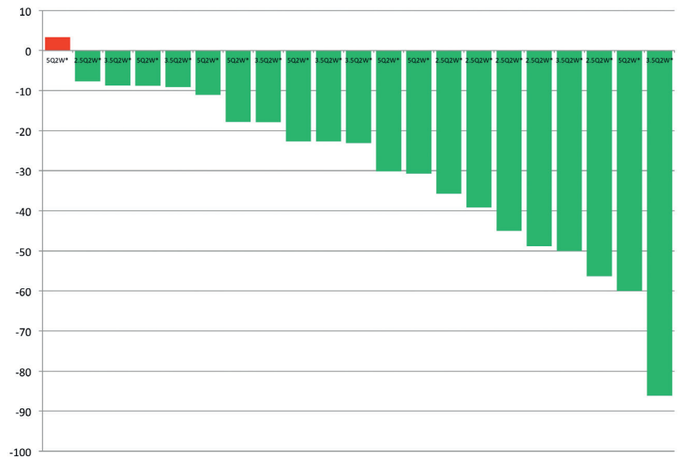

Figure 2 Waterfall plot of: Gemcitabine and *Nab*-paclitaxel plus Demcizumab in patients with PDA. Preliminary efficacy data of the triple combination in patients with advanced PDA showing promising response rates.

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- **AWARDS AND RECOGNITION**

- The 2013 "*el Talento*" Prize in the Academic Talent Category endowed by the Spanish business daily *Cinco Días*.
- Science Committee Member, Cancer Research UK.

BREAST CANCER JUNIOR CLINICAL RESEARCH UNIT

Miguel Quintela-Fandino
Junior Clinical Research Unit Head

Staff Scientists
María José Bueno, Juan Manuel Funes, Paloma Navarro

OVERVIEW

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

Specifically, our research areas are:

- → Discovery of new targets for breast cancer prevention: role of fatty acid synthase (FASN).
- → Breast cancer functional taxonomy: via 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.

Graduate Student
Ivana Zagorac

Technicians
Verónica Jiménez, Tamara Mondejar, Jesús Sánchez

Clinical Research Fellow
Elena Hernández (until February)

Research Associate
Ramón Colomer

RESEARCH HIGHLIGHTS

Role of FASN in breast cancer

We generated a triple transgenic mouse line with tamoxifen-inducible deletion of FASN in breast tissue expressing the PyMT oncogene. The lack of FASN abrogates the generation of breast tumours. We are currently tackling the mechanistic explanation for the observed phenomenon; our data points towards a metabolic disruption of the Warburg effect.

Breast cancer taxonomy

We have focused our efforts on triple-negative breast cancer. Taking advantage of high-throughput phospho-proteomics – using *in vivo* models and clinical samples from patients with an adverse clinical course – we have observed several similarities between cell lines with aggressive behaviour. The phosphoprofiles revealed that a few "driver" kinases accounted for the differential profiles between more aggressive and mildly aggressive cell lines, and showed a spectacular overlap with those differentially driving the profiles in patients with an adverse dismal prognosis versus patients who were cured. JAK/STAT proteins, MAPK kinases, AXL receptor and EPHRB1 were some of the hits; we are currently studying their functional relevance.

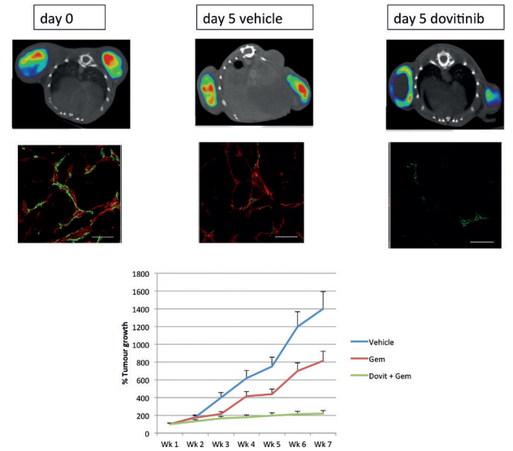

Figure Breast tumours were primed with the antiangiogenic dovitinib. ^{18}F-misonidazole, a probe that binds to hypoxic areas, was infused and its uptake was assayed by positron emission tomography (PET), under the hypothesis that it would track vessel normalisation. The example shows a tumour that, after 5 days, normalises the hypoxia (up) and the vasculature (down); red: dextran extravasation; green: vessels. Normalisation was coupled to chemo-resistance reversion.

Resistance to targeted therapies

We have completed a thorough study of acquired resistance against small-molecule multikinase-inhibitor antiangiogenics, coupled with a preclinical study focused on the development of biomarkers that can track the responding patients and segregate them from the remainder of the patients. We have shown that acquired resistance against this class of antiangiogenics is mediated by a switch from the Warburg effect to mitochondrial metabolism. This switch renders mitochondrial metabolism essential for tumour survival and is therapeutically targetable.

Clinical trials

We have completed 3 investigator-sponsored clinical trials enrolling more than 200 patients. More information about clinical/translational collaborative CNIO/BR trials can be found in our group website. ■

PUBLICATIONS

- Quintela-Fandino M *et al.* (incl. Bueno MJ, Lombardía L, Lopez A, Colomer R) (2014). Selective activity over a constitutively active RET-variant of the oral multikinase inhibitor dovitinib: Results of the CNIO-BR002 phase I-trial. *Mol Oncol* 8, 1719-1728.
- Quintela-Fandino M *et al.* (incl. Hernandez Agudo E) (2014). Phase I clinical trial of nintedanib plus paclitaxel in early HER-2-negative breast cancer (CNIO-BR-01-2010/GEICAM-2010-10 study). *Br J Cancer* 111, 1060-1064.

CRIS FOUNDATION-CNIO PROSTATE CANCER JUNIOR CLINICAL RESEARCH UNIT

David Olmos
Junior Clinical Research Unit Head

Clinical Investigator
Elena Castro

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 (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.

Clinical Research Fellow
Nuria Romero

Graduate Students
Elena R. Cutting (since September),
Paz Nombela, Floortje Van De Poll

Technicians
M. Mercedes Alonso,
Patricia Cozar (since March)

Research Associate
Maria I. Pacheco

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 anaemia 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. *BRCA2, BRCA1, PALB2,* and *NSB1*) or DNA damage sensors/check points (e.g. *CHK2*) that directly activate HR. We have investigated the effect of inherited *BRCA* mutations on conventional treatments for localised and locally advanced PrCa as a model of sporadic aggressive PrCa. We have shown that *BRCA* carriers have worse outcomes than non-carriers, when conventionally treated with radiotherapy or prostatectomy, as they relapsed and progressed earlier to lethal metastatic disease. We are currently working on the molecular characterisation of *BRCA* mutated PrCa, in collaboration with the Institute of Cancer Research (UK), KConFab and Peter McCallum Cancer Centre (Australia), as well as several Spanish centres.

Circulating biomarkers 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. PROSTAC and PROSABI are two prospective, multicentre, and parallel biomarker studies in patients receiving docetaxel, cabazitaxel or abiraterone acetate. With these studies, we aim to analytically qualify and clinically validate a series of blood-borne biomarkers including: ctDNA, ctRNA, exosomes and CTCs. ■

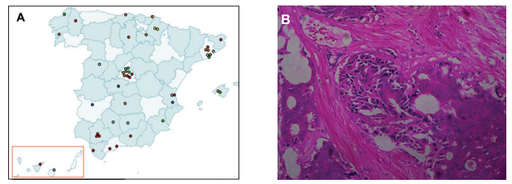

Figure (**A**) Distribution of the 47 centres participating in the PROSTAC/PROSABI studies. (**B**) H&E stained microscope image showing a focus of high grade prostate cancer in a *BRCA2* mutation carrier.

PUBLICATIONS

- Castro E *et al*. (incl. Olmos D). Effect of *BRCA* Mutations on Metastatic Relapse and Cause-specific Survival After Radical Treatment for Localised Prostate Cancer. *Eur Urol.* PMID: 25454609.
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- Postel-Vinay S *et al*. (incl. Olmos D) (2014). Towards new methods for the determination of dose limiting toxicities and the assessment of the recommended dose for further studies of molecularly targeted agents – Dose-Limiting Toxicity and Toxicity Assessment Recommendation Group for Early Trials of Targeted therapies, an European Organisation for Research and Treatment of Cancer-led study. *Eur J Cancer* 50, 2040-2049.
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- Romero-Laorden N *et al.* (incl. Olmos D)(2014). Circulating and disseminated tumor cells in ovarian cancer: a systematic review. *Gynecol Oncol* 133, 632-639.
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- Molife LR *et al*. (incl. Olmos D) (2014). Randomized Phase II trial of nintedanib, afatinib and sequential combination in castration-resistant prostate cancer. *Future Oncol* 10, 219-231.
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AWARDS AND RECOGNITION

- The Stewart Rahr-Prostate Cancer Foundation Young Investigator Award 2014, Prostate Cancer Foundation, USA.

MOLECULAR DIAGNOSTICS UNIT

Luis Lombardía
Unit Head

Technician
Diana Romero

OVERVIEW

The Molecular Diagnostics Unit (MDU) is first and foremost entrusted to supply the hospitals of the National Health System (NHS) with a wide range of sensitive, specific, reliable and updated assays. We routinely identify alterations in the sequence or expression levels of key genes involved in cancer, which could be used in the diagnosis and/or prognosis of patients, the detection of minimal residual disease in patients in clinical remission, or for monitoring response to therapy. Furthermore, the Unit is also entrusted to support CNIO's Clinical Research Units by developing and implementing novel solutions for their research needs. We work in partnership with several international and national groups dedicated to standardising and improving molecular diagnostics in cancer. Finally, the MDU remains fully committed to promoting laboratory training and the mentoring of students, technicians and medical residents.

RESEARCH HIGHLIGHTS

Fostering our support

In 2014, we developed 4 new assays that complement previously implemented tests for a more comprehensive assessment of patients.

Thus, Acute Myeloid Leukaemia (AML) patients have been shown to form a heterogeneous group in terms of neoplasms. Proper care of these patients depends on their cytogenetic characterisation, which stratifies them into 3 main groups associated with good, intermediate and bad prognosis. The intermediate-risk group (40-50% of AML patients), lacking an altered karyotype, is considered to be cytogenetically normal (CN-AML). Three of the tests we implemented this year aim to evaluate CN-AML patients by detecting mutations:

- In the whole sequence of the *CEBPA* (CCAAT/enhancer binding protein alpha) gene, since biallelic mutations are indicators of good prognosis in CN-AML patients previously found to have native *NPM1* and *FLT3* genes.
- In exons 7 and 9 of *WT1* (Wilms tumour 1). These mutations, coupled to an internal tandem repeat alteration in the *FLT3* gene, have been associated with a worse clinical course in young adult patients with CN-AML.
- In exon 4 of *IDH1* and *IDH2* (isocitrate dehydrogenase 1 and 2) genes. Mutations in these genes have been described to be good prognostic markers in CN-AML patients with a mutated *NPM1* and a native *FLT3*. *IDH1/2* mutations are also useful in supporting the diagnosis of low-grade gliomas and secondary glioblastoma.

Moreover, we have implemented an assay to detect mutations in the *CALR* (calreticulin) gene, which allows the diagnosis of patients with essential thrombocythaemia or primary myelofibrosis. The addition of *CALR* to the panel of biomarkers already in use has provided, probably for the first time in the field of Molecular Diagnostics of Cancer, the possibility to classify more than 90% of the patients affected by Chronic Myeloproliferative Neoplasms (FIGURE).

Partnering and training

MDU continues to participate in several initiatives geared towards the personalised medicine segment. During 2014, our Unit trained a laboratory technician, a resident and two undergraduate students. ■

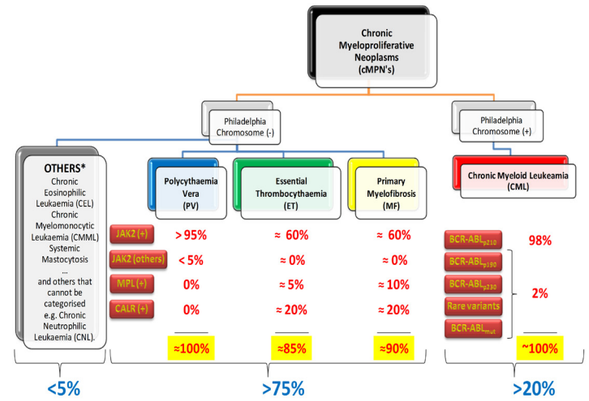

Figure The discovery of mutations in the *CALR* gene, along with other diagnostics markers already in use, such as the *BCR-ABL* fusion gene, *JAK2* and *MPL*, has allowed us, in 2014, to diagnose and classify more than 90% of the patients affected by a Chronic Myeloproliferative Neoplasm.

PUBLICATIONS

- Quintela-Fandino M, Bueno MJ, Lombardía L, Gil M, Gonzalez-Martin A, Marquez R, Bratos R, Guerra J, Tan E, Lopez A, Colomer R, Salazar R (2014). Selective activity over a constitutively active RET-variant of the oral multikinase inhibitor dovitinib: Results of the CNIO-BR002 phase I-trial. *Mol Oncol* 8, 1719-1728.
- Alvarez-Larrán A, Bellosillo B, Pereira A, Kerguelen A, Hernández-Boluda JC, Martínez-Avilés L, Fernández-Rodríguez C, Gómez M, Lombardía L, Angona A, Ancochea A, Senín A, Longarón R, Navarro B, Collado M, Besses C (2014). *JAK2V617F* monitoring in polycythemia vera and essential thrombocythemia: clinical usefulness for predicting myelofibrotic transformation and thrombotic events. *Am J Hematol* 89, 517-523.
- Manso R, Rodríguez-Pinilla SM, Lombardía L, Ruiz de Garibay G, Del Mar López M, Requena L, Sánchez L, Sánchez-Beato M, Piris MA (2014). An A91V SNP in the *perforin* gene is frequently found in NK/T-cell lymphomas. *PLoS One* 9, e91521.
- Rodríguez-Lombardero S, Vizoso-Vázquez A, Lombardía LJ, Becerra M, González-Siso MI, Cerdán ME (2014). Sky1 regulates the expression of sulfur metabolism genes in response to cisplatin. *Microbiology* 160, 1357-1368.

AWARDS AND RECOGNITION

- Editorial board Member, *Advances in Genomics*.

TRANSLATIONAL BIOINFORMATICS UNIT

Fátima Al-Shahrour
Unit Head

Post-Doctoral Fellow
Hector Tejero

Graduate Student
Javier Perales

Technician
Elena Piñeiro

OVERVIEW

The genomics medicine revolution brings new hope in the fight against cancer. Thousands of tumours from different cancer types have been sequenced and genomically characterised, confirming the complexity of cancer genomes and providing a new perspective in tackling cancer. Computational approaches have the ability to decipher cancer genome marks, but there are still many challenges to face in order to translate cancer genome discoveries into clinical medicine.

The Translational Bioinformatics Unit (TBU) uses computational methodologies to perform genomic analyses of cancer patients' data, in order to identify new biomarkers and mechanisms of drug response. Our main goal is to translate this knowledge into effective treatments for cancer patients.

RESEARCH HIGHLIGHTS

During this third year, our main research activity has been focused on the development of a novel computational approach 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) from the following large-scale projects: the Genomics of Drug Sensitivity in Cancer (Wellcome Trust *Sanger Institute, Massachusetts General Hospital*), the Cancer Cell Line Encyclopedia (CCLE, Broad Institute of MIT and Harvard) and the Achilles Project (Broad Institute of MIT and Harvard). Applying this analysis, we obtain a ranked list of biologically relevant genes, some of them candidate drivers of the tumour phenotype. We then use the PanDrugs database – recently developed in our lab – that integrates several public pharmacological resources with clinical information, in order to propose and prioritise those that may have therapeutic implications. We have validated this approach by applying this methodology to publicly available data (ICGC and TCGA cancer genome projects) as well as data generated within our institution.

Sequencing analysis into the clinic

Since 2013, we have collaborated with the *Hospital de Madrid-San Chinarro* to analyse next-generation sequencing data from patients' tumours. During this period, we applied our analytical pipeline for the categorisation and interpretation of patients' tumours; this enabled us to match them to effective drugs or treatments based on their genomic alterations. The result is a ranked list of genetic variants that could serve as potential therapeutic targets and thereby also help guide treatment decisions for patients. So far, we have analysed more than 200 patients and this new pipeline has facilitated the identification of actionable mutations in nearly half of the patients. ■

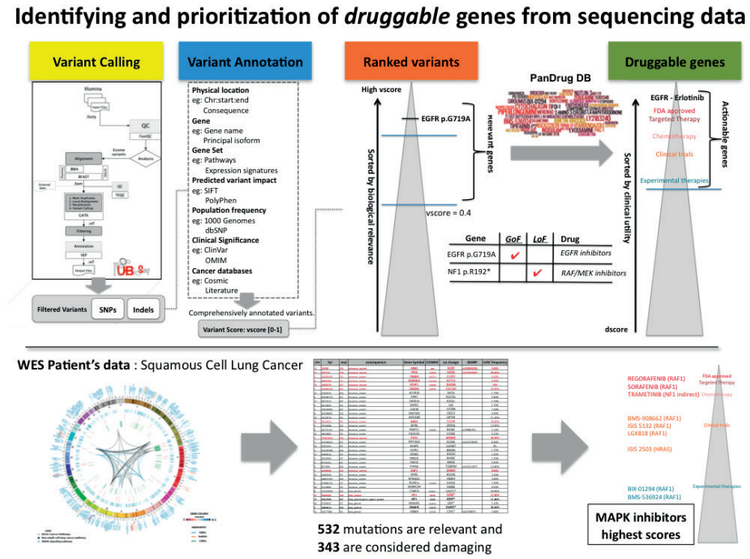

Figure The figure shows the new analytical pipeline developed at TBU for the identification of putatively actionable tumour- specific genomic alterations and score calculation. The result is a ranked list of drug-gene associations based on their biological and clinical relevance.

PUBLICATIONS

- Christensen CL *et al.* (incl. Al-Shahrour F) (2014). Targeting Transcriptional Addictions in Small Cell Lung Cancer with a Covalent CDK7 Inhibitor. *Cancer Cell* 26, 909-922.
- Park SM *et al.* (incl. Al-Shahrour F) (2014). Musashi-2 controls cell fate, lineage bias, and TGF-ß signaling in HSCs. *J Exp Med* 211, 71-87.
- Järås M *et al.* (incl. Al-Shahrour F) (2014). Csnk1a1 inhibition has p53-dependent therapeutic efficacy in acute myeloid leukemia. *J Exp Med* 211, 605-612.
- Rampal R*et al.* (incl. Al-Shahrour F) (2014). Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood* 123, e123-e133.
- Garralda E *et al.* (incl. López-Casas PP, Al-Sahrour F, Valencia A, Hidalgo M) (2014). Integrated next generation sequencing and avatar mouse models for personalized cancer treatment. *Clin Cancer Res* 20, 2476-2484.

H12O-CNIO HAEMATOLOGICAL MALIGNANCIES CLINICAL RESEARCH UNIT

Joaquín Martínez-López (since November)
Head of Clinical Research Unit

OVERVIEW

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 study ingminimal 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. ■

PUBLICATIONS AT OTHER INSTITUTIONS

- Goede V *et al.* (incl. de la Serna J) (2014). Obinutuzumab plus Chlorambucil in Patients with CLL and Coexisting Conditions. *N Engl J Med* 370, 1101-1110.
- Papaemmanuil E *et al.* (incl. Rapado I) (2014). RAG-mediated recombination is the predominant driver of oncogenic rearrangement in *ETV6-RUNX1* acute lymphoblastic leukemia. *Nat Genet* 46, 116-125.
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- Ribera JM *et al.* (incl. Grande C) (2014). Treatment of high-risk Philadelphia Chromosome-Negative Acute Lymphoblastic Leukemia in Adolescents and Adults According to Early Cytologic Response and Minimal Residual Disease After Consolidation Assessed by Flow Cytometry: Final Results of the PETHEMA ALL-AR-03 Trial. *J Clin Oncol* 32, 1595-1604.
- Mateos MV *et al.* (incl. Martínez-López J). Update of the GEM2005 trial comparing VMP/VTP as induction in elderly multiple myeloma patients: do we still need alkylators?. *Blood.* PMID: 25102853.
- Martinez-Lopez J *et al.* (incl. Lahuerta JJ, Barrio S, Ayala R, Montalban MA, Sopena M, Cedena T, Rapado I) (2014). Prognostic value of deep sequencing method for minimal residual disease detection in multiple mieloma. *Blood* 123, 3073-3079.
- Puig N *et al.* (incl. Martínez J, Montalbán MA, Lahuerta JJ) (2014). Critical evaluation of ASO RQ-PCR for minimal residual disease evaluation in multiple myeloma. A comparative analysis with flow cytometry. *Leukemia* 28, 391-397.
- Martínez N *et al.* (incl. Martinez-Lopez J) (2014).Whole exome sequencing in splenic marginal 1 zone lymphoma reveals mutations in 2 genes involved in marginal zone differentiation. *Leukemia* 28,1334-1340.
- Menezes J *et al.* (incl. Acquadro F, Gómez-López G, Salgado RN, Ayala R, Pisano DG, Piris MA, Alvarez S, Cigudosa JC) (2014). Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. *Leukemia* 28, 823-829.
- Hernandez-Boluda JC *et al.* (incl. Martinez-Lopez J) (2014). The International Prognostic Scoring System does not accurately discriminate different risk categories in patients with post-essential thrombocythemia and post-polycythemia vera myelofibrosis. *Haematologica* 99, e55-e57.
- Lippert E *et al.* (incl. Martinez-Lopez J) (2014). Clinical and biological characterization of patients with low (0.1-2%) *JAK2V617F* allele burden at diagnosis. *Haematologica* 99, e98-e101.
- Martino A *et al.* (incl. Martinez-Lopez J) (2014). Genetic variants and multiple myeloma risk: Immense validation of the best reported associations**.** *Cancer Epidemiol Biomarkers Prev* 23, 670-674.

H12O-CNIO LUNG CANCER CLINICAL RESEARCH UNIT

Luis Paz-Ares (since November)
Head of Clinical Research Unit

OVERVIEW

Cancer is a major public health problem that is responsible for 25% of global mortality worldwide. Although vital prognosis is strongly linked to early diagnosis, most cancer patients are diagnosed at advanced stages, thus limiting the possibilities of curative resection and significantly worsening the prognosis. The Group focuses on two main research areas: the early diagnosis of the disease and the development of effective systemic therapies.

Identification of molecular biomarkers in solid tumours

This line of research is focused on the search for alterations in the origin and progression of several solid tumours. Specifically, we are analysing the differential expression profile of genes, proteins and microRNAs. All these profiles are then associated with the histological subtype of different neoplasms and their tumour stage. In turn, we also analyse the mechanisms of activation of different molecular markers to validate their potential value, not only in the diagnosis and prognosis of the disease, but also as possible therapeutic targets.

Development of new models for the evaluation of cancer therapeutic strategies

In collaboration with other Research Groups at the Centre, we carry out pre-clinical studies monitoring the biological effect of the treatments *in situ*. To this end, we develop animal models that reproduce, in a reliable manner, the behaviour of several tumours. Solid tumour-derived xenografts are established in animal models in order to predict the effectiveness of anti-tumour agents and to analyse the involved molecular biomarkers. ■

PUBLICATIONS AT OTHER INSTITUTIONS

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BIOBANK

Manuel M. Morente
Director

Staff Scientist
Lydia Sánchez (until March)

Technicians
M. Jesús Artiga, Francisco De Luna, M. Cruz Marín, Inmaculada Almenara (since November)

OVERVIEW

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 main objective of the CNIO Biobank is to facilitate the access of human biological samples for researchers at the CNIO and other collaborating institutions, and to ensure that they meet the scientific and ethical requirements in compliance with Spanish legislation."

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 B.000848.

RESEARCH HIGHLIGHTS

Biobanking

- → 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. All recommendations and advice are in accordance with the current Spanish legal framework.
- → Collaboration with CNIO researchers in human pathology.
- → Collaboration in diagnostic activities as specialists in human pathology.

CNIO's Biobank was authorised by the Health Authorities of the Community of Madrid in October, 2013. From this date onwards, we have supported 22 tissue requests from 12 scientific research projects. Additionally, as the Spanish National Biobank Network Coordination Office, we have managed 17 scientific research projects.

The mean impact factor of the 23 publications published in 2014, for which our Unit provided support was 7.25. We also provided sample and/or documental support for the familial cancer activities of the CNIO Human Cancer Genetics Programme (46 cases requested and 83 diagnostic cases).

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). 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 IARC-WHO/NCI initiative, EurocanPlatform (7th FP), *Plan de Cáncer Familiar de la Comunidad de Madrid*, and the *Sociedad Española de Anatomía Patológica* (*SEAP*). ■

PUBLICATIONS

- Zapatero A (incl. Morente M, Artiga MJ) (2014). Predictive value of PAK6 and PSMB4 expression in patients with localized prostate cancer treated with dose-escalation radiation therapy and androgen deprivation therapy. *Urol Oncol* 32, 1327-1332.
- Zapatero A (incl. Morente M, González MJ). HIF1A expression in localized prostate cancer treated with dose escalation radiation therapy. *Cancer Biomark*. PMID: 25524941.

AWARDS AND RECOGNITION

- Coordinator, Spanish National Biobank Network – ISCIII (2014-2017).
- Executive Committee (Directive Bureau) Member, the European, Middle Eastern & African Society for Biopreservation and Biorepositories (ESBB).
- Scientific Advisory Committee Member, Biobanques (BBMRI-ERIC.fr)-INSERM, France.
- External Ethics Advisory Committee Member, TransBioBC (7FP).

STEM CELLS AND CANCER GROUP

Christopher Heeschen
(in Transition)
Group Leader

Staff Scientists
Alexandra Aicher (until June), Susana García (until June), Bruno Sainz (until April), Patricia Sancho (until May)

Post-Doctoral Fellow
Sara Trabulo (until June)

Graduate Students
Michele Cioffi (until November), Anja Fries (until May), Irene Miranda (until September), Marina Roy (until September), Alejandra Tavera, Sladjana Zagorac (until July)

Technicians
Sonia Alcalá, Emma Burgos (until May), Magdalena Choda (until May), Catarina L. Reis (until November), Marianthi Tatari (until June), Mireia Vallespinos

Visiting Scientist
Morten Draeby Sorensen

OVERVIEW

The purpose of our Group is to improve the still dismal outcome of patients with pancreatic ductal carcinoma – the most common and aggressive form of pancreatic cancer – by developing targeted therapies against exclusively tumourigenic and metastatic cancer stem cells. It has now become evident that cancer heterogeneity is not only generated by distinct subclones within each tumour, but is also driven by phenotypic and functional heterogeneity and plasticity within each subclone. So-called cancer stem cells (CSCs), or tumour-promoting cells, are responsible for this intraclonal functional heterogeneity. Specifically, CSCs represent a subset of cancer cells, for which we and others have now provided conclusive evidence down to the single cell that they represent the root of the disease by giving rise to all differentiated progenies within each cancer subclone. Even more importantly, these cells are essential drivers of metastasis and represent an important source for disease relapse following treatment, which is still the case for the vast majority of patients with pancreatic ductal adenocarcinoma (PDAC). Based on these now well-defined features of CSCs that drive PDAC progression and resistance to therapy, CSCs shou ld represent a crucial component for any new treatment approach. Indeed, our proof-of-concept data clearly demonstrate that the additional targeting of CSCs using genetic and pharmacological tools, respectively, drastically improves cancer therapy efficacy over standard care. Thus, developing effective CSC-targeting therapies bears significant clinical value and provides the conceptual basis for our proposal. Building on our recent achievements, we are now planning comprehensive studies to functionally interrogate cancer stem cells.

"Our research should ultimately allow us to develop novel multimodal therapies in order to eliminate cancer stem cells as the root of PDAC as well as their differentiated progenies. Targeted delivery of new therapies in combination with advanced imaging technologies will be achieved by nanoparticle technology and will be tested in well-designed clinical trials."

RESEARCH HIGHLIGHTS

Development of a novel CSC-centred screening platform

We have identified an intrinsic autofluorescent phenotype in PDAC CSCs and have implemented this marker as a novel and functionally relevant tool for studying CSCs. This distinct inherent property represents a novel biological feature that is traceable and provides unprecedented robustness to identify and purify CSCs without the use of antibodies or any kind of manipulation, thus drastically reducing experimental errors and artefacts. Not only does this allow us to more accurately capture the dynamic complexity of pancreatic CSCs and to develop new pancreatic CSC-tailored therapies – e.g. using tailored shRNA screens – but it is now also being utilised for our novel and proprietary CSC-centred compound screening platform (ScanCSC™). Our platform is unique in that it utilises freshly isolated primary cancer cells derived directly from patients in the clinic (resection, biopsy, or circulating blood) and that it provides drug response data for both CSCs and non-CSCs. Thus, this platform now enables us to define drug response profiles for each individual patient in real time (<5 days without *in vivo* propagation). Building on these innovative findings, we are now further developing our ScanCSC™ platform in the direction of Precision Medicine approaches.

The cancer stem cell niche

Understanding the CSC microenvironment. The PDAC microenvironment not only provides structural support for tumour development, but more importantly, it provides cues to CSCs that regulate self-renewal and metastasis (Lonardo et al., *Cell cycle*, 2012). Here we show that the immuno-modulatory antimicrobial peptide LL-37 is strongly and specifically secreted by tumour-associated macrophages in response to CSC-secreted TGFβ and Nodal/Activin. Moreover, LL-37 increases pluripotency-associated gene expression, self-renewal, invasion, and *in vivo* tumourigenicity via the P2X7 purinoceptor and, thus, in an ATP- and Ca^{2+}-dependent fashion (Sainz et al., *Cancer Cell*, 2013), which can be reversed by genetic or pharmacological targeting of this receptor. Thus, hCAP-18/LL-37 represents a previously unrecognised tumour microenvironment factor that plays a critical role in CSC-mediated tumourigenesis.

Nicotine triggers initiation and progression of K-Ras-driven pancreatic cancer via a Gata6 acinar de-differentiation programme

Although smoking has been identified as a leading risk factor for PDAC, the molecular mechanisms remain unknown. We used 2 different mouse models of *K-Ras*-driven pancreatic tumourigenesis and a wide variety of *in vitro* systems in order to examine the effects of nicotine on the pancreas. We show that nicotine perpetuates pancreatic oncogenic transformation by inducing acinar de-differentiation via AKT/ERK/MYC-mediated downregulation of the key acinar regulator, which then allows for subsequent RAS hyperactivation when mutant *K-Ras* is present. Apart from accelerating early pancreatic carcinogenesis in 2 distinct genetically engineered PDAC mouse models, we also show that downregulation of *Gata6* enhances nicotine-mediated tumour progression via promotion of CSC signatures, including epithelial-to-mesenchymal transition, resulting in increased numbers of circulating cancer cells and liver dissemination. Intriguingly, the latter could be reversed by overexpressing *Gata6* or by co-treatment with the anti-diabetic drug metformin, which prevented nicotine-induced pancreatic carcinogenesis and progression by promoting an acinar differentiation programme via upregulation of *Gata6*. Altogether, our findings reveal for the first time, that by altering the acinar cell compartment, nicotine accelerates *K-Ras*-initiated PDAC development/progression.

Nano-volume well array chip for large-scale propagation and high-resolution analysis of individual CSCs

To address the issue of cellular heterogeneity at a high-throughput level, we have developed a nano-volume (0.4nL) well array chip that allows large-scale isolation and propagation of single cells. Notably, the chip enables single-cell analysis of freshly isolated primary cells at a high-resolution and is compatible with both adherent as well as 3D suspension cultures. Simultaneous time-lapse imaging of thousands of nano-volume wells enables the monitoring of cell division, as well as the tracking of cell fate, and/or alterations in the microscopic cellular morphology and/or markers expression. CSCs can be monitored for up to 7 days by time-lapse high-resolution imaging at the single-cell level. We utilised this novel platform to conclusively demonstrate that non-CSCs do not de-differentiate into CSCs, while CSCs were able to give rise to both CSCs and non-CSCs by undergoing symmetric and asymmetric division, respectively. Moreover, the small scale allowed for the efficient *in vitro* propagation of rare cells, including the expansion and characterisation of circulating tumour cells. ■

PUBLICATIONS

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Direction of Innovation

ANABEL SANZ
Director of Technology Transfer

"At CNIO we are committed to fulfil one of our major strategic goals; the translation of discoveries into practical solutions for the benefit of cancer patients and society in general."

CNIO's comprehensive approach to cancer research integrates excellent frontier research and translational capabilities with applied programmes. This set up enhances the opportunities to discover novel and more effective strategies to prevent and control cancer. In close collaboration with CNIO's scientists, the Direction of Innovation has been very active in attracting partners to commercialise or further develop CNIO's technologies.

The highlights of the Direction of Innovation's achievements were threefold in 2014:

- During 2014, we devoted significant efforts to strengthen and expand our collaborations with partners in the value chain leading towards the market. We have consolidated the relationship with Roche under their Extended Innovation Network and with another pharmaceutical company, Eli Lilly & Company, with whom CNIO has had a long standing partnership. Furthermore, we have extended our portfolio of relationships to other major companies and stakeholders such as Pfizer and the Flemish Institute for Biotechnology. This initiative enables us to share some of our ideas and combine our expertise and resources. We are not just simply licensing ideas and technologies to companies; we are actually bringing academia and industry groups together to jointly work on assessing, advancing and introducing a myriad of ideas into the clinic. Furthermore, the income generated by collaborative research adds to the sustainability of the centre.
- During 2014 the CNIO adopted regulations with the aim of incentivising CNIO scientists to collaborate by sharing their knowledge and innovative ideas for the benefit of commercialisation. For the first time, the CNIO has adopted a policy to distribute royalties generated from license contracts amongst its innovators.
- A third key component is the fostering of an innovation culture. In particular, we believe young researchers are instrumental towards the generation and diffusion of new technologies, and for that reason we foster innovation training initiatives in collaboration with the *Instituto de Empresa* Business School. Thanks to the support of the *Fundación Banco Santander*, a group of young researchers received training on managerial and entrepreneurial skills, which enabled them to develop their ideas into potential commercial opportunities. In the last 3 years, more than 20 CNIO researchers have pursued this training.

BIOTECHNOLOGY PROGRAMME

FERNANDO PELÁEZ Programme Director

The main mission of the Biotechnology Programme is to provide expert technical support and advice to CNIO Research Groups in a number of disciplines and technologies widely used in biomedical research, as well as to implement and develop state-of-the-art biotechnological tools and reagents for cancer research. The Programme is currently composed of nine Core Units covering major areas in Biotechnology, such as 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 services and collaborate with groups from other institutions, both public and private.

In early 2014, the Programme underwent significant staff cuts that affected most of the Units. However, with an extraordinary effort from everyone in the entire Programme, and with the collaboration of the CNIO Research Groups, we have been able to manage this new situation, maintaining the quality of the delivered services. On a more positive note, the search for a new Head of the Histopathology Unit – a position that had been vacant since July 2013 – culminated with the recruitment of Alba de Martino; this young veterinarian pathologist, from the Campus Science Support Facilities of the Vienna Biocenter Campus, joined the CNIO in April this year. We wish her every success in leading the Unit in the forthcoming years.

This year, the Core Units were particularly active in their search for funding from external sources. Two of the Units (the Proteomics and Transgenic Mice Core Units) have been granted projects from the Spanish *Plan Nacional*. Also, the royalties derived from the sales of the antibodies produced by the Monoclonal Antibodies Unit reached a historical maximum.

"Despite the difficulties, the Biotechnology Programme has been able to maintain its competitive edge and keeps being an essential player in achieving the CNIO's scientific goals."

The Programme has been involved in the organisation of workshops and specialised meetings, such as the Core Technologies for Life Sciences Meeting held in Paris in June. Furthermore, the Flow Cytometry and Confocal Microscopy Core Units organised the III Core Management Workshop at the CNIO, as well as the 2nd Congress of the Spanish Network of Advanced Optical Microscopy (REMOA) in October.

Last but not least, 2014 has been a very productive year scientifically for the Programme. The contribution of the Units to the overall scientific performance of the CNIO is reflected in nearly 30 publications, including several papers in top journals. ■

GENOMICS CORE UNIT

Orlando Domínguez
Core Unit Head

Technicians
Purificación Arribas, Martha L. Campo (Until February), Ana Díez (Until April), Guadalupe Luengo, Jorge Monsech, Maria Montalvo (June-November), David B. Rodríguez (Until March), Ángeles Rubio

OVERVIEW

The genome is the compendium of the genetic material that maintains the identity and the biological essence of the species. Any given cell in an individual stores a copy of the genome. Each cell's genome functions like a computer's hard disk; different programmes and instructions are read and executed on it and from it. Chemically made up of linear DNA macromolecules, the genome is distributed into chromosomes, and packed with and interpreted by a myriad of protein cohorts. Protein-coding genes hardly represent a 2% fraction of the mammalian genome. New functional non-protein coding genes are increasingly being mapped and characterised. Our genome also features large deserts, fossils, and even mobile pieces that are able to jump and randomly integrate elsewhere. The genome is not immutable; changes can be either neutral, positive, or contribute to a genetic disease like cancer. Cancer originates from the accumulation of genetic changes, which affect genes that then begin to play some different tune, either a melody or a damaging noise. The field of genomics sheds light on these complexities. It deals with both the structure and the dynamics of the genome, it deals with its activities, with the interactions of the genes with one another and with their environment.

"As a core facility service provider for CNIO scientists, the Genomics Unit provides a toolbox for DNA and RNA analyses that are dedicated to an array of applications, either at the single locus or at any more global genomic level."

RESEARCH HIGHLIGHTS

Each cancer genome is different. Even an individual tumour harbours a number of different subclonal genomes, each with some different alteration. By analysing this heterogeneity, genomics reveals the genetic diversity and helps to dissect cancer mechanisms. It employs a distinct set of powerful methodologies, with the capacity to interrogate a wide number of genetic loci, or even a whole genome in a single experiment. Some tools can detect modifications at a structural level: mutations, binding of protein factors, or variations in chromatin folding. Others are suitable to examine functional choreographies; the complex network of gene activity in response to treatments, which may uncover therapeutic targets and prognostic biomarkers.

The Genomics Unit provides services at two levels of coverage:

→ The genome-wide level is addressed by both deep-sequencing and microarray technologies. Deep-sequencing permits a variety of applications, including transcriptome analyses such as RNAseq and small RNAseq, genome-wide location of interacting protein factors on chromosomal DNA by ChIPseq, as well as whole-genome or whole-exome tumour sequencing. 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 also be addressed with DNA microarrays.

→ At the single locus level other offers are available. A traditional DNA capillary sequencing service, based on a 3730xl DNA Analyser from Applied Biosystems, is being used to find mutations in candidate genes, or 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.

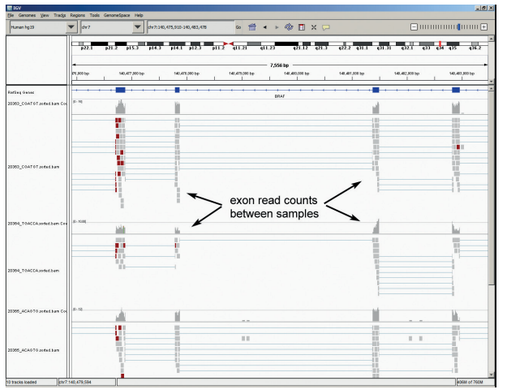

Figure This figure shows an intermediate step in a transcriptomic analysis by RNAseq. cDNA sequencing reads are mapped to the genome – clusters correspond to gene exons – and counted. Counts reflect transcript abundance. Comparison among samples highlights transcriptional differences.

The Unit also provides a transgenic mouse genotyping service that implements dual-labelled, allele-specific, fluorescent probe technology for a quick and efficient turnaround time. ■

PUBLICATIONS

- Piazzolla D, Palla AR, Pantoja C, Cañamero M, de Castro IP, Ortega S, Gómez-López G, Dominguez O, Megías D, Roncador G, Luque-Garcia JL, Fernandez-Tresguerres B, Fernandez AF, Fraga MF, Rodriguez-Justo M, Manzanares M, Sánchez-Carbayo M, García-Pedrero JM, Rodrigo JP, Malumbres M, Serrano M (2014). Lineage-restricted function of the pluripotency factor NANOG in stratified epithelia. *Nat Commun* 5, 4226.
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TRANSGENIC MICE CORE UNIT

Sagrario Ortega
Core Unit Head

Technicians
M. Carmen Gómez, Jaime A. Muñoz, Patricia Prieto, Marta S. Riffo (Until March), Pier Francesco Vargiu

OVERVIEW

The laboratory mouse is the organism of choice for studying the basis of human disease and for generating animal models of human genetic disorders. Cancer has a strong genetic component, and genetically modified mice are an essential tool for studies of gene function in cancer, the elucidation of disease mechanisms, and for drug discovery and target validation. The Transgenic Mice Unit at the CNIO offers state-of-the-art technology for the manipulation of the mouse genome and for the cryopreservation of genetically modified mouse strains. The Unit also provides support to CNIO researchers in many aspects related to research with embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, as well as embryo- and mouse model-based research. Finally, the Unit leads its own research projects that are focused on the generation of mouse models for studies of tumour biology and on the screening of cancer-related genes.

RESEARCH HIGHLIGHTS

Figure Creating a knockin Tyr-GFP reporter allele by CRISPR. The gRNA and Cas9 RNA were injected into the cytoplasm of B6.CBA zygotes (agouti). Note the loss of hair pigmentation in this representative litter, indicating the inactivation of the Tyr locus by CRISPR/Cas9; produced in collaboration with Davide Seruggia and Lluis Montoliu, *Centro Nacional de Biotecnología* (CSIC), Madrid.

During 2014, the Transgenic Mice Core Unit focused on 2 technological developments: 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 genome-wide forward- and reverse- genetic screenings in mice using transposons or lentiviral gene-trap vectors for genome-wide mutagenesis. The haploid karyotype allows 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 the HaploESCancer collection, derived from CNIO mice. 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. These libraries will help us to expand the repertoire of genetic screenings with a specific focus on cancer.

The year 2014 has also witnessed a revolution in mammalian genome editing. The CRISPR/Cas9 system, imported from archaeal and other bacterial genomes, has expanded the currently available set of mammalian genome engineering tools, thereby 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 started testing the system directly in mouse zygotes in order to generate conditional alleles as well as knockin reporter alleles. The FIGURE shows the result of a zygote injection of CRISPR/Cas9 reagents to create a GFP reporter allele in the endogenous mouse Tyrosinase locus (Tyr-GFP knockin). ■

PUBLICATIONS

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MONOCLONAL ANTIBODIES CORE UNIT

Giovanna Roncador
Core Unit Head

Technicians
Aroa García (Since August), M. Mar López (Until February), Lorena Maestre, Ana I. Reyes

OVERVIEW

During the last 40 years, the development of monoclonal antibody (mAb) technology has led to the generation of large panels of highly specific reagents that have had a tremendous impact in basic research, diagnostics and applied medicine. Indeed, mAb technology enables a better understanding of life processes, and can help to discover and aid the investigation of new pathways for the diagnosis, prevention and treatment of disease.

"The Unit produces novel and high-quality mAbs that are used in basic research 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."

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 mAbs production in gene-inactivated mice, mAb characterisation and validation, medium-scale mAb production, as well as a service of *Mycoplasma* testing for the cell culture facility.

RESEARCH HIGHLIGHTS

During the last 14 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, generating revenues through 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 58 thoroughly validated, high-quality mAbs (accessible at http://www.cnio.es/es/grupos/plantillas/personalizable.asp?pag=763).

In 2008, in collaboration with Oxford University, we founded EuroMAbNet; the first European non-profit organisation of laboratories specialised in mAb production. We provide an arena to exchange knowledge, share cutting-edge methodologies and materials, as well as create common strategies to standardise and improve Abs production and validation. The EuroMabNet web page can be accessed through this link: www.euromabnet.com.

In collaboration with J. F. García-García, Head of the Pathology Department at the MD Anderson Cancer Center, we have produced and characterised a new mAb against human CSF1R protein. CSF1R is a tyrosine-kinase that plays an essential role in promoting the differentiation of myeloid progenitors into monocytes, macrophages and dendritic cells. In recent years, several studies have highlighted the presence of macrophages in the microenvironment (TAMs) of a variety of lymphomas, stressing the importance of the identification of this cell type as an additional tool for lymphoma diagnosis. Using a variety of tissue microarrays, we have identified and documented the expression of CSF1R protein at the single cell level in lymphoid tissue, showing that CSF1R mAb is a specific marker of the normal monocytes/macrophages lineage.

CSF1R+ TAMs were less frequent in B-cell lymphocytic leukaemia and lymphoblastic B-cell lymphoma than in diffuse large B-cell lymphoma, peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma and cHL (classical Hodgkin lymphoma). Hodgkin Reed–Sternberg cells (HRS) in cHL and the neoplastic cells in the NHLs studied (with the exception of two cases of anaplastic large cell lymphoma) lacked detectable CSF1R protein. A CSF1R+ enriched microenvironment in cHL was associated with shorter survival. CSF1R pathway activation was evident in cHL, and inactivation of this pathway could be a potential therapeutic strategy in cHL cases. ■

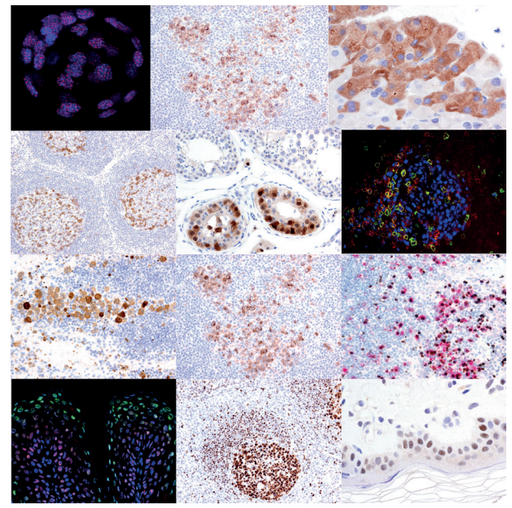

Figure Immunohistochemistry with several mAbs produced by the Monoclonal Antibodies Unit.

PUBLICATIONS

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- Martín-Sánchez E *et al.* (incl. Odqvist L, Roncador G, Blanco-Aparicio C, Real FX) (2014). PIM kinases as potential therapeutic targets in a subset of peripheral T cell lymphoma cases. *PLoS One* 9, e1121148.
- Metcalf RA *et al.* (incl. Roncador G) (2014). Myeloid cell nuclear differentiation antigen is expressed in a subset of marginal zone lymphomas and is useful in the differential diagnosis with follicular lymphoma. *Hum Pathol* 45, 1730-1736.
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- Patel S *et al.* (incl. Roncador G) (2014). Increased Expression of Phosphorylated FADD in Anaplastic Large Cell and Other T-Cell Lymphomas. *Biomark Insights* 3, 77-84.

MOLECULAR IMAGING CORE UNIT

Francisca Mulero
Core Unit Head

Technicians
Elena Andrés, Juan A. Cámara, Silvia Sánchez, Coral Velasco (Until June), Gloria Visdominé (Since October)

"Clearly, molecular imaging is a rapidly growing, wide-ranging field of study that has tremendous potential to elucidate biological processes and pathways at the cellular and molecular levels, and to translate scientific discoveries made in the research laboratory into the clinical setting. The important insights gained from preclinical small animal molecular imaging and their translation into clinical trials promises to accelerate the development of individualised therapies tailored to a patient's genetic makeup."

OVERVIEW

Molecular imaging offers significant advantages to the scientist over traditional research paradigms. First, molecular imaging procedures can be conducted in the living organism in a non-invasive way. Whereas studies of tumour responsiveness, for example to a new therapeutic agent, would have traditionally involved a large cohort of animals from which subsets would be analysed histologically at multiple time points, molecular imaging allows characterisation of tumour development and response to a therapeutic – and even response to discontinuation of the therapeutic – within the same small set of animals imaged longitudinally at multiple time points. This example illustrates two associated advantages of molecular imaging: 1) a study can be conducted with significantly fewer animals (thereby minimising animal usage and reducing animal costs) than would otherwise be necessary; and 2) the statistical power is increased because each animal serves as its own control. Other advantages include the ability of molecular imaging procedures to interrogate the whole body, besides from focusing on specific regions, and to visualise the molecular target of interest in 3-dimensional space. Finally, molecular imaging is becoming a key bridging technology to translate experimental preclinical findings into the clinical environment.

RESEARCH HIGHLIGHTS

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

This year, in collaboration with the CNIO Breast Cancer Clinical Research Unit, we have completed the study using ^{18}F-fluoromisonidazole for assessing tumour hypoxia in patients – as we previously carried out in mouse models of breast cancer – adapting the scan protocol, and positioning this positron emission tomography (PET) probe to be used in human patients (FIGURE). Tumour hypoxia is a key factor in predicting tumour response to treatment; the compound gets trapped into the hypoxic cell acting as a biomarker of hypoxic tissues, and this enables us to image it.

In 2014, we continued our grant project with the Massachusetts Institute of Technology (MIT) titled "Improved Molecular Imaging by Multi-tracer PET", which focuses on using dual isotopes to assess different biological changes simultaneously. We also started a new project in collaboration with the CNIO Melanoma Group, "Early Assessment of Treatment Response in Advanced Melanoma Patients". Additionally, our Unit provides imaging support in clinical trials sponsored by the CNIO Clinical Research Programme.

Furthermore, we continue to actively participate in international consortia: "m+visión" led by the MIT, and Euro-BioImaging, a large-scale pan-European research infrastructure project of the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. ■

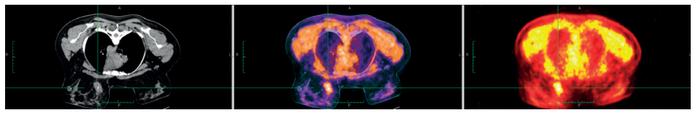

Figure Positron emission tomography-computed tomography (PET-CT) scan of a patient with a right breast carcinoma using ^{18}F-MISO; the scan shows higher uptake in the hypoxic areas within the tumour. CT images (left), fused images PET-CT (middle) and PET images (right).

PUBLICATIONS

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AWARDS AND RECOGNITION

- Co-chair of the 2014 Fellows Selection Committee, Scientific Advisory Board Member and Faculty, the Madrid-MIT "m+vision" Consortium, Spain.
- Project Evaluator of the *Agencia Nacional de Promoción Científica y Tecnológica*, *Ministerio de Ciencia, Tecnología e Innovación Productiva de Argentina*.

FLOW CYTOMETRY CORE UNIT

Lola Martinez
Core Unit Head

Technicians
Ultan P. Cronin, Elena Garrido, Miguel Ángel Sánchez

OVERVIEW

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

We provide CNIO groups with the necessary technical and scientific advice regarding flow cytometry technologies and assays, collaborating with them in the design and acquisition, as well as for data analysis and interpretation.

"Mammalian Haploid cells have recently emerged as a valuable research tool for genetic screenings. Our Unit actively collaborates with different CNIO groups to help them maintain haploid cell cultures, by sorting them based on their DNA content and analysing the progression of the cell population in those cultures."

The Unit has 4 analysers and 2 high-speed cell sorters with different configurations of lasers and detectors. Analysers are available to users who have undergone 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 for cell sorting, in compliance with biosafety regulations.

RESEARCH HIGHLIGHTS

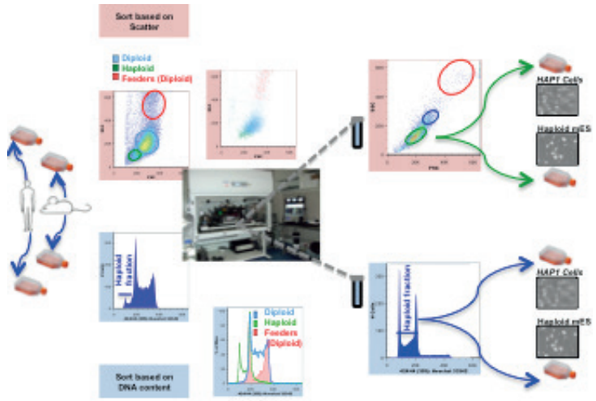

Figure A schematic example of the aseptic enrichment and purification of haploid cells from mouse and human samples, based either on their scatter pattern and/or DNA content profile, after live staining with Hoechst 33342 and cell sorting.

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 applications that have been developed and validated at our Unit include:

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

We have developed several new multicolour panels for the detection of different cellular progenitor subtypes, T, B and inflammatory cells obtained from samples such as haematopoetic tissues, pancreas, skin, liver, lung, etc., and have combined these panels with the detection of proliferation and cell death. In keeping with our commitment, we have assessed new fluorochromes, brilliant violet, brilliant blue and eFluor dyes, among others, and have incorporated them into certain panels, improving the resolution of the subpopulations of interest.

The 561nm excitation line acquired for our cell sorter and analyser has proven to be a great investment and it is constantly being used for the detection and isolation of red fluorescent protein-expressing cells; recently, several mouse models expressing Katushka as a reporter were generated at the CNIO. We are also putting our 407nm line to work for the quantification and sorting of samples expressing the Blue Fluorescent Protein (BFP).

The Unit continually develops comprehensive training courses for the application of flow cytometry techniques in different fields. This year, in collaboration with the CNIO Confocal Microscopy Core Unit, we organised the III International Core Management Workshop, which was highly attended and was very positively evaluated by all the participants. ■

PUBLICATIONS

- Rodríguez-Díez E, Quereda V, Belluti, F, Prchal-Murphy M, Partida D, Eguren M, Kollmann K, Gómez de Cedrón M, Dubus P, Cañamero M, Martinez D, Sexl V, Malumbres M (2014). Cdk4 and Cdk6 cooperate in counteracting the INK4 family of inhibitors during murine leukemogenesis. *Blood*. 124, 2380-290.
- Montserrat J, Sánchez MA, de Paz R, Díaz D, Mur S, Reyes E, Prieto A, de la Hera A, Martínez-A C, Alvarez-Mon M (2014). Distinctive patterns of naïve/memory subset distribution and cytokine expression in CD4 T lymphocytes in ZAP-70 B-chronic lymphocytic patients. *Cytometry B Clin Cytometry* 86, 32-43.

CONFOCAL MICROSCOPY CORE UNIT

Diego Megías
Core Unit Head

Technicians
Manuel Pérez, Joaquim Soriano

OVERVIEW

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

"The Confocal Microscopy Unit is fully committed to the implementation of advanced microscopy methodologies in cancer research, with the aim of creating a benefit for society by increasing our understanding of the biology and disorders of cells that cause cancer."

RESEARCH HIGHLIGHTS

The Confocal Microscopy Core Unit is equipped with 3 laser scanning confocal systems (Leica SP2 and SP5) that incorporate UV and multiphoton excitation, as well as a white light laser and a Hybrid Detector. We also have 2 wide field systems, namely, a Deltavision 4D deconvolution station and a Leica DMRI6000 system, equipped with microinjection. All the microscopes are automated and equipped with incubators for live-cell imaging.

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

- → An Opera (Perkin Elmer) High-Content Screening (HCS) system, which allows running HCS experiments on fixed and live cells in multi-well plates, and enables the monitoring of cell dynamics (translocation, cell division, etc.) by means of fluorescence markers.
- → A Matrix Screening Application integrated into the SP5 confocal systems, allowing HT feeding of the instrument, not only in multi-well plates but also in tissue sections.

These technological advances increase the level of information obtained from a sample and allow for the automated HT screening of cell behaviour in response to different treatments.

During 2014, the Confocal Microscopy Unit significantly refurbished its computational capacity for image analysis by upgrading its HTS Opera microscope and the Unit's servers; we have developed new programmed routines that have now made it possible for us to manage, stitch and analyse a huge amount of images; something that was previously impossible.

Additionally, we have upgraded our laser scanning confocals with a new time gating application that has now made it possible for us to run acquisitions, not only based on intensity, but also taking fluorophore life time into account, thus allowing us to control contamination from light reflection and autofluorescence. We have incorporated new flow control devices for more accurate dynamic live cell-based assays in perfusion chambers.

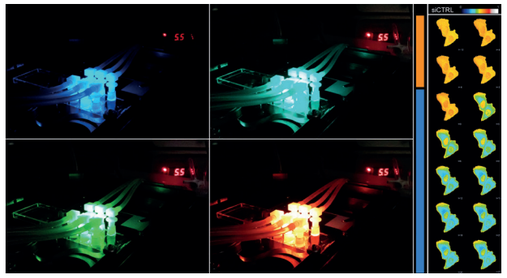

Figure Microfluidic experiment under different excitation wavelengths dedicated to a FRET assay (right). The Figure shows Fret activity before and after induction of glucose stimuli.

We are very proud to have co-organised 2 international conferences:

- → The 2nd Spanish National Advanced Microscopy Network Meeting, which took place at the CNIO, brought together a large number of international speakers and attendees. This event explored new advances and techniques in microscopy.
- → The III Core Management Workshop, focused on the general management, funding and stability of core units for all disciplines. ■

PUBLICATIONS

- Miranda-Lorenzo I, Dorado J, Lonardo E, Alcala S, Serrano AG, Clausell-Tormos J, Cioffi M, Megias D, Zagorac S, Balic A, Hidalgo M, Erkan M, Kleeff J, Scarpa A, Sainz B Jr, Heeschen C (2014). Intracellular autofluorescence: a biomarker for epithelial cancer stem cells. *Nat Methods* 11, 1161-1169.
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PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Staff Scientist
Jorge L. Martínez

OVERVIEW

Proteomics is uniquely positioned as one of the most powerful technologies to help understand cellular processes regulated at the protein level. State-of-the-art mass spectrometers have significantly improved in sensitivity and dynamic range, allowing sequencing of more than 20 peptides per second (10 times more than 5 years ago). In fact, in 2014, 2 groups have reported the first drafts of the human proteome, which consists of more than 19,000 proteins and 27,000 isoforms, opening up new avenues for biomedical research. Furthermore, when combined with other highly-specific enrichment techniques, mass spectrometry (MS) is capable of monitoring several thousands of phosphorylation, ubiquitination and acetylation sites, among other post-translational modifications, in a quantitative manner. The generation of these vast amounts of data will enhance our understanding of the mechanisms that govern signalling pathways.

Graduate Student
Silvia L. Gomes (Since February)

Technicians
Fernando García, Rut González (Until March), Nuria Ibarz, Jaime Martínez (Since June), M. Isabel Ruppen, Pilar Ximénez De Embún

RESEARCH HIGHLIGHTS

Throughout 2014, the Unit has implemented 2 enrichment strategies for the analysis of the phosphoproteome and ubiquitinome in biological samples. These methods can be applied to identify post-translational modifications, regulated by kinases/phosphatases and ubiquitin ligases/deubiquitinases, and then study their implication in different mechanisms of cancer. We have also successfully used click chemistry to identify, by mass spectrometry, secreted proteins and exosomes that could help to determine the metastatic potential of tumour cells. In addition, the Unit has continued to apply quantitative proteomics workflows (e.g. SILAC and iTRAQ), such as for the identification of ubiquitin ligase substrates, CDH1 and CUL4A, that will allow us to study their role in cancer. Several label-free approaches are also being used for the identification of novel protein interactions involved in cancer development.

Regarding our work on the production of recombinant proteins, it is worth highlighting, among other projects, the production of functionally active TRF1, which was carried out in collaboration with Maria Blasco from the CNIO Telomeres and Telomerase Group. This recombinant protein has allowed us to generate highly-specific monoclonal antibodies and to carry out an in-depth functional and biochemical characterisation of this component of the shelterin complex. Finally, in collaboration with the CIEMAT, the CNIO Molecular Imaging Core Unit and the Seve Ballesteros Foundation-CNIO Brain Tumour Group, we are currently developing engineered antibodies for positron emission tomography (PET) as a non-invasive tool for the diagnosis, response prediction and treatment monitoring of glioblastoma multiforme (GBM). ■

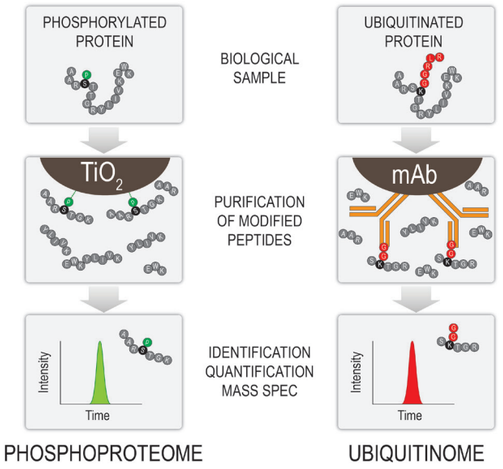

Figure Two enrichment strategies have been implemented in the Proteomics Unit to analyse the phosphoproteome and ubiquitinome in biological samples.

PUBLICATIONS

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AWARDS AND RECOGNITION

- The CNIO Proteomics Unit has joined the Spanish consortium ProteoRed-ISCIII (www.proteored.com).

HISTOPATHOLOGY CORE UNIT

Alba De Martino (Since April)
Core Unit Head

Technicians
Virginia Álvarez (Until March), Núria Cabrera (Until June), María Gómez, Patricia González, María Lozano, Raquel Pajares (Until March), Maria Udriste (Since August), Zaira Vega

OVERVIEW

Pathology is devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs that underlie disease. By using molecular, immunological and morphological techniques, pathology serves as the bridge between the basic sciences and clinical medicine. The Histopathology Core Unit offers knowledge and expertise through a full range of services including processing, embedding, cutting of paraffin embedded samples (FFPE), as well as the construction of tissue microarrays (TMAs). We also provide our users with histological stains upon request, research and diagnostic immunohistochemistry testing, antibody validation, *in situ* hybridisation (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 also collaborates with researchers at any stage of their career, helping them with the characterisation of genetically and phenotypically relevant animal models of disease, and providing them with the pathology expertise required for the success of their research projects.

"The Unit collaborates with researchers at any stage of their career, helping them with the characterisation of genetically and phenotypically relevant animal models of disease, and providing them with expertise in histological techniques and pathology evaluation."

RESEARCH HIGHLIGHTS

Aware of the importance that our researchers place on *in situ* detection of protein expression and the standardised antibody validation process, the Unit has added 35 new antibodies to the currently available antibody panel. This panel includes more than 1,000 antibodies that have been optimised for human and mouse tissue samples and around 3,000 antibodies that have been tested since the Unit was created. In addition, we routinely perform multiple protein labelling by chromogenic immunohistochemistry for the detection and co-localisation of different targets on the same slide.

Furthermore, we work in close collaboration with the CNIO Monoclonal Antibodies Core Unit in order to develop new mAbs against proteins that are the subject of study of several CNIO Research Groups. 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.

Likewise, during 2014, the Unit introduced several changes including: 1) the optimisation of available techniques to improve the quality and reproducibility of *in situ* hybridisation with ALU probes for a better and brighter detection of human cells in murine tissue (xenografts), as well as 2) the TUNEL assay for the detection of DNA fragmentation, testing the sensitivity and specificity of new reagents, and making these new protocols available to our researchers. As a new service, the Unit has also started to develop automated immunofluorescence techniques for FFPE tissue sections, as well as new algorithms for automated quantification on digital slides.

Our Unit participates in several External Quality Assessment Schemes, such as NordiQC and UK NEQAS, which periodically evaluate the quality of the stains performed at the Unit. ■

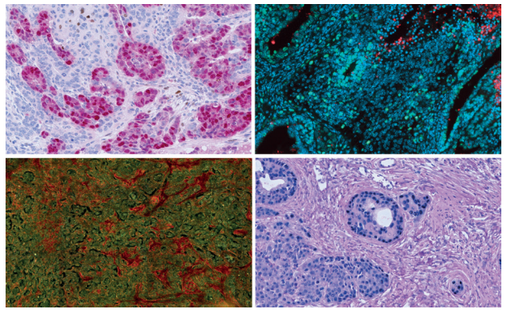

Figure Examples of techniques developed at the Histopathology Core Unit in support of different research projects. P21 and Oct-4 double immunohistochemistry in mouse teratoma (top left); Ki67 (green) and Cleaved Caspase 3 (red) double immunofluorescence in mouse teratoma (top right); Sirius red under polarised light in a mouse model of pulmonary fibrosis (bottom left); ALU *in situ* hybridisation for the detection of human cells (blue) in a mouse xenograft.

PUBLICATIONS

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ANIMAL FACILITY

Isabel Blanco
Core Unit Head

Management
Charles River Laboratories International, Inc. (until October), Vivotecnia Management & Services (since October)

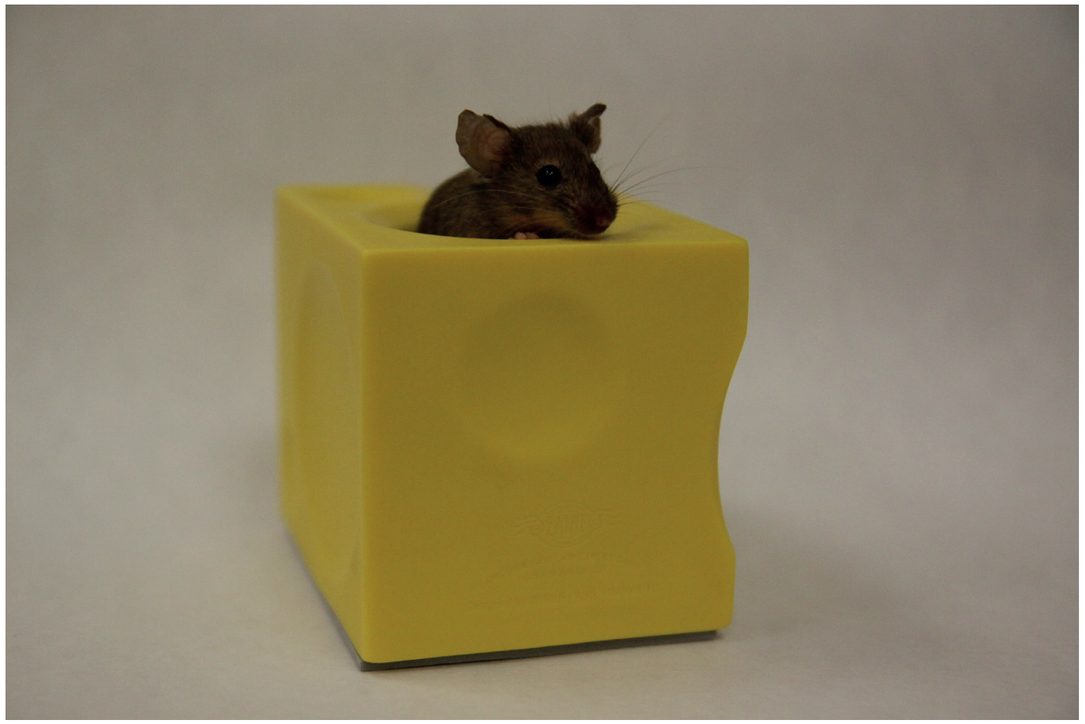

Figure 1 Agouti mouse.

The CNIO has a state-of-the-art Animal Facility. The facility was managed by Charles River Laboratories until September 2014, and then by Vivotecnia Management & Services from October 2014 onwards. The Animal Facility's primary responsibility is the supply, husbandry and quality control of laboratory animals used by the CNIO Research Programmes in their experimental protocols. The strict compliance to national, European Union 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 closely with 16 Research Groups from within both the Basic Research Programmes (Molecular Oncology, BBVA Foundation-CNIO Cancer Cell Biology) and the Translational Research Programmes (Clinical Research), as well as with several Sections and Units from the Experimental Therapeutics and Biotechnology Programmes.

"The ability to carry out projects and procedures involving animal experimentation in line with the highest standards of excellence, and in compliance with the complex existing regulation, is one of the key factors that place the CNIO at the forefront of cancer research worldwide."

Our Animal Facility has the capacity to house 19,000 type IIL cages (each with an average capacity for 3.5 mice). More than 1,500 different 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 to 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 integrated Individually Ventilated Caging (IVC) units in the building ventilation systems. Mice are manipulated in Type II biosafety cabins.

Daily operations and husbandry procedures are highly automated in order to safe-guard our personnel from any associated risks; robotic devices perform any 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 on the premises, including the use of gamma irradiation, UV light and volatile carcinogenic agents, as well as surgical procedures. This year the Facility has implemented a lab animal monitoring system (Oxylet) that enables the measuring of a number of physiological parameters for metabolic profiling and phenotyping of mouse models.

Figure 2 Oxylet, a modular system for respiratory metabolism, food/drink intake and activity in rodents.

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 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), which has been set up to comply with the new European Directive 2010/63/UE.

The Royal Decree RD53/2013 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. To abide with the former requirement, the Animal Facility offers CNIO staff an official Category C Qualification Annual Training Course on the education and training of personnel performing work with laboratory animals. This course has been internationally accredited by FELASA (Federation of European Laboratory Animal Science Associations), being one of the only two courses awarded with this accreditation in Spain. ■

EXPERIMENTAL THERAPEUTICS PROGRAMME

JOAQUÍN PASTOR Programme Director

The main research areas of the Experimental Therapeutics Programme (ETP) are as follows:

For the *Early-Drug Discovery* projects, we have progressed to reach *in vivo proof-of-concept* (PoC) and /or have entered into licensing agreements with our advanced lead compounds; i.e. phosphatidylinositol 3-kinase (PI3K α/δ), proto-oncogene serine/threonine protein kinase Pim (PIM), combined PIM/ PI3K/ mammalian target of rapamycin (mTOR), and ataxia telangiectasia and Rad3-related protein (ATR) inhibitors. The ATR inhibition project is the result of a successful model of collaboration between CNIO Basic Research Groups, the Genomic Instability Group, and the ETP. The ATR inhibitors discovered at the CNIO have been licensed to Merck Serono for further clinical development. In particular, one of the ETP-CNIO inhibitors has already been validated and selected by the pharmaceutical company as a candidate for further development, thereby reaching a crucial milestone within the licensing agreement. We have also been working on our CDK8 inhibition project. The validation of CDK8 as an "oncotarget" in different cell lines using biological tools such as "kinase dead" constructs is underway. In the meantime, our Medicinal Chemistry Group has successfully generated and optimised novel CDK8 inhibitors, yielding highly potent and selective compounds that are currently under characterisation for their Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties.

We have continued the development of *Kinase X** inhibitors. The ETP has generated highly potent compounds that have demonstrated general selectivity against off-target kinases. Some representative inhibitors have displayed excellent ADMET properties.

During 2014, we participated in several *Exploratory Projects* in collaboration with CNIO researchers, or continued with providing support for their various research activities: Marcos Malumbres (microtubule-associated serine/threonine protein kinase-like (MASTL)/Haspin), Manuel Serrano (Gluconeogenesis), Manuel Hidalgo and M. del Pozo (CNIC) (Caveolin 1), Miguel Quintela (analysis of drugs from *in vivo* samples), Maria Blasco (TRF1), and Daniel Lietha (Allosteric focal adhesion kinase (FAK) inhibitors). As a major example, the CNIO Telomeres and Telomerase Group has validated telomeric repeat binding factor 1 (TRF1) as a potential oncotarget *in vivo*, and has developed a screening assay to identify TRF1 inhibitors. The screening campaign of a selected library of ETP compounds has yielded several hits that are currently under characterisation, *in vitro* and *in vivo*, as potential novel therapeutics for cancer treatment.

Finally, we have started a second collaborative project with the VIB Department of Transgene Technology and Gene Therapy (KU Leuven, Belgium), *target X***. The ETP-Biology Group has been working on assay development activities for this project. ■

"The Experimental Therapeutics Programme is at present a well-established early-Drug Discovery Group (e-DD) at the CNIO. The integration of e-DD activities, alongside the Centre's existing 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."

* Kinase x (confidential), in collaboration with B. Lambrecht from the VIB Inflammation Research Centre, University of Gent (Belgium).

** Target x (confidential), in collaboration with P. Carmeliet from the VIB Department of Transgene Technology and Gene Therapy, KU Leuven (Belgium).

MEDICINAL CHEMISTRY SECTION

Sonia Martínez
Section Head

Staff Scientists
Ana Belén García, Cristina Gómez, Esther González, Ana Isabel Hernández, María Del Rosario Rico, R. Concepción Riesco, Sonsoles Rodríguez, Carmen Varela

Technician
Virginia Rivero

OVERVIEW

The role of Medicinal Chemistry in the Drug Discovery Process is to optimise, not only the pharmacological properties of the initial hits, potency and selectivity, but also the drug-like properties of these molecules in order to achieve leads and drug candidates that can become good drugs at the end of the process.

The most active or selective compound may not make the best drug product because of property limitations that cause poor pharmacokinetics (PK) or safety issues. A less potent compound with better properties may produce a better *in vivo* therapeutic response and therefore develop into a better drug. Optimisation of these pharmacological and drug-like properties is done through an iterative process in close collaboration with the Biology Section of the Experimental Therapeutics Programme (ETP).

Chemical modifications of the molecules and their biological characterisation at the biochemical and cellular level, including *in vitro* ADME (Absorption, Distribution, Metabolism, and Excretion) assays, will allow us to establish Structure-Activity-Relationships (SAR) and Structure-Property Relationships (SPRs) that will help the medicinal chemist to understand how structural modifications impact the potency, selectivity and properties of a particular scaffold, and therefore improve them in a next round of chemical modifications.

Additionally, compounds should be in a "free chemical space" and susceptible to be included in patent applications.

"During 2014, one of our ATR inhibitors licensed to Merck Serono was validated and selected by the pharmaceutical company as a candidate for further clinical development, thereby reaching a crucial milestone within the licensing agreement."

RESEARCH HIGHLIGHTS

During 2014, our Section was involved in several projects:

Ataxia telangiectasia and Rad-3 related protein inhibitors (ATRi)

ATRi was a successful project in which we obtained lead compounds from 2 different chemical series, generated by our Section, with demonstrated *in vivo proof-of-concept* (the regulation of associated biomarkers and an anti-tumor effect in the allo-Eμ-myc model after oral administration). This project was licensed to Merck Serono in 2013. During this year, we finalised the synthesis of more analogues (57 compounds) from one of the licensed chemical series in order to strengthen the patent application. In March 2014, the patent application achieved the level of PCT filing, and in September, it went public with publication number WO2014140644. This patent was filed jointly by the CNIO Genomic Instability Group and the ETP.

Cyclin-dependent protein kinase 8 inhibitors (CDK8i) project

During the first 10 months of the year, we synthesised 73 compounds within the context of this project. After exploration of several chemical series at the Hit-to-Lead (HtL) phase, we prioritised one of them based on the selectivity profile of the compound against a panel of 456 kinases. This chemical series has shown a very clean profile, with only 1 kinase having significant off-target activity (>90% binding affinity), and some of the other kinases having less relevant activities (< 20% binding affinity), (FIGURE).

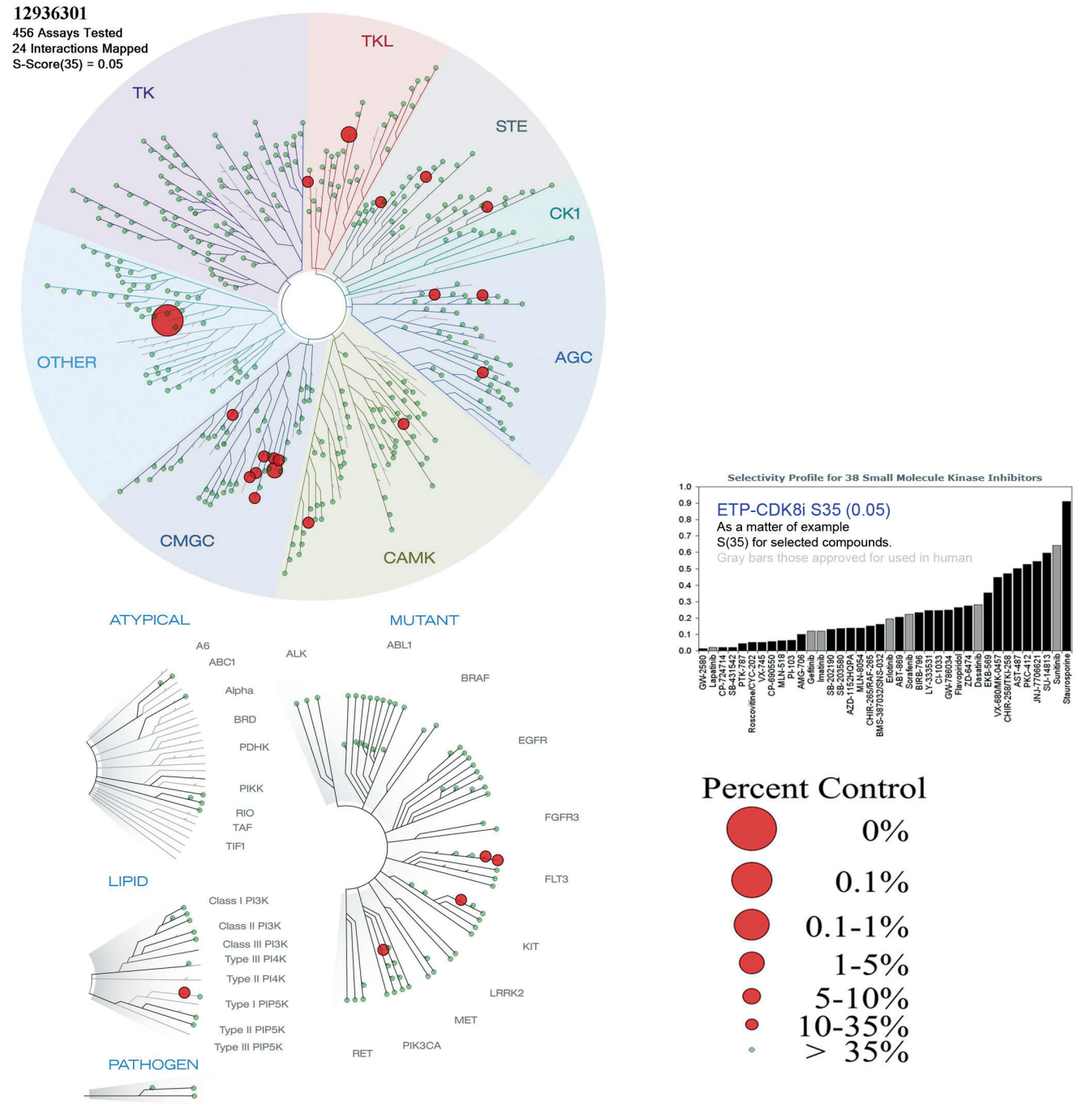

Figure A selected CDK8 inhibitor tested in a 456 kinase panel (KINOMEscan). The selectivity score of the compound S(35) is 0.05. A representation of the selectivity profile of S(35) for 38 small molecule kinase inhibitors indicates the selectivity of our compound. Kinases that are found to bind are marked with red circles -whereas larger circles indicate higher binding affinity. Only 1 kinase can be considered to be significantly off target (>90% affinity), the other red dots represent affinities of less than 20%. Our CDK8 inhibitor has CDK8 binding affinity (2 nM) but this activity is not represented in the panel because the KINOMEscan binding assay is performed in the absence of cyclin.

During 2014, we focused our efforts on optimising the synthetic route to facilitate the synthesis of more analogues and to explore all the positions of the molecule in order to build the SARs. We have actually generated a good set of compounds with low nanomolar biochemical activity range. Additionally, the compounds have been characterised for their *in vitro* ADME properties, in terms of solubility, permeability, metabolic stability and cytochrome inhibition. These data have allowed us to establish SPRs that will guide a second round of exploration in order to improve any of these parameters.

Kinase X* inhibitors

The Kinase x inhibitor project is carried out in collaboration with the VIB Inflammation Research Centre (UGent, Belgium). During 2013, and as a result of a High-Throughput Screening (HTS) campaign, complemented with compounds coming from literature searches of molecules with reported off-target Kinase x activity, we obtained a hit with inhibitory activity in the low nanomolar range (25 nM). A broad exploration around the hit was performed in an HtL phase, generating a patentable chemical series. During this phase, we faced some off-target selectivity issues and, therefore, every set of active compounds generated in the project was characterised internally by the Biology Section in a set of off-target kinases (a selected panel of 24 kinases) using a KINOMEscan screening platform. This has allowed us to understand the parts of the molecule involved in the lack of selectivity and also to learn about the modifications that are responsible for a more selective profile in our molecules. Additionally, 2 other chemical series are currently being explored. At this point of the project, we have been able to identify some parts of the molecule that are crucial to achieve potency and selectivity against other targets, and we have been able to significantly improve the selectivity. *In vitro* ADME characterisation of some selected compounds has provided us with information about the potential metabolic stability of these compounds and their clean profile in terms of cytochrome inhibition and hERG (the human Ether-a-go-go-Related Gene) binding.

During this period, a homology model of the protein kinase X, based on the reported structure of one of its isoforms, was created for computational studies. The docking of representative inhibitors in the homology model helped us to understand the binding mode of our molecules in the ATP binding pocket of kinase x and has guided part of our chemical exploration. During the first 10 months of the year, 222 compounds were synthesised.

Focal Adhesion kinase inhibitors (FAKi)

This is a collaborative project carried out with the CNIO Cell Signalling and Adhesion Group with the aim of finding allosteric Focal Adhesion Kinase (FAK) inhibitors. We have provided analogues for the identified hit from our library. Additionally, we are currently carrying out a small chemical exploration around this hit through the synthesis of some analogues, in order to better understand the binding mode of this chemical class within an identified allosteric pocket and to try to improve the activity of the initial hit. ■

* Kinase x (confidential), in collaboration with B. Lambrecht from the VIB Inflammation Research Centre, University of Gent (Belgium).

PUBLICATION

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PATENTS

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- Pastor Fernández J, Fernández-Capetillo Ruiz O, Martínez González S, Blanco Aparicio C, Rico Ferreira MR, Toledo Lázaro LI, Rodríguez Arístegui S, Murga Costa M, Varela Busto C, López Contreras AJ, Renner O, Nieto Soler M, Cebrián Muñoz DA (2014). Preparation of fused tricyclic oxazinopyrimidine derivatives as inhibitors of ATR kinase useful in the treatment of cancer. *PCT Int. Appl. WO2014/140644 A1.*

BIOLOGY SECTION

Carmen Blanco
Section Leader

Staff Scientists
Oliver Renner, Manuel Urbano

Post-Doctoral Fellows
Borja Barrera (Since October), David Á. Cebrián (Until September)

Technicians
Enara Aguirre, Nuria Ajenjo, M. Isabel Albarrán, Antonio Cebriá, Elena Gómez-Casero, Belen Pequeño (Until February), M. Carmen Rodríguez de Miguel

OVERVIEW

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

The group possesses automated liquid-handling instruments that allow rapid, accurate, and reproducible compound dispensing and assay plate setup. Additionally, the group has incorporated suitable tracking software to enable efficient sample tracking and recording.

We have proven experience in developing and optimising biochemical assays with a main focus on the kinase field. The Section incorporates 3 different multiparametric plate readers, 2 of them for biochemical and cellular measurements and a High Content Screening plate reader for confocal *in vivo* real-time image capture. We can perform phenotypic assays as well as biomarker modulation measurements. We routinely run ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) assays to decipher the molecular profile of newly prepared compounds: measurement of cytochrome P450 activity, HT (High-Throughput) hERG (the human Ether-a-go-go-Related Gene) binding, and HT metabolic stability. For studies such as *in vivo* proof-of-concept, we perform pharmacokinetic and phamacodynamic experiments to analyse the modulation of different biomarkers in the whole animal in order to establish correlations with the exerted biological effect of a given compound. We use murine cancer models, which are selected according to tumour target expression, *in vitro* activity of the molecules and clinical relevance to validate antitumour efficacy.

"During 2014, one of our ATR inhibitors, licensed to Merck Serono has already been validated and selected by the pharmaceutical company as a candidate for further development, thereby reaching a crucial milestone within the licensing agreement."

RESEARCH HIGHLIGHTS

During 2014, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

Deregulation of the Wnt/β-catenin pathway plays a central role in colon cancer and CDK8 has been identified as an oncogene in these types of tumours. CDK8 kinase activity is necessary for β-catenin-driven tumour formation.

We seek to obtain selective CDK8 inhibitors against other CDKs. CDK7, CDK8, CDK9 and CDK19 regulate gene expression through different interactions with the transcription machinery. We have, therefore, developed and validated CDK8/CycC, CDK9/CycT, CDK7/cyclin H and CDK19, binding assays. The latter, shows high homology to CDK8 and is also coupled to Cyclin C, thus being an alternative component of the Mediator complex that could be relevant in several situations. The knowledge of the selectivity of our CDK8 inhibitors has allowed a better interpretation of the modulation of cellular β-catenin-dependent transcriptional activity exerted by these compounds.

We have tested 73 newly prepared compounds in the biochemical assay for CDK8; 48 of them were also tested for CDK9 and CDK7, and subsequently in the cellular assay. Taking into account all these activities, we have established a Partial Least Squares Regression (PLS) model in order to find a correlation between the biochemical and cellular activities. We were able to establish a PLS model with a q2 of 0.63 and 2 factors: factor 1 (PLS1) composed by CDK9 and CDK7 (related activities), and factor 2 with CDK8 activities. Both factors contribute to the cellular activity, therefore, in this setting, very selective CDK8 inhibitors do not show cellular activities bellow 1 μM (FIGURE). We are currently working on the development of a novel cell-based assay, in order to have a more direct read out of CDK8-dependent kinase cellular activity.

Among the different chemical series, we have identified very selective CDK8 inhibitors, dual CDK8/CDK19 inhibitors, and others, with 100x selectivity versus CDK9. Some of these compounds have been tested in a panel of 456 kinases showing striking selectivity. These compounds are going to be used to validate the role of CDK8 in various aspects of cancer biology.

Kinase X

After a screening of an ETP-library of 5K compounds and the identification of hits with medium and low nanomolar activities, we started the Hit- to- Lead (HtL) phase. As kinase x belongs to a family of proteins with three different isoforms, we set up biochemical assays for all 3 isoforms. So far, the identified compounds behave as pan–isoform inhibitors. We have also characterised tumour cell lines for the expression of the 3 isoforms by qRT-PCR, selecting some of them to evaluate the cellular activity of representative inhibitors from the different chemical classes developed at ETP. We have tested 234 compounds in the primary biochemical assay and 67 were screened in off-target activities.

Ataxia telangiectasia and Rad-3 related protein (ATR)

In collaboration with Merck Serono, we have characterised 57 compounds that were synthesised for patent reinforcement. For all of them, we have generated data for the cellular inhibition of ATR, as well as biochemical data for 3 off-targets.

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

This is a collaborative project with the CNIO Cell Division and Cancer and Macromolecular Crystallography Groups. After a phenotypic screening of a diverse ETP-library of 5K compounds, we identified 5 hits with EC50 values that ranged between 5.0 and 0.5 μM. Three of them did not inhibit CDK1 at the biochemical level and were further characterised for the inhibition of P-ENSA – a target of MASTL – in synchronised cells arrested in mitosis. The cellular inhibition of MASTL ranged from 2.5 μM to 0.5 μM for selected hits. The actual validation of these hits as putative MASTL inhibitors will need a direct biochemical assay. In this direction, we are purifying the protein from insect cells.

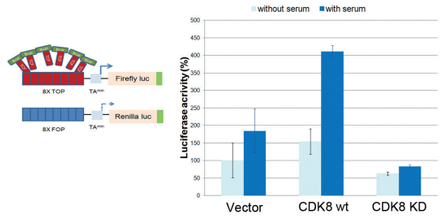
Telomeric repeat binding factor 1 (TRF1)

This is a collaborative project with the CNIO Telomeres and Telomerase Group. Our Section has contributed by providing technical support for the systematic implementation of the assay developed by M. Méndez.

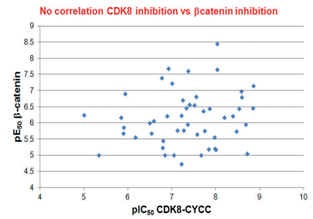
Support to other CNIO groups

We have provided support to 2 Clinical Research groups (the Gastrointestinal Cancer Clinical Research Unit and the Breast Cancer Junior Clinical Research Unit) by analysing, with liquid chromatography-tandem mass spectrometry (LC-MS/MS), the levels of several standard-of-care-drugs in tumour and host-mouse plasma samples from different mouse models of cancer. ■

A) Cellular assay to validate CDK8 inhibitors



B) Experimental data



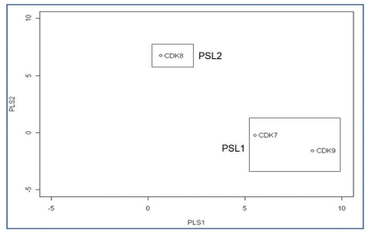
C) Data set to establish Partial Least Regresion (PLS) model

CHEMISTRY	CDK8-CYCC IC50	CDK9/CYCT IC50	CDK7/CYCH/MAT1 IC50	BETA_CATENIN EC50
ETP-753	1.41E-09	1.36E-08	1.20E-07	7.19E-08
ETP-175	1.44E-09	3.00E-08	3.78E-06	3.49E-07
ETP-927	1.96E-09	6.71E-07	1.00E-05	8.87E-06
ETP-749	2.10E-09	2.67E-07	1.00E-05	1.13E-06
ETP-669	2.58E-09	6.22E-08	3.08E-07	1.58E-07
ETP-745	2.62E-09	4.66E-09	3.39E-06	1.05E-07
ETP-799	2.96E-09	1.24E-07	1.11E-06	3.65E-07
ETP-002	3.46E-09	4.05E-07	1.00E-05	1.86E-06
ETP-926	4.18E-09	9.62E-08	1.00E-05	6.10E-07
ETP-586	6.39E-09	1.95E-06	8.86E-06	6.94E-07
ETP-003	9.32E-09	4.70E-08	1.00E-05	3.82E-09
ETP-006	9.48E-09	5.74E-10	1.19E-07	2.28E-08
ETP-738	9.96E-09	1.00E-05	1.00E-05	6.82E-06
ETP-176	1.05E-08	4.20E-07	1.00E-05	6.27E-06
ETP-875	1.09E-08	6.43E-08	1.00E-05	2.84E-06
ETP-185	1.39E-08	9.82E-09	2.35E-06	3.58E-07
ETP-775	1.48E-08	1.00E-05	1.00E-05	6.62E-06
ETP-891	1.82E-08	1.18E-08	2.46E-06	1.74E-06
ETP-609	1.92E-08	1.11E-08	6.33E-06	4.32E-07
ETP-776	2.17E-08	1.00E-05	1.00E-05	1.00E-05
ETP-019	2.72E-08	9.38E-06	7.73E-06	2.26E-06
ETP-767	2.84E-08	9.01E-09	1.86E-06	1.56E-07
ETP-887	3.08E-08	5.41E-09	1.47E-06	2.78E-07
ETP-780	4.04E-08	1.41E-09	3.23E-08	2.75E-07
ETP-850	4.30E-08	4.15E-10	1.34E-08	2.50E-08

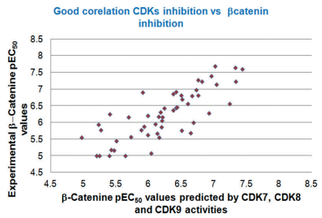
CHEMISTRY	CDK8-CYCC IC50	CDK9/CYCT IC50	CDK7/CYCH/MAT1 IC50	BETA_CATENIN EC50
ETP-729	4.44E-08	1.00E-05	1.00E-05	1.10E-06
ETP-796	4.76E-08	2.49E-08	6.47E-07	3.53E-07
ETP-633	5.38E-08	6.90E-07	1.36E-06	1.71E-06
ETP-734	5.68E-08	1.50E-08	5.06E-07	2.02E-07
ETP-983	6.11E-08	4.73E-06	1.00E-05	1.91E-05
ETP-999	6.13E-08	1.41E-09	1.70E-08	5.93E-07
ETP-756	7.63E-08	6.60E-06	1.00E-05	1.69E-06
ETP-906	9.22E-08	1.00E-05	1.00E-05	1.00E-05
ETP-832	9.90E-08	4.36E-09	8.94E-08	6.01E-08
ETP-794	1.21E-07	2.25E-09	2.03E-08	2.07E-08
ETP-677	1.31E-07	3.02E-08	1.00E-05	6.15E-07
ETP-750	1.46E-07	1.00E-05	1.00E-05	1.00E-05
ETP-917	1.60E-07	2.52E-07	1.00E-05	3.56E-06
ETP-004	1.62E-07	1.00E-05	1.00E-05	5.85E-06
ETP-831	1.70E-07	2.80E-09	2.27E-08	4.09E-08
ETP-893	2.59E-07	2.39E-08	1.33E-06	8.82E-07
ETP-827	2.96E-07	5.48E-08	8.45E-07	2.14E-06
ETP-792	3.23E-07	5.25E-09	1.29E-07	1.01E-06
ETP-726	6.77E-07	1.00E-05	1.00E-05	2.80E-06
ETP-866	1.16E-06	3.22E-08	1.93E-06	1.28E-07
ETP-852	1.27E-06	2.54E-08	3.93E-07	1.39E-06
ETP-865	1.30E-06	2.39E-09	2.30E-07	2.10E-06
ETP-890	1.46E-06	2.80E-08	3.73E-07	6.79E-07
ETP-781	4.64E-06	8.67E-08	6.08E-06	1.00E-05
ETP-078	1.00E-05	4.06E-08	1.00E-05	5.75E-07

Figure Interpretation of CDK8 cellular inhibition based on the biochemical CDK profile. (**A**) Validation of the cellular assay based on the transcriptional activity of β catenin in HCT116 cells. (**B**) Representation of the biochemical versus the cellular inhibition of CDK8. (**C**) Set of biochemical data for the inhibition of CDK8, CDK9 and CDK7 and cellular data for the inhibition of the β catenin reporter. (**D**) Data from figure C was used to make a partial regression model (PLS) that determines the contribution of each CDK to the cellular assay. (**E**) Validation of the PLS model.

D) PLS model with 2 factors: factor 1 (PLS1) composed by CDK9 & CDK7 (related activities) and factor 2 with CDK8 activities



E) Predictec vs Experimental Data



βcatenin data do not show a good correlation with CDK8 inhibition, a more direct read out is required

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- Morgado-Palacin L, Llanos S, Urbano M, Blanco-Aparicio C, Megias D, Pastor J, Serrano M (2014). Non-genotoxic activation of p53 through the RPL11-dependent ribosomal stress pathway. *Carcinogenesis* 35, 2822-2830.
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PATENTS

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- Pastor Fernández J, Fernández-Capetillo Ruiz O, Martínez González S, Blanco Aparicio C, Rico Ferreira MR, Toledo Lázaro LI, Rodríguez Arístegui S, Murga Costa M, Varela Busto C, López Contreras AJ, Renner O, Nieto Soler M, Cebrián Muñoz DA (2014). Preparation of fused tricyclic oxazinopyrimidine derivatives as inhibitors of ATR kinase useful in the treatment of cancer. *PCT Int. Appl. WO2014/140644 A1.*

CNIO - LILLY CELL SIGNALLING THERAPIES SECTION

Susana Velasco
Section Head

Staff Scientists
Marta I. Barradas, Ana Cerezo, Carmen M. Pérez

Technicians
Laura Diezma, Scherezade Jiménez-Villa, Eva P. Lospitao, Sandra Peregrina

SCOPE OF THE ELI LILLY-CNIO PARTNERSHIP

Eli Lilly and CNIO are collaborating on the identification and validation of novel targets in cancer metabolism. The CNIO-Lilly Cell Signalling Therapies Section – funded through a research contract with Eli Lilly – 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. We are using a combination of *in vitro* and *in vivo* approaches in order to obtain a complete understanding of the metabolic reprogramming by oncogenes such as *RAS* (Barradas et al., 2014;

"An altered metabolic programme in tumour cells may be at the root of the malignant transformation process. We are using a combination of state-of-the-art *in vitro* and *in vivo* approaches to obtain a complete characterisation of the metabolic status of tumours."

this work was presented at the Beatson International Conference on *Cancer and Metabolism* in Glasgow, July 2014), as well as the characterisation of the metabolic status of tumours (Barradas et al. and Cerezo et al., 2014; this work was presented at the 26th EORTC-NCI-AACR Symposium on *Molecular Targets in Cancer Therapeutics* in Barcelona, November 2014). 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 (FIGURE, A and B). Finally, each drug 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 (FIGURE, C) and the immunohistochemical characterisation of tumours based on different metabolic and tumour markers (FIGURE, D).

SCIENTIFIC CONTEXT

The observation of an altered metabolic state in cancer cells dates back to the early 20th century when Otto Warburg observed that cancer cells preferentially utilise glycolysis over oxidative phosphorylation for growth, even in the presence of normal oxygen levels; a phenomenon known as the "Warburg effect" (Warburg 1956). Warburg argued that this altered metabolic state was the underlying cause of 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, confer an altered metabolic phenotype to cancer cells by regulating genes involved in central metabolic pathways, such as glycolysis, fatty acid metabolism, oxidative phosphorylation, and the one carbon pool (FIGURE, A). In addition to oncogenes, several metabolic enzymes have been found to be mutated or deregulated in different tumour types, including succinate dehydrogenase (SDH), fumarate hydratase (FH), isocitrate dehydrogenase (IDH), phosphoglycerate dehydrogenase (PHGDH). Cellular metabolism is a fine tuned process; tumours may rely heavily on specific metabolic pathways to obtain their energy while using other pathways to grow in order to give tumour cells a growth advantage. This situation, however, 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. Thus, the mechanistic understanding of cancer metabolism has led to renewed interest in developing therapeutics that target key enzymes in this process. ■

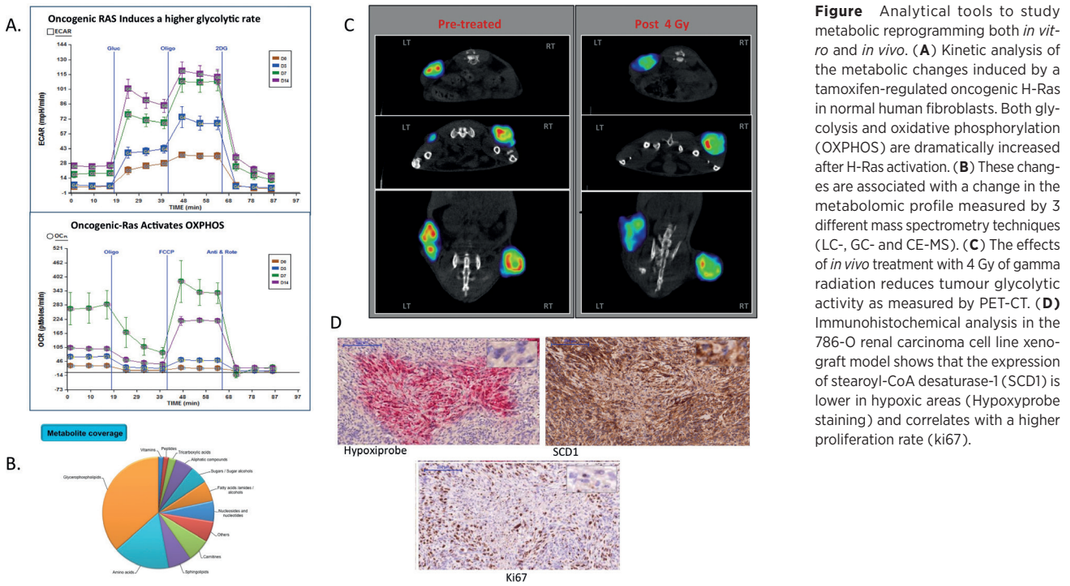


Figure Analytical tools to study metabolic reprogramming both *in vitro* and *in vivo*. (**A**) Kinetic analysis of the metabolic changes induced by a tamoxifen-regulated oncogenic H-Ras in normal human fibroblasts. Both glycolysis and oxidative phosphorylation (OXPHOS) are dramatically increased after H-Ras activation. (**B**) These changes are associated with a change in the metabolomic profile measured by 3 different mass spectrometry techniques (LC-, GC- and CE-MS). (**C**) The effects of *in vivo* treatment with 4 Gy of gamma radiation reduces tumour glycolytic activity as measured by PET-CT. (**D**) Immunohistochemical analysis in the 786-O renal carcinoma cell line xenograft model shows that the expression of stearoyl-CoA desaturase-1 (SCD1) is lower in hypoxic areas (Hypoxyprobe staining) and correlates with a higher proliferation rate (ki67).

CNIO - LILLY EPIGENETICS SECTION

María José Barrero
Section Head

Staff Scientist
Sergio Ruíz

Technicians
Verónica García, Jacinto Sarmentero

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. 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.

"Our goal is to identify epigenetic events that contribute to tumourigenesis and that might be susceptible to modulation by therapeutic agents. Targeting the appropriate epigenetic effectors can help to restore the proper patterns of gene expression in cancer cells and to revert the cancer phenotype."

SCIENTIFIC CONTEXT

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

Over the last few years, several studies including our own, have suggested that the deregulation of the chromatin-modifying machineries can lead to aberrant gene expression causing cancer and other human diseases. The epigenome is regulated in a highly dynamic fashion by the coordinated action of regulators that are able to write, erase, and read histone and DNA modifications (FIGURE). Thus, contrary to genetic mutations, epigenetic aberrations can be reversed by targeting the appropriate epigenetic regulators. Indeed, drugs that target DNA methyltransferases and histone deacetylases have successfully demonstrated anticancer properties and are currently being used in the clinic. Therefore, identifying the molecular function of critical epigenetic regulators and their complex relationship with the cancer epigenome, in addition to the development of small molecular inhibitors of their activities, holds great promise for cancer therapy (FIGURE). ■

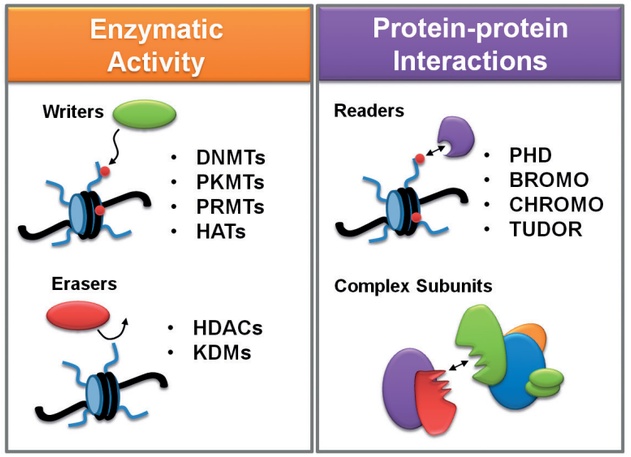


Figure Strategies for targeting epigenetic regulators. The enzymatic activities of DNA methyltransferases (DNMTs), protein lysine methyltransferases (PKMTs), protein arginine methyltransferases (PRMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), or lysine demethylases (KDMs), are amenable to inhibition by small molecules. Additionally, molecular probes can be used to block the interactions of readers containing PHD, Bromo, Chromo or Tudor domains, with modified histones, or to disrupt the interaction between critical core components of chromatin-related complexes.

TECHNOLOGY TRANSFER AND VALORISATION OFFICE

ANABEL SANZ Director

In direct partnership with CNIO's faculty, the main activity of CNIO's Technology Transfer and Valorisation Office (CNIO-TTO) is the commercial exploitation of research results obtained at the CNIO through the submission of patents, collaboration with industry and the creation of innovative companies. To this end, CNIO-TTO promotes, coordinates and manages the relationships of the researchers with the companies and other public and private stakeholders.

The main activities of the CNIO-TTO include:

- Evaluation of the commercial potential of inventions, research tools and software developments originating from CNIO's research endeavours.
- Negotiation of material transfer, confidentiality, sponsored cooperative research and development agreements with other organisations.
- Licensing of intellectual property rights.
- Supporting and fostering a culture of innovation at the CNIO.

Following the consolidation of strategic partnerships with industry, CNIO's patent portfolio has been profoundly re-structured under efficient portfolio management and budget control criteria. As a result, the patent portfolio of the CNIO is composed of 22 patent families that cover numerous territories. The cost of about two thirds of the portfolio has been deferred to our partners. This allows us to concentrate our efforts on new inventions.

The CNIO-TTO implements a rational intellectual property protection strategy based on technological dossiers that address key issues regarding the patentability of the results and their commercial viability. In 2014, besides from several national and international patent application extensions, the CNIO-TTO filed for patent protection of 3 novel inventions with potential commercial application in the cancer diagnostics and therapeutics markets.

"Thanks to the newly approved policy on royalty distribution, our inventors and innovators are getting recognition for their efforts."

We do our utmost to ensure the development of the protected technologies. Through partnerships with different stakeholders in the value chain we are bridging the innovation gap to the point where those technologies could be taken up by the biotech and pharmaceutical industry.

Besides from our focus on novel therapeutics and diagnostics for cancer treatment, the CNIO is very accomplished at developing a number of novel technologies, such as antibodies and research tools. These technologies are licensed to companies for their distribution. In 2014, a total of 23 technologies were commercialised and the revenues are increasing at a steady rate.

Another key component of the technology transfer process is the promotion of our portfolio at technology fairs. During 2014, the CNIO presented its technologies at two major fairs: BioSpain 2014 and at the South Summit Start Up 2014.

Furthermore, the technology transfer potential of the CNIO has been successfully recognised by renowned stakeholders such as the Botín Foundation, which has renewed its support to 2 Research Groups and extended it to a third one. This collaboration enables the Research Groups to undertake innovative projects and facilitates access to additional investments for the projects in order to reach the required decision point to determine whether or not the technology could be introduced to the market.

PRIVATE SPONSORS

"We would like to thank all our sponsors and donors for the generous support that we received from them in 2014. They play an inherent role in our present and future successes."

One of the ***Fundación "la Caixa's"*** 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 **Spanish Association Against Cancer *(Asociación Española Contra el Cáncer, AECC)***, in addition to funding research projects at the CNIO through a competitive grant process, awards grants aimed at supporting cancer research through its Science Foundation. These grants provide support to scientists and clinicians who work intensively in the field of oncology.

The ***Fundación BBVA*** generously supports the BBVA Foundation – CNIO Cancer Cell Biology Programme, headed by Professor Erwin Wagner, since mid 2009. This Programme focuses on research into tumour processes, covering all aspects of tumour cell biology from the molecular level to the analysis of gene functions in normal and pathological conditions.

Fundación CRIS is dedicated to the promotion and development of research with the aim of eliminating the serious health threat of cancer. *Fundación CRIS* generously supports the *CRIS* Foundation – CNIO Prostate Cancer Clinical Research Unit, headed by David Olmos, since 2012. This group focuses on translating advances in prostate cancer research into improvements in patient care.

AVON, funds the Breast Cancer Clinical Research Unit, led by Miguel Quintela, since 2010. The Research Project "Avon-CNIO" on breast cancer research has the main goal of advancing personalised treatment for breast cancer patients.

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

The ***Fundación Banco Santander*** funds the Banco Santander Foundation – CNIO Fellowships for Young Researchers. Kurt Whittemore, from the University of Arizona (USA), was the recipient of a Santander Foundation-CNIO Fellowship in 2014. Additionally, thanks to the support of the *Fundación Banco Santander*, a group of young researchers received training on managerial and entrepreneurial skills, in collaboration with the IE Business School.

The ***Fundación Marcelino Botín*** and the ***Banco Santander*** are committed to supporting scientific research and knowledge transfer from academia to the market through science programmes. These two well-recognised organisations collaborate with the CNIO in this regard by supporting the research groups led by Manuel Serrano and Maria A. Blasco. This year, they have also further extended their support to a third CNIO researcher, Óscar Fernández-Capetillo.

The ***Fundación Jesús Serra-Catalana Occidente*** continues to fund the Visiting Scientists Programme that was established to support prestigious international professors for short stays at the CNIO. The recipients of the *Jesús Serra* Foundation's Visiting Scientist Award in 2014 were Eva Nogales from the University of California in Berkeley (USA), Andre Nussenzweig from the National Cancer Institute in Bethesda (USA) and Peter Petzelbauer from the Medical University of Vienna (Austria).

AXA Research Fund (ARF), a global initiative of scientific philanthropy run by the insurance group AXA, awarded an AXA-CNIO Permanent Chair in Molecular Oncology to Mariano Barbacid as part of its 2011 call. This type of sponsorship allows long-term support to the investigator thanks to the annual interest obtained from the capital (2 million EUR) assigned to this AXA-CNIO Permanent Chair.

OTHER SPONSORS

Our activities and seminars are also supported through individual donations – citizens who wish to contribute personally to the battle against cancer – as well as via external fundraising from the following local associations: ***the French Embassy,*** **Fressia Group,** ***the Fundación Antoni Serra, and the Fundación Banco Sabadell.***

We would also like to thank our anonymous benefactors who, with their decision to donate their legacy to support cancer research at the CNIO, have made a very meaningful contribution to the community for generations to come.

Communication

COMMUNICATIONS

NURIA NORIEGA Head of Communications

Vanessa Pombo (since September)

Establishing a recognised expertise in cancer research implies sharing our goals, achievements, challenges and insights with society; our first and ultimate interlocutor.

The Communications Department has now been operational for three years. We are proud to say that in 2014 we surpassed last year's excellent media exposure, achieving around 2,300 mentions worldwide. This means an increase by about 41% compared with 2013, and by 200% when compared with 2012.

Throughout the year, CNIO research was featured not only in national digital, printed and broadcast media, but also in international media, such as Fox News, *The Huffington Post*, *New Scientist* and *The Scotsman*, among many others. One of the top stories of the year was published in July in *Cell Metabolism*, under the title "A Switch from White to Brown Fat Increases Energy Expenditure in Cancer-Associated Cachexia," by the researcher Erwin F. Wagner, Director of the BBVA Foundation-CNIO Cancer Cell Biology Programme. The story was featured on the front page of *El País*, one of the most popular newspapers in Spain, as well as in other national and international media channels, such as TVE, *The Boston Globe* or Fox News. It was also commented on by *Science* and *Nature*. According to Altmetric, this article is amongst the highest ever scored in *Cell Metabolism* (ranked #11 of 799). It is also in the top 5% of all articles ever tracked by Altmetric: around 3 million across all journals so far.

We must recognise the constant increase of our visibility in social media networks. We have around 6,400 followers interacting with our information on Twitter. This platform has revealed itself to be an essential channel of direct communications with journalists and, more particularly, with patients, families and associations. Regarding YouTube, our channel is followed by 155 subscribers and our videos have been viewed over 10,000 times in 2014: 528 hours of content in total.

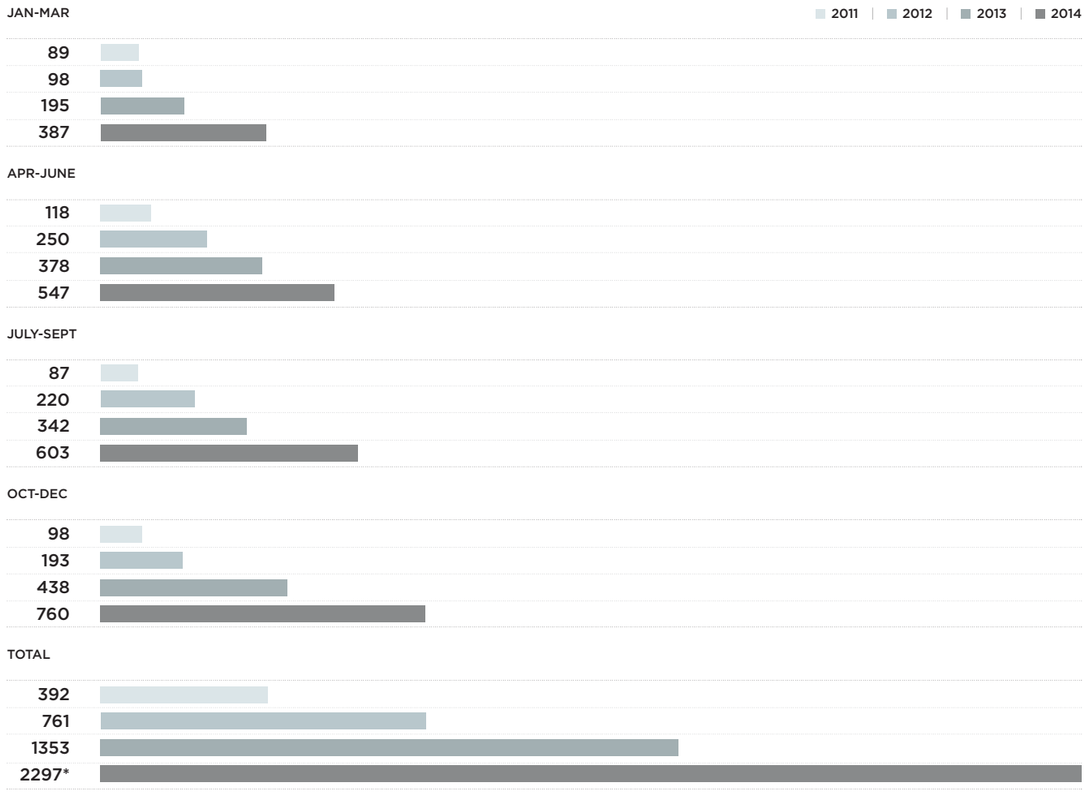
In 2014, CNIO submitted 32 press releases to the global news service EurekAlert!, which is the same number as in 2013. Throughout the year, these stories received over 100,000 hits, representing an average of 8,500 page views per month. This means the total annual number of hits rose by 22% when compared with last year's hits.

> **"Raising awareness of the key role of research in the fight against cancer."**

We are also aware of the need to communicate about what it is that we do directly to the patients themselves. That's the reason why the CNIO has participated for the second time in the annual meeting of the Spanish Group of Patients with Cancer (GEPAC); an enriching experience that helps us to assess the human relevance of our mission as a Research Centre of Excellence.

The interest of the media and society in the work carried out by our scientists is constantly growing. Knowledge is key when it comes to raising awareness about the relevance of basic and applied research in the fight against cancer. We contribute to this awareness by sharing rigorous information about our scientists' work, and by keeping ourselves committed to delivering transparent communications.

CNIO APPEARANCES IN PRINT AND DIGITAL MEDIA



*More than 41 % when compared to 2013.

PRESS CLIPPINGS

1 *El País*, January 16, 2014
2 *Los Desayunos de TVE*, TVE, February 4, 2014
3 *El País*, February 21, 2014
4 *Fox News*, February 28, 2014
5 *ABC*, March 15, 2014
6 *ABC*, March 16, 2014
7 *Diario Médico*, March 31, 2014
8 *Comando Actualidad*, TVE, April 2, 2014
9 *El País*, April 15, 2014

10

SELECCIÓN ESPAÑOLA de ciencia
¡exclusiva!
Quo

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Producen un modelo celular humano con translocaciones de dos tipos de cáncer
CNIC y CNIO emplean tecnología RGEN para generar marcos específicos de LMA y sarcoma de Ewing

12

Diálogo galardona al Institut Curie y al CNIO

13

«Tenía un amigo con un tumor y al secuenciarlo había 4.000 mutaciones»

14

El científico extremo

15

Una científica gallega halla la llave que hace vulnerable al melanoma

16

Hallazgo de científicos españoles del CNIO
Nuestro genoma, más pequeño de lo esperado
El ser humano cuenta con 19.000 genes, 1.700 menos de lo que se pensaba
Un poco más humanos

17

La grasa 'buena' causa la extrem delgadez de los pacientes de cánc
La gran esperanza contra la ob

18

#MilleniumTVE
CÁNCER: LA GRAN BATALLA

19

MEDICINA
La senescencia comienza en el desarrollo embrionario y ayuda a remodelar tejidos

20

THIS WEEK
Centenarian genes offer longevity clues

21

Un medicamento en estudio logra la remisión en el 50% de casos de cáncer de mama

22

SOCIEDAD
Una viguesa descubre que las células madre de los tumores se identifican por su brillo

23

Una terapia esperanzadora

24

JUEVES 06 NOVIEMBRE 2014
Desarrollan un modelo mur que imita el síndrome Noona

25

CULTURAS Y SOCIEDAD
«Dentro de diez años los tratamientos contra el cáncer serán personalizados»

26

Ratones «avatar» para combatir el cáncer

27

cnio

28

sinc
Descubierto un derivado de la vitamina B3 que previene el cáncer de hígado en ratones

29

Ciencias
Células contra la calvicie

10 *QUO*, May 29, 2014
11 *Gaceta Médica*, June 9, 2014
12 *El Economista*, June 10, 2014
13 *El Progreso*, June 21, 2014
14 *La Vanguardia*, June 22, 2014
15 *La Voz de Galicia*, June 27, 2014
16 *La Razón*, July 4, 2014
17 *El País* (front page), July 18, 2014
18 *Millennium*, La 2, July 20, 2014
19 *Diario Médico* (front page), September 1, 2014
20 *New Scientist*, September 6, 2014
21 *El Día de Córdoba*, September 13, 2014
22 *Faro de Vigo* (front page), September 30, 2014
23 *La Razón*, November 2, 2014
24 *Diario Médico*, November 6, 2014
25 *La Rioja*, November 8, 2014
26 El Mundo, November 10, 2014
27 *La Sexta Columna*, La Sexta, November 14, 2014
28 *SINC*, November 20, 2014
29 *La Razón*, December 24, 2014

INVITED GUEST SPEAKERS (Distinguished Seminar Series)

Ada Yonath, January 10, 2014

Emmanuel Barillot, June 13, 2014

Victoria Seewaldt, June 27, 2014

Jean-Pierre Changeux, November 4, 2014

Shahragim Tajbakhsh, November 7, 2014

Bruce Stillman, November 21, 2014

SOCIAL EVENTS

LEFT (UP) Jérôme Bonnafont, French Ambassador to Spain, and Maria Blasco, Director of the CNIO, signed the letter of intent to strengthen the cooperation between CNIO and French research centres. February 6, 2014.

LEFT (DOWN) *"Meet the researchers, be a researcher"* was the slogan of the European Researcher's Night at the CNIO, with around 200 visitors. September 26, 2014.

ABOVE CNIO and Institut Curie received the 11th *Diálogo* Award for Franco-Hispanic Friendship, which recognises their world-leading work in the prevention, diagnosis and treatment of cancer. June 10, 2014.

Simon Manley, British Ambassador to Spain, facilitated discussions on a bilateral R&D agreement and joint projects between CNIO and Cancer Research UK. October 13, 2014.

The *Asociación Española de Fundaciones* visited the CNIO to learn about the Centre's cancer research. October 27, 2014.

The Dean's Office and the CNIO pre-doctoral (CNIOSA) and postdoctoral (CNIO-PDA) researchers organised Lab Day 2014, conceived to promote interactions between the Centre's research groups. December 9, 2014.

CNIO Offices

DEAN'S OFFICE

MARÍA S. SOENGAS
Dean for Academic Affairs

At the CNIO we are proud of being at the forefront of cancer research. 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 places considerable emphasis on career development, supported in part by highly competitive PhD and Postdoctoral programmes. Agreements are also in place with active medical centres in order to help bridge the gap between academic and clinical environments.

Importantly, the activities of the Dean's Offices are not just for our trainees. Indeed, the inspiring series of seminars and workshops that we host are organised by the CNIO Student Association (CNIOSA) and Postdoc Association (CNIOPDA). In this context, we are very excited about our ongoing efforts to expand on scientific reasoning, grant writing, time management and interviewing, topics that we consider important to cover in our curriculum. We also acknowledge the relevance of preparing our personnel beyond the bench; therefore, we pay special attention to aspects such as (inter)active job searching, public communications, management of intellectual property, and the creation of *start ups* or *spin offs*. These are addressed in concert with CNIO's Training Programmes and the Innovation and Communication Offices, which are all 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 cancer awareness-raising campaigns and were involved in the annual meeting of the Spanish Group of Patients with Cancer (*GEPAC*). The open-doors day activities that we hosted, such as the *European Researchers' Night*, were also highly attended, with over 160 participants involved in hands-on experiments.

Finally, I would like to thank the many volunteers (trainees, staff and principal investigators) who contributed to making our Annual CNIO Lab Day a truly inspirational event. We were fortunate to have Glenn Merlino (NCI Cancer Institute, US) who spoke about the *National Cancer Research Institute* (US) and about state-of-the-art mouse models that are revolutionising gene discovery and drug response in cancer. It was an equal pleasure to learn from Angel Sánchez (*Everis Foundation*) about the relevance of concise and precise presentations geared towards the industry and possible investors. We also had seven outstanding talks given by CNIO trainees, which covered exciting discoveries in the fields of crystallography, computational biology, drug development, proteomics, tumour suppression, genetic risk factors and metabolism. Progress in other basic and translational aspects of cancer were discussed in over sixty posters, which together exemplified the breadth of research represented by our different Scientific Programmes. Finally, we also had the pleasure of announcing the Second Series of the "CNIO Awards for Publications by PhD Students". This year the awards went to Irene Miranda-Lorenzo (*Nat Methods*), Direna Alonso-Curbelo (*Cancer Cell*), Krishna Tummala (*Cancer Cell*), Iñaki Comino-Méndez (*J Natl Cancer Inst*) and Stefanie Wurm (*Genes & Dev*). These are just some of the few examples of the outstanding work that is being carried out at the CNIO by a vibrant community of young and active investigators, mentored by a committed faculty that is on the frontline of cancer research. ■

"At the CNIO we aim high: to perform the most innovative and competitive basic and translational research, and to prepare our trainees for the future, so that they can fulfil their potential as influential leaders."

CNIO-WOMEN IN SCIENCE OFFICE (WISE OFFICE)

Lola Martínez
(since October)
Mirna Pérez-Moreno
(until October)
Coordinators

Members
Francisca Mulero: Networking and Seminars Coordinator; Águeda Tejero: Work-Life Balance Coordinator; Pablo Fernández, Diego Megías, Fernando Peláez

The growing number of female PhD graduates in the European Union is not reflected in the number of women taking up senior science research positions. Although several gender equality policies and political strategies for equal opportunities and gender mainstreaming have been established in Spain, a more systematic implementation of these actions to ensure equality in research is still needed.

The CNIO-Women in Science (WISE) Office aims to promote awareness about these important aspects and to foster equal opportunities for the career advancement of individuals in the CNIO community.

In 2014, WISE activities were directed towards stimulating institutional gender awareness through the organisation of conferences, seminars, and lectures with gender experts and role models, including networking and coaching sessions. In addition, the Office proposed several initiatives to the CNIO Direction in relation to work-life balance issues and has elaborated and submitted, in collaboration with the CNIO Direction and Management, a grant proposal for the call for "promoting gender equality in research and innovation" of the Horizon 2020 framework programme.

Some of our activities in 2014 include the following:

- → 15/12/2014: WISE seminar – "Women in Finance" by Paula Inés Paap. Partner of IFA (International Financial Agency).
- → 30/09/2014:WISE seminar – "Women leadership, Advocacy or Diversity Management" Dr. Margarita Alonso. Director *Fundación Instituto de Empresa (IE Foundation)*. Madrid, Spain.
- → 15/07/2014: WISE seminar – "What is it like to be a girl professor?" Dr. Martha Gray. Harvard-MIT HST; MIT EECS; Research Lab of Electronics; IMES, Cambridge, USA.
- → 27/05/2014: WISE seminar - "Networking and Career Building in Science". Dr. Capitolina Díaz. University of Valencia. President of the Spanish Association of Women in Science and Technology (AMIT), Valencia, Spain.
- → 08/05/2014: WISE coaching workshop - "The enzyme of the change" by Beatriz Ajenjo and Carlos Ferrari. International Coach Federation. Spain.
- → 06/03/2014: WISE meeting - International Women's day - Coffee meeting. ■

"More countries have understood that women's equality is a prerequisite for development (Annan, 2001). At CNIO, the awareness of the gender dimension in science has resulted in an increased affiliation of men to the WISE office in 2014. This has contributed towards achieving our common goals, and towards the continuation of making the CNIO a Centre of Excellence that ensures gender equality."

Facts & Figures

COMPETITIVE FUNDING

The CNIO attracts a substantial proportion of its funding from external sources. Most of this funding comes from national and international public funding agencies, as well as from private entities. In 2014, researchers at the CNIO were involved in 134 projects that received extramural funding.

Of these, 27 were international collaborative projects (4 of which are coordinated by the CNIO) and 30 collaborative projects with other groups in Spain. The international collaborative projects were funded by institutions, such as the European Commission through the 7th Framework Programme, The Melanoma Research Alliance 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 2014, 17 of these projects received international funding and 60 received national funding. Individual research projects are also funded by the European Research Council (ERC) Advanced and Starting Grants, the NIH, the European Commission ("People" Programme), the Association for International Cancer Research (AICR), US National Institutes of Health (NIH), the European Science Foundation (ESF), the Howard Hughes Medical Institute (HHMI) and the European Foundation for the Study of Diabetes (EFSD).

INTERNATIONAL GRANTS COLLABORATIVE PROJECTS

AXA RESEARCH FUND

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A. (coordinator) Serrano, Manuel	Identification and manipulation of molecular pathways relevant for age-dependent tissue regeneration

EUROPEAN COMMISSION 7th FRAMEWORK PROGRAMME

COLLABORATIVE PROJECTS (CP)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Heeschen, Christopher	CAM-PaC: Integrative Analysis of Gene Functions in Cellular and Animal Models of Pancreatic Cancer
Malats, Núria	TransBioBC: Translation of novel Biomarkers for Bladder Cancer for clinical outcome prediction

COST ACTION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria (coordinator)	COST Action BM1204 EU Pancreas: An integrated European platform for pancreas cancer research: from basic science to clinical and public health interventions for a rare disease

EURATOM

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Serrano, Manuel	RISK-IR: Risk, Stem Cells and Tissue Kinetics-Ionising Radiation

INNOVATIVE MEDICINES INITIATIVE JOINT UNDERTAKING (IMI JU)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	e-TOX: Integrating bioinformatics and chemoinformatics approaches for the development of expert systems allowing the *in silico* prediction of toxicities
Valencia, Alfonso	Open PHACTS: An open, integrated and sustainable chemistry, biology and pharmacology knowledge resource for drug discovery

INTEGRATED PROJECT

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	COGS: Collaborative oncological gene-environment study
Heeschen, Christopher	MULTIFUN: Multifunctional nanotechnology for selective detection and treatment of cancer
Malumbres, Marcos	MitoSys: Systems biology of mitosis
Valencia, Alfonso	BLUEPRINT: A BLUEPRINT of haematopoietic epigenomes
Valencia, Alfonso	ASSET: Analysing and striking the sensitivities of embryonal tumours
Valencia, Alfonso	RD-CONNECT: An integrated platform connecting registries, biobanks and clinical bioinformatics for rare disease research

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	ITN aDDRess: Joint training and research network on chromatin dynamics and the DNA damage response

NETWORKS OF EXCELLENCE (NOE)

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
Blasco, Maria A.	EuroBATS: Identifying biomarkers of ageing using whole transcriptomic sequencing
Heeschen, Christopher Real, Francisco X.	EPC-TM-Net: Targeting the tumour microenvironment to improve pancreatic cancer prognosis
Malats, Núria Real, Francisco X. (coordinator)	CANCERALIA: Development of novel diagnostic and therapeutic approaches to improve patient outcome in lung and pancreatic tumours
Robledo, Mercedes	ENS@T- CANCER: European network for the study of adrenal tumours-structuring clinical research on adrenal cancers in adults

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (MIT)

M+VISION

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Hidalgo, Manuel	Team Albumin: Monitoring of stroma-targeting pancreatic cancer treatments with MRI
Mulero, Francisca	Team PET: Improved molecular imaging by multi-tracer PET
Olmos, David	Team Cell: Development and testing of a biological use case of technology-rare cell detection
Pérez, Mirna Alicia	Team Leuko: Home-based neutrophil blood testing to tailor chemotherapy regimens to personal toxicity limits
Soengas, María S.	Team TxResponse: Early assessment of treatment response in advanced melanoma patients

MELANOMA RESEARCH ALLIANCE (MRA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S. (coordinator)	Imaging and therapeutic targeting of lymphangiogenesis in melanoma

US NATIONAL INSTITUTES OF HEALTH (NIH)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Valencia, Alfonso	GENCODE 2: Integrated human genome annotation: generation of a reference gene set

INTERNATIONAL GRANTS INDIVIDUAL PROJECTS

BAYER HC

PROGRAMME: "GRANTS FOR TARGETS"

Malumbres, Marcos	Inhibiting mitosis and restoring PP2A by targeting Mastl, a new kinase for cancer therapy

EUROPEAN COMMISSION 7th FRAMEWORK PROGRAMME

MARIE CURIE ACTIONS (MCA)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Al-Shahrour, Fátima	PERSMEDOMICS: Bioinformatics and integrative genomics for a novel personalized cancer therapy
Malumbres, Marcos	Mastl CDC: Role of the protein kinase Mastl in cell division and cancer
Montoya, Guillermo	SMARTBREAKER: Rational designing of new meganucleases as molecular scissors for genomic tailoring
Squatrito, Massimo	GLIDD: DNA Damage Response (DDR) signalling in tumour formation and therapeutic resistance of gliomas

EUROPEAN RESEARCH COUNCIL (ERC)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ERC Advanced Grant RAS AHEAD: Ras genes in health and disease
Blasco, Maria A.	ERC Advanced Grant TEL STEM CELL: From telomere chromatin to stem cell biology
Fernández-Capetillo, Óscar	ERC Consolidator Grant RSHEALTH: Investigating the causes and consequences of replication stress in mammalian health
Heeschen, Christopher	ERC Advanced Grant Pa-CSC: Molecular characterization and targeted elimination of metastatic pancreatic cancer stem cells
Serrano, Manuel	ERC Advanced Grant CANCER&AGEING: Common mechanisms underlying cancer and ageing
Wagner, Erwin F.	ERC Advanced Grant AP-1-FUN: AP-1(Fos/Jun) functions in physiology and disease

EUROPEAN FOUNDATION FOR THE STUDY OF DIABETES (EFSD)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Role of URI in obesity/type 2 diabetes-mediated hepatic metabolic dysfunctions

HOWARD HUGHES MEDICAL INSTITUTE (HHMI)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Fernández-Capetillo, Óscar	Exploring the role of replicative stress in cancer and ageing

US NATIONAL INSTITUTES OF HEALTH (NIH)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	The unfolded protein response in melanoma progression and chemoresistance

WORLDWIDE CANCER RESEARCH (WCR, FORMERLY AICR)

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Djouder, Nabil	Defining the oncogenicity of URI in hepatocellular carcinoma (HCC) development
Fernández-Capetillo, Óscar	Exploiting oncogene-induced replicative stress for the selective killing of cancer cells
Wagner, Erwin F.	Dissecting the roles of Fra proteins in lung adenocarcinoma progression and metastasis

NATIONAL GRANTS COLLABORATIVE PROJECTS

COMMUNITY OF MADRID / *COMUNIDAD AUTÓNOMA DE MADRID (CAM)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano Malumbres, Marcos (coordinator)	*Programa ONCOCYCLE: El ciclo celular y los microRNAs en la autorenovación y diferenciación de células progenitoras*
Blasco, Maria A. Serrano, Manuel (coordinator)	*Programa ReCaRe: Reprogramación en cáncer y regeneración*
Campos-Olivas, Ramón Lietha, Daniel	*Programa BIPEDD 2: Plataforma integrada de bioinformática para el descubrimiento de nuevos fármacos basado en la estructura del receptor*
González-Neira, Anna	*Programa VISIONANIMAL: Modelos animales para el estudio de enfermedades de la visión*
Martínez, Jorge L.	*Programa ANGIOBODIES 2: Desarrollo de anticuerpos recombinantes para uso terapéutico y diagnostico en angiogénesis patológica y para la identificación de nuevos marcadores angiogénicos*
Montoya, Guillermo	*Programa INTERACTOMICS: Interactómica del centrosoma*
Real, Francisco X.	*Programa CEL-DD: Linajes y competición celular en el desarrollo y la enfermedad*
Robledo, Mercedes	*Programa TIRONET: Fisiopatología tiroidea: Mecanismos implicados en cáncer, autoinmunidad y mecanismo de acción de hormonas tiroideas*
Soengas, María S.	*Programa NANODENMED: Nanosistemas dendríticos como agentes y vectores terapéuticos en distintas aplicaciones biomédicas*

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

SUB-PROGRAMME OF THEMATIC NETWORKS FOR COOPERATIVE RESEARCH/*SUBPROGRAMA DE REDES TEMÁTICAS DE INVESTIGACIÓN COOPERATIVA (RETICS)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Cigudosa, Juan C.	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Malats, Núria	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Morente, Manuel M. (coordinator)	*RETIC Biobancos*
Real, Francisco X.	*Red Temática de Investigación Cooperativa en Cáncer (RTICC)*
Valencia, Alfonso	*Red Temática de Investigación Cooperativa en Biomedicina Computacional (COMBIOMED)*

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Benítez, Javier	*Plataforma de recursos biomoleculares y bioinformáticos, PRB2*
Morente, Manuel M. (coordinator)	*Plataforma de Biobancos*
Muñoz Peralta, Javier	*Plataforma de recursos biomoleculares y bioinformáticos, PRB2*
Valencia, Alfonso	*Plataforma de recursos biomoleculares y bioinformáticos, PRB2*

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Gómez, Carlos Jesús	Chemosensitivity profiles for the personalized therapy of advanced colorectal cancer
Hidalgo, Manuel	Personalized treatment for pancreatic cancer patients

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

NATIONAL R&D&I PLAN 2008-2011

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Cigudosa, Juan C.	*Proyecto INNPACTO PROCARDIO: Desarrollo de tecnologías avanzadas de producción y validación de un producto celular alogénico para el tratamiento de la enfermedad cardiovascular*
Heeschen, Christopher	*Proyecto FCCI NANOTECNOLOGÍA: Nanopartículas multifuncionales para tratamiento dirigido e imagen in vivo de células troncales tumorales*
Hidalgo, Manuel	*Proyecto INNPACTO ORALBEADS: Desarrollo de dispersiones sólidas micro/nanoestructuradas para administración oral de compuestos marinos*
Liébanes, María Dolores	*Programa EUROCIENCIA: Plan Estratégico de participación en el 7º Programa Marco*
Soengas, María S.	*Programa CONSOLIDER RNAREG: Una aproximación integrada a la regulación post-transcripcional de la expresión génica y su papel en enfermedad*

NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION (2013-2016)

Networks of Excellence/*Redes de Excelencia*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano (Coordinator)	*ONCObio: Biología del Cáncer*
Malumbres, Marcos (Coordinator)	CellSYS: Functional and Systems Biology of Cell Proliferation
Serrano, Manuel (Coordinator)	SENESTHERAPY: Cell senescence in cancer therapy

Challenges-Collaboration/*Retos-Colaboración*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Soengas, María S.	*Ensayo Clínico Fase I de BO-110: un nuevo tratamiento para melanoma avanzado y otros tumores*

NATIONAL GRANTS INDIVIDUAL PROJECTS

BBVA FOUNDATION / *FUNDACIÓN BBVA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Jiménez, Alberto	*Desarrollo de nuevas herramientas diagnósticas no invasivas por imagen para el diagnóstico del glioblastoma multiforme, el tumor cerebral más maligno*

SPANISH GROUP OF NEUROENDOCRINE TUMOURS / *GRUPO ESPAÑOL DE TUMORES NEUROENDOCRINOS (GETNE)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Robledo, Mercedes	*ParafeOMICS: Identificación de marcadores diagnósticos y pronósticos en feocromocitomas y paragangliomas a través de la integración de cuatro plataformas de análisis masivo*

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

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Álvarez, Sara	Identification of biomarkers that predict the clinical response to DNA hypomethylating therapies on myelodisplastic syndromes
Benítez, Javier	Biologic and genetic bases of telomere shortening in hereditary breast cancer. Searching for new high susceptibility genes in *BRCAX* families with short telomeres
Cascón, Alberto	Exome sequencing of trios, mother-father-proband, 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
Colomer, Ramón	Structural destabilisation for breast cancer oncogenes addiction due to the fatty acid synthase action
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, Anna	Personalizing breast cancer treatment: prediction model construction for taxanes and anthracyclines efficacy thought the integration of different genomic approaches
Guerra, Carmen	Preventive and therapeutic strategies in noonan, costello and cardio facio cutaneous syndromes
Heeschen, Christopher	Development of novel therapeutic strategies for the targeted elimination of metastatic cancer stem cells
Hidalgo, Manuel	Targeting Pancreatic Cancer Stroma
Malats, Núria	Aetiology of pancreas cancer: Application of "omics" technologies in the assessment of risk factors
Olmos, David	Homologous recombination DNA repair deficiency related chromosomal instability in aggressive prostate cancer
Quintela, Miguel Angel	From systems biology to clinical trials: high-throughput studies and definition of predictive factors and resistance mechanisms against breast cancer drugs
Robledo, Mercedes	Use of massive analysis platforms on endocrine tumor studies: from OMICS to patients
Squatrito, Massimo	Investigating the role of Fra1 and Fra2 in glioma tumor formation and treatment response

LA MARATÓ TV3 FOUNDATION / *FUNDACIÓN LA MARATÓ TV3*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	Molecular analysis of Capicua, a novel tumor suppressor involved in RTK signaling and transcriptional repression
Fernández-Capetillo, Óscar	Exploring synthetic lethal interactions between PARP and the DNA damage response in cancer treatment
Soengas, María S.	Role of RNA binding proteins in melanoma progression: searching for new diagnostic markers and therapeutic targets

MADRI+D FOUNDATION / *FUNDACIÓN MADRI+D*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Dean's Office for Academic Affairs	*Ven a conocer a los científicos, ¡conviértete en un científico!* European Researchers' Night 2014, organised by the Madri+d Foundation and founded by European Commission under the Framework Programme H2020

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

NATIONAL R&D&I PLAN 2008-2011

Sub-programme: non-targeted fundamental research projects/*Subprograma de Proyectos de Investigación Fundamental no Orientada*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Barbacid, Mariano	ONCORAS: Inhibition of oncogenic K-Ras signaling in cancer
Blasco, Maria A.	TELOMERE: Mammalian telomeres and telomerase: from chromatin structure to stem cell biology
Djouder, Nabil	Papel Urica: Decoding the URI role in the hepatocellular carcinoma (HCC) development
Fernández-Capetillo, Óscar	RS/CANCER-AGEING: Exploring the role of replication stress in cancer and ageing
Guinea-Viniegra, Juan	PsorTACEmiR21: Investigating the role of microRNA21/TIMP-3/TACE in psoriasis - evaluating the potential therapeutic implications
Lietha, Daniel	FAKBasicsToDrugs: From the molecular study of growth signaling and cellular adhesion to the drug discovery
Losada, Ana	COHESIN: Animal models for the study of cohesin functions
Malumbres, Marcos	MitoSYS: Physiological and therapeutic relevance of mitotic kinases and phosphatases
Méndez, Juan	MCMREPLICA: MCM complex functions in the DNA replication and the genetic stability
Montoya, Guillermo	Macromachines: Structural biology of macromolecular machines involved in chromosome dynamics
Pérez, Mirna Alicia	CrosSkin: Intercellular crosstalk in skin physiology and disease
Real, Francisco X.	Pancreatic adenocarcinoma: role of the acinar and ductal components and development of animal models
Rodríguez, Cristina	Identification of markers predictive of paclitaxel severe neurotoxicity using genome-wide platforms
Soengas, María S.	Cellular stress in melanoma progression and chemoresistance
Tress, Michael	AlteredDynamics: An experimentally validated computational approach to study protein conformational plasticity and its alteration
Valencia, Alfonso	Development of biocomputing systems and subjacent 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

Sub-programme of support for centres and units of excellence 'Severo Ochoa'/*Subprograma de apoyo a centros y unidades de excelencia 'Severo Ochoa'*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	*Acreditación del CNIO como Centro de Excelencia "Severo Ochoa"*

Sub-programme of support for the technology transfer function in research centres/*Subprograma de apoyo a la función transferencia en centros de investigación (INNCIDE)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Quintero, Marisol	*Proyecto INNCIDE-CNIO para favorecer la creación de valor económico de los conocimientos derivados de los descubrimientos científicos y de los resultados de investigación y desarrollo del CNIO*

NATIONAL PLAN FOR SCIENTIFIC AND TECHNICAL RESEARCH AND INNOVATION (2013-2016)

R&D Projects of Excellence/*Proyectos de I+D Excelencia*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Méndez, Juan	REPLICON: Molecular mechanisms that control eukaryotic DNA replication
Ramón-Campos, Santiago	CADstructure: Structural determination of the architecture of CAD, an antitumor target that controls the biosynthesis of pyrimidines
Ruiz, Sergio	RSHIPS: Replicative stress during somatic cell reprogramming

Acquisition of scientific and technical equipment/*Adquisición de equipamiento científico-técnico*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Gonzalez, David	*Infraestructura de almacenamiento científico*

Challenges-Research/*Retos-Investigación*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Blasco, Maria A.	TeloHealth: Telomeres, telomerase and disease
Djouder, Nabil	MILC: Metabolic inflammation in liver cancer
Losada, Ana	COHESIN: Cohesin function and regulation: a multidisciplinary approach
Muñoz, Javier	steMS: Understanding ground state pluripotency of embryonic stem cells through mass spectrometry-based proteomics
Ortega, Sagrario	HaploEScancer: Haploid ES cells for cancer research
Pastor, Joaquín	CDK8eDD: CDK8 a novel target in cancer therapy. Relevance of CDK8 kinase activity, discovery and optimization of selective orally bioavailable CDK8 inhibitor
Serrano, Manuel	CANCERAGE: Cancer and ageing-associated diseases: new frontiers and new strategies

MUTUA MADRILEÑA FOUNDATION / *FUNDACIÓN MUTUA MADRILEÑA*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Rodriguez, Cristina	*Estudio de microRNAs como predictores de respuesta a Sunitinib en pacientes con carcinoma renal*
Guinea-Viniegra, Juan/María Jiménez	*JunB/AP-1, supresor tumoral en la piel. Mecanismos moleculares e interacción funcional con p53*

PROSTATE CANCER FOUNDATION / *FUNDACIÓN PARA EL CÁNCER DE PRÓSTATA*

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

RAMON ARECES FOUNDATION / *FUNDACIÓN RAMÓN ARECES*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Montoya, Guillermo	*Desarrollo de bisturíes moleculares para la reparación de genes implicados en enfermedades monogénicas*
Serrano, Manuel	*Reprogramación nuclear in vivo e interrelación funcional entre p27 y Sox2*

SPANISH ASSOCIATION AGAINST CANCER / *ASOCIACIÓN ESPAÑOLA CONTRA EL CÁNCER (AECC)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Malats, Núria Real, Francisco X. (coordinator)	*Cáncer de vejiga invasivo: hacia una medicina de precisión*

SPANISH SOCIETY OF MEDICAL ONCOLOGY / *SOCIEDAD ESPAÑOLA DE ONCOLOGÍA MÉDICA (SEOM)*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Olmos, David	*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*

VOLKSWAGEN FOUNDATION / *FUNDACIÓN VOLKSWAGEN*

PRINCIPAL INVESTIGATOR	PROJECT TITLE
Lietha, Daniel	Nanoapertures loaded with individual molecules

EDUCATION & TRAINING PROGRAMMES

One of the principal goals of the CNIO is to increase its training capacity in order to give students and professionals the opportunity to advance their careers in the healthcare sector. During 2014, the CNIO has signed several new agreements with Spanish Universities and other institutions, namely with the *Fundación Banco Santander, Universidad de Barcelona, Universidad Europea de Madrid, Universidad de Zaragoza, Universidad Complutense de Madrid, Universidad de Valladolid, Universidad Pompeu Fabra,* and the *Fundación Estudios Médicos de Molina del Segura.*

TRAINING PROGRAMMES	PARTICIPANTS IN EDUCATION AND TRAINING PROGRAMMES				
	2010	**2011**	**2012**	**2013**	**2014**
Training of PhD students	132	123	121	116	**108**
Post-doctoral training	95	83	81	67	**55**
Training for MDs	25	20	16	21	**14**
Laboratory training for MSc/BSc students	54	46	42	36	**73**
Laboratory training for technicians	29	26	26	19	**21**
Master's Degree in Molecular Oncology	26	37	37	37	**34**

TRAINING OF BSC/MSC STUDENTS

The CNIO is committed to training junior scientists at the onset of their careers. To this end, the Centre has established a programme that offers BSc and MSc students the opportunity to obtain hands-on practical laboratory experience by working on ongoing research projects in one of the CNIO groups. The CNIO offers 2 types of short-term laboratory training:

- → An annual Summer Training Programme for undergraduate students, from any country, who are in their last 2 years of study in the biomedical field. The Programme encompasses 8 weeks of full-time laboratory training (312 hours). During this time the students actively participate in research projects in one of the CNIO groups. During 2014, 8 students from 6 countries participated in this programme.
- → Additionally, students can apply for laboratory training throughout the academic year by directly contacting the Heads of CNIO individual Research Groups or Units. This year, 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, 21 students obtained their PhD degrees in 2014 and 21 more joined the CNIO in that same year. More than one third of the 108 students working at the CNIO in 2014 were graduates from foreign universities, thus contributing to the internationalisation of the Centre.

Since 2008, the *Fundación "la Caixa"* offers international fellowships to PhD students to enable them to carry out their thesis projects in biomedical research in Spanish centres of excellence. The CNIO was chosen, as one of 4 such centres, to launch a programme for outstanding young pre-doctoral students from all over the world who have an interest in pursuing an ambitious PhD project. Since 2013, the Ministry of Economy 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 second call of this new "*la Caixa*"-Severo Ochoa International PhD Programme was very successful, attracting around 100 eligible applications from undergraduates from 24 different countries. During 2014, 4 pre-doctoral students received one of these recognised fellowships.

The distribution of students across the CNIO's Research Programmes in 2014 was as follows: 42.6% of students worked in the Molecular Oncology Programme, 13.0% in the BBVA Foundation- CNIO Cancer Cell Biology Programme, 13.9% in the Structural Biology and Biocomputing Programme, 15.7% in the Human Cancer Genetics Programme, 8.3% in the Clinical Research Programme, 0.9% in the Biotechnology Programme and the remaining 5.6% in the Stems Cells and Cancer Group.

FUNDING OF PHD TRAINING	NO.
SPANISH ENTITIES	84
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (I+D Projects)	6
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (Predoctoral fellowships)	24
Ministry of Education, Culture and Sport / *Ministerio de Educación, Cultura y Deporte* (Predoctoral fellowships)	10
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)* (I+D Projects)	2
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)* (Predoctoral fellowships)	2
Spanish Association Against Cancer (AECC) / *Fundación Científica de la AECC* (I+D Projects)	1
Cellectis Agreement	1
Centre for Industrial Technological Development / *Centro para el Desarrollo Tecnológico Industrial* (I+D Projects)	1
CIBERER	1
"la Caixa" Foundation / *Fundación "la Caixa"* (Predoctoral fellowships)	34
Botín Foundation / *Fundación Botín*	1
Hospital de Madrid	1
INTERNATIONAL ENTITIES	24
European Commission Framework Programme	5
Marie Skłodowska-Curie actions of the European Commission	1
European Research Council	8
European Urology Association (Predoctoral fellowship)	1
Melanoma Research Alliance	1
Pfizer	2
Fulbright Spain / *Fulbright España* (Predoctoral fellowships)	3
Prostate Cancer Foundation Young Investigator Award	1
Bayer	1
Hoffmann-La Roche	1
TOTAL	**108**

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 2014, 55 postdoctoral fellows worked at the CNIO. Notably, more than 45% of these fellows were from outside of Spain, many coming from very prestigious international institutions.

In 2014, the *Fundación Banco Santander* signed a new agreement with the CNIO to continue 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 University of Arizona, was awarded a Santander Foundation-CNIO Fellowship in 2014.

FUNDING SOURCES OF POST-DOCTORAL CONTRACTS	NO.
SPANISH ENTITIES	31
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (I+D Projects)	3
Ministry of Economy and Competitiveness / *Ministerio de Economía y Competitividad* (Posdoctoral Fellowships)	3
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)* (I+D Projects)	1
Institute of Health Carlos III / *Instituto de Salud Carlos III (ISCIII)* (Posdoctoral Fellowships)	7
Community of Madrid / *Comunidad Autónoma de Madrid* (I+D Projects)	2
Spanish Association Against Cancer (AECC) / *Fundación Científica de la AECC* (Posdoctoral Fellowships)	6
Botín Foundation / *Fundación Botín*	1
CIBERER	2
Madrid-MIT M+Visión (Posdoctoral Fellowship)	1
Cris Foundation / *Fundación Cris*	1
CNIO	4
INTERNATIONAL ENTITIES	24
European Commission Framework Programme	8
European Research Council	6
Association for International Cancer Research	3
Daiichi Sankyo Agreement	1
European Association for the Study of Diabetes	2
Hoffmann-La Roche	1
Clinical trials Boehringer	1
Federation of European Biochemical Societies (Posdoctoral Fellowship)	1
Leukemia and Lymphoma Society (Posdoctoral Fellowship)	1
TOTAL	**55**

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, Masters in Molecular and Cell Biology, Masters in Molecular Biomedicine, and Masters in Biotechnology.

Master's Degree in Biocomputing and Computational Biology

The Master in *Bioinformática y Biología Computacional* – directed by Alfonso Valencia, Director of CNIO's Structural Biology and Biocomputing Programme – is organised together with the National School of Health of the National Institute of Health Carlos III (*Escuela Nacional de Sanidad del Instituto de Salud Carlos III, ENS-ISCiii*), and the Madrid Science Park (*Parque Científico de Madrid*, PCM).

Official Master's Degree in Clinical and Applied Cancer Research

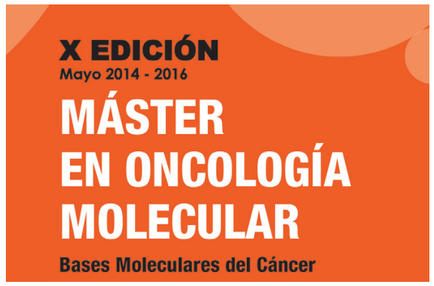
Manuel Hidalgo, CNIO's Vice-Director of Translational Research codirects – in collaboration with the CEU-San Pablo University in Madrid (USP-CEU) – a Postgraduate Training Programme in Clinical and Applied Cancer Research: the *Máster Universitario en Investigación Clínica y Aplicada en Oncología*.

Official Master's Degree in Therapeutic Targets, Research and Development

The CNIO collaborates with the Biochemistry and Molecular Biology Department at the University of *Alcala* (*UAH*) for the *Máster Oficial en Dianas Terapéuticas, Investigación y Desarrollo.*

Master's Degree in Molecular Oncology

The main objective of this Master's degree, organised in collaboration with the Centre for Biomedical Studies (*Centro de Estudios Biosanitarios, CEB*), is to offer training in molecular oncology with emphasis on the latest findings in translational research that are essential for state-of-the art oncological clinical practice. Upon successful completion of the 500 hours of training, a certificate for a Master's degree in Molecular Oncology – recognised by the European School of Oncology (ESO) – is awarded.



LABORATORY TRAINING FOR TECHNICIANS

This training programme has been developed for students in Anatomical Pathology and is organised through agreements with 10 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 (710 hours) of laboratory training for students. Additionally, the CNIO offered real-life work experience to 1 student of Analytical Assays and Quality Control for 13 weeks (370 hours); 1 student of Clinical Diagnosis for 13 weeks (380 hours); 1 student of Diagnosis Imaging for 8 weeks (224 hours); and 2 students of Medical Archiving and Recording for 14 weeks (440 hours). Of the 21 students who participated in this programme in 2014, 4 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 and also counts with an Onco-MIR Rotation Programme; this initiative is a collaborative effort with the Spanish Ministry of Health (now *Ministerio de Sanidad, Servicios Sociales e Igualdad*). Training usually consists of a 3-month period during residency. In 2014, 14 medical residents from 12 different hospitals enjoyed the benefits of rotations within the different Groups and Units of the CNIO.

ADVANCED TRAINING OF SCIENTISTS THROUGH EXTRAMURAL PROGRAMMES

During 2014, 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 (4 contracts) and Río Hortega (3 contracts) programmes, Juan de la Cierva programme (Spanish Ministry of Economy and Competitiveness, 2 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 2014, Eva Nogales from the University of California in Berkeley (USA), Andre Nussenzweig from the National Cancer Institute in Bethesda (USA) and Peter Petzelbauer from the Medical University of Vienna (Austria) were beneficiaries of the Jesús Serra Foundation's Visiting Researcher Programme.

Fundación Jesús Serra
Catalana Occidente

SCIENTIFIC EVENTS

MEETINGS & CONFERENCES

The CNIO annually hosts various international meetings and conferences. Within this category, the 4 international events held in 2014 focused on recent advances in the areas of familial cancer, Von Hippel-Lindau syndrome, lung cancer targets and early drug discovery, as well as on new developments in microscopy.

CRUK-CNIO EARLY DRUG DISCOVERY MEETING
MARCH 13, 2014

ORGANISERS
- **Sara Cebrián**, UK Science and Innovation Network, British Embassy in Madrid, Spain
- **Robert Williams**, Cancer Research UK
- **Joaquín Pastor**, CNIO, Spain

SPEAKERS
- **Anabel Sanz**, CNIO, Spain
- **Dr. Spiros Linardopoulos**, Institute for Cancer Research, ICR, UK
- **Dr. Oskar Fernandez Capetillo**, CNIO, Spain
- **Dr. Marcos Malumbres**, CNIO, Spain
- **Dr. Donald Ogilvie**, Paterson Institute, UK
- **Dr. Joaquin Pastor**, CNIO, Spain
- **Dr. Steve Wedge**, Newcastle University, UK
- **Dr. Martin Drysdale**, Beatson Institute, UK
- **Dr. Robert Williams**, Drug Development Office, CRUK, UK
- **Dr. Ryan Anderson**, Oxford Radiobiology Centre, UK
- **Dr. Manuel Hidalgo**, CNIO, Spain
- **Phil L'Huillier**, Cancer Research Technology, UK
- **Mr. Simon Manley**, British Ambassador to Spain

6TH ESO-CNIO FAMILIAL CANCER CONFERENCE
JUNE 5-6, 2014

ORGANISERS
- **Javier Benítez**, CNIO, Madrid, Spain
- **Rosalind Eeles**, The Royal Marsden Hospital, Sutton, UK
- **Hans Vasen**, Leiden University Medical Centre, Leiden, The Netherlands

SESSIONS
- Common cancers
- Other hereditary cancer syndromes
- New technologies applied to familial cancer studies

SPEAKERS
- **Lauri A. Aaltonen**, University of Helsinki, Finland
- **Javier Benítez**, Spanish National Cancer Research Centre, Madrid, Spain
- **Gabriel Capellà**, Catalan Institute of Oncology, Barcelona, Spain
- **Peter Devilee**, Leiden University Medical Center, Netherlands
- **Diana Eccles**, Southampton University Hospital Trust, Hampshire, UK
- **Rosalind Eeles**, The Royal Marsden Hospital, Sutton, UK
- **Thierry Frebourg**, Rouen University Hospital, France
- **Shirley Hodgson**, St. George's Hospital Medical School, London, UK
- **Eamonn Richard Maher**, University of Birmingham, UK
- **Fred H. Menko**, VU University Medical Center, Amsterdam, Netherlands
- **Roger Milne**, Cancer Epidemiology Centre, Melbourne, Australia
- **Julia Newton-Bishop**, University of Leeds, St James' University Hospital, UK
- **Ana Osorio**, Spanish National Cancer Research Centre, Madrid, Spain
- **Mark Pritchard**, Institute of Translational Medicine, University of Liverpool, UK
- **Mercedes Robledo**, Spanish National Cancer Research Centre, Madrid, Spain
- **Jordi Surrallés**, Autonoma University of Barcelona, Spain
- **Miguel Urioste**, Spanish National Cancer Research Centre, Madrid, Spain
- **Hans Vasen**, Leiden University Medical Centre, Netherlands

- **Alberto Villanueva**, Catalan Institute of Oncology, Barcelona, Spain

In addition, 6 short talks were selected among participants' contributions and 30 posters were presented.

11TH INTERNATIONAL VHL SYMPOSIUM
OCTOBER 23-25, 2014

ORGANISERS
- **Dr. Mercedes Robledo**, CNIO, Spain
- **Dr. José María de Campos**, *Fundación Jiménez Díaz*, Spain
- **Dr. Jesús García-Donas**, *Centro Integral Oncologico Clara Campal*, Spain
- **Dr. James Gnarra**, University of Pittsburgh Cancer Institute, USA
- **Dr. Eric Jonasch**,The University of Texas MD Anderson Cancer Center, USA
- **Dr. Mª Elena Kusak**, *Hospital Ruber Internacional*, Spain
- **Dr. Susi Martinez**, VHL Alliance, Spain
- **Dr. Ilene Sussman**, VHL Alliance, USA
- **Dr. Karina Villar**, VHL Alliance, Spain

SPEAKERS
- **Karel Pacak**, National Institutes of Health, Bethesda, United States
- **Othon Iliopoulos**, Center for Cancer Research, Massachusetts General Hospital Cancer Center, Charlestown, United States
- **Amato Giaccia**, Department of Radiation Oncology, Center for Clinical Sciences Research, Stanford University School of Medicine, Stanford, California, United States
- **Eamonn R Maher**, Department of Medical Genetics, University of Cambridge, United Kingdom
- **Rathmell WK**, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, United States
- **Wilhelm Krek**, Institute of Molecular Health Sciences, ETH Zurich, Zurich, Switzerland
- **Yannick Arlot**, Institut de Génétique et Developpement de Rennes, Rennes, France
- **Marie Louise Mølgaard Binderup.**, Dept. of Cellular and Molecular Medicine, University of Copenhagen, Denmark
- **Jacques Lenders**, Radboud University Medical Center Nijmegen, The Netherlands
- **Ignacio Blanco**, Genetic Counseling and Clinical Genetics Program, Germans Trias Hospital, Barcelona, Spain
- **Eric Jonasch**, Department of Genitourinary Medical Oncology, University of Texas MD, Anderson Cancer Center, Houston, United States
- **Rachel Giles**, University Medical Center Utrecht, The Netherlands
- **Ian Frew**, Institute of Physiology, University of Zurich, Zurich, Switzerland
- **Jaume Capdevila**, Hospital Vall d'Hebron, Barcelona, Spain
- **Cristina Rodríguez-Antona**, Hereditary Endocrine Cancer Group, CNIO, Madrid, Spain
- **José García Arumi**, Department of Ophthalmology, Hospital Valle de Hebrón, Barcelona, Spain
- **Sven Gläsker**, Department of Neurosurgery, Freiburg University Medical Center, Freiburg, Germany
- **José María de Campos**, Department of Neurosurgery, VHL Unit Care, Fundación Jiménez Díaz, Madrid, Spain
- **Mª Elena Kusak**, Department of Neurosurgery, Radiosurgery Unit, Hospital Ruber Internacional, Madrid, Spain
- **Martin K. Walz**, Kliniken Essen-Mitte, Klinik für Chirugie und Zentrum für Minimal Invasive Chirugie, Essen, Germany
- **Carlos Cenjor**, Fundación Jiménez Díaz, Madrid, Spain
- **Jean-Jacques Patard**, CHU, Bicêtre, France
- **Samuel Sommaruga**, Yale University, New Haven, United States
- **Jean-Michel Correas**, AP-HP, Hôpital Bicêtre, Le Kremlin Bicêtre, France
- **Carmen Ayuso**, Department of Genetics, FJD, Chair of the IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain
- **Karina Villa**, SESCAM. Health Service of Castilla-La Mancha, Toledo, Spain. Vice President of *Alianza Española* VHL
- **José M. de Campos**, Department of Neurosurgery, VHL Care Unit, *IIS-Fundación Jiménez Díaz*, Madrid, Spain
- **Roberto Álvarez**, *Fundación Instituto San José. Hermanos de San Juan de Dios*, Madrid, Spain
- **Ilene Sussman**, VHL Family Alliance, USA
- **Susi Martínez**, President of the Spanish Alliance VHL, Spain

In adition, 17 short talks were selected among participants' contributions and 25 posters were presented.

2ND CONGRESS OF THE SPANISH NETWORK OF ADVANCED OPTICAL MICROSCOPY
OCTOBER, 13-15, 2014

ORGANISERS
- CNIO; CBMSO; CSIC with the collaboration of *Fundación Severo Ochoa*

SPEAKERS
- **Peter Friedl**, Radboud University Medical Center
- **Paul French**, Imperial College London
- **Javier Adur**, LAMAE, Microscopy Laboratory Applied to Molecular and Cellular Studies, National University of Entre Rios
- **Joaquim Soriano Felipe**, CNIO
- **Manel Bosch**, CCiT, University of Barcelona
- **Miguel Galarraga**, Center for Applied Medical Research Univeristy of Navarra
- **Petr Walczysko**, College of Life Sciences, University of Dundee
- **Javier García Sancho**, Instituto de Biología y Genética Molecular
- **Augusto Silva**, Althia
- **Jordi Andilla**, The Institute of Photonic Sciences
- **Francisco Porto**, Leica
- **José Rino**, Insituto de Medicina Molecular, *Faculdade de Medicina da Universidade de Lisboa*
- **Nickolaos Nikiforos Giakoumakis**, University of Patras
- **Maria Anna Rapsomaniki**, University of Patras
- **José Requejo-Isidro**, University of the Basque Country, National Research Council of Spain
- **Pablo Loza**, University of St. Andrews
- **Alberto Diaspro**, *Istituto Italiano di Tecnologia* (IIT)
- **Angela d'Esposito**, University College London
- **Gabriel Cristóbal**, Institute of Optics "Daza de Valdés" (IO-CSIC)
- **Sébastien Tosi**, Institute for Research in Biomedicine
- **Julien Colombelli**, Institute for Research in Biomedicine
- **Jorge Ripoll**, *Hospital General Universitario Gregorio Marañón*
- **Daniel Jaque**, Faculty of Sciences, UAM
- **Jordi Sobrino**, Hamamatsu Photonics
- **Corinne Lorenzo**, Paul Sabatier University
- **Jesús Lancis**, Jaume I University
- **Mónica Marro**, The Institute for Photocnic Sciences
- **Rafael Fritz**, University of Basel
- **Javier Díez Guerra**, CBMSO
- **Nuno Moreno**, Gulbenkian Science Institute
- **Cristina Flors**, Institute IMDEA
- **Timo Zimermann**, Centre for Genomic Regulation
- **Spencer Shorte**, Pasteur Institute
- **Tim Bushnell**, University of Rochester Medical Center

TRAINING COURSES AND WORKSHOPS

The CNIO is committed to disseminating the results of state-of-the-art cancer research to the wider community, including medical professionals and junior scientists, 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.

COURSE OF LABORATORY ANIMAL SCIENCE FOR RESEARCHERS CATEGORY C
MARCH 3-14, 2014

ORGANISERS
- **Isabel Blanco**, CNIO, Madrid, Spain
- **Ignacio Álvarez**, Complutense University of Madrid, Spain
- **José M. Orellana**, University of Alcalá, Spain

SPEAKERS
- **Ignacio Álvarez**, Complutense University of Madrid, Spain
- **Javier Benito**, Complutense University of Madrid, Spain
- **Isabel Blanco**, CNIO, Madrid, Spain
- **Argelia Castaño**, Carlos III Institute, Madrid, Spain
- **Ernesto de la Cueva**, Charles River, Madrid, Spain
- **Colin Dunn**, Charles River, UK / Laboratory Animals Ltd
- **Ricardo Feinstein**, National Veterinary Department, Sweden
- **Michael Festing**, Animal Procedures Committee, UK
- **Javier Guillén**, AAALAC International
- **Bryan Howard**, University of Sheffield, UK
- **Marcos Malumbres**, CNIO, Madrid, Spain
- **Antonio Martinez**, GSK, Madrid, Spain
- **Jesús Martínez**, CIEMAT, Madrid, Spain

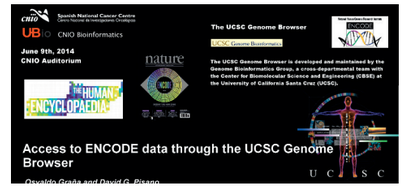
URO-ONCOLOGICAL PATHOLOGY TUTORIAL: A 2-DAY "MEET DE EXPERT" KIDNEY AND BLADDER CANCERS
JUNE 9-10, 2014

ORGANISERS
- **Francisco X. Real and Núria Malats**, CNIO, Madrid, Spain

SPEAKERS
- **Yves Allory**, Henr Modor Hospital & University Paris Est, France
- **Ferran Algaba**, *Fundació Puigvert & Universitat Autonoma de Barcelona*, Spain
- **José M. S. Morgado**, CNIC, Madrid, Spain
- **David Morton**, University of Birmingham, UK
- **Francisca Mulero**, CNIO, Madrid, Spain
- **José Mª Orellana**, University of Alcalá, Spain
- **Sagrario Ortega**, CNIO, Madrid, Spain
- **Belén Pintado**, CNB, Madrid, Spain
- **Sergio Salazar**, Charles River, Paris, France
- **Salvador Nieves**, Cajal Neuroscience Institute, Madrid, Spain
- **Graham Tobin**, Animal Welfare Consultant, UK
- **Patri Vergara**, Autónoma University of Barcelona, Spain

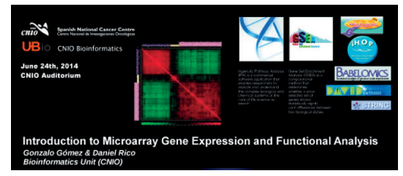
ACCESS TO ENCODE DATA THROUGH THE UCSC GENOME BROWSER
JUNE 9, 2014

ORGANISERS
- **Osvaldo Graña and David G. Pisano**, CNIO, Madrid, Spain



INTRODUCTION TO MICROARRAY GENE EXPRESSION AND FUNCTIONAL ANALYSIS
JUNE 24, 2014

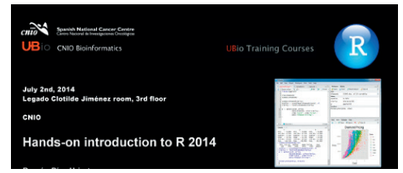
ORGANISERS
- **Gonzalo Gómez and Daniel Rico**, CNIO, Madrid, Spain



HANDS-ON INTRODUCTION TO R 2014
JULY 2, 2014

ORGANISERS
- **Osvaldo Graña and David G. Pisano**, CNIO, Madrid, Spain

SPEAKER
- **Ramón Díaz Uriarte**, "Alberto Sols" Biomedical Research Institute, UAM-CSIC, Madrid, Spain



ACCESS TO GENES AND GENOMES WITH ENSEMBL
SEPTEMBER 23, 2014

ORGANISERS
- **Osvaldo Graña and David G. Pisano**, CNIO, Madrid Spain

SPEAKER
- **Denise Carvalho-Silva**, Ensembl team European Bioinformatics Institute (EBI)

III CORE MANAGEMENT WORKSHOP
OCTOBER 16-17, 2014

ORGANISERS
- **Diego Megias and Lola Martínez**, CNIO, Madrid, Spain

SPEAKERS
- **Mónica Morales**, Centre for Genomic Regulation, Spain
- **Joanne Lannigan**, Virginia University, USA
- **Sebastián Montero**, Leica Microsystems
- **Ernesto de la Cueva Bueno**, Charles River
- **Andreas Sommer**, CSF-Vienna Biocenter, Austria
- **Ray Lannigan**, Cytek Development Inc.
- **Jorge Ripoll**, Bioengineering Dept Carlos III University, Spain
- **Anna Petrunkina**, NIHR Cambridge BRC Cell Phenotyping Hub
- **José María Alonso**, IZASA
- **Owen Hughes**, Millipore MERCK
- **Spencer Shorte**, Pasteur Institute, France
- **Giovanna Roncador**, CNIO, Spain
- **Tiago P. Carvalho**, LabOrders
- **Lindsey Ward/ Herbert Auer**, iLab Solutions LLC
- **Manuel Morente**, CNIO, Spain
- **Tim Bushnell**, ExpertCytometry
- **Jean-Yves Tinevez**, Stratocore
- **Elena Trovesi**, Core Vision in Science

CHANGING ONCOLOGY THROUGH TECHNOLOGY
OCTOBER 28-29, 2014

COURSE COORDINATOR
- **Francisca Mulero**, CNIO, Madrid, Spain

COURSE FACULTY
- **Francisca Mulero, Marisol Soengas and Massimo Squatrito**, CNIO, Madrid, Spain
- **Aitana Calvo, Tatiana Massarrah and Iván Márquez Rodas**, *Hospital General Gregorio Marañón*, Madrid, Spain
- **Soraya Casla**, Technical University of Madrid, Spain
- **Norberto Malpica**, *Universidad Rey Juan Carlos*, Madrid, Spain

PATHOLOGY OF THE EXOCRINE PANCREAS-OF MICE AND MEN
DECEMBER 4-6, 2014

ORGANISERS
- **Fiona Campbell**, Royal Liverpool University Hospital, UK
- **Irene Esposito**, University of Munich, Germany
- **Núria Malats and Francisco X. Real**, CNIO, Madrid, Spain

OTHER COURSES (HELD IN SPANISH)

CNIO researchers also organised several meetings, training courses and workshops in the Spanish language for the local research and public health community.

CURSO TEÓRICO PRÁCTICO DE ONCOLOGÍA TRASLACIONAL
JANUARY 24-25, 2014

ORGANISERS
- **CNIO - Springer**

COORGANISERS
- **Dr. Ramón Colomer**, *Hospital Universitario La Princesa*, Spain
- **Dr. Miguel A. Quintela**, CNIO, Spain

SPEAKERS
- **Dr. Ramón Colomer**, *Hospital Universitario La Princesa*, Spain
- **Dr. Miguel A. Quintela**, CNIO, Spain
- **Dr. Aleix Prat**, *Hospital Universitario de la Vall d'Hebron*, Spain
- **Dr. Juan de la Haba**, *Hospital Universitario Reina Sofía*, Spain
- **Dr. Luis Manso**, *Hospital Universitario 12 de Octubre*, Spain

IV EDICIÓN NUEVAS APLICACIONES Y NUEVAS TECNOLOGÍAS
MAY 6-8, 2014

ORGANISERS
- **Unidad de Microscopia Confocal –CNIO y Leica Microsystems**

SPEAKERS
- **Nathalie Garin**, Leica Microsystem, Switzerland
- **Timo Zimmermann**, CRG, Spain
- **Ralf Jacob**, Philipps University of Marburg, Germany

REUNIÓN PARA LA CREACIÓN DE GRUPOS DE TRABAJO Y DEFINICIÓN DE PROYECTOS DE INVESTIGACIÓN EN IMAGEN MÉDICA
JUNE 4, 2014

ORGANISERS
- **M+VISION CONSORTIUM - CNIO**

SPEAKERS
- **Francisca Mulero**, CNIO, Spain
- **Eduardo Fraile**, *Unidad Central de Radiodiagnóstico*, Spain
- **Mario Moya**, Madrid-MIT M+Vision Consortium, Spain
- **Germán González**, Madrid-MIT M+Vision Consortium, Spain
- **Mª Jesús Ledesma**, *Universidad Politécnica de Madrid*, CIBERBBN, Spain
- **Raúl Sanjosé**, Harvad Medical School, USA

TALLER CEGEN-PRB2: DISEÑO Y ANÁLISIS DE DATOS DE ESTUDIOS DE ASOCIACIÓN CON SNP_S
NOVEMBER 24, 2014

ORGANISERS
- **Dr. Javier Benítez**, CNIO, Spain
- **Dr. Ángel Carracedo**, NODO CeGen, Spain

SPEAKERS
- **Dr. Javier Benítez**, CNIO and *Nodo del CeGen-ISCIII*, Spain
- **Dr. Mercedes Robledo**, CNIO, Spain
- **D. Guillermo Pita**, CeGen-CNIO, Spain
- **Tais Moreno**, CeGen-CNIO, Spain
- **Dr. Pablo Fernández**, *Centro Nacional de Epidemiología*, ISCIII and CIBERESP, Spain
- **Ana Osorio**, CNIO, Spain
- **Dr. David González Pisano**, CNIO, Spain
- **Sara Ruiz**, CNIO, Spain
- **Veronika Mancikova**, 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, three of these seminars were sponsored by the French Embassy.

The purpose of this international seminar series is not limited to bringing outstanding cancer researchers to the CNIO, but also serves to annually invite 3 to 4 opinion leaders from other areas of science, technology, and literature; the overarching goal is to enable the CNIO to present its know-how as well as its vision on contemporary and future technological, societal and cultural challenges. These "out-of-the-box" seminars are sponsored by the "Fundación Banco Sabadell". The breadth of expertise and topics covered creates a multidisciplinary and intellectually challenging environment that goes far beyond the frontiers of cancer research.

In total, the CNIO hosted 30 distinguished speakers in 2014, among which, 1 Nobel Prize winner.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
10/01/2014	**Ada Yonath**	Weizmann Institute of Science, Rehovot, Israel	From basic science to species-specific resistance to antibiotics
17/01/2014	**Caterina Biscari**	Director of CELLS-ALBA Barcelona, Spain	Particle accelerators from fundamental physics to Oncologic Therapies
31/01/2014	**Jens Siveke**	III Medical Clinic, Technical University Munich, Germany	Novel targeting strategies in pancreatic cancer - a GEMM-based approach
FEBRUARY			
21/02/2014	**Eyal Gottlieb**	Beatson Institute, Glasgow, Scotland	Alternating essential metabolic requirements of cancer cells
MARCH			
14/03/2014	**James A. Fagin**	Memorial Sloan-Kettering Cancer Center, NY, USA	Development and Implementation of Mechanism-Based Therapies for Thyroid Cancer
21/03/2014	**Gioacchino Natoli**	European Institute of Oncology, Milan, Italy	Transcriptional and epigenetic mechanisms in inflammation and inflammation-driven cancer
28/03/2014	**Peter Sicinski**	Dana-Farber Cancer Institute, Boston, USA	Cell cycle machinery in development and in cancer
APRIL			
10/04/2014	**Richard Flavell**	Yale School of Medicine, New Haven, USA	Inflammasomes in health, dysbiosis, and disease
11/04/2014	**Stephen Blacklow**	Harvard Medical School - Dana Farber Cancer Institute, Boston, USA	Unraveling Mechanisms of Normal and Oncogenic Notch Activation
25/04/2014	**Albert J. R. Heck**	Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences Utrecht University, The Netherlands	Novels insights into Systems Biology by Proteogenomics, Phosphoproteomics and Immunoproteomics
29/04/2014	**Josep LLovet**	ICREA-IDIBAPS, Clinic Hospital, Barcelona, Spain	Molecular pathogenesis and targeted therapies
MAY			
09/05/2014	**Stefano Piccolo**	University of Padova Institute of Histology and Embryology Viale, Padova, Italy	The Hippo pathway from organ size to malignancy: functions and regulations of YAP/TAZ
30/05/2014	**Peter Nussbaumer**	Lead Discovery Center GmbH, Dortmund, Germany	The Discovery and Potential Application of Selective Inhibitors of Rab GeranylGeranylTransferase

JUNE				
13/06/2014	**Emmanuel Barillot**	Institut Curie, Paris, France	Precision medicine and network modeling in oncology	
20/06/2014	**Matthias Hebrok**	Diabetes Center, University of California, San Francisco, USA	The role of Chromatin remodeling in pancreatic cancer	
27/06/2014	**Victoria Seewaldt**	Duke University School of Medicine, Durham, USA	Is breast cancer risk determined by what our mothers ate? Imprinting and aggressive biology of triple-negative breast cancer in African American women	
SEPTEMBER				
19/09/2014	**Rafael Rosell**	Catalan Institute of Oncology; Hospital Germans Trias i Pujol, Barcelona, Spain	Moving from singly targeted therapy to co-targeted therapy in lung cancer	
26/09/2014	**Alberto Bardelli**	Institute for Cancer Research of Candiolo - IRCCS, Torino,Italy	Molecular-driven therapies for colorectal tumors	
OCTOBER				
03/10/2014	**Sam M. Hanash**	The University of Texas MD Anderson Cancer Center, Houston, USA	Integrating biomarkers into lung cancer screening	
17/10/2014	**Félix M. Goñi**	Biophysics Unit - CSIC-UPV/ EHU, Vizcaya, Spain	A biochemist in the kitchen	Fundación BancoSabadell
24/10/2014	**Iain Cheeseman**	Whitehead Institute for Biomedical Research, Cambridge, USA	Guarding genomic stability during mitosis	
31/10/2014	**Hendrik Stunnenberg**	Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen, The Netherlands	Epigenomics: functional indexing genomes in health and disease	
NOVEMBER				
04/11/2014	**Jean-Pierre Changeux**	*Collège de France Institut Pasteur*, Paris, France	Allosteric mechanisms of signal transduction investigated with pentameric ligand gated ion channels: consequences for drug design	
07/11/2014	**Shahragim Tajbakhsh**	Pasteur Institute, Paris, France	Molecular determinants of stem cells during development and regeneration	
14/11/2014	**Rosalind Eeles**	Cancer Research UK, London, UK	Genetic predisposition to prostate cancer and its clinical implications	
21/11/2014	**Bruce Stillman**	Cold Spring Harbor Laboratory, NY, USA	The mechanism and control of the initiation of chromosome DNA replication	
28/11/2014	**Anja Groth**	Biotech Research and Innovation Centre, University of Copenhagen, Denmark	Chromatin Replication and Epigenome Maintenance	
DECEMBER				
05/12/2014	**François Burgat**	The National Center for Scientific Research; Institute for Research and Study on the Arab and Muslim Worlds (IREMAM) Aix en Provence, France	"Tunis" or "Mosoul": Which way out of the Arab Spring?	Fundación BancoSabadell
12/12/2014	**Enrique Dans**	IE Business School, Madrid, Spain	Health and analytics: rethinking potential scenarios	Fundación BancoSabadell
19/12/2014	**Joan Margarit**	Poet and Architect, Barcelona, Spain	It wasn't far away or difficult / No estaba lejos, no era difícil	Fundación BancoSabadell

AD-HOC SEMINARS

In addition to the CNIO Distinguished Seminar Series, the CNIO also hosts numerous *ad-hoc* seminars throughout the year. A total of 41 *ad-hoc* seminars were organised by CNIO researchers in 2014.

DATE	SPEAKER	ORGANISATION	TITLE
JANUARY			
03/01/2014	**Gianlucca Varetti**	Dana-Farber Cancer Institute, Boston, USA	Understanding the Molecular Causes of Micronuclei Defects
21/01/2014	**Thomas Helleday**	Torsten and Ragnar Söderberg Professor of Translational Medicine Science for Life Laboratory, Karolinska Institutet, Stockholm, Sweden	MTH1 inhibitors for a depersonalised treatment of cancer
24/01/2014	**Lluís Morey**	Centre for Genomic Regulation (CRG), Barcelona, Spain	Role of Polycomb complexes in embryonic stem cells pluripotency and differentiation
FEBRUARY			
20/02/2014	**Zachary A. Gurard-Levin**	*Institut Curie - Centre de Recherche, Unité "Dynamique du Noyau"*, Paris, France	Chromatin regulators: new opportunities for biomarkers and therapeutic targets
25/02/2014	**Stephen Hague**	Bio-Rad Laboratories Ltd, Warrington, UK	Droplet Digital PCR Demo
27/02/2014	**Sara Kozma**	IDIBELL, Barcelona, Spain	Translating basic mTOR signaling knowledge into hepatocellular carcinoma treatment
MARCH			
11/03/2014	**Madalena Tarsounas**	The CR-UK/MRC Gray Institute for Radiation Oncology and Biology University of Oxford Oxford - UK	Roles of BRCA1 and BRCA2 tumour suppressors in telomere maintenance and genome integrity
24/03/2014	**Christian Speck**	MRC Clinical Sciences Center, London, UK	Structure and loading mechanism of the replicative helicase
APRIL			
02/04/2014	**Johannes Walter**	Harvard Medical School, Boston, USA	Mechanisms of DNA Replication-Coupled Repair
04/04/2014	**Manuel Valiente**	Memorial Sloan Kettering Cancer Center, New York, USA	Deconstructing metastatic disease in the brain
21/04/2014	**Inke Nathke**	The University of Dundee, Scotland, UK	Changes in the organisation of cells and tissue at early stages of colon cancer

MAY				
12/05/2014	**Antonio del Sol**	Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg	Systems Biology Approaches to Cellular Reprogramming and Cellular Disease Models	
12/05/2014	**Roberto Ferrari**	University of California, Los Angeles, USA	Epigenomics of Cell Transformation and Development Fate Decision	
13/05/2014	**James Brugarolas**	The University of Texas Southwestern Medical Center, Dallas, USA	Translating cancer genome discoveries into patient care	
27/05/2014	**Capitolina Diaz**	University of Valencia, Spanish Association of Women in Science and Technology, Valencia, Spain	Networking and Career building in Science	WISE OFFICE
JUNE				
06/06/2014	**Nuria Gago López**	University of Washington, Seattle, USA	Identification of Multipotent Adult ProgenitorCells from Human Cardiospheres with Divergent Differentiation Potential	
12/06/2014	**David Torrents**	ICREA Research Professor; BSC-IRB Research Programme in Computational Biology, Barcelona, Spain	Identification of complex Karyotypes in cancer genomes	
12/06/2014	**Alexandre Darmoise**	Field Applications Scientis, Fluidigm Sciences	Massively Informative: Fundamentals and Applications of CyTOF* Mass Cytometry	
16/06/2014	**Mikhail Nikiforov**	Roswell Park Cancer Institute, NY, USA	Unsuspected role of nucleotide metabolism in melanoma	
24/06/2014	**Lars Zender**	University Hospital Tübingen, Germany	Direct in vivo RNAi Screening for accelerated Cancer Gene Discovery	
JULY				
03/07/2014	**Francesco Acquadro**	Clinical Application Consultant at Thermo Fisher Scientific	Flexibility: the key aptitude - Moving between countries and Jobs	Jesús Serra Seminar
10/07/2014	**Christian Dillon**	Cancer Research Technology Limited, London, UK	Targeting aberrant polarity in cancer: an atypical (PKC) approach	
14/07/2014	**Andreas M. Beyer**	Medical College of Wisconsin, Assistant Professor of the Department of Medicine, Division of Cardiology, Cardiovascular Research Center, Milwaukee, Wisconsin, USA	Protective Role of Telomerase in the Mechanism of Flow Mediated Dilation in Resistance Vasculature	
15/07/2014	**Martha Gray**	Harvard-MIT HST; MIT EECS; Research Lab of Electronics; IMES, Cambridge, USA	What is it like to be a girl professor?	WISE OFFICE
16/07/2014	**Alexander Fleischmann**	Center for Interdisciplinary Research in Biology (CIRB) Collège de France Paris, France	Genes, circuits, and behavior: from the nose to the brain	
16/07/2014	**Oscar Llorca**	Spanish National Research Council (CSIC); Center of Biological Research (CIB),Madrid, Spain	Molecular and Structural basis for DNA repair and RNA degradation by the PI3K-like kinases	
17/07/2014	**Maria Grazia Ruocco**	Immunologie, Immunopatholgie, Immnunothérapie. Pierre and Marie Curie University, Paris, France	Immune tolerance during pregnancy and its implications for cancer	
18/07/2014	**Eva Nogales**	Bioenergy/GTL & Structural Biology, University of California, Berkeley, USA	Structural Studies into the Mechanistic Origin of Microtubule Dynamic Instability	
24/07/2014	**Angeles de Cara**	Musem National d'Histoire Naturelle, Paris, France	Estimation of identity by descent from genomic data: Runs of homozigosity	
28/07/2014	**Jose Luis Ambite**	University of Southern California, Information Sciences Institute, USA	Data Integration in BioInformatics: Three (not so) easy pieces	
SEPTEMBER				
22/09/2014	**Gianluca Varetti**	Dana-Farber Cancer Institute, Boston, USA	Understanding the Molecular Causes of Micronuclei Defects	
30/09/2014	**Margarita Alonso**	Director of IE Foundation, Madrid, Spain	Women leadership, Advocacy or Diversity Management	WISE OFFICE
OCTOBER				
22/10/2014	**Eduardo Vilar-Sánchez**	The University of Texas MD Anderson Cancer Center, Houston, USA	Genomic characterization of intestinal polyps and targeted chemoprevention drug development in Hereditary Colorectal Cancer Syndromes	
23/10/2014	**Peter Petzelbauer**	University of Vienna Medical School, Austria	Endothelial barrier function and its role in disease	
23/10/2014	**Inder M. Verma**	Salk Institute for Biological Studies, La Jolla, USA	Cancer Stem Cells: Lessons From Glioblastoma	
27/10/2014	**Maximina H Yun**	University College London, Institute of Structural and Molecular Biology, UK	Molecular mechanisms of salamander limb regeneration	
30/10/2014	**Kaustuv Basu**	Cancer Research, Uppsala University, Sweden	Importance of hyaluronan in age related disease and cancer	
NOVEMBER				
06/11/2014	**Karlene Cimprich**	Stanford University, School of Medicine, USA	Novel Mechanisms for the Maintenance of Genome Stability	
06/11/2014	**Alexander James Roy Bishop**	Greehey Children's Cancer Research Institute, The University of Texas Health Science Center at San Antonio, USA	Alkylation: mechanisms of cytotoxicity, inflammation and strategies to augment chemotherapy efficacy in basal breast cancers	
18/11/2014	**Véronique Orian-Rousseau**	Karlsruhe Institute of Technology (KIT), Germany	Targeted CD44 therapy in pancreatic cancer	
DECEMBER				
15/12/2014	**Paula Inés Papp**	AFI, Madrid, Spain	Women in Finance	WISE OFFICE

SCIENTIFIC DIVULGATION EVENTS

RESEARCHERS' NIGHT
SEPTEMBER 26, 2014

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 26, 2014) 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.

OPEN DOORS DAY: INVESTIGATING TO DISARM CANCER
NOVEMBER 3-16, 2014

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 (*XIII Semana de la Ciencia*, November 3 –16, 2014). In 2014, approximately 100 people participated in guided visits to the Centre's research facilities over the course of 2 days.

GEPAC
NOVEMBER 7-9, 2014

This year, the CNIO participated for a second 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, AEAL, 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 second year running. The idea was to be present so that we could answer people's questions about cancer research and the latest developments.

GUIDED VISITS

Throughout the year, the CNIO provides tailor-made opportunities to visit its installations and to learn about the essentials of cancer research. During 2014, more than 315 people participated in such guided visits; most of them were ESO and Bachillerato student groups, but also professionals in the health sector.

BCNMOMENTS
JULY 22-25, 2014

During this year, the company bcnmoments organised the 'Leading Program Madrid'; a programme sponsored by the "*la Caixa*" Foundation that awards the 20 highest *selectividad* test scores within the Community of Madrid. The selected students had the opportunity to get to know different success stories in a broad range of companies and institutions, including the CNIO. During their "Business Experience" at the CNIO, the students had the chance to visit the labs guided by young scientists, and to attend two master classes given by a senior Group Leader and the Director of Innovation.

MUNCYT SUMMER SCIENCE CAMP 2014
JUNE 30 TO AUGUST 15, 2014

In line with the Centre's commitment to science education, the CNIO also collaborates with the *Instituto de Salud Carlos III* to host some of the activities of the MUNCYT Summer Science Camp; a summer programme promoted by the National Museum of Science and Technology (Muncyt) and the Spanish Foundation for Science and Technology (FECYT), aimed at children between the ages of 6 and 12. This activity provides children an enriching educational experience by fostering their learning and creativity through play, hands-on science experimentation and scientific thinking. This year, the CNIO hosted 7 half-day camps coordinated by the Confocal Microscopy and Flow Cytometry Units. Approximately 150 children participated in these activities.

ADMINISTRATION

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* In accordance with the Spanish Transparency Legislation (Spanish Royal Decree 451/2012, of March 5), the following information is hereby provided:
- At the close of the financial year, the accumulated remuneration received by the Top Management of the Foundation – the CNIO's Director plus the Managing Director – has amounted to a total of 254,944 Euros. This amount was received as base salary, seniority, small bonuses and variable component salary.
- Members of the CNIO Board of Trustees are not remunerated.

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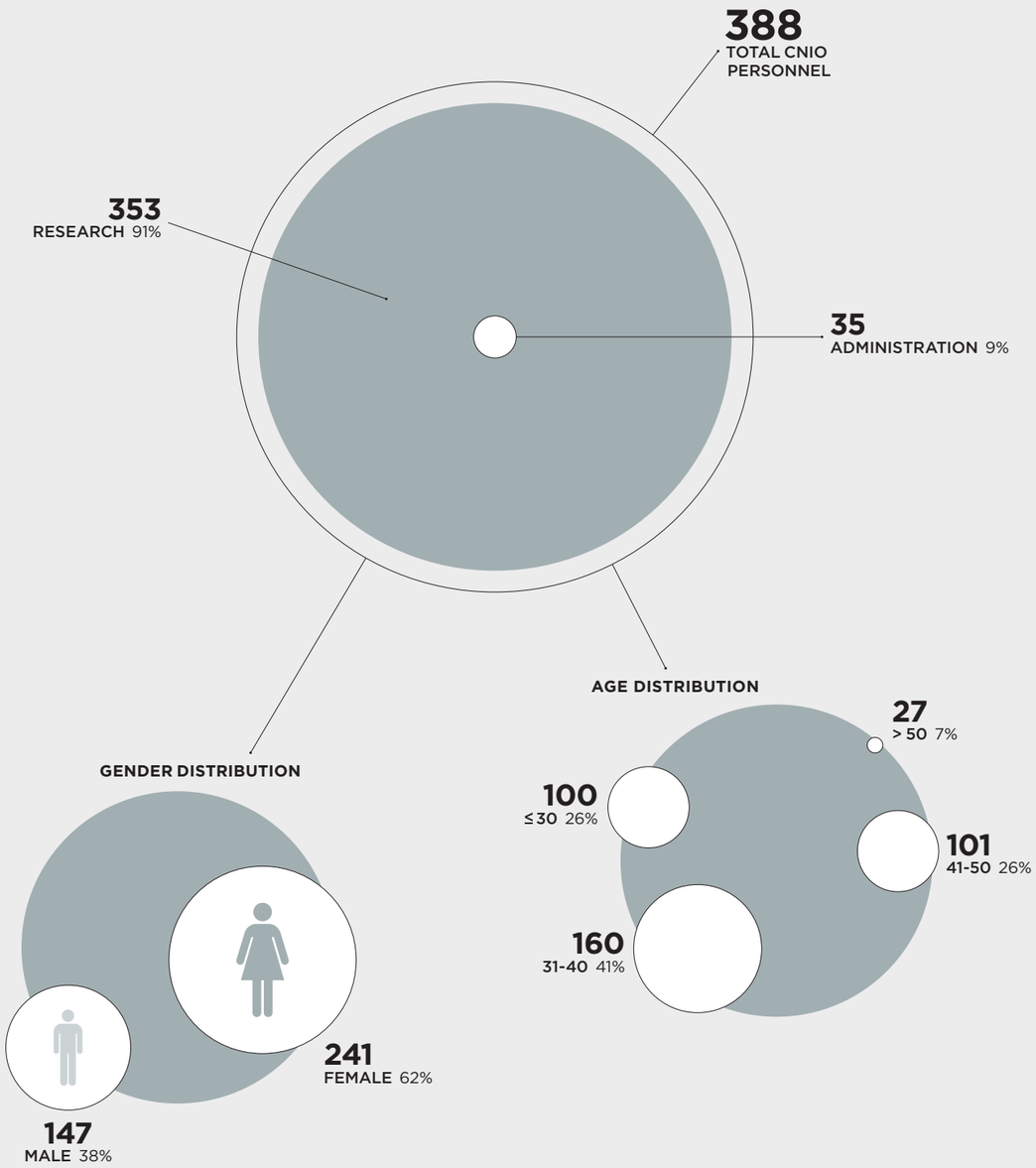
CNIO PERSONNEL 2014



SCIENTIFIC PERSONNEL 2014

TOTAL SCIENTIFIC PERSONNEL 100% **353**

DISTRIBUTION BY PROGRAMMES

STRUCTURAL BIOLOGY AND BIOCOMPUTING 13%	44
BIOTECHNOLOGY 10%	36
BBVA FOUNDATION-CNIO CANCER CELL BIOLOGY 13%	45
HUMAN CANCER GENETICS 13%	47
CLINICAL RESEARCH 12%	41
MOLECULAR ONCOLOGY 30%	107
EXPERIMENTAL THERAPEUTICS 9%	33

DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 13%	45
GRADUATE STUDENTS 23%	82
STAFF SCIENTISTS 18%	64
PRINCIPAL INVESTIGATORS 13%	46
TECHNICIANS 33%	116

GENDER DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS	FEMALE	47%	**21**
	MALE	53%	**24**
GRADUATE STUDENTS	FEMALE	72%	**59**
	MALE	28%	**23**
STAFF SCIENTISTS	FEMALE	64%	**41**
	MALE	36%	**23**
PRINCIPAL INVESTIGATORS	FEMALE	37%	**17**
	MALE	63%	**29**
TECHNICIANS	FEMALE	73%	**85**
	MALE	27%	**31**
TOTAL SCIENTIFIC PERSONNEL	FEMALE		**223**
	MALE		**130**

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: BASIC RESEARCH

TOTAL SCIENTIFIC PERSONNEL 100% **353**

POST-DOCTORAL FELLOWS 18%	35
GRADUATE STUDENTS 32%	62
STAFF SCIENTISTS 19%	38
PRINCIPAL INVESTIGATORS 10%	19
TECHNICIANS 21%	42
TOTAL 100%	196

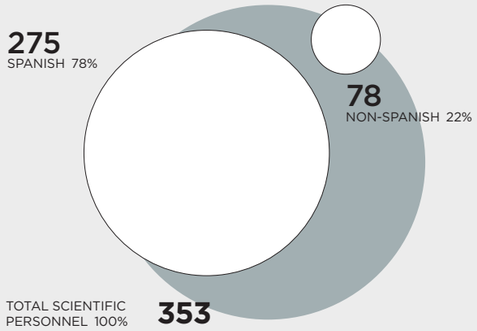
DISTRIBUTION BY PROFESSIONAL CATEGORY IN: TRANSLATIONAL RESEARCH

POST-DOCTORAL FELLOWS 10%	9
GRADUATE STUDENTS 22%	19
STAFF SCIENTISTS 12%	11
PRINCIPAL INVESTIGATORS 15%	13
TECHNICIANS 41%	36
TOTAL 100%	88

DISTRIBUTION BY PROFESSIONAL CATEGORY IN: INNOVATION

POST-DOCTORAL FELLOWS 1%	1
GRADUATE STUDENTS 1%	1
STAFF SCIENTISTS 22%	15
PRINCIPAL INVESTIGATORS 20%	14
TECHNICIANS 56%	38
TOTAL 100%	69

SCIENTIFIC PERSONNEL: NATIONAL ORIGIN

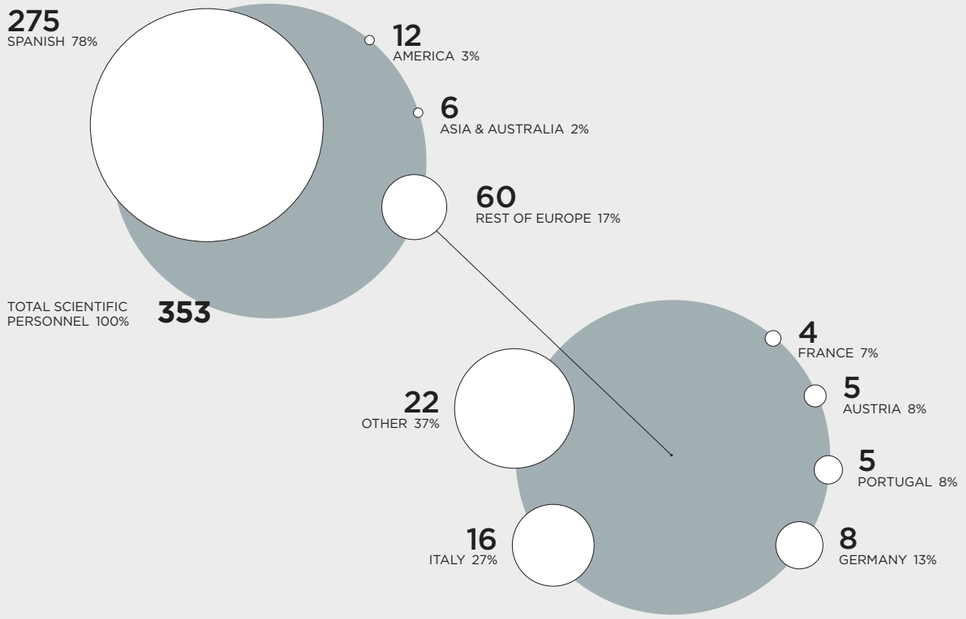


FOREIGN SCIENTIFIC PERSONNEL: DISTRIBUTION BY PROFESSIONAL CATEGORY

POST-DOCTORAL FELLOWS 44%	20
GRADUATE STUDENTS 38%	31
STAFF SCIENTISTS 17%	11
PRINCIPAL INVESTIGATORS 13%	6
TECHNICIANS 9%	10

Total foreign scientific personnel 78
Percent values represent percentages of foreign employees of the total CNIO personnel in each category

DISTRIBUTION OF SCIENTIFIC PERSONNEL BY NATIONAL ORIGIN



CREATIVE TEAM

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

AMPARO GARRIDO PHOTOGRAPHY

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

Underbau is a design studio that emerged in 2008 from the partnership of two freelance designers with 10 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. Underbau's total-design approach puts the emphasis on e ciency and coherency. To achieve that, the studio assumes full responsibility for the entire creative process, from the initial concept to the final product. The working team for the 2014 Scientific Report is formed by Nicolás García, Tania Martínez, Javier Pividal, Pablo Suárez and Juanjo Justicia.

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Supervision **Isabel Barthelemy**
Text, data and figures **CNIO Faculty and Management**
Publication database **Victoria López**
Photography **Amparo Garrido**
Design **underbau**

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